# Pharmacokinetic Drug-Drug Interaction Studies of Antiplatelet Agents with Antidiabetic Agents in Rats

**THESIS**

Submitted in partial fulfilment

of the requirements for the degree of

**DOCTOR OF PHILOSOPHY**

By

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**PILANI (RAJASTHAN) INDIA.**

**2008**

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# CERTIFICATE

This is to certify that the thesis entitled "**Pharmacokinetic Drug-Drug Interaction Studies of Antiplatelet Agents with Antidiabetic Agents in Rats**" and submitted by **Varanasi Venkata Sesha Kanthi Kiran** ID No **2004PHXF015** for award of Ph. D. Degree of the Institute embodies original work done by him under my supervision.

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# ACKNOWLEDGEMENTS

This work was carried out at the Department of Pharmacokinetics and Drug Metabolism, Glenmark Research Centre, during the years 2004 to 2008. I am grateful to all those who helped me during this period.

I am especially grateful to Dr. Swaroop Kumar VS Vakkalanka, President-Drug Discovery and Clinical Development, for his encouraging supervision. His broad knowledge in Drug Discovery and in science has made a great impression on me. He has created with his warmth an inspiring environment for productive scientific work.

I am sincerely thankful to Prof. L.K.Maheshwari, Vice chancellor, BITS, Pilani for providing me an opportunity to do my doctoral thesis work. I express my heartfelt gratitude to Prof. Ravi Prakash, Dean, Research & Consultancy division, and the staff at BITS for their kind and continous support for making this work as success.

I am very grateful to Mr. Glenn Saldhana, Chief Executive officer, Glenmark Pharmaceutical Limited, India for providing this opportunity to do my work at Glenmark Research Centre, Navi Mumbai, India and also for his encouragement through out the period.

I wish to express my sincere thanks to Mr. Satheeshmanikandan TRS, Mr. Suresh Potharaju, Mr. Sridhar Veeraraghavan and Ms. Shraddha Rajak for their continuous support throughout this work. I also express my gratitude to Dr. Srinivas Gullapalli, Dr. Srinivasa Raju, Dr. Kasiram and Dr. Chandra Vemavarapu for their thorough review and constructive criticism concerning this work.

I also thank my junior colleagues Ms. Pankajini Mallick, Mr. Sivakumar SPN, Mr. Sabhapathi, Mr. Suresh Ponniyan, Mr. Anand Patil, Ms. Aparna and Mr. Rambabu Arla for their skillful technical assistance. I wish to thank all my colleagues at the Glenmark Research Centre for their support.

My warmest thanks are due to my parents, my wife, my children and my family for their love and moral support through out this research work with which this work is successfully completed.

(Kanthi Kiran)

# ABSTRACT

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The aims of this work were to investigate the drug-drug interaction effects of antiplatelet agents, cilostazol (CLZ) and pentoxifylline (PTX) as model drugs, on the pharmacokinetics and pharmacodynamics of the oral antidiabetic drugs, rosiglitazone (RSG), metformin (MET), nateglinide (NTG) and glipizide (GLP), and also to investigate the effects of these antidiabetic drugs on the pharmacokinetics and pharmacodynamics of antiplatelet agents vice versa in male wistar rats. In addition, the inhibition effects of these drugs on different CYP isozymes responsible for the metabolism of other drugs were also studied. The plasma protein binding interactions were also studied for all these drugs.

A minimum of 6 rats were used in each study, except in pharmacodymic group where 3 rats were used to estimate the antiplatelet activity. All the studies were sequential or parallel studies with 3 to 4 groups in each drug-drug interaction study. Pretreatment with the single dose or multiple dose of effector drug was followed by a single dose of test drug, where after blood samples were collected for the determination of plasma drug concentrations.

CLZ has moderately increased the plasma concentrations of all these oral antidiabetic agents except NTG. CLZ has significantly increased the maximum observed plasma concentrations ($C_{max}$) of RSG, MET and GLP but decreased that of NTG. It has also elevated the systemic exposure (AUC) of MET. It has increased the glucose lowering activity of GLP in normal rats and thus alarming the chances of hypoglycemia in clinical settings. The changes caused by CLZ on all these drugs could be due to complex mechanisms interacting at absorption, distribution and excretion but not metabolism to major extent.

All the antidiabetic agents investigated in this study have moderately increased plasma levels of CLZ up on their multiple doses pretreatment. The $C_{max}$ and AUC of CLZ were increased significantly in presence of RSG, MET and GLP. It was accompanied by decrease in the $C_{max}$ of DCLZ, an active metabolite of CLZ in presence of RSG and MET but increased in presence of GLP. The antiplatelet activity of CLZ in rats was improved in presence of GLP that may be beneficial during clinical settings. However, the increased

levels of CLZ in presence of GLP is highly alarming and dosage adjustment may be required if similar findings are established in humans.

PTX has overall decreased the plasma levels of all these antidiabetic agents studied. PTX has decreased the $C_{max}$ of RSG and NTG but has reduced the AUC of MET and NTG. PTX has also increased the glucose lowering activity of GLP in normal rats though there was slight drop in $C_{max}$ and thus alarming the hypoglycemia in clinical settings. However, it did not change the glucose lowering activity of NTG and other drugs. The changes caused by PTX on all these drugs could also be due to complex mechanism interacting at absorption, metabolism to major extent and distribution, excretion to minor extent.

The antidiabetic agents investigated in this study have moderately decreased plasma levels of PTX up on their multiple doses pretreatment. The $C_{max}$ and AUC of PTX were decreased in presence of RSG and MET but increased in presence of NTG and GLP. The antiplatelet activity of PTX was improved in presence of GLP but decreased in presence of MET. These mechanisms of these interactions could not be identified due to complex mechanisms. Overall the dosage adjustment of PTX needs to checked due to its short half-life and decreased levels in presence of most of the antidiabetic drugs studied.

The pharmacokinetics of antidiabetic agents studied in this research was largely influenced by the antiplatelet agents and hence the dosage adjustments may be required for RSG, MET and GLP in presence of CLZ and PTX if they mimic the same in clinical conditions. At the same time the pharmacokinetics of CLZ and PTX were also influenced by all these antidiabetic drugs upon multiple doses and are needed to be investigated further.

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# LIST OF SYMBOLS AND ABBREVIATIONS

| Symbol/Abbreviation | Full form/Meaning |
|---|---|
| % | percent |
| ~ | approximate |
| °C | degree Celsius (centigrade) |
| µ | micron |
| µg | microgram |
| µL | micro liter |
| a | slope |
| ABI | ankle-brachial index |
| ADA | Anti Diabetic Agents |
| ANOVA | Analysis of Variance |
| AQ | aqueous |
| $AUC_{0-\infty}$ | The area under the plasma concentration versus time curve, from zero to infinity. |
| $AUC_{0-t}$ | The area under the plasma concentration versus time curve from time zero to the last measurable concentration, as calculated by the linear trapezoidal method. |
| b | intercept |
| BLQ | Below Limit of Quantitation/Quantification |
| CAD | coronary artery disease |
| CC | Calibration Curve |
| CDER | Center for Drug Evaluation and Research |
| CFR | Code for federal regulation |
| CLI | critical limb ischemia |
| CLZ | Cilostazol |
| cm | Centimetre |
| $C_{max}$ | Maximum Measured Plasma Concentration |
| Conc. | concentration |
| CRP | C-reactive protein |
| CV | Coefficient of Variance |
| DCLZ | 3,4-dehydro-cilostazol |
| G/g | gram |
| GLP | Glipizide |
| HCl | hydrochloride/hydrochloric acid |
| HPLC | high performance liquid chromatography |

| Symbol/Abbreviation | Full form/Meaning |
|---|---|
| HQC | high quality control |
| hr | Hour |
| Hr | hour |
| i.e. | That is |
| ID | identification |
| IS | Internal Standard |
| IU | International Unit |
| $K_3$EDTA | Tripotassium ethylene diamine tetraacetic acid |
| $K_{el}$ | Apparent first order elimination or terminal rate constant calculated from a semi-Log plot of the plasma concentration versus time curve |
| Kg | Kilogram |
| LC-MS/MS | liquid chromatography coupled with tandem mass spectroscopy |
| LDL | low-density lipoprotein |
| LOQ | limit of quantitation/quantification |
| LQC | low quality control |
| M | Missing samples |
| Max | Maximum |
| MD | Multiple dose |
| MET | Metformin |
| MI | myocardial infarction |
| Min | minute |
| mL | milliliter |
| mM | milli molar |
| MQC | medium quality control |
| MRM | multiple reaction monitoring |
| MV | method validation |
| MW | molecular weight |
| n | number of samples |
| No./no. | count/number |
| NAP | Not Applicable |
| ng | nanogram |
| NO | nitric oxide |
| No. | Number |
| NTG | Nateglinide |
| p | Probability |
| P & A | Precision and Accuracy |

| Symbol/Abbreviation | Full form/Meaning |
|---|---|
| p.a.r. | peak area ratio |
| PAD | peripheral arterial disease |
| PIO | Pioglitazone |
| PK | Pharmacokinetics |
| PRP | Platelet Rich Plasma |
| PPP | Platelet Poor Plasma |
| Psi | pounds per square inch |
| PTX | Pentoxifylline |
| QA | Quality Assurance |
| QC | Quality Control |
| Qty | Quantity |
| $r^2$ | coefficient of determination |
| rpm | revolutions per minute |
| RSD | Relative Standard Deviation |
| RSG | Rosiglitazone |
| RT | Retention Time |
| SD | Single Dose |
| S.D | Standard Deviation |
| Sec | seconds |
| SOP | Standard Operating Procedure |
| Std. | Standard |
| $t_{1/2}$ | Time required for the plasma drug concentration to decrease to half of the drug concentration |
| $T_{max}$ | Time of maximum measured plasma concentration. |
| UKPDS | United Kingdom Prospective Diabetes Study |
| ULOQ | Upper Limit of Quantitation/Quantification |
| USFDA | United states Food and Drug Administration |
| USP | United States Pharmacopoeia |
| v/v | volume/volume |
| Vs | Versus |
| w/v | weight/volume |

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# Chapter 1

# INTRODUCTION

# 1 INTRODUCTION

Therapeutic treatment of chronic diseases often involves concomitant medications that include two or more pharmacological agents. Some drug products contain several pharmacologically active ingredients. Furthermore some patients may suffer from more than one disease and will be treated accordingly with different classes of drugs simultaneously. Increasingly, more than one physician is responsible for the care for each patient and this may lead to the prescription of more drugs at a time. These entire factors are responsible for potential drug - drug interaction which may have beneficial or adverse effects. So physicians need to have precise data on not only the existence of interactions but on the frequency of occurrence and biological importance of the event. The clinical significance of these drug interactions is very important, in the therapeutic scenario (Ament et al., 2000). Drug interactions are important cause of adverse drug reactions. They are likely to cause serious consequences when they affect elderly or seriously ill patients. Patients at particular risk includes those with renal or hepatic disease, those on long term therapy for chronic disease, for example those with HIV infection, epilepsy or diabetes, those in intensive care, transplant recipients, patients undergoing complicated surgical procedures and those with more than one prescribing doctor.

Drug interactions are of two type's viz. drug-drug interactions, drug-non drug interactions (Food interactions). Drug interactions concern only with drug-drug explains the ways in which drugs may interact with each other eg: altering absorption, biotransformation, excretion, protein binding, action at receptor sites etc. Some drugs may interact with food (eg. furafylline inhibits caffeine clearance) or foodstuff which contain potent pharmacological principles may interact with drugs (eg. components of grapefruit juice can block terfenadine metabolism). These drug-drug interactions and drug-non drug interactions may be either beneficial or harmful to the patient. While studying the various drug interactions, a care should be exercised especially on those drug interactions where the likely to be clinically significant. Eg. Hypertensive crisis, serious drops in blood pressure, haemorrhage, seizures, cardiac arrhythmias, hypoglycemia and central disorders.

The investigations on such drug-drug interactions are used to be a part of later phases of clinical development. Usually they are based on the empirical observation of patients subjected to varying drug therapies in a clinical setting. However, in vitro data can give certain knowledge about these interactions during the preclinical phase of drug

development only. Especially when mechanism of action to novel, these pre-clinical studies may have uncertain pharmacodynamic or toxicity relevance to humans and for those reasons overall studies should be designed very carefully by the pharmaceutical companies. These represent the most expensive phase of development and consequently, failures at that stage will complete jeopardize substantial investments. Hence some in vitro studies and preclinical studies will predict well about these possible interactions and there by reduces the further cost of drug development.

An early indicator of risk likely to encountered during clinical trials will be valuable in directing the design of the studies, present in appropriate administration of the candidate drug and then avoid unnecessary risk to patient participates in the trials. Preclinical and clinical data are also contained on the label of new medicine and therefore available to prescribe for benefit of patient in the healthcare. If conducted early enough in drug discovery, clinically predictive interaction studies may provide data to assist the design of a new molecule with a reduced potential to cause adverse events of 194 new drug products approved by USFDA between 1992-97 (Yuan et al., 1999). Advanced experimental techniques and improved understanding of the mechanism underlying drug-drug interaction have enabled some relatively simple approaches to the rationalized therapy against clinical out courses in humans. In enviably much of validation of such an approach has come from retrospective examinations of clinical interaction of existing drugs compared with in vitro data obtained subsequent to the drug being approved.

However there is lack of enough information available in literature for existing drugs in market and it is essential to understand a lot about their interaction potential especially when they are used in combination. In this study we attempted to investigate the pharmacokinetic drug-drug interaction of different drugs which are prescribed in combination in major disorders like diabetes.

## 1.1 Diabetes and its complications

Diabetes mellitus is a chronic disease in which blood glucose levels remain high resulting from defects in insulin secretion, insulin action, or both. The hormone insulin is responsible for regulating glucose levels in the blood. Abnormally high levels of glucose can damage the small and large blood vessels, leading to diabetic blindness, kidney disease, amputations of limbs, stroke, and heart disease.

There are three common types of diabetes.

- Type 1 diabetes is usually (but not always) diagnosed in children and young adults. Patients with type 1 diabetes are deficient in insulin and their glucose load is corrected by insulin therapy.
- Type 2 diabetes is usually (but not always) diagnosed in adults over the age of 45. In type 2 diabetes, either the pancreatic beta cell does not produce enough insulin or the receptors are resistant to insulin thus cannot facilitate its action.
- Gestational diabetes occurs during pregnancy: 2-4 percent of all pregnant women have gestational diabetes. If a woman has gestational diabetes, she has about a 40 percent chance of having type 2 diabetes later in her life.

The first treatment for type 2 diabetes is often diet control for blood glucose correction, weight loss, and exercising. Sometimes these measures are not enough to bring blood glucose levels down to the normal range. In those cases taking a medicine that lowers blood glucose levels is essential. Patients with type 1 diabetes cannot produce optimum insulin levels. For them, insulin shots are the only way to keep blood glucose levels down. Patients with type 2 diabetes tend to have two problems, there could be problems associated with either insulin secretion or insulin resistance that results in hyperglycemia.
All oral medications currently existing in market for type 2 diabetes are members of five classes of drugs: These five classes of drugs work in different ways to lower blood glucose levels

- sulfonylureas
- meglitinides
- biguanides
- thiazolidinediones and
- alpha-glucosidase inhibitors..

Diabetes mellitus magnifies the risk of cardiovascular morbidity and mortality (Cerveny et al., 1998). Besides the well-recognized microvascular complications of diabetes, such as nephropathy and retinopathy, there is a growing epidemic of macrovascular complications, including diseases of coronary arteries, peripheral arteries, and carotid vessels, particularly in the burgeoning type 2 diabetic population. Some of the complications reported are as follows.

Heart Disease and Stroke

Diabetes carries an increased risk for heart attack, stroke, and complications related to poor circulation that leads to peripheral arterial disorders (PAD).

Kidney Disease

Diabetes can cause nephropathy and damage the kidneys, which not only cause them to fail, but can also make them lose their ability to filter out waste products.

Eye Complications

Diabetes can cause eye problems like retinopathy and may lead to blindness. People with diabetes will have a higher risk of blindness than people without diabetes.

Diabetic Neuropathy and Nerve Damage

One of the most common complications of diabetes is diabetic neuropathy. Excessive blood glucose levels damage the nerves that run throughout the body, connecting the spinal cord to muscles, skin, blood vessels, and other organs.

Foot Complications

People with diabetes can develop many different foot problems. Foot problems most often happen when there is nerve damage in the feet or when blood flow is poor.

Skin Complications

Some of the patients with diabetes will have a skin disorder caused or affected by diabetes at some time in their lives. In fact, such problems are sometimes the first sign that a person has diabetes. Luckily, most skin conditions can be prevented or easily treated if caught early.

Other complications

Diabetes also causes other complications like Gastroparesis and depression.

## 1.2 Vascular Complications and PAD in Diabetics

Diabetes mellitus is increasing throughout the world. 75% of the early deaths in diabetics are related to coronary artery disease. The mechanisms of arterial disease in diabetic patients appear to be multiple but increased levels of glucose and fructose play havoc with blood vessel walls. A cascading series of adverse events follows the onset of high blood sugar and can only be controlled by reduction of free sugar in the diet and comprehensive diet revision associated with increased exercise. Pathogenic mechanisms include the accumulation of glucose, fructose and sorbitol in tissues and the modification of proteins by glycation.

Some of the vascular diseases are attributed to abnormalities in blood fats. A high sugar and fat in the diet of diabetics is a lethal combination. More than 60 % of non-insulin dependent diabetes has elevated blood fat levels. The general adverse consequences of Diabetes are cardiovascular complications like heart diseases, stroke and high blood pressure. The risk of atherosclerotic disease is markedly increased among individuals with diabetes. The increased risk is independent of, and additive to, other cardiovascular risk factors. Atherosclerosis causes most of the deaths or disability in patients with diabetes, particularly in the type 2 diabetic patient population (Beckman et al., 2002, Lowel et al., 2000). A lot more can be done to limit or prevent the cardiovascular complications of diabetes.

The Verona Diabetes Study showed that cardiovascular disease is responsible for 44% of all-cause fatalities in the diabetic patient population (Brun et al., 2000). The duration of diabetes increases the risk of mortality from cardiovascular disease, independent of co-existing risk factors (Fox et al., 2004). Insulin resistance is a key factor in the pathogenesis of diabetes. Insulin resistance and its attendant metabolic abnormalities may cause much of the increased cardiovascular risk of diabetes (Watson et al., 2003). The clinical consequences of atherosclerosis include coronary artery diseases (CAD), cerebrovascular disease, Peripheral arterial disease (PAD) are potentially life threatening. Epidemiological studies have confirmed an association between diabetes and an increased prevalence of Peripheral arterial disease (Pyorala et al., 1987; Donahue and Orchard, 1992). Peripheral arterial disease (PAD) is usually characterized by occlusive arterial disease of the lower extremities.

Platelet activation and thrombosis are key contributing factors in disease development and progression of PAD. Some of the studies in clinics provide evidence of enhanced platelet activity in patients with diabetes. The levels of Platelet Contractile force (PCF) and clot elastic modulus (CEM) are increased in diabetic patients with chest pain (Carr et al., 2002). Elevated PCF™ in diabetics may correlate with increased thrombotic risk in these patients. Platelet function and clot structure are generally altered in diabetes. Levels of platelet-derived microparticles (PMPs), platelet activation markers (P-selectin expressed on, or annexin V binding to, platelets (plt:P-selectin or plt-annexin V, respectively chemokines (IL-8, monocyte chemotactic peptide-l (MCP-I), and regulated on activation normally T-cell expressed and secreted (RANTES», and soluble P- and Eselectins were compared in peripheral blood from diabetic and control patients in order to develop a better understanding of their potential contribution to diabetic vascular complications. Significant

increases were found for PMPs, plt:P-selectin, MCP-I, RANTES and soluble P- and E-selectins in diabetic individuals, whereas IL-8 levels were similar. Furthermore, after ticlopidine treatment, most of these factors receded to baseline levels observed in non-diabetic patients. From these findings it is indicated that antiplatelet drugs might be able to prevent or reduce vascular complications in diabetic patients.

## 1.3 Anti-Platelet drugs and their use in Diabetic Patients

Diabetes is associated with considerably higher risks of developing peripheral arterial disease (PAD) which, when it occurs, is more severe and progresses more rapidly than in nondiabetics (Hittel and Donnelly, 2002). Moreover, the presence of PAD is a potent marker of increased cardiovascular risk. If PAD is identified on the basis of ankle brachial index (ABI) of < 0.90, its prevalence in patients with diabetes may be as high as 29%. Early detection of PAD in the diabetic patient is therefore important, but may be complicated by the presence of neuropathy and calcification of the arteries such that ischemic symptoms are not felt by the patient and ankle pressures are not reduced. Toe pressures are an alternative diagnostic tool in these patients. Good glycaemic control, while an essential part of diabetes management, does not appear to bring more than modest benefits in preventing the peripheral vascular complications of diabetes. Therefore, attention to other risk factors is needed.

Risk management includes lifestyle modifications, treating associated conditions (diabetes, dyslipidemia, and hypertension), and preventing ischemic events with aggressive antiplatelet theraypy such as clopidogrel. Most of the drugs available in use as anti-platelet drugs are cilostazol, pentoxifylline, clopidogrel, aspirin, dypyridamole, and GP IIb/IIIa inhibitors (abciximab and tirofiban), etc. Pharmacological therapies to improve symptomatic PAD include cilostazol and pentoxifylline. Treatment with the phosphodiesterase III inhibitor, cilostazol, has been shown to improve walking distances significantly in diabetes patients with intermittent claudication and also appears to improve plasma lipid profiles (Chapman and Goa, 2003). Further, cilostazol has an antiplatelet action, which may prove to be of benefit in diabetes because hyperglycaemia is associated with increased platelet aggregability (Barnett et al., 2004). Revascularization in diabetes patients with critical leg ischemia is complex and associated with poorer outcomes than in non-diabetes patients. More over cilostazol is also having a positive role of enhancing insulin sensitivity in diabetes conditions (Nakaya et al., 1999). All these evidences

indicates the possible use of anti-platelets simultaneously with other antidiabetic agents in diabetic patients

## 1.4 Drug interactions of Anti-platelet drugs with antidiabetic drugs

Since the management of type 2 diabetes mellitus usually involves combined pharmacological therapy to obtain adequate glucose control and treatment of concurrent pathologies (especially dyslipidaemia and arterial hypertension), drug-drug interactions must be carefully considered with antihyperglycaemic drugs. Additive glucose-lowering effects have been extensively reported when combining sulphonylureas (or the new insulin secretagogues, meglitinide derivatives, i.e. nateglinide and repaglinide) with metformin, sulphonylureas (or meglitinide derivatives) with thiazolidinediones (also called glitazones) and the biguanide compound metformin with thiazolidinediones

Relevant pharmacological agents are those that are widely coadministered in diabetic patients (e.g. lipid-lowering agents, antihypertensive agents); those that have a narrow efficacy/toxicity ratio (e.g. digoxin, warfarin); or those that are known to induce (rifampicin) or inhibit (fluconazole) the cytochrome P450 (CYP) system. Metformin is currently a key compound in the pharmacological management of type 2 diabetes, used either alone or in combination with other antihyperglycaemics. There are no clinically relevant metabolic interactions with metformin, because this compound is not metabolised and does not inhibit the metabolism of other drugs. In contrast, sulphonylureas, meglitinide derivatives and thiazolidinediones are extensively metabolised in the liver via the CYP system and thus, may be subject to drug-drug metabolic interactions. A lot of interaction studies have been carried with other pharmacological agents like statins, lipid lowering agents and anti-hypertensive drugs. The effects of inducers or inhibitors of CYP isoenzymes on the metabolism and pharmacokinetics of the glucose-lowering agents of each pharmacological class has been tested.

Few studies only have been done to evaluate the pharmacokinetic drug-drug interactions though the prescription combinations are common in diabetics with PAD. There is a lack of pharmacovigilance report on adverse events after simultaneous treatment of statins and Clopidogrel (Hansten, 2003; Saw et al., 2003). Still not many studies have been performed so far with other anti-platelet drugs apart from aspirin (Anderson and Nawarskas, 2001; Takanohashi et al., 2007). Most of the drugs are metabolized by hepatic microsomal enzyme system which changes the non polar active compound to more polar metabolite. Many drugs counteracts in this aspects by either enzyme induction or enzyme inhibition, if

they are following the same metabolic pathways. For example, Thiazolidinediones which are common in use as antidiabetic agents inhibit CYP450 enzymes significantly (Sahi et al., 2003). Cilostazol is known to be effected by CYP34 enzyme to more extent (Suri et al., 1999; Abbas et al., 2000). Significant drug interactions are observed when cilostazol is co-administered with other agents that inhibit cytochrome P450 (CYP) 3A4 (e.g. erythromycin or diltiazem) or CYP2C19 (e.g. omeprazole). As a result, in Europe cilostazol is contraindicated in patients receiving CYP3A4 or CYP2C19 inhibitors and in the US, it is recommended that dosage reduction for cilostazol be considered during co-administration of cilostazol and CYP3A4 or CYP2C19 inhibitors. Co-administration of cilostazol with aspirin or warfarin did not result in any clinically significant changes to coagulation parameters, bleeding time or platelet aggregation but dipyridamole has improved the antiplatelet activity of cilostazol without changing its bleeding time (Li et al., 2005). Pentoxifylline is metabolized by CYP1A2 and its metabolites are largely excreted through urine. The compounds interacting with this enzyme are of potentially changing the pharmacokinetic characteristics and its antiplatelet activity of pentoxifylline.

Though antiplatelet agents are prescribed commonly in diabetic populations but there is no evidence of such investigations on the pharmacokinetic interactions of these agents with antidiabetic drugs. As part of understanding pharmacokinetic drug-drug interaction in these aspects, we wanted to investigate the possible drug interaction effect of anti-platelet drug with different classes of anti diabetic drugs viz. sulphonylureas, biguanides, meglitinide anologues and thiazolidinediones. We have studied the effect of two antiplatelet agents viz. cilostazol and pentoxifylline on the pharmacokinetics of four antidiabetic drugs (glipizide, metformin, nateglinide and rosiglitazone) and their blood glucose lowering activity in rats. We also studied the effect of these antidiabetic agents on the pharmacokinetics of antiplatelet agents and their antiplatelet activity. The present data in this thesis is indicative of a potential therapeutic monitoring required for the simultaneous administration of antiplatelet agents in presence of antidiabetic drugs in humans.

## 1.5 Objectives of the present research work

The proposed research aimed to:

i) Study the potential pharmacokinetic drug - drug interaction effect of two of the existing anti-platelet drugs with four different classes of Anti diabetic drugs in rats using single and multiple dose designs.

ii) Study the potential pharmacokinetic drug - drug interaction effect of above four different classes of Anti diabetic drugs on two of the above anti-platelet drugs with in rats using single and multiple dose designs.

iii) Evaluation of these effects using in vitro methods to identify the mechanism of interaction wherever possible.

iv) Determination of feasible pharmacodynamic parameters of these study drugs in combination which has shown interaction above in rats using multiple dose design.

v) Comparative evaluation of the significant drug interactions in search of the safe combinations for humans

# Chapter 2

# LITERATURE REVIEW

# 2 LITERATURE REVIEW

## 2.1 Over view of drug-drug interactions

Drug-drug interactions can occur when two or more drugs administered simultaneously mutually interact with each other in a manner that results in alteration of the pharmacokinetic profile and/or pharmacological or toxicological response to one or both of the compounds. These interactions may result from multitude of factors viz. peculiar properties of each drug, the physical and physiological status of the patient and the total number of drugs to which the patient is exposed. Considerations of three main phases in drug action and their essential aspects with regard to therapeutical efficacy is required while studying the drug interactions. The pharmaceutical phase, the first phase where the disintegration and distribution of the active compounds may interfere to determine the quality of drug available for the absorption. It is followed by pharmacokinetic phase where drugs may interact via inhibition of absorption or elimination process or induction of enzyme responsible for metabolism of drug. The consequences of such interaction may range from those of purely academic interest, such as minor changes as elimination or metabolic profiles, to overt toxicity or even total clinical outcomes. The final phase, the pharmacodynamics phase, concerns with the interaction of drugs and the specific receptors and there with the induction of a stimulus and the generation of the effect. Drugs which interact with each other may cause loss of or exaggeration of pharmacological effect, or confer an altered phenotype on the patient thus expanding the consequences of drug-drug interaction to apparently unrelated xenobiotic or endogenous metabolism. Interactions need not to show only effect on each other, they can also show the effect on endogenous metabolic process (eg. 6B-hydroxylate of cortisol increase with CYP4503A4 induction)

### 2.1.1 Types of drug-drug interactions

The major types of drug-drug interactions that take place outside and inside the body are

- Pharmacokinetic interactions.
- Pharmacodynamic interactions.

**Pharmacokinetic Interactions**

The drug interactions may take place at the stage of absorption, distribution, metabolism and excretion.

a) Interactions at the level of absorption

Interactions may lead to the altered absorption that may be due to alteration of the gastric pH, gastrointestinal motility, complexation and effect on gastrointestinal flora.

Eg: Sodium bicarbonte decreases the absorption of tetracycline by altering the gastric pH (Elliott and Armstrong, 1972). The absorption of lincomycin is decreased when administered along with kaolin-pectin mixture due to physical adsorption of the antibiotic(Wagner et al., 1968). Cholistyramine decreases the absorption of warfarin by complex formation whereas sorbitol enhances the absorption of vitamin C (Lawendel, 1956; Serlin and Breckenridge, 1983). Iron preparations form complexes with tetracycline, oxytetracycline, methacycline and doxycycline and decrease the absorption of these antibiotics (Neuvonen et al., 1970).

There pharmaceutical interactions occur outside the body. Physical incompatibility may influence when two drugs are being combined together in single formulation. Certain drugs may form complexation/chelation when formulated together with metal ions or EDTA so that the complexation causes decrease of absorption of those drugs(Levy, 1971)

Divalent cations or trivalent cations like calcium, magnesium, zinc and aluminium, milk and milk products, antacids, vitamins, mineral products and cathartics containing divalent and trivalent cations decrease bioavailability of tetracyclines. Antacids delay the absorption of phenobarbital with decreased hypnotic effect (Azarnoff and Hurwitz, 1970).

Certain drugs alter the motility/rate of gastric emptying so that it could result in decreased or increased absorption of other drugs. Cathartics by increasing GI motility, may increase the rate at which another drug passes through the GI tract. Anticholinergics, by decreasing GI motility may influence drug absorption of other drugs by keeping them in longer contact with the intestine so that the drug absorption may be increased. Metoclopropamide increases the motility of upper GI tract, it should be anticipated that it may influence the absorption f other drugs administered concurrently.

b) Interactions by modifying distribution

The pharmacological or toxicological activity of drugs is often a function of the free drug concentration in the plasma and may relate to the unbound area under the plasma concentration-time curve (AUC) or steady state unbound plasma concentrations. Some drugs are strongly bound to plasma proteins and in this state they are pharmacologically inactive. Many drugs are highly plasma protein bound, with >99 percent of the plasma drug concentration being associated with protein, and, moreover, drugs have the potential to compete with each other for plasma protein binding sites. Certain groups of drugs seem to

share a limited number of protein binding sites and can be displaced from the binding sites by others. This results in an increase in the unbound and pharmacologically active form of one of the drugs leading to toxicity. Some drugs displace the other drugs from binding sites in the tissues as well as plasma proteins.

Eg: phenylbutazone, oxyphenbutazone can displace warfarin sodium from protein binding sites leading to bleeding tendency. Phenylbutazone, sulphaphenazole and salicylates can displace tolbutamide (Pond et al., 1977; Seltzer and Donoso, 1989). Sulfonamides and vitamin K can displace bilirubin and may cause kernicterus (Seltzer and Donoso, 1989) in neonates. Salicylates can displace methotrexate ((Zuik and Mandel, 1975). Quinidine displaces digoxin from binding sites in tissues and plasma proteins (McEvoy, 1999).

For a highly bound drug, the volume of distribution ($V_d$) can become restricted in a way that reflects the distribution of albumin namely, drug and albumin are confined to the plasma and extravascular fluids ($V_d$ ~ 0.04-0.3 L/kg). Incase of a drug with low volume of distribution and where only the unbound fraction of the drugs is pharmacologically active, then displacement of the the bound-free equilibrium in favour of free drug by co-administration of highly protein-bound therapeutic agent can increase the free fraction of the drug in plasma water. Where the therapeutic is low, disturbance of the equilibrium can lead to toxicity, or where the concentration-response slope is sleep, an exaggerated pharmacological response may ensue. an increase in the free fraction of drug through displacements from proteins also tend to result in increased clearance so that the bound-free equilibrium is reapidly established. However, in a converse situation where the protein binding is high and $V_d$ is also high, then any free drug originating from displacement of bound drug will re-equilibrate into the tissues so that the fraction unbound in plasma remains essentially unaffected and interactions at the plasma protein binding level become irrelevant (Ito et al., 1998).

c) Interactions by modifying the metabolism of drugs

Most clinically important drug interactions involve the effect of one drug on the metabolism of another drug. Most lipid soluble drugs are eliminated by oxidation in the liver by involvement of hepatic cytochrome P450 enzyme system of microsomal mixed function oxidases. Some drugs by altering the activity of these mixed function oxidases cause enzyme induction or enzyme inhibition.

Drugs like barbiturates, griseofulvin, phenytoin, rifampicin, ethanol (chronic) enhance the metabolism of other drugs by enzyme induction and decrease the duration of action. Allopurinol, chloramphenicol, cimetidine, ciprofloxacin, isoniazid, metronidazole, alcohol

(acute) decrease the metabolism of other drugs by microsomal enzyme inhibition and increase their duration of action. Chloramphenicol decreases the metabolism of tolbutamide (Christensen and Skovsted, 1969).Antibiotics like erythromycin, cyclosporin, isoniazid and ketoconazole reduce the metabolic clearance of glucocorticoids (Zurcher et al., 1989) (LaForce et al., 1983)

d) Interactions at the excretion level

Most of the interactions at the excretion level occur in kidneys. Interference with renal excretion is the mechanism behind some drug interactions. Example: Acidic drugs filtered into the urine are present in unionized form in the urine pH and are reabsorbed back into the blood stream with consequent prolongation of half-life. Acidifiers like ammonium chloride prolong the half-life of acidic drugs by the above mechanism. Similarly sodium bicarbionate and fruit juices produce alkaline urine and prolong the half-life of basic drugs. Some of the less soluble sulpha drugs precipitate crystallurea in the acidic urinary pH. Concomitant administration of sodium bicarbonate reduces the chances of formation of crystallurea with sulpha drugs (Levy and Regardh, 1971).

Concomitant administration of quinidine, amiodarone, diltiazem and verapamil can bring about increase in serum concentration of digoxin due to inhibition of its renal and non-renal clearance. Thiazide diuretics and loop diuretics decrease the excretion of lithium by increasing reabsorption (Baer et al., 1971) (Horowitz and Fisher, 1969)

**Pharmacodynamic Interactions**

Pharmacodynamic interactions are those where the effects of one drug are changed by the presence of another drug at its site of action.

a) Antagonistic type interactions

It is expected that a drug with an agonist action at a particular receptor type will be antagonized at that receptor. Example: Spironolactone antagonizes the effects of carbenoxolone through action at aldosterone receptors in ulcer patients. Antihypertensive effect of guanethidine, bethanidine and debrisoquine is prevented or reversed by antidepressant drugs(Mitchell et al., 1967). The thiazide diuretics are known to elevate the blood glucose levels but contraindicated when these are used in diabetic agents who are taking antidiabetic drugs. They oppose the action of antidiabetic agents when given together.

b) Additive type interactions

When two drugs which have similar pharmacological effects are given together, the effects are additive. Example: Salicylates and anticoagulants. Aspirin and paracetamol. Nitrous oxide and ether, ephedrine and theophylline.

c) Interactions due to changes in drug transport mechanisms

The antihypertensive effect of adrenergic neurone blocking drugs such as bethenidine and debrisoquine is prevented or reversed by indirectly acting amines and the tricyclic antidepressants. Tricyclic antidepressants also prevent the re-uptake of noradrenaline into the peripheral adrenargic neurons. (Kwon et al., 2003)

d) Interaction due to disturbances in fluid and electrolyte balance

Drugs that alter the concentration of plasma potassium level alter the therapeutic effect of cardiac glycosides, such as digoxin as well as other antiarrhythmic drugs. Angiotensin converting enzyme inhibitors have a potassium sparing effect, such that concurrent use of potassium supplements or potassium sparing diuretics may lead to dangerous hyperkalemia.

### 2.1.2 The mechanism based drug- drug interaction

Drug molecules have the potential to be transported or metabolized by different mechanisms. Several mechanisms may underlie clinical drug-drug interactions which involves various clearance pathways (Figure 2.1). One or more of these mechanisms may operate at any point of time. Induction by xenobiotics can lead to increase metabolic activity of enzymes thus increase the, metabolism of the induced compound (autoinduction) or other unrelated drugs and endogenous substrates. Reversible or irreversible inhibition of a single drug metabolic enzyme will lead to a reduction in the capacity of that enzyme to clear drugs from the body. If the enzyme were key determinate of drug clearance, then it would be reasonable to expect an increase or decrease in plasma levels, unless other enzymes capable for interaction in this key enzyme's intrinsic clearance.

**Drug metabolizing enzymes**

The CYPs are a diverse supper family of enzymes which taken together, are capable of metabolizing wide variety of endogenous and Xenobiotic substances including drug molecules (Parkinson and Knight, 1969) Some different CYPS have been identified in

human liver and majority th commonly prescribed drugs in the US are primarily cleared by CYP mediated metabolism (Bertz and Granneman, 1997). There are 30 distinct P 450 genes in human & 40 in rat (Nelson et al., 1993). Indeed 350 of new P450 are cloned and sequenced.

In general biotransformation is the conversion of lipophilic xenobiotic into more hydrophilic water soluble metabolites thereby serving to

- reduce the biological half life of the xenobiotics.
- reduce the duration of the exposure to xenobiotics
- avoid accumulation of parent xenobiotics in the organism
- change the biological activity of the xenobiotics
- change the duration of the biological activity of the xenobiotics

Although other organs and tissues are also relevant, the liver is quantitative the most important organ in the process of biotransformation, a process which may be divide into phase-I, phase-II and phase-III metabolism.

- Phase I metabolic biotransformation include oxidation, reduction and hydrolysis reactions in order to reveal or introduce functional or reactive groups in the molecule which are majorly governed by CYP450 and non CYP450 enzymes.

- Phase-II transformations are generally conjugation reactions of parent xenobiotics or phase –I metabolites with for example inorganic sulphate, amino acids, glucuronic acids or glutathione. They facilitate transport and enhance elimination via the renal and biliary routes.

- Phase-III constitutes further metabolism of products derived from phase-II conjugation reaction. These reactions are catalyzed by enzymes which are also active in phase-I and Phase-II reactions. Basically phase-III reactions different from Phase-I and Phase-II reactions only in the fact that substrates are products of previous phase-I or Phase-II metabolic reaction.

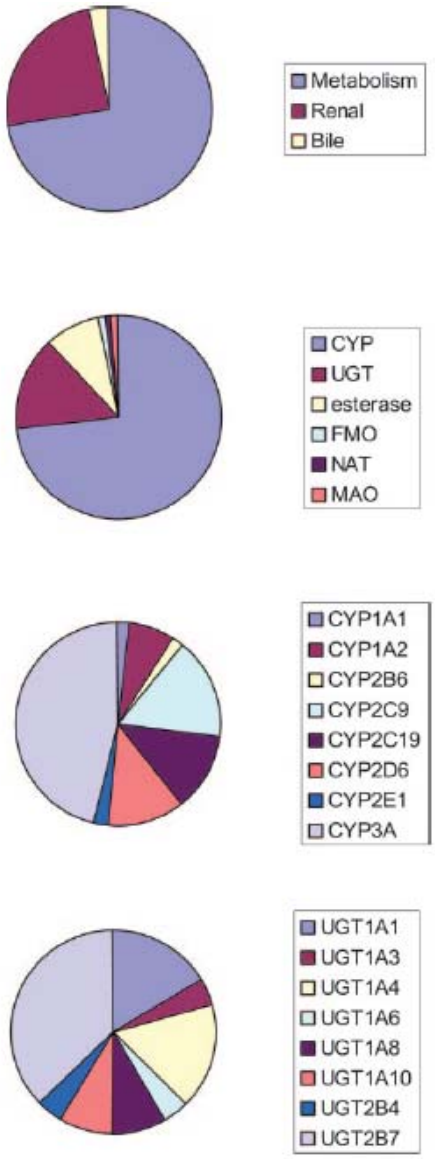



*Figure 2.1 Clearance mechanisms for the top 200 drugs prescribed in the United States in 2002*

CYP- Cytochrome P450; FMO-Flavin Mono Oxidase; UGT-UDP-Glucoronidyltranferase;
NAT- N-acetyltransferase; MAO-Mono Amino Oxidase

This figure was adapted from Williams et al., 2004. Metabolism is a listed clearance mechanism for three quarters of the top 200 prescribed drugs in the United States (top panel). Top panel, listed clearance mechanisms; second panel from top, listed enzymes contributing to clearance for metabolized drugs; second panel from bottom, proportion of cytochrome P450 substrates in the top 200 metabolized by each listed member of that subfamily; bottom panel, proportion of UGT substrates in the top 200 metabolized by each listed member of that subfamily

**Cytochrome P450**

The most important enzyme system catalyzing phase-I metabolic reactions are the Cytochrome P-450 (P450) system. In 1950, it was evident that it is not a single protein but consists of number of different proteins. In 1958, it was discovered that when pig or rat liver microsomes were treated with dithionate and purged with CO, a strong absorption had occurred at 450 nm that was most unusual for the then-k pigments (Klingenberg, 1958), the pigment responsible for this phenomenon was called P ( for pig t) 450. It was named "cytochrone P450" and further characterized as P450 hemoprotein (Omura and Sato, 1964)

Human beings have 17 known CYP gene families (http://drnelson.utmem.edu/human.p450s.html), of which only the first 3 seem to be important for drug metabolism (Wrighton and Stevens, 1992). Apart from being involved in drug metabolism, the CYP enzymes also play an important role in cholesterol biosynthesis, vitamin D metabolism, bile acid metabolism, and biosynthesis of steroids and thromboxane A2 (Nelson et al., 1993). CYP enzymes can be induced and inhibited by various drugs and other xenobiotics (Pelkonen et al., 1998), and also be affected by some diseases.

The major CYP 450 enzyme are classified as

Table 2.1 Cytochrome P-450 and its sub family of enzymes.

| CYP family | CYP sub family |
|---|---|
| CYP1A | 1, 2 |
| CYP2A | 1,2,3,4,5,6,7,8,9,10,11,12, Mouse $P450_{tu}$, Baboon $P450_{F1}$, Cynomolgus monkey P450CLM6 |
| CYP2B | 1, 2, 6 |
| CYP2C | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 |
| CYP2D | 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17, 18, 19, 20, 21, 7P, 8P |
| CYP2E | 1, 2 |
| CYP3A | 1,2,3,4,5,6,7,8,9,10,11,12,13 |

Porphyrias are a group of diseases with genetic deficiencies in heme biosynthesis. Patients with certain forms of porphyria can have markedly decreased metabolism of certain drugs. Interestingly, in patients with variegate porphyria the prolonged t½ of antipyrine (about 30

hours) can be shortened to 6 hours by a 3-day treatment with intravenous heme (Tokola et al., 1988), and in these patients, even a single infusion of heme can reduce the t½ of antipyrine to 13 hours (Mustajoki et al., 1992).

The **CYP1A** subfamily comprises two members, CYP1A1 and CYP1A2; they share 68% amino acid sequence identity. CYP1A1 is expressed primarily in extrahepatic tissues, such as lungs, small intestine, and placenta, whereas CYP1A2 is expressed primarily in the liver (Wrighton and Stevens, 1992), where it constitutes more than 10% of the total liver CYP content (Guengerich et al., 1994).

CYP1A1 and CYP1A2 are inducible by xenobiotics, and this induction is mediated generally by the aryl hydrocarbon (Ah) receptor. Cigarette smoke and charcoal grilled meat are typical inducers of CYP1A enzymes. Human CYP1A induction shows marked heterogeneity, which may be caused by polymorphisms in the Ah receptor. In addition, the CYP1A1 gene shows structural polymorphisms; furthermore the great variability in CYP1A2 activity is suggestive of genetic polymorphism. CYP1A enzymes are capable of activating procarcinogenic xenobiotics. In addition, CYP1A2 is an important drug-metabolizing enzyme in the human liver. Substrates for CYP1A2 include caffeine, clozapine, theophylline, and R-warfarin (Bertilsson et al., 1994). Fluvoxamine and ciprofloxacin are known inhibitors of CYP1A2 (Fuhr et al., 1992; Rasmussen et al., 1995).

**CYP2A6** comprises 1% to 4% of the total human liver CYP protein (Guengerich et al., 1994) and is polymorphically expressed (Oscarson, 2001). CYP2A6 protein is induced in cell cultures by rifampicin (Dalet-Beluche et al., 1992), and results from an in vivo study indicate that also the antiepileptic drugs carbamazepine and phenytoin induce CYP2A6 (Sotaniemi et al., 1995). CYP2A6 is the major coumarin 7-hydroxylase in human liver and is involved in the metabolism also of nicotine, halothane, valproic acid, and disulfiram (Pelkonen et al., 1998; Oscarson, 2001). Defective CYP2A6 alleles are associated with deficient nicotine metabolism (Nakajima et al., 2001). According to some studies, these alleles tend to delay the age of starting regular smoking, reduce the number
of cigarettes smoked, and increase the likelihood of quitting smoking ((Pianezza et al., 1998; Gu et al., 2000). Interestingly, the CYP2A6 inhibitor methoxsalen (Kharasch et al., 2000) inhibits nicotine first-pass metabolism and in a laboratory setting reduces smoking (Sellers et al., 2000).

The **CYP2C** subfamily, the most complex mammalian subfamily, has 4 known human members: CYP2C8, CYP2C9, CYP2C18, and CYP2C19. CYP2C enzymes are primarily hepatic, but significant quantities of CYP2C protein exist in the small intestine, as well

(Zhang et al., 1999). CYP2C8, CYP2C9, and CYP2C19 are inducible in cell cultures by rifampicin, phenobarbital, and dexamethasone (Gerbal-Chaloin et al., 2001), and in vivo data indicate that, in addition, carbamazepine and phenytoin induce CYP2C enzymes (Anderson, 1998). CYP2C9 is the most abundant enzyme of the CYP2C subfamily in the liver and in one study accounted for more than 30% of the total human liver CYP content (Lasker et al., 1998). CYP2C9 and CYP2C19 are polymorphically expressed, with approximately 3% of Caucasians and 20% of Asians being poor metabolizers of CYP2C19 substrates (Wedlund et al., 1984). CYP2C9 has at least 3 important allelic variants, Arg144/Ile359 (CYP2C9*1; wild-type), Cys144/Ile359 (CYP2C9*2), and Arg144/Leu359 (CYP2C9*3) (Miners and Birkett, 1998). The CYP2C9*3 allele (in both heterozygous and homozygous individuals) is associated with decreased clearance of CYP2C9 substrates such as S-warfarin, celecoxib, phenytoin, and glipizide (Takahashi et al., 1998; Kidd et al., 1999; Tang et al., 2001). CYP2C9 polymorphisms also affect warfarin and phenytoin dose requirements (Aithal et al 1999, van der Weide et al 2001) and risk of bleeding complications during warfarin therapy (Aithal et al., 1999). Approximately 10% to 15% of Caucasians are heterozygous and 1% homozygous for the CYP2C9*3 allele (Yasar et al., 1999).

Taxol (paclitaxel) is the prototypic substrate for CYP2C8 (Rettie and Lang, 2000), and CYP2C8 plays an important role in the metabolism of cerivastatin, pioglitazone, and rosiglitazone (Muck, 1998; Mudaliar and Henry, 2001). CYP2C8 is also capable of metabolizing benzphetamine, retinoic acid, tolbutamide, benzo(a)pyrene, carbamazepine, and R-ibuprofen, although it is probably not the main enzyme responsible for the in vivo metabolism of these compounds (Rettie and Lang, 2000). Many drug substrates for CYP2C9 have narrow therapeutic indexes (Miners and Birkett, 1998), including S-warfarin, phenytoin, and the first generation sulfonylurea tolbutamide (Table I). It seems that most of the sulfonylurea antidiabetic drugs are metabolized primarily by CYP2C9 (Relling et al., 1990; Kidd et al., 1999; Yoon et al., 2001). Other substrates for CYP2C9 include many nonsteroidal anti-inflammatory drugs (NSAIDs): ibuprofen, diclofenac, naproxen, piroxicam, and tenoxicam, as well as losartan (Miners and Birkett, 1998). There seems to be no convincing evidence that CYP2C18 contributes to the in vivo metabolism of any drugs, although in vitro data show that it may contribute to the metabolism of diazepam, omeprazole and lansoprazole (Rettie and Lang, 2000). S-mephenytoin is the prototypic substrate of CYP2C19 (Rettie and Lang, 2000). Omeprazole and diazepam are

also metabolized by CYP2C19, and the active cycloguanil metabolite of proguanil seems to be produced by CYP2C19.

Currently no known specific inhibitors of CYP2C8 or CYP2C18 exist (Rettie and Lang, 2000). Ketoconazole and diethyldithiocarbamate inhibited CYP2C8 at concentrations often thought specifically to inhibit CYP3A4 or CYP2E1, and some CYP3A substrates such as midazolam, quinine, terfenadine, and triazolam can also inhibit CYP2C8 in vitro (Ong et al., 2000). Sulfaphenazole is a prototype inhibitor of CYP2C9 (Rettie and Lang, 2000). The antiarrhythmic drug amiodarone and the azole antifungals fluconazole and miconazole are potent CYP2C9 inhibitors both in vitro and in vivo (Miners and Birkett, 1998). Fluvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, can also inhibit CYP2C9 in vitro and in vivo (Miners and Birkett, 1998), but only slightly affects the pharmacokinetics of the CYP2C9 substrates glibenclamide and tolbutamide in patients with NIDDM. S-mephenytoin can competitively inhibit CYP2C19 in vitro only at high concentrations. The CYP2C19 substrate omeprazole seems capable of inhibiting CYP2C19 in vivo (Funck-Brentano et al., 1997), and fluvoxamine inhibits CYP2C19, among other CYP enzymes (Rasmussen et al., 1995).

**CYP2D6** is the only functionally active isozyme of the CYP2D subfamily in humans, but its expression constitutes only about 2% to 5% of the total hepatic CYP content (Yamazaki et al., 1994). It is, however, polymorphically expressed, and its expression varies more than 100-fold between the poor metabolizers and the most active extensive metabolizers – the ultrarapid CYP2D6 metabolizers (Raimundo et al., 2000). Approximately 7% of Caucasians are poor metabolizers of CYP2D6. In contrast to all other CYP enzymes involved in human drug metabolism, CYP2D6 seems not to be inducible.

Several widely used drugs are metabolized by CYP2D6. These include antiarrhythmics, α-adrenergic receptor antagonists, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), neuroleptics, opiates, anticancer agents, and amphetamines (Raimundo et al., 2000). Quinidine, the SSRI fluoxetine, the antiarrhythmic flecainide, and the antimycotic terbinafine are known inhibitors of CYP2D6 (Abdel-Rahman et al., 1999).

**CYP3A** is the most important drug-metabolizing CYP subfamily in man. It has been estimated that CYP3A enzymes are involved in the metabolism of more than 50% of all clinically used drugs (Wrighton et al., 2000). The most prominently expressed CYP enzyme in the human liver may be CYP3A4. In one study, CYP3A4 accounted for almost 30% of the total CYP in the liver (Nakamura et al., 1994), and is the dominant CYP enzyme in the small intestinal mucosa (Kivisto et al., 1996; Zhang et al., 1999), with its

greatest activity in the proximal small intestine. Hepatic and intestinal CYP3A4 can be induced by several widely used drugs, such as rifampicin, dexamethasone, carbamazepine, phenytoin, and St John's wort (*Hypericum Perforatum*) (Backman et al., 1996; Wrighton et al., 2000). CYP3A4 catalyzes the metabolism of a wide variety of commonly prescribed drugs, such as the psychotropic drugs buspirone, alprazolam, midazolam, and triazolam, the HMG-CoA reductase inhibitors atorvastatin, lovastatin, and simvastatin, the calcium-channel blockers felodipine, nifedipine, and verapamil, and the gastroprokinetic cisapride (Dresser et al., 2000) The CYP3A4 enzyme has two substrate binding sites and is allosterically regulated (Ueng et al., 1997). The drug interactions caused by inhibition of CYP3A4 exhibit substrate dependency (Wang et al., 2000), and one should be careful in extrapolating drug-drug interactions studied for one CYP3A4 substrate to another substrate. CYP3A5 is the second functionally active member of the CYP3A subfamily. It has an amino acid identity of 88% with CYP3A4. CYP3A5 is present at readily detectable levels in only about 30% of human livers (Wrighton et al., 2000), but it is the most abundant CYP3A isozyme in the human kidney, where it may be important in the hydroxylation of endogenous molecules. It also exists in other extrahepatic tissues, such as the gastrointestinal tract, lung, and pancreas (Wrighton et al., 2000). CYP3A5 is inducible in hepatocyte cultures by rifampicin and phenobarbital (Wrighton et al., 2000) and in one human lung adenocarcinoma cell line by glucocorticoids and phenobarbital (Hukkanen et al 2000). The substrate specificity of CYP3A5 seems to be in general similar to that of CYP3A4 (Wrighton et al., 2000). CYP3A7 is found mainly in fetal tissues and comprises about 50% of the total CYP expressed in the human fetal liver. It is not present in significant quantities in adult liver, but can be found in appreciable quantities in the endometrium and placenta (Wrighton et al., 2000). CYP3A7 has an amino acid identity of 88% with CYP3A4, and the metabolic capabilities of CYP3A7 and CYP3A4 seem to be similar.

Apart from being involved in the metabolism of a great number of drugs, CYP3A enzymes can also be inhibited by a considerable number of drugs. Known inhibitors of CYP3A include the macrolide antibiotics erythromycin and clarithromycin, the calcium-channel blockers diltiazem, mibefradil, and verapamil, the azole antimycotics ketoconazole and itraconazole, the HIV protease inhibitors ritonavir, indinavir, and saquinavir, the novel antidepressant nefazodone, and grapefruit juice (Dresser et al., 2000; Wrighton et al., 2000).

As we discussed here, the major CYP enzymes is CYP3A4 which is in high abundance in the liver and small intestine. Thus CYP3A4 present in the both interactive liver offers significant opportunities for D-DI. (eg CSA and KTZ) and represent fundamental barrier to drug absorption and systemic exposure Another phase I enzyme with the roll to play as flavin containing monooxygenase a distinct class of flavo protease capable of biotransformation relative few form FMO (FMO 2 & 5) were actively contributes to drug metabolism. Phase II were typically UDPGA (UGT) and (SULT) sulphotransfer. Apart these enzymes other enzymes involves in xenobiotic acetylase ,methylase and acetylase transferase, MAO and esterase may be involved in metabolism of some drugs. 5-HT re-uptake inhibitor is metabolized by monoamineoxidase (MAO-A), an enzyme normally responsible for noradrenaline, adrenaline and serotonin catabolism. Given such mechanistic knowledge, contra-indications for sumatriptan include MAO-inhibitors, ergotamines and other selective serotonin re-uptake inhibitors but there appears to be scant evidence that moclobemide has these type of clinically significant interactions.

**Enzyme inhibition**

The mechanisms of CYP inhibition can be roughly divided into 2 groups: reversible inhibition and irreversible inhibition, with the former being probably the more common mechanism (Lin and Lu, 1998). Reversible inhibition can be divided, on a kinetic basis, into competitive, noncompetitive, and uncompetitive inhibition. In competitive inhibition, the inhibitor competes with the substrate for the same binding site within a CYP enzyme. In noncompetitive inhibition, the inhibitor binds to the same enzyme as does the substrate, but the binding site differs. In uncompetitive inhibition, the inhibitor binds only to an enzyme that forms a complex with the substrate. Potent reversible inhibitors of CYP enzymes include, for example, itraconazole (CYP3A4), fluoxetine (CYP2D6), miconazole (CYP2C9), and ciprofloxacin (CYP1A2) (Fuhr et al., 1992).

Irreversible inhibition also includes what can be considered quasi-irreversible inhibition. In quasi-irreversible inhibition, the inhibitor undergoes metabolic activation by the CYP enzymes to form inhibitory intermediate metabolites. These metabolites form stable inactive complexes with the prosthetic heme of CYP. In vitro, the metabolic activity of the inactive CYP can be reversed during incubation with highly lipophilic compounds that displace the metabolic intermediate from the active site, or by irradiation, or by oxidation to the ferric state by the addition of potassium ferricyanide. In vivo, these complexes are, however, so stable that the CYP enzymes involved are unavailable for drug metabolism, and synthesis of new enzymes is required to overcome the inhibition; hence the name

quasi-irreversible inhibition (Lin and Lu, 1998). In irreversible inhibition, certain drugs with functional groups are oxidized by the CYP enzymes to form reactive intermediate metabolites that covalently bind and irreversibly inactivate CYP. Because metabolic activation is needed, the inhibitors are often called mechanism-based inactivators or suicide substrates (Lin and Lu, 1998). The metabolic intermediates of the macrolide antibiotics erythromycin and clarithromycin form complexes with the iron of the heme of CYP3A4 and thus inactivate it. Erythromycin and clarithromycin are potent inhibitors of CYP3A4 (Lindstrom et al., 1993). In addition, furafylline (CYP1A2), gestodene (CYP3A4), and grapefruit juice (CYP3A4) are known irreversible inhibitors of CYP enzymes (Kunze and Trager, 1993; Lown et al., 1997).

**Enzyme induction**

The induction of CYP enzymes can be caused by at least 5 different mechanisms. Ethanol selectively induces CYP2E1 primarily by stabilizing the enzyme protein (Fuhr, 2000). Other types of induction of CYP enzymes seem to be mediated by intracellular receptors, namely the Ah receptor, the constitutive androstane receptor (CAR), the PXR, and the peroxisome proliferator-activated receptor (PPAR) (Fuhr, 2000). The Ah receptor is a transcription factor that belongs to the basic-helix-loop-helix-PAS (bHLH-PAS) family, whereas CAR, PXR, and PPAR are orphan nuclear receptors (Waxman, 1999). Polycyclic aromatic hydrocarbons, found for instance in tobacco smoke and charcoal grilled meat, bind to the Ah receptor. The inducer-Ah receptor complex, together with the Ah receptor nuclear translocator (Arnt), binds to a deoxyribonucleic acid (DNA) response element and increases protein synthesis.

The most important enzyme induced through this mechanism is CYP1A2. Other enzymes induced by this mechanism include CYP1A1 and some phase II enzymes such as glutathione S-transferases (GSTs) and UDPglucuronosyltransferases (UGTs) (Fuhr, 2000). A clear dose dependency is evident between smoking and caffeine clearance (an indicator of CYP1A2 activity) with a 1.22-fold increase in caffeine clearance in subjects who smoke 1 to 5 cigarettes per day and a 1.72-fold increase in subjects who smoke more than 20 per day (Tantcheva-Poor et al., 1999). Smoking also raises the systemic elimination of other CYP1A2 substrates, such as theophylline, tacrine, and clozapine (Fuhr, 2000).

The mechanism of the induction of protein synthesis by the nuclear receptors CAR, PXR, and PPAR is essentially similar. An inducer binds to CAR, PXR, or PPAR, and the inducer-receptor complex forms a heterodimer with the retinoid X receptor (RXR). This heterodimer binds to a DNA response element and enhances DNA transcription and

eventually protein synthesis (Waxman, 1999). Phenobarbital binds to CAR and affects the expression of approximately 50 genes. Of the CYP enzymes, phenobarbital seems to have the greatest effect on CYP2B6, but clearly also induces CYP1A2, CYP2C8, CYP2C9, and CYP3A4 and also some UGTs (Fuhr, 2000). Phenobarbital also induces CYP2C19, although to a smaller extent than CYP2C8 or CYP2C9 (Gerbal-Chaloin et al., 2001).

PXR is activated by a number of compounds that are known to induce CYP3A4, such as rifampicin, phenobarbital, dexamethasone, and St John's wort (Lehmann et al., 1998; Moore and Kliewer, 2000). Recent studies suggest that PXR is also involved in the induction of CYP2C8 and CYP2C9 (Gerbal-Chaloin et al., 2001). Interestingly, apart from activating the PXR receptor, dexamethasone can induce CAR, PXR, and RXR protein synthesis through its effects on the glucocorticoid receptor and thus can potentiate the inducing effects of CAR and PXR activators (Pascussi et al., 2000). A new group of antidiabetic drugs, the thiazolidinediones or glitazones, are known to bind to the $PPAR_{\gamma}$, whereas the fibrate drugs bind to the $PPAR_{\alpha}$. $PPAR_{\alpha}$ activates the transcription of genes that encode for proteins involved in lipoprotein and fatty acid metabolism, and $PPAR_{\gamma}$ controls adipocyte differentiation and adipogenesis (Fuhr, 2000). $PPAR_{\gamma}$ is involved in the induction of CYP4A enzymes (Waxman, 1999).

**Drug transport systems**

Other systems, which do not themselves metabolise drugs, but which are critical to modulating systemic exposure can also be source of drug-drug interactions (Sasabe et al., 1997). There are so many transporter systems involved in absorption, distribution and elimination of drugs from various organs. The transport protein, p-glycoprotein (P-gp), is also present in especially high concentrations in the small intestine, and is often co-located with CYP3A4. Many substrates for CYP3A4 are also substrates or inhibitors of P-gp, although this may be a fortuitious association (Thummel and Wilkinson, 1998) and inducers of CYP3A4 can also induce P-gp (Ito et al., 1998). In the extreme cases, combination of P-gp and CYP3A4 inhibition could lead to gross elevation of the systemic exposure (reduced first pass effect), or conversely, combined P-gp and CYP3A4 induction could dramatically reduce exposure by increasing metabolism in the gut and liver (Ito et al., 1998).

Facilitated uptake of drugs into the target tissue or eliminating organ also represents a potential source of drug-drug interactions. Carrier-mediated uptake of cimetidine in to the liver can be inhibited by grepafloxacin (Sasabe et al., 1997), similarly, non-steroidal anti-inflammatory compounds can inhibit the elimination of methotrexate via the kidney (Ito et

al., 1998). Some drugs which reduce hepatic blood flow can affect the pharmacokinetics of co-administered high clearance drugs like cimetidine reduces propranolol clearance.

Table 2.2 Major Human Transporters, substrates and its inhibitors

| Gene | Aliases | Tissue | Substrate | Inhibitor |
|---|---|---|---|---|
| *ABCB1* | P-gp, MDR1 | intestine, liver, kidney, brain, placenta, adrenal, testes | digoxin, fexofenadine, indinavir, vincristine, colchicine. topotecan, paclitaxel | ritonavir, cyclosporine, verapamil, erythromycin, ketocoanzole, itraconazole, quinidine, elacridar (GF120918) |
| *ABCB4* | MDR3 | liver | digoxin, paclitaxel, vinblastine | |
| *ABCB11* | BSEP | liver | vinblastine | |
| *ABCC1* | MRP1 | intestine, liver, kidney, brain | adefovir, indinavir | |
| *ABCC2* | MRP2, CMOAT | intestine, liver, kidney, brain | Indinavir, cisplatin, | cyclosporine |
| *ABCC3* | MRP3, CMOAT2 | intestine, liver, kidney, placenta, adrenal | etoposide, methotrexate, tenoposide | |
| *ABCC4* | MRP4 | | | |
| *ABCC5* | MRP5 | | | |
| *ABCC6* | MRP6 | liver, kidney | cisplatin, daunorubicin | |
| *ABCG2* | BCRP | intestine, liver, breast, placenta | daunorubicin, doxorubicin, topotecan | Elacridar (GF120918) |

Table 2.2 continued….

| Gene | Aliases | Tissue | Substrate | Inhibitor |
|---|---|---|---|---|
| *SLCO1B1* | OATP1B1, OATP-C OATP2 | liver | rifampin, rosuvastatin, methotrexate, pravastatin, thyroxine | cyclosporine rifampin |
| *SLCO1B3* | OATP1B3, OATP8, | liver | digoxin, methotrexate, rifampin, | |
| *SLCO2B1* | SLC21A9, OATP-B | intestine, liver, kidney, brain | pravastatin | |
| *SLC10A1* | NTCP | liver, pancreas | | |
| *SLC10A2* | ASBT | ileum, kidney, biliary tract | | |
| *SLC15A1* | PEPT1 | intestine, kidney | ampicillin, amoxicillin, captopril, valacyclovir | |
| *SLC15A2* | PEPT2 | kidney | ampicillin, amoxicillin, captopril, valacyclovir | |
| *SLC22A1* | OCT-1 | liver | acyclovir, amantadine, desipramine, ganciclovir metformin | disopyramide, midazolam, verapamil, phenformin, phenoxy-benzamine quinidine, quinine, ritonavir |

Table 2.2 continued ….

| Gene | Aliases | Tissue | Substrate | Inhibitor |
|---|---|---|---|---|
| *SLC22A2* | OCT2 | kidney, brain | amantadine, cimetidine, memantine | desipramine, phenoxy-benzamine quinine |
| *SLC22A3* | OCT3 | skeletal muscle, liver, placenta, kidney, heart | cimetidine | desipramine, prazosin, phenoxy-benzamine |
| *SLC22A4* | OCTN1 | kidney, skeletal muscle, placenta, prostate, heart | quinidine, verapamil | |
| *SLC22A5* | OCTN2 | kidney, skeletal muscle, prostate, lung, pancreas, heart, small intestine, liver | quinidien, verapamil | |
| *SLC22A6* | OAT1 | kidney, brain | acyclovir, adefovir, methotrexate, zidovudine | probenecid, cefadroxil, cefamandole, cefazolin, |
| *SLC22A7* | OAT2 | liver, kidney | zidovudine | |
| *SLC22A8* | OAT3 | kidney, brain | cimetidine, methotrexate, zidovudine | probenecid, cefadroxil, cefamandole, cefazolin |

ABC:ATP-binding cassette transporter superfamily; SLC: solute-linked carrier transporter family; SLCO: solute-linked carrier organic anion transporter family; MDR1: multi-drug resistance; MRP: multi-drug resistance related protein; BSEP:bile salt export pump; BCRP: breast cancer resistance protein; OAT: organic anion transporter; OCT: organic cation transporter; NTCP: sodium taurocholate co-transporting polypeptide; ASBT: apical sodium-dependent bile salt transporter

**P-glycoprotein**

The impact of drug transporters on pharmacokinetics has been widely recognized in the past few years. Several transporters with different functions have been characterized in various organs. P-glycoprotein, a product of the multiple drug resistance 1 (MDR1) gene, is one of the most studied drug transporters and belongs to the superfamily of adenosine triphosphate (ATP)- binding cassette (ABC) proteins (Sharom et al., 1999). It was originally recognized as a cause of the multidrug resistance in cancer chemotherapy. Like the CYP enzymes, P-glycoprotein is also able to interact with a large number of structurally distinct drugs and xenobiotics.

P-glycoprotein is a transmembrane efflux protein that actively transports drugs, other xenobiotics, and cellular metabolites out of the cells. P-glycoprotein is expressed in the epithelial cells on the luminal surfaces of many organs with an excretory or barrier function: the liver, kidneys, and small intestine, and the endothelial cells of the blood-brain and blood-testes barriers. Therefore, P-glycoprotein can affect drug disposition, for instance by inhibiting drug absorption from the gastrointestinal tract and by facilitating drug excretion into the bile and urine.

Table 2.3 Major acceptable in vitro substrates and inhibitors of P-glycoprotein

| Substrate | Inhibitor |
|---|---|
| Digoxin | Cyclosporine A [a], Ketoconazole [a] |
| Loperamide | Nelfinavir [a] |
| Quinidine | Quinidine [b] |
| Vinblastine [a] | Ritonavir [a] |
| Talinolol | Saquinavir [a] |
| | Tacrolimus , LY335979 |
| | Valspodar (PSC833) , Verapamil |
| | Elacridar, (GF120918) |
| | (GG 918) , Reserpine |

[a] also CYP3A inhibitor [b] also CYP2D6 inhibitor

The known drug substrates for P-glycoprotein include a number of anticancer agents and other drugs, such as digoxin. Of the antidiabetic drugs studied, glibenclamide is a substrate for the P-glycoprotein (Golstein et al., 1999), and verapamil, an inhibitor of P-glycoprotein,

elevates the plasma concentrations of glibenclamide (Semple et al., 1986). It is not yet known whether glipizide, glimepiride, or repaglinide is a substrate for the P-glycoprotein.

**Induction and inhibition of P-glycoprotein**

Several studies provide convincing evidence that P-glycoprotein is inducible by some of the same drugs that are known to induce drug-metabolizing enzymes (Schuetz et al., 1996; Greiner et al., 1999; Dunn and Faulds, 2000). Recently, P-glycoprotein induction by rifampicin was shown to be mediated by the orphan nuclear receptor PXR (Geick et al 2001), which is involved in CYP3A4 induction. A number of PXR activators induce either CYP3A4 or P-glycoprotein or both (Lehmann et al., 1998; Moore and Kliewer, 2000). PXR messenger ribonucleic acid (mRNA) is present in marked quantities in the liver and the small intestine but to a much lesser extent, if at all, in the kidneys (Moore and Kliewer, 2000). Consequently, rifampicin seems to induce P-glycoprotein mainly in the small intestine and the liver (Greiner et al., 1999).

Several widely used drugs inhibit the P-glycoprotein and may therefore cause drug interactions with P-glycoprotein substrates. Vinblastine is a substrate of CYP3A4 and P-gp, where as nifedepine is an inducer of CYP3A4 but an inhibitor of CYP3A4. on the other hand, cyclosporine A (CsA) has been characterized as substrates for CYP3A4 but a substrate and inducer of P-gp. The net result of P-gp acts to prevent free accesss of some durg modlecules, such as CsA, to the systemic circulation. Inhibition of P-gp by co-administered agents, such as the antifungal Ketoconazole can lead to elevated systemic exposure to CsA and potential toxicity. Inhibition of the intestinal P-glycoprotein may increase the systemic availability of P-glycoprotein substrates, whereas inhibition of the P-glycoprotein in the kidneys may reduce the renal excretion of P-glycoprotein substrates. For example, itraconazole raises plasma digoxin concentrations by reducing its renal clearance (Jalava et al., 1997). On the other hand, inhibition of the P-glycoprotein at the blood-brain barrier may lead to an increase in the permeation of drugs through the blood-brain barrier and to an increase in their effects on the central nervous system, as is seen with the antidiarrheal opioid loperamide after quinidine administration (Sadeque et al., 2000). Apart from its role as a substrate for the P-glycoprotein, glibenclamide is also an inhibitor of the P-glycoprotein (Golstein et al., 1999). Furthermore, recent studies suggest that glibenclamide may be a general inhibitor of the ABC transporters (Payen et al., 2001).

## 2.2 History and overview of diabetes

### Chronology of some important events in the history of diabetes

- 11th century – diagnosis of diabetes commonly made by 'water tasters', who drank the urine of those suspected of having diabetes. Urine taste of diabetics was thought to be sweet, and the Latin word 'mellitus' (meaning 'honey) was added to the term diabetes
- 19th century –
  - ⇒ development of chemical tests to indicate and measure the presence of sugar in the urine
  - ⇒ Claude Bernard, a French researcher, studied the functioning of the pancreas and the glycogen metabolism of the liver
  - ⇒ Czech researcher, I.V. Pavlov, discovered the links between the nervous system and gastric secretion, making an important contribution to science's knowledge of the physiology of the digestive system
  - ⇒ 1869 - Paul Langerhans, a German medical student, announced in a dissertation that the pancreas contains two systems of cells. One set secretes the normal pancreatic juice, the function of the other was unknown. Several years later, these cells are identified as the 'islets of Langerhans.'
  - ⇒ 1889 - Oskar Minkowski and Joseph von Mering at the University of Strasbourg, France, first remove the pancreas from a dog to determine the effect of an absent pancreas on digestion
- 1908 - German scientist, Georg Zuelzer develops the first injectable pancreatic extract to suppress glycosuria; however, there are extreme side effects to the treatment
- 1921 - Isolation of insulin from pancreas in 1921 by Banting and Best
- 1922 – Mass production of insulin by Eli Lilly and company in collaboration with the University of Toronto in North America
- 1944 – Development of standard insulin syringe
- 1955 – Introduction of oral drugs for lowering blood glucose levels
- 1959 – Recognition of type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes
- 1966 – First pancreas transplantation at University of Manitoba
- 1970 – Development of blood glucose meters and insulin pumps
- 1983 – Introduction of biosynthetic human insulin
- 1986 – Introduction of insulin pen delivery system

### 2.2.1 Classification and diagnosis of diabetes

As knowledge of diabetes continued to develop, an International Expert Committee was formed in 1995 to revise the nomenclature, diagnostic criteria and classification of diabetes developed by the National Diabetes Data Group (NDDG) in 1979 (2003b). The salient features of the changes in NDDG/WHO classification are as below:

*Definition and description of diabetes*:

- Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels
- The committee recommended the recognition of two major forms of diabetes, which they termed type 1 and type 2 diabetes to include evidence that diabetes mellitus was an etiologically and clinically heterogeneous group of disorders that share hyperglycaemia in common. Broadly, the committee proposed classification of diabetes based on aetiology into four types, *viz*., type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency), type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance), Gestational diabetes mellitus (GDM), and other specific types that include 8 sub-types. The class termed 'malnutrition-based related diabetes has been eliminated
- Patients with any form of diabetes may require insulin treatment at some stage of their disease. Such use of insulin does not, of itself, classify the patient
- The terms insulin-dependent diabetes mellitus and non–insulin-dependent diabetes mellitus and their acronyms, IDDM and NIDDM, are eliminated. The terms type 1 and type 2 diabetes are retained, with Arabic numerals being used rather than roman numerals
- Type 2 diabetes includes the most prevalent form of diabetes, which results from insulin resistance with an insulin secretory defect
- The stage termed impaired glucose tolerance (IGT) has been retained. The analogous intermediate stage of fasting glucose is named impaired fasting glucose (IFG). Selective rather than universal screening for glucose intolerance in pregnancy is now recommended

- The degree of hyperglycaemia may change over time and reflects the severity of underlying metabolic process and its treatment more than the nature of the process itself (**Figure 2.2**)
- For the clinician and patient, it is less important to label the particular type of diabetes than is to understand the pathogenesis of the hyperglycaemia and to treat it effectively.

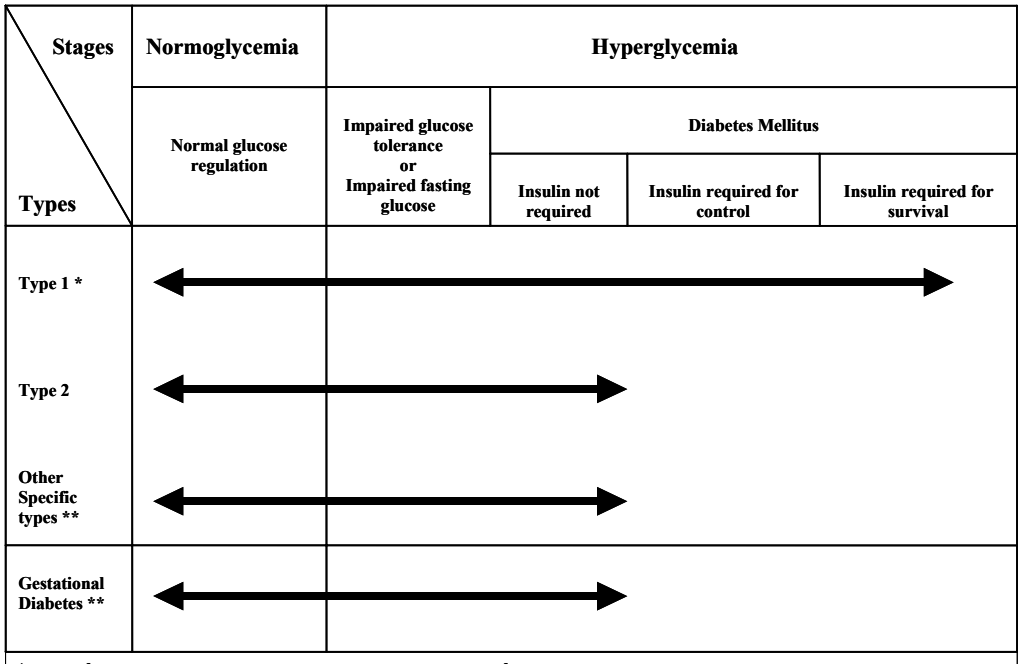



*Figure 2.2 Disorders of glycaemia: Etiologic types and stages*

* Even after presenting in ketoacidosis, these patients can briefly return to normoglycemia without requiring continuous therapy (i.e, "honeymoon remission"); ** In rare instances, patients in these categories (e.g., Vacor toxicity, Type 1 diabetes presenting in pregnancy) may require insulin for survival.

Impaired glucose tolerance and impaired fasting glucose:

The terms impaired glucose tolerance (**IGT**) and impaired fasting glucose (**IFG**) that refer to a metabolic stage intermediate between normal glucose homeostasis and diabetes, now referred to as pre-diabetes, are defined as described below:

Criteria for the diagnosis of diabetes mellitus in epidemiological studies:

- Symptoms of diabetes plus casual plasma glucose concentration ≥ 200 mg/dL (11.1 mmol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydypsia, and unexplained weight loss

**Or**

- Fasting plasma glucose (FPG) ≥ 126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h

**Or**

- 2-h post-load glucose (2-h PG) ≥ 200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by WHO (2003b), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water

In the absence of unequivocal hyperglycaemia with acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day. The third measure (OGTT) is not recommended for routine clinical use

Criteria for diagnosis of intermediate group of subjects whose FPG levels / 2-h PG values in OGTT are below those mentioned in epidemiological studies, but are too high to be considered altogether normal:

⇒ FPG < 110 mg/dL (6.1 mmol/L) = normal fasting glucose

⇒ FPG ≥ 110 mg/dL (6.1 mmol/L) and < 126mg/dL (7.0 mmol/L) = **IFG**

⇒ FPG ≥ 126 mg/dL (7.0 mmol/L) = provisional diagnosis of diabetes (the diagnosis must be confirmed, as described above)

The corresponding OGTT values are as below:

⇒ 2-h PG < 140 mg/dL (7.8mmol/L) = normal glucose tolerance

⇒ 2-h PG ≥ 140 mg/dL and < 200 mg/dL (11.1 mmol/L) = **IGT**

⇒ 2-h PG ≥ 200 mg/dL (11.1 mmol/L) = provisional diagnosis of diabetes (the diagnosis must be confirmed, as described above)

Criteria for testing of diabetes in asymptomatic, undiagnosed individuals:

⇒ Testing for diabetes should be considered in individuals at age 45 years and above, particularly in those with a BMI ≥ 25 kg/$m^2$†; if normal, it should be repeated at 3-year intervals

⇒ Testing should be considered at a younger age or be carried out more frequently in individuals who are overweight (BMI ≥ 25 kg/$m^2$**;** this may not be correct for all ethnic groups) and have additional risk factors:

- have a first-degree relative with diabetes
- are habitually physically inactive

- are members of a high-risk ethnic population (e.g., African-American, Hispanic American, Native American, Asian American, Pacific Islander)
- have delivered a baby weighing > 9 lb or have been diagnosed with GDM
- are hypertensive (≥ 140/90)
- have an HDL cholesterol level ≤ 35 mg/dL (0.90 mmol/L) and/or a triglyceride level ≥ 250 mg/dL (2.82 mmol/L)
- have PCOS
- on previous testing, had IGT or IFG
- have a history of vascular disease

The OGTT or FPG test may be used to diagnose diabetes; however, in clinical settings the FPG test is greatly preferred because of ease of administration, convenience, acceptability to patients, and lower cost

### 2.2.2 Pathophysiology of type 2 diabetes

The development of type 2 diabetes is characterized by progression from normal glucose tolerance to impaired glucose tolerance (IGT) to diabetes. The pathophysiology of type 2 diabetes encompasses progressive pancreatic β-cell dysfunction and insulin resistance in all major target tissues, such as skeletal muscle, kidney, liver and adipose tissue. Most of the newly diagnosed type 2 diabetic subjects already suffer from "late complications of diabetes" at the time of diagnosis, highlighting the fact that it represents only the "tip of the iceberg" of long existing metabolic disturbances with deleterious effects on the vascular system, tissues and organs (Matthaei et al., 2000). Pathogenesis of type 2 diabetes is schematically represented in **Figure 2.3** (Moneva and Dagogo-Jack, 2002). The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that non-pharmacological treatments, such as reduced caloric intake and increased physical activity, as the primary option is sufficient only in 25% of patients with a 3- year history (after the diagnosis) of diabetes (Turner et al., 1999). Though their efficacy has been demonstrated in many studies (Schneider et al., 1995), the actual number of patients sufficiently treated without pharmacological agents is comparatively low. As the disease duration advanced with associated progressive deterioration in β-cell function (Levy et al., 1998), the percentage of responders to non-pharmacological treatments fell down to 10% after 9 years. These data clearly indicate that pharmacological treatment is required in the vast majority of type 2 diabetic population.

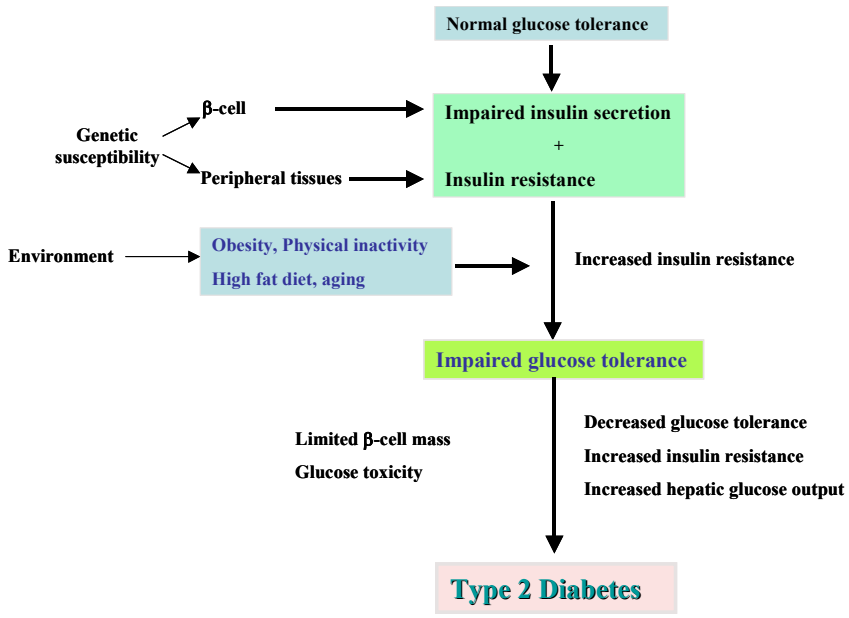



*Figure 2.3 Pathogenesis of type 2 diabetes.*

The evolution from normal glucose tolerance to clinically overt type 2 diabetes is associated with progressive insulin resistance and β-cell insulin secretory deficiency.

### Insulin resistance

Skeletal muscle, liver and adipose tissue are some of the major metabolic sites for insulin action. Insulin resistance is defined as a steady-state plasma glucose level that is higher than would be expected for the prevailing plasma insulin. Insulin resistance is commonly associated with obesity and type 2 diabetes. The term is often used synonymously with impaired insulin-stimulated glucose disposal, as measured with the hyperinsulinaemic-euglycaemic clamp technique (DeFronzo et al., 1979; Moller and Flier, 1991; Garvey and Birnbaum, 1993). Insulin-induced inhibition of lipolysis is an exquisitely sensitive measure, with $EC_{50}$ values that fall in the physiological insulinaemic range (Stumvoll and Jacob, 1999). Overproduction of nonesterified fatty acids and over-expression of tumour necrosis factor-alpha ($TNF_{\alpha}$) by adipocytes have been implicated in the pathogenesis of insulin resistance (Boden, 1997; Hotamisligil, 1999). Mechanistically, insulin resistance is a complex syndrome that involves a number of molecular defects at different levels along the insulin signalling cascade (Le Roith and Zick, 2001). Excessive basal glucose production in the presence of fasting hyperinsulinaemia is the key feature of type 2 diabetes (DeFronzo et al., 1982; Bogardus et al., 1984). Defective suppression of endogenous

glucose production by normal or elevated insulin levels is observed in type 2 diabetes (Mitrakou et al., 1990). Involvement of insulin resistance of glucose production in the pathogenesis of type 2 diabetes becomes evident with these observations (**Figure 2.4**).

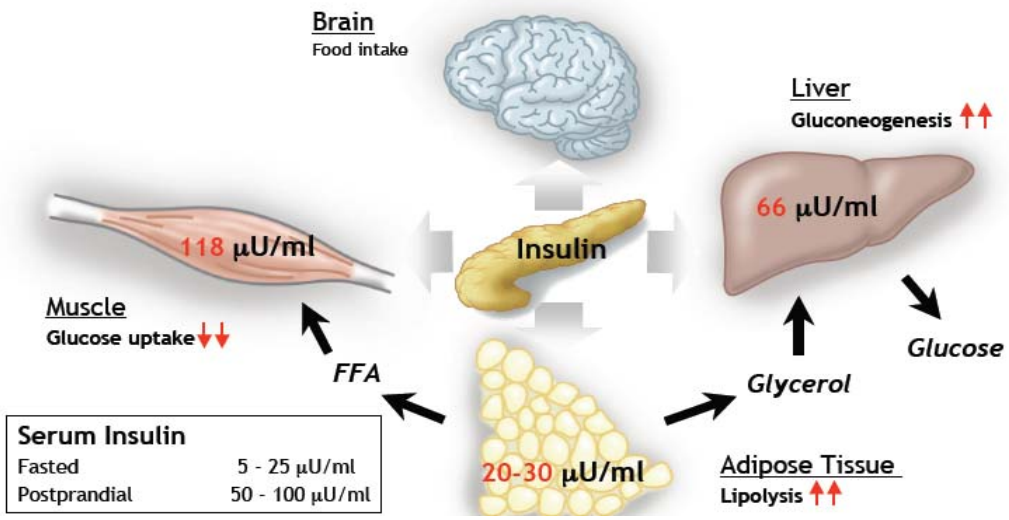



*Figure 2.4 Insulin resistance and pathogenesis of type 2 diabetes*

**β-cell dysfunction**

The pancreatic β-cells secrete insulin in response to glucose stimulation through a series of transmembrane electrical reactions. It has been suggested that the final common pathway responsible for the development of type 2 diabetes is the failure of the pancreatic β-cell to compensate for insulin resistance. An absolute decompensation in β-cell function is seen during transition from IGT to diabetes (**Figure 2.5**). It is not clear whether the progressive β-cell dysfunction results from pre-programmed genetic abnormalities, acquired defects, or combination of both. A loss of β-cell differentiation can be found in the early stage of type 2 diabetes (Weir et al., 2001). The β-cell mass is lower in type 2 diabetic patients compared with obese control subjects, but the reduction is modest and does not completely explain the hyperglycaemia. Thus the functional loss (i.e. impaired glucose-mediated insulin secretion) exceeds the expected impact of a 20-50% loss of β-cell mass, reported in autopsy studies of type 2 diabetes patients.

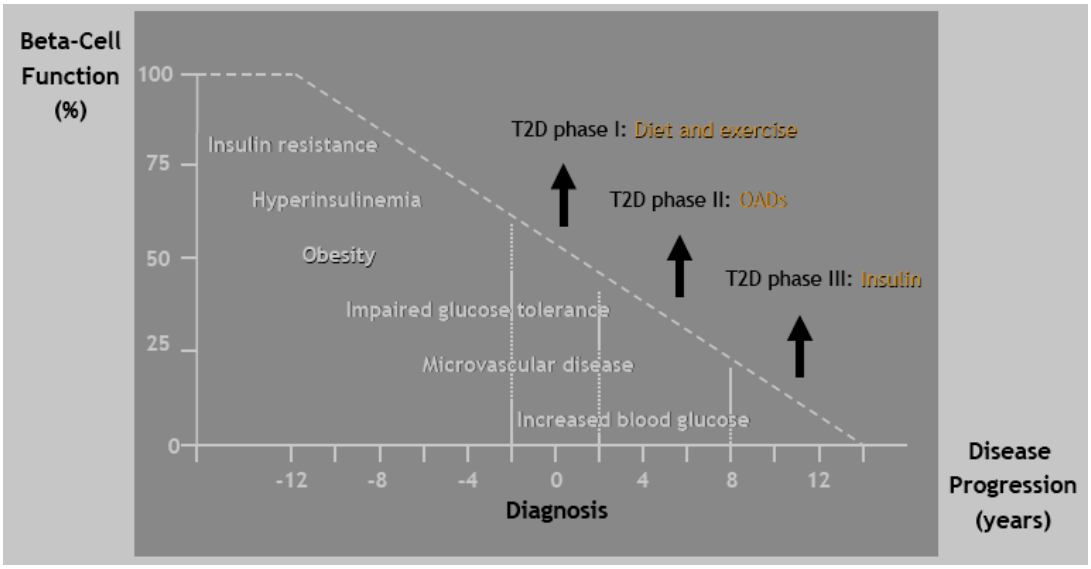



*Figure 2.5 Beta-cell function and diabetes disease progression*

**Hepatic glucose output**

Basal rates of hepatic glucose production (HGP) are variably increased in patients with type 2 diabetes but are normal in their normoglycaemic first-degree relatives. The ability of insulin to suppress HGP also is normal in first-degree relatives, suggesting that dysregulated HGP is probably acquired late in the pathogenesis. The increased endogenous glucose output, triggered by an increased flux of gluconeogenic precursors (such as glycerol, FFAs, and alanine), is a result, at least in part, of hepatic insulin resistance. The diabetic state increases fat utilization as alternative fuel; products of the resultant lipolysis and fatty acid oxidation, such as acetyl-coenzyme A, are potent stimuli for hepatic gluconeogenesis. There are no abnormalities in the activities of the principal enzymes of gluconeogenesis such as phosphoenolpyruvate carboxykinase, fructose 1,6-biphosphatase, and glucose-6-phosphatase in type 2 diabetes. Hepatic insulin resistance is associated with a decrease in glucokinase activity, which catalyzes a crucial step in hepatic glucose metabolism. The liver in type 2 diabetes thus appears to be programmed to overproduce and underutilize glucose.

### 2.2.3 Complications of diabetes

Diabetes (both type 1 and type 2) is a debilitating and true multisystem disease. In addition to creating day-to-day challenges in glycaemic control, it causes gradual breakdown of vital bodily functions leading to disabling, and in some cases, life-threatening complications. In both types of diabetes, abnormally high levels of blood glucose, as well as other

metabolites, damage both the small and large blood vessels, producing microvascular and macrovascular complications respectively, that affect almost each and every organ of the body.

**Microvascular complications**

The term 'microvascular complications' encompasses the effects of diabetes on the small blood vessels throughout the body that lead to severe damage to the eye, kidney and nervous system. Each of these complications has distinct pathophysiological features and may require distinct therapeutic approaches.

**Ocular related complications**

'Retinopathy', the most common form of diabetic eye disease that damages the retina, develops when small blood vessels that supply the retina with oxygen and other nutrients are damaged. Virtually all people with Type 1 diabetes have retinal damage, with 30 percent having the most severe form. Approximately, 80% of the type 2 diabetics who take insulin have retinopathy after 15 years and 10 to 15 percent have proliferative retinopathy.

**Kidney related complications**

Nephropathy occurs as a result of damage to kidneys that affects their functional ability. The damage is usually a silent process, gradually progress over a long period and manifests itself only when <25% of renal function remains active. After 5 years of diagnosis of diabetes, approximately, 10% of type 2 diabetics develop clinically detectable proteinuria and 20% show signs of renal damage after 20 years.

**Nervous system related complications**

Diabetes affects many parts of the nervous system. The damage is partly due to the effect on the small blood vessels and also probably due to direct effect on the nerve tissue itself, resulting in the damage of both the peripheral nerves (nerves involved in sensation and movement) –*Peripheral neuropathy*, and the autonomic nerves (nerves that control many internal functions, such as heart rate, gastric motility, bladder function, normal sexual response, etc.) –*Autonomic neuropathy*. While peripheral neuropathy causes pain and loss of sensation, contributing to the increased risk for ulceration, limb infection and amputation, autonomic neuropathy may lead to heart arrhythmias, poor control of blood pressure, and digestive and sexual dysfunction.

**Oral complications of diabetes**

Oral complications of diabetes are extremely common, difficult to treat within poorly controlled patients and reduce the quality of life. They include mucosal infections, salivary gland dysfunction leading to difficulty swallowing and speaking.

**Macrovascular complications**

Macrovascular complications (damage to the large blood vessels) is the most common cause of death in type 1 and type 2 diabetic patients and diabetes is a major cause of cardiac, cardiovascular disease (CVD) and peripheral vascular damage. In diabetes, the supply of nutrients and oxygen to tissues is impaired by the combination of peripheral vascular disease of the large blood vessels (atherosclerosis) and by the microvascular damage to the small blood vessels and capillaries, which nourish the same area. Individuals with diabetes also have some damage to the heart muscle (cardiomyopathy) and to the nerves that supply the heart (cardiac autonomic neuropathy) as a result of the abnormal metabolism present in this disease. A diabetic patient with existing or incipient macrovascular diseases requires multiple modifications of lifestyle and diet, as well as a poly-pharmaceutical approach to address the needs for glucose control, optimization of lipid levels and blood pressure, and other disease risk factors.

### 2.2.4 Oral antidiabetic drugs

The first attempts to treat human diabetes by orally active drugs were made between 1925 and 1930 with synthalines and their derivatives. However, because of their toxicity, these compounds were never used in clinical practice. The hypoglycemic activity of some of the antibacterial sulfonamides was discovered in the 1940's, with carbutamide and tolbutamide the first sulfonamide derivative antidiabetic drugs in clinical use. The second-generation sulfonylurea, glibenclamide, has been in clinical use since 1969 and is per milligram 500 times as active as tolbutamide.

The oral antidiabetic drugs form the basis of the modern pharmacological treatment of NIDDM. There are currently 5 groups of oral antidiabetic drugs in clinical use: the sulfonylureas, meglitinide analogues, biguanides, thiazolidinediones, and α-glucosidase inhibitors. In addition, also the dietary fiber guar gum can be used to treat hyperglycemia in patients with diabetes (Nuttall, 1993). On the basis of their primary mechanism of action, the oral antidiabetic drugs can be divided into those that act by enhancing insulin secretion

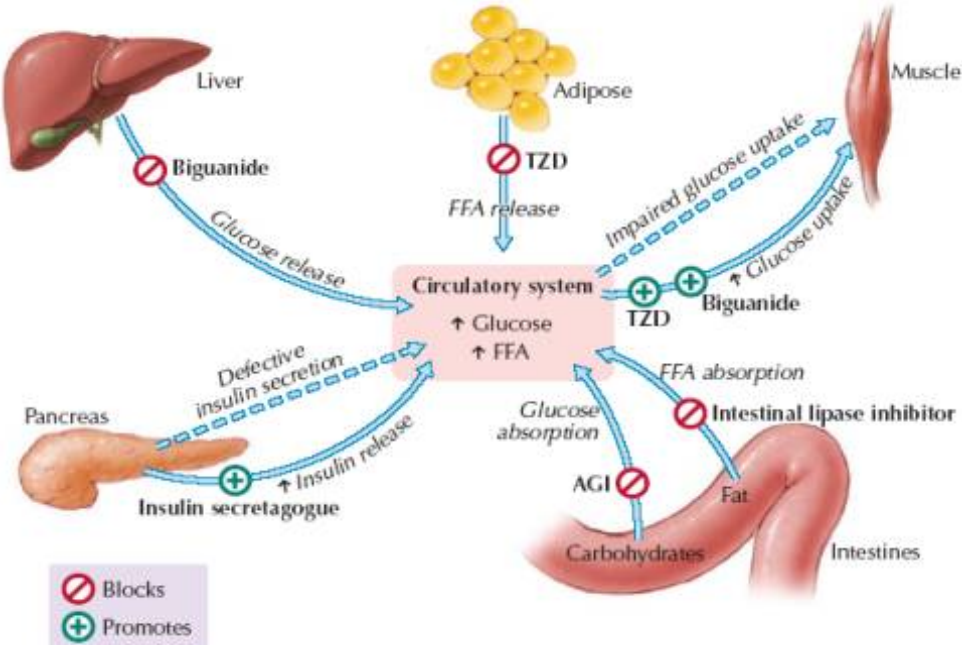
from the pancreas and those that act through extrapancreatic effects. The former include the sulfonylureas;

The oral antidiabetic drugs glimepiride, glibenclamide, glipizide, and repaglinide all act primarily by stimulating glucose-induced insulin release from pancreatic β-cells (Creutzfeldt, 1969; Wensing, 1989; Guay, 1998; Langtry and Balfour, 1998).The sulfonylureas and repaglinide block ATP-sensitive potassium channels in the β -cells (Sturgess et al., 1985), which leads to membrane depolarization, influx of calcium into the cells, and eventually, to release of insulin. The ATP-sensitive potassium channel is a eteromultimeric protein comprising 2 subunits: the sulfonylurea receptor (SUR) and the inwardly rectifying potassium channel (KIR6.x) subunits, in 4:4 stoichiometry (Aguilar-Bryan et al., 1998). The KIR6.x has 2 and the SUR has 3 subtypes (KIR6.1, KIR6.2, SUR1, SUR2A, SUR2B). The combination of KIR6.2 and SUR1 forms the pancreatic ATP-sensitive potassium channel, whereas the combination of KIR6.2 and either of the 2 SUR2 subtypes forms the ATP-sensitive potassium channels expressed in the heart(SUR2A) and smooth muscle (SUR2B) (Aguilar-Bryan et al., 1998). The first generation sulfonylurea tolbutamide has been proposed to bind relatively selectively to SUR1 (Gribble et al., 1998), whereas glibenclamide, glimepiride, and repaglinide seem to bind both SUR1 and SUR2A with approximately similar affinities (Gribble et al., 1998; Dabrowski et al., 2001; Song and Ashcroft, 2001). However, glimepiride and repaglinide bind to different sites on SUR than does glibenclamide (Kramer et al., 1994; Fuhlendorff et al., 1998), and recent evidence suggests that glimepiride has fewer effects on the cardiovascular ATP sensitive potassium channel and thereby possibly fewer cardiovascular adverse effects than glibenclamide has (Mocanu et al., 2001). The sulfonylureas and repaglinide may, in part, act also via extrapancreatic effects, and some evidence suggests that they may enhance glycogen synthesis and inhibit glycogenolysis and gluconeogenesis in the liver; they may also improve peripheral glucose uptake by the muscles ((Wensing, 1989; Guay, 1998; Langtry and Balfour, 1998).

The α-glucosidase inhibitors slow down carbohydrate absorption from the gut (Balfour and McTavish, 1993). The first of the α-glucosidase inhibitors in clinical use was acarbose. It is eliminated through cleavage by the intestinal digestive enzymes and through biotransformation by the intestinal bacteria. Only trace amounts of unaltered acarbose are absorbed from the gastrointestinal tract into the systemic circulation (Balfour and McTavish, 1993). Miglitol is a newer α-glucosidase inhibitor, and in contrast to acarbose, it

is almost completely absorbed from the gastrointestinal tract after low doses, but its absorption is saturable (Scott & Spencer 2000). Miglitol is not metabolized and is excreted very rapidly unaltered (Scott and Spencer, 2000).

Currently available oral antihyperglycaemia agents (OHAs) for type 2 diabetes are presented in **Figure 2.6** and **Figure 2.7** and **Table 2.4** and **Table 2.5** (Cheng and Fantus, 2005).




TZD = thiazolidinedione; FFA = free fatty acid; AGI = α-glucosidase inhibitor.

*Figure 2.6 Major target organs and actions of orally administered antihyperglycemic agents in type 2 diabetes mellitus.*

**Combinations to be avoided**

- Sulfonylurea and nonsulfonylurea insulin secretagogue
- Insulin secretagogue and pre-prandial insulin
- Thiazolidinedione and insulin

Combinations of sub-maximal doses of different classes of OHAs may be equally effective as or more effective than maximum dose of monotherapy in improving glucose control with fewer adverse effects.

Table 2.4 Food and Drug Administration -approved indications for oral antidiabetic agents

| Drug | FDA-approved Indications |
|---|---|
| Sulfonylureas | Monotherapy or with metformin, alpha-glucosidase inhibitors, thiazolidinediones, or insulin |
| Biguanides (Metformin) | Monotherapy or with sulfonylurea or insulin |
| Alpha-glucosidase inhibitors | Monotherapy or with sulfonylurea, metformin, or insulin (Miglitol only ap proved for use with sulfonylurea) |
| Thiazolidinediones | Monotherapy or with sulfonylurea, metformin, or insulin (Rosiglitazone not approved for use with insulin) |
| Nonsulfonylurea secretagogues | Monotherapy or with metformin |
| Glucovance® (Glyburide + Metformin) | Monotherapy or in combination with a thiazolidinedione |
| Metaglip® (Glipizide + Metformin) | Monotherapy |
| Avandamet® (Rosiglitazone + Metformin) | Monotherapy |

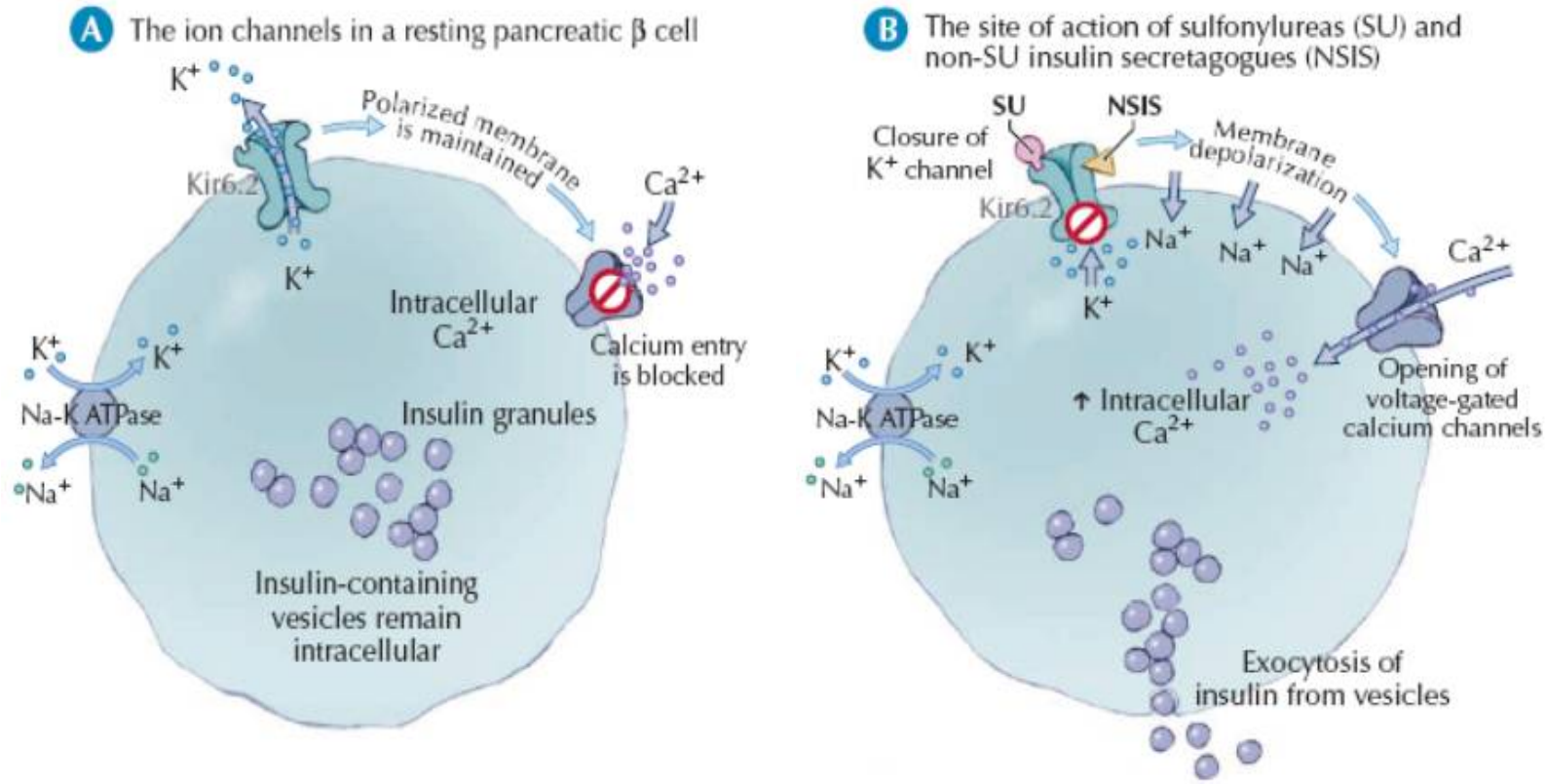



*Figure 2.7 Insulin secretagogues mimic glucose to close adenosine triphosphate sensitive potassium channels and stimulate insulin secretion.*

(A) shows the ion channel in a resting pancreatic β cell. (B) shows the action of insulin secretagogues on the cell. (This figure was adapted from Cheng and Fauntus et al., 2005).

Table 2.5 Orally administered antihyperglycemic agents (OHAs) for the treatment of diabetes

| Drug class | Mechanism of action | Primary site of action | Dosage | Decrease in HbA1c concentration* | Main side effects | Drug interactions | Contraindications |
|---|---|---|---|---|---|---|---|
| **α-Glucosidase inhibitor** | Delays intestinal | Small intestines | 25 mg once daily, titrated to | 0.5%–1.0% | Gastrointestinal | - | Irritable bowel syndrome, severe |
| **Biguanide**† (metformin) | Increases liver and muscle | Liver, peripheral tissue | 500 mg once daily, titrated to 1000 mg twice | 1.0%–1.5% | Gastrointestinal, lactic acidosis (rare) | Alcohol (↑ risk of lactic acidosis) | Moderate to severe liver or cardiac dysfunction, mild |
| **Insulin secretagogue** | | | | | | Many | |
| **Sulfonylureas** (gliclazide, glimepiride, glyburide) | Increases insulin secretion | | Gliclazide: 40–160 mg twice daily, 30–120 mg once daily (MR form) | 1.0%–1.5% | Hypoglycaemia, weight gain | ↑ effect by CYP 450 3A4 inhibitos; ↓ effect by CYP 450 3A4 inducers | Moderate to severe liver dysfunction; adjust dose in the presence of severe kidney dysfunction. |
| **Non-sulfonylureas** (repaglinide, nateglinide) | | | Glimepiride: 1–8 mg once daily | Repaglinide: 1.0%–1.5% Nateglinide: 0.5%–1.0% | Hypoglycaemia, weight gain | | Avoid use of glyburide in elderly patients or patients with kidney dysfunction |
| | | Pancreas | Glyburide: ≤ 5 mg once daily, titrated to > 5 mg twice daily | | | | Severe liver or kidney dysfunction; avoid concomitant use of repaglinide with gemfibrozil |
| | Acute increase of insulin secretion | | Repaglinide:0.5-4 mg 3 times aily Nateglinidie: 60-120 mg 3 times daily | | | | |
| **Insulin sensitizer or thiazolidinedione**§ (rosiglitazone, | Increases adipose and muscle insulin | Peripheral tissue, liver | Rosiglitazone: 2–8 mg once daily Pioglitazone: | 1.0%–1.5% | Weight gain, edema, anemia, pulmonary edema, CHF | ↑ effect by CYP 450 2C8 and 2C9 | Severe liver dysfunction, NYHA class II-IV CHF |
| **Intestinal lipase inhibitor**¶ (orlistat) | Decreases intestinal fat absorption (weight loss) | Intestinal tract | 120 mg 3 times daily | 0.3%–0.9% | Gastrointestinal, reduced absorption of fat-soluble vitamins | | Malabsorption syndrome, cholestasis |

**Note: MR** = modified release, **CHF** = congestive heart failure, **NYHA** = New York Heart Association, *****Indicated average decreases in hemoglobin A1cconcentrations after 3–6 months of monotherapy, †Preferred primary agent for overweight patients, ‡ Use with caution or avoid in the presence of any elevation in serum creatinine levels, § 6–12 weeks are required to achieve the full glucose-lowering effect, ¶ Suitable for obese patients only.

## Novel targets for the treatment of diabetes

Irrespective of the underlying cause of diabetes, goal of any kind of treatment is directed at normalization of blood glucose. For normal metabolism insulin must be released from the pancreas in an exquisitely exact amount, at the right time and in a right pattern. The normal pancreas also senses the fasting and fed state as well as the energy content of the meals eaten. At any point of time glucose homeostasis is maintained by a balance between insulin secretion and insulin action. The robustness of the physiological system existing in non-diabetic people takes care of the alterations in any of these parameters. None of the available pharmacological agents can either take the place of this exquisite sensing capacity or restore the pattern of insulin kinetics precisely. This is evident from the presence of so many compounds to treat type 2 diabetes.

In light of recent progress made in understanding insulin resistance and pancreatic β-cell dysfunction, the two major defects in type 2 diabetes, several novel therapeutic approaches have been evolved to address both defects. Some of the important targets are presented in **Table 2..6** (Lenhard and Gottschalk, 2002; Wagner, 2002). All these targets focus on improving insulin sensitivity and augmenting glucose-dependent insulin secretion.

Table 2.6 Molecular targets in type 2 diabetes

| Mechanism of action | Target | Human proof of concept* |
|---|---|---|
| Hepatic glucose production | Glucagon receptor | Yes |
| | Glycogen phosphorlyase | Yes |
| Glucose stimulated secretion | GLP-1 receptor | Yes |
| | DPP-4 enzyme | Yes |
| Insulin signalling | Insulin receptor | No |
| | PTP-1B enzyme | Yes |
| | GSK-3 | No |
| | SHIP-2 | No |
| | IκB kinase | No |
| | Protein kinase C-8 | No |

PTP-1B, Protein tyrosine phosphatase 1B; GSK-3, glycogen synthetase-3;
SH2 domain containing inositol-5-phosphatase 2
* proof of concept demonstrated in healthy subjects, but not in patient with type-2 diabetes

Most of the novel targets mentioned in **Table 2.6** is in early stages of development and some of them even require a proof of concept in human/type 2 diabetic subjects. In a few of these targets, GLP-1 receptor agonists and DPP-IV inhibitors in particular, have been extensively studied both in preclinical and clinical phases and are showing lot of promise. The present work is based on DPP-IV target and attempts have been made to provide preclinical evidence to the therapeutic utility of a DPP-IV inhibitor in the management of type 2 diabetes.

In this study we have chosen the following commonly used oral antidiabetic drugs to study the drug interaction potential with the antiplatelet agents in rats. The literature reviews of the following are described in subsequent sections of this chapter.

- sulphonyl urea (glipizide)
- insulin secretogogue (nateglinide)
- biguanide (metformin)
- thiazolidinedione analogues (rosiglitazone)

### 2.2.3.1 Glipizide

Glipizide is a second-generation sulfonylurea indicated for the treatment of NIDDM. The dosage of glipizide is between 2.5 mg and 15 mg once daily. The Chemical Abstracts name of glipizide is 1-cyclohexyl-3-[[p-[2-(5-methylpyrazinecarboxamido) ethyl] phenyl]sulfonyl]urea. The molecular formula is $C_{21}H_{27}N_5O_4S$; the molecular weight is 445.55; the structural formula is shown below:

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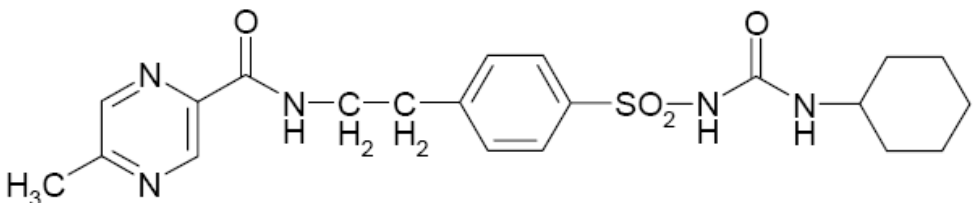


In humans, it is recommended that if the daily dose exceeds 15 mg (maximum 20 mg) it should be given in 2 or 3 doses

**Mechanism of Action**

The primary mode of action of glipizide in experimental animals appears to be the stimulation of insulin secretion from the beta cells of pancreatic islet tissue and is thus dependent on functioning beta cells in the pancreatic islets. In humans glipizide appears to lower the blood glucose acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets. The mechanism by which glipizide lowers blood glucose during long-term administration has not been clearly established. In man, stimulation of insulin secretion by glipizide in response to a meal is undoubtedly of major importance. Fasting insulin levels are not elevated even on long-term Glipizide administration, but the postprandial insulin response continues to be enhanced after at least 6 months of treatment. The insulinotropic response to a meal occurs within 30 minutes after an oral dose of Glipizide in diabetic patients, but elevated insulin levels do not persist beyond the time of the meal challenge. Extrapancreatic effects may play a part in the mechanism of action of oral sulfonylurea hypoglycemic drugs.

**Other Effects**

It has been shown that glipizide therapy was effective in controlling blood sugar without deleterious changes in the plasma lipoprotein profiles of patients treated for NIDDM.

In a placebo-controlled, crossover study in normal volunteers, Glipizide had no anti-diuretic activity, and, in fact, led to a slight increase in free water clearance.

**Pharmacokinetics**

Absorption

Glipizide is completely absorbed from the gastrointestinal tract and has an oral bioavailability of nearly 100% (Pentikainen et al., 1983). Its peak plasma concentration is nearly 200 ng/ml after the oral administration of 2.5 mg glipizide, and is generally reached within 1 to 2 hours (Huupponen et al., 1982).

Distribution

Glipizide is also highly bound to plasma protein (92% to 99%) (Pentikainen et al., 1983; Wensing, 1989)and has a low volume of distribution (about 10 liters).

Metabolism

Glipizide is extensively metabolized in the liver, and its main metabolites are 4-trans-ydroxyglipizide, NH 3-cis-hydroxyglipizide, and N-(2-acetyl-amino-ethyl-phenyl-sulfonyl) N-cyclohexylurea (DCAA) (Wensing, 1989). CYP2C9 is probably the main CYP enzyme involved in the oxidative metabolism of glipizide, because in an individual homozygous for the defective CYP2C9*3 allele, the oral clearance of glipizide was drastically lower than in other subjects (Kidd et al., 1999).

Excretion

The metabolites of glipizide are pharmacologically inactive and are excreted primarily in the urine (Pentikainen et al., 1983; Wensing, 1989); its t½ is between 2 and 4 hours. Glipizide pharmacokinetics are unaffected by aging (Kradjan et al., 1989).

**Adverse effects**.

The main adverse effect of glipizide is hypoglycemia (Wensing, 1989), which is potentially dangerous (Seltzer, 1972). Other common adverse effects include gastrointestinal reactions such as nausea, vomiting, or epigastric pain, and skin reactions such as pruritus, erythema, urticaria, or morbilliform or maculopapular rash. Hepatic, renal, and hematological adverse effects are rare.

**Pharmacokinetic interactions**

The hypoglycemic action of sulfonylureas may be potentiated by certain drugs including nonsteroidal anti-inflammatory agents, some azoles and other drugs that are highly protein bound, salicylates, sulfonamides, chloramphenicol, probenecid, coumarins, monoamine oxidase inhibitors, and beta-adrenergic blocking agents. When such drugs are administered to a patient receiving glipizide, the patient should be observed closely for hypoglycemia. When such drugs are withdrawn from a patient receiving glipizide, the patient should be observed closely for loss of control. In vitro binding studies with human serum proteins indicate that Glipizide binds differently than tolbutamide and does not interact with salicylate or dicumarol. However, caution must be exercised in extrapolating these findings to the clinical situation and in the use of glipizide with these drugs.

Certain drugs tend to produce hyperglycemia and may lead to loss of control. These drugs include the thiazides and other diuretics, corticosteroids, phenothiazines, thyroid products, estrogens, oral contraceptives, phenytoin, nicotinic acid, sympathomimetics, calcium channel blocking drugs, and isoniazid. When such drugs are administered to a patient receiving glipizide, the patient should be closely observed for loss of control. When such drugs are withdrawn from a patient receiving glipizide, the patient should be observed closely for hypoglycemia.

Glipizide is very similar to glibenclamide with respect to its chemical structure, pharmacokinetic profile, and metabolism. One would therefore expect to see similar metabolic drug-drug interactions. However, relatively few have been reported. The CYP2C9 inhibitor fluconazole, 100 mg daily for 7 days, raised the AUC of glipizide by 49%. As could be expected on the basis of current knowledge of the metabolism of glipizide, a single 20-mg oral dose of the CYP3A4 inhibitor nifedipine failed to affect significantly the pharmacokinetics of glipizide (Connacher et al., 1987). In a study in patients with NIDDM, cimetidine (400 mg) and ranitidine (150 mg), taken together with a normal morning dose of glipizide, raised the AUC of glipizide by 23% and 34%, respectively (Feely et al., 1993). In that study, the hypoglycemic activity of glipizide was also significantly increased. The cyclooxygenase inhibitor indobufen (200 mg twice daily for 5 days) elevated the AUC of glipizide by 25% (Elvander-Stahl et al., 1984). Magnesium hydroxide (850 mg) taken together with glipizide accelerated the absorption of glipizide and enhanced the early insulin and glucose responses(Kivisto and Neuvonen, 1991a). Sodium bicarbonate, but not aluminum hydroxide, may also accelerate glipizide absorption (Kivisto and Neuvonen, 1991b). The dietary fiber guar gum used for NIDDM patients to treat hyperglycemia does not affect the absorption of glipizide (Huupponen et al., 1985).

The 5-day treatment with 600 mg of rifampicin once daily significantly affected the pharmacokinetics of glipizide. Rifampicin reduced the mean $AUC_{0-\infty}$ of glipizide by 22% ($P < .05$), but elevated the $C_{max}$ by 18% ($P < .05$). The mean $t_{½}$ of glipizide was shortened from 3.0 to 1.9 hours ($P < 0.01$) by rifampicin. A reduction in the $t_{½}$, but not in the $AUC_{0-\infty}$, was seen in every subject. The relative decrease in the $AUC_{0-\infty}$ of glibenclamide was significantly greater than that in the $AUC_{0-\infty}$ of glipizide ($P < .05$). Statistically non-

significant decreases in the decremental $AUC_{0-7}$ and maximum decrease in blood glucose were obvious during the rifampicin phase compared to those of the placebo phase None of the subjects experienced severe hypoglycemic symptoms (Niemi et al., 2001).

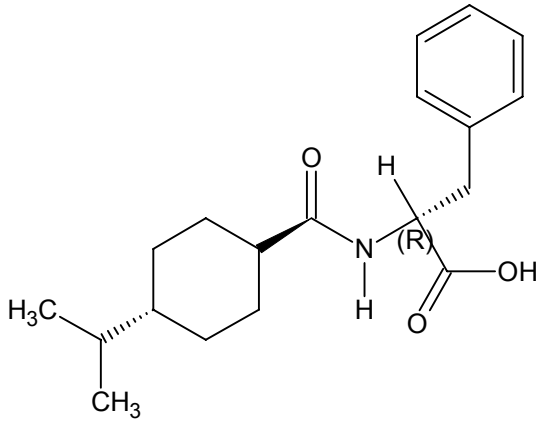
**Overdosage**

There is no well documented experience with glipizide overdosage. The acute oral toxicity was extremely low in all species tested (LD50 greater than 4 g/kg).

Overdosage of sulfonylurea including glipizide can produce hypoglycemia. Mild hypoglycemic symptoms without loss of consciousness or neurological findings should be treated aggressively with oral glucose and adjustments in drug dosage and/or meal patterns. Close monitoring should continue until the physician is assured that the patient is out of danger. Severe hypoglycemic reactions with coma, seizure, or other neurological impairment occur infrequently, but constitute medical emergencies requiring immediate hospitalization. If hypoglycemic coma is diagnosed or suspected, the patient should be given a rapid intravenous injection of concentrated (50%) glucose solution. This should be followed by a continuous infusion of a more dilute (10%) glucose solution at a rate that will maintain the blood glucose at a level above 100 mg/dL. Patients should be closely monitored for a minimum of 24 to 48 hours since hypoglycemia may recur after apparent clinical recovery. Clearance of glipizide from plasma would be prolonged in persons with liver disease. Because of the extensive protein binding of Glipizide, dialysis is unlikely to be of benefit.

### 2.2.3.2 Nateglinide

Nateglinide, a D-phenylalanine derivative, a potent insulin secretogogue designed to restore the early phase insulin secretion (Dunn and Faulds, 2000) used in NIDDM. The Chemical Abstracts name of nateglinide is (-)-N-[(trans-4-isopropylcyclohexane) carbonyl]-D-phenylalanine, is structurally unrelated to the oral sulfonylurea insulin secretogogue. Nateglinide is a white powder. It is freely soluble in methanol, ethanol, and chloroform, soluble in ether, sparingly soluble in acetonitrile and octanol, and practically insoluble in water. The molecular formula is $C_{19}H_{27}NO_3$; the molecular weight is 317.43; the structural formula is shown below:




In humans, it is recommended that if the daily dose exceeds 60 mg (maximum 120 mg) it should be given in 2 doses.

**Mechanism of Action**

Nateglinide is an amino-acid derivative that lowers blood glucose levels by stimulating insulin secretion from the pancreas. This action is dependent upon functioning beta-cells in the pancreatic islets. Nateglinide interacts with the ATP-sensitive potassium ($K^+ATP$) channel on pancreatic beta-cells. The subsequent depolarization of the beta cell opens the calcium channel, producing calcium influx and insulin secretion. The extent of insulin release is glucose dependent and diminishes at low glucose levels. Nateglinide is highly tissue selective with low affinity for heart and skeletal muscle. $K_{ATP}$ channels comprise a pore of inwardly rectifying $K^+$ channels subunits encased by sulphonylurea receptor (SUR) subunits with ATP-ase activity. Nateglinide binds competitively to SURs, thereby inhibiting $K_{ATP}$ channels and stimulating insulin secretion ((Hu, 2002; Quast et al., 2004). Nateglinide shows a greater degree of specificity for SUR1 over SUR2, as compared to glibenclamide and repaglinide (Hu et al., 2000; Hu, 2002).

**Other Effects**

Nateglinide has rapid onset and short duration of insulinotropic effect that results in reduction of mealtime glucose riseand lowers the postabsorptive potential for hypoglycemia in humans and experimental animals. (Fujitani et al., 1996; Ikenoue et al., 1997; Hu et al., 1998; Karara et al., 1999; Hanefeld et al., 2000; Keilson et al., 2000). In contrast to sulphonylureas, nateglinide increases pancreatic β-cell sensitivity to ambient glucose without increase in basal insulin secretion (Morimoto et al., 1998).In addition,

nateglinide has little effect on β-cell apoptosis, as compared with the induction associated with sulfonylureas (Maedler et al., 2005). In clinical studies, treatment with nateglinide resulted in an improvement in glycemic control, as measured b glycosylated hemoglobin A $1_C$ ($HbA_{1C}$) and post-meal glucose. Fasting plasma glucose (FPG) levels were also reduced. Nateglinide has also shown an inhibition of DPP IV and augments the antidiabetic activity of glucagons-like-peptide (Duffy et al., 2007)

**Pharmacokinetics**

Absorption

Following oral administration immediately prior to a meal, nateglinide is rapidly absorbed with mean peak plasma drug concentrations ($C_{max}$) generally occurring within 1 hour ($T_{max}$) after dosing (McLeod, 2004). When administered to patients with Type 2 diabetes over the dosage range 60 to 240 mg three times a day for one week, nateglinide demonstrated linear pharmacokinetics for both AUC (area under the time/plasma concentration curve) and $C_{max}$. $T_{max}$ was also found to be independent of dose in this patient population. Absolute bioavailability is estimated to be approximately 73%. When given with or after meals, the extent of nateglinide absorption (AUC) remains unaffected(Weaver et al., 2001). However, there is a delay in the rate of absorption characterized by a decrease in $C_{max}$ and a delay in time to peak plasma concentration ($T_{max}$). Plasma profiles are characterized by multiple plasma concentration peaks when nateglinide is administered under fasting conditions. This effect is diminished when nateglinide is taken prior to a meal. Nateglinide is known to inhibit PEPT1 and other transporters Furthermore nateglinide competitively inhibited $H^+$- driven ceftibuten transporter-mediated ceftibuten uptake reflecting that it inhibits ceftibuten/$H^+$ cotransport system. The uptake of nateglinide from apical to basal was greater than that of reverse uptake when studied in vitro using $^{14}$-C-nateglinide suggesting that it may not be substrate for P-glycoprotein but its uptake from apical side is mediated by the proton dependent transporter system distinct from MCT1(Okamura et al., 2002).

Distribution

Based on data following intravenous (IV) administration of nateglinide, the steady state volume of distribution of nateglinide is estimated to be approximately 10 liters in healthy subjects. Nateglinide is extensively bound (98%) to serum proteins, primarily serum

albumin, and to a lesser extent to α-1 acid glycoprotein. The extent of serum protein binding is independent of drug concentration over the test range of 0.1-10 (g/mL).

Metabolism

Nateglinide is metabolized by the mixed-function oxidase system prior to elimination. Nateglinide is primarily metabolized to hydroxy and glucoronide conjugated products and CYP2C9 is involved in these pathways (Weaver et al., 2001). Nateglinide is metabolized in major through CYP2C9 and to a lesser extent via CYP3A4. The major metabolites are less potent antidiabetic agents than nateglinide. The isoprene minor metabolite possesses potency similar to that of the parent compound nateglinide. In vitro data demonstrate that nateglinide is predominantly metabolized by cytochrome P450 isoenzymes CYP2C9 (70%) andCYP3A4 (30%). Nateglinide is also known to be a potent inhibitor of its self enzyme CYP2C9(Sahi et al., 2004)

Excretion

Nateglinide and its metabolites are rapidly and completely eliminated following oral administration(Weaver et al., 2001; McLeod, 2004) in humans. Within 6 hours after dosing, approximately 75% of the administered $^{14}$-C-nateglinide was recovered in the urine. Eighty-three percent of the $^{14}$-C-nateglinide was excreted in the urine with an additional 10% eliminated in the feces. Approximately 16% of the 14C-nateglinide was excreted in the urine as parent compound. In all studies of healthy volunteers and patients with Type 2 diabetes, nateglinide plasma concentrations declined rapidly with an average elimination half-life of approximately1.5 hours. Consistent with this short elimination half-life, there was no apparent accumulation of nateglinide upon multiple dosing of up to 240 mg three times daily for 7 days.

**Adverse effects**

Hypoglycemia was relatively uncommon in all treatment arms of the clinical trials. Only 0.3% of nateglinide patients discontinued due to hypoglycemia. Gastrointestinal symptoms, especially diarrhea and nausea, were no more common in patients using the combination of nateglinide and metformin than in patients receiving metformin alone. The other effects by treatment of nateglinide alone were upper respiratory infections, back pain, dizziness and accidental trauma.

**Pharmacokinetic interactions**

The meglitinide analogues repaglinide and nateglinide are new short-acting insulin secretagogues (Landgraf, 2000). Nateglinide differs from repaglinide in that it is even shorter-acting (Kalbag et al., 2001) and is eliminated by metabolism by both CYP2C9 and CYP3A4 (Dunn and Faulds, 2000).

The hypoglycemic action of nateglinide may be potentiated by certain drugs which inhibit CYP2C9 and CYP3A4. In vitro drug metabolism studies indicate that nateglinide is predominantly metabolized by the cytochrome p450 isozyme CYP2C9 (70%) and to a lesser extent CYP3A4 (30%). Many drugs have been studied to predict the interaction effect on invitro metabolism of nateglinide(Takanohashi et al., 2007). Nateglinide is a potential inhibitor of the CYP2C9 isoenzyme *in vivo* as indicated by its ability to inhibit the *invitro* metabolism of tolbutamide. Inhibition of CYP 3A4 metabolic reactions was not detected in in vitro experiments.

Fluconazole affected the disposition of nateglinide in humans, probably by the inhibition of CYP2C9, leading to increasing nateglinide concentrations that could increase the efficacy of nateglinide or its adverse events (Niemi et al., 2001). Rifampicin modestly decreased the exposure of nateglinide probably by inducing its oxidative biotransformation (Niemi et al., 2001). The coadministration of gemfibrozil and itraconazole has limited influence on pharmacokinetics of nateglinide in humans (Niemi et al., 2001). In another study sulfinpyrazone, a selective CYP2C9 inhibitor has modestly increased the systemic exposure of nateglinide by 28% and is well tolerated (Sabia et al., 2004).

In a randomized, multiple-dose crossover study, patients with type 2 diabetes were administered 120 mg nateglinide three times a day before meals for 1 day in combination with glyburide 10 mg daily. There were no clinically relevant alterations in the pharmacokinetics of either agent (Barnett et al., 2004). When nateglinide 120 mg three times daily before meals was administered in combination with metformin 500 mg three times daily to patients with type 2 diabetes, there were no clinically relevant changes in the pharmacokinetics of either agent (Ristic et al., 2007). When nateglinide 120 mg before meals was administered in combination with a single 1 mg dose of digoxin to

healthy volunteers there were no clinically relevant changes in the pharmacokinetics of either agent.

When healthy subjects were administered nateglinide 120 mg three times daily before meals for four days in combination with a single dose of warfarin 30 mg on day 2, there were no alterations in the pharmacokinetics of either agent. Prothrombin time was not affected (Anderson et al., 2002b). Administration of morning and lunch doses of nateglinide 120 mg in combination with a single 75 mg dose of diclofenac in healthy volunteers resulted in no significant changes to the pharmacokinetics of either agent (Anderson et al., 2002a).

Nateglinide is highly bound to plasma proteins (98 %), mainly albumin. In vitro displacement studies with highly protein-bound drugs such as furosemide, propranolol, captopril, nicardipine, pravastatin, glyburide, warfarin, phenytoin, acetylsalicylic acid, tolbutamide, and metformin showed no influence on the extent of nateglinide protein binding. Similarly, nateglinide had no influence on the serum protein binding of propranolol, glyburide, nicardipine, warfarin, phenytoin, acetylsalicylic acid, and tolbutamide in vitro. However, prudent evaluation of individual cases is warranted in the clinical setting.

Certain drugs, including nonsteroidal anti-inflammatory agents (NSAIDs), salicylates, monoamine oxidase inhibitors, and non-selective beta-adrenergic-blocking agents may potentiate the hypoglycemic action of nateglinide and other oral antidiabetic drugs.

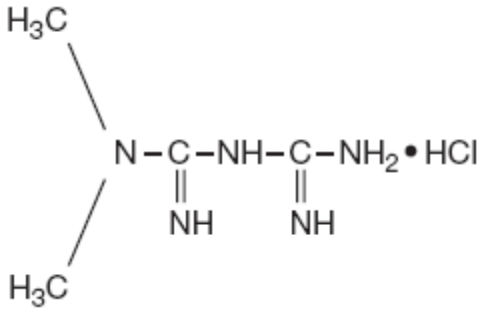
Certain drugs including thiazides, corticosteroids, thyroid products, and sympathomimetics may reduce the hypoglycemic action of nateglinide and other oral antidiabetic drugs.

**Overdosage**

Overdose may result in an exaggerated glucose-lowering effect with the development of hypoglycemic symptoms. Hypoglycemic symptoms without loss of consciousness or neurological findings should be treated with oral glucose and adjustments in dosage and/or meal patterns. Severe hypoglycemic reactions with coma, seizure, or other neurological symptoms should be treated with intravenous glucose. As nateglinide is highly protein bound, dialysis is not an efficient means of removing it from the blood.

### 2.2.3.3 **Metformin**

Metformin hydrochloride is an oral antihyperglycemic drug used in the management of type 2 diabetes. Metformin hydrochloride (N, N-dimethylimidodicarbonimidic diamide hydrochloride) is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents. Metformin hydrochloride is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5$.HCI and a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether and chloroform. The structural formula is shown below:




**Mechanism of Action**

Metformin is an antihyperglycemic agent which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Biguanides are an effective treatment in hyperglycemia, acting mainly by promoting glucose utilization and reducing hepatic glucose production (Dunn and Peters, 1995). Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease.

**Other Effects**

Metformin, an insulin-sensitizing biguanide derivative, reduces hepatic glucose output primarily through inhibition of gluconeogenesis and to a lesser extent glucogenolysis. It increases insulin-stimulated glucose uptake in skeletal muscles and adipocytes (Luna et al., 2006) used in NIDDM

**Pharmacokinetics**

Absorption

As per the USFDA docket for metformin hydrochloride tablets, the absolute bioavailability of a metformin hydrochloride 500 mg tablet given under fasting conditions is approximately 50-60 % and there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alteration in elimination. Food decreases the extent of and slightly delays the absorption of metformin, by approximately a 40% lower mean peak concentration ($C_{max}$) and 25% lower area under the plasma concentration versus time curve (AUC), and a 35 minute prolongation of time to peak plasma concentration (Tmax) following administration of a single 850 mg tablet of metformin with food, compared to the same tablet strength administered fasting in a clinical study. The clinical relevance of these decreases is unknown.

Metformin could be absorbed from the whole intestine, with the main absorption site at duodenum. This concentration-dependent permeability behavior in the duodenum indicates that metformin is transported by both passive and active carrier-mediated saturable mechanism (Choi et al., 2006). The P(eff) value can not be increased by co-perfusion with verapamil, indicating that absorption of metformin is not efficiently transported by P-gp in the gut wall. Furthermore metformin is neither a substrate nor an inducer of P-gp. Based on the P(eff) values obtained in the present study and using established relationships, the human fraction dose absorbed for metformin is estimated to be 74%-90% along human intestine (Song et al., 2006). Metformin is a substrate for plasma membrane monoamine transporter (PMAT) at intestine and thus affected by pH (Zhou et al., 2007).

Distribution

The apparent volume of distribution (V/F) of metformin following single oral doses of 850 mg averaged 654 + 358 L. Metformin is negligibly bound to plasma proteins in

contrast to sulfonylurea which are more than 90% protein bound, Metformin partitions into erythrocytes, most likely as a function of time.

It is a substrate for some of the transporters involved in hepatic and renal distribution. OCT1 is majorly responsible for hepatic and intestinal uptake of metformin while the renal distribution and excretion are governed by other transporter mechanisms (Kumura and Masuda et al., 2005).OCT1 genotypes are major determinants of metformin pharmacokinetics where those having low expression of this gene have shown to elevated $C_{max}$ and AUC in humans (Shu et al., 2008)

The in vivo distribution of Metformin in rats revealed that the expression level of renal OCT2 was a key factor in the control of the concentrative accumulation of metformin in the kidney. These findings suggest that metformin is superior substrate for renal OCT2 than hepatic OCT1 and renal OCT2 plays a dominant role on its pharmacokinetics (Kimura and Masuda et al., 2005). Hence the renal distribution and accumulation is high compared to that of hepatic accumulation with metformin.

Metabolism

It is already known from literature that metformin is not metabolized through any of the enzymes physiologically. Metformin was metabolized mainly via CYP2C11, 2D1, and 3A1/2 in rats. This result could contribute to understanding of the possible changes in metformin pharmacokinetics in disease models where CYP2C11 and/or 3A1/2 are altered (Choi and Lee, 2006)

Excretion

Metformin is excreted unchanged in the urine and does not undergo either hepatic metabolism (no metabolites have been identified in humans) or biliary excretion (Dunn & Peters 1995). Renal clearance is approximately 3.5 times greater than creatinine clearance which indicates that tubular secretion is the major route of metformin elimination. Following oral administration, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life of approximately 6.2 hours. In blood, the elimination half-life is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution.

It is being primarily excreted through urine and is a substrate for some of the transporters involved in hepatic and renal distribution apart from glomerural filtration (Kimura and Okuda et al., 2005) . OCT1 is majorly responsible for hepatic and intestinal uptake of metformin while the renal distribution and excretion are governed by other transporter mechanisms (Wang et al., 2002).

**Adverse effects**

The main adverse effect of Metformin is Lactic acidosis and diarrhea, which are potentially dangerous. Other common adverse effects include gastrointestinal reactions such as nausea, vomiting, Flatulence, Asthenia, indigestion and Abdominal discomfort.

**Pharmacokinetic interactions**

A single-dose, metformin-furosemide drug interaction study in healthy subjects demonstrated that pharmacokinetic parameters of both compounds were affected by co-administration. Furosemide increased the metformin plasma and blood $C_{max}$ by 22% and blood AUC by 15%, without any significant change in metformin renal clearance. When administered with metformin, the $C_{max}$ and AUC of furosemide were 31% and 12% smaller, respectively, than when administered alone, and the terminal half-life was decreased by 32%, without any significant change in furosemide renal clearance. No information is available about the interaction of metformin and furosemide when co-administered chronically (Alivanis et al., 2006, Bryant et al., 2004).

A single-dose, metformin -nifedipine drug interaction study in normal healthy volunteers demonstrated that co-administration of nifedipine increased plasma metformin $C_{max}$ and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. Tmax and half-life were unaffected. Nifedipine appears to enhance the absorption of metformin. Metformin had minimal effects on nifedipine.

Cationic Drugs - Cationic drugs (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, and vancomycin) that are eliminated by renal tubular secretion theoretically have the potential for interaction with metformin by competing for common renal tubular transport systems (Kimura et al., 2005). Such interaction between metformin and oral cimetidine has been observed in normal healthy volunteers in both single- and multiple-dose, metformin -cimetidine drug interaction studies, with a 60% increase in peak metformin plasma and whole blood

concentrations and a 40% increase in plasma and whole blood metformin AUC. There was no change in elimination half-life in the single-dose study. Metformin had no effect on cimetidine pharmacokinetics (Somogyi et al., 1987).

Although such interactions remain theoretical (except for cimetidine), careful patient monitoring and dose adjustment of metformin and/or the interfering drug is recommended in patients who are taking cationic medications that are excreted via the proximal renal tubular secretory system.

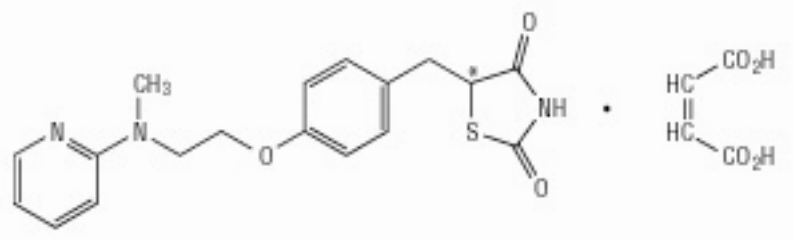
**Overdosage**

Hypoglycemia has not been seen with ingestion of up to 85 grams of metformin, although lactic acidosis has occurred in such circumstances. Metformin is dialyzable with a clearance of up to 170 mL/min under good hemodynamic conditions. Therefore, hemodialysis may be useful for removal of accumulated drug from patients in whom metformin overdosage is suspected

### 2.2.3.4 **Rosiglitazone**

Rosiglitazone maleate is (±)-5-[[4-[2-(methyl-2-pyridinylamino) ethoxy] phenyl] methyl]-2, 4-thiazolidinedione, (Z)-2-butenedioate (1:1) with a molecular weight of 473.52 (357.44 free base). The molecule has a single chiral center and is present as a racemate. Due to rapid interconversion, the enantiomers are functionally indistinguishable. The molecular formula is $C_{18}H_{19}N_3O_3S \bullet C_4H_4O_4$. Rosiglitazone maleate is a white to off-white solid and is readily soluble in ethanol and a buffered aqueous solution with pH of 2.3; solubility decreases with increasing pH in the physiological range

The structural formula of rosiglitazone maleate is:



**Mechanism of action**

Rosiglitazone, a member of the thiazolidinedione class of antidiabetic agents, improves glycemic control by improving insulin sensitivity. Rosiglitazone is a highly selective and potent agonist for the peroxisome proliferator-activated receptor-gamma (PPARγ). In humans, PPAR receptors are found in key target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPARγ nuclear receptors regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization. In addition, PPARγ-responsive genes also participate in the regulation of fatty acid metabolism.

Insulin resistance is a common feature characterizing the pathogenesis of type 2 diabetes. The antidiabetic activity of rosiglitazone has been demonstrated in animal models of type 2 diabetes in which hyperglycemia and/or impaired glucose tolerance is a consequence of insulin resistance in target tissues. Rosiglitazone reduces blood glucose concentrations and reduces hyperinsulinemia in the ob/ob obese mouse, db/db diabetic mouse, and *fa/fa* fatty zucker rat.

In animal models, rosiglitazone antidiabetic activity was shown to be mediated by increased sensitivity to insulin's action in the liver, muscle, and adipose tissues. The expression of the insulin-regulated glucose transporter GLUT-4 was increased in adipose tissue. Rosiglitazone did not induce hypoglycemia in animal models of type 2 diabetes and/or impaired glucose tolerance.

**Other effects**

Rosiglitazone increases total, low- and high-density lipoprotein (LDL and HDL) cholesterol, and triglycerides (Doggrell, 2008). Rosiglitazone decreased inflammatory markers. Furthermore, it rosiglitazone may cause a small decrease in blood pressure, improve endothelial function and reduce restenosis. Microalbuminuria is also reduced by rosiglitazone. Despite the improvements in surrogate end points, there is little clear evidence that rosiglitazone cause major improvements in cardiovascular outcomes. Thus, rosiglitazone has no effect or may even increase cardiovascular outcomes

**Pharmacokinetics**

Absorption

Maximum plasma concentration ($C_{max}$) and the area under the curve (AUC) of rosiglitazone increase in a dose-proportional manner over the therapeutic dose range. The elimination half-life is 3 to 4 hours and is independent of dose (Cox et al., 2000).

The absolute bioavailability of rosiglitazone is 99%. Peak plasma concentrations are observed about 1 hour after dosing. Administration of rosiglitazone with food resulted in no change in overall exposure (AUC), but there was an approximately 28% decrease in $C_{max}$ and a delay in Tmax (1.75 hours).

Distribution

The mean (CV %) oral volume of distribution ($V_{ss/F}$) of rosiglitazone is approximately 17.6 (30%) liters, based on a population pharmacokinetic analysis. Rosiglitazone is approximately 99.8% bound to plasma proteins, primarily albumin.

Metabolism

Rosiglitazone is extensively metabolized with no unchanged drug excreted in the urine. The major routes of metabolism were N-demethylation and hydroxylation, followed by conjugation with sulfate and glucuronic acid (Cox et al., 2000) in humans. All the circulating metabolites are considerably less potent than parent and, therefore, are not expected to contribute to the insulin-sensitizing activity of rosiglitazone.

Phase I metabolism resulted in ring hydroxylation, N-demethylation and oxidative removal of the pyridinylamino function to yield a phenoxyacetic acid derivative. Sulphation of phase I metabolites occurred in both rat and dog, but glucuronidation was only observed in the rat.. The parent compound was the major circulating component in both species at early times, but at later times sulphate conjugates of phase 1 metabolites were predominant (Bolton et al., 1996). In vitro data demonstrate that rosiglitazone is predominantly metabolized by cytochrome P450 (CYP) isoenzyme 2C8, with CYP2C9 contributing as a minor pathway.

Excretion

Following oral or intravenous administration of [$^{14}$C] rosiglitazone maleate, approximately 64% and 23% of the dose was eliminated in the urine and in the feces, respectively. The plasma half-life of [$^{14}$C] related material ranged from 103 to 158 hours

(Cox et al., 2000). Clearance was almost exclusively by metabolism, with only small amounts of unchanged BRL 49653 being excreted following oral administration of [14C]-rosiglitazone to rat and dogs

**Adverse effects**

Rosiglitazone causes swelling or fluid retention, especially in the ankles or legs, shortness of breath or trouble breathing, fast increase in weight, unusual tiredness. Rosiglitazone may increase the risk of heart problems related to reduced blood flow to the heart. These include possible increases in the risk of angina (heart-related chest pain) or myocardial infarction ("heart attack"). This risk appeared higher in patients who took this drug with insulin or with nitrate medication.

**Pharmacokinetic interactions**

In vitro drug metabolism studies suggest that rosiglitazone does not inhibit any of the major P450 enzymes at clinically relevant concentrations. In vitro data demonstrate that rosiglitazone is predominantly metabolized by CYP2C8, and to a lesser extent, 2C9.

Concomitant administration of gemfibrozil (600 mg twice daily), an inhibitor of CYP2C8, and rosiglitazone (4 mg once daily) for 7 days increased rosiglitazone AUC by 127%, compared to the administration of rosiglitazone (4 mg once daily) alone. Given the potential for dose-related adverse events with rosiglitazone, a decrease in the dose of rosiglitazone may be needed when gemfibrozil is introduced.

Rifampin administration (600 mg once a day), an inducer of CYP2C8, for 6 days is reported to decrease rosiglitazone AUC by 66%, compared to the administration of rosiglitazone (8 mg) alone.

Rosiglitazone (4 mg twice daily) was shown to have no clinically relevant effect on the pharmacokinetics of nifedipine (Harris et al., 1999) and oral contraceptives (ethinyl estradiol and norethindrone), which are predominantly metabolized by CYP3A4.

Sixteen healthy volunteers with the homozygous SLCO1B1 c.521TT genotype (controls), 12 with the heterozygous c.521TC genotype and four with the homozygous c.521CC genotype ingested a single 4-mg dose of rosiglitazone and SLCO1B1 polymorphism had no statistically significant effect on any of the pharmacokinetic variables of rosiglitazone

indicating that OATP1B1 plays no significant role in the disposition of these drugs (Kalliokoski et al., 2008)

Rosiglitazone (2 mg twice daily) taken concomitantly with glyburide (3.75 to 10 mg/day) for 7 days did not alter the mean steady-state 24-hour plasma glucose concentrations in diabetic patients stabilized on glyburide therapy. Repeat doses of rosiglitazone (8 mg once daily) for 8 days in healthy adult Caucasian subjects caused a decrease in glyburide AUC and $C_{max}$ of approximately 30%. In Japanese subjects, glyburide AUC and $C_{max}$ slightly increased following coadministration of rosiglitazone (Lin et al., 2004).

Single oral doses of glimepiride in 14 healthy adult subjects had no clinically significant effect on the steady-state pharmacokinetics of rosiglitazone. No clinically significant reductions in glimepiride AUC and $C_{max}$ were observed after repeat doses of rosiglitazone (8 mg once daily) for 8 days in healthy adult subjects (Feher and Lengyel, 2007).

Concurrent administration of rosiglitazone (2 mg twice daily) and metformin (500 mg twice daily) in healthy volunteers for 4 days had no effect on the steady-state pharmacokinetics of either metformin or rosiglitazone (Di Cicco, Allen et al., 2000).

Coadministration of acarbose (100 mg three times daily) for 7 days in healthy volunteers had no clinically relevant effect on the pharmacokinetics of a single oral dose of rosiglitazone (Miller et al., 2001)

Repeat oral dosing of rosiglitazone (8 mg once daily) for 14 days did not alter the steady-state pharmacokinetics of digoxin (0.375 mg once daily) in healthy volunteers (Di Cicco, Miller et al., 2000).

Repeat dosing with rosiglitazone had no clinically relevant effect on the steady-state pharmacokinetics of warfarin enantiomers.
A single administration of a moderate amount of alcohol did not increase the risk of acute hypoglycemia in type 2 diabetes mellitus patients treated with rosiglitazone.
Pretreatment with ranitidine (150 mg twice daily for 4 days) did not alter the pharmacokinetics of either single oral or intravenous doses of rosiglitazone in healthy

volunteers (Miller et al., 2002). These results suggest that the absorption of oral rosiglitazone is not altered in conditions accompanied by increases in gastrointestinal pH

Rosiglitazone and pioglitazone are both eliminated by extensive metabolism in the liver, mainly by the CYP2C8 enzyme (Mudaliar and Henry, 2001). CYP2C9 catalyzes a minor pathway in the metabolism of rosiglitazone, and secondary metabolic enzymes for pioglitazone include CYP3A4, CYP2C9, and CYP1A1/2 . The effects of inducers or inhibitors of CYP enzymes on the pharmacokinetics and pharmacodynamics of rosiglitazone and pioglitazone have not been studied (Balfour and Plosker, 1999)

Overdosage

Limited data are available with overdosage of rosiglitazone is available till date. The maximum usage of rosiglitazone up to 20 mg is used in humans and no overdosage related symptoms were observed. It is recommended that in case any overdosage related symptoms appears in patients treated with rosiglitazone, supportive treatments should be initiated simultaneously.

## 2.3 Peripheral vascualar disorders and Diabetes

Atherosclerosis is a progressive process affecting multiple vascular beds; its clinical consequences, which include coronary artery disease (CAD), cerebrovascular disease, and peripheral arterial disease (PAD), are potentially life-threatening (Hawkins, 2004). These vascular beds are interdependent, and subsequent disease progressios are manifestations of the same process. Therefore they are associated with the same clinical events as the disease process overlap. The rate of atherosclerosis development can vary among vascular beds. Platelet acitivation and thrombosis are key contributing factors in disease development and progression. Increase in low-density lipoproteins (LDL) cholesterol levels, endothelial dysfunction, oxidative stress, and risk factors (e. cigarette smoking, obesity) also contribute to disease progression (Faxon, 2004).

Peripheral arterial disease in patients with diabetes adversely affects quality of life (Khaira et al., 1996) and is associated with substantial functional impairment (Vogt et al., 1994). The reduced walking speed and distance associated with intermittent claudication may result in progressive loss of function and long-term disability (Adam et al., 2003). With more severe disease, critical limb ischemia (CLI) may develop, resulting in

ischemic ulceration of the foot and risk of limb loss (Adler et al., 1999; Hiatt, 2002). Importantly, PAD is associated with a substantial increase in the risk of fatal and non-INSULINfatal cardiovascular and cerebrovascular events, including myocardial infarction (MI) and strok. Patients with diabetes and PAD are at higher risk of lower extremity amputation than those without diabetes (van der Eb et al., 2001). Furthermore, cardiovascular and cerebrovascular event rates are higher in diabetic individuals with PAD than in comparable non-diabetic populations (Adam et al., 2003).

Although much is known about PAD in the general population, the management of PAD in those with diabetes is less clear. Recently, the American Diabetes Association (ADA) issued a consensus statement that provides guidelines for the diagnosis and management of PAD in patients with diabetes (Adam et al., 2003)

### 2.3.1 Epidemiology

Peripheral arterial disease affects approximately 12 million people in the U.S.; approximately 20% to 30% of these patients have diabetes (Mohler, 2007). However, accurate assessment of the preva- lence of PAD in the diabetic population is confounded by various factors: the condition is often asymptomatic; peripheral neuropathy may alter pain perception; and two of the common clinical findings, the absence of peripheral pulses and the presence of claudication, are inadequate diagnostic indicators (Adam et al., 2003). In studies using the ankle-brachial index (ABI), which is the preferred screening technique, the prevalence of PAD (defined as an ABI _0.90) in diabetic individuals ranges from 20% to 30% (Elhadd et al., 1999). The duration and severity of diabetes correlates with the incidence and extent of PAD (van der Eb et al., 2001). In a prospective cohort study, (Al-Delaimy et al., 2004) found a strong positive association between the duration of diabetes and the risk of developing PAD. The association was particularly strong among men with hypertension or who were current smokers. (Adler et al., 2002) estimated the prevalence of PAD up to 18 years after the diagnosis of diabetes in 4,987 subjects (United Kingdom Prospective Diabetes Study [UKPDS]). The data showed a higher prevalence of PAD in those with longer duration of diabetes. The degree of diabetic control is an independent risk factor for PAD; with every 1% increase in glycosylated hemoglobin, the risk of PAD has been shown to increase by 28% (Selvin et al., 2004). The risk of PAD is associated with advancing age and the presence of

peripheral neuropathy (2003a). In addition, the risk is higher in those of African-American or Hispanic descent compared with non- Hispanic white individuals, even after adjustment for other known risk factors and the increased prevalence of diabetes in these populations. In a community-based study, African- American subjects had a lower mean ABI and a greater prevalence of PAD than their non-Hispanic white counterparts (Kullo et al., 2003). Patients with diabetes more commonly develop symptomatic PAD. In the Framingham study, the presence of diabetes increased the risk of intermittent claudication by 3.5-fold in men and 8.6-fold in women (Kannel and McGee, 1985). Furthermore, patients with diabetes and PAD are more likely to present with an ischemic ulcer or gangrene than patients without diabetes, increasing the risk of lower-extremity amputation (Faglia et al., 1998; Jude et al., 2001) observed a positive trend between PAD severity and amputation rate in patients with diabetes. People with diabetes are 15 times more likely to have an amputation than those without (Bild et al., 1989), and an annual amputation incidence rate of 0.6% has been reported in these patients (Holzer et al., 1998; Gonzalez and Oley, 2000).

### 2.3.2 Pathophysiology

The pathophysiology of PAD in the diabetic population is similar to that in the non-diabetic population. However, the distribution of peripheral atherosclerosis in patients with PAD and diabetes is often more distal than in patients without diabetes, and commonly involves the tibial vessels (Haltmayer et al., 2001). The abnormal metabolic state that accompanies diabetes directly contributes to the development of atherosclerosis; proatherogenic changes include increases in vascular inflammation and alterations in multiple cell types (Beckman et al., 2002). Inflammation is an established risk factor for the development of atherosclerosis. Elevated levels of C-reactive protein (CRP) are strongly associated with the development of PAD. Furthermore, CRP levels are abnormally elevated in patients with impaired glucose tolerance (Ridker et al., 1998). In addition to being a marker of atherosclerosis, elevated levels of CRP may also be a risk factor for PAD. C-reactive protein has procoagulant effects related to its ability to enhance expression of tissue factor (Cermak et al., 1993). C-reactive protein also inhibits endothelial cell nitric oxide (NO) synthase, resulting in abnormal regulation of vascular tone, and increases production of plasminogen activator inhibitor-1, which inhibits the

formation of fibrinolytic plasmin from plasminogen (Vinik et al., 2001). Most patients with diabetes and PAD demonstrate generalized endothelial cell dysfunction. In healthy vessels, endothelial cells synthesize NO, a potent vasodilator that
inhibits platelet activation and vascular smooth muscle cell migration. Diabetes impairs NO-mediated vasodilatation (Williams et al., 1996). A number of mechanisms contribute to the decreased bioavailability of endothelium-derived NO in diabetes, including hyperglycemia, excess free fatty acids, and insulin resistance (De Vriese et al., 2000). The effects of endothelial cell dysfunction increase arterial susceptibility to atherosclerosis. In addition to reducing NO oncentrations, diabetes increases the production of vasoconstrictors, such as endothelin-1, which increase vascular tone and vascular smooth muscle cell growth and migration. Diabetes also stimulates other atherogenic pathways in vascular smooth muscle cells. For example, hyperglycemia activates protein kinase C and nuclear factor kappa-B, increasing the production of reactive oxygen species that promote the formation of atherosclerotic lesions (Inoguchi et al., 2000). Vascular smooth muscle cells cultured from patients with diabetes demonstrate enhanced migration, an important step in the progression to advanced plaque formation (Suzuki et al., 2001). These cells strengthen the atheroma, making it less likely to rupture and cause thrombosis. However, Fukumoto et al. (Fukumoto et al., 1998) demonstrated that advanced plaques in diabetic individuals have fewer smooth muscle cells than healthy controls; it is believed that hyperglycemia-induced lipid modifications regulate the apoptosis of vascular smooth muscle cells in advanced atherosclerotic lesions, promoting plaque instability and precipitation of clinical events (Geng and Libby, 2002). Platelet aggregation is enhanced in diabetes. Elevated glucose levels activate protein kinase C, decrease production of platelet-derived NO, and increase oxidative stress. In diabetes, platelets also have increased expression of glycoprotein Ib and IIb/IIIa receptors, enhancing their thrombotic potential (Cermak et al., 1993). In addition to potentiating platelet aggregation, diabetes augments blood coagulability by increasing the expression of tissue factor and decreasing levels of anticoagulants, such as antithrombin III. Consequently, it is more likely that atherosclerotic plaque rupture will result in thrombus formation (Carr, 2001). Thus, alterations in metabolism in diabetes adversely affect multiple cell types within the vascular wall. The increased tendency towards coagulation, coupled with impaired fibrinolysis, contributes to the enhanced thrombotic

potential characteristic of diabetes. Diagnosis The main reasons to diagnose PAD in diabetic individuals are to initiate therapies that decrease the risk of atherothrombotic events, improve quality of life, and decrease disability. A diagnosis of PAD indicates the presence of systemic atherosclerosis that confers additional cardiovascular risk to the patient with diabetes, and gives further impetus to aggressively manage vascular risk factors in this high-risk group. A typical protocol for the diagnosis of PAD is shown in **Figure 2.8**.

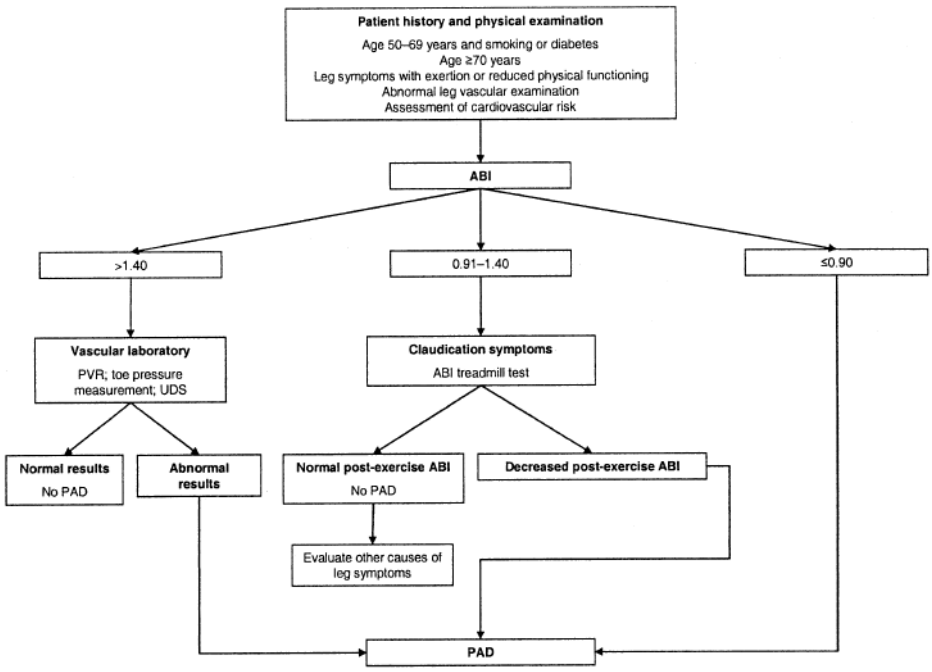



*Figure 2.8 Typical protocol for the diagnosis of peripheral arterial disease in patients with diabetes*

ABI -ankle-brachial index; PAD-peripheral arterial disease; PVR-pulse-volume recording; UDS-ultrasonic duplex scanning. (This figure was adapted from Marso and Hiatt et al., 2006)

A thorough medical history and physical examination are of primary importance in evaluating a diabetic individual for the presence of PAD. Information about the onset and duration of symptoms, pain characteristics, and any alleviating factors is helpful. The clinical stage of symptomatic PAD can be classified using the Fontaine staging system (Dormandy and Rutherford, 2000). Fontaine stage I represents those who have PAD but are asymptomatic; stages IIa and IIb include patients with mild and moderate-to-severe intermittent claudication, respectively; those with ischemic rest pain are classified in Fontaine stage III; and patients with distal ulceration and gangrene represent Fontaine

stage IV. A typical history of claudication has a low sensitivity, but a high specificity for PAD (Criqui et al., 1985). Physical examination should include bloodpressure measurement, palpation of peripheral pulses, and auscultation of pulses and bruits. Palpation of peripheral pulses should include an assessment of the femoral, popliteal, and pedal vessels (2003a); pulses should be graded as absent, diminished, or normal. Dorsalis pedis pulse abnormalities are less sensitive for PAD, since up to 30% of these abnormalities may be due to a congenital absence of the dorsalis pedis artery (Mohler, 2003). The absence of both the dorsalis pedis pulse and the posterior tibial pulse strongly suggests the presence of PAD, but further diagnostic testing is required to confirm the diagnosis.

**ABI screening**

Although physical examination provides important information, additional non-invasive testing is necessary to ensure the diagnosis. The ABI is a reproducible and reasonably accurate measurement for the detection of PAD. The ABI is defined as the ratio of the ankle systolic blood pressure divided by the brachial systolic blood pressure, and is normally between 1.00 and 1.40 (Hiatt, 2001). In PAD, the ankle systolic blood pressure is less than the brachial systolic blood pressure, and the ABI is reduced to _1.00; PAD is defined as an ABI _0.90. Lower ABI values indicate more severe PAD and a higher risk of cardiovascular events. In the primary care setting, Mohler et al. (Mohler et al., 2004) assessed perceptions of the ABI among 886 clinicians; most believed the ABI was useful in the diagnosis of both symptomatic (96%) and asymptomatic (89%) PAD (Mohler et al., 2004). The ADA consensus statement recommends that a screening ABI be performed in all diabetic individuals ~50 years of age. If normal (0.91 to 1.40), the test should be repeated every five years. A screening ABI should be performed in any patient with symptoms of PAD (2003a). Ankle-brachial index determinations may be of limited value in some patients with diabetes, because calcification of the tibial arteries may render them non-compressible, resulting in unusually high ABI values (1.40) (Hiatt et al., 1995). Under these conditions, the ABI cannot distinguish patients who have arterial occlusion from those who do not, making the ABI unreliable (Mohler et al., 2004). However, an elevated ABI is still predictive of an increased risk of cardiovascular events, and other non-invasive vascular tests should be considered to make the diagnosis of PAD (Resnick et al., 2004). The ABI screening recommendations from the ADA

consensus statement should be incorporated into clinical practice for the following reasons. First, an abnormal ABI is strongly associated with heightened risk for coronary heart disease mortality and morbidity. If not performed previously, screening for concomitant CAD should be considered on the basis of other clinical indicators of cardiac ischemia (e.g., angina). Second, if PAD is confirmed, an aggressive secondary prevention medical strategy is warranted. The National Cholesterol Education Program/Adult Treatment Panel III guidelines classify diabetes as a coronary heart disease equivalent and recommend a targeted low-density lipoprotein (LDL) cholesterol level of _100 mg/dl A recent update to these guidelines suggests a target LDL level of _70 mg/dl for very high-risk patients (Grundy et al., 2004)This recommendation was based on emerging data from the Heart Protection Study and Pravastatin or Atorvastatin Evaluation and Infection Therapy (Cannon et al., 2004)) which suggest a lower LDL target in patients with established coronary vascular disease and a major additional risk factor, such as diabetes. On the basis of this recommendation, it is reasonable to suggest that diabetic individuals with documented PAD, independent of symptoms, be treated to an LDL target of 70 mg/dl, but specific clinical trials would need to be performed to fully substantiate this recommendation. Third, PAD is underdiagnosed in the primary care setting, and this is an important barrier for the optimal secondary prevention of ischemic cardiovascular disease Lastly, many individuals have rather atypical claudication symptoms. A large-scale PAD screening study demonstrated that only one-third of patients with documented PAD had classical claudication symptoms (Criqui et al., 1985)The remaining patients either had atypical symptoms or were asymptomatic. These data suggest that classical claudication symptoms are not a reliable indicator for PAD and are inadequate in determining a person's health status due to PAD. In the patient with a confirmed PAD diagnosis in whom further investigation is required (usually in the context of planning a revascularization procedure), the next step would be a vascular laboratory evaluation for segmental pressure and pulse volume recordings. Both hemodynamic tests aid in the localization of arterial occlusive lesions (Mohler, 2003)Other non-invasive imaging techniques, such as ultrasonic duplex scanning or magnetic resonance angiography (MRA), can be used when more precise measurements of the morphological features of occlusions are required (i.e., when considering various revascularization options). Ultrasonic duplex scanning can directly visualize vessels, providing information on artery

wall thickness, degree of flow turbulence, and changes in blood flow velocity (Hiatt and Jones, 1992)Comprehensive imaging of the peripheral vasculature has traditionally been possible only with invasive conventional angiography. However, with the introduction of MRA and computed tomographic angiography (CTA), non-invasive imaging is now a reality (Bashir and Cooper, 2003) contrast-enhanced MRA produces images that are comparable with conventional angiography (Ouriel, 2001). Recently, the development of CTA has dramatically improved image quality and expanded the applications for non-invasive angiography; consequently, The CTA is replacing conventional angiography in many PAD imaging studies (Ek et al., 2004)

Management

Once diabetic individuals with PAD have been identified, the aim of medical management is to aggressively modify cardiovascular risk factors and to prescribe antiplatelet therapy. It is also important to relieve the symptoms of intermittent claudication in order to improve functional status and quality of life.

**Risk factor modification**

Atherosclerotic risk factors for PAD include cigarette smoking, diabetes, dyslipidemia, and hypertension.

Cigarette Smoking

Cigarette smoking is the most important risk factor for the development and progression of PAD. The amount and duration of tobacco use correlate directly with the development and progression of PAD (Freund et al., 1993)Smoking cessation increases long-term survival in patients with PAD. In one study, the 10-year survival rate was 82%

in former smokers compared with 46% in continuing smokers (Jonason and Bergstrom, 1987)An effective method of smoking cessation is nicotine replacement therapy in combination with the oral antidepressant bupropion Jorenby et al. (Jorenby et al., 1999)conducted a double-blind, placebo-controlled comparison of sustainedrelease bupropion (244 subjects), a nicotine patch (244

subjects), bupropion and a nicotine patch (245 subjects), and placebo (160 subjects) for smoking cessation. The abstinence rates at 12 months were 15.6% in the placebo group, compared with 16.4% in the nicotine-patch group, 30.3% in the bupropion group (p _ 0.001), and 35.5% in the group given bupropion and the nicotine patch (p _ 0.001). Although abstinence rates were higher with combination therapy than with bupropion

alone, the difference was not statistically significant. In a recent trial, a combination of physician advice, nicotine replacement therapy, and counseling has been shown to improve long-term mortality (Anthonisen et al., 2005)).

Diabetes

Data to support aggressive glycemic control to reduce the cardiovascular risk associated with PAD are lacking. In the UKPDS, there was evidence that intensive glycemic control with a sulphonylurea or insulin produced a non-significant 16% risk reduction for MI and sudden death compared with conventional diabetic control with diet However, there were no significant differences in diabetesrelated mortality or all-c). ause mortality between the intensive and conventional groups. Most of the risk eduction in the "any diabetes-related" aggregate end point was due to a 25% reduction in microvascular end points (retinopathy, vitreous hemorrhage, or renal failure) rather than macrovascular end points (MI, sudden death, stroke, amputation, or death due to PAD). Current guidelines from the ADA recommend a target glycosylated hemoglobin level of _7.0% in diabetic individuals in order to prevent microvascular complications. It should be noted that the current recommendations for glucose control from the ADA consensus statement are not based on clinical trial evidence in patients with diabetes and PAD. Further study is warranted in this population, in particular with agents that improve

insulin sensitivity.

Dyslipidemia

Several large trials with 3-hydroxy-3- methylglutaryl coenzyme A reductase inhibitors have demonstrated significant reductions in cardiovascular event rates in patients with PAD and co-existing CAD (Pedersen et al., 1998; Cannon et al., 2004). Lipidlowering therapy also decreases cardiovascular events in diabetes. Indeed, patients with diabetes may experience greater risk reduction by lipid lowering than non-diabetic individuals. In the Scandinavian Simvastatin Survival Study, simvastatin reduced the risk of total mortality by 43% in patients with diabetes compared with 29% in those without the disease (Pyorala et al., 1997)Although there are no direct data on treating dyslipidemia in patients with both diabetes and PAD, published guidelines recommend a target LDL cholesterol level of _70 mg/dl in this very high-risk group (Grundy et al., 2004).The ADA consensus statement recommends a target LDL cholesterol level of _100 mg/dl. As previously stated, we acknowledge the discrepancy between the published guidelines and

the ADA consensus statement, but in the absence of clinical trial data, we would recommend an LDL cholesterol target of 70 mg/dl in patients with diabetes and PAD. Recently, the Collaborative AtoRvastatin Diabetes Study (CARDS)(Colhoun et al., 2004) evaluated the efficacy of atorvastatin for the primary prevention of cardiovascular events in 2,838 patients with diabetes and at least one other risk factor for CAD, but without elevated LDL cholesterol levels. The risk of reaching the primary end point of a first acute major cardiovascular event was reduced by 37% with atorvastatin relative to placebo. The CARDS study validated an aggressive approach to lipid management for the primary prevention of cardiovascular events in patients with diabetes at risk for CAD, irrespective of pre-treatment LDL cholesterol levels.

Hypertension

Hypertension increases the high risk of cardiovascular disease associated with diabetes. However, the role of intensive blood pressure control in patients with diabetes and PAD has not been established. In a recent study, blood-pressure lowering in normotensive patients with diabetes and PAD was particularly effective in preventing cardiovascular events (Mehler et al., 2003). The UKPDS showed that although diabetes end points were significantly reduced by tight blood-pressure control, there was no effect on the risk of amputation due to PAD). Nevertheless, a marked reduction in vascular events with aggressive hypertension management has been demonstrated in diabetic individuals (Hansson et al., 1998)The ADA consensus supports aggressive bloodpressure control (_130/80 mm Hg) in patients with diabetes and PAD to reduce cardiovascular risk . Studies, such as the Hypertension Optimal Treatment trial and the Appropriate Blood Pressure Control in Diabetes (ABCD) trial, have suggested that a lower target blood pressure may be beneficial .The ABCD trial demonstrated improved outcomes (particularly non-fatal MI) for patients with PAD and diabetes who achieved a blood pressure of _125/75 mm Hg compared with 135/85 mm Hg (Estacio et al., 1998; Hansson et al., 1998; Weir et al., 2001)

**Antiplatelet therapy**

In addition to the established risk factors, the risk of cardiovascular morbidity and mortality in PAD patients with diabetes also relates to platelet activity and inflammation. Platelet activity can be modified by the use of antiplatelet agents. The treatment goal is to prevent thrombus formation and the resultant vascular events. Aspirin has been shown to

significantly improve vascular graft patency in 3,000 patients with PAD treated with bypass surgery or peripheral angioplasty .Aspirin at dosages of 80 to 325 mg/day is recommended for all diabetic individuals 21 years of age ,but this recommendation is also not fully supported by clinical trial evidence. Thus, aspirin has an established role in secondary prevention in patients at high risk, with clinical evidence of either CAD or stroke. However, the role of aspirin in other populations, such as in patients with either PAD or diabetes, without clinical evidence of CAD or stroke, has not been established. Clopidogrel, an adenosine diphosphate receptor antagonist, has potent antiplatelet activity. The Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) study (1996) was the first to evaluate aspirin versus clopidogrel in patients with recent stroke, recent MI, or established PAD. The study compared clopidogrel at 75 mg/day with aspirin at 325 mg/day in 19,000 patients (approximately 20% with diabetes). Patients treated with clopidogrel had an annual 5.32% risk of stroke, MI, or vascular death compared with a 5.83% risk in those treated with aspirin. This represented a significant 8.7% relative-risk reduction in favor of clopidogrel (p=0.043). In a subset analysis of 6,452 patients with PAD, clopidogrel recipients had a 23.8% relative-risk reduction compared with aspirin recipients (p=0.0028) (Fig. 2), with an annual event rate of 3.71% compared with 4.86%. Furthermore, in the PAD subgroup, approximately one-third of the patients had diabetes; in these patients, clopidogrel was also superior to aspirin .On the basis of these results, clopidogrel was approved by the FDA for the reduction of ischemic events in patients with PAD. The ADA consensus recommends that patients with diabetes should be on an antiplatelet agent, and that those with PAD may benefit more by taking clopidogrel than aspirin.

**Symptomatic PAD**

To relieve the symptoms of intermittent claudication, patients should exercise regularly. The best results with exercise therapy are achieved under supervision, and should consist of repetitive daily walks with intermittent periods of rest and weekly increases in walking time and distance (Hiatt, 2002; Stewart et al., 2002). Drug therapy can be added as adjunctive treatment to an exercise program, although this combination has not been well studied (Ouriel, 2001). Currently, two medications are approved in the U.S. for the symptomatic treatment of intermittent claudication: pentoxifylline and cilostazol. Pentoxifylline, a hemorrheological agent, decreases blood viscosity and improves

erythrocyte flexibility (Angelkort et al., 1985).The results of clinical trials demonstrating the efficacy of pentoxifylline in improving treadmill-walking distance have been equivocal, and there are insufficient data to justify generalized use in PAD (Jackson and Clagett, 2001). Cilostazol, a phosphodiesterase inhibitor, is probably the most effective agent available in the U.S. Cilostazol (100 mg twice daily) has been shown to improve maximal walking distance by 40% to 50% compared with placebo (Dawson et al., 1998; Money et al., 1998). In a direct comparison, the mean maximal walking distance in PAD patients treated with cilostazol for 24 weeks was significantly greater compared with that of patients who received pentoxifylline or placebo (107, 64, and 65 m, respectively) (Dawson et al., 1998). Because of concerns about the potential risk of mortality, cilostazol is contraindicated if any degree of systolic or diastolic heart failure is present. The mean maximal walking distance increase from baseline in patients with peripheral arterial disease after 24 weeks of treatment with placebo (n= 239), pentoxifylline 400 mg three times daily (n = 232), or cilostazol 100 mg twice daily (n = 227) *p< 0.001 vs. pentoxifylline (this figure was adapted from Loosemore et al., 1994)

Various studies have evaluated prostacyclin, prostacyclin analogues (iloprost and beraprost), and intravenous infusion of prostaglandin E1 for the treatment of intermittent clau-dication. The results of these studies have been inconsistent. In a meta-analysis of five placebo-controlled trials, iloprost demonstrated a 21% increase in ulcer healing rates in patients with Fontaine stage 4 PAD compared with placebo (Loosemore et al., 1994). Furthermore, in another pooled analysis, patients with intermittent claudication who were randomized to receive oral prostanoids demonstrated a 30% improvement in mean maximum walking distance compared with subjects receiving the placebo (Johannessen et al., 2004). However, in a study by (Mohler et al., 2003) in which 897 patients with intermittent claudication were randomized to receive beraprost or placebo in a double-blinded manner for one year, there was no significant improvement in maximum walking distance in the beraprost group compared with the placebo group. Administration of beraprost did not improve the pain-free walking distance, and there was no improvement in the quality-of life measures between the treatment groups. Therefore, prostaglandins cannot be recommended for the treatment of either claudication or critical limb ischemia (CLI.). CLI is the precursor of limb loss and requires urgent treatment. Conservative management includes limited debridement of ulcers, the provision of appropriate

footwear, use of non-adherent dressings, institution of adjunctive wound-healing techniques, and treatment of infection (unloading of the foot and administration of antibiotics). Surgical drainage and debridement are often required to resolve the infection, and revascularization is usually indicated. Indeed, revascularization is the preferred treatment in patients with CLI, as surgical debridement without revascularization tends to lead to larger non-healing wounds. Successful management of CLI requires not only treatment of the presenting symptoms (ulceration and infection), but also of the underlying ischemia.

**Revascularization**

Many patients may not experience optimal improvement in symptoms with medical therapy alone (Dawson et al., 1998). Over recent years, revascularization has emerged as an important strategy for management of these patients. Two general evascularization techniques exist: endovascular interventions and open surgical procedures. Endovascular revascularization has increased in popularity in recent years; data available from the U.S. reveals a more than five-fold increase in endovascular interventions from 1980 to 2000 (Anderson et al., 2004). In general, endovascular revascularization is more appropriate in patients with relatively focal disease in arteries above the knee; however, short-term success rates for opening long totally occluded vessels and below-the-knee arteries are improving. To date, the best results have been achieved in the aortoiliac vessels, where one-year patency rates of 80% to 90% have been demonstrated (Sullivan et al., 1997). In diabetes, open surgical revascularization tends to have greater durability than endovascular procedures. Bypass to the tibial or pedal vessels with autogenous vein is the most predictable method of improving blood flow to the threatened limb. Indeed, surgical bypass with greater saphenous vein is the procedure of choice for patients with diabetes and tibial disease. Revascularization is the definitive therapy for the management of patients with CLI, with the aim of healing ischemic ulcers and preventing limb loss. Surgical revascularization is generally superior to endovascular procedures (Beckman et al., 2002). Although most ischemic limbs can be revascularized, lack of a target vessel, unavailability of an autogenous vein, or irreversible gangrene may mean that some cannot. In these patients, amputation may be a better option than prolonged medical treatment.

**Amputation**

A careful program of medical and surgical interventions can prevent most limb amputations. However, amputation may represent an acceptable option for patients facing a prolonged course of treatment and a poor prognosis for a successful outcome. Amputation is indicated when there is overwhelming infection that threatens the patient's life, or when necrosis secondary to a major arterial occlusion has destroyed the foot.

### 2.3.4 Antiplatelet agents (APA) used in peripheral arterial disorders

Most of the drugs available in use as anti-platelet drugs are cilostazol, pentoxifylline, clopidogrel, aspirin, dypyridamole, anaplag and GP IIb/IIIa inhibitors (abciximab and tirofiban) etc.

Importance of Anti-Platelet drugs in Cardiovascular Patients

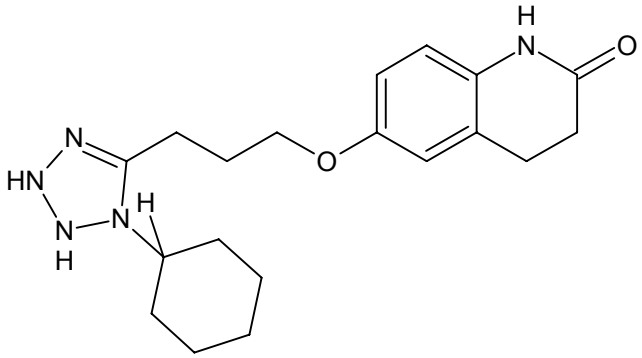
Heart failure (HF) is a significant health problem in the United States, with a prevalence of 5 million patients and 500 000 new diagnoses each year. Heart failure is also significant health care–dollar expenditure, with 5.4% of the health care budget contributing to its treatment. Furthermore, it is a disease of the elderly, affecting 6% to 10% of those older than 65 years. Since the elderly with concomitant disease states are highly affected, polypharmacy may be more problematic in this subpopulation. In these conditions metformin and thiazolidinediones are being used as diabetic medications. In similar conditions anti-hypertensive agents or anti-arrythmic agents can be used simultaneously with hematological medications viz. anagrelide and cilostazol etc.

Anticoagulants and antithrombotic drugs have played a key role in the prophylaxis, treatment and surgica/interventional management of thrombotic and cardiovascular disorders. Some of the findings suggest that cilostazol improves vascular endothelial dysfunction in smokers. The increased vasodilatory response to reactive hyperemia by cilostazol was reduced after cessation of the drug.

#### 2.3.4.1 Cilostazol

Cilostazol is a quinolinone derivative, 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1H)-quinolinone, that inhibits cellular phosphodiesterase (more specific for phosphodiesterase III). Cilostazol is indicated for the reduction of symptoms of intermittent claudication, as indicated by an increased walking distance. Cilostazol occurs

as white to off-white crystals or as a crystalline powder that is slightly soluble in methanol and ethanol, and is practically insoluble in water, 0.1 N HCl, and 0.1 N NaOH. The structural formula of cilostazol is C20H27N5O2, and its molecular weight is 369.46. The structure of cilostazol has shown below.




**Mechanism of Action**

Cilostazol is a selective inhibitor of phosphodiesterase type III, an enzyme that breaks down cyclic AMP in turn suppresses the platelet aggregation (Kimura et al., 1985). The antiplatelet action of cilostazol takes effect in vivo within 6 hours of oral ingestion, and platelet aggregation ability returns to normal within 48 hours after drug withdrawal in patients. In patients with peripheral arterial disease, cilostazol also improves skin blood flow and clinical signs as a result of its vasodilating or antiplatelet effects (Dawson et al., 1998). Cilostazol enhances the activity of lipoprotein lipase, resulting in decreases in serum triglyceride levels (Schumacher et al., 1995). The major common adverse events of cilostazol are palpitations and headache. Cilostazol has positive ionotropic activity, as do other PDE III inhibitors.

The mechanism of the effects of cilostazol on the symptoms of intermittent claudication is not fully understood. cilostazol and several of its metabolites are cyclic AMP (cAMP) phosphodiesterase III inhibitors (PDE III inhibitors), inhibiting phosphodiesterase activity and suppressing cAMP degradation with a resultant increase in cAMP in platelets and blood vessels, leading to inhibition of platelet aggregation and vasodilation, respectively. Cilostazol reversibly inhibits platelet aggregation induced by a variety of

stimuli, including thrombin, ADP, collagen, arachidonic acid, epinephrine, and shear stress.

**Other Effects**

Effects on circulating plasma lipids have been examined in patients taking cilostazol. After 12 weeks, as compared to placebo, cilostazol 100 mg b.i.d. produced a reduction in triglycerides of 29.3 mg/dL (15%) and an increase in HDL-cholesterol of 4.0 mg/dL (≡ 10%).

Cilostazol affects both vascular beds and cardiovascular function. It produces non-homogeneous dilation of vascular beds, with greater dilation in femoral beds than in vertebral, carotid or superior mesenteric arteries. Renal arteries were not responsive to the effects of cilostazol.

In dogs or cynomolgous monkeys, cilostazol increased heart rate, myocardial contractile force, and coronary blood flow as well as ventricular automaticity, as would be expected for a PDE III inhibitor. Left ventricular contractility was increased at doses required to inhibit platelet aggregation. A-V conduction was accelerated. In humans, heart rate increased in a dose-proportional manner by a mean of 5.1 and 7.4 beats per minute in patients treated with 50 and 100 mg b.i.d., respectively. In 264 patients evaluated with Holter monitors, numerically more cilostazol-treated patients had increases in ventricular premature beats and non-sustained ventricular tachycardia events than did placebo-treated patients; the increases were not dose-related.

**Pharmacokinetics**

Absorption

In man, the plasma concentrations of cilostazol reduced per the same dose were higher than in these animals and the AUC showed a good dose-dependence at doses of 25 to 300 mg/body (Akiyama et al., 1985). Unchanged cilostazol was not detected at any doses in human urine. The plasma concentrations increased rapidly after administration and reached their maximum concentrations in 3-4 hr, and then declined rapidly. In other study (Akiyama et al., 1985) the plasma concentrations of cilostazol after oral administration at a dose of 100 mg/body reached to a maximum of 763.9 ng/ml at 3 hr and resulting in two compartmental analysis showed half-lives of 2.2 hr in the α-phase and 18.0 hr in the β-phase. The $AUC_{0-\infty}$ was 13097.8 ng.hr/ml. As per the innovators

information about cilostazol (pletal prescribing information) a high fat meal increases absorption, with an approximately 90% in $C_{max}$ and 25% increase in AUC.

Some in vitro studies (Toyobuku et al., 2003) showed that absorption of cilostazol is region dependent, decreasing in the order of duodenum ~ jejunum > ileum and is transported by both secretory and absorptive transporters. P-Glycoprotein contributes in part to the intestinal secretion, while the absorptive mechanism has not been identified. Interactions of these mechanisms, and possibly also metabolism, could cause the large interindividual fluctuation in bioavailability of cilostazol. However, the high absorption of cilostazol is due to its high absorptive permeability and the participation of the efflux transporter p-glycoprotein does not retard the apparent intestinal absorption because of high absorptive membrane permeability by passive and/or carrier-mediated transported mechanisms. The effect of cilostazol on [$^3$H]daunomycin transport in LLC-GA5-COL150 cells strongly suggested that cilostazol interacts with P-gp.

Distribution

Cilostazol is 95 - 98% protein bound, predominantly to albumin. The mean percent binding for 3,4-dehydro-cilostazol is 97.4% and for 4´-trans-hydroxy-cilostazol is 66%. Mild hepatic impairment did not affect protein binding. The free fraction of cilostazol was 27% higher in subjects with renal impairment than in normal volunteers. The displacement of cilostazol from plasma proteins by erythromycin, quinidine, warfarin, and omeprazole was not clinically significant.

Metabolism

It is highly metabolized by hepatic cytochrome p-450 enzymes, mainly 3A4 and to a lesser extent via 2C19 (Abbas et al., 2000) with metabolites largely excreted through urine. The major metabolite, 3, 4-dehydro cilostazol, was primarily metabolized via CYP3A and inhibition of CYP3A resulted in increased levels of the other active metabolite, 4-‘trans-hydroxy-cilostazol, in humans reflected the role of non CYP3A enzymes responsible for this metabolite (Bramer and Suri, 1999). Two metabolites are active with one metabolite (3, 4-dehydro cilostazol) appearing to accounting for at least 50% of the pharmacologic proportional and the other metabolite (4-‘trans-hydroxy-cilostazol) is accounting for at least 25% of the pharmacologic proportional.

Pharmacokinetics are approximately dose proportional. Cilostazol and its active metabolites have apparent half-lives of about 11-13 hours. Cilostazol and its active metabolites accumulate about 2-fold with chronic administration and reach steady state blood levels within a few days. The pharmacokinetics of cilostazol and its two major active metabolites were similar in healthy normal subjects and patients with intermittent claudication due to peripheral arterial disease (PAD) (Bramer and Suri, 1999). (Mallikaarjun and Bramer, 1999)

Excretion

Following oral administration of 100 mg radiolabeled cilostazol, 56% of the total analytes in plasma was cilostazol, 15% was 3,4-dehydro-cilostazol (4-7 times as active as cilostazol), and 4% was 4′-trans-hydroxy-cilostazol (one fifth as active as cilostazol). The primary route of elimination was via the urine (74%), with the remainder excreted in feces (20%). No measurable amount of unchanged cilostazol was excreted in the urine, and less than 2% of the dose was excreted as 3,4-dehydro-cilostazol. About 30% of the dose was excreted in urine as 4′-trans-hydroxy-cilostazol. The remainder was excreted as other metabolites, none of which exceeded 5%. There was no evidence of induction of hepatic microenzymes.

After the oral administration of $^{14}$C-Cilostazol(Akiyama et al., 1985) at a dose of 10 mg/kg.b.wt in male and female rats and rabbits, and at a dose of 3 mg/kg.b.wt in dogs, the $T_{max}$, $C_{max}$ and $T_{1/2}$ of the radioactivity were 2 hr, 710.5 ng.eq/ml and 2.5 hr in male rats, 4 hr, 110028 ng.eq/ml and 3.7 hr in female rats, 8 hr, 358.1 ng.eq/ml and 6.9 hr rabbits, 3 hr, 559.4 ng.eq/ml and 1.6 hr in dogs, respectively. The blood and tissue concentrations of radioactivity showed a marked sex difference in rats and female were shown to have more exposure to the drug. The urinary and fecal excretions of radioactivity were 42.7 and 61.7% in male rats, 31.1 and 55.7% in female rats. Biliary excretion of radioactivity for 48 hr was 31.7% in male rats. The binding of $^{14}$C-Cilostazol to rat, dog and human plasma protein in vitro was more than 93% each, and the binding of radioactivity to plasma protein at 4 hr post dose in male rat was 69-91%. Considering that the absorption rate of cilostazol was about 88% in rats the total clearance was attributable mainly due to its metabolic clearance.

**Adverse effects**

The adverse effects of cilostazol are abdominal pain, bachache, infection, palpitaion, tachycardia, diarrhea, dyspepsia, flatulence, nausea, peripheral edema and dizziness. Congestive heart failure is the common problem associated with this drug in high doses**.**

**Pharmacokinetic interactions**

As per the platel information, cilostazol could have pharmacodynamic interactions with other inhibitors of platelet function and pharmacokinetic interactions of effect of drugs on its metabolism by CYP3A4 and CYP2C19. Short-term coadministration of aspirin with cilostazol showed a 23%-35% increase in inhibition of ADP-induced ex vivo platelet aggregation compared to aspirin alone (Mallikaarjun and Bramer, 1999). Erythromycin coadministration increased the $C_{max}$ and AUC by 87% and 73% in healthy humans and this followed by reduction of the formation of 3, 4-dehydro-cilostazol by decreasing its $C_{max}$ by 24% and increased the formation of other metabolite 4'-trans-hydroxy-cilostazol by 29% in $C_{max}$ and 141% in AUC (Suri et al., 1999). This also reflected that the CYP3A is involved in the formation of 3, 4-dehydro cilostazol but not for other metabolite.

Coadministration of omeprazole did not significantly affect the metabolism of cilostazol, but the systemic effect to 3, 4-dehyro-cilostazol was increased by 69%, probably the result of omeprazole's potent inhibition of CYP2C19 (Suri and Bramer, 1999)

Coadministration of quinidine did not significantly affect the pharmacokinetics of cilostazol showed that CYP2D6 inhibition did not affect the metabolism of cilostazol(Bramer and Suri, 1999).

Coadministration of warfarin with twice daily administration of cilostazol 100mg did not alter (R)- and (S)-warfarin pharmacokinetics, prothrombin time, partial thromboplastin time, Ivy bleeding times, or cilostazol protein binding (Suri et al., 1999). From this it is being observed that cilostazol did not inhibit the CYP3A4, CYP1A2, CYP2C19 (R-warfarin) and also CYP2C9 (S-warfarin).

Coadministration of a single dose of lovastatin 80 mg with cilostazol at steady state did not result in clinically significant increases in lovostatin and its hydroxyacid metabolite plasma concentrations. Lovastatin and metabolite exposure is increased only by up to 2-fold when cilostazol is coadministered, which is considerably less than that observed for potent CYP3A inhibitors such as itraconazole and grapefruit juice. Absorption of

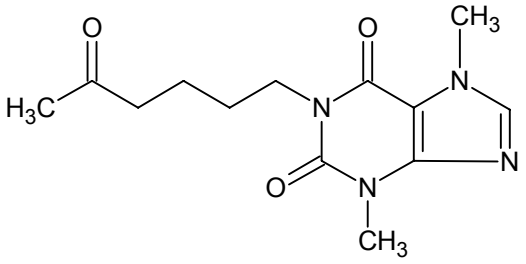
cilostazol decreased approximately 15% when it was given with lovastatin. No dosage adjustments are necessary for cilostazol when coadministered with lovastatin, whereas lovastatin dose reductions may be needed when the 2 drugs are given together (Suri et al., 1998).

**Overdosage**

Information on overdosage with cilostazol in human is limited. The signs and symptoms of an acute overdose can be anticipated to be those of excessive pharmacologic effect: severe headache, diarrhea, hypotension tachycardia, and possibly cardiac arrhythmias. Hence the drug interactions of various drugs need to be studied carefully in those having diabetes and cardiovascular disorders. The patient should be carefully observed and given supportive treatment.

### 2.3.4.2 Pentoxifylline

Pentoxifylline is a tri-substituted xanthine derivative designated chemically as 1-(5-oxohexyl)-3, 7-dimethylxanthine that, unlike theophylline, is a hemorrheologic agent, i.e. an agent that affects blood viscosity. Pentoxifylline is soluble in water and ethanol, and sparingly soluble in toluene. The CAS Registry Number is 6493-05-6. The chemical structure is:




**Mechanism of action**

Pentoxifylline and its metabolites improve the flow properties of blood by decreasing its viscosity. In patients with chronic peripheral arterial disease, this increases blood flow to the affected microcirculation and enhances tissue oxygenation. The precise mode of action of pentoxifylline and the sequence of events leading to clinical improvement are still to be defined. Pentoxifylline administration has been shown to produce dose-related hemorrheologic effects, lowering blood viscosity, and improving erythrocyte flexibility. Leukocyte properties of hemorrheologic importance have been modified in animal and in

vitro human studies. Pentoxifylline has been shown to increase leukocyte deformability and to inhibit neutrophil adhesion and activation. Tissue oxygen levels have been shown to be significantly increased by therapeutic doses of pentoxifylline in patients with peripheral arterial disease.

Pentoxifylline (Oxpentifylline) is a trisubstituted xanthine derivative with potent hemorrheologic properties (Samlaska and Winfield, 1994; Frampton and Brogden, 1995) currently beiIt is introduced as a hemorrheologic agent for the treatment of intermittent claudication putatively by decreasing the blood viscosity through enhanced deformability of erythrocytes (Ernst, 1994). Increased red blood cell flexibility, reduction in blood visocosity, and decreased potential of platelet aggregation are the basic actions of pentoxifylline resulting in therapeutic benefits due to improved microcirculation and tissue oxygenation. Pentoxifylline's generally accepted mechanism of action is the inhibition of phosphodiesterases, leading to increased intracellular levels of cyclic adenosine monophosphate (cAMP). It is useful in treatment of prophylaxis of chemotherapy-induced organ toxicity (Blanco JA et al and Thompson JA et al), treatment of wasting syndrome of AIDS (Vroegindeweij et al., 1992; Dezube et al., 1995) the complications of diabetes (Campbell, 1993) Pentoxifylline is used for the treatment of diabetes-induced peripheral vascular disease, improving the symptoms such as claudication. Since there is an increase in generation of free radicals has been observed in diabetes, and accordingly the microvascular (mcv) permeability changes during diabetes has been studied (Bonnardel-Phu and Vicaut, 2000). A five week treatment in diabetic rats with pentoxifylline which inhibits the leukocyte activation did not have any effect on the increase of albumin extravasation reflecting that reactive oxygen species are responsible for an increase in mcv permeability likely by leucocyte-independent mechanisms. One possible mechanism for these activities could be the inhibitory effect of this compound on glycation and AGE-formation. (Samuelsson et al., 1992)

**Other Effects**

A number of studies have shown pentoxifylline's effect on cytokine network. Antidiabetic effect of pentoxifylline is associated with systemic and target tissue modulation of cytokines and NO production (Stosic-Opincal et al., 2005) The most relevant clinical results are the therapeutic benefits of pentoxifylline in attenuating the effects of tumor necrosis factor-alpha (TNF- alpha) in conditions such as septic shock.

pentoxifylline improves survival and reduces blood concentrations of TNF-alpha, IL-6, Lactate, and endothelin-1 in fulminant intra-abdominal sepsis in rats (Lundblad and Giercksky, 1995). pentoxifylline also has been found to exert antifibrogenic actions, using cultered fibroplasts or animal models of fibrosis, including liver fibrosis (Windmeier and Gressner, 1997). it also helpful in increasing the sperm motility and is useful in reproductive system as well (Tesarik and Mendoza, 1993)

There is not much evidence to assess the effectiveness and safety of methylxanthines viz (Yuan et al., 2005) pentoxifylline after ischaemic stroke (Bath et al., 2000). But it has been reported to exert beneficial in cardiac surgery and these cardioprotective effects of pentoxifylline against I/R injury may be dAue to reduction in the activation of NF-kappa B and the production of TNF-alpha content T (Yuan et al., 2005) Pentoxifylline has shown to beneficially influence a large number of inflammatory skin diseases (Bruynzeel et al., 1998). Pentoxifylline characteristics show that it is an ideal agent to be used as adjunct in therapy of chronic proliferative glomerulonephritides (Wardle, 1998). Pentoxifylline has proven effects on sperm motility, increasing the proportion of hyperactivated spermatozoa (Tournaye et al., 1994a; Tournaye et al., 1994b). It can also acrosome reaction and this may be the more relevant function for clinical prediction and thus it is useful in reproductive system. (Yovich et al,, 1993). Pentoxifylline increases the splanchnic perfusion independent of any change in cardiac outflow in humans (Suren et al., 1991).

One of the minor metabolite of pentoxifylline, Lisofyline prevents the autoimmune diabetic recurrence after islet transplantation (Yang et al., 2004) and it suppress proinflammatory cytokines and prevents β-cell from inflammation in STZ induced diabetes in mice(Yang et al., 2003a; Yang et al., 2003b). This also prevents type-I diabetes in non-obese diabetes mice (Yang et al., 2002). It protects the β-cell damage by promoting the mitochondrial metabolism (Chen et al., 2002). FMLP induces acute inflammatory responses through activation of neutrophils independent of endogenous histamine release, and that pentoxifylline inhibits these responses through elevated intracellular Camp (Nakagawa et al., 1995).

Pentoxifylline by PDE-III inhibition attenuates the macroscopic intestinal ulceration produced by diclofenac administration and this mechanism is independent of TNF-alpha synthesis inhibition (Verghese et al., 1995).

Pentoxifylline is indicated for the treatment of patients with intermittent claudication on the basis of chronic occlusive arterial disease of the limbs. Pentoxifylline can improve function and symptoms but is not intended to replace more definitive therapy, such as surgical bypass, or removal of arterial obstructions when treating peripheral vascular disease.

The usual dosage of pentoxifylline in controlled-release tablet form is one tablet (400 mg) three times a day with meals. Efficacy has been demonstrated in double-blind clinical studies of 6 months duration.

Digestive and central nervous system side effects are dose related. If patients develop these effects it is recommended that the dosage be lowered to one tablet twice a day (800 mg/day). If side effects persist at this lower dosage, the administration of pentoxifylline should be discontinued

**Pharmacokinetics**

Absorption

After the oral administration of $^{14}C$-pentoxifylline (Bryce et al., 1989) to three healthy male subjects, the radiolabeled drug was rapidly absorbed and also rapidly eliminated. The peak plasma concentrations were reached within 0.25 to 0.75 hr after oral administration. The total recovery of radioactive material was 93% in urine and 3% in feces by 24 hr post dosing out of which 89% of radioactivity was observed within 6 hr post dosing reflecting the rapid elimination of pentoxifylline. The radio activity was decayed in biphasic manner.

After oral administration of non-radiolabeled compound in aqueous solution to humans (as per Trental prescribing information), pentoxifylline is almost completely absorbed. It undergoes a first-pass effect and the various metabolites appear in plasma very soon after dosing. Peak plasma levels of the parent compound and its metabolites are reached within 1 hour.

Distribution

Pentoxifylline binds in low to plasm proteins however it has high binding to erythrocyte membranes. Both parent and metabolite drug distributes in to breast milk.

Metabolism

Pentoxifylline is highly metabolized by hepatic cytochrome p-450 enzymes, mainly via CYP1A2 and to a lesser extent via CYP2E11 (Peterson et al., 2004) with metabolites largely excreted through urine. In humans, pentoxifylline is transformed in to at least 7 phase I metabolites, of which two major metabolites M-I & M-V are active. The major metabolites are Metabolite l (1-[5-hydroxyhexyl]-3,7-dimethylxanthine) and Metabolite V (1-[3-carboxypropyl]-3,7-dimethylxanthine), and plasma levels of these metabolites are 5 and 8 times greater, respectively, than pentoxifylline. The reduction of the keto group of pentoxifylline to a secondary alcohol in M I takes place chiefly in erythrocytes, is rapidly reversible, and creates a chiral centre. At therapeutic blood concentrations, in erythrocytes the rate of formation of (S)-M I is 15 times greater than that of (R) –M I enantiomer. Back conversion of (S)-M I to pentoxifylline was 3-4 times faster than that of (R)-enantiomer. The another minor metabolite, R(-)isomer of M-1, Lysofylline is known to be converted back to pentoxifylline by majorly via CYP1A2 at high $K_m$ but not at low $K_m$ value (Lee and Slattery, 1997).

Following oral administration of aqueous solutions containing 100 to 400 mg of pentoxifylline, the pharmacokinetics of the parent compound and Metabolite l are dose-related and not proportional (non-linear), with half-life and area under the blood-level time curve (AUC) increasing with dose. The elimination kinetics of Metabolite V are not dose-dependent. The apparent plasma half-life of pentoxifylline varies from 0.4 to 0.8 hours and the apparent plasma half-lives of its metabolites vary from 1 to 1.6 hours. There is no evidence of accumulation or enzyme induction (Cytochrome P450) following multiple oral doses.

Excretion

Excretion is almost totally urinary; the main biotransformation product is Metabolite V. Essentially no parent drug is found in the urine. Despite large variations in plasma levels of parent compound and its metabolites, the urinary recovery of Metabolite V is consistent and shows dose proportionality. Less than 4% of the administered dose is recovered in feces. Food intake shortly before dosing delays absorption of an immediate-release dosage form but does not affect total absorption. The pharmacokinetics and metabolism of pentoxifylline have not been studied in patients with renal and/or hepatic dysfunction, but AUC was increased and elimination rate decreased in an older population (60-68 years) compared to younger individuals (22-30 years).

After administration of the 400 mg controlled-release pentoxifylline tablet, plasma levels of the parent compound and its metabolites reach their maximum within 2 to 4 hours and remain constant over an extended period of time. Coadministration of pentoxifylline tablets with meals resulted in an increase in mean $C_{max}$ and AUC by about 28% and 13% for pentoxifylline, respectively. $C_{max}$ for metabolite 1 also increased by about 20%. The controlled release of pentoxifylline from the tablet eliminates peaks and troughs in plasma levels for improved gastrointestinal tolerance

The pharmacokinetics and bioavailability of pentoxifylline has been established in broiler chicken reflecting that the half-life of pentoxifylline was 1.05hr and the oral $C_{max}$ was around 4µg/mL when dosed at 100 mg/kg.b.wt. Metabolites I, IV and V were seen in oral plasma where V was predominant. (De Boever et al., 2005).

Inter conversion and tissue distribution of pentoxifylline and lisofylline in mice has been studied (Wyska et al., 2006). The conversion of lisofylline to pentoxifylline was greater than that of reverse. The conversion of pentoxifylline to (S)-isomer of M I is 16 times greater than that of the conversion of pentoxifylline to (R)-isomer of M I. After pentoxifylline oral administration the tissue to serum concentration ratio ranged from 0.1 for liver and 0.32 for brain where as the tissue to serum concentration ratio -(R)-M I were very high (more than 1) and lung was showing that highest (4.98). Where as lisofylline was given as parent and is in reverse way. Hence the reversible metabolism plays a modest role in disposition of pentoxifylline and –(R)-M I.

In another study in rabbits (Hry HY et al), pentoxifylline showed a rapid absorption and metabolism after either oral or subcutaneous administration. In comparison to mice, rabbits has shown slow metabolism. In contrast to man, it had lower metabolites than the plasma drug it self.

A dosing regimen and hemotologic effects of pentoxifylline and its active metabolites in normal dogs have been studied (Rees et al., 2003). Pentoxifylline was readily metabolized and bioavailable (50% at 30 mg/kg.b.wt/po). Both M I and M V were present in plasma where M V was predominant through out the study.

The physical activity also can influence the absorption and metabolism of orally administered pentoxifylline in humans (Adam et al., 1999)

The smoking tend to reduce the plasma levels of pentoxifylline and its active metabolite M I compared to that of the non smokers and hence the dosage administration needs to be titrated accordingly (Mauro et al., 1992)

Liver cirrhosis profoundly increases the levels of pentoxifylline after oral treatment of PTX in cirrhosis patients. However the formation of hydroxy metabolite M I is not modified in the patients suggesting the extrahepatic metabolism of pentoxifylline (Rames et al., 1990).

**Adverse effects**

The adverse events observed with this drug are angina/chest pain, arrhythmia/palpitation, Flushing. Abdominal discomfort, belching/flatus/bloating, dyspepsia, nausea and vomiting. Dizziness was also observed as adverse effect.

**Pharmacokinetic interactions**

Although a causal relationship has not been established, there have been reports of bleeding and/or prolonged prothrombin time in patients treated with pentoxifylline with and without anticoagulants or platelet aggregation inhibitors. Patients on Warfarin should have more frequent monitoring of prothrombin times, while patients with other risk factors complicated by hemorrhage (e.g., recent surgery, peptic ulceration) should have periodic examinations for bleeding including hematocrit and/or hemoglobin.

Pentoxifylline was found to reverse the vincristine resistance of the L1250/VCR mouse leukemic cell subline by overcoming P-gp. Thus pentoxifylline is considered as reversal agent in multi drug resistance (Breier et al., 1994; Stefankova et al., 1996).

In an in vitro study pentoxifylline decreases mdr1 mRNA by 2 fold in presence of 100 mg/l pentoxifylline and there by enhanced the doxorubicin (DOX) accumulation in resistant L1210/VCR cell lines. Thus pentoxifylline is down regulating mdr1 and P-gp protein and can enhance the permeability of drugs are effected by these transporters (Drobna et al., 2002).

Endotoxins are known to decrease the P-gp mediated biliary and renal excretion of rhodamine-123 in rats by decreaseing the expression of mdr1a, which is likely due to increased plasma TNF-alpha levels. Pentoxifylline (150 mg/kg) significantly inhibited

endotoxin-induced reduction of the biliary excretion of rhodamine-123 (Ando et al., 2001).

M-1 and PTX had similar absorption and elimination rates. M-1 was rapidly converted to PTX, while very little PTX was converted to M-1 in vivo. The peak concentration of biologically active drug (PTX+M-1) was 36% higher when M-1 was administered compared to PTX. Combination of ciprofloxacin and PTX significantly increased serum concentrations of both PTX and M-1 (2-fold) compared to controls in mice. The combination of M-1 and ciprofloxacin significantly increased serum concentration of M-1 (3-fold) and PTX (2-fold). The ciprofloxacin/M-1 combination produced a significantly higher sera concentration of bioactive drug compared to all other groups suggesting that this combination may enhance the anti-fibrogenic effect (Raoul et al., 2007).

Pentoxifylline caused a time-dependant influence on the pharmacokinetics of orally administered carbamazepine (CBZ) in healthy subjects. Pentoxifylline reduced the rate of absorption ($T_{max}$ and $K_a$) but not the extent of CBZ absorption at 22.00 hr administration compared to that of 10.00 hr administration. No significant changes observed in other parameters (Poondru et al., 2001)

Concomitant administration of pentoxifylline and theophylline by intravenous route did not effect the metabolism and excretion of the either of them in rats despite the existance of similar sites for metabolism on the xanthine nucleus of each compound (Rocci et al., 1987). Where as concomitant administration of pentoxifylline and theophylline-containing drugs by oral route leads to increased theophylline levels and theophylline toxicity in some individuals. Such patients should be closely monitored for signs of toxicity and have their theophylline dosage adjusted as necessary.

When pentoxifylline was coadministered with cyclosporin A (CSA) in rats, no difference in CSA pharmacokinetics was found (Brunner et al., 1989).

When cimetidine (50 mg/kg/iv) administered along with pentoxifylline (1 mg/kg/iv) in rats, it resulted in 37% decrease in pentoxifylline clearance (23.8 ml/min to 15 ml/min) and no change in AUC was observed. This decrease in clearance is probably due to the

inhibition of hepatic microsomes by cimetidine (Luke et al., 1986). it is also supported by the interaction study of cimetidine and pentoxifylline in humans where the cimetidine elevates pentoxifylline plasma levels primarily by decreasing its oral clearance and no change in AUC. Hence the increase of gastric absorption in this case cannot be ruled out (Mauro et al., 1988).

Pentoxifylline has been used concurrently with antihypertensive drugs, beta blockers, digitalis, diuretics, and antiarrhythmics, without observed problems. Small decreases in blood pressure have been observed in some patients treated with pentoxifylline; periodic systemic blood pressure monitoring is recommended for patients receiving concomitant antihypertensive therapy. This reflects that if indicated, dosage of the antihypertensive agents should be reduced. But no pharmacokinetic data is available so far in literature citing the drug interaction of the above combinations either in animals or humans.

# Chapter 3

# MATERIALS AND METHODS

# 3 MATERIALS AND METHODS

## 3.1 Materials

Cilostazol, pentoxifylline, rosiglitazone, metformin, nateglinide, and glipizide were obtained from Glenmark Pharmaceuticals Ltd, India. The recombinant human liver CYP enzymes were purchased from CYPEX, UK and stored at -80$^0$C until use. The ultra filtration units (Amicon 30 kDa) used for protein binding studies was purchased from Millipore, USA. Tween-80, Methanol, $KH_2PO_4$ and Methylcellulose were purchased from Merck chemicals, Mumbai, India. Acetonitrile and Methanol (both HPLC grade) was purchased from E.Merck India Ltd., Mumbai and butyl t-methyl ether were purchased from J.T.Baker, New Jersey, USA. Ammonium acetate (GR-grade) was procured from E Merck (India) Ltd., India. Formic acid was obtained from Sigma Aldrich, Germany. Ultra pure water of 18 MΩ/cm was obtained from Milli-Q PLUS purification system (Millipore, Bradford, USA). Blank rat plasma was collected from healthy, drug free Wistar rats at the Glenmark research unit, Navi Mumbai (India). Plasma was obtained by centrifuging the $K_2$-EDTA (di-potassium ethylene diamine tetra acetic acid, Sigma Aldrich, Steinheim, Germany) blood at 3000 rpm for 10 min and stored at -70$^0$C until use.

## 3.2 Animals

Male Wistar rats weighing 170 to 250 g were inbred in animal house facility of Glenmark Pharmaceuticals Ltd., at R&D Centre, MIDC Mahape, Navi Mumbai that has been registered for “Research & Breeder of Animals” by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Govt. of India) with the Registration number 231/2000/CPCSEA. Animal experiments were approved by Institutional Animal Ethics Committee of this centre. Animals were housed at a temperature (22±1°C) and relative humidity (55±10 %) controlled room and were exposed to a controlled 12-hr light / dark cycle for minimum of 3 days before being used for experiments. Food and water were made available at *ad libitum*.

## 3.3 In vitro studies

### 3.3.1 Preparation of rat liver microsomes:

Two male Wistar rats were given a daily dose of the effector compound for seven days as per the dosing schedule given below and were sacrificed by cervical dislocation at 24 hr post dose (day 8). The tissues were perfused to drain the blood and liver was excised from both the rats. The microsomal fractions were prepared from the above liver tissues using the standard differential centrifugation techniques (Rettie et al., 1989) and both the samples were mixed together to attain a concentration of 10 mg protein/ml in 100 mM potassium buffer (pH 7.4) containing 1 mM EDTA.. Control liver microsomes were prepared in similar manner by using two rats given prior daily doses of 0.5% methyl cellulose solution containing Tween 80 ( 2.5µl per ml of the solution) for seven days. All these microsomal preparations were stored at -70 $^{0}$ C until further experiments.

### 3.3.2 Rat microsomal incubation for estimating metabolic stability

The study design of the rat microsomal studies is presented in table 3.1. The rat liver mcirosomes (0.2 or 1 mg protein/ml) prepared as described above were thawed on wet ice and the aliquots were incubated with reaction mixture (total volume 0.5 ml) consisted of 100 mM Tris buffer (pH 7.4) and test drug (at a concentration of 10 µM) in triplicates. Pre incubation was performed in a shaking water bath (SW-22, Julabo, Switzerland) at 37$^{0}$C for 5 min. The enzymatic reaction was initiated by adding the 2 mM NADPH and allowed to proceed at 37$^{0}$C in the shaking water bath for 60 min, and then terminated by addition of 25µL trifluroaceticacid. The supernatant collected after centrifuging the above reaction mixture was estimated for test drug and the percent drug remaining (metabolic stability) was calculated based on the peak area ratios observed in both 0 and 60 min samples

### 3.3.3 Incubation studies with CYP bactosomes (recombinant human liver CYP isoforms) for estimating inhibition potential

Microsomal incubation mixtures (total volume 0.5 ml) consisted of 100 mM Tris buffer (pH 7.4), 2 mM NADPH, specific recombinant human liver CYP isoforms (CYPEX,

UK), test drug as substrate (at a concentration of 10 μM) and either vehicle or interacting drug as inhibitor (at a concentration of 10 μM) in triplicates. These details of experimental conditions are presented in table 3.2. The enzymes were thawed on wet ice and the aliquots were pre incubated with reaction mixture containing inhibitor drug in a shaking water bath (SW-22, Julabo, Switzerland) at $37^0$C for 15 min before the addition of substrate. And then the reaction was allowed to proceed at $37^0$C in the shaking water bath for 60 min, and then terminated by addition of 25μL TFA. The supernatant collected after centrifuging the above reaction mixture was estimated for substrate drug and the percent substrate remaining (metabolic stability) was calculated based on the peak area ratios observed in both 0 and 60 min samples

### 3.3.4 Incubation studies with pooled human liver microsomes for estimating the effect of ADAs on pentoxifylline metabolism

Microsomal incubation mixtures (total volume 0.5 ml) consisted of 100 mM Tris buffer (pH 7.4), 2 mM NADPH, pooled human liver microsomes (1mg/ml protein, In Vitro Technologies, USA), test drug as inhibitor (at a concentration of 10 μM) and vehicle or pentoxifylline (at a concentration of 10 μM) in triplicates. The enzymes were thawed on wet ice and the aliquots were pre incubated with reaction mixture containing inhibitor drug in a shaking water bath (SW-22, Julabo, Switzerland) at $37^0$C for 15 min before the addition of substrate. And then the reaction was allowed to proceed at $37^0$C in the shaking water bath for 60 min, and then terminated by addition of 25μL TFA. The supernatant collected after centrifuging the above reaction mixture was estimated for pentoxifylline and the percent drug remaining (metabolic stability) was calculated based on the peak area ratios observed in both 0 and 60 min samples

Table 3.1 Study details of rat microsomal incubations to evaluate the effect of test drugs on metabolic stability of substrate compounds

| Test Compound Treated for 7 days | Category | Protein Conc. | *substrate | *substrate Conc. | Preincubation for 5 min | NADPH | Incubation for 60 min | Quenching solvent | Analyte of interest |
|---|---|---|---|---|---|---|---|---|---|
| Vehicle | RLM | 0.2 mg/ml | CLZ | 10 | | 2 mM | | TFA | CLZ |
| | RLM | 0.2 mg/ml | DCLZ | 10 | | 2 mM | | TFA | DCLZ |
| | RLM | 1.6 mg/ml | PTX | 10 | | 2 mM | | TFA | PTX |
| | RLM | 0.2 mg/ml | RSG | 10 | | 2 mM | | TFA | RSG |
| | RLM | 1mg/ml | MET | 10 | | 2 mM | | TFA | MET |
| | RLM | 0.2 mg/ml | NTG | 10 | | 2 mM | | TFA | NTG |
| | RLM | 2 mg/ml | GLP | 10 | | 2 mM | | TFA | GLP |
| RSG | RLM | 0.2 mg/ml | CLZ | 10 | | 2 mM | | TFA | CLZ |
| | RLM | 0.2 mg/ml | DCLZ | 10 | | 2 mM | | TFA | DCLZ |
| | RLM | 1.6 mg/ml | PTX | 10 | | 2 mM | | TFA | PTX |
| MET | RLM | 0.2 mg/ml | CLZ | 10 | | 2 mM | | TFA | CLZ |
| | RLM | 0.2 mg/ml | DCLZ | 10 | | 2 mM | | TFA | DCLZ |
| | RLM | 1.6 mg/ml | PTX | 10 | | 2 mM | | TFA | PTX |
| NTG | RLM | 0.2 mg/ml | CLZ | 10 | | 2 mM | | TFA | CLZ |
| | RLM | 0.2 mg/ml | DCLZ | 10 | | 2 mM | | TFA | DCLZ |
| | RLM | 1.6 mg/ml | PTX | 10 | | 2 mM | | TFA | PTX |
| GLP | RLM | 0.2 mg/ml | CLZ | 10 | | 2 mM | | TFA | CLZ |
| | RLM | 0.2 mg/ml | DCLZ | 10 | | 2 mM | | TFA | DCLZ |
| | RLM | 1.6 mg/ml | PTX | 10 | | 2 mM | | TFA | PTX |
| CLZ | RLM | 0.2 mg/ml | RSG | 10 | | 2 mM | | TFA | RSG |
| | RLM | 1 mg/ml | MET | 10 | | 2 mM | | TFA | MET |
| | RLM | 0.2 mg/ml | NTG | 10 | | 2 mM | | TFA | NTG |
| | RLM | 1 mg/ml | GLP | 10 | | 2 mM | | TFA | GLP |
| PTX | RLM | 0.2 mg/ml | RSG | 10 | | 2 mM | | TFA | RSG |
| | RLM | 1 mg/ml | MET | 10 | | 2 mM | | TFA | MET |
| | RLM | 0.2 mg/ml | NTG | 10 | | 2 mM | | TFA | NTG |
| | RLM | 1 mg/ml | GLP | 10 | | 2 mM | | TFA | GLP |

Table 3.2 Study details of rat microsomal incubations to evaluate the inhibitory effect of test drugs on metabolic stability of substrates

| Category | Protein Conc (pmol/ml) | Inhibitor | *inhibitor Conc | NADPH | | Substrate | *substrate Conc (µM) | | Quenching solvent | Analyte |
|---|---|---|---|---|---|---|---|---|---|---|
| CYP3A4 | 2.4 pmol | RSG | 10 µM | 2 mM | | CLZ | 10 | | TFA | CLZ & DCLZ |
| CYP3A4 | 2.4 pmol | MET | 10 µM | 2 mM | | CLZ | 10 | | TFA | CLZ & DCLZ |
| CYP3A4 | 2.4 pmol | NTG | 10 µM | 2 mM | | CLZ | 10 | | TFA | CLZ & DCLZ |
| CYP3A4 | 2.4 pmol | GLP | 10 µM | 2 mM | | CLZ | 10 | | TFA | CLZ & DCLZ |
| CYP2C8 | 16 pmol | CLZ | 10 µM | 2 mM | | RSG | 10 | | TFA | RSG |
| CYP2C8 | 16 pmol | PTX | 10 µM | 2 mM | Pre-incubation for 15 min | RSG | 10 | Incubation for 60 min | TFA | RSG |
| CYP2C9 | 8 pmol | CLZ | 10 µM | 2 mM | | NTG | 5 | | TFA | NTG |
| CYP2C9 | 8 pmol | PTX | 10 µM | 2 mM | | NTG | 5 | | TFA | NTG |
| CYP2C9 | 8 pmol | CLZ | 10 µM | 2 mM | | GLP | 5 | | TFA | GLP |
| CYP2C9 | 8 pmol | PTX | 10 µM | 2 mM | | GLP | 5 | | TFA | GLP |

Table 3.3 Study details of pooled human liver microsomes to evaluate the inhibitory effect of ADAs on metabolic stability of pentoxifylline (PTX)

| Test Compound | Category | Protein Conc | *inhibitor Conc ( 10 µM) | NADPH | | *substrate Conc (µM) | | Quenching solvent | Analyte of interest |
|---|---|---|---|---|---|---|---|---|---|
| Vehicle | PHLM | 1mg/ml | | 2 mM | | 10 | | TFA | PTX |
| Rosiglitazone | PHLM | 1mg/ml | 10 | 2 mM | | 10 | | TFA | PTX |
| Metformin | PHLM | 1mg/ml | 10 | 2 mM | | 10 | | TFA | PTX |
| Nateglinide | PHLM | 1mg/ml | 10 | 2 mM | Pre-incubation 5 min | 10 | Incubation 60 min | TFA | PTX |
| Glipizide | PHLM | 1mg/ml | 10 | 2 mM | | 10 | | TFA | PTX |

### 3.3.5 Plasma protein binding studies

Different sets of rat plasma (1 ml in duplicate) containing test drug (at a concentration of 1 µg/ml) were incubated with and without another test drug for interaction (at a concentration of 1 µg/ml) for 30 min in a constant heat water bath at $37^0$C. After this incubation, these samples were aliquot in to 30kDa Amicon micro centrifuge tubes and processed for the supernatant by ultra filtration for 10 min at room temperature and then samples obtained from both the buffer and plasma wells were submitted to analysis. The study design of the plasma protein binding studies are presented in table 3.4

Table 3.4 Study design of in vitro plasma protein binding studies to evaluate the displacement potential of test drugs in rat plasma

| Test drug | Rat plasma | Control | CLZ | PTX | Analytes |
|---|---|---|---|---|---|
| Control | 500 µl | - | 1 µg/ml | - | CLZ |
| | 500 µl | - | - | 1 µg/ml | PTX |
| RSG | 500 µl | 1 µg/ml | - | - | RSG |
| | 500 µl | 1 µg/ml | 1 µg/ml | - | RSG & CLZ |
| | 500 µl | 1 µg/ml | - | 1 µg/ml | RSG & PTX |
| MET | 500 µl | 1 µg/ml | - | - | MET |
| | 500 µl | 1 µg/ml | 1 µg/ml | - | MET & CLZ |
| | 500 µl | 1 µg/ml | - | 1 µg/ml | MET & PTX |
| NTG | 500 µl | 1 µg/ml | - | - | NTG |
| | 500 µl | 1 µg/ml | 1 µg/ml | - | NTG & CLZ |
| | 500 µl | 1 µg/ml | - | 1 µg/ml | NTG & PTX |
| GLP | 500 µl | 1 µg/ml | - | - | MET |
| | 500 µl | 1 µg/ml | 1 µg/ml | - | MET & CLZ |
| | 500 µl | 1 µg/ml | - | 1 µg/ml | MET & PTX |

## 3.4 Analysis of samples

All samples from metabolism, protein binding and in vivo pharmacokinetic studies were analyzed using either HPLC (Waters 2690 Alliance module) coupled with UV/PDA detector or LC-MS/MS (Perkin-Elmer series 200 LC coupled with AB/MDS-SCIEX API 3200/QTRAP in positive ESI system using Multiple reaction monitoring (MRM) mode.

The following drugs were estimated in the biological samples during the course of this thesis work either using High Performance Liquid Chromatography (HPLC) coupled with UV detector or Tandem Mass Spectometry (MS/MS). The samples from metabolism studies were estimated using the respective plasma analytical methods and all of them have shown more than 80% recovery.

- Cilostazol (CLZ)
- 3, 4-dehydro-cilostazol (DCLZ)
- Pentoxifylline (PTX)
- Rosiglitazone (RSG)
- Metformin (MET)
- Nateglinide (NTG)
- Glipizide (GLP)

### 3.4.1 Analytical methods for cilostazol and 3,4-dehydro-cilostazol in rat plasma

*3.4.1.1 Methods using HPLC-UV*

A new rapid, specific, sensitive and simple liquid-liquid extraction method using HPLC-UV for the simultaneous estimation of CLZ, DCLZ and rosiglitazone (RSG) in rat plasma using pioglitazone (PIO) as internal standard was developed and validated. This method was used for estimation CLZ & DCLZ in samples during drug interaction studies between CLZ and RSG/MET. This method has not shown any interference for metformin during respective studies.

The following conditions were set to check the simultaneous analysis of CLZ & DCLZ and RSG using PIO as internal standard.

Chromatographic conditions

| | | |
|---|---|---|
| Instrument | : | HPLC (Waters 2695 Alliance module) |
| Detector | : | UV detector (Waters 2487) |
| Wave length | : | 257 nm (CLZ) |
| | | 226 nm (DCLZ, RSG, PIO) |
| Flow rate | : | 1.2 ml/min |
| Column | : | Hypersil BDS $C_{18}$ (150 X 4.6 mm X 5µ) |
| Column temperature | : | 25°C ± 2°C |
| Sample temperature | : | 10°C ± 2°C |
| Injection Volume | : | 50 µL |
| Mobile Phase | : | 50mM $KH_2PO_4$ Buffer (pH~5): Acetonitrile |
| | | (65:35, % v/v) |
| Retention time | : | CLZ ~ 9.6 ± 0.2 min |
| | | DCLZ ~ 7.2 ± 0.2 min |
| | | RSG ~ 8.5 ± 0.2 min |
| | | PIO (IS) ~ 14.9 ± 0.2 min |

Sample processing:

To each 75 µl of plasma sample ($K_2$ EDTA as a anticoagulant), IS (10 µl of 20 µg/ml of PIO) was added and extracted with 1.5 ml of methyl t-butyl ether, mixed to vortex for 10 min and centrifuged at 10000 rpm for 10 min. The supernatant was collected and evaporated to dryness under a stream of nitrogen. The dried residue was reconstituted immediately before analysis with 100 µl of mobile phase, vortexed for 1 min and 75µl was injected onto HPLC.

*3.4.1.2 Methods using LC-MS/MS*

A new rapid, specific and simple liquid-liquid extraction method using LC-MS/MS for the simultaneous determination of CLZ, DCLZ and nateglinide (NTG) using repaglinide as internal standard (IS) in rat plasma have been developed in our lab and this method was used to estimate CLZ & DCLZ in samples from drug interactions studies between CLZ and NTG.

The following conditions were set to check the analysis of CLZ, DCLZ & NTG using repaglinide as internal standard.

Chromatographic conditions

| | | |
|---|---|---|
| Instrument | : | Perkin-Elmer 200 micro LC pump system (Norwalk, CT, USA) coupled with API 3200/Q Trap LC-MS/MS |
| Flow rate | : | 0.4 ml/min |
| Column | : | Hypurity $C_{18,}$ 50 x4.6 mm, 5µ |
| Column temperature | : | 40°C ± 2°C |
| Sample temperature | : | 10°C ± 2°C |
| Injection Volume | : | 5 µL |
| Mobile Phase | : | 2mMAmmoniumacetatebuffer(pH~3.4):Acetonitrile (10:90, %v/v) |
| Retention time | : | NTG ~ 2.12 ± 0.2 min<br>CLZ ~ 1.87 ± 0.2 min<br>DCLZ ~ 1.86 ± 0.2 min<br>Repaglinide (IS) ~ 2.25 ± 0.2 min |

Sample processing:

To 50 µl of rat plasma, 25µl of of repaglinide solution 2 ppm was added and then 50µL of 0.1N Hcl has been added to vortex with mix for 30 sec. To this mixture, 1.5 ml of above TBME was added and mixed with vortex for 10 min. These samples were centrifuged at 10000 rpm for 5 min and supernatant was collected and dried under nitrogen stream. Reconstitute the residue with 100 µl of mobile phase and mixed with vortex for 1 min and 5 µl of injection volume was injected onto chromatographic column.

Another new rapid, specific and simple liquid-liquid extraction method using LC-MS/MS for the simultaneous determination of CLZ, DCLZ and Glipizide (GLP) using glimepiride as internal standard (IS) in rat plasma have been developed in our lab and this method was used to estimate CLZ & DCLZ in samples from drug interactions studies between CLZ and GLP.

The following conditions were set to check the analysis of CLZ, DCLZ & GLP using glimepiride as internal standard.

Chromatographic conditions

| | | |
|---|---|---|
| Instrument | : | Perkin-Elmer 200 micro LC pump system (Norwalk, CT, USA) coupled with API 3200/Q Trap LC-MS/MS |
| Flow rate | : | 0.4 ml/min |
| Column | : | Hypurity $C_{18,}$ 50 x4.6 mm, 5µ |
| Column temperature | : | 40°C ± 2°C |
| Sample temperature | : | 10°C ± 2°C |
| Injection Volume | : | 5 µL |
| Mobile Phase | : | 2mMAmmoniumacetatebuffer(pH~3.4):Acetonitrile (10:90, %v/v) |
| Retention time | : | GLP ~ 2.1 ± 0.2 min<br>CLZ ~ 1.87 ± 0.2 min<br>DCLZ ~ 1.86 ± 0.2 min<br>Glimepiride (IS) ~ 2.2 ± 0.2 min |

Sample processing:

To 50 µl of rat plasma, 25µl of IS solution (2ppm) was added and then 50µL of 0.1N Hcl has been added to vortex with mix for 30 sec. To this mixture, 1.5 ml of above TBME was added and mixed with vortex for 10 min. These samples were centrifuged at 10000 rpm for 5 min and supernatant was collected and dried under nitrogen stream. Reconstitute the residue with 100 µl of mobile phase and mixed with vortex for 1 min and 5 µl of injection volume was injected onto chromatographic column.

### 3.4.2 Analytical method for pentoxifylline

A simple and sensitive High Performance Liquid Chromatographic method for the determination of pentoxifylline (PTX) in Wistar Rat plasma using estimation linezolid as internal standard has been developed in our lab and is being used for of PTX in samples from drug interaction studies between pentoxifylline and RSG/ MET/ NTG/ GLP.

The following conditions were set to check the analysis of PTX using linezolid as internal standard.

Chromatographic conditions

| | | |
|---|---|---|
| Instrument | : | HPLC (Waters 2695 Alliance module) |
| Detector | : | UV detector (Waters 2487) |
| Wave length | : | 273nm |
| Column | : | Hypersil BDS $C_{18}$, 250 x 4.6 mm, 5µm. |
| Mobile phase | : | 0.25%TriethylamineBuffer (pH 3.25): Acetonitrile (78: 22 % v/v) |
| Flow rate | : | 1.0 mL/minute |
| Injection volume | : | 75 µL |
| Column oven temp | : | 30°C ± 2°C |
| Sample cooler temp | : | 10°C ± 2°C |
| Retention time | : | PTX ~ 6.2 ± 0.2 min<br>Linezolid (IS) ~ 8.1 ± 0.2 min |

Sample processing:

To 100 µl of plasma (test sample) of rat was mixed with 10 µl of IS, 25 µl of 1N NaOH was added and vortex for 2 minutes followed by addition of a pinch of Sodium chloride. And then 1.5 ml of DCM was added and mixed with vortex for 5 min. These samples were centrifuged at 10000 rpm for 10 min and supernatant was withdrawn into evaporating glass tubes for evaporation. Evaporation was carried out at 40° C under stream of nitrogen gas. Reconstituted the residue with 100 µl of mobile phase and mixed with vortex for 1 min and 75 µl of injection volume was injected onto chromatographic column.

### 3.4.3 Analytical methods for rosiglitazone

As discussed above, a simultaneous estimation of CLZ, DCLZ and rosiglitazone (RSG) in rat plasma using pioglitazone (PIO) as internal standard was developed and validated. This method was used for estimation RSG in samples from drug interaction studies during pentoxifylline and cilostazol.

The following conditions were set to check the analysis of RSG using PIO as internal standard.

Chromatographic conditions

| | | |
|---|---|---|
| Instrument | : | HPLC (Waters 2695 Alliance module) |
| Detector | : | UV detector (Waters 2487) |
| Wave length | : | 226 nm (, RSG, PIO) |
| Flow rate | : | 1.2 ml/min |
| Column | : | Hypersil BDS $C_{18}$ (150 X 4.6 mm X 5µ) |
| Column temperature | : | 25°C ± 2°C |
| Sample temperature | : | 10°C ± 2°C |
| Injection Volume | : | 50 µL |
| Mobile Phase | : | 50mM $KH_2PO_4$ Buffer (pH~5.2): Acetonitrile (65:35, % v/v) |
| Retention time | : | RSG ~ 8.5 ± 0.2 min<br>PIO (IS) ~ 14.9 ± 0.2 min |

Sample processing:

To each 75 µl of plasma sample ($K_2$ EDTA as a anticoagulant), I.S. (10 µl of 20 µg/ml PIO) was added and extracted with 1.5 ml of methyl t-butyl ether, mixed to vortex for 10 min and centrifuged at 10000 rpm for 10 min. The supernatant was collected and evaporated to dryness under a stream of nitrogen. The dried residue was reconstituted immediately before analysis with 100 µl of mobile phase, vortexed for 1 min and 75µl was injected onto HPLC.

### 3.4.4 Analytical methods for metformin

A new rapid, specific, sensitive and simple protein precipitation method using HPLC-UV for the determination of metformin (MET) in rat plasma suitable for pharmacokinetic studies have been developed in our lab and this method was used in samples from drug interaction studies between MET and CLZ/PTX.

The following conditions were set to check the analysis of metformin using nicorandil as internal standard.

Chromatographic conditions

| | | |
|---|---|---|
| Instrument | : | HPLC (Waters 2690 Alliance module) |
| Detector | : | UV detector (Waters 2487) |
| Wave length | : | 233 nm |
| Flow rate | : | 1.5 ml/min |
| Column | : | Kromosil $C_{18}$ (250 X 4.6 mm X 5µ) |
| Column temperature | : | 25°C ± 2°C |
| Sample temperature | : | 10°C ± 2°C |
| Injection Volume | : | 50 µL |
| Mobile Phase | : | 50Mm $KH_2PO_4$ Buffer (pH~6.0): Acetonitrile (89:11) |
| Retention time | : | MET ~ 9.0 ± 0.2 min<br>Nicorandil ~ 13.0 ± 0.2 min |

Sample processing:

95 µl of blank plasma of rat was mixed with vortex by adding 5 µl of solution containing metformin for 30 sec and then 10µl of Nicorandil solution 50 ppm has been added to that. To this mixture, 25µl of 30% perchloric acid was added and mixed with vortex for 1 min. These samples were centrifuged for 5 min at 15000 rpm for 5 min and clear aqueous solution was transferred into disposable inserts for injection.

### 3.4.5 Analytical methods for nateglinide

As discussed above, a LC-MS/MS for the simultaneous determination of CLZ, DCLZ and nateglinide (NTG) using repaglinide as internal standard (IS) in rat plasma have been developed in our lab and this method was used to estimate NTG in samples from drug interactions studies between NTG and CLZ/PTX.

The following conditions were set to check the analysis of NTG using repaglinide as internal standard.

Chromatographic conditions

| | | |
|---|---|---|
| Instrument | : | Perkin-Elmer 200 micro LC pump system (Norwalk, CT, USA) coupled with API 3200/Q Trap LC-MS/MS |
| Flow rate | : | 0.4 ml/min |
| Column | : | Hypurity $C_{18,}$ 50 x4.6 mm, 5µ |
| Column temperature | : | 40°C ± 2°C |
| Sample temperature | : | 10°C ± 2°C |
| Injection Volume | : | 5 µL |
| Mobile Phase | : | 2mMAmmoniumacetatebuffer(pH~3.4):Acetonitrile (10:90, %v/v) |
| Retention time | : | NTG ~ 2.12 ± 0.2 min<br>repaglinide (IS) ~ 2.25 ± 0.2 min |

Sample processing:

To 50 µl of rat plasma, 25µl of of repaglinide solution 2 ppm was added and then 50µL of 0.1N Hcl has been added to vortex with mix for 30 sec. To this mixture, 1.5 ml of above TBME was added and mixed with vortex for 10 min. These samples were centrifuged at 10000 rpm for 5 min and supernatant was collected and dried under nitrogen stream. Reconstitute the residue with 100 µl of mobile phase and mixed with vortex for 1 min and 5 µl of injection volume was injected onto chromatographic column.

### 3.4.6 Analytical methods for Glipizide

As discussed above, a LC-MS/MS method for the simultaneous determination of CLZ, DCLZ and Glipizide (GLP) using glimepiride as internal standard (IS) in rat plasma have been developed in our lab and this method was used to estimate GLP in samples from drug interactions studies between GLP and CLZ/PTX.

The following conditions were set to check the analysis of GLP using glimepiride as internal standard.

Chromatographic conditions

| | | |
|---|---|---|
| Instrument | : | Perkin-Elmer 200 micro LC pump system (Norwalk, CT, USA) coupled with API 3200/Q Trap LC-MS/MS |
| Flow rate | : | 0.4 ml/min |
| Column | : | Hypurity $C_{18,}$ 50 x4.6 mm, 5µ |
| Column temperature | : | 40°C ± 2°C |
| Sample temperature | : | 10°C ± 2°C |
| Injection Volume | : | 5 µL |
| Mobile Phase | : | 2mMAmmoniumacetatebuffer(pH~3.4):Acetonitrile (10:90, %v/v) |
| Retention time | : | GLP ~ 2.1 ± 0.2 min<br>Glimepiride (IS) ~ 2.2 ± 0.2 min |

Sample processing:

To 50 µl of rat plasma, 25µl of IS solution (2ppm) was added and then 50µL of 0.1N Hcl has been added to vortex with mix for 30 sec. To this mixture, 1.5 ml of above TBME was added and mixed with vortex for 10 min. These samples were centrifuged at 10000 rpm for 5 min and supernatant was collected and dried under nitrogen stream. Reconstitute the residue with 100 µl of mobile phase and mixed with vortex for 1 min and 5 µl of injection volume was injected onto chromatographic column.

## 3.5 In vivo studies

### 3.5.1. Single dose pharmacokinetic study of antidiabetic agent (ADA) in rats:

Male Wistar rats (n=6) were administered with single dose ADA (doses as per the table 3.5) in 0.5 % methylcellulose suspension through oral gavages in overnight fasted (12hrs before dose) condition. These rats were further administered simultaneously the vehicle at 5 ml/kg.b.wt/p.o keeping in view of the design of simultaneous drug-drug interaction studies and fasting was continued till 4 hr post dose. The blood samples were collected at

definite time intervals as per the table (predose, 10, 15, 30 min and 1, 2, 3, 4, 6, 8, 12 and 24 hrs post dose) and were kept on ice bath till further processing. These samples were further separated for plasma by centrifuging at 4ºC for 10 min at 1000 x g and then stored at –70ºC till further analysis. These samples were analyzed for estimating the levels of ADA using respective analytical methods.

### 3.5.2. Single dose pharmacokinetic study of cilostazol in rats:

Male Wistar rats (n=6) were administered with single dose 10 mg/kg.b.wt/p.o cilostazol in 0.5 % methylcellulose suspension through oral gavages in overnight fasted (12hrs before dose) condition. These rats were further administered simultaneously the vehicle at 5 ml/kg.b.wt/p.o keeping in view of the design of simultaneous drug-drug interaction studies and fasting was continued till 4 hr post dose. The blood samples were collected at predose, 10, 15, 30 min and 1, 2, 4, 6, 8, 12 and 24 hrs post dose and were kept on ice bath till further processing. These samples were further separated for plasma by centrifuging at 4ºC for 10 min at 1000 x g and then stored at –70ºC till further analysis. These samples were analyzed for estimating the levels of cilostazol as well as its major metabolite 3, 4-dehydro-cilostazol by HPLC-UV.

### 3.5.3. Effect of single dose of ADA on pharmacokinetics of cilostazol in rats

Five groups of male Wistar rats (n=6 per group) were administered with single dose simultaneous treatment of 10 mg/kg.b.wt/p.o cilostazol and a single dose of one of the ADA in 0.5 % methylcellulose suspension at 5 ml/kg.b.wt/p.o volume through oral gavages in overnight fasted (12hrs before dose) condition and fasting was continued till 4 hr post dose. The blood samples were collected at predose, 10, 15, 30 min and 1, 2, 4, 6, 8, 12 and 24 hrs post dose and were kept on ice bath till further processing. These samples were further separated for plasma by centrifuging at 4ºC for 10 min at 1000 x g and then stored at –70ºC till further analysis. These samples were analyzed for estimating the levels of cilostazol as well as its major metabolite 3, 4-dehydro-cilostazol from one group and ADA levels from second group.

### 3.5.4. Effect of multiple doses of ADA on pharmacokinetics of cilostazol in rats

Five groups of male Wistar rats (n=6 in per group) were administered with seven daily single doses of respective ADA and 0.5 % w/v methylcellulose solution containing 2.5 µL/mL tween 80 (vehicle) respectively. These were simultaneously administered with single dose of cilostazol (10 mg/kg.b.wt/p.o) on seventh day through oral gavages in overnight fasted (12hrs before dose) condition and fasting was continued till 4 hr post dose. The blood samples were collected at predose, 10, 15, 30 min and 1, 2, 4, 6, 8, 12 and 24 hrs post dose on seventh day and were kept on wet ice bath till further processing. These samples were further harvested for plasma by centrifuging at 40C for 10 min at 1000g and then stored at $-70^0$C till further analysis. These samples were analyzed for estimation of the levels of cilostazol as well as its major metabolite 3, 4-dehydro-cilostazol from all groups.

### 3.5.5. Effect of multiple doses of cilostazol on pharmacokinetics of ADA in rats

Five groups of male Wistar rats (n=6 per group) were administered with seven daily single doses of cilostazol (10 mg/kg.b.wt/day/p.o) and 0.5 % w/v methylcellulose solution containing 2.5 µL/mL tween 80 (vehicle) respectively. These were simultaneously administered with single dose of respective ADA in each group on seventh day through oral gavages in overnight fasted (12hrs before dose) condition and fasting was continued till 4 hr post dose. The blood samples were collected at predose, 10, 15, 30 min and 1, 2, 4, 6, 8, 12 and 24 hrs post dose on seventh day and were kept on wet ice bath till further processing. These samples were further harvested for plasma by centrifuging at 4ºC for 10 min at 1000g and then stored at $-70^0$C till further analysis. These samples were analyzed for estimation of the levels of ADA from their respective groups.

### 3.5.6. Single dose pharmacokinetic study of pentoxifylline in rats:

Male Wistar rats (n=6) were administered with single dose 10 mg/kg.b.wt/p.o pentoxifylline in 0.5 % methylcellulose suspension through oral gavages in overnight fasted (12hrs before dose) condition. These rats were further administered simultaneously the vehicle at 5 ml/kg.b.wt/p.o keeping in view of the design of simultaneous drug-drug interaction studies and fasting was continued till 4 hr post dose.. The blood samples were collected at predose, 10, 15, 30, 45 min and 1.0, 1.5 2.0, 2.5, 3.0 and 4.0 hr post dose and were kept on ice bath till further processing. These samples were further separated for plasma by centrifuging at 4ºC for 10 min at 1000 x g and then stored at –70ºC till further analysis. These samples were analyzed for estimating the levels of pentoxifylline.

### 3.5.7. Effect of single dose ADA on pharmacokinetics of pentoxifylline in rats:

Five groups of Wistar rats (n=6 per group) were administered with single dose simultaneous treatment of 10 mg/kg.b.wt/p.o pentoxifylline and a single dose of one of the ADA in 0.5 % methylcellulose suspension at 5 ml/kg.b.wt/p.o volume through oral gavages in overnight fasted (12hrs before dose) condition. The fasting was continued till 4 hr post dose. The blood samples were collected at predose, 10, 15, 30, 45 min and 1.0, 1.5 2.0, 2.5, 3.0 and 4.0 hr post dose and were kept on ice bath till further processing. These samples were further separated for plasma by centrifuging at 4ºC for 10 min at 1000 x g and then stored at –70ºC till further analysis. These samples were analyzed for estimating the levels of pentoxifylline.

### 3.5.8. Effect of single dose pentoxifylline on pharmacokinetics of ADA in rats:

Five groups of male Wistar rats (n=6 per group) were administered with single dose simultaneous treatment of 10 mg/kg.b.wt/p.o/t.i.d. pentoxifylline and single dose of respective ADA in 0.5 % methylcellulose suspension at 5 ml/kg.b.wt/p.o volume through oral gavages in overnight fasted (12hrs before dose) condition. The fasting was continued till 4 hr post first dose.The blood samples were collected at pre dose, 10, 15, 30 min and 1.0, 1.5 2.0, 2.5, 3.0 and 4.0 hr from group 1 for pentoxifylline and at predose, 10, 15, 30

min and 1, 2, 4, 6, 8, 12 and 24 hr post dose from group 2 for rosiglitazone. These were kept on ice till further processing. These samples were further harvested for plasma by centrifuging at $4^0$C for 10 min at 1000 x g and then stored at $-70^0$C till further analysis. These samples were analyzed for estimation of the levels of respective ADA.

### 3.5.9. Effect of multiple doses of ADA on pharmacokinetics of pentoxifylline in rats

Five groups of male Wistar rats (n=6 in per group) were administered with seven daily single doses of respective ADA and 0.5 % w/v methylcellulose solution containing 2.5 µL/mL tween 80 (vehicle) respectively. These were simultaneously administered with single dose of pentoxifylline (10 mg/kg.b.wt/p.o) on seventh day through oral gavages in overnight fasted (12hrs before dose) condition and fasting was continued till 4 hr post dose. The blood samples were collected at predose, 10, 15, 30, 45 min and 1.0, 1.5 2.0, 2.5, 3.0 and 4.0 hr post dose on seventh day and were kept on wet ice bath till further processing. These samples were further harvested for plasma by centrifuging at 40C for 10 min at 1000g and then stored at $-70^0$C till further analysis. These samples were analyzed for estimation of the levels of pentoxifylline from all groups.

### 3.5.10. Effect of multiple doses of pentoxifylline on pharmacokinetics of ADA in rats

Five groups of male Wistar rats (n=6 per group) were administered with seven daily doses of pentoxifylline (10 mg/kg.b.wt/t.i.d/p.o) and 0.5 % w/v methylcellulose solution containing 2.5 µL/mL tween 80 (vehicle) respectively. These were simultaneously administered with single dose of ADA on seventh day through oral gavages in overnight fasted (12hrs before dose) condition and fasting was continued till 4 hr post dose. The blood samples were collected at predose, 10, 15, 30 min and 1, 2, 4, 6, 8, 12 and 24 hrs post dose on seventh day and were kept on wet ice bath till further processing. These samples were further harvested for plasma by centrifuging at 4ºC for 10 min at 1000g and then stored at $-70^0$C till further analysis. These samples were analyzed for estimation of the levels of ADA from their respective groups.

### 3.5.11. Effect of ADA on anti platelet activity of cilostazol using ex vivo platelet aggregation method

(i) **Antiplatelet activity of cilostazol in presence of multiple dose anti - diabetic agent treated rats:** Seven groups of Male Wistar rats (n=3 in each group) were administered with seven daily single doses of vehicle or ADA in 0.5 % methylcellulose suspension and also simultaneously administered with single dose (10 mg/kg.b.wt/p.o) of either cilostazol or pentoxifylline in all the animals except the group 1 on seventh day through oral gavages in overnight fasted (12hrs before dose) condition. The blood samples were collected at 1 hrs post dose on seventh day in to the microcentrifuge tubes containing sodium citrate as anti-coagulant. and these were kept on ice till further processing to prepare the plasma.

(ii) **Preparation of platelet rich and plate poor plasma.** These samples were further harvested for platelet rich plasma (PRP) by centrifuging at 150g for 10 min at room temperature and then stored ice till further analysis. These samples were reanalyzed for platelet poor plasma (PPP) by re-centrifuging at 1500g for 10 min at room temperature and then transferred in to other tube for dilution of PRP.

**(iii) Induction of platelet aggregation by ADP:** 180µl of PRP was added to a 96 well microplate in duplicate, then 20µl of adenosine diphosphate (ADP) was added as a platelet aggregating stimulus, immediately started taking reading using kinetic mode in Spectra max 190 for 5 min at a wavelength of 560nm at an interval of 30seconds.

### 3.5.12. Assessment of whole blood glucose activity

The assessment of whole blood glucose activity was done from the groups which were administered with ADA pretreated with vehicle, cilostazol or pentoxifylline during PK experiments. Estimation of whole blood glucose (WBG) was done using a hand-held glucometer (Ascensia Entrust®, Bayer, Germany) with blood glucose strips. Blood drops were collected from PK samples immediately for measurement of WBG after administration of single dose of ADA on day-1 or seventh day in groups pretreated with

vehicle or cilostazol or pentoxifylline in overnight fasted animals A drop of blood was placed in the area specified on the blood glucose measuring strip to record the WBG value. The Ascensia Entrust ® gave accurate quantitative measurement of glucose in blood from 30 to 550 mg/dL (1.6 to 30.5 mmol/L, 1 mmol/L being equivalent to18 mg/dL) and displays the result in mg/dL, the conventional format for measuring blood glucose. Reduction in blood glucose produced by the compound was calculated using the area under the curve (AUC).

## 3.6 Data analysis

### 3.6.1 Data processing:

The concentration data was acquired either using the Empower software supplied by the Waters manufacturer for HPLC analysis or Analyst software Version 1.3 (MDS/SCIEX) from LC-MS/MS. The concentration of the samples is calculated from the equation using regression analysis that suits with weighting factor ($1/x^2$).

$$y=ax + b$$

Where, x = Concentration of analyte

y = Peak area ratio of analyte to IS

a = Slope of the calibration curve

b = Intercept of the calibration curve

The concentration data was used in vitro plasma protein binding studies and in vivo pharmacokinetic studies. The peak area ratios of drug to IS were considered for calculating percent remaining of drugs during analysis of metabolism study samples

### 3.6.2 Pharmacokinetic analysis

The plasma concentration-time data were analyzed using the WinNonlin Software (Version 5.01 from Pharsight Corporation, USA) based on non linear regression analysis. The concentrations observed below the limit of quantification (LOQ) in pharmacokinetic studies were considered as zero for estimating the pharmacokinetic parameters. The

pharmacokinetic parameters were estimated using the non compartmental model analysis. The peak plasma concentration ($C_{max}$ ) for each analyte and the corresponding time of its occurrence ($t_{max}$ ) were obtained by visual by visual inspection of the plasma concentration-time curves. The elimination rate constant ($k_{el}$ ), elimination half-life ($t_{1/2}$ ), apparent volume of distribution ($V_{d\_F}$ ) and clearance ($CL_F$) were estimated from the non linear regression analysis. At least three non zero concentration at the elimination phase of semilog plot were considered for the estimation of elimination rate constant ($K_{el}$).The total areas under the curves from time zero to the last quantifiable time point ($AUC_{0-t}$) and from time zero to infinity ($AUC_{0-\infty}$) were estimated using the linear trapezoidal approximation.

### 3.6.3 Statistical analysis

Data analysis was performed using Graph Pad Prism 3.02 (Graph Pad software Inc., USA). Sample data were expressed as either as mean ± standard error of mean (SEM) for metabolism studies and mean ± standard deviation (S.D) for pharmacokinetic studies. The median values for $T_{max}$ and harmonic mean values for elimination half-life were used in pharmacokinetic studies. Comparison of means of related samples and from two independent groups were made using the student's unpaired t-test. Comparisons of three or more groups were made by one way ANOVA using bonifferoni's multiple comparison test compared to that of control group during antiplatelet activity and random blood glucose assessment. A *p* value of less than 0.05 was adopted as statistically significant and the observed table values were reported in tables and figures.

Table 3.5 Study design of in vivo pharmacokinetic studies to evaluate the drug interaction potential of ADA on cilostazol in rats

| Study No. | Study | Pretreatment | Pretreatment Dose (ml*or mg/kg.b.wt) | Treatment | Treatment Dose (ml* or mg/kg.b.wt) | Analytes |
|---|---|---|---|---|---|---|
| | Control | - | - | Vehicle + CLZ | 5* + 10 | CLZ & DCLZ |
| I | RSG vs CLZ | - | - | RSG + CLZ | 3 + 10 | CLZ & DCLZ |
| | | Vehicle + RSG for 6 days | 5* + 3 | RSG + CLZ on day-7 | 3 + 10 | CLZ & DCLZ |
| II | MET vs CLZ | - | - | MET + CLZ | 300 + 10 | CLZ & DCLZ |
| | | Vehicle + MET for 6 days | 5* + 300 | MET + CLZ on day-7 | 300 + 10 | CLZ & DCLZ |
| III | NTG vs CLZ | - | - | NTG + CLZ | 10 + 10 | CLZ & DCLZ |
| | | Vehicle +NTG for 6 days | 5* + 10 | NTG + CLZ on day-7 | 10 + 10 | CLZ & DCLZ |
| IV | GLP vs CLZ | - | - | GLP + CLZ | 10 + 10 | CLZ & DCLZ |
| | | Vehicle +GLP for 6 days | 5* + 10 | GLP + CLZ on day-7 | 10 + 10 | CLZ & DCLZ |

Table 3.6 Study design of in vivo pharmacokinetic studies to evaluate the drug interaction potential of ADA on pentoxifylline in rats

| Study No. | Study | Pretreatment | Pretreatment Dose (ml*or mg/kg.b.wt) | Treatment | Treatment Dose (ml* or mg/kg.b.wt) | Analytes |
|---|---|---|---|---|---|---|
| | Control | - | - | Vehicle + PTX | 5* + 10 | PTX |
| V | RSG vs PTX | - | - | RSG + PTX | 3 + 10 | PTX |
| | | Vehicle + PTX for 6 days | 5* + 3 | RSG + PTX on day-7 | 3 + 10 | PTX |
| VI | MET vs PTX | - | - | MET + PTX | 300 + 10 | PTX |
| | | Vehicle + PTX for 6 days | 5* + 300 | MET + PTX on day-7 | 300 + 10 | PTX |
| VII | NTG vs PTX | - | - | NTG + PTX | 10 + 10 | PTX |
| | | Vehicle +PTX for 6 days | 5* + 10 | NTG + PTX on day-7 | 10 + 10 | PTX |
| VIII | GLP vs PTX | - | - | GLP + PTX | 10 + 10 | PTX |
| | | Vehicle + PTX for 6 days | 5* + 10 | GLP + PTX on day-7 | 10 + 10 | PTX |

Table 3.7 Study design of in vivo pharmacokinetic studies to evaluate the drug interaction potential of cilostazol on ADA in rats

| Study No. | Study | Pretreatment | Pretreatment Dose (ml*or mg/kg.b.wt) | Treatment | Treatment Dose (ml* or mg/kg.b.wt) | Analytes |
|---|---|---|---|---|---|---|
| | Control (RSG) | - | - | Vehicle + RSG | 5* + 3 | RSG |
| | Control (MET) | - | - | Vehicle + MET | 5* + 300 | MET |
| | Control (NTG) | - | - | Vehicle + NTG | 5* + 10 | NTG |
| | Control (GLP) | - | - | Vehicle + GLP | 5* + 10 | GLP |
| IX | CLZ vs RSG | - | - | CLZ + RSG | 10 + 3 | RSG |
| | | Vehicle + CLZ for 6 days | 5* + 10 | CLZ + RSG on day-7 | 10 + 3 | RSG |
| X | CLZ vs MET | - | - | CLZ + MET | 10 + 300 | MET |
| | | Vehicle + CLZ for 6 days | 5* + 10 | CLZ + MET on day-7 | 10 + 300 | MET |
| XI | CLZ vs NTG | - | - | CLZ + NTG | 10 + 10 | NTG |
| | | Vehicle + CLZ for 6 days | 5* + 10 | CLZ + NTG on day-7 | 10 + 10 | NTG |
| XII | CLZ vs GLP | - | - | CLZ + GLP | 10 + 10 | GLP |
| | | Vehicle + CLZ for 6 days | 5* + 10 | CLZ + GLP on day-7 | 10 + 10 | GLP |
| | | Vehicle + CLZ for 6 days | 5* + 10 | CLZ + RSG on day-7 | 10 + 3 | RSG |
| XIII | PTX vs RSG | - | - | PTX + RSG | 10 + 3 | RSG |
| | | Vehicle + PTX for 6 days | 5* + 10 | PTX + RSG on day-7 | 10 + 3 | RSG |

| Study No. | Study | Pretreatment | Pretreatment Dose (ml*or mg/kg.b.wt) | Treatment | Treatment Dose (ml* or mg/kg.b.wt) | Analytes |
|---|---|---|---|---|---|---|
| XIV | PTX vs MET | - | - | PTX + MET | 10 + 300 | MET |
| | | Vehicle + PTX for 6 days | 5* + 10 | PTX + MET on day-7 | 10 + 300 | MET |
| XV | PTX vs NTG | - | - | PTX + NTG | 10 + 10 | NTG |
| | | Vehicle + PTX for 6 days | 5* + 10 | PTX + NTG on day-7 | 10 + 10 | NTG |
| XVI | PTX vs GLP | - | - | PTX + GLP | 10 + 10 | GLP |
| | | Vehicle + GLP for 6 days | 5* + 10 | PTX + GLP on day-7 | 10 + 10 | GLP |

* vehicle was administered as ml/kg.b.wt

Table 3.8 Study Design of effect of ADA on antiplatelet activity of cilostazol and pentoxifylline

| Group | Treatment | Dose | No.of animals |
|---|---|---|---|
| 1 | control | vehicle10 ml/kg.b.wt | 3 |
| 2 | vehicle + CLZ | vehicle 5 ml/kg.b.wt + 10 mg/kg.b.wt | 3 |
| 3 | RSG + CLZ | 3 mg/kg.b.wt for 7 days + 10 mg/kg on day-7 | 3 |
| 5 | MET + CLZ | 300 mg/kg.b.wt for 7 days + 10 mg/kg on day-7 | 3 |
| 6 | NTG + CLZ | 10 mg/kg.b.wt for 7 days + 10 mg/kg on day-7 | 3 |
| 7 | GLP + CLZ | 10 mg/kg.b.wt for 7 days + 10 mg/kg on day-7 | 3 |
| 8 | vehicle + PTX | vehicle 5 ml/kg.b.wt + 10 mg/kg.b.wt | 3 |
| 9 | RSG + PTX | 3 mg/kg.b.wt for 7 days + 10 mg/kg on day-7 | 3 |
| 11 | MET + PTX | 300 mg/kg.b.wt for 7 days + 10 mg/kg on day-7 | 3 |
| 12 | NTG + PTX | 10 mg/kg.b.wt for 7 days + 10 mg/kg on day-7 | 3 |
| 13 | GLP + PTX | 10 mg/kg.b.wt for 7 days + 10 mg/kg on day-7 | 3 |

# Chapter 4

# RESULTS

# 4 RESULTS

## 4.1 In vitro Metabolism studies with liver microsomes

### 4.1.1 Effect of antidiabetic agents on cilostazol metabolism in rat liver microsomes

The in vitro inhibitory effect of ADA on percent substrate remaining (metabolic stability) of the cilostazol and its metabolite, 3, 4-dehydro-cilostazol was studied in rat liver microsomes prepared from rats pretreated with ADA and the results are presented in **Table 4.1 & 4.2**.

The metabolic stability of cilostazol was changed from 13.46 % (vehicle treated) to 60.04 % (rosiglitazone treated), 37.18 % (metformin treated), 91.05 % (nateglinide treated), and 54.00 % (glipizide treated). Thus ADAs investigated in this studies are significantly improved the metabolic stability ($p<0.001$) of cilostazol in rat liver microsomes.

Table 4.1 Effect of antidiabetic agents on cilostazol metabolism in rat liver microsomes

| S.No. | Group | % CLZ remaining (mean ± SEM) | Difference in mean compared to vehicle (%) |
|---|---|---|---|
| 1 | Vehicle | 13.46 ± 0.76 | |
| 2 | Rosiglitazone | 60.04 ± 4.16 | 46.58 |
| 3 | Metformin | 37.48 ± 0.45 | 24.01 |
| 4 | Nateglinide | 91.05 ± 2.11 | 75.49 |
| 5 | Glipizide | 54.00 ± 1.60 | 40.54 |

n=3 in each set

Table 4.2 Effect of antidiabetic agents on 3, 4-dehydro-cilostazol metabolism in rat liver microsomes

| S.No. | Group | % DCLZ remaining (mean ± SEM) | Difference in mean compared to vehicle (%) |
|---|---|---|---|
| 1 | Vehicle | 35.73 ± 0.81 | |
| 2 | Rosiglitazone | 75.76 ± 2.02 | 40.03 |
| 3 | Metformin | 49.16 ± 1.07 | 13.43 |
| 4 | Nateglinide | 91.65 ± 1.23 | 55.92 |
| 5 | Glipizide | 73.65 ± 0.69 | 37.92 |

n=3 in each set

The metabolic stability of 3, 4-dehydro-cilostazol was also changed from 35.73 % (vehicle treated) to 75.76 % (rosiglitazone treated), 50.84 % (metformin treated), 91.65 % (nateglinide

treated), 73.65 % (glipizide treated). Thus ADAs ($p<0.001$) significantly improved the metabolic stability of 3, 4-dehydro-cilostazol in rat liver microsomes.

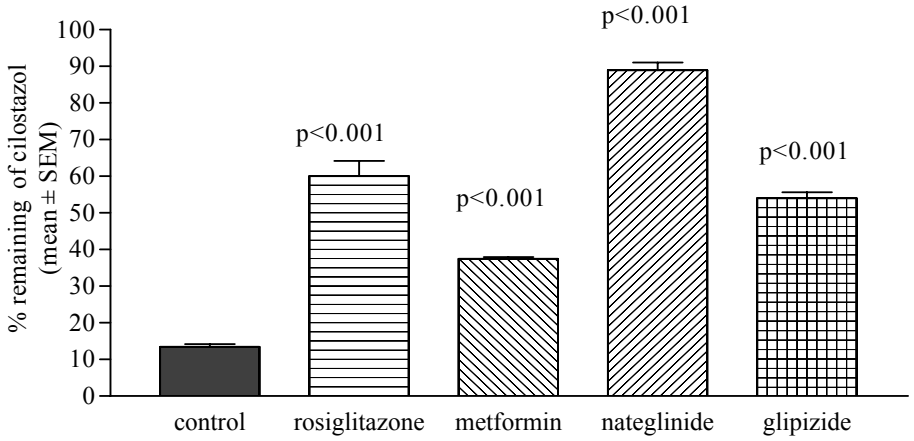



*Figure 4.1 Effect of ADA on cilostazol metabolism in rat liver microsomes*
n=3 in each set; p values were calculated using student unpaired t-test compared to control group

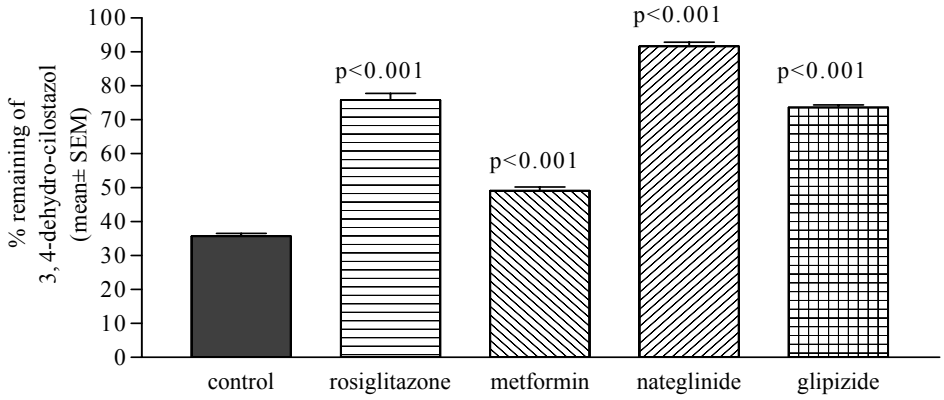



*Figure 4.2 Effect of ADA on cilostazol metabolism in rat liver microsomes*
n=3 in each set; p values were calculated using student unpaired t-test compared to control group

### 4.1.2 Effect of antidiabetic agents on pentoxifylline metabolism in rat liver microsomes

The in vitro inhibitory effect of ADA on percent remaining (metabolism stability) of the pentoxifylline was studied in rat liver microsomes prepared from rats pretreated with ADA and the results are presented in **Table 4.3**.

The metabolic stability of pentoxifylline was changed from 92.91 % (vehicle treated) to 79.15 % (rosiglitazone treated), 81.08 % (nateglinide treated), 88.45 % (glipizide treated). Thus

rosiglitazone ($p<0.001$), nateglinide ($p<0.05$) and glipizide ($p<0.05$) significantly reduced the metabolic stability of pentoxifylline in rat liver microsomes**.**

Table 4.3 Effect of antidiabetic agents on pentoxifylline metabolism in rat liver Microsomes

| S.No. | Group | % PTX remaining (mean ± SEM) | Difference in mean compared to vehicle (%) |
|---|---|---|---|
| 1 | Vehicle | 92.91 ± 0.98 | |
| 2 | Rosiglitazone | 79.15 ± 0.56 | -13.76 |
| 3 | Metformin | 96.40 ± 1.99 | +3.49 |
| 4 | Nateglinide | 81.08 ± 0.75 | -11.83 |
| 5 | Glipizide | 88.45 ± 0.43 | -4.45 |

n=3 in each set

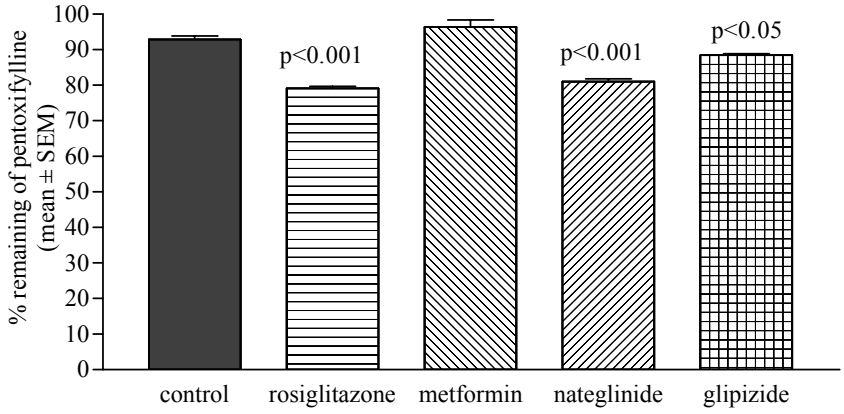



*Figure 4.3 Effect of ADA on pentoxifylline metabolism in rat liver microsomes*
n=3 in each set; p values were calculated using student unpaired t-test compared to control group

### 4.1.3 Effect of antidiabetic agents on cilostazol metabolism in CYP3A4 human recombinant enzymes

The metabolic stability of cilostazol was changed from 74.11 % (vehicle treated) to 84.34 % (rosiglitazone treated), 76.37 % (metformin treated), 77.21 % (nateglinide treated), 92.44 % (glipizide treated). Thus rosiglitazone and glipizide improved the metabolic stability of cilostazol in CYP3A4 but were not significant.

The formation of 3, 4-dehydro-cilostazol in CYP3A4 up on incubation with cilostazol was also changed from 2.71 µmoles/mg protein/min (vehicle treated) to 2.24 (rosiglitazone treated), 2.69

(metformin treated), 2.74 (nateglinide treated) and 2.49 (glipizide treated). Thus rosiglitazone ($p<0.001$) and glipizide decreased the formation of 3, 4-dehydro-cilostazol in CYP3A4.

Table 4.4 Effect of antidiabetic agents on cilostazol metabolism in CYP3A4 human recombinant enzymes

| S.No. | Group | % CLZ remaining (mean ± SEM) | Difference in mean compared to vehicle (%) | Mean DCLZ formation (µmoles/mg protein/min) |
|---|---|---|---|---|
| 1 | Vehicle | 74.11 ± 0.18 | 23.89 | 2.71 |
| 2 | Rosiglitazone | 84.34 ± 4.79 | 10.24 | 2.24 |
| 3 | Metformin | 76.37 ± 0.82 | 2.26 | 2.69 |
| 4 | Nateglinide | 77.21± 1.23 | 3.18 | 2.74 |
| 5 | Glipizide | 92.44 ± 5.69 | 18.33 | 2.49 |

n=3 in each set

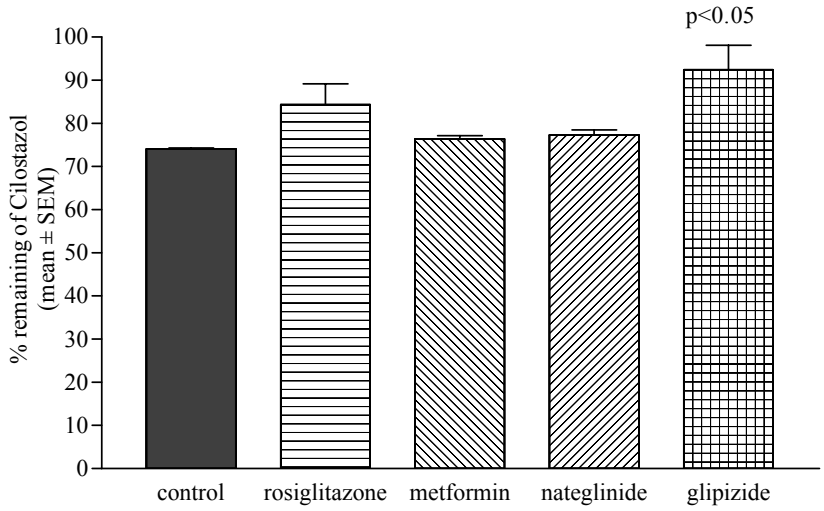



*Figure 4.4 Effect of ADA on cilostazol metabolism in CYP3A4 bactosomes*
n=3 in each set; p values were calculated using student unpaired t-test compared to control group

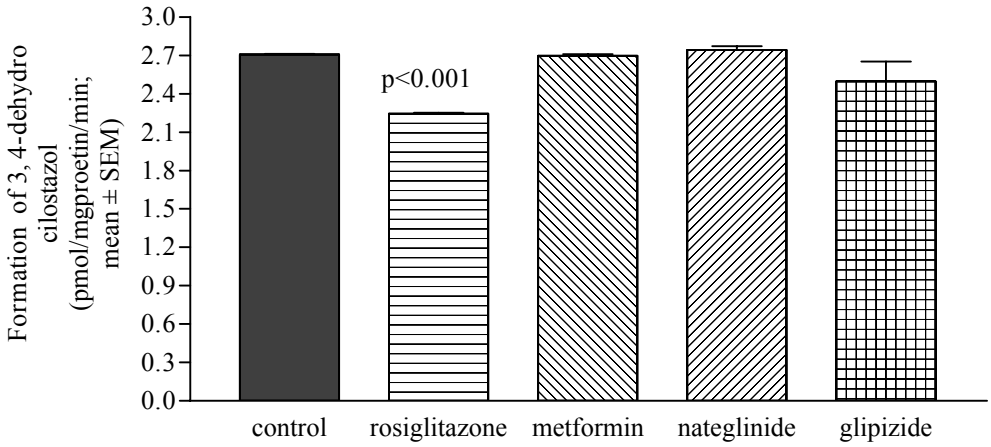



*Figure 4.5 Effect of ADA on 3, 4-dehydro-cilostazol formation in CYP3A4 human recombinant enzymes*

n=3 in each set; p values were calculated using student unpaired t-test compared to control group

### 4.1.4 Effect of antidiabetic agents on cilostazol metabolism in CYP2C19 human recombinant enzymes

The metabolic stability of cilostazol in CYP2C19 bactosomes was not changed based on the disappearance of the molecule. However there was a metabolite peak which was predominantly observed and was attributed to be 4'trans-hydroxy-cilostazol (authentic standard was not available to match with) based on literature. This peak area was not significantly altered in rosiglitazone group compared to that of control group. Thus rosiglitazone has not shown to be inhibited the metabolism of cilostazol in CYP2C19. However, it was formed less in glipizide group compared to that of control group. Thus glipizide might have inhibited the formation of this minor metabolite for cilostazol through CYP2C19 but it need to be investigated further.

### 4.1.5 Effect of antidiabetic agents on pentoxifylline metabolism in pooled human liver microsomes

The in vitro inhibitory effect of ADA on percent remaining (metabolic stability) of the pentoxifylline was studied in pooled human liver microsomes prepared from rats pretreated with ADA and the results are presented in **Table 4.5**.

The metabolic stability of pentoxifylline was changed from 59.49 % (vehicle treated) to 77.62 % (rosiglitazone treated), 61.01 % (metformin treated), 70.18 % (nateglinide treated), 68.12 %

(glipizide treated). Thus rosiglitazone ($p<0.05$), and glipizide ($p<0.05$) significantly increased the metabolic stability of pentoxifylline in pooled human liver microsomes.

Table 4.5 Effect of antidiabetic agents on pentoxifylline metabolism in pooled human liver microsomes

| S.No. | Group | % PTX remaining (mean ± SEM) | Difference in mean compared to vehicle (%) |
|---|---|---|---|
| 1 | Vehicle | 59.49 ± 1.83 | |
| 2 | Rosiglitazone | 77.62 ± 3.96 | 18.13 |
| 3 | Metformin | 61.01± 2.56 | 1.51 |
| 4 | Nateglinide | 70.18± 3.87 | 10.89 |
| 5 | Glipizide | 68.12 ± 0.85 | 8.63 |

n=3 in each set

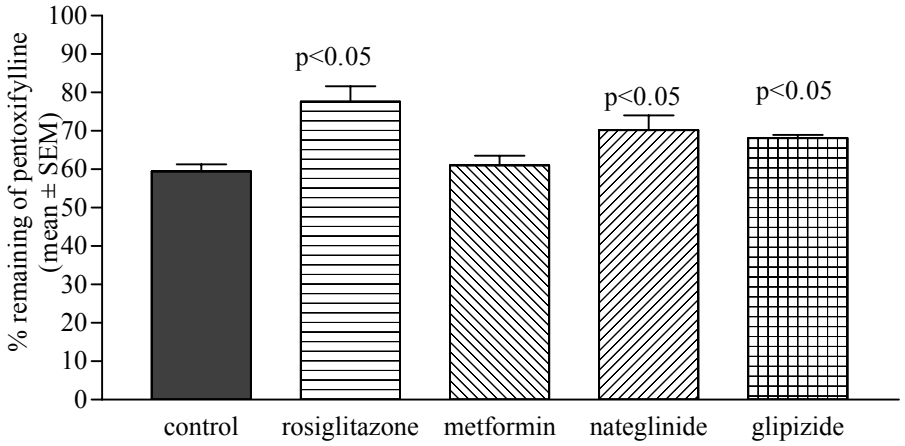



*Figure 4.6 Effect of ADA on pentoxifylline metabolism in human liver microsomes*
n=3 in each set; p values were calculated using student unpaired t-test compared to control group

### 4.1.6 Effect of antiplatelet agents on antidiabetic agent's metabolism in rat liver microsomes

The in vitro inhibitory effect of antiplatelet agents, cilostazol and pentoxifylline, on metabolic stability of the ADA was studied separately in rat liver microsomes prepared from rats pretreated with cilostazol or pentoxifylline and the results are presented below.

The metabolic stability of rosiglitazone was changed from 70.57 % (vehicle treated) to 77.12 % (cilostazol treated), and 78.96 % (pentoxifylline treated) groups. Thus cilostazol ($p<0.05$), and pentoxifylline ($p<0.05$) significantly increased the metabolic stability of rosiglitazone in rat liver microsomes. The metabolic stability of metformin was high and was not altered in presence of

either cilostazol or pentoxifylline treated groups. The metabolic stability of nateglinide was changed from 42.43 % (vehicle treated) to 62.97 % (cilostazol treated), and 13.25 % (pentoxifylline treated) groups. Thus cilostazol ($p<0.01$) incrased and pentoxifylline ($p<0.01$) decreased significantly the metabolic stability of nateglinide in rat liver microsomes. The metabolic stability of glipizide was changed from 77.99 % (vehicle treated) to75.78 % (cilostazol treated), and 65.79 % (pentoxifylline treated) groups. Thus pentoxifylline ($p<0.01$) significantly decreased the metabolic stability of nateglinide in rat liver microsomes.

Table 4.6 Effect of antiplatelet agents on antidiabetic agent's metabolism in rat liver microsomes

| S.No. | Substrate | % substrate remaining (mean ± SEM) in presence of vehicle | % substrate remaining (mean ± SEM) in presence of CLZ | % substrate remaining (mean ± SEM) in presence of PTX |
|---|---|---|---|---|
| 1 | Rosiglitazone | 70.57 ± 0.44 | 77.12 ± 1.47 | 78.96 ± 0.40 |
| 2 | Metformin | 98.28 ± 0.21 | 97.35 ± 0.43 | 97.56 ±0.29 |
| 3 | Nateglinide | 42.43 ± 0.19 | 62.97± 0.65 | 13.25 ± 5.07 |
| 4 | Glipizide | 77.99 ± 0.47 | 75.78 ± 0.82 | 65.79 ± 0.82 |

n=3 in each set

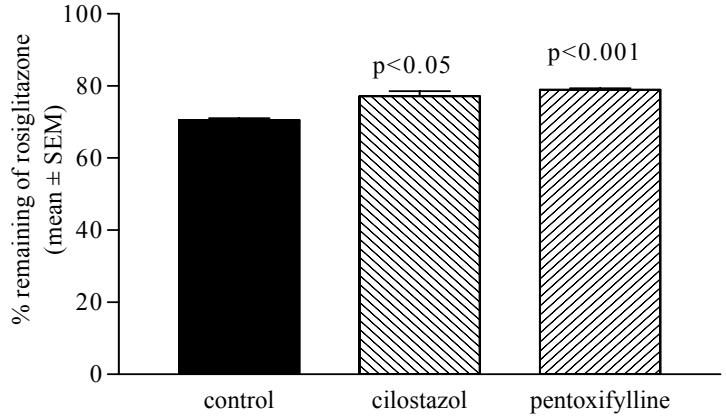



*Figure 4.7 Effect of cilostazol and pentoxifylline on rosiglitazone in rat liver microsomes*
n=3 in each set; p values were calculated using student unpaired t-test compared to control group

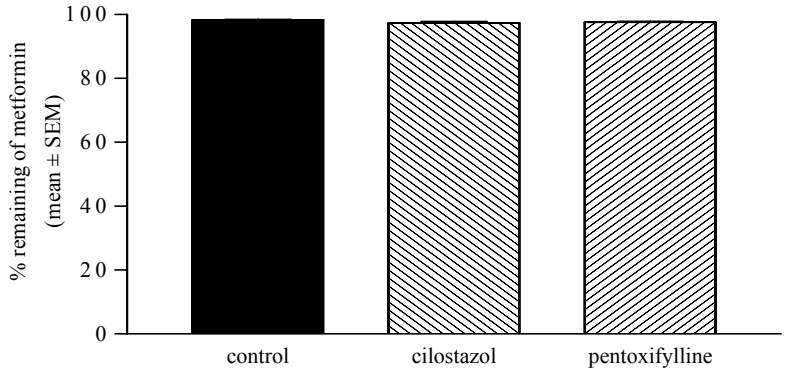



*Figure 4.8 Effect of cilostazol and pentoxifylline on metformin in rat liver microsomes*
n=3 in each set;

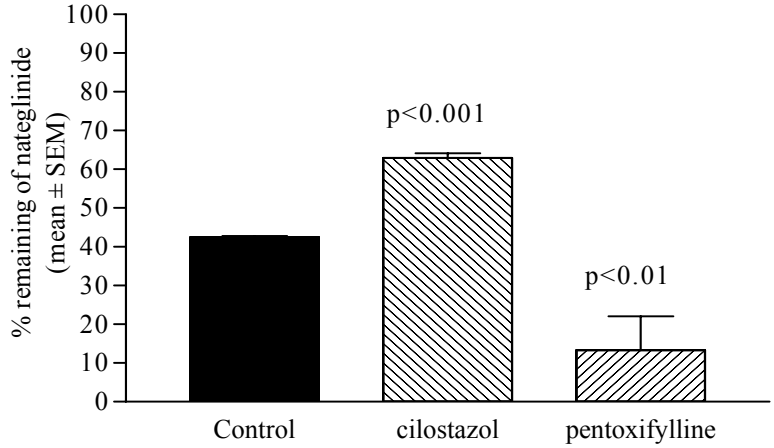



*Figure 4.9 Effect of cilostazol and pentoxifylline on nateglinide in rat liver microsomes*
n=3 in each set; p values were calculated using student unpaired t-test compared to control group

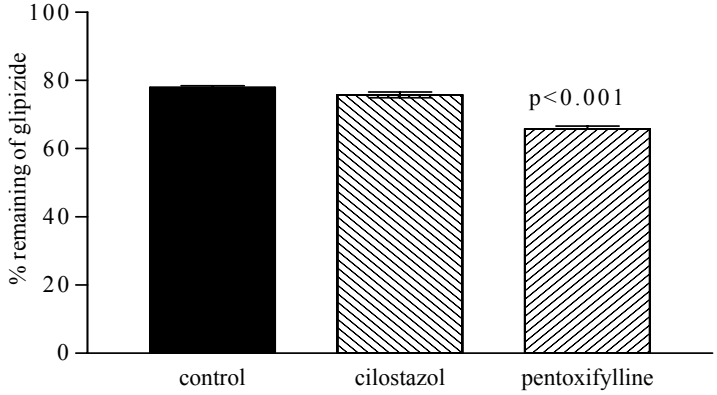



*Figure 4.10 Effect of cilostazol and pentoxifylline on glipizide in rat liver microsomes*
n=3 in each set; p values were calculated using student unpaired t-test compared to control group

### 4.1.7 Effect of antiplatelet agents on antidiabetic agent's metabolism in recombinant human liver microsomes (CYP450)

The in vitro inhibitory effect of antiplatelet agents, cilostazol and pentoxifylline, on metabolic stability of the ADA was studied separately in recombinant human liver microsomes (specific CYP450 bactosomes) prepared from rats pretreated with either cilostazol or pentoxifylline and the results are presented below.

Table 4.7 Effect of antiplatelet agents on antidiabetic agent's metabolism in recombinant human liver microsomes (CYP450)

| S.No. | Substrate | CYP450 enzyme | % substrate remaining (mean ± SEM) in presence of vehicle | % substrate remaining (mean ± SEM) in presence of CLZ | % substrate remaining (mean ± SEM) in presence of PTX |
|---|---|---|---|---|---|
| 1 | Rosiglitazone | CYP2C8 | 42.34 ± 0.96 | 32.68 ± 2.53 | 50.88 ± 0.94 |
| 2 | Nateglinide | CYP2C9 | 48.41 ± 0.35 | 72.99 ± 1.59 | 45.05 ± 3.33 |
| 3 | Glipizide | CYP2C9 | 69.59 ± 0.43 | 69.14 ± 1.52 | 70.53 ± 2.24 |

n=3 in each set

Pentoxifylline has significantly increased the metabolic stability of rosiglitazone in CYP2C8 enzymes where as cilostazol did not. Cilostazol had increased the metabolic stability of nateglinide in CYP2C9 enzymes but pentoxifylline did not the show it. Neither cilostazol nor pentoxifylline has shown effect on glipizide in CYP2C9 enzymes.

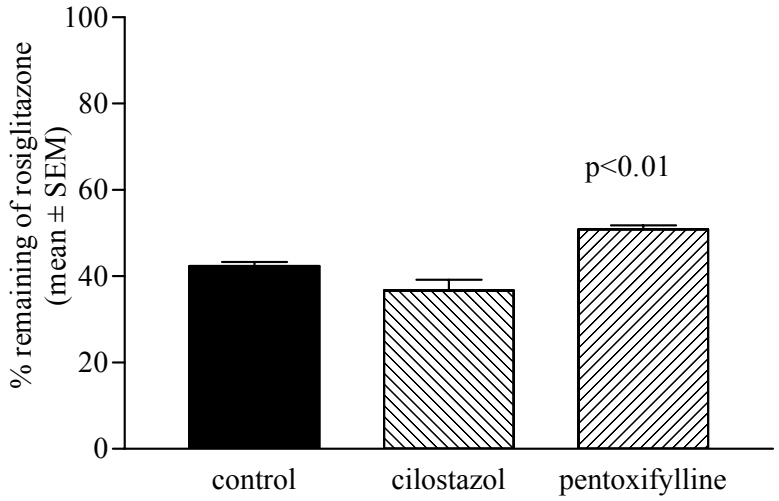



*Figure 4.11 Effect of cilostazol and pentoxifylline on rosiglitazone metabolism in CYP2C8*
n=3 in each set; p values were calculated using student unpaired t-test compared to control group

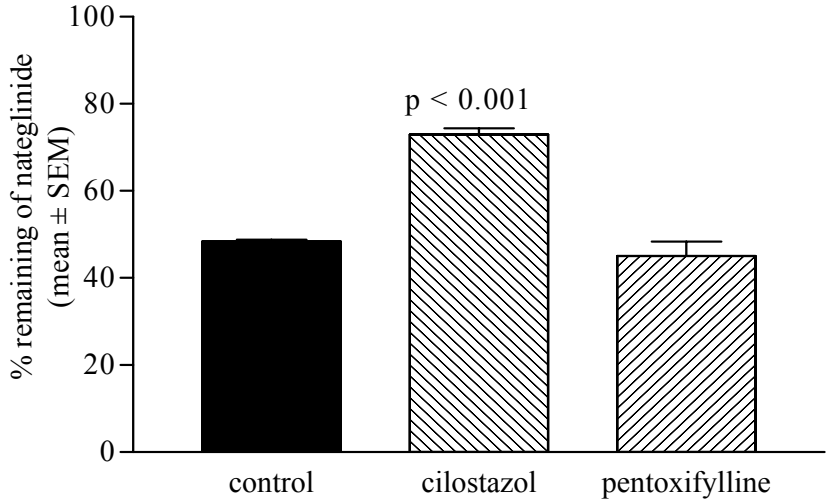



*Figure 4.12 Effect of cilostazol and pentoxifylline on nateglinide metabolism in CYP2C9*
n=3 in each set; p values were calculated using student unpaired t-test compared to control group

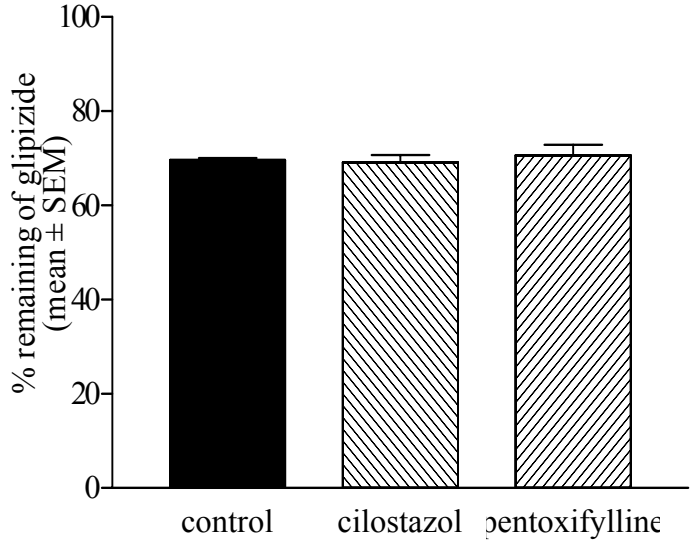



*Figure 4.13 Effect of cilostazol and pentoxifylline on glipizide metabolism in CYP2C9 human recombinant enzymes*
n=3 in each set

## 4.2 In vitro plasma protein binding studies

Effects of antidiabetic agents on plasma protein binding (PPB) of antiplatelet agents

The percent protein binding of cilostazol alone was > 98% and in presence of ADA was also > 99% reflecting the no role of any displacement potential for the ADAs studied on cilostazol in rat plasma. The SEM values for all samples were observed within 2%.

Table 4.8 Effect of antidiabetic agents on *in-vitro* protein binding of cilostazol in rat plasma

| S.No. | Group | % Mean PPB of CLZ |
|---|---|---|
| **1** | Vehicle | >98 |
| 2 | Rosiglitazone | >98 |
| 3 | Metformin | >99 |
| 4 | Nateglinide | >99 |
| 5 | Glipizide | >99 |

n=3 in each set

Table 4.9 Effect of antidiabetic agents on *in-vitro* protein binding of pentoxifylline in rat plasma

| S.No. | Group | % Mean PPB of PTX |
|---|---|---|
| 1 | Vehicle | 37 |
| 2 | Rosiglitazone | 38 |
| 3 | Metformin | 45 |
| 4 | Nateglinide | 38 |
| 5 | Glipizide | 41 |

n=3 in each set

Table 4.10 Effects of antiplatelet agents on plasma protein binding of antidiabetic agents

| S.No. | Group | % Mean PPB of RSG | % Mean PPB of MET | % Mean PPB of NTG | % Mean PPB of GLP |
|---|---|---|---|---|---|
| 1 | Vehicle | >90 | 5 | >99 | >98 |
| 2 | Cilostazol | >99 | 12 | >99 | >97 |
| 3 | Pentoxifylline | >93 | 18 | >99 | >99 |

n=3 in each set

## 4.3 Analytical Methods

The following analytes were estimated in the biological samples during the course of work using High Performance Liquid Chromatography (HPLC) coupled with either UV detector or Tandem Mass Spectrometry (MS/MS)

- Cilostazol (CLZ)
- 3,4-dehydro-cilostazol (DCLZ)
- Pentoxifylline (PTX)
- Rosiglitazone (RSG)
- Metformin (MET)
- Nateglinide (NTG)
- Glipizide (GLP)

### 4.3.1 Analysis of Cilostazol, 3, 4-dehydro-cilostazol and Rosiglitazone rat plasma

The method developed for the simultaneous estimation of CLZ, DCLZ and RSG in wistar rat plasma using PIO as internal standard was validated before study sample analysis. The validation results are summarized in **Table 4.11** and were found to be within acceptable criterial as per ICH guidelines.

### 4.3.2 Analysis of Cilostazol, 3, 4-dehydro-cilostazol and Nateglinide rat plasma

The method developed for the simultaneous estimation of CLZ, DCLZ and NTG in wistar rat plasma using Repaglinide as internal standard was validated before study sample analysis. The validation results are summarized in **Table 4.12** and were found to be within acceptable criterial as per ICH guidelines.

### 4.3.3 Analysis of Cilostazol, 3, 4-dehydro-cilostazol and Glipizide in rat plasma

The method developed for the simultaneous estimation of CLZ, DCLZ and GLP in wistar rat plasma using glimepiride as internal standard was validated before study sample analysis. The validation results are summarized in **Table 4.14** and were found to be within acceptable criterial as per ICH guidelines.

Table 4.11 Validation summary of CLZ, DCLZ and RSG in wistar rat plasma

| | Validation Parameters | Results CLZ | DCLZ | RSG | IS | Acceptance Criteria |
|---|---|---|---|---|---|---|
| Linearity | Range | 25-2500 ng/ml | 20-2000 ng/ml | 25-2500 ng/ml | NA | NA |
| | Goodness of fit | ≥0.999 | ≥0.999 | ≥0.999 | NA | ≥ 0.98 |
| Sensitivity (LOQ) | Limit of quantification | 25 ng/ml | 20 ng/ml | 25 ng/ml | NA | NA |
| Accuracy | Within batch (LLOQ, L, M & H - QCs) | 94.6% to 102.7% | 101.2% to 107.1% | 94.4% to 105.1% | NA | 85-115 % except LOQ QC & ≤ 80-120% for LOQ QC |
| | Between batch (LLOQ, L, M & H - QCs) | 95.5% to 105.0% | 100.3% to 105.2% | 93.1% to 106.1% | NA | |
| Precision (RSD) | Within batch (LLOQ, L, M & H - QCs) | 3.7% to 6.6% | 4.3% to 6.6% | 2.9% to 8.4% | NA | ≤ 15 % except LOQ QC & ≤ 20% for LOQ QC |
| | Between batch (LLOQ, L, M & H - QCs) | 1.2% to3.0% | 1.1% to 5.9% | 1.0% to 4.2% | NA | |
| Recovery | Accuracy (L, M & H- QCs) | 91.9 %, 94.5% & 96.7% | 98.5 %, 97.0% & 97.0% | 94.7 %,102.8%& 104.7% | 84.50% | NA |
| | Precision (L, M & H- QCs) | 3.7%, 6.9% & 2.0% | 0.7%, 1.0% & 1.0% | 3.0%, 3.3% & 4.6% | 2.50% | % CV ≤ 20 % |
| Dilution integrity | Diluted concentration ¼ | | | | | |
| | Accuracy | 97.50% | 98.40% | 98.10% | NA | 80 - 120% |
| | Precision | 2.80% | 1.20% | 1.60% | NA | ≤ 20% |
| | Diluted concentration 1/8 | | | | | |
| | Accuracy | 97.20% | 98.30% | 97.80% | NA | 80 to 120% |
| | Precision | 1.80% | 0.80% | 1.50% | NA | ≤ 20% |
| Stability | Bench top at 25$^0$C (L & H - QCs) - 6.0 hours | 92.5 % & 97.9% | 85.6 % & 93.1% | 93.0 % & 99.8% | NA | 85 -115% |
| | Autosamplerat 10$^0$C (L & H- QCs) - 24.0 hous | 95.0 % & 99.4% | 94.0 % & 97.3% | 94.9 % & 96.2% | NA | 85 -115% |
| | Freeze thaw (L & H- QCs) - after 3$^{rd}$ Cycle | 96.4 % & 98.9% | 86.9 % & 96.3% | 94.2 % & 98.3% | NA | 85 -115% |
| | Long term at -70$^0$C (L & H- QCs) -15 days | 92.2 % & 99.1% | 107.1 % & 97.0% | 92.3 % & 95.2% | NA | 85 -115% |

Table 4.12 Validation summary of CLZ, DCLZ and NTG in wistar rat plasma

| | Validation Parameters | Results CLZ | DCLZ | NTG | IS | Acceptance Criteria |
|---|---|---|---|---|---|---|
| Linearity | Range | 21-2100 ng/ml | 21-2100 ng/ml | 21-2100 ng/ml | NA | NA |
| | Goodness of fit | ≥0.992 | ≥0.995 | ≥0.996 | NA | ≥ 0.98 |
| Sensitivity (LOQ) | Limit of quantification | 21 ng/ml | 21 ng/ml | 21 ng/ml | NA | NA |
| Accuracy | Within batch (LLOQ, L, M & H - QCs) | 98.7% to 107.8% | 98.5% to 107.7% | 99.5% to 110.6% | NA | 85-115 % except LOQ QC & ≤ 80-120% for LOQ QC |
| | Between batch (LLOQ, L, M & H - QCs) | 97.9% to 108.2% | 99.2% to 108.4% | 100.1% to 111.1% | NA | |
| Precision (RSD) | Within batch (LLOQ, L, M & H - QCs) | 6.3% to 12.2% | 5.9% to11.3% | 6.5% to 11.8% | NA | ≤ 15 % except LOQ QC & ≤ 20% for LOQ QC |
| | Between batch (LLOQ, L, M & H - QCs) | 6.4% to 11.7% | 5.5% to 10.4% | 6.1% to 11.9% | NA | |
| Recovery | Accuracy (L, M & H- QCs) | 83.2 %,91.0% & 85.9% | 82.5 %, 81.2% & 88.1% | 79.5 %, 86.5% & 87.6% | 83.2% | NA |
| | Precision (L, M & H- QCs) | 4.4%,5.3% & 6.9% | 3.1%, 4.1% & 7.5% | 8.1%,7.1% & 5.0% | 5.6% | % CV ≤ 20 % |
| Dilution integrity | Diluted concentration ¼ | | | | | |
| | Accuracy | 99.8% | 99.5% | 100.1% | NA | 80 - 120% |
| | Precision | 0.5% | 0.6% | 0.6% | NA | ≤ 20% |
| | Diluted concentration 1/8 | | | | | |
| | Accuracy | 100.0% | 99.9% | 99.9% | NA | 80 to 120% |
| | Precision | 1.1% | 0.4% | 0.6% | NA | ≤ 20% |
| Stability | Bench top at 25$^0$C (L & H - QCs) - 6.0 hours | 103.1 % & 99.3 % | 102.0 % & 99.0 % | 98.9 % & 99.3% | NA | 85 -115% |
| | Autosamplerat 10$^0$C (L & H- QCs) - 24.0 hous | 107.8 % &103.5 % | 98.9 % & 108.0 % | 103.7 % & 106.3 % | NA | 85 -115% |
| | Freeze thaw (L & H- QCs) - after 3$^{rd}$ Cycle | 102.4 % & 98.7 % | 100.6 % & 99.1 % | 97.1 % & 99.8 % | NA | 85 -115% |
| | Long term at -70$^0$C (L & H- QCs) -30 days | 97.5 % & 98.0 % | 07.1 % & 97.2 % | 99.6 % & 98.7 % | NA | 85 -115% |

Table 4.13 Validation summary of CLZ, DCLZ and GLP in wistar rat plasma

| | Validation Parameters | Results CLZ | DCLZ | GLP | IS | Acceptance Criteria |
|---|---|---|---|---|---|---|
| Linearity | Range | 24.3-1980 ng/ml | 22.90-1870 ng/ml | 25.4-2080 ng/ml | NA | NA |
| | Goodness of fit | ≥0.9967 | 0.997 | 0.997 | NA | ≥ 0.98 |
| Sensitivity (LOQ) | Limit of quantification | 24.3 ng/ml | 22.9 ng/ml | 25.4 ng/ml | NA | NA |
| Accuracy | Within batch (LLOQ, L, M & H - QCs) | 90.9% to 111.0% | 95.5-108.2 | 93.7-106.3 | NA | 85-115 % except LOQ QC & ≤ 80-120% for LOQ QC |
| | Between batch (LLOQ, L, M & H - QCs) | 97.0% to 108.7% | 99.6-106.1 | 97.4-101.9 | NA | |
| Precision (RSD) | Within batch (LLOQ, L, M & H - QCs) | 1.6% to 10.7% | 3.4-14.6 | 1.1-8.9 | NA | ≤ 15 % except LOQ QC & ≤ 20% for LOQ QC |
| | Between batch (LLOQ, L, M & H - QCs) | 0.4% to2.7% | 2.1-4.3 | 1.3-3.5 | NA | |
| Recovery | Accuracy (L, M & H- QCs) | 80.1%,80.15%,80.61% | 76.01%,78.00%,76.23% | 87.31%,91.72%,91.81% | 70.3% | NA |
| | Precision (L, M & H- QCs) | 1.0%, 1.0% & 0.7% | 1.8%,3.6%,8.0% | 4.4%,2.4%,4.0% | 5.6% | % CV ≤ 20 % |
| Dilution integrity | Diluted concentration ¼ | 1980ng/ml | 1870 ng/ml | 2680 ng/ml | | |
| | Accuracy | 98.00% | 106.20% | 90.50% | NA | 80 - 120% |
| | Precision | 3.00% | 2.70% | 2.60% | NA | ≤ 20% |
| | Diluted concentration 1/8 | 990 ng/ml | 935 ng/ml | 1040 ng/ml | | |
| | Accuracy | 98.10% | 95.40% | 91.80% | NA | 80 to 120% |
| | Precision | 3.20% | 1.70% | 4.20% | NA | ≤ 20% |
| Stability | Bench top at $25^0$C (L & H - QCs) - 6.0 hours | 105.3 % & 100.7% | 98.6% & 102.4% | 97.6% & 96.6% | NA | 85 -115% |
| | Autosamplerat $10^0$C (L & H- QCs) - 24.0 hous | 106.5 % & 101.8% | 97.8% & 97.3% | 99.7% & 99.2% | NA | 85 -115% |
| | Freeze thaw (L & H- QCs) - after 3rd Cycle | 111.3 % & 108.3% | 96.2% & 98.7% | 104.9% & 99.3% | NA | 85 -115% |
| | Long term at -$70^0$C (L & H- QCs) -15 days | 92.8 % & 92.0% | 103.6% & 93.8% | 99.3% & 111.2% | NA | 85 -115% |

### 4.3.5 Analysis of Metformin in rat plasma

The method developed for the estimation of Metformin in wistar rat plasma using nicornadil as internal standard was validated before study sample analysis. The validation results are summarized in **Table 4.15** and were found to be within acceptable criterial as per ICH guidelines.

Table 4.14 Validation summary of Metformin in Wistar rat plasma

| | Validation Parameters | Results MET | IS | Acceptance Criteria |
|---|---|---|---|---|
| Linearity | Range | 51.36 - 5257.45ng/ml | NA | NA |
| | Goodness of fit | | NA | ≥ 0.98 |
| Sensitivity (LOQ) | Limit of quantification | 51.36 ng/mL | NA | NA |
| Accuracy | Within batch (LLOQ, L, M & H - QCs) | 0.8% to 5.7% | NA | 85-115 % except LOQ QC & ≤ 80-120% for LOQ QC |
| | Between batch (LLOQ, L, M & H - QCs) | 1.2% to 4.8% | NA | |
| Precision (RSD) | Within batch (LLOQ, L, M & H - QCs) | 93.5% to 102.7% | NA | ≤ 15 % except LOQ QC & ≤ 20% for LOQ QC |
| | Between batch (LLOQ, L, M & H - QCs) | 95.1% to 99.8% | NA | |
| Recovery | Accuracy (L, M & H- QCs) | 100.43%, 99.15% & 92.24%. | 93.7% | NA |
| | Precision (L, M & H- QCs) | 1.2%, 0.2% & 0.3% | 1.5% | % CV ≤ 20 % |
| Dilution integrity | Diluted concentration $_{1/4}$ | | | |
| | Accuracy | 101.6% | NA | 80 - 120% |
| | Precision | 0.7% | NA | ≤ 20% |
| | Diluted concentration $_{1/8}$ | | | |
| | Accuracy | 110.7% | NA | 80 - 120% |
| | Precision | 0.6% | NA | ≤ 20% |
| Stability | Bench top at 25$^0$C (L & H - QCs) - 6.0 hours | 91.2 %& 101.3 % | NA | 85 -115% |
| | Autosamplerat 10$^0$C (L & H- QCs) - 24.0 hous | 92.4 % & 100.7 % | NA | 85 -115% |
| | Freeze thaw (L & H- QCs) - after 3$^{rd}$ Cycle | 101.3 % & 98.9 % | NA | 85 -115% |
| | Long term at -70$^0$C (L & H- QCs) -28 days | 93.1 % & 98.7% | NA | 85 -115% |

### 4.3.6 Analysis of Pentoxifylline in rat plasma

The method developed for the estimation of Pentoxifylline in wistar rat plasma using linezolid as internal standard was validated before study sample analysis. The validation results are summarized in table no- 4.16 and were found to be within acceptable criterial as per ICH guidelines.

Table 4.15 Validation summary of Pentoxifylline in wistar rat plasma

| | Validation Parameters | Results PTX | Results IS | Acceptance Criteria |
|---|---|---|---|---|
| Linearity | Range | 25.0-2034.0 ng/ml | NA | NA |
| | Goodness of fit | ≥0.992 | NA | ≥ 0.98 |
| Sensitivity (LOQ) | Limit of quantification | 25.0 ng/ml | NA | NA |
| Accuracy | Within batch (LLOQ, L, M & H - QCs) | 92.3% to 110.7% | NA | 85-115 % except LOQ QC & ≤ 80-120% for LOQ QC |
| | Between batch (LLOQ, L, M & H - QCs) | 98.8% to 104.7% | NA | |
| Precision (RSD) | Within batch (LLOQ, L, M & H - QCs) | 1.6% to 4.2% | NA | ≤ 15 % except LOQ QC & ≤ 20% for LOQ QC |
| | Between batch (LLOQ, L, M & H - QCs) | 2.0% to 8.5% | NA | |
| Recovery | Accuracy (L, M & H- QCs) | 94.35 %, 97.0% & 101.43% | 97.29% | NA |
| | Precision (L, M & H- QCs) | 3.9%, 3.5% & 4.9% | 3.20% | % CV ≤ 20 % |
| Dilution integrity | Diluted concentration 1/4 | | | |
| | Accuracy | 103.6% | NA | 80 - 120% |
| | Precision | 9.1% | NA | ≤ 20% |
| | Diluted concentration 1/8 | | | |
| | Accuracy | 100.1% | NA | 80 - 120% |
| | Precision | 2.0% | NA | ≤ 20% |
| Stability | Bench top at 25$^0$C (L & H - QCs) - 6.0 hours | 102.9 %,98.2% | NA | 85 -115% |
| | Autosamplerat 10$^0$C (L & H- QCs) - 24.0 hous | 102.6% & 94.8% | NA | 85 -115% |
| | Freeze thaw (L & H- QCs) - after 3$^{rd}$ Cycle | 99.2% & 94.4% | NA | 85 -115% |
| | Long term at -70$^0$C (L & H- QCs) -28 days | 93.3% & 88.8% | NA | 85 -115% |

## 4.4 In vivo drug-drug interaction studies

### 4.4.1 Effect of Antidiabetic agents on cilostazol pharmacokinetics in rats

The effect of antidiabetic agents on single dose cilostazol pharmacokinetics was investigated by pretreatment with either single dose or multiple doses (seven daily doses) of respective antidiabetic agents. These studies were monitored for both cilostazol (CLZ) and its major metabolite 3,4-dehydro-cilostazol (DCLZ) concentrations. The summary results from four antidiabetic agents investigated are presented in **Table 4.16, 4.17, 4.18, and 4.19**. The individual study results are explained in the subsequent sections.

Table 4.16 Single dose effect of ADA on pharmacokinetic parameters of cilostazol by simultaneous administration of ADA and cilostazol in Wistar rats

| Parameters of CLZ | Units | Vehicle treated (mean ± S.D) | Rosiglitazone treated (mean ± S.D) | Metformin treated (mean ± S.D) | Nateglinide treated (mean ± S.D) | Glipizide treated (mean ± S.D) |
|---|---|---|---|---|---|---|
| $C_{max}$ | µg/ml | 1.52 ± 0.36 | 1.93 ± 0.52 | 1.10 ± 0.30 | 1.59 ± 0.39 | 1.90 ± 0.51 |
| $AUC_{0-24}$ | µg.hr/ml | 3.61 ± 0.59 | 4.41 ± 1.18 | 4.55 ± 2.01*** | 4.58 ± 0.69* | 5.35 ± 1.11** |
| $AUC_{0-inf}$ | µg.hr/ml | 4.12 ± 0.68 | 4.78 ± 1.26 | 5.34 ± 1.63*** | 5.12 ± 0.891* | 5.84 ± 1.16* |
| $T_{max}$ | hr | 0.17 ± 0.04 | 0.25 ± 0.32 | 0.25 ± 0.33 | 0.25 ± 0.72 | 0.25 ± 0.72 |
| $t_{½}$ | hr | 3.10 ± 1.14 | 2.54 ± 2.65 | 4.52 ± 2.78 | 3.26 ± 1.08 | 3.02 ± 1.47 |
| $K_{el}$ | $hr^{-1}$ | 0.223 ± 0.056 | 0.27 ± 0.2 | 0.156 ± 0.067 | 0.213 ± 0.053 | 0.230 ± 0.066 |
| $V_{z\_F}$ | L/kg | 12.04 ± 5.0 | 11.87 ± 6.92 | 15.82 ± 9.26 | 9.91 ± 2.92 | 8.50 ± 3.80 |
| $Cl_{z\_F}$ | L/hr/kg | 2.48 ± 0.42 | 2.26 ± 0.78 | 1.99 ± 0.48 | 2.00 ± 0.33 | 1.76 ± 0.31 |

n=6 in each treatment group; *p<0.05; **p<0.01; ***p<0.001 using student's unpaired t-test compared to vehicle

Table 4.17 Single dose effect of ADA on pharmacokinetic parameters of 3,4-dehydro-cilostazol by simultaneous administration of ADA and cilostazol in Wistar rats

| Parameters of DCLZ | Units | Vehicle treated (mean ± S.D) | Rosiglitazone treated (mean ± S.D) | Metformin treated (mean ± S.D) | Nateglinide treated (mean ± S.D) | Glipizide treated (mean ± S.D) |
|---|---|---|---|---|---|---|
| $C_{max}$ | µg/ml | 0.85 ± 0.25 | 0.55 ± 0.07* | 0.83 ± 0.30 | 0.93 ± 0.16 | 1.39 ± 0.30* |
| $AUC_{0-24}$ | µg.hr/ml | 3.16 ± 0.59 | 1.98 ± 0.45** | 3.93 ± 0.69* | 4.31 ± 0.69* | 4.69 ± 0.65** |
| $AUC_{0-inf}$ | µg.hr/ml | 3.70 ± 0.59 | 2.12 ± 0.56*** | 6.37 ± 2.76* | 4.76 ± 0.72* | 5.37 ± 0.99* |
| $T_{max}$ | hr | 0.5 ± 0.25 | 1.00 ± 0.20* | 1.00 ± 0.27 | 2.00 ± 1.02 | 2.00 ± 0.41 |
| $t_{1/2}$ | hr | 3.09 ± 5.04 | 1.90 ± 1.52 | 5.71 ± 4.41 | 3.29 ± 1.66 | 3.94 ± 1.95 |
| $K_{el}$ | $hr^{-1}$ | 0.224 ± 0.126 | 0.364 ± 0.24 | 0.120 ± 0.074 | 0.211 ± 0.076 | 0.176 ± 0.065 |

n=6 in each treatment group;*p<0.05; **p<0.01; ***p<0.001 using student's unpaired t-test compared to vehicle

Table 4.18 Multiple doses effect of ADA on pharmacokinetic parameters of cilostazol by simultaneous administration of ADA and cilostazol in Wistar rats

| Parameters of CLZ | Units | Vehicle treated (mean ± S.D) | Rosiglitazone treated (mean ± S.D) | Metformin treated (mean ± S.D) | Nateglinide treated (mean ± S.D) | Glipizide treated (mean ± S.D) |
|---|---|---|---|---|---|---|
| $C_{max}$ | µg/ml | 1.52 ± 0.36 | 2.80 ± 0.64** | 1.73 ± 0.53 | 1.41 ± 0.31 | 5.63 ± 2.87** |
| $AUC_{0-24}$ | µg.hr/ml | 3.61 ± 0.59 | 6.07 ± 1.25** | 8.59 ± 1.62*** | 4.90 ± 0.92** | 17.29 ± 14.52* |
| $AUC_{0-inf}$ | µg.hr/ml | 4.12 ± 0.68 | 6.43 ± 1.44** | 10.93 ± 3.89*** | 5.56 ± 0.04* | 18.04 ± 14.74* |
| $T_{max}$ | hr | 0.17 ± 0.04 | 0.21 ± 0.04 | 0.21 ± 0.04 | 0.25 ± 0.11 | 0.25 ± 0.32 |
| $t_{½}$ | hr | 3.10 ± 1.14 | 2.46 ± 1.29 | 7.64 ± 5.64 | 4.25 ± 3.34 | 2.64 ± 0.90 |
| $K_{el}$ | hr -1 | 0.223 ± 0.056 | 0.33 ± 0.13 | 0.091 ± 0.004 | 0.149 ± 0.066 | 0.263 ± 0.070 |
| $V_{z\_F}$ | L/kg | 12.04 ± 5.0 | 5.32 ± 1.72 | 12.05 ± 2.83 | 15.05 ± 7.70 | 3.35 ± 1.97 |
| $Cl_{z\_F}$ | L/hr/kg | 2.48 ± 0.42 | 1.63 ± 0.39 | 1.01 ± 0.34 | 1.85 ± 0.32 | 0.79 ± 0.39 |

n=6 in each treatment group;*p<0.05; **p<0.01; ***p<0.001 using student's unpaired t-test compared to vehicle

Table 4.19 Multiple doses effect of ADA on pharmacokinetic parameters of 3,4-dehydro-cilostazol by simultaneous administration of ADA and cilostazol in Wistar rats

| Parameter of DCLZ | Units | Vehicle treated (mean ± S.D) | Rosiglitazone treated (mean ± S.D) | Metformin treated (mean ± S.D) | Nateglinide treated (mean ± S.D) | Glipizide treated (mean ± S.D) |
|---|---|---|---|---|---|---|
| $C_{max}$ | µg/ml | 0.85 ± 0.25 | 0.50 ± 0.09** | 0.72 ± 0.14 | 1.20 ± 0.38 | 1.91 ± 0.34*** |
| $AUC_{0-24}$ | µg.hr/ml | 3.16 ± 0.59 | 1.62 ± 0.30*** | 6.96 ± 2.30* | 5.59 ± 1.54* | 11.09 ± 4.87** |
| $AUC_{0-inf}$ | µg.hr/ml | 3.70 ± 0.59 | 1.76 ± 0.24*** | 10.30 ± 5.20* | 6.32 ± 1.76* | 13.03 ± 6.47* |
| $T_{max}$ | hr | 0.5 ± 0.25 | 1.00 ± 0.20* | 2.00 ± 2.84 | 1.00 ± 0.55 | 2.00 ± 0.41 |
| $t_{1/2}$ | hr | 3.09 ± 5.04 | 2.14 ± 0.50 | 8.23 ± 5.43 | 5.48 ± 1.77 | 3.89 ± 1.50 |
| $K_{el}$ | hr -1 | 0.224 ± 0.126 | 0.324 ± 0.074 | 0.084 ± 0.038 | 0.127 ± 0.032 | 0.178 ± 0.058 |

n=6 in each treatment group; *p<0.05; **p<0.01; ***p<0.001 using student's unpaired t-test compared to vehicle

#### 4.4.1.1 Effect of rosiglitazone on cilostazol and its metabolite pharmacokinetic parameters by simultaneous administration in rats (Study I)

Cilostazol and 3, 4-dehydro-cilostazol were undetectable in the predose samples in all rats, indicating that rosiglitazone does not interfere with the cilostazol assay. **Figure 4.14 & 4.15** shows the plasma concentration-time curve of cilostazol and 3, 4-dehydro-cilostazol in rats receiving cilostazol alone or concurrent treatment with rosiglitazone. Visual inspection of the plasma concentration-time curve for cilostazol showed that the mean plasma concentrations of cilostazol tended to be increased and while those of 3, 4-dehydro-cilostazol tended to be decreased.

The estimated pharmacokinetic parameters of cilostazol in the absence and presence of rosiglitazone are summarized in **Table 4.20**. The maximum plasma concentration reached ($C_{max}$) for cilostazol in presence of Vehicle, single dose rosiglitazone and multiple doses of rosiglitazone are in the ratio of 1.00: 1.27:1.84 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.22: 1.68. Though the maximum absorption and systemic exposure of cilostazol were slightly increased more than 20 % these were not significantly affected in presence of single dose rosiglitazone. And these parameters were significantly increased ($C_{max}$ by 84% and $AUC_{0-24}$ by 68%, $p<0.01$) in presence of multiple doses of rosiglitazone. The time

to reach maximum absorption ($T_{max}$) was not effected in presence of either single dose or multiple doses of rosiglitazone.

Table 4.20 Effect of rosiglitazone on single dose pharmacokinetic parameters of cilostazol by simultaneous administration of cilostazol and rosiglitazone in Wistar rats

| **Parameters of cilostazol** | **Units** | **Vehicle treated** (mean ± S.D) | **Rosiglitazone treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 1.52 ± 0.36 | 1.93 ± 0.52 | 2.80 ± 0.64** |
| **$AUC_{0-24}$** | µg.hr/ml | 3.61 ± 0.59 | 4.41 ± 1.18 | 6.07 ± 1.25** |
| **$AUC_{0-inf}$** | µg.hr/ml | 4.12 ± 0.68 | 4.78 ± 1.26 | 6.43 ± 1.44** |
| **$T_{max}$** | hr | 0.17 ± 0.04 | 0.25 ± 0.32 | 0.21 ± 0.04 |
| **$t_{½}$** | hr | 3.10 ± 1.14 | 2.54 ± 2.65 | 2.46 ± 1.29 |
| **$K_{el}$** | $hr^{-1}$ | 0.223 ± 0.056 | 0.27 ± 0.2 | 0.33 ± 0.13 |
| **$V_{z\_F}$** | L/kg | 12.04 ± 5.0 | 11.87 ± 6.92 | 5.32 ± 1.72 |
| **$Cl_{Z\_F}$** | L/hr/kg | 2.48 ± 0.42 | 2.26 ± 0.78 | 1.63 ± 0.39 |

N=6 in each treatment group; **p<0.01 using student's unpaired t-test compared to vehicle

The estimated pharmacokinetic parameters of 3, 4-dehydro-cilostazol in the absence and presence of rosiglitazone are summarized in **Table 4.21**. The maximum plasma concentration reached ($C_{max}$) for 3, 4-dehydro-cilostazol after administration of cilostazol in presence of vehicle, single dose rosiglitazone and multiple doses of rosiglitazone are in the ratio of 1.00: 0.65:0.58 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 0.62: 0.51. The maximum formation of 3, 4-dehydro-cilostazol by administration of cilostazol and its systemic exposure was not affected in presence of single dose rosiglitazone. And these parameters were very significantly decreased ($C_{max}$ by 35% and $AUC_{0-24}$ by 38%, $p<0.05$) and also in presence of multiple doses of rosiglitazone ($C_{max}$ by 42% and AUC 0-24 by 49%, $p<0.01$). The time to reach maximum formation of metabolite ($T_{max}$) was slightly delayed by 0.5 hr in presence of either single dose or multiple doses of rosiglitazone. The extent of formation of metabolite by administration of cilostazol was greatly reduced in presence of either single dose or multiple dose administration of rosiglitazone (42% and 61%).

Table 4.21 Effect of rosiglitazone on single dose pharmacokinetic parameters of 3, 4-dehydro-cilostazol by simultaneous administration of cilostazol and rosiglitazone in Wistar rats

| **Parameters of 3, 4-dehydro-cilostazol** | **Units** | **Vehicle treated** (mean ± S.D) | **Rosiglitazone treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 0.85 ± 0.25 | 0.55 ± 0.07* | 0.50 ± 0.09** |
| **AU $_{0-24}$** | µg.hr/ml | 3.16 ± 0.59 | 1.98 ± 0.45** | 1.62 ± 0.30*** |
| **AUC $_{0-inf}$** | µg.hr/ml | 3.70 ± 0.59 | 2.12 ± 0.56*** | 1.76 ± 0.24*** |
| **$T_{max}$** | hr | 0.5 ± 0.25 | 1.00 ± 0.20* | 1.00 ± 0.20* |
| **$t_{½}$** | Hr | 3.09 ± 5.04 | 1.90 ± 1.52 | 2.14 ± 0.50 |
| **$K_{el}$** | $hr^{-1}$ | 0.224 ± 0.126 | 0.364 ± 0.24 | 0.324 ± 0.074 |

N=6 in each treatment group; *p<0.05; **p<0.01; ***p<0.001 using student's unpaired t-test compared to vehicle

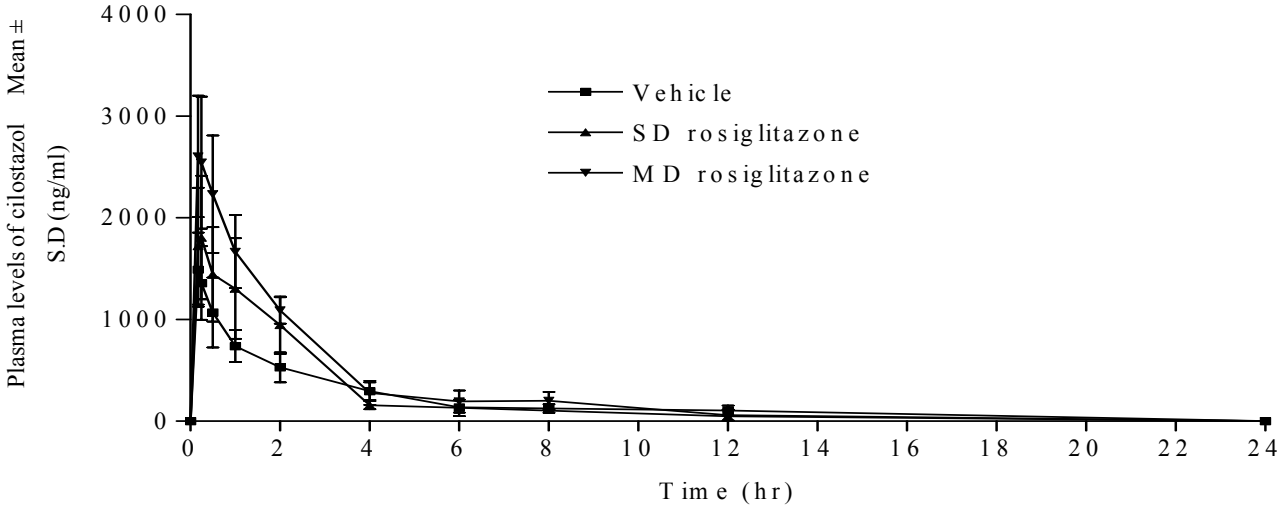



*Figure 4.14 Effect of rosiglitazone on plasma concentration-time plot of cilostazol in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses

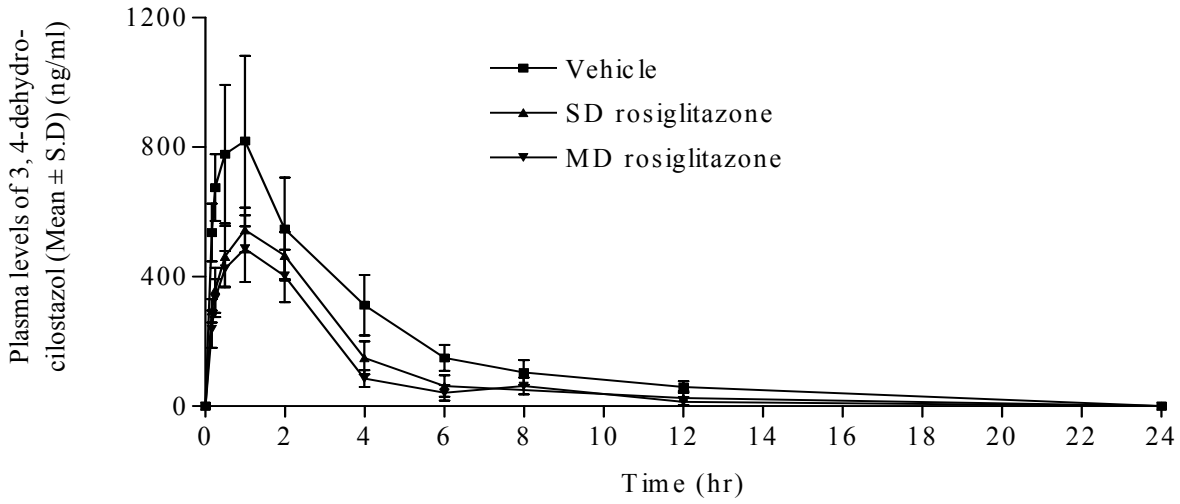



*Figure 4.15 Effect of rosiglitazone on plasma concentration-time curve of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses

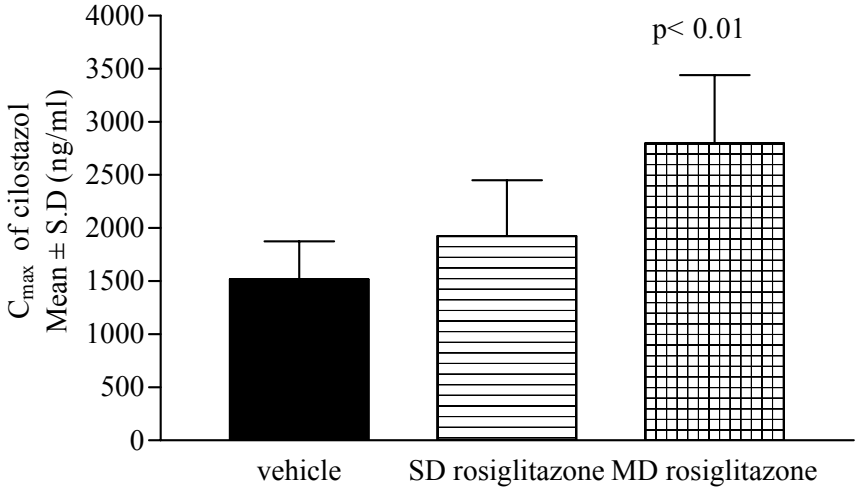



*Figure 4.16 Effect of rosiglitazone on plasma $C_{max}$ of single dose cilostazol in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

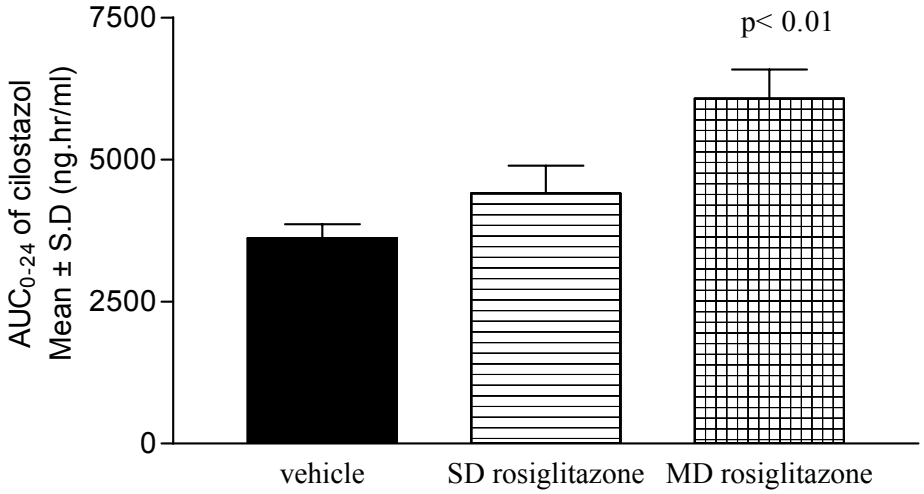



*Figure 4.17 Effect of rosiglitazone on plasma AUC $_{0\text{-}24}$ of single dose cilostazol in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

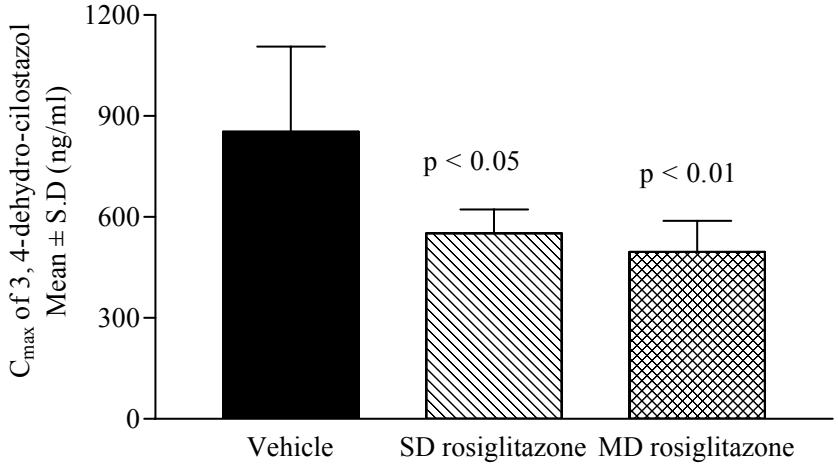



*Figure 4.18 Effect of rosiglitazone on plasma $C_{max}$ of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

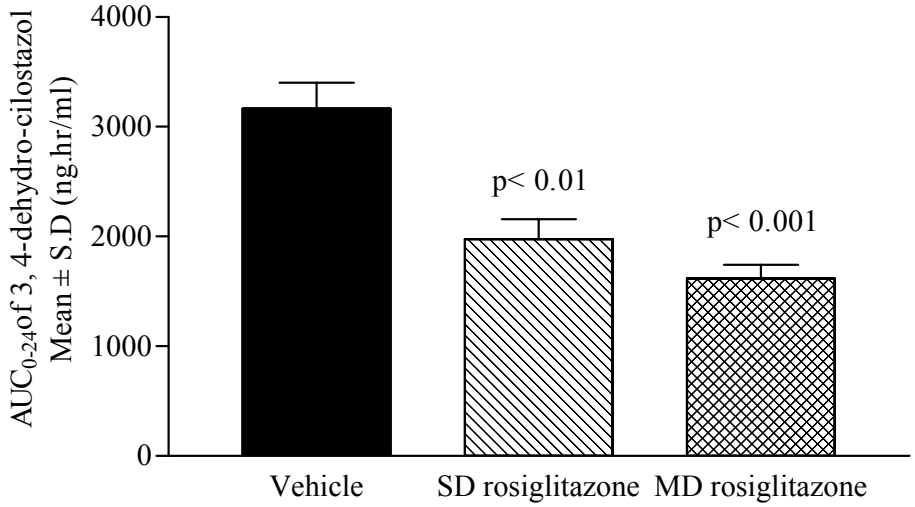



*Figure 4.19 Effect of rosiglitazone on plasma $AUC_{0-24}$ of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

#### 4.4.1.2 Effect of metformin on cilostazol and its metabolite pharmacokinetic parameters by simultaneous administration in rats (Study II)

Cilostazol and 3, 4-dehydro-cilostazol were undetectable in the predose samples in all rats, indicating that metformin does not interfere with the cilostazol assay. **Figure 4.20 & Figure 4.21** shows the plasma concentration-time curve of cilostazol and 3, 4-dehydro-cilostazol in rats receiving cilostazol alone or concurrent treatment with metformin. Visual inspection of the plasma concentration-time curve for cilostazol showed that the mean plasma concentrations of cilostazol tended to be slightly increased and where as of 3, 4-dehydro-cilostazol levels tended to be decreased significantly.

The estimated pharmacokinetic parameters of cilostazol in the absence and presence of metformin are summarized in **Table 4.22**. The maximum plasma concentration reached ($C_{max}$) for cilostazol in presence of Vehicle, single dose metformin and multiple doses of metformin are in the ratio of 1.00: 1.06:1.14 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.12: 2.38. Though the maximum absorption of cilostazol was not changed, the systemic exposure of cilostazol was slightly increased more than 12 % but this was not significantly affected in presence of single dose metformin. And this parameter was significantly increased

($AUC_{0-24}$ by 138%, $p<0.0001$) in presence of multiple doses of metformin. The time to reach maximum absorption ($T_{max}$) was not effected in presence of either single dose or multiple doses of metformin. The half-life was slightly increased from 3.10 to 7.60 hr for cilostazol in presence of multiple doses of metformin and is statistically significant ($p<0.05$).

Table 4.22 Effect of metformin on single dose pharmacokinetic parameters of cilostazol by simultaneous administration of cilostazol and metformin in Wistar rats

| **Parameters of cilostazol** | **Units** | **Vehicle treated** (mean ± S.D) | **Metformin treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 1.52 ± 0.36 | 1.10 ± 0.30 | 1.73 ± 0.53 |
| **$AUC_{0-24}$** | µg.hr/ml | 3.61 ± 0.59 | 4.55 ± 2.01*** | 8.59 ± 1.62*** |
| **$AUC_{0-inf}$** | µg.hr/ml | 4.12 ± 0.68 | 5.34 ± 1.63*** | 10.93 ± 3.89*** |
| **$T_{max}$** | hr | 0.17 ± 0.04 | 0.25 ± 0.33 | 0.21 ± 0.04 |
| **$t_{1/2}$** | hr | 3.10 ± 1.14 | 4.52 ± 2.78 | 7.64 ± 5.64 |
| **$K_{el}$** | $hr^{-1}$ | 0.223 ± 0.056 | 0.156 ± 0.067 | 0.091 ± 0.004 |
| **$V_{z\_F}$** | L/kg | 12.04 ± 5.0 | 15.82 ± 9.26 | 12.05 ± 2.83 |
| **$Cl_{z\_F}$** | L/hr/kg | 2.48 ± 0.42 | 1.99 ± 0.48 | 1.01 ± 0.34 |

n=6 in each treatment group; ***$p<0.0001$ compared to vehicle using student's unpaired t-test;

The estimated pharmacokinetic parameters of active metabolite of cilostazol in the absence and presence of metformin are summarized in **Table 4.23**. The maximum plasma concentration reached ($C_{max}$) for 3, 4-dehydro-cilostazol after administration of cilostazol in presence of vehicle, single dose metformin and multiple doses of metformin are in the ratio of 1.00: 0.96:0.84 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.33: 2.20. The maximum formation of 3, 4-dehydro-cilostazol by administration of cilostazol and its systemic exposure was reduced but not significantly affected in presence of single dose and multiple dose metformin. Though the maximum formation of 3, 4-dehydro-cilostazol was not changed, the systemic exposure of cilostazol was slightly increased more than 33 % and this was significantly ($p<0.05$) affected in presence of single dose metformin. And this parameter was also significantly increased ($AUC_{0-24}$ by 120%, $p<0.01$) in presence of multiple doses of metformin. The time to reach maximum absorption ($T_{max}$) was not significantly effected in presence of either single dose or multiple doses of metformin The time to reach maximum formation of metabolite

($T_{max}$) was slightly delayed by 0.5 hr and 1.5 hr in presence of either single dose or multiple doses of metformin. Though it was not statistically significant, the half-life was greatly increased from 3.09 to 8.23 hr for this metabolite after administration of cilostazol in presence of multiple doses of metformin. The extent of formation of metabolite by administration of cilostazol was not affected in presence multiple dose administration of metformin.

Table 4.23 Effect of metformin on single dose pharmacokinetic parameters of 3, 4-dehydro-cilostazol by simultaneous administration of cilostazol and metformin in Wistar rats

| **Parameters of 3, 4-dehydro-cilostazol** | **Units** | **Vehicle treated** (mean ± S.D) | **Metformin treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 0.85 ± 0.25 | 0.83 ± 0.30 | 0.72 ± 0.14 |
| **$AUC_{0-24}$** | µg.hr/ml | 3.16 ± 0.59 | 3.93 ± 0.69* | 6.96 ± 2.30* |
| **$AUC_{0-inf}$** | µg.hr/ml | 3.70 ± 0.59 | 6.37 ± 2.76* | 10.30 ± 5.20* |
| **$T_{max}$** | hr | 0.5 ± 0.25 | 1.00 ± 0.27 | 2.00 ± 2.84 |
| **$t_{½}$** | hr | 3.09 ± 5.04 | 5.71 ± 4.41 | 8.23 ± 5.43 |
| **$K_{el}$** | hr -1 | 0.224 ± 0.126 | 0.120 ± 0.074 | 0.084 ± 0.038 |

n=6 in each treatment group; *p<0.05 compared to vehicle using student's unpaired t-test;

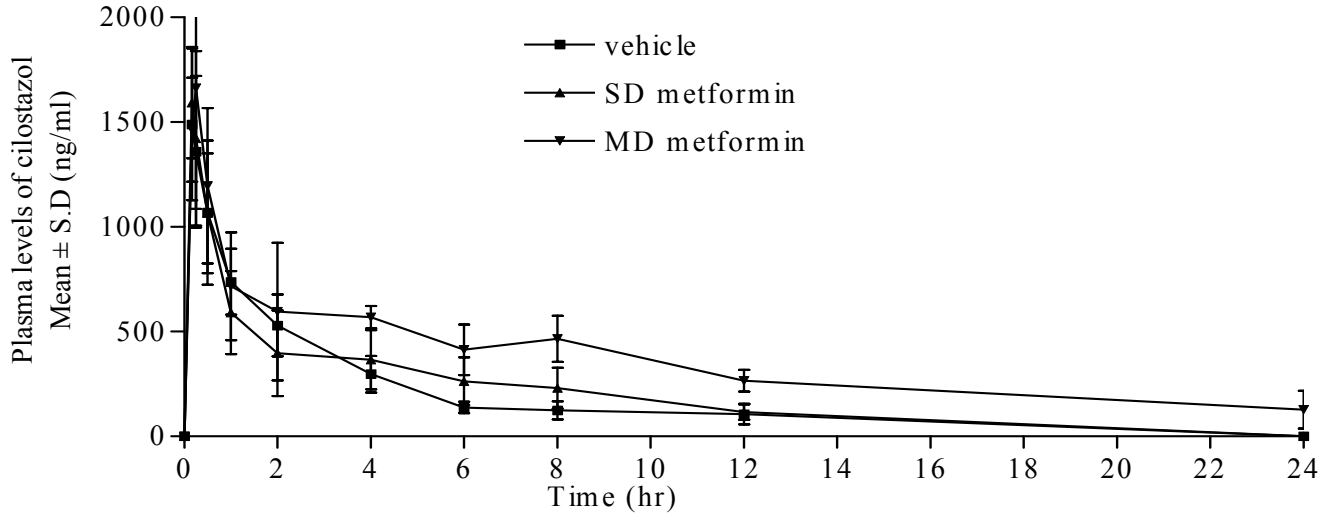



*Figure 4.20 Effect of metformin on plasma concentration-rime plot of cilostazol in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses

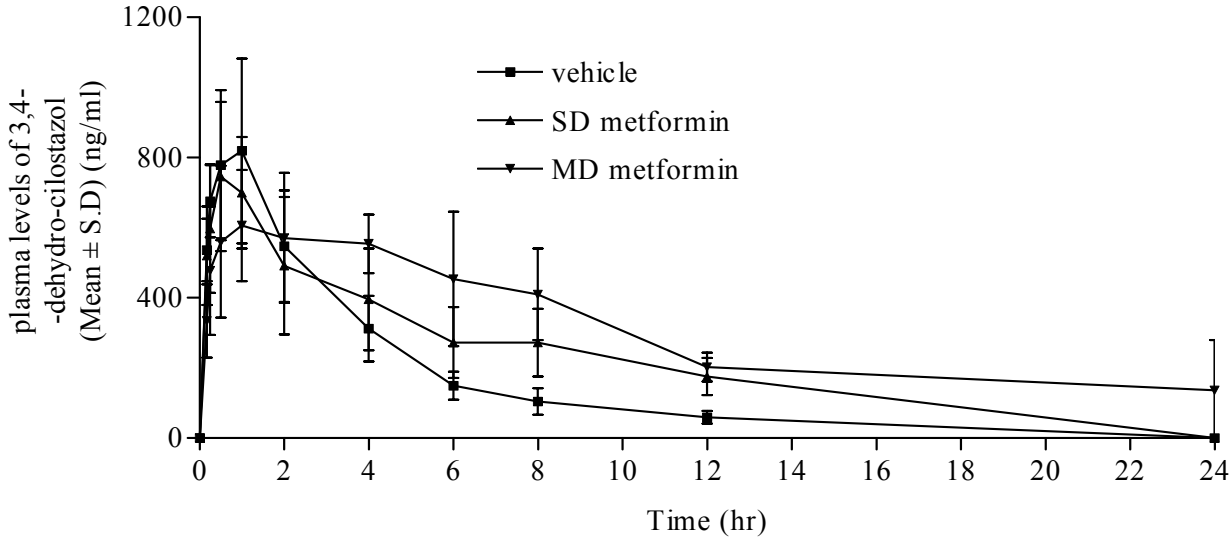



*Figure 4.21 Effect of metformin on plasma concentration-time curve of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses

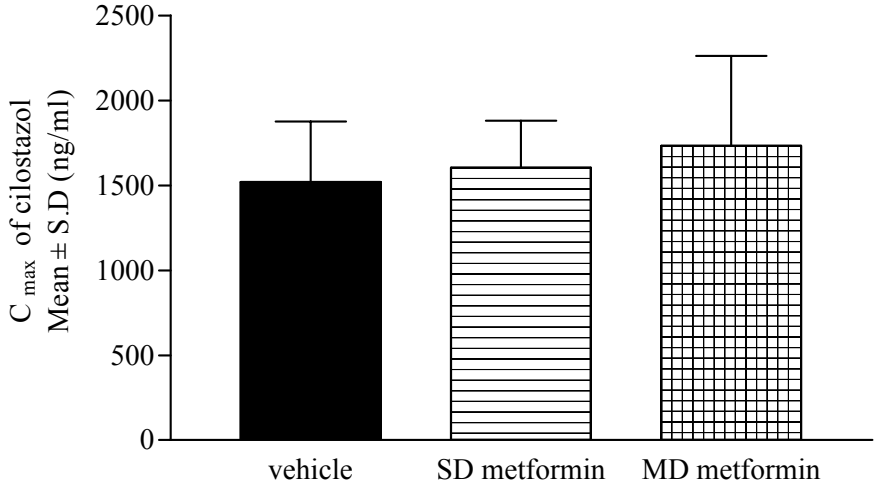



*Figure 4.22 Effect of metformin on plasma $C_{max}$ of single dose cilostazol in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses;

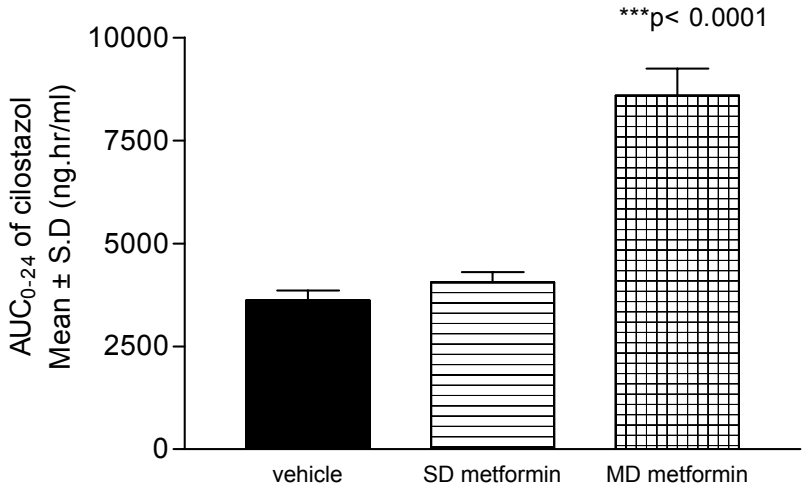



*Figure 4.23 Effect of metformin on plasma $AUC_{0-24}$ of single dose cilostazol in Wistar rats*

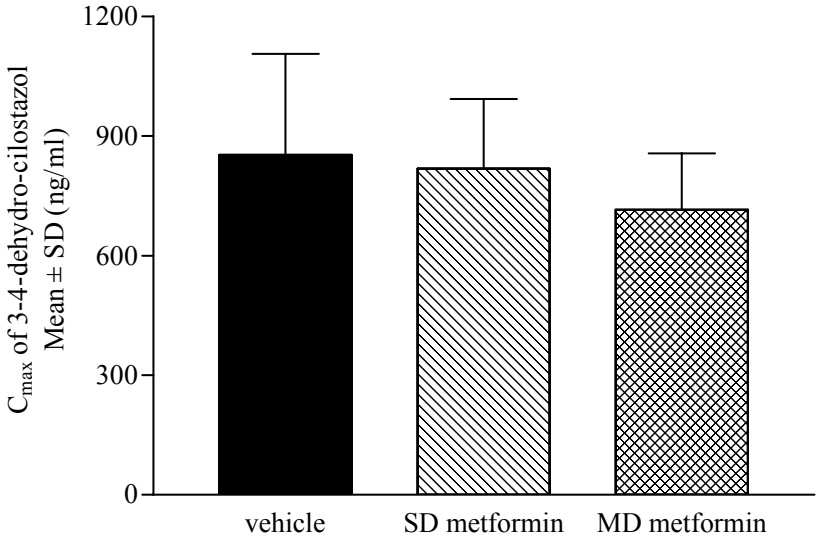



*Figure 4.24 Effect of metformin on plasma $C_{max}$ of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses;

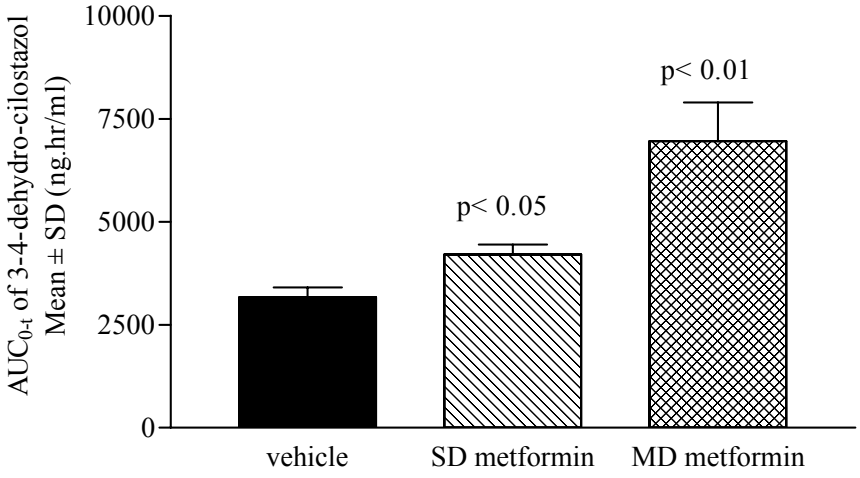



*Figure 4.25 Effect of metformin on plasma $AUC_{0-24}$ of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

#### 4.4.1.3 Effect of nateglinide on cilostazol and its metabolite pharmacokinetic parameters by simultaneous administration in rats (Study III)

Cilostazol and 3, 4-dehydro-cilostazol were undetectable in the predose samples in all rats, indicating that nateglinide does not interfere with the cilostazol assay. **Figure 4.26 & Figure 4.27** shows the plasma concentration-time curve of cilostazol and 3, 4-dehydro-cilostazol in rats receiving cilostazol alone or concurrent treatment with nateglinide. Visual inspection of the plasma concentration-time curve for cilostazol showed that the mean plasma concentrations of cilostazol tended to be slightly increased and where as of 3, 4-dehydro-cilostazol levels tended to be increased significantly. However, no statistically significant differences in the mean plasma concentrations of either cilostazol or its active metabolite were found between the vehicle and nateglinide co administered groups at any point of time.

The estimated pharmacokinetic parameters of cilostazol in the absence and presence of nateglinide are summarized in **Table 4.24**. The maximum plasma concentration reached ($C_{max}$) for cilostazol in presence of Vehicle, single dose nateglinide and multiple doses of nateglinide are in the ratio of 1.00: 1.09:0.93 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.27: 1.36. Though the maximum absorption of cilostazol was not changed, the systemic exposure of cilostazol was slightly increased more than 27 % and this was significantly ($p<0.05$)

affected in presence of single dose nateglinide. And this parameter was also significantly increased ($AUC_{0-24}$ by 36%, $p<0.01$) in presence of multiple doses of nateglinide. The time to reach maximum absorption ($T_{max}$) was not effected in presence of either single dose or multiple doses of nateglinide. The half-life was slightly increased from 3.10 to 4.25 hr for cilostazol in presence of multiple doses of nateglinide but it was not statistically significant.

Table 4.24 Effect of nateglinide on single dose pharmacokinetic parameters of cilostazol by simultaneous administration of cilostazol and nateglinide in Wistar rats

| **Parameters of cilostazol** | **Units** | **Vehicle treated** (mean ± S.D) | **Nateglinide treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 1.52 ± 0.36 | 1.59 ± 0.39 | 1.41 ± 0.31 |
| **$AUC_{0-24}$** | µg.hr/ml | 3.61 ± 0.59 | 4.58 ± 0.69* | 4.90 ± 0.92** |
| **$AUC_{0-inf}$** | µg.hr/ml | 4.12 ± 0.68 | 5.12 ± 0.891* | 5.56 ± 0.04* |
| **$T_{max}$** | hr | 0.17 ± 0.04 | 0.25 ± 0.72 | 0.25 ± 0.11 |
| **$t_{1/2}$** | hr | 3.10 ± 1.14 | 3.26 ± 1.08 | 4.25 ± 3.34 |
| **$K_{el}$** | hr -1 | 0.223 ± 0.056 | 0.213 ± 0.053 | 0.149 ± 0.066 |
| **$V_{z\_F}$** | L/kg | 12.04 ± 5.0 | 9.91 ± 2.92 | 15.05 ± 7.70 |
| **$Cl_{Z\_F}$** | L/hr/kg | 2.48 ± 0.42 | 2.00 ± 0.33 | 1.85 ± 0.32 |

n=6 in each treatment group; *p<0.05; **p<0.01 compared to vehicle using student's unpaired t-test;

Table 4.25 Effect of nateglinide on single dose pharmacokinetic parameters of 3, 4-dehydro-cilostazol by simultaneous administration of cilostazol and nateglinide in Wistar rats

| **Parameters of 3, 4-dehydro-cilostazol** | **Units** | **Vehicle treated** (mean ± S.D) | **Nateglinide treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 0.85 ± 0.25 | 0.93 ± 0.16 | 1.20 ± 0.38 |
| **$AUC_{0-24}$** | µg.hr/ml | 3.16 ± 0.59 | 4.31 ± 0.69* | 5.59 ± 1.54* |
| **$AUC_{0-inf}$** | µg.hr/ml | 3.70 ± 0.59 | 4.76 ± 0.72* | 6.32 ± 1.76* |
| **$T_{max}$** | hr | 0.5 ± 0.25 | 2.00 ± 1.02 | 1.00 ± 0.55 |
| **$t_{1/2}$** | hr | 3.09 ± 5.04 | 3.29 ± 1.66 | 5.48 ± 1.77 |
| **$K_{el}$** | hr -1 | 0.224 ± 0.126 | 0.211 ± 0.076 | 0.127 ± 0.032 |

n=6 in each treatment group; *p<0.05 compared to vehicle using student's unpaired t-test

The estimated pharmacokinetic parameters of active metabolite of cilostazol in the absence and presence of nateglinide are summarized in **Table 4.25**. The maximum plasma concentration reached ($C_{max}$) for 3, 4-dehydro-cilostazol after administration of cilostazol in presence of vehicle, single dose nateglinide and multiple doses of nateglinide are in the ratio of 1.00: 1.09:1.40 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.36: 1.77. The maximum formation of 3, 4-dehydro-cilostazol by administration of cilostazol was not affected in presence of single dose nateglinide. Though the maximum formation of 3, 4-dehydro-cilostazol was not changed, the systemic exposure of cilostazol was slightly increased more than 36 % and this was significantly ($p<0.05$) affected in presence of single dose nateglinide. And this parameter was also significantly increased ($AUC_{0-24}$ by 77%, $p<0.01$) in presence of multiple doses of nateglinide. The time to reach maximum absorption ($T_{max}$) was not effected in presence of either single dose or multiple doses of nateglinide The time to reach maximum formation of metabolite ($T_{max}$) was slightly delayed by 0.5 hr in presence of either single dose or multiple doses of nateglinide. Though it was not statistically significant, the half-life was greatly increased from 3.09 to 5.48 hr for this metabolite after administration of cilostazol in presence of multiple doses of nateglinide. The extent of formation of metabolite (AUC of metabolite/AUC of parent drug) by administration of cilostazol was slightly increased in presence multiple dose administration of nateglinide (26%).

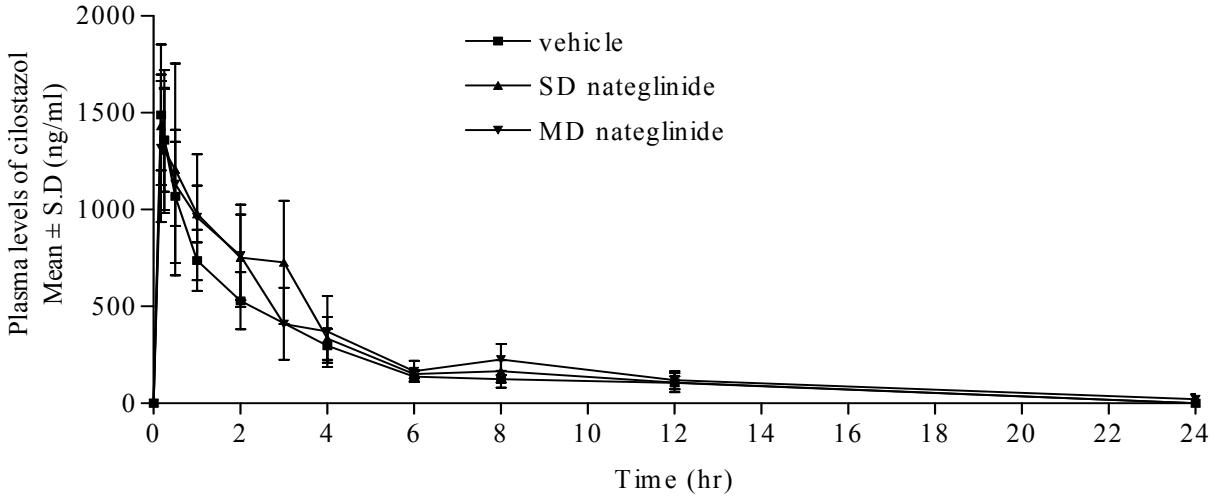



*Figure 4.26 Effect of nateglinide on plasma concentration-time plot of cilostazol in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses

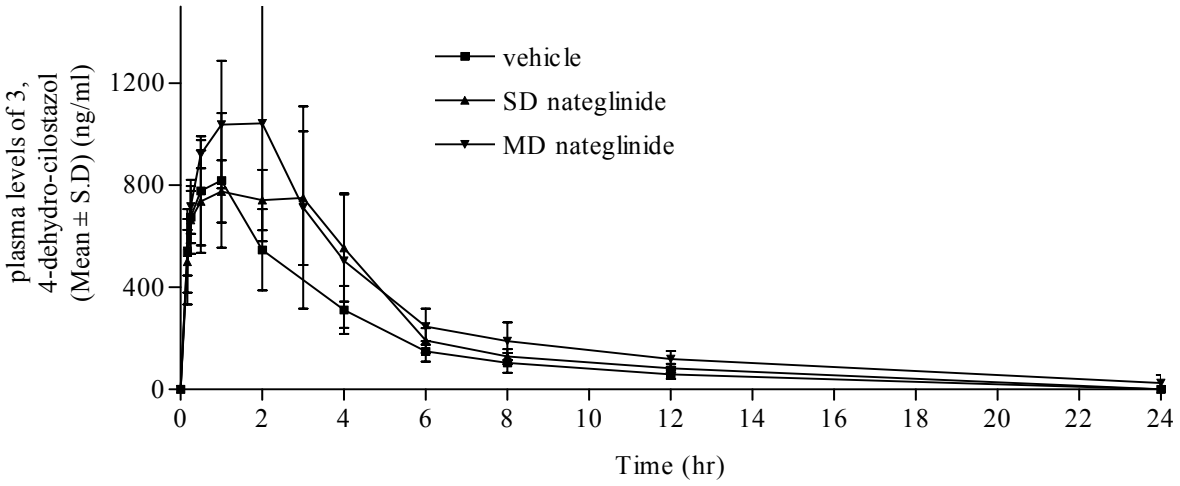



Figure 4.27 *Effect of nateglinide on plasma concentration-time curve of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses

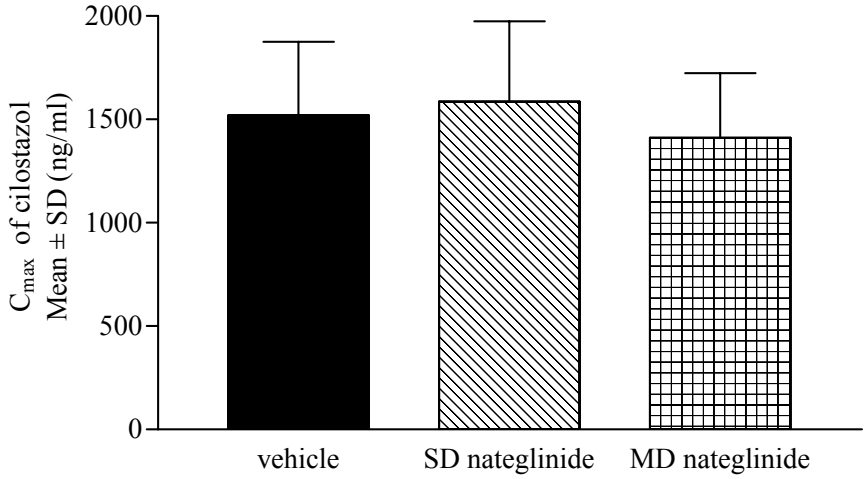



*Figure 4.28 Effect of nateglinide on plasma $C_{max}$ of single dose cilostazol in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses;

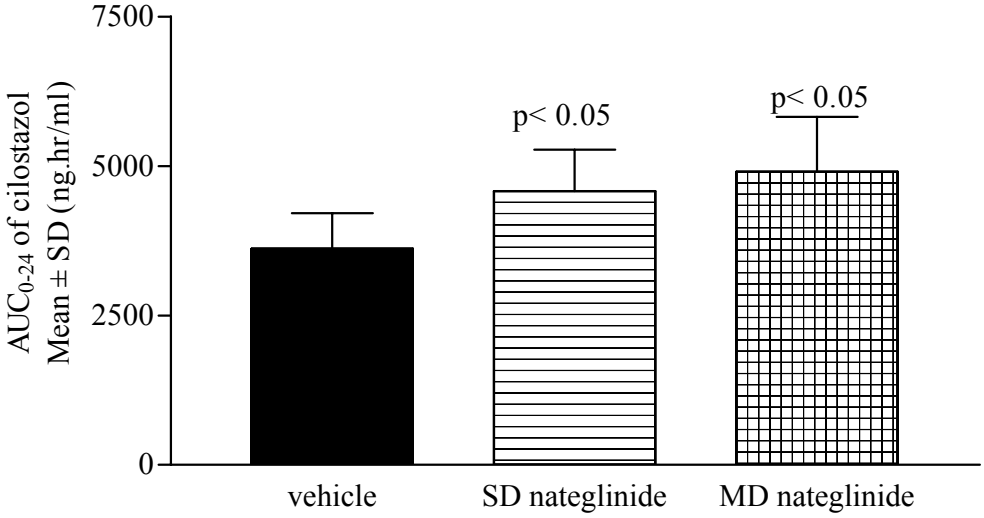



*Figure 4.29 Effect of nateglinide on plasma $AUC_{0-24}$ of single dose cilostazol in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

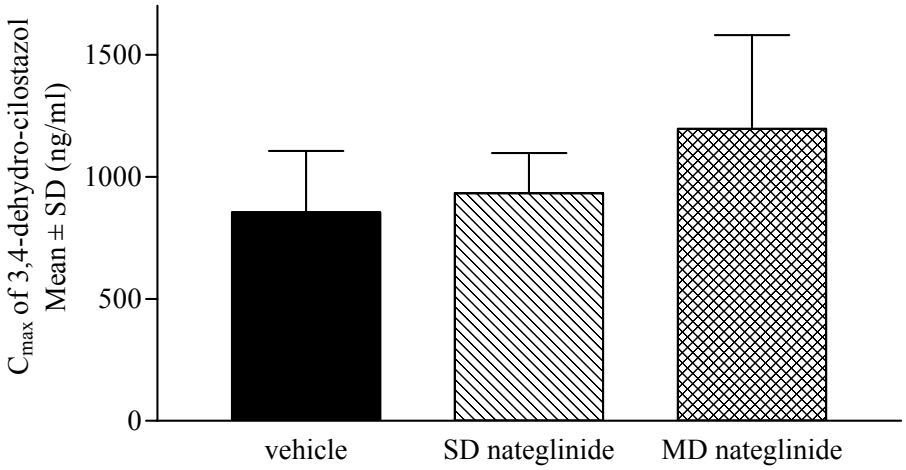



*Figure 4.30 Effect of nateglinide on plasma $C_{max}$ of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses;

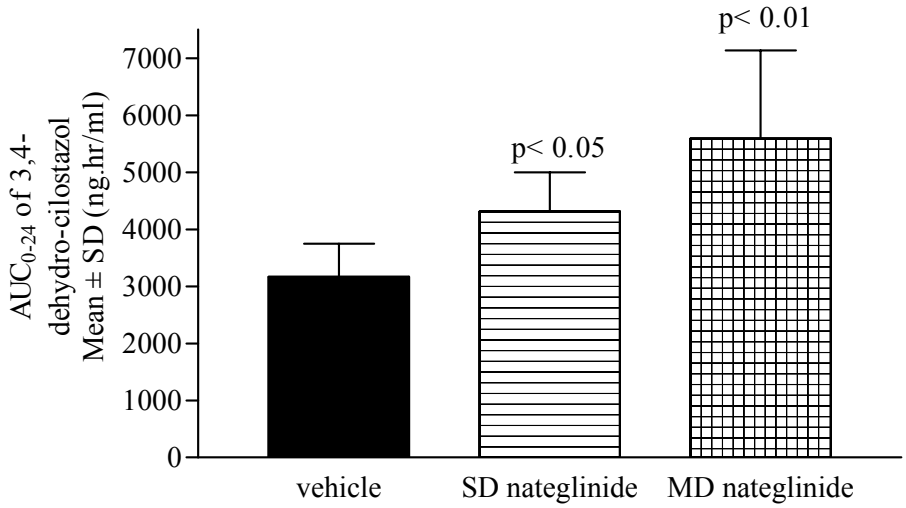



*Figure 4.31 Effect of nateglinide on plasma $AUC_{0-24}$ of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

#### 4.4.1.4 Effect of glipizide on cilostazol and its metabolite pharmacokinetic parameters by simultaneous administration in rats (Study IV)

Cilostazol and 3, 4-dehydro-cilostazol were undetectable in the predose samples in all rats, indicating that glipizide does not interfere with the cilostazol assay. **Figure 4.32 & Figure 4.33** shows the plasma concentration-time curve of cilostazol and 3, 4-dehydro-cilostazol in rats receiving cilostazol alone or concurrent treatment with glipizide. The intra group variability observed these groups were high and were reproduced upon repeatition. Visual inspection of the plasma concentration-time curve for cilostazol showed that the mean plasma concentrations of cilostazol tended to be increased and also of 3, 4-dehydro-cilostazol levels tended to be increased significantly.

The estimated pharmacokinetic parameters of cilostazol in the absence and presence of glipizide are summarized in **Table 4.26**. The maximum plasma concentration reached ($C_{max}$) for cilostazol in presence of Vehicle, single dose glipizide and multiple doses of glipizide are in the ratio of 1.00: 1.25:3.70 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.48: 4.78. Though the maximum absorption of cilostazol was changed slightly but not significant, the systemic exposure of cilostazol was greatly increased by 48 % and this was significantly ($p<0.01$) affected in presence of single dose glipizide. And these parameters were also

significantly increased ($C_{max}$ by 270%, $p<0.01$ and $AUC_{0-24}$ by 378%, $p<0.05$) in presence of multiple doses of glipizide. The time to reach maximum absorption ($T_{max}$) was increased slightly but not significant in presence of either single dose or multiple doses of glipizide. The half-life was slightly decreased from 3.10 to 2.64 hr for cilostazol in presence of multiple doses of glipizide but it was not statistically significant.

Table 4.26 Effect of glipizide on single dose pharmacokinetic parameters of cilostazol by simultaneous administration of cilostazol and glipizide in Wistar rats

| **Parameters of cilostazol** | **Units** | **Vehicle treated** (mean ± S.D) | **Glipizide treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 1.52 ± 0.36 | 1.90 ± 0.51 | 5.63 ± 2.87** |
| **$AUC_{0-24}$** | µg.hr/ml | 3.61 ± 0.59 | 5.35 ± 1.11** | 17.29 ± 14.52* |
| **$AUC_{0-inf}$** | µg.hr/ml | 4.12 ± 0.68 | 5.84 ± 1.16* | 18.04 ± 14.74* |
| **$T_{max}$** | hr | 0.17 ± 0.04 | 0.25 ± 0.72 | 0.25 ± 0.32 |
| **$t_{1/2}$** | hr | 3.10 ± 1.14 | 3.02 ± 1.47 | 2.64 ± 0.90 |
| **$K_{el}$** | hr -1 | 0.223 ± 0.056 | 0.230 ± 0.066 | 0.263 ± 0.070 |
| **$V_{z\_F}$** | L/kg | 12.04 ± 5.0 | 8.50 ± 3.80 | 3.35 ± 1.97 |
| **$Cl_{z\_F}$** | L/hr/kg | 2.48 ± 0.42 | 1.76 ± 0.31 | 0.79 ± 0.39 |

n=6 in each treatment group; *p<0.05; **p<0.01 compared to vehicle using student's unpaired t-test;

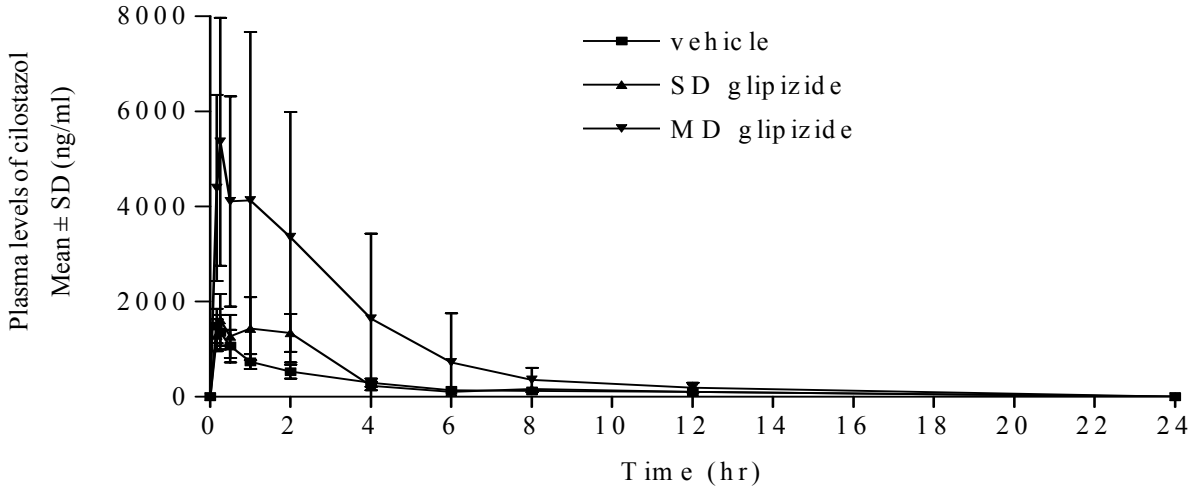



*Figure 4.32 Effect of glipizide on plasma concentration-time plot of cilostazol in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses

The estimated pharmacokinetic parameters of active metabolite of cilostazol in the absence and presence of glipizide are summarized in **Table 4.27**. The maximum plasma concentration reached ($C_{max}$) for 3, 4-dehydro-cilostazol after administration of cilostazol in presence of vehicle, single dose glipizide and multiple doses of glipizide are in the ratio of 1.00: 1.63: 2.64 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.48: 3.50. The maximum formation of 3, 4-dehydro-cilostazol by administration of cilostazol and its systemic exposure increased by 63% in presence of single dose glipizide ($p<0.05$ )and also the systemic exposure of cilostazol was increased more than 48 % and this was significantly ($p<0.05$) affected in presence of single dose glipizide. And these parameters were also significantly increased ($C_{max}$ by 164%, $p<0.0001$ and $AUC_{0-24}$ by 250%, $p<0.01$) in presence of multiple doses of glipizide. The time to reach maximum absorption ($T_{max}$) was not effected in presence of either single dose or multiple doses of glipizide The time to reach maximum formation of metabolite ($T_{max}$) was significantly delayed by 1.5 hr in presence of either single dose or multiple doses of glipizide ($p<0.0001$). Though it was not statistically significant, the half-life was very slightly increased from 3.09 to 3.89 hr for this metabolite after administration of cilostazol in presence of multiple doses of glipizide. The extent of formation of metabolite by administration of cilostazol was slightly decreased in presence multiple dose administration of glipizide (26%).

Table 4.27 Effect of glipizide on single dose pharmacokinetic parameters of 3, 4-dehydro-cilostazol by simultaneous administration of cilostazol and glipizide in Wistar rats

| **Parameters of 3, 4-dehydro-cilostazol** | **Units** | **Vehicle treated** (mean ± S.D) | **Glipizide treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 0.85 ± 0.25 | 1.39 ± 0.30* | 1.91 ± 0.34*** |
| **$AUC_{0-24}$** | µg.hr/ml | 3.16 ± 0.59 | 4.69 ± 0.65** | 11.09 ± 4.87** |
| **$AUC_{0-inf}$** | µg.hr/ml | 3.70 ± 0.59 | 5.37 ± 0.99* | 13.03 ± 6.47* |
| **$T_{max}$** | hr | 0.5 ± 0.25 | 2.00 ± 0.41 | 2.00 ± 0.41 |
| **$t_{1/2}$** | hr | 3.09 ± 5.04 | 3.94 ± 1.95 | 3.89 ± 1.50 |
| **$K_{el}$** | hr -1 | 0.224 ± 0.126 | 0.176 ± 0.065 | 0.178 ± 0.058 |

n=6 in each treatment group; *p<0.05; **p<0.01; ***p<0.001 compared to vehicle using student's unpaired t-test

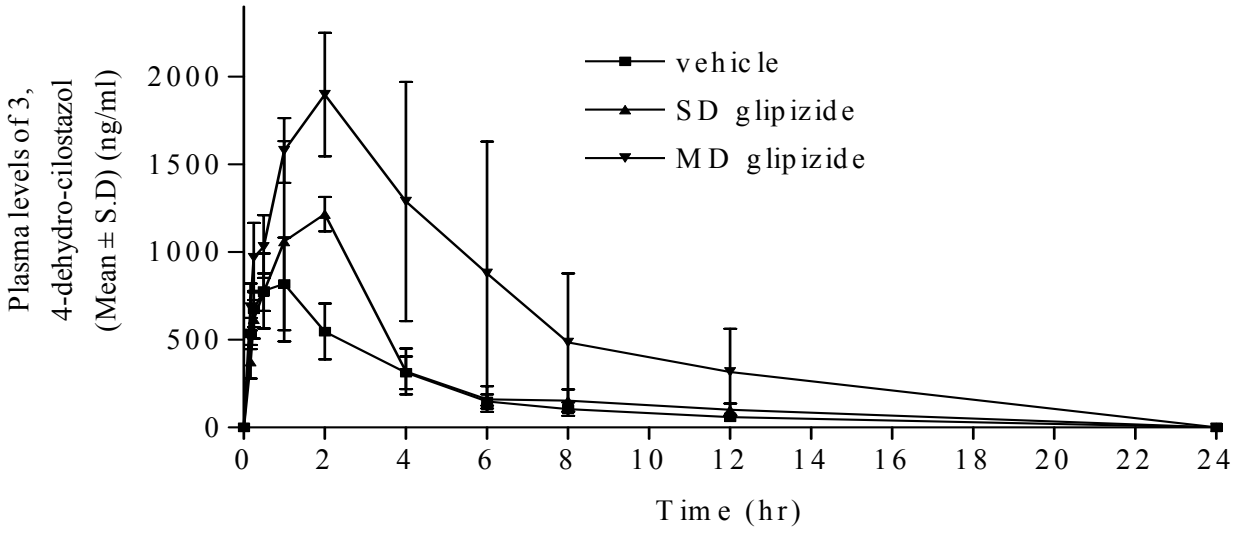



*Figure 4.33 Effect of glipizide on plasma concentration-time curve of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses

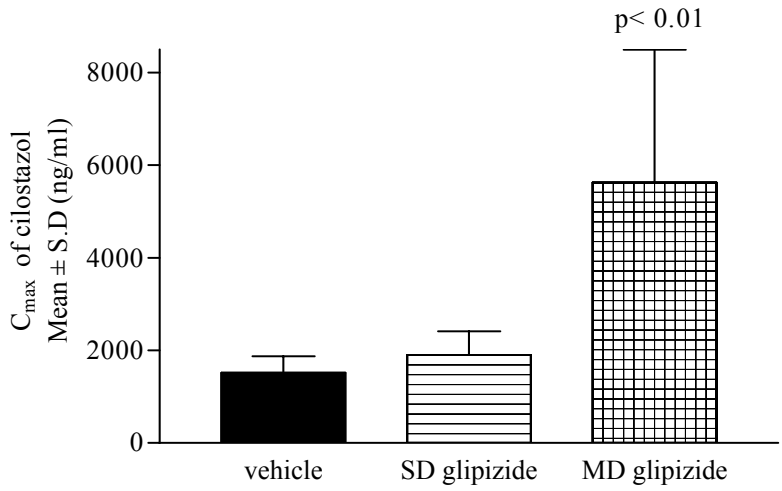



*Figure 4.34 Effect of glipizide on plasma $C_{max}$ of single dose cilostazol in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

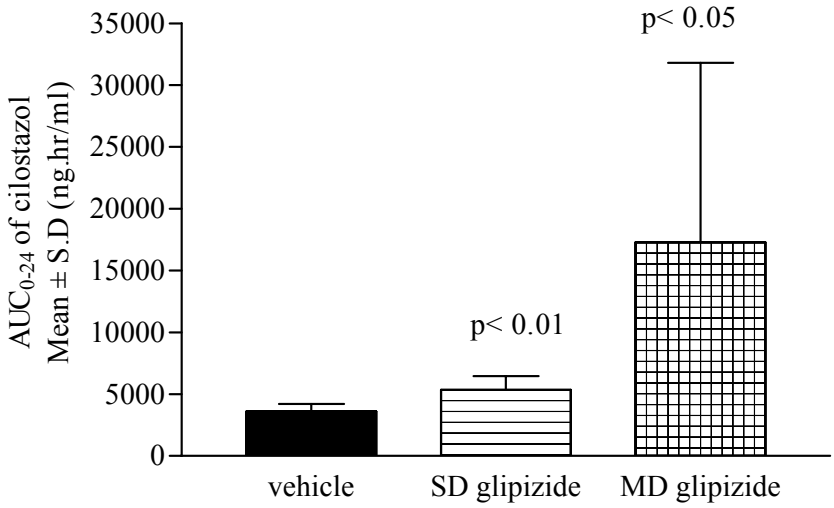



*Figure 4.35 Effect of glipizide on plasma $AUC_{0-24}$ of single dose cilostazol in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

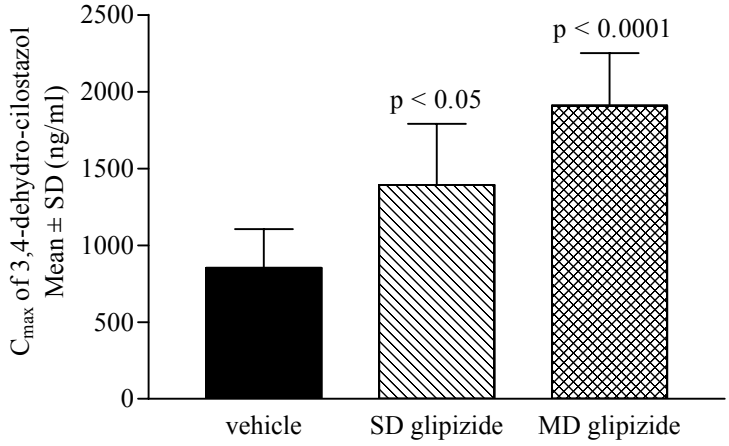



*Figure 4.36 Effect of glipizide on plasma $C_{max}$ of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

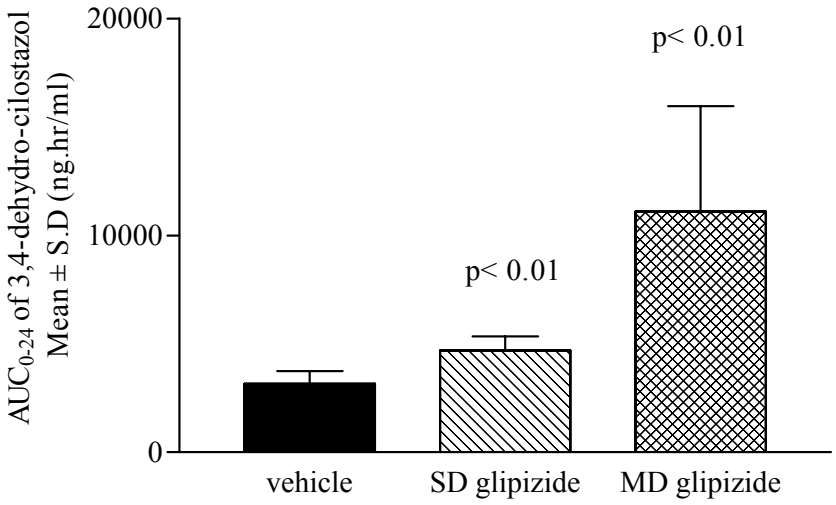



*Figure 4.37 Effect of glipizide on plasma $AUC_{0-24}$ of cilostazol metabolite (3, 4-dehydro-cilostazol) in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

### 4.4.2 Effect of Antidiabetic agents on pentoxifylline pharmacokinetics in rats

Table 4.28 Single dose effect of ADA on pharmacokinetic parameters of pentoxifylline by simultaneous administration of ADA and pentoxifylline in Wistar rats

| Parameters of PTX | Units | Vehicle treated (mean ± S.D) | Rosiglitazone treated (mean ± S.D) | Metformin treated (mean ± S.D) | Nateglinide treated (mean ± S.D) | Glipizide treated (mean ± S.D) |
|---|---|---|---|---|---|---|
| $C_{max}$ | µg/ml | 1.12 ± 0.35 | 0.79 ± 0.34 | 0.89 ± 0.25 | 1.09 ± 0.39 | 1.75 ± 0.25** |
| $AU_{0-24}$ | µg.hr/ml | 0.67 ± 0.12 | 0.60 ± 0.23 | 0.51 ± 0.19 | 0.55 ± 0.10 | 0.79 ± 0.11 |
| $AUC_{0-inf}$ | µg.hr/ml | 0.72 ± 0.13 | 0.65 ± 0.23 | 0.52 ± 0.20 | 0.61 ± 0.11 | 0.83 ± 0.12 |
| $T_{max}$ | hr | 0.17 ± 0.03 | 0.25 ± 0.03* | 0.17 ± 0.00 | 0.17 ± 0.04 | 0.17 ± 0.00 |
| $t_{½}$ | hr | 0.37 ± 0.23 | 0.43 ± 0.26 | 0.38 ± 0.09 | 0.32 ± 0.17 | 0.28 ± 0.10 |
| $K_{el}$ | $hr^{-1}$ | 1.895 ± 0.771 | 1.587 ± 0.910 | 1.830 ± 0.370 | 2.17 ± 0.78 | 2.53 ± 0.78 |
| $V_{z\_F}$ | L/kg | 8.97 ± 4.41 | 14.80 ± 9.56 | 21.28 ± 10.57 | 9.45 ± 5.74 | 5.17 ± 1.52 |
| $Cl_{z\_F}$ | L/hr/kg | 14.38 ± 2.66 | 17.36 ± 6.57 | 40.36 ± 12.63 | 16.84 ± 3.31 | 12.19 ± 1.67 |

n=6 in each treatment group; *p<0.05; **p<0.01 compared to vehicle using student's unpaired t-test;

The effect of antidiabetic agents on single dose pentoxifylline pharmacokinetics was investigated by pretreatment with either single dose or multiple doses (seven daily doses) of respective antidiabetic agents. These studies were monitored for pentoxifylline (PTX) concentrations. The summary results from four antidiabetic agents investigated are presented in **Table 4.28 and 4.29**. The individual study results are explained in the subsequent sections.

Table 4.29 Multiple doses effect of ADA on pharmacokinetic parameters of pentoxifylline by simultaneous administration of ADA and pentoxifylline in Wistar rats

| Parameters of PTX | Units | Vehicle treated (mean ± S.D) | Rosiglitazone treated (mean ± S.D) | Metformin treated (mean ± S.D) | Nateglinide treated (mean ± S.D) | Glipizide treated (mean ± S.D) |
|---|---|---|---|---|---|---|
| $C_{max}$ | µg/ml | 1.12 ± 0.35 | 0.79 ± 0.20 | 0.40 ± 0.15*** | 1.75 ± 0.53* | 1.95 ± 0.44** |
| $AU_{0-24}$ | µg.hr/ml | 0.67 ± 0.12 | 0.45 ± 0.08** | 0.25 ± 0.10*** | 0.91 ± 0.22* | 0.77 ± 0.18 |
| $AUC_{0-inf}$ | µg.hr/ml | 0.72 ± 0.13 | 0.51 ± 0.09** | 0.27 ± 0.10*** | 0.93 ± 0.22 | 0.79 ± 0.17 |
| $T_{max}$ | hr | 0.17 ± 0.03 | 0.17 ± 0.04 | 0.25 ± 0.11 | 0.21 ± 0.04 | 0.17 ± 0.03 |
| $t_{½}$ | hr | 0.37 ± 0.23 | 0.29 ± 0.03 | 0.31 ± 0.24 | 0.24 ± 0.09 | 0.17 ± .02* |
| $K_{el}$ | $hr^{-1}$ | 1.895 ± 0.771 | 2.408 ± 0.244 | 2.207 ± 0.809 | 3.18 ± 1.00 | 4.18 ± 0.37 |
| $V_{z\_F}$ | L/kg | 8.97 ± 4.41 | 2.41 ± 0.24 | 11.84 ± 4.03 | 3.92 ± 1.5 | 3.13 ± 0.47 |
| $Cl_{z\_F}$ | L/hr/kg | 14.38 ± 2.66 | 8.41 ± 1.62 | 21.85 ± 9.12 | 11.21 ± 2.26 | 13.14 ± 2.52 |

n=6 in each treatment group; *p<0.05; **p<0.01; ***p<0.001 compared to vehicle using student's unpaired t-test;

#### 4.4.2.1 Effect of rosiglitazone on pentoxifylline pharmacokinetic parameters by simultaneous administration in rats (Study V)

Pentoxifylline were undetectable in the predose samples in all rats, indicating that rosiglitazone does not interfere with the pentoxifylline assay. **Figure 4.38** shows the plasma concentration-time curve of pentoxifylline receiving pentoxifylline alone or concurrent treatment with rosiglitazone. Visual inspection of the plasma concentration-time curve for pentoxifylline showed that the mean plasma concentrations of pentoxifylline tended to be increased. However,

no statistically significant differences in the mean plasma concentrations of pentoxifylline were found between the vehicle and rosiglitazone co administered groups at any point of time.

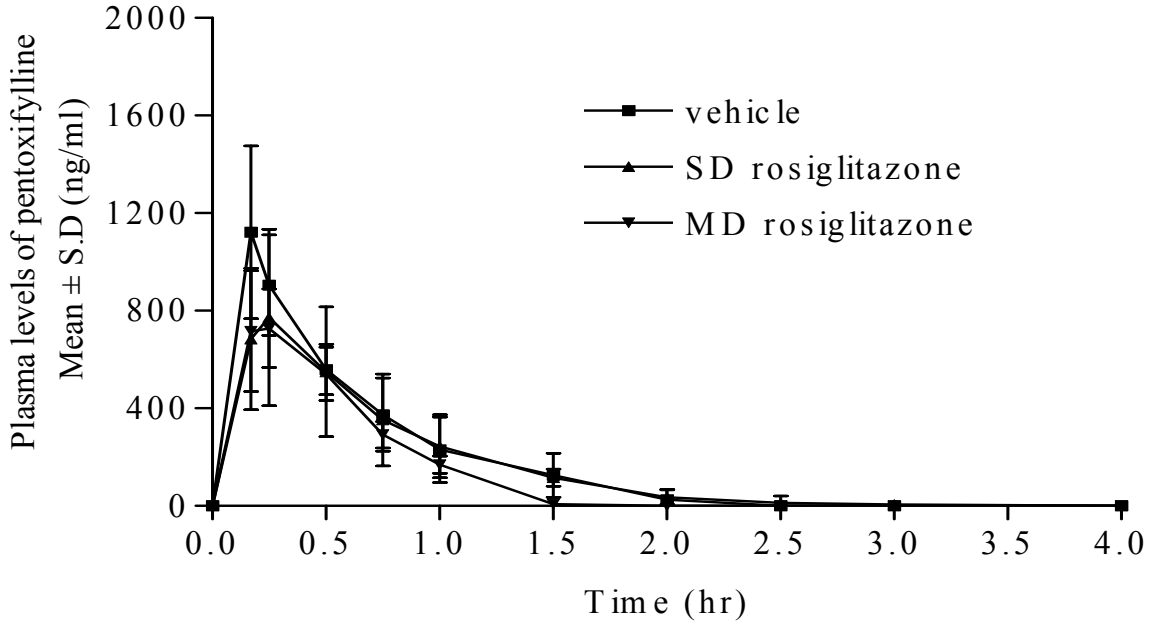



*Figure 4.38 Effect of rosiglitazone on plasma concentration-time plot of pentoxifylline in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses

The estimated pharmacokinetic parameters of pentoxifylline in the absence and presence of rosiglitazone are summarized in **Table no. 4.30**. The maximum plasma concentration reached ($C_{max}$) for pentoxifylline in presence of Vehicle, single dose rosiglitazone and multiple doses of rosiglitazone are in the ratio of 1.00: 0.70:0.70 where as the systemic exposures ($AUC_{0-4}$) are in the ratio of 1.00: 0.90: 0.67. Though the maximum absorption and systemic exposure of pentoxifylline were slightly decreased more than 30 % these were not significantly affected in presence of single dose rosiglitazone. And these parameters were significantly decreased ($C_{max}$ by 30% and $AUC_{0-4}$ by 33%, $p<0.01$) in presence of multiple doses of rosiglitazone. The time to reach maximum absorption ($T_{max}$) was delayed by 5 min in presence of single dose significantly but not in multiple doses of rosiglitazone. But the half life was not affected either in presence of single or multiple doses of rosiglitazone.

Table 4.30 Effect of rosiglitazone on single dose pharmacokinetic parameters of pentoxifylline by simultaneous administration of pentoxifylline and rosiglitazone in Wistar rats

| Parameters of pentoxifylline | Units | Vehicle treated (mean ± S.D) | Rosiglitazone treated | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| $C_{max}$ | µg/ml | 1.12 ± 0.35 | 0.79 ± 0.34 | 0.79 ± 0.20 |
| $AUC_{0-4}$ | µg.hr/ml | 0.67 ± 0.12 | 0.60 ± 0.23 | 0.45 ± 0.08** |
| $AUC_{0-inf}$ | µg.hr/ml | 0.72 ± 0.13 | 0.65 ± 0.23 | 0.51 ± 0.09** |
| $T_{max}$ | hr | 0.17 ± 0.03 | 0.25 ± 0.03* | 0.17 ± 0.04 |
| $T_{½}$ | hr | 0.37 ± 0.23 | 0.43 ± 0.26 | 0.29 ± 0.03 |
| $K_{el}$ | $hr^{-1}$ | 1.895 ± 0.771 | 1.587 ± 0.910 | 2.408 ± 0.244 |
| $V_{z\_F}$ | L/kg | 8.97 ± 4.41 | 14.80 ± 9.56 | 2.41 ± 0.24 |
| $Cl_{z\_F}$ | L/hr/kg | 14.38 ± 2.66 | 17.36 ± 6.57 | 8.41 ± 1.62 |

n=6 in each treatment group; *p<0.05; **p<0.01 compared to vehicle using student's unpaired t-test

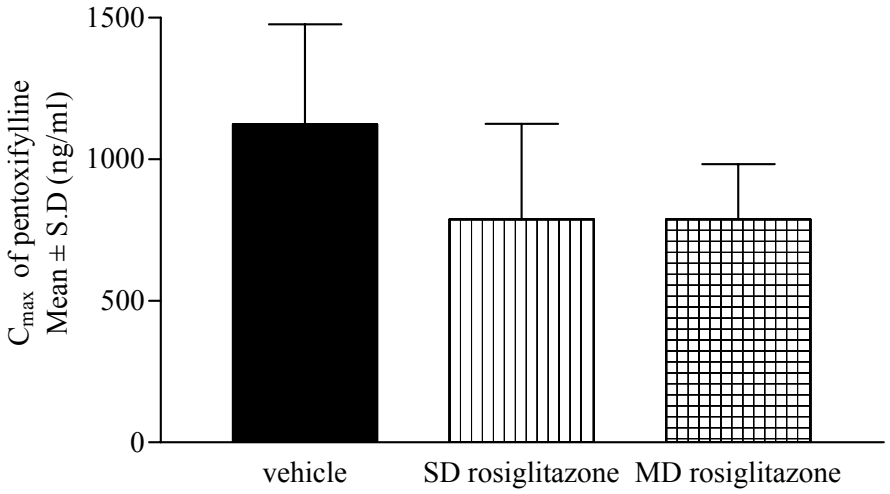



*Figure 4.39 Effect of rosiglitazone on plasma $C_{max}$ of single dose pentoxifylline in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

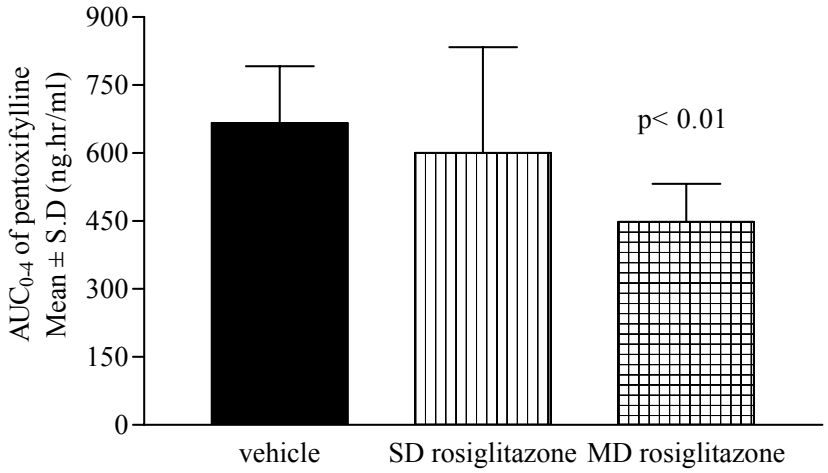



*Figure 4.40 Effect of rosiglitazone on plasma $AUC_{0-4}$ of single dose pentoxifylline in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

### *4.4.2.2* Effect of metformin on pentoxifylline pharmacokinetic parameters by simultaneous administration in rats (Study VI)

Pentoxifylline were undetectable in the predose samples in all rats, indicating that metformin does not interfere with the pentoxifylline assay. **Figure 4.41** shows the plasma concentration-time curve of pentoxifylline receiving pentoxifylline alone or concurrent treatment with metformin. Visual inspection of the plasma concentration-time curve for pentoxifylline showed that the mean plasma concentrations of pentoxifylline tended to be decreased. There seems to be statistically significant differences in the mean plasma concentrations of pentoxifylline found between the vehicle and metformin co administered groups at most of the points of time.

The estimated pharmacokinetic parameters of pentoxifylline in the absence and presence of metformin are summarized in **Table 4.31**. The maximum plasma concentration reached ($C_{max}$) for pentoxifylline in presence of Vehicle, single dose metformin and multiple doses of metformin are in the ratio of 1.00: 0.80:0.35 where as the systemic exposures ($AUC_{0-4}$) are in the ratio of 1.00: 0.76: 0.37. Though the maximum absorption and systemic exposure of pentoxifylline were decreased by more than 20%, they were not significant in presence of single dose metformin. Where as these parameters were significantly decreased ($C_{max}$ by 65 % and $AUC_{0-4}$ by 63 %, $p<0.0001$) in presence of multiple doses of metformin. The time to reach maximum absorption

($T_{max}$) was delayed by 5 min in presence of multiple dose of metformin but not significant. But the half life was not affected either in presence of single or multiple doses of metformin though there seems to a slight reduction in presence of multiple dose of metformin.

Table 4.31 Effect of metformin on single dose pharmacokinetic parameters of pentoxifylline by simultaneous administration of pentoxifylline and metformin in Wistar rats

| **Parameters of pentoxifylline** | **Units** | **Vehicle treated** (mean ± S.D) | **Metformin treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 1.12 ± 0.35 | 0.89 ± 0.25 | 0.40 ± 0.15*** |
| **$AUC_{0-4}$** | µg.hr/ml | 0.67 ± 0.12 | 0.51 ± 0.19 | 0.25 ± 0.10*** |
| **$AUC_{0-inf}$** | µg.hr/ml | 0.72 ± 0.13 | 0.52 ± 0.20 | 0.27 ± 0.10*** |
| **$T_{max}$** | Hr | 0.17 ± 0.03 | 0.17 ± 0.00 | 0.25 ± 0.11 |
| **$t_{½}$** | Hr | 0.37 ± 0.23 | 0.38 ± 0.09 | 0.31 ± 0.24 |
| **$K_{el}$** | hr -1 | 1.895 ± 0.771 | 1.830 ± 0.370 | 2.207 ± 0.809 |
| **$V_{z\_F}$** | L/kg | 8.97 ± 4.41 | 21.28 ± 10.57 | 11.84 ± 4.03 |
| **$Cl_{Z\_F}$** | L/hr/kg | 14.38 ± 2.66 | 40.36 ± 12.63 | 21.85 ± 9.12 |

n=6 in each treatment group; ***p<0.001 compared to vehicle using student's unpaired t-test;

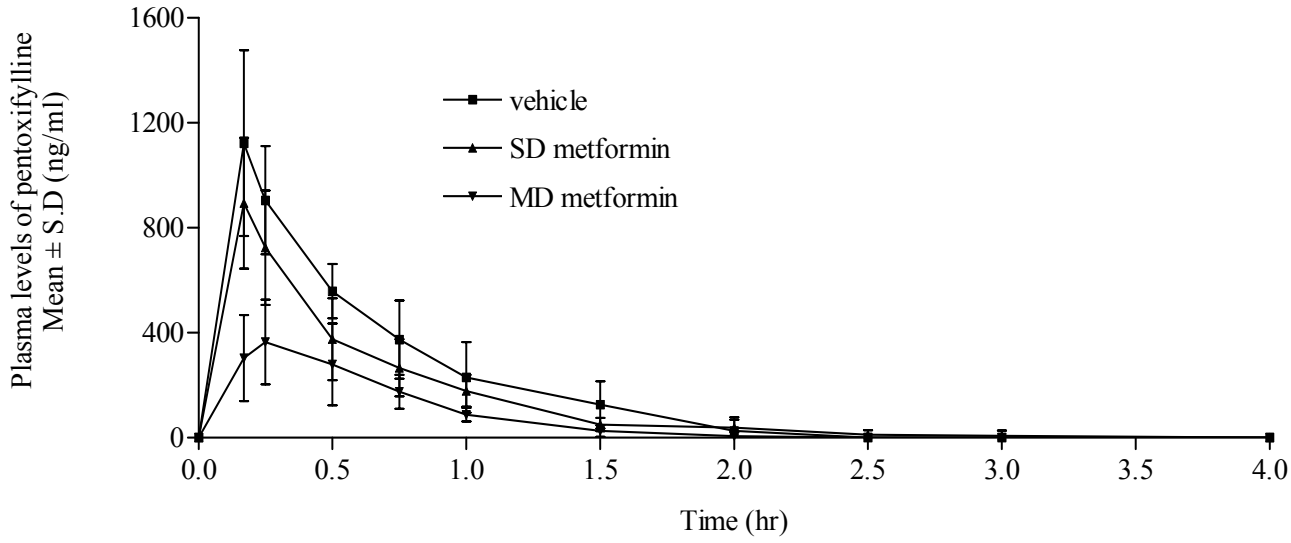



*Figure 4.41 Effect of metformin on plasma concentration-time plot of pentoxifylline in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses

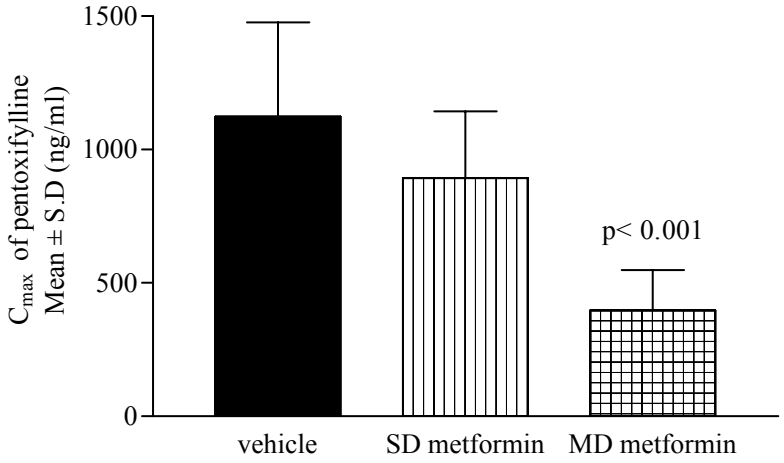



*Figure 4.42 Effect of metformin on plasma $C_{max}$ of single dose pentoxifylline in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

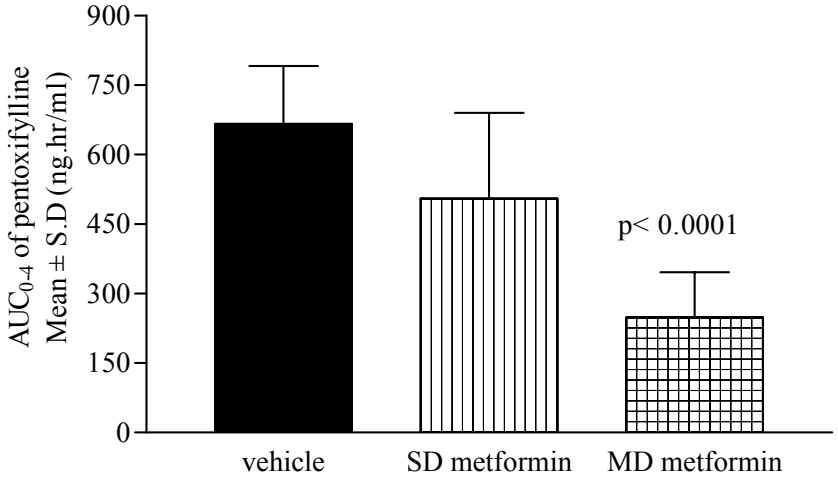



*Figure 4.43 Effect of metformin on plasma $AUC_{0-4}$ of single dose pentoxifylline in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

### *4.4.2.3* Effect of nateglinide on pentoxifylline pharmacokinetic parameters by simultaneous administration in rats (Study VII)

Pentoxifylline levels were undetectable in the predose samples in all rats, indicating that nateglinide does not interfere with the pentoxifylline assay. **Figure 4.44** shows the plasma concentration-time curve of pentoxifylline receiving pentoxifylline alone or concurrent treatment with nateglinide. Visual inspection of the plasma concentration-time curve for pentoxifylline showed that the mean plasma concentrations of pentoxifylline tended to be increased. However, no statistically significant differences in the mean plasma concentrations of pentoxifylline were found between the vehicle and nateglinide co administered groups at any point of time.

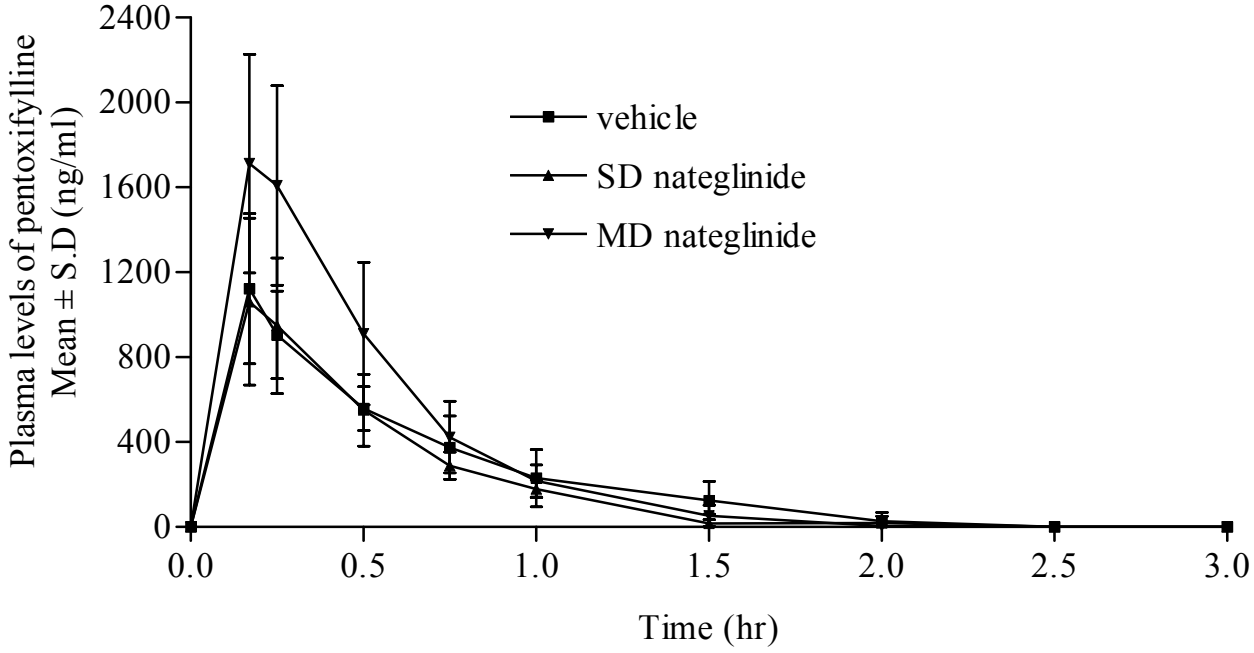



*Figure 4.44 Effect of nateglinide on plasma concentration-time plot of pentoxifylline in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses

The estimated pharmacokinetic parameters of pentoxifylline in the absence and presence of nateglinide are summarized in **Table 4.32**. The maximum plasma concentration reached ($C_{max}$) for pentoxifylline in presence of Vehicle, single dose nateglinide and multiple doses of nateglinide are in the ratio of 1.00: 0.97: 1.56 where as the systemic exposures ($AUC_{0-4}$) are in the ratio of 1.00: 0.83: 1.36. Though the systemic exposure of pentoxifylline was decreased slightly around 17 %, the AUC and the maximum absorption were not significantly affected in presence of single dose nateglinide. Where as these parameters were significantly increased ($C_{max}$ by 56 % and $AUC_{0-4}$ by 36 %, $p < 0.05$) in presence of multiple doses of nateglinide. The time to reach maximum absorption ($T_{max}$) was slightly delayed by 5 min in presence of multiple dose of nateglinide but not significant. But the half life was not affected significantly either in presence of single or multiple doses of nateglinide though there seems to a slight reduction in presence of multiple dose of nateglinide.

Table 4.32 Effect of nateglinide on single dose pharmacokinetic parameters of pentoxifylline by simultaneous administration of pentoxifylline and nateglinide in Wistar rats

| **Parameters of pentoxifylline** | **Units** | **Vehicle treated** (mean ± S.D) | **Nateglinide treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 1.12 ± 0.35 | 1.09 ± 0.39 | 1.75 ± 0.53* |
| **$AUC_{0-4}$** | µg.hr/ml | 0.67 ± 0.12 | 0.55 ± 0.10 | 0.91 ± 0.22* |
| **$AUC_{0-inf}$** | µg.hr/ml | 0.72 ± 0.13 | 0.61 ± 0.11 | 0.93 ± 0.22* |
| **$T_{max}$** | hr | 0.17 ± 0.03 | 0.17 ± 0.04 | 0.21 ± 0.04 |
| **$t_{½}$** | hr | 0.37 ± 0.23 | 0.32 ± 0.17 | 0.24 ± 0.09 |
| **$K_{el}$** | $hr^{-1}$ | 1.90 ± 0.77 | 2.17 ± 0.78 | 3.18 ± 1.00 |
| **$V_{z\_F}$** | L/kg | 8.97 ± 4.14 | 9.45 ± 5.74 | 3.92 ± 1.5 |
| **$Cl_{Z\_F}$** | L/hr/kg | 14.38 ± 2.66 | 16.84 ± 3.31 | 11.21 ± 2.26 |

n=6 in each treatment group; *p<0.05 compared to vehicle using student's unpaired t-test;

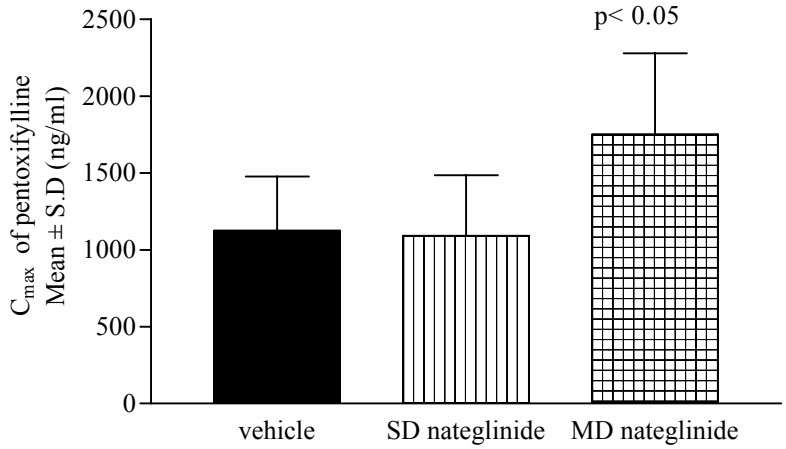



*Figure 4.45 Effect of nateglinide on plasma $C_{max}$ of single dose pentoxifylline in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

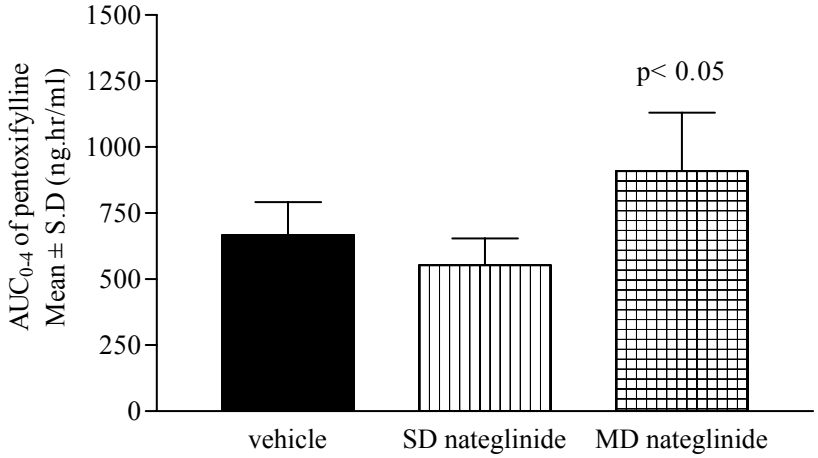



*Figure 4.46 Effect of nateglinide on plasma $AUC_{0-4}$ of single dose pentoxifylline in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

#### 4.4.2.4 Effect of glipizide on pentoxifylline pharmacokinetic parameters by simultaneous administration in rats (Study VIII)

Pentoxifylline levels were undetectable in the predose samples in all rats, indicating that glipizide does not interfere with the pentoxifylline assay. **Figure 4.47** shows the plasma concentration-time curve of pentoxifylline receiving pentoxifylline alone or concurrent treatment with glipizide. Visual inspection of the plasma concentration-time curve for pentoxifylline showed that the mean plasma concentrations of pentoxifylline tended to be decreased. However, no statistically significant differences in the mean plasma concentrations of pentoxifylline were found between the vehicle and glipizide co administered groups at any point of time.

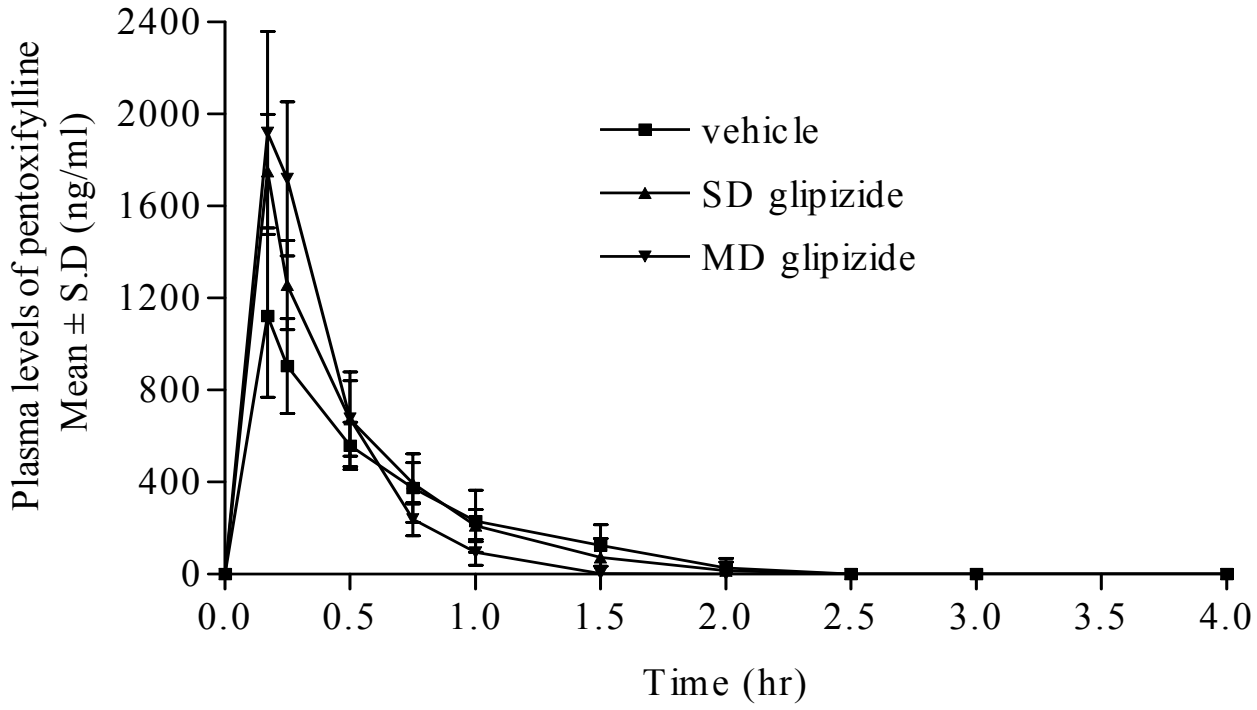



*Figure 4.47 Effect of glipizide on plasma concentration-time plot of pentoxifylline in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses

The estimated pharmacokinetic parameters of pentoxifylline in the absence and presence of glipizide are summarized in **Table 4.33**. The maximum plasma concentration reached ($C_{max}$) for pentoxifylline in presence of Vehicle, single dose glipizide and multiple doses of glipizide are in the ratio of 1.00: 1.56:1.74 where as the systemic exposures ($AUC_{0-4}$) are in the ratio of 1.00: 1.19: 1.16. Though the maximum absorption of pentoxifylline was increased significantly ($p<0.01$) more than 50 % the systemic exposure was not significantly affected in presence of single dose glipizide. The similar trend was also observed up in presence of the multiple doses of glipizide where a significant increase in $C_{max}$ by 74% ($p< 0.01$) was observed but not the systemic exposure, $AUC_{0-4}$ by 19%). The time to reach maximum absorption ($T_{max}$) was not changed in presence of multiple dose of glipizide. The half life was not affected either in presence of single dose of glipizide but there seems to be a reduction in presence of multiple doses of glipizide.

Table 4.33 Effect of glipizide on single dose pharmacokinetic parameters of pentoxifylline by simultaneous administration of pentoxifylline and glipizide in Wistar rats

| **Parameters of pentoxifylline** | **Units** | **Vehicle treated** (mean ± S.D) | **Glipizide treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 1.12 ± 0.35 | 1.75 ± 0.25** | 1.95 ± 0.44** |
| **$AUC_{0-4}$** | µg.hr/ml | 0.67 ± 0.12 | 0.79 ± 0.11 | 0.77 ± 0.18 |
| **$AUC_{0-inf}$** | µg.hr/ml | 0.72 ± 0.13 | 0.83 ± 0.12 | 0.79 ± 0.17 |
| **$T_{max}$** | hr | 0.17 ± 0.03 | 0.17 ± 0.00 | 0.17 ± 0.03 |
| **$t_{½}$** | hr | 0.37 ± 0.23 | 0.28 ± 0.10 | 0.17 ± .02 |
| **$K_{el}$** | $hr^{-1}$ | 1.895 ± 0.771 | 2.53 ± 0.78 | 4.18 ± 0.37 |
| **$V_{z\_F}$** | L/kg | 8.97 ± 4.41 | 5.17 ± 1.52 | 3.13 ± 0.47 |
| **$Cl_{Z\_F}$** | L/hr/kg | 14.38 ± 2.66 | 12.19 ± 1.67 | 13.14 ± 2.52 |

n=6 in each treatment group; **p<0.01 compared to vehicle using student's unpaired t-test;

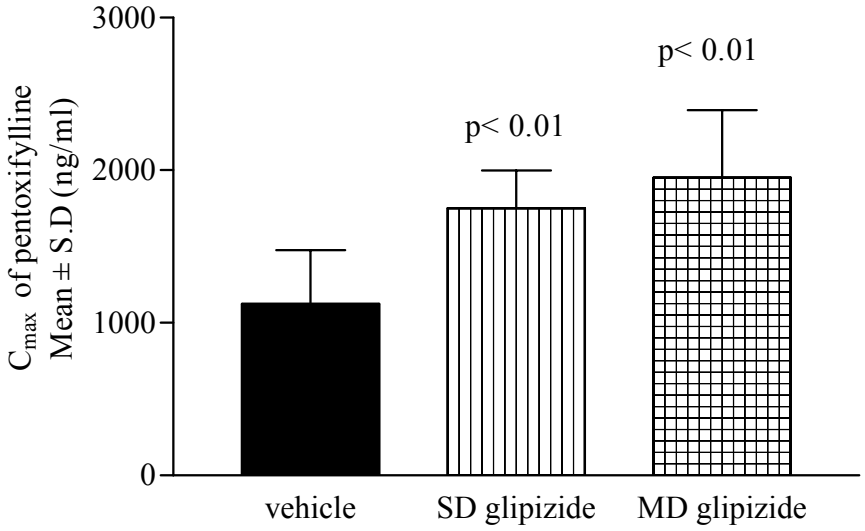



*Figure 4.48 Effect of glipizide on plasma $C_{max}$ of single dose pentoxifylline in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

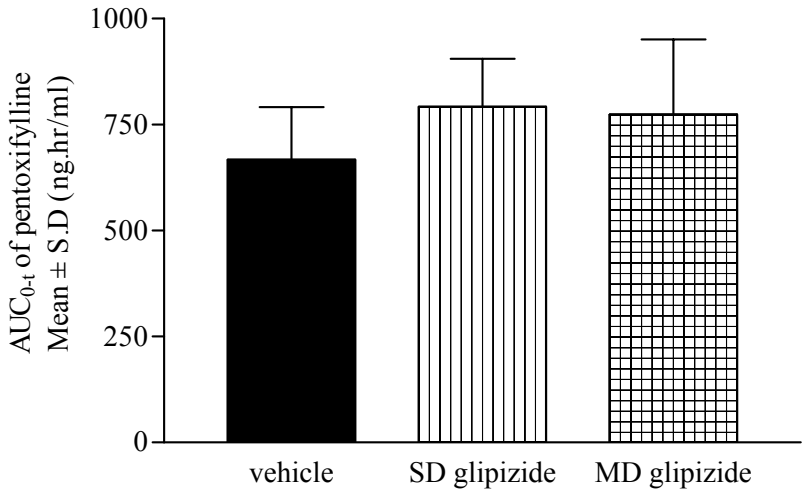



*Figure 4.49 Effect of glipizide on plasma $AUC_{0-4}$ of single dose pentoxifylline in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses;

### 4.4.3 Effect of antiplatelet agents on antidiabetic agent's pharmacokinetics in rats

The effect of antiplatelet agents on single dose ADA pharmacokinetics was investigated by pretreatment with either single dose or multiple doses (seven daily doses) of respective antiplatelet agents. These studies were monitored for respective ADA (RSG/MET/NTG/GLP) concentrations. The summary results from two antiplatelet agents investigated are presented in **Table 4.34, 4.35, 4.36 and 4.37**. The individual study results are explained in the subsequent sections.

Table 4.34 Effect of APA on pharmacokinetic parameters of rosiglitazone by simultaneous administration of APA and rosiglitazone in Wistar rats

| **Parameters of RSG** | **Units** | **Vehicle treated** (mean ± S.D) | **Cilostazol treated** | | **Pentoxifylline treated** | |
|---|---|---|---|---|---|---|
| | | | **Single dose** (mean ± S.D) | **Multiple dose** (mean ± S.D) | **Single dose** (mean ± S.D) | **Multiple dose** (mean ± S.D) |
| $C_{max}$ | µg/ml | 11.98 ± 1.46 | 14.23 ± 1.38* | 13.67 ± 1.14* | 10.43 ± 1.11 | 9.65 ± 1.91* |
| $AUC_{0-24}$ | µg.hr/ml | 19.83 ± 3.48 | 24.34 ± 3.87 | 21.14 ± 2.89 | 20.37 ± 2.02 | 21.26 ± 6.18 |
| $AUC_{0-inf}$ | µg.hr/ml | 19.88 ± 3.50 | 24.42 ± 3.87 | 21.36 ± 2.82 | 20.53 ± 2.01 | 21.46 ± 6.11 |
| $T_{max}$ | hr | 0.25 ± 0.10 | 0.17 ± 0.04 | 0.25 ± 0.12 | 0.75 ± 0.38* | 0.75 ± 0.38* |
| $T_{½}$ | hr | 0.77 ± 0.16 | 0.79 ± 0.08 | 0.85 ± 0.07 | 0.89 ± 0.18 | 0.99 ± 0.18* |
| $K_{el}$ | $hr^{-1}$ | 0.90 ± 0.19 | 0.87 ± 0.09 | 0.82 ± 0.07 | 0.78 ± 0.14 | 0.70 ± 0.12 |
| $V_{z\_F}$ | L/kg | 0.17 ± 0.02 | 0.14 ± 0.01 | 0.17 ± 0.02 | 0.19 ± 0.04 | 0.21 ± 0.03 |
| $Cl_{z\_F}$ | L/hr/kg | 14.38 ± 2.66 | 17.36 ± 6.57 | 40.36 ± 12.63 | 16.84 ± 3.31 | 12.19 ± 1.67 |

n=6 in each treatment group; *$p<0.05$ compared to vehicle using student's unpaired t-test;

Table 4.35 Effect of APA on pharmacokinetic parameters of metformin by simultaneous administration of APA and metformin in Wistar rats

| Parameters of MET | Units | Vehicle treated (mean ± S.D) | Cilostazol treated | | Pentoxifylline treated | |
|---|---|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| $C_{max}$ | µg/ml | 10.31 ± 1.72 | 10.87 ± 2.14 | 7.31 ± 2.35 | 14.76 ± 1.09 | 10.38 ± 1.90 |
| $AUC_{0-24}$ | µg.hr/ml | 34.52 ± 3.43 | 51.82 ± 5.12*** | 49.05 ± 4.40*** | 27.85 ± 6.95 | 24.39 ± 2.76 |
| $AUC_{0-inf}$ | µg.hr/ml | 38.35 ± 5.17 | 57.62 ± 5.96*** | 56.26 ± 7.29*** | 29.25 ± 7.08 | 25.12 ± 2.80 |
| $T_{max}$ | hr | 1.00 ± 0.00 | 1.00 ± 1.22 | 0.75 ± 0.27 | 0.50 ± 0.16 | 0.38 ± 0.17 |
| $t_{½}$ | hr | 9.08 ± 3.80 | 8.52 ± 3.22 | 7.96 ± 3.06 | 2.00± 3.22 | 2.37 ± 0.80 |
| $K_{el}$ | $hr^{-1}$ | 0.08 ± 0.02 | 0.08 ± 0.03 | 0.09 ± 0.03 | 0.277 ± 0.100 | 0.293 ± 0.078 |
| $V_{z\_F}$ | L/kg | 110.20 ± 29.53 | 70.19 ± 21.22 | 65.94 ± 18.22 | 43.49 ± 19.66 | 44.27 ± 15.51 |
| $Cl_{z\_F}$ | L/hr/kg | 7.95 ± 1.12 | 5.26 ± 0.58 | 5.41 ± 0.74 | 10.70 ± 2.32 | 12.07 ± 1.38 |

n=6 in each treatment group; ***p<0.001 compared to vehicle using student's unpaired t-test;

Table 4.36 Effect of APA on pharmacokinetic parameters of nateglinide by simultaneous administration of APA and nateglinide in Wistar rats

| Parameters of NTG | Units | Vehicle treated (mean ± S.D) | Cilostazol treated | | Pentoxifylline treated | |
|---|---|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| $C_{max}$ | µg/ml | 10.60 ± 1.91 | 11.53 ± 2.30 | 7.31 ± 2.35* | 4.24 ± 1.45**** | 2.80 ± 0.72**** |
| $AUC_{0-24}$ | µg.hr/ml | 2.71 ± 0.50 | 2.85 ± 0.55 | 2.86 ± 0.51 | 1.35 ± 0.36*** | 1.24 ± 0.24**** |
| $AUC_{0-inf}$ | µg.hr/ml | 2.73 ± 0.50 | 2.87 ± 0.54 | 2.88 ± 0.51 | 1.37 ± 0.37*** | 1.27 ± 0.25**** |
| $T_{max}$ | hr | 0.17 ± 0.00 | 0.17 ± 0.00 | 0.17 ± 0.00 | 0.17 ± 0.00 | 0.17 ± 0.04 |
| $t_{½}$ | hr | 0.16 ± 0.08 | 0.19 ± 0.11 | 0.32 ± 0.11 | 0.21 ± 0.11 | 0.25 ± 0.09 |
| $K_{el}$ | $hr^{-1}$ | 4.434 ± 1.184 | 3.630 ± 1.502 | 2.155 ± 0.727 | 3.200 ± 1.770 | 2.795 ± 1.052 |
| $V_{z\_F}$ | L/kg | 0.92 ± 0.29 | 1.13 ± 0.56 | 1.79 ± 0.64 | 3.13 ± 1.91 | 3.30 ± 1.41 |
| $C_{z\_F}$ | L/hr/kg | 3.76 ± 0.63 | 3.59 ± 0.72 | 3.55 ± 0.51 | 7.93 ± 1.65 | 8.16 ± 1.65 |

n=6 in each treatment group;*p<0.05; **p<0.01, ***p<0.001,****p<0.0001 compared to vehicle using student's unpaired t-test;

Table 4.37 Effect of APA on pharmacokinetic parameters of glipizide by simultaneous administration of APA and glipizide in Wistar rats

| Parameters of GLP | Units | Vehicle treated (mean ± S.D) | Cilostazol treated | | Pentoxifylline treated | |
|---|---|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| $C_{max}$ | µg/ml | 32.15 ± 8.50 | 59.73 ± 16.78 | 42.68 ± 6.27* | 36.45 ± 7.92 | 27.18 ± 4.55 |
| $AUC_{0-24}$ | µg.hr/ml | 289.46 ± 64.90 | 399.69 ± 50.38 | 242.44 ± 34.68 | 336.16 ± 51.08 | 282.24 ± 64.48 |
| $AUC_{0-inf}$ | µg.hr/ml | 325.62 ± 97.01 | 428.32 ± 71.58 | 247.46 ± 36.81 | 353.25 ± 49.22 | 301.56 ± 75.36 |
| $T_{max}$ | hr | 2.00 ± 0.61 | 2.00 ± 0.41 | 2.50 ± 0.82 | 2.00 ± 1.03 | 3.00 ± 1.10 |
| $t_{½}$ | hr | 6.97 ± 1.78 | 6.67 ± 1.78 | 2.91 ± 0.96 | 5.52 ± 0.39 | 5.57 ± 0.83 |
| $K_{el}$ | $hr^{-1}$ | 0.099 ± 0.020 | 0.104 ± 0.026 | 0.238 ± 0.100 | 0.13 ± 0.01 | 0.12 ± 0.02 |
| $V_{z\_F}$ | L/kg | 0.33 ± 0.04 | 0.23 ± 0.05 | 0.21 ± 0.07 | 0.23 ± 0.04 | 0.28 ± 0.04 |
| $Cl_{z\_F}$ | L/hr/kg | 0.03 ± 0.01 | 0.02 ± 0.00 | 0.04 ± 0.01 | 0.03 ± 0.00 | 0.03 ± 0.01 |

n=6 in each treatment group; *p<0.05 compared to vehicle using student's unpaired t-test;

#### 4.4.3.1 Effect of cilostazol on rosiglitazone pharmacokinetic parameters by simultaneous administration in rats (Study IX)

Rosiglitazone were undetectable in the predose samples in all rats, indicating that cilostazol does not interfere with the rosiglitazone assay. **Figure 4.49** shows the plasma concentration-time curve of rosiglitazone in rats receiving rosiglitazone alone or concurrent treatment with cilostazol. Visual inspection of the plasma concentration-time curve for rosiglitazone showed that the mean plasma concentrations of rosiglitazone tended to be increased.

The estimated pharmacokinetic parameters of rosiglitazone in the absence and presence of cilostazol are summarized in **Table 4.38**. The maximum plasma concentration reached ($C_{max}$) for rosiglitazone in presence of vehicle, single dose cilostazol and multiple dose cilostazol are in the ratio of 1.00: 1.19:1.14 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.26: 1.06. The maximum absorption of rosiglitazone was increased significantly ($p<0.05$) in presence of either single or multiple dose administration of cilostazol. Though the systemic exposure of rosiglitazone was slightly increased in presence of cilostazol but these parameters were not

significantly affected either in presence of single dose or multiple doses of cilostazol. The time to reach maximum absorption ($T_{max}$) was slightly reduced in presence of single dose of cilostazol but it did not show the similar effect in presence of multiple doses of cilostazol. Hence it could be due the variation in animals but not due to cilostazol.

Table 4.38 Effect of cilostazol on single dose pharmacokinetic parameters of rosiglitazone by simultaneous administration of cilostazol and rosiglitazone in Wistar rats

| Parameters of rosiglitazone | Units | Vehicle treated | Cilostazol treated | |
|---|---|---|---|---|
| | | | Single dose | Multiple dose |
| **$C_{max}$** | µg/ml | 11.98 ± 1.46 | 14.23 ± 1.38* | 13.67 ± 1.14* |
| **$AUC_{0-24}$** | µg.hr/ml | 19.83 ± 3.48 | 24.34 ± 3.87 | 21.14 ± 2.89 |
| **$AUC_{0-inf}$** | µg.hr/ml | 19.88 ± 3.50 | 24.42 ± 3.87 | 21.36 ± 2.82 |
| **$T_{max}$** | hr | 0.25 ± 0.10 | 0.17 ± 0.04 | 0.25 ± 0.12 |
| **$t_{½}$** | hr | 0.77 ± 0.16 | 0.79 ± 0.08 | 0.85 ± 0.07 |
| **$K_{el}$** | $hr^{-1}$ | 0.90 ± 0.19 | 0.87 ± 0.09 | 0.82 ± 0.07 |
| **$V_{z\_F}$** | L/kg | 0.17 ± 0.02 | 0.14 ± 0.01 | 0.17 ± 0.02 |
| **$Cl_{z\_F}$** | L/hr/kg | 0.15 ± 0.02 | 0.13 ± 0.02 | 0.14 ± 0.02 |

n=6; in each treatment group * $p<0.05$ compared to vehicle using student's unpaired t-test;

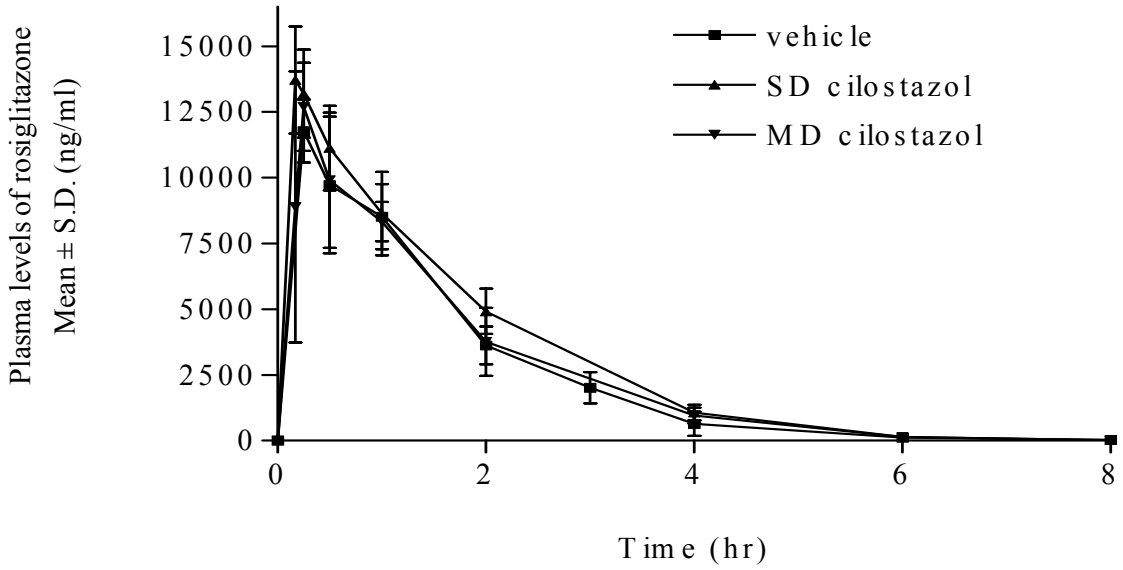



*Figure 4.50 Effect of cilostazol on plasma concentration-time plot of rosiglitazone in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses

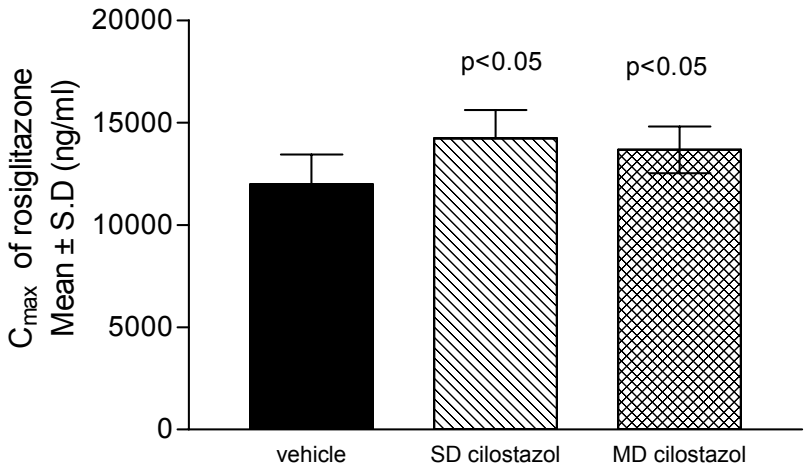



*Figure 4.51 Effect of cilostazol on plasma $C_{max}$ of single dose rosiglitazone in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

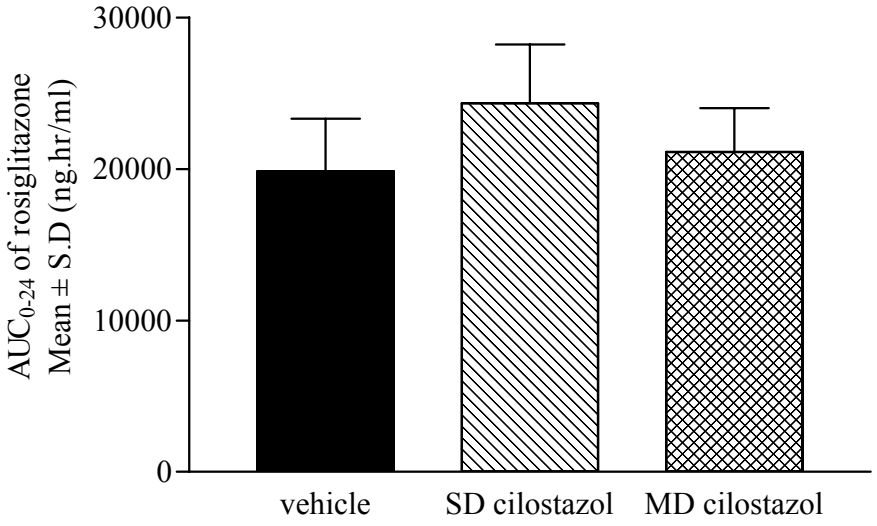



*Figure 4.52 Effect of cilostazol on plasma $AUC_{0-24}$ of single dose rosiglitazone in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

#### *4.4.3.2* Effect of pentoxifylline on rosiglitazone pharmacokinetic parameters by simultaneous administration in rats (Study X)

Rosiglitazone were undetectable in the predose samples in all rats, indicating that pentoxifylline does not interfere with the rosiglitazone assay. **Figure 4.53** shows the plasma concentration-time curve of rosiglitazone in rats receiving rosiglitazone alone or concurrent treatment with pentoxifylline. Visual inspection of the plasma concentration-time curve for rosiglitazone showed that the mean plasma concentrations of rosiglitazone tended to be very slightly decreased. However, no statistically significant differences in the mean plasma concentrations of rosiglitazone were found between the vehicle and pentoxifylline co administered groups at any point of time.

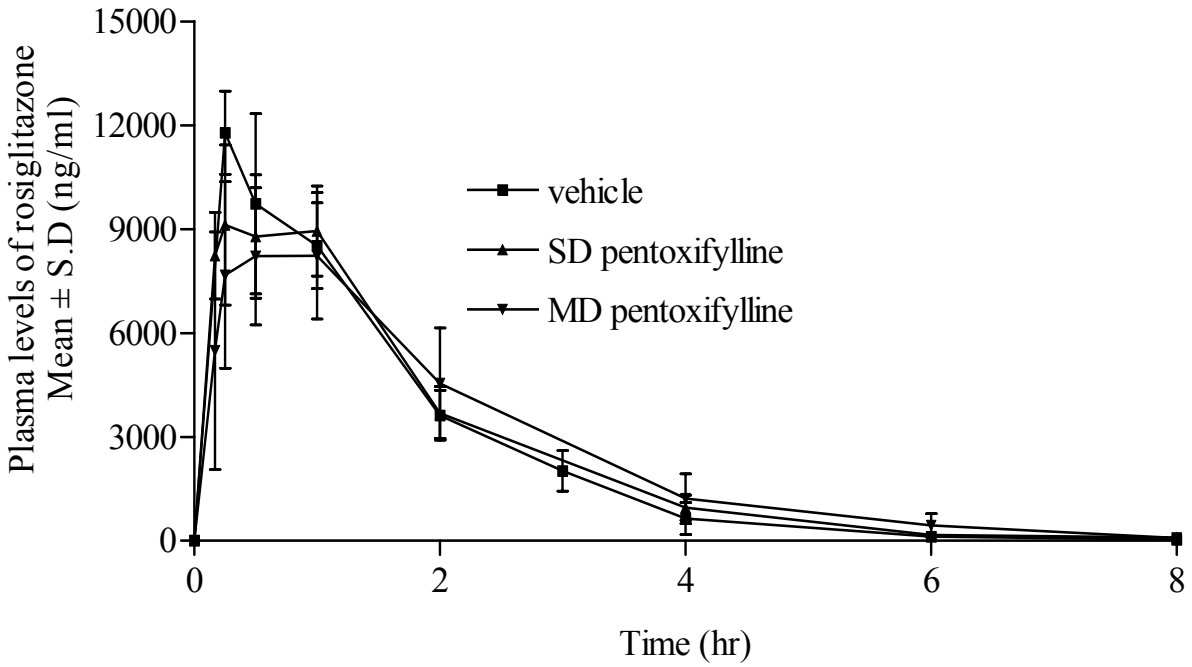



*Figure 4.53 Effect of pentoxifylline on plasma concentration-time plot of rosiglitazone in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses

The estimated pharmacokinetic parameters of rosiglitazone in the absence and presence of pentoxifylline are summarized in **Table 4.39**. The maximum plasma concentration reached ($C_{max}$) for rosiglitazone in presence of vehicle, single dose pentoxifylline and multiple dose pentoxifylline are in the ratio of 1.00: 0.87:0.81 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.03: 1.07. The maximum absorption of rosiglitazone was decreased slightly in presence of single dose pentoxifylline but it was significant ($p<0.05$) only in presence of multiple dose administration of pentoxifylline. Though the systemic exposure of rosiglitazone was slightly increased in presence of pentoxifylline but these parameters were not significantly affected either in presence of single dose or multiple doses of pentoxifylline. The time to reach maximum absorption ($T_{max}$) was slightly delayed in presence of multiple doses of pentoxifylline. The elimination half-life ($t_{1/2}$) was slightly increased significantly ($p<0.05$) in presence of multiple doses of pentoxifylline could be due to the slight delay in absorption.

Table 4.39 Effect of pentoxifylline on single dose pharmacokinetic parameters of rosiglitazone by simultaneous administration of pentoxifylline and rosiglitazone in Wistar rats

| **Parameters of rosiglitazone** | **Units** | **Vehicle treated** (mean ± S.D) | **Pentoxifylline treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 11.98 ± 1.46 | 10.43 ± 1.11 | 9.65 ± 1.91* |
| **$AUC_{0-24}$** | µg.hr/ml | 19.83 ± 3.48 | 20.37 ± 2.02 | 21.26 ± 6.18 |
| **$AUC_{0-inf}$** | µg.hr/ml | 19.88 ± 3.50 | 20.53 ± 2.01 | 21.46 ± 6.11 |
| **$T_{max}$** | Hr | 0.25 ± 0.10 | 0.75 ± 0.38* | 0.75 ± 0.38* |
| **$t_{½}$** | Hr | 0.77 ± 0.16 | 0.89 ± 0.18 | 0.99 ± 0.18* |
| **$K_{el}$** | hr -1 | 0.900 ± 0.190 | 0.782 ± 0.140 | 0.698 ± 0.125 |
| **$V_{z\_F}$** | L/kg | 0.17 ± 0.02 | 0.19 ± 0.04 | 0.21 ± 0.03 |
| **$Cl_{Z\_F}$** | L/hr/kg | 0.15 ± 0.02 | 0.15 ± 0.02 | 0.15 ± 0.04 |

n=6 in each treatment group; * $p<0.05$ compared to vehicle using student's unpaired t-test;

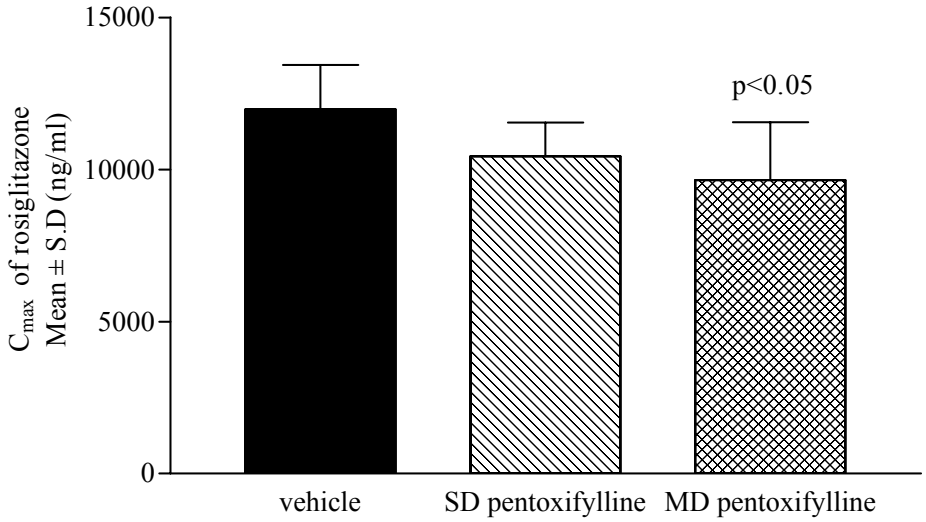



*Figure 4.54 Effect of pentoxifylline on plasma $C_{max}$ of single dose rosiglitazone in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

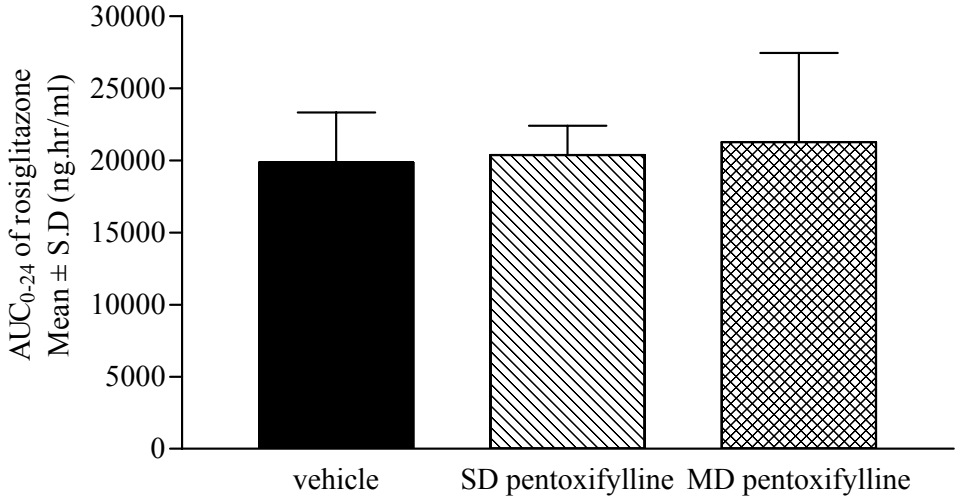



*Figure 4.55 Effect of pentoxifylline on plasma $AUC_{0-24}$ of single dose rosiglitazone in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses;

#### *4.4.3.3* Effect of cilostazol on metformin pharmacokinetic parameters by simultaneous administration in rats (Study XI)

Metformin were undetectable in the predose samples in all rats, indicating that cilostazol does not interfere with the metformin assay. **Fig. 4.56** shows the plasma concentration-time curve of metformin in rats receiving metformin alone or concurrent treatment with cilostazol. Visual inspection of the plasma concentration-time curve for metformin showed that the mean plasma concentrations of metformin tended to be increased.

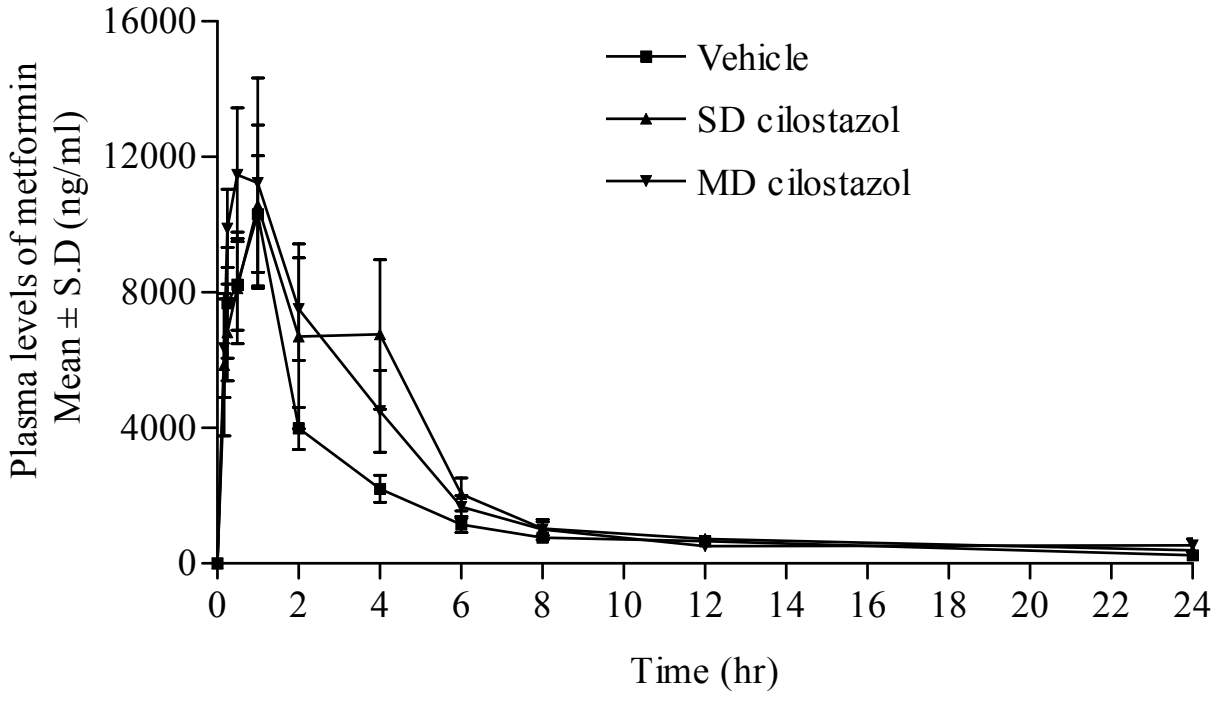



*Figure 4.56 Effect of cilostazol on plasma concentration-time plot of metformin in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses

The estimated pharmacokinetic parameters of cilostazol in the absence and presence of metformin are summarized in **Table 4.40**. The maximum plasma concentration reached ($C_{max}$) for metformin in presence of vehicle, single dose cilostazol and multiple dose cilostazol are in the ratio of 1.00: 1.05:1.18 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.50: 1.42. The maximum absorption of metformin was increased but not significantly in presence of single and multiple doses of cilostazol. The systemic exposures of metformin were significantly increased around 50% ($p<0.0001$) either in presence of single dose or multiple doses of cilostazol. The time to reach maximum absorption ($T_{max}$) was also not effected in presence of either single or multiple doses of cilostazol. The half-life of metformin was significantly decreased from 9.08 hr to 7.96 hr after multiple dose administration of cilostazol.

Table 4.40 Effect of cilostazol on single dose pharmacokinetic parameters of metformin by simultaneous administration of cilostazol and metformin in Wistar rats

| **Parameters of metformin** | **Units** | **Vehicle treated** (mean ± S.D) | **Cilostazol treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 10.31 ± 1.72 | 10.87 ± 2.14 | 12.19 ± 2.27 |
| **$AUC_{0-24}$** | µg.hr/ml | 34.52 ± 3.43 | 51.82 ± 5.12*** | 49.05 ± 4.40*** |
| **$AUC_{0-inf}$** | µg.hr/ml | 38.35 ± 5.17 | 57.62 ± 5.96*** | 56.26 ± 7.29*** |
| **$T_{max}$** | hr | 1.00 ± 0.00 | 1.00 ± 1.22 | 0.75 ± 0.27 |
| **$t_{½}$** | hr | 9.08 ± 3.80 | 8.52 ± 3.22 | 7.96 ± 3.06 |
| **$K_{el}$** | $hr^{-1}$ | 0.08 ± 0.02 | 0.08 ± 0.03 | 0.09 ± 0.03 |
| **$V_{z\_F}$** | L/kg | 110.20 ± 29.53 | 70.19 ± 21.22 | 65.94 ± 18.22 |
| **$Cl_{Z\_F}$** | L/hr/kg | 7.95 ± 1.12 | 5.26 ± 0.58 | 5.41 ± 0.74 |

n=6 in each treatment group; *** $p<0.001$ compared to vehicle using student's unpaired t-test;

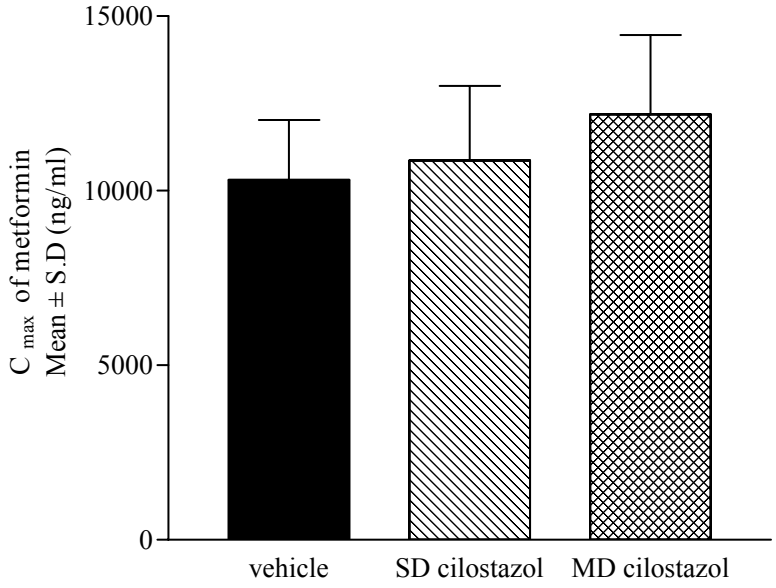



*Figure 4.57 Effect of cilostazol on plasma $C_{max}$ of single dose metformin in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

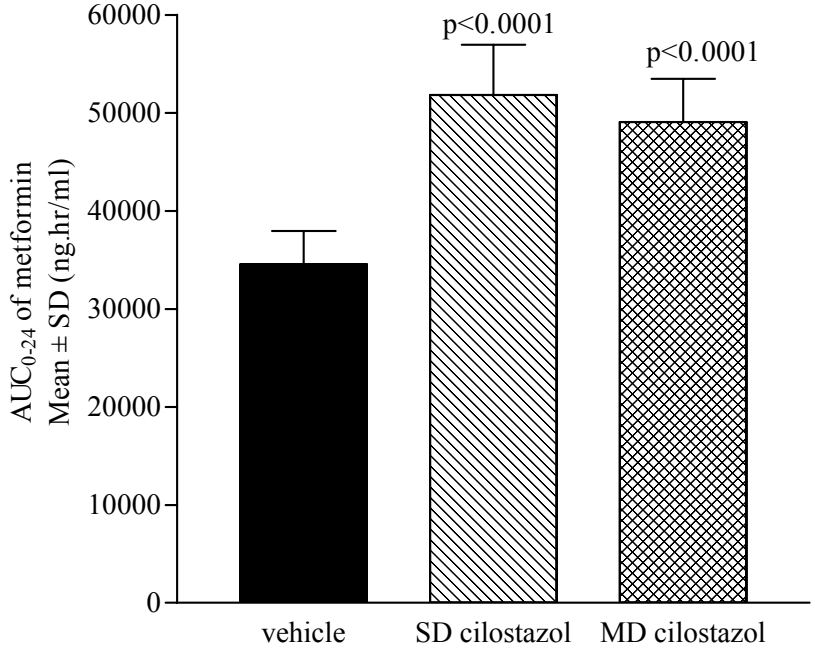



*Figure 4.58 Effect of cilostazol on plasma $AUC_{0-24}$ of single dose metformin in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

#### *4.4.3.4* Effect of cilostazol on nateglinide pharmacokinetic parameters by simultaneous administration in rats (Study XII)

Nateglinide were undetectable in the predose samples in all rats, indicating that cilostazol does not interfere with the nateglinide assay. **Fig. 4.59** shows the plasma concentration-time curve of nateglinide in rats receiving nateglinide alone or concurrent treatment with cilostazol. Visual inspection of the plasma concentration-time curve for nateglinide showed that the mean plasma concentrations of nateglinide tended to be decreased.

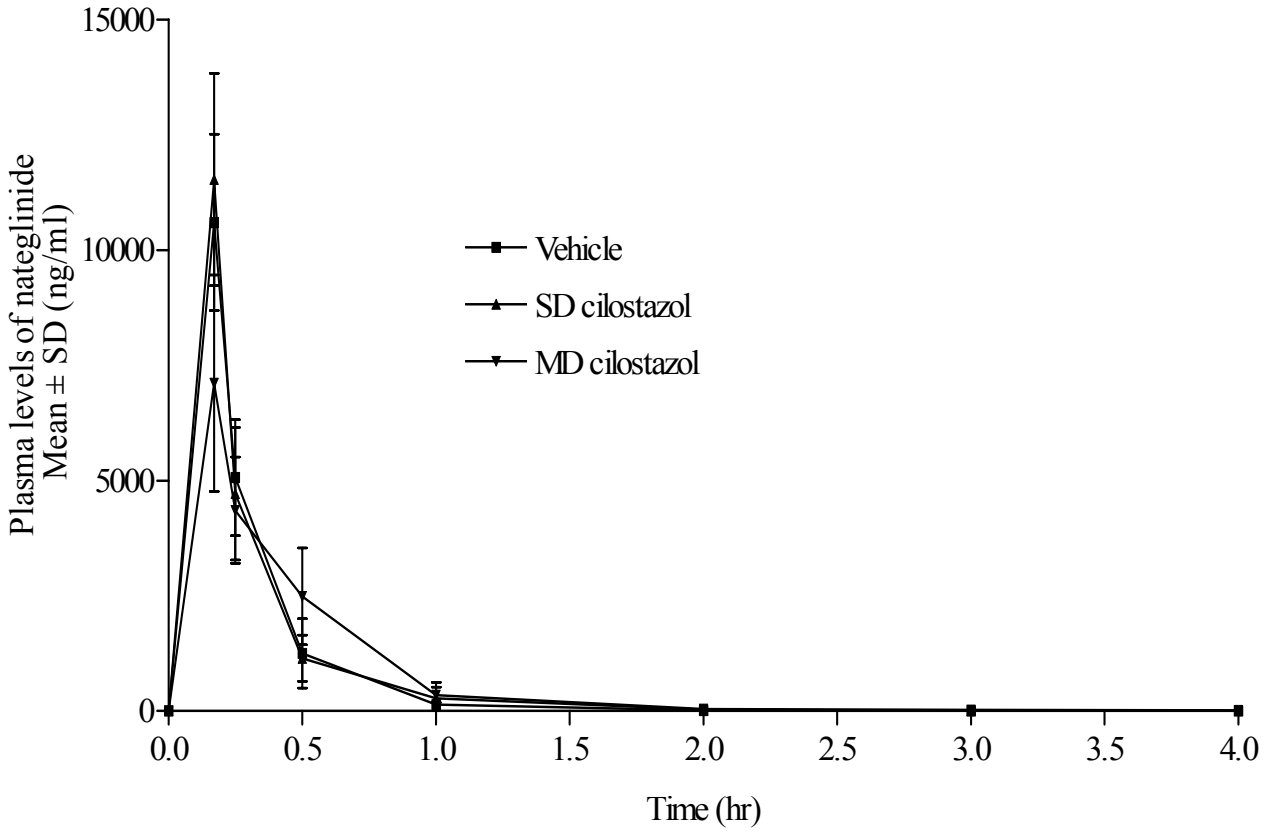



*Figure 4.59 Effect of cilostazol on plasma concentration-time plot of nateglinide in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses

The estimated pharmacokinetic parameters of nateglinide in the absence and presence of cilostazol are summarized in **Table 4.41**. The maximum plasma concentration reached ($C_{max}$) for nateglinide in presence of vehicle, single dose cilostazol and multiple dose cilostazol are in the ratio of 1.00: 1.09:0.67 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.05: 1.06. The maximum absorption of nateglinide was decreased significantly ($p<0.05$) in presence of multiple dose but not with single dose administration of cilostazol. The systemic exposure of nateglinide was not significantly changed either in presence of single dose or multiple doses of cilostazol. The time to reach maximum absorption ($T_{max}$) was also not effected in presence of either single or multiple doses of cilostazol. The half-life of nateglinide was significantly increased from 0.16 hr to 0.32 hr after multiple dose administration of cilostazol.

Table 4.41 Effect of cilostazol on single dose pharmacokinetic parameters of nateglinide by simultaneous administration of cilostazol and nateglinide in Wistar rats

| **Parameters of nateglinide** | **Units** | **Vehicle treated** (mean ± S.D) | **Cilostazol treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 10.60 ± 1.91 | 11.53 ± 2.30 | 7.31 ± 2.35* |
| **$AUC_{0-24}$** | µg.hr/ml | 2.71 ± 0.50 | 2.85 ± 0.55 | 2.86 ± 0.51 |
| **$AUC_{0-inf}$** | µg.hr/ml | 2.73 ± 0.50 | 2.87 ± 0.54 | 2.88 ± 0.51 |
| **$T_{max}$** | hr | 0.17 ± 0.00 | 0.17 ± 0.00 | 0.17 ± 0.00 |
| **$t_{½}$** | hr | 0.16 ± 0.08 | 0.19 ± 0.11 | 0.32 ± 0.11 |
| **$K_{el}$** | $hr^{-1}$ | 4.434 ± 1.184 | 3.630 ± 1.502 | 2.155 ± 0.727 |
| **$V_{z\_F}$** | L/kg | 0.92 ± 0.29 | 1.13 ± 0.56 | 1.79 ± 0.64 |
| **$Cl_{z\_F}$** | L/hr/kg | 3.76 ± 0.63 | 3.59 ± 0.72 | 3.55 ± 0.51 |

n=6 in each treatment group; * $p<0.05$ compared to vehicle using student's unpaired t-test;

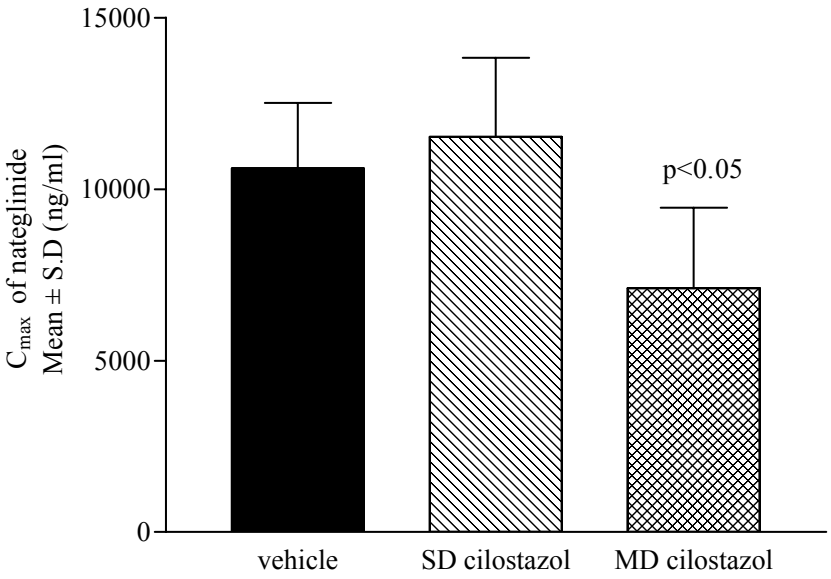



*Figure 4.60 Effect of cilostazol on plasma $C_{max}$ of single dose nateglinide in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

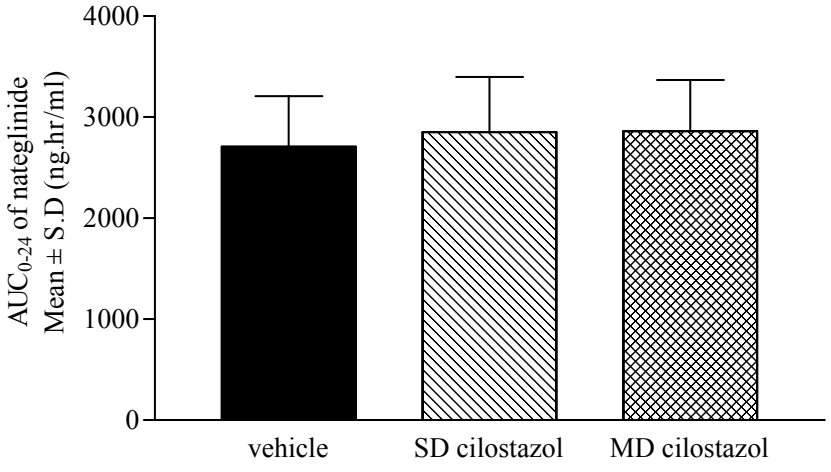



*Figure 4.61 Effect of cilostazol on plasma $AUC_{0-24}$ of single dose nateglinide in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

#### 4.4.3.5 Effect of cilostazol on glipizide pharmacokinetic parameters by simultaneous administration in rats (Study XIII)

Glipizide were undetectable in the predose samples in all rats, indicating that cilostazol does not interfere with the glipizide assay. **Fig. 4.62** shows the plasma concentration-time curve of glipizide in rats receiving glipizide alone or concurrent treatment with cilostazol. Visual inspection of the plasma concentration-time curve for glipizide showed that the mean plasma concentrations of glipizide tended to be decreased.

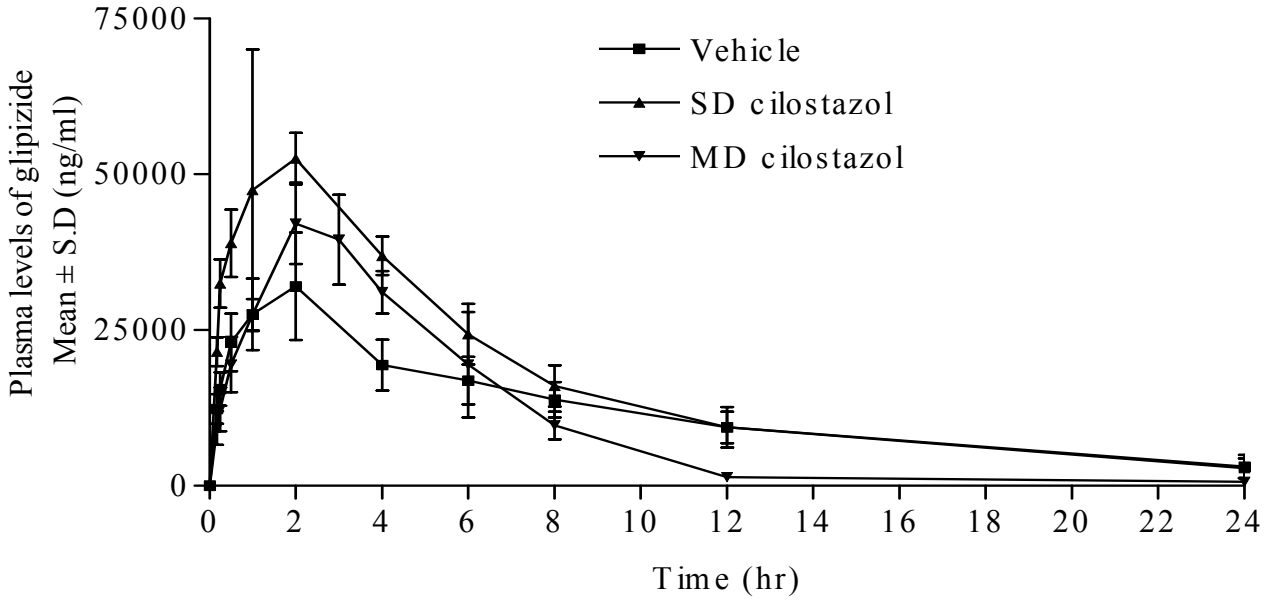



Figure 4.62 Effect of cilostazol on plasma concentration-time plot of glipizide in Wistar rats n=6 in each treatment group; SD-single dose; MD-multiple doses

The estimated pharmacokinetic parameters of cilostazol in the absence and presence of glipizide are summarized in **Table 4.42**. The maximum plasma concentration reached ($C_{max}$) for glipizide in presence of vehicle, single dose cilostazol and multiple dose cilostazol are in the ratio of 1.00: 1.86:1.33 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.36:0.84. The maximum absorption of glipizide was increased significantly ($p<0.05$) in presence of multiple dose but not with single dose administration of cilostazol. Though the systemic exposures of

glipizide was significantly increased in presence of single dose of cilostazol ($p<0.01$) but not changed significantly in presence of multiple doses of cilostazol. The time to reach maximum absorption ($T_{max}$) was also not effected in presence of either single or multiple doses of cilostazol. The half-life of glipizide was significantly decreased from 6.97 hr to 4.85 hr after multiple dose administration of cilostazol ($p<0.05$).

Table 4.42 Effect of cilostazol on single dose pharmacokinetic parameters of glipizide by simultaneous administration of cilostazol and glipizide in Wistar rats

| **Parameters of glipizide** | **Units** | **Vehicle treated** (mean ± S.D) | **Cilostazol treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 32.15 ± 8.50 | 59.73 ± 16.78 | 42.68 ± 6.27* |
| **$AUC_{0-24}$** | µg.hr/ml | 289.46 ± 64.90 | 399.69 ± 50.38 | 242.44 ± 34.68 |
| **$AUC_{0-inf}$** | µg.hr/ml | 325.62 ± 97.01 | 428.32 ± 71.58 | 247.46 ± 36.81 |
| **$T_{max}$** | Hr | 2.00 ± 0.61 | 2.00 ± 0.41 | 2.50 ± 0.82 |
| **$t_{½}$** | Hr | 6.97 ± 1.78 | 6.67 ± 1.78 | 2.91 ± 0.96 |
| **$K_{el}$** | hr -1 | 0.099 ± 0.020 | 0.104 ± 0.026 | 0.238 ± 0.100 |
| **$V_{z\_F}$** | L/kg | 0.33 ± 0.04 | 0.23 ± 0.05 | 0.21 ± 0.07 |
| **$Cl_{Z\_F}$** | L/hr/kg | 0.03 ± 0.01 | 0.02 ± 0.00 | 0.04 ± 0.01 |

n=6 in each treatment group; * $p<0.05$ compared to vehicle using student's unpaired t-test;

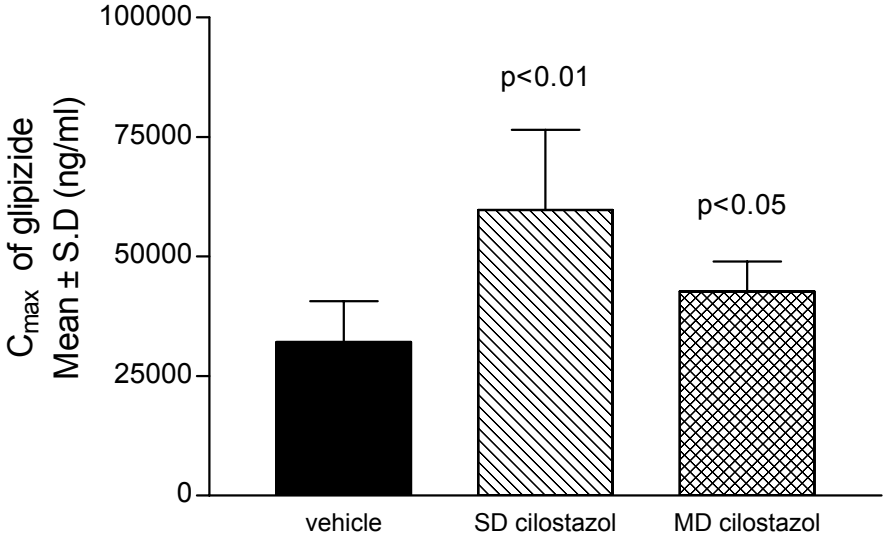



*Figure 4.63 Effect of cilostazol on plasma $C_{max}$ of single dose glipizide in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

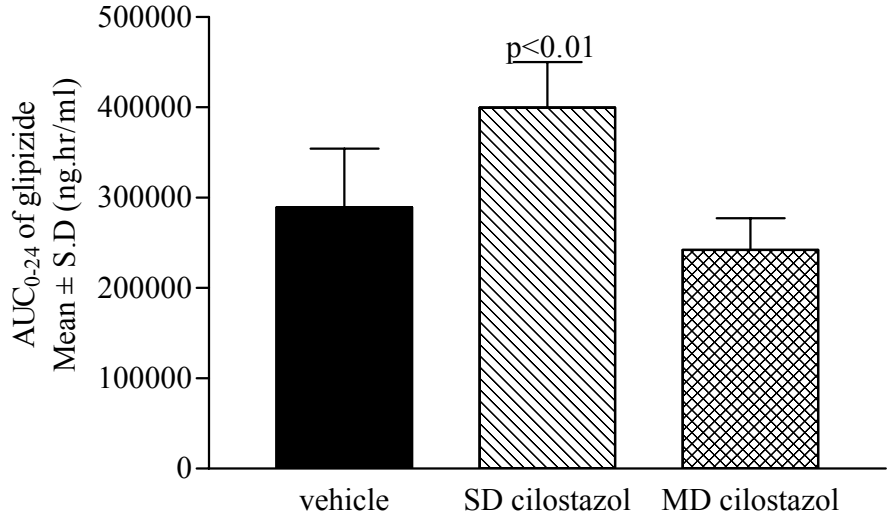



*Figure 4.64 Effect of cilostazol on plasma $AUC_{0-24}$ of single dose glipizide in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

#### *4.4.3.6* Effect of pentoxifylline on metformin pharmacokinetic parameters by simultaneous administration in rats (Study XIV)

Metformin were undetectable in the predose samples in all rats, indicating that pentoxifylline does not interfere with the metformin assay. **Fig. 4.65** shows the plasma concentration-time curve of metformin in rats receiving metformin alone or concurrent treatment with pentoxifylline. Visual inspection of the plasma concentration-time curve for metformin showed that the mean plasma concentrations of metformin tended to be increased but not through out the study.

The estimated pharmacokinetic parameters of metformin in the absence and presence of pentoxifylline are summarized in **Table 4.43**. The maximum plasma concentration reached ($C_{max}$) for metformin in presence of vehicle, single dose pentoxifylline and multiple dose pentoxifylline are in the ratio of 1.00: 1.43: 1.01 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 0.81: 0.71. The maximum absorption of metformin was increased significantly ($p<0.0001$) in presence of single dose pentoxifylline but it was not changed in

Table 4.43 Effect of pentoxifylline on single dose pharmacokinetic parameters of metformin by simultaneous administration of pentoxifylline and metformin in Wistar rats

| Parameters of metformin | Units | Vehicle treated (mean ± S.D) | Pentoxifylline treated | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| $C_{max}$ | µg/ml | 10.31 ± 1.72 | 14.76 ± 1.09*** | 10.38 ± 1.90 |
| $AUC_{0-24}$ | µg.hr/ml | 34.52 ± 3.43 | 27.85 ± 6.95 | 24.39 ± 2.76**** |
| $AUC_{0-inf}$ | µg.hr/ml | 38.35 ± 5.17 | 29.25 ± 7.08 | 25.12 ± 2.80**** |
| $T_{max}$ | Hr | 1.00 ± 0.00 | 0.50 ± 0.16 | 0.38 ± 0.17 |
| $t_{½}$ | Hr | 9.08 ± 3.80 | 2.00± 3.22 | 2.37 ± 0.80 |
| $K_{el}$ | hr -1 | 0.08 ± 0.02 | 0.277 ± 0.100 | 0.293 ± 0.078 |
| $V_{z\_F}$ | L/kg | 110.20 ± 29.53 | 43.49 ± 19.66 | 44.27 ± 15.51 |
| $Cl_{Z\_F}$ | L/hr/kg | 7.95 ± 1.12 | 10.70 ± 2.32 | 12.07 ± 1.38 |

n=6 in each treatment group; ***p<0.001, *** p<0.0001 compared to vehicle using student's unpaired t-test;

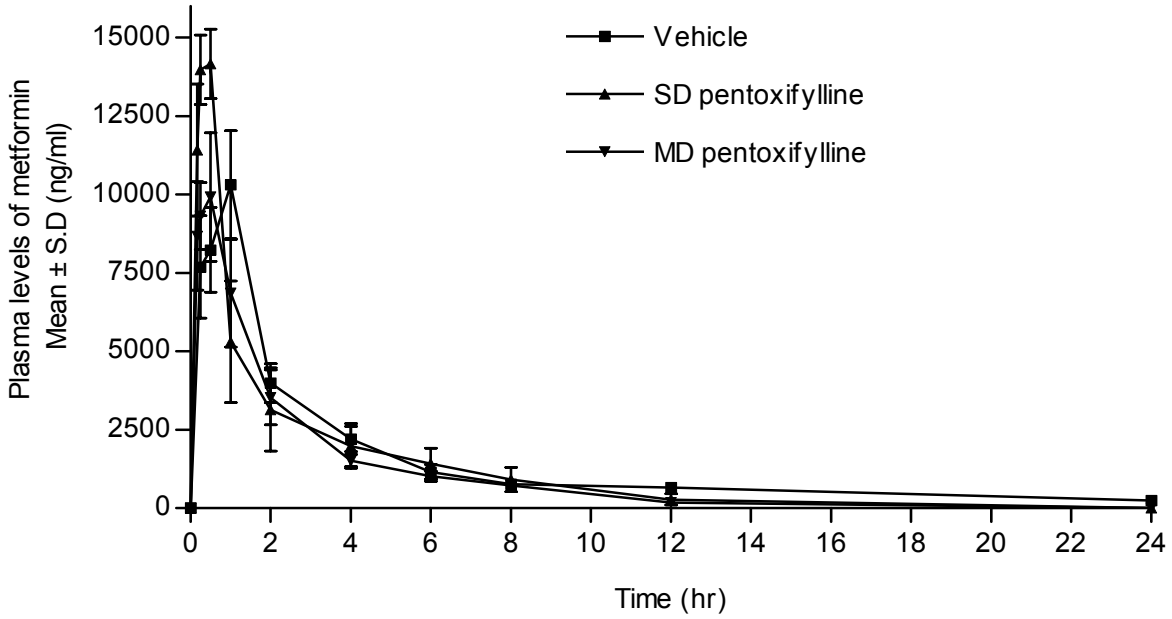



Figure 4.65 *Effect of pentoxifylline on plasma concentration-time plot of metformin in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses

presence of multiple dose administration of pentoxifylline. Though the systemic exposure of metformin was slightly decreased in presence of pentoxifylline it decreased the AUC by 29% significantly ($p<0.0001$) in presence of multiple doses of pentoxifylline. The time to reach maximum absorption ($T_{max}$) was significantly reduced ($p<0.05$) in presence of multiple doses of pentoxifylline. The elimination half-life ($t_{1/2}$) was significantly decreased around 3 times lower ($p<0.05$) in presence of both single and multiple doses of pentoxifylline.

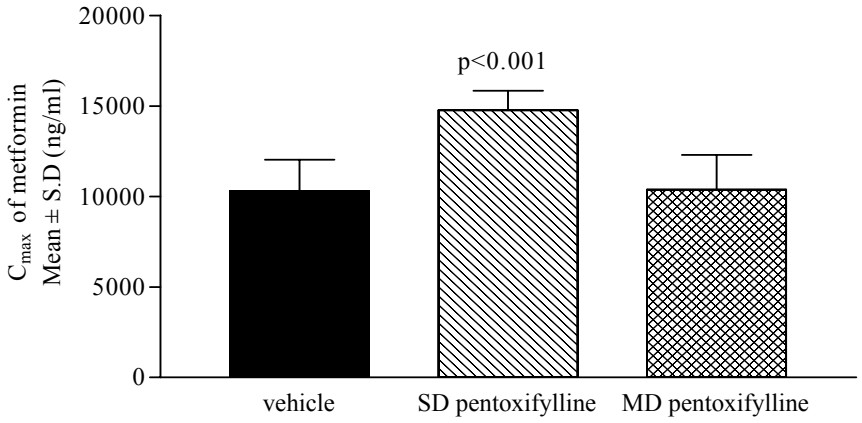



*Figure 4.66 Effect of pentoxifylline on plasma $C_{max}$ of single dose metformin in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

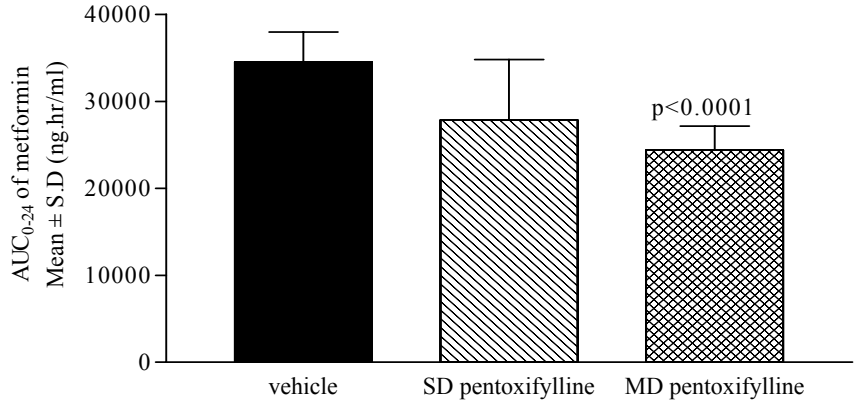



*Figure 4.67 Effect of pentoxifylline on plasma $AUC_{0-24}$ of single dose metformin in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

### *4.4.3.7* Effect of pentoxifylline on nateglinide pharmacokinetic parameters by simultaneous administration in rats (Study XV)

Nateglinide levels were undetectable in the predose samples in all rats, indicating that pentoxifylline does not interfere with the nateglinide assay. **Fig. 4.68** shows the plasma concentration-time curve of nateglinide in rats receiving nateglinide alone or concurrent treatment with pentoxifylline. Visual inspection of the plasma concentration-time curve for nateglinide showed that the mean plasma concentrations of nateglinide tended to be decreased.

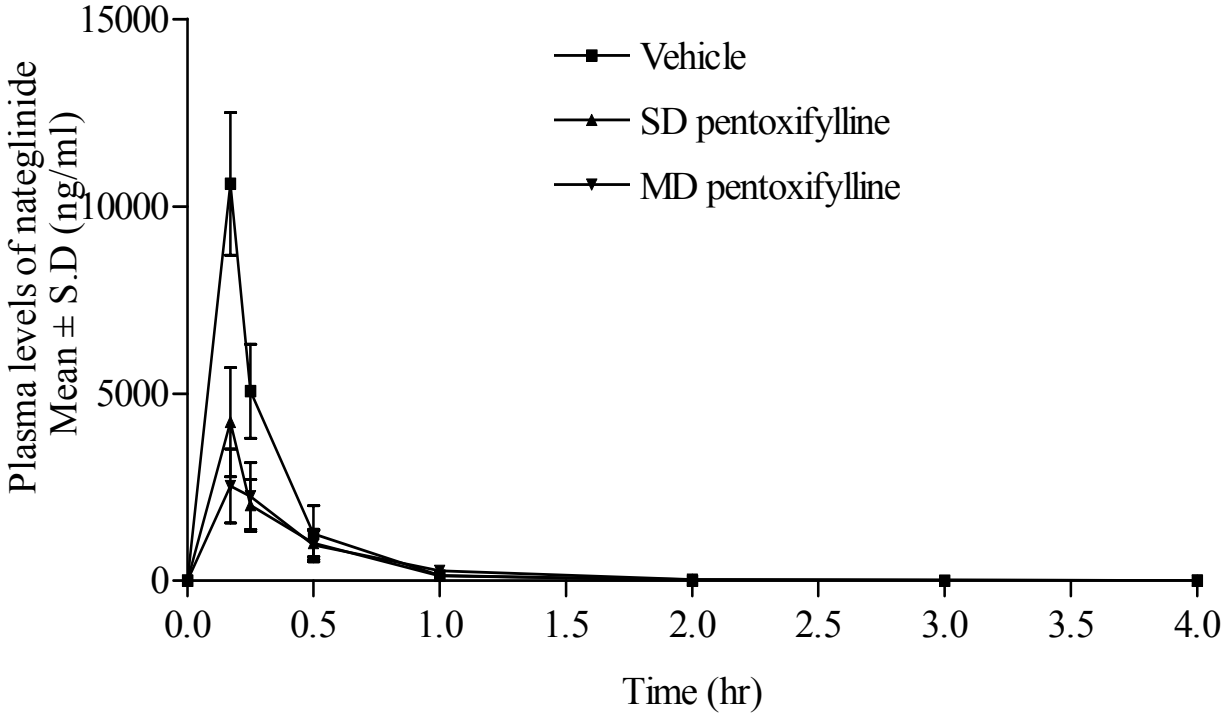



*Figure 4.68 Effect of pentoxifylline on plasma concentration-time plot of nateglinide in Wistar rats*

n=6 in each treatment group; SD-single dose; MD-multiple doses

The estimated pharmacokinetic parameters of nateglinide in the absence and presence of pentoxifylline are summarized in **Table 4.44**. The maximum plasma concentration reached ($C_{max}$) for nateglinide in presence of vehicle, single dose pentoxifylline and multiple dose pentoxifylline are in the ratio of 1.00: 0.40: 0.26 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 0.50: 0.46. The maximum absorption of nateglinide was significantly decreased ($p<0.0001$) in presence of single dose (by 60%) and multiple dose (by74%) administration of pentoxifylline. The systemic exposure was also significantly decreased by around 50% ($p<0.0001$) in presence of both single dose multiple dose administration of pentoxifylline. Though the systemic exposure of nateglinide was decreased in presence of pentoxifylline but the time to reach maximum absorption ($T_{max}$) and the elimination half-life ($t_{1/2}$) were slightly increased but not significant.

Table 4.44 Effect of pentoxifylline on single dose pharmacokinetic parameters of nateglinide by simultaneous administration of pentoxifylline and nateglinide in Wistar rats

| **Parameters of nateglinide** | **Units** | **Vehicle treated** (mean ± S.D) | **Pentoxifylline treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 10.60 ± 1.91 | 4.24 ± 1.45*** | 2.80 ± 0.72**** |
| **$AUC_{0-24}$** | µg.hr/ml | 2.71 ± 0.50 | 1.35 ± 0.36*** | 1.24 ± 0.24**** |
| **$AUC_{0-inf}$** | µg.hr/ml | 2.73 ± 0.50 | 1.37 ± 0.37*** | 1.27 ± 0.25**** |
| **$T_{max}$** | Hr | 0.17 ± 0.00 | 0.17 ± 0.00 | 0.17 ± 0.04 |
| **$t_{½}$** | Hr | 0.16 ± 0.08 | 0.21 ± 0.11 | 0.25 ± 0.09 |
| **$K_{el}$** | hr -1 | 4.434 ± 1.184 | 3.200 ± 1.770 | 2.795 ± 1.052 |
| **$V_{z\_F}$** | L/kg | 0.92 ± 0.29 | 3.13 ± 1.91 | 3.30 ± 1.41 |
| **$Cl_{Z\_F}$** | L/hr/kg | 3.76 ± 0.63 | 7.93 ± 1.65 | 8.16 ± 1.65 |

n=6 in each treatment group; *** $p<0.001$, ****$p<0.0001$ compared to vehicle using student's unpaired t-test;

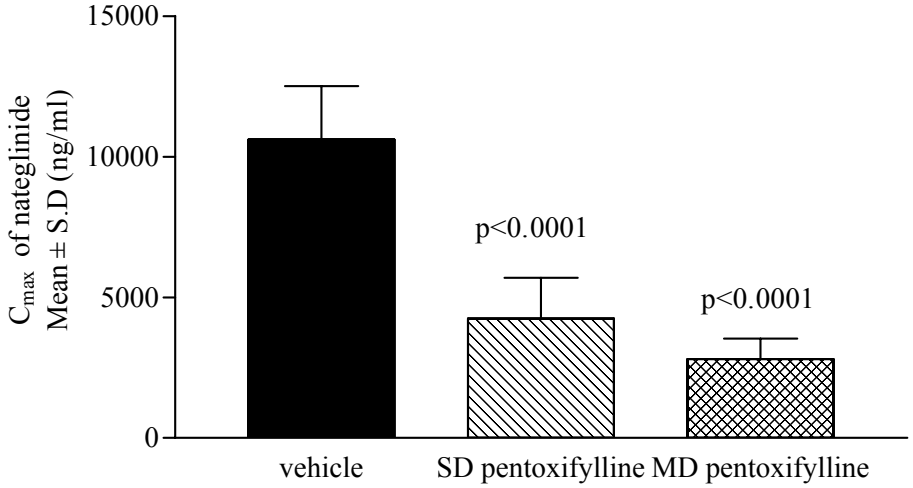



*Figure 4.69 Effect of pentoxifylline on plasma $C_{max}$ of single dose nateglinide in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

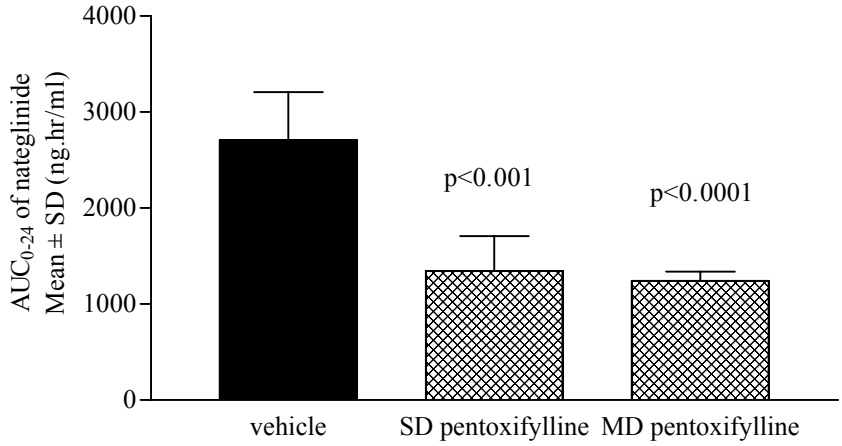



*Figure 4.70 Effect of pentoxifylline on plasma $AUC_{0-24}$ of single dose nateglinide in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

### *4.4.3.8* Effect of pentoxifylline on glipizide pharmacokinetic parameters by simultaneous administration in rats (Study XVI)

Glipizide were undetectable in the predose samples in all rats, indicating that pentoxifylline does not interfere with the glipizide assay. **Fig. 4.71** shows the plasma concentration-time curve of glipizide in rats receiving glipizide alone or concurrent treatment with pentoxifylline. Visual inspection of the plasma concentration-time curve for glipizide showed that the mean plasma concentrations of glipizide tended to be decreased but not through out the study.

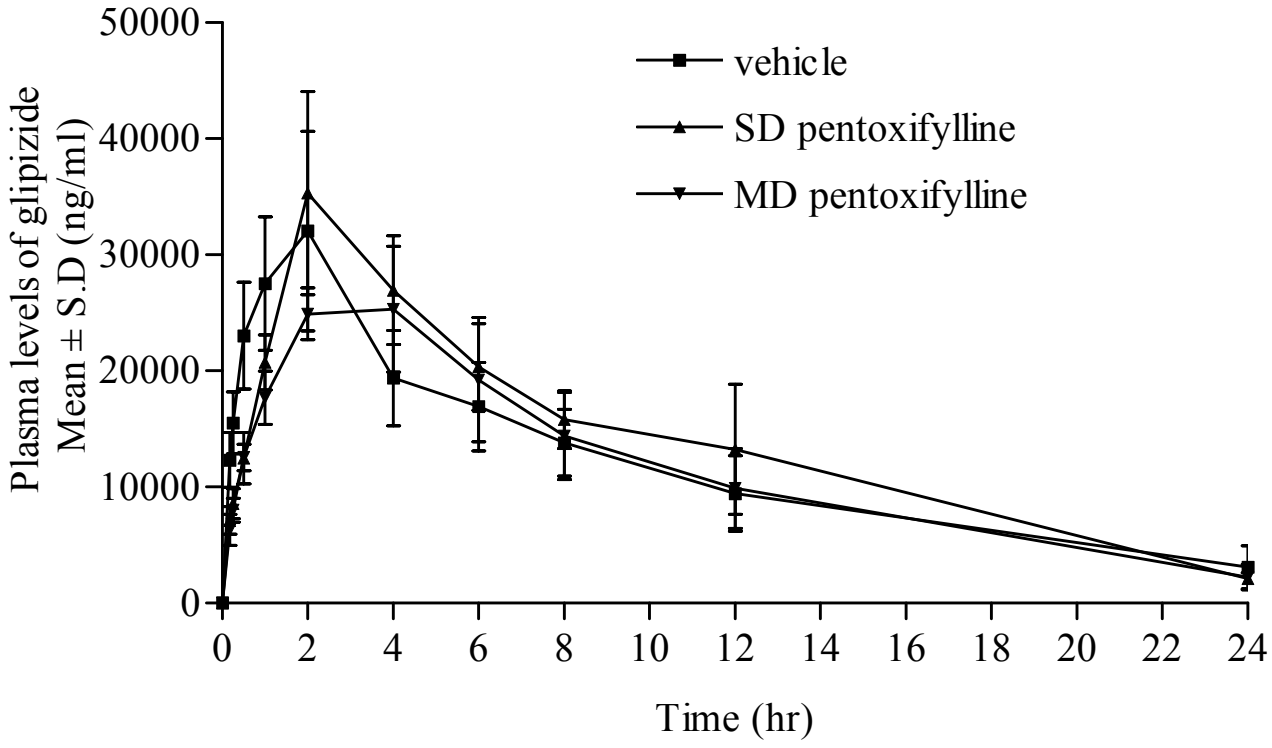



*Figure 4.71 Effect of pentoxifylline on plasma concentration-time plot of glipizide in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses

The estimated pharmacokinetic parameters of glipizide in the absence and presence of pentoxifylline are summarized in **Table 4.45**. The maximum plasma concentration reached ($C_{max}$) for glipizide in presence of vehicle, single dose pentoxifylline and multiple dose pentoxifylline are in the ratio of 1.00: 1.13: 0.85 where as the systemic exposures ($AUC_{0-24}$) are in the ratio of 1.00: 1.16: 0.98. The maximum absorption of glipizide was decreased slightly increased in presence of single dose pentoxifylline but it was decreased slightly in presence of multiple dose administration of pentoxifylline and these were not significant. Though the systemic exposure of glipizide was slightly increased in presence of single dose pentoxifylline but these parameters were not significantly affected in presence of multiple doses of pentoxifylline. The time to reach maximum absorption ($T_{max}$) was slightly increased in presence of multiple doses of pentoxifylline. The elimination half-life ($t_{1/2}$) was slightly decreased significantly ($p<0.05$) in presence of single dose but not significant in multiple doses of pentoxifylline.

Table 4.45 Effect of pentoxifylline on single dose pharmacokinetic parameters of glipizide by simultaneous administration of pentoxifylline and glipizide in Wistar rats

| **Parameters of glipizide** | **Units** | **Vehicle treated** (mean ± S.D) | **Pentoxifylline treated** | |
|---|---|---|---|---|
| | | | Single dose (mean ± S.D) | Multiple dose (mean ± S.D) |
| **$C_{max}$** | µg/ml | 32.12 ± 8.50 | 36.45 ± 7.92 | 27.18 ± 4.55 |
| **$AUC_{0-24}$** | µg.hr/ml | 289.46 ± 64.90 | 336.16 ± 51.08 | 282.24 ± 64.48 |
| **$AUC_{0-inf}$** | µg.hr/ml | 325.62 ± 97.01 | 353.25 ± 49.22 | 301.56 ± 75.36 |
| **$T_{max}$** | Hr | 2.00 ± 0.67 | 2.00 ± 1.03 | 3.00 ± 1.10 |
| **$t_{½}$** | Hr | 6.96 ± 1.76 | 5.52 ± 0.39 | 5.57 ± 0.83 |
| **$K_{el}$** | hr -1 | 0.10 ± 0.02 | 0.13 ± 0.01 | 0.12 ± 0.02 |
| **$V_{z\_F}$** | L/kg | 0.33 ± 0.04 | 0.23 ± 0.04 | 0.28 ± 0.04 |
| **$Cl_{Z\_F}$** | L/hr/kg | 0.03 ± 0.01 | 0.03 ± 0.00 | 0.03 ± 0.01 |

n=6 in each treatment group;

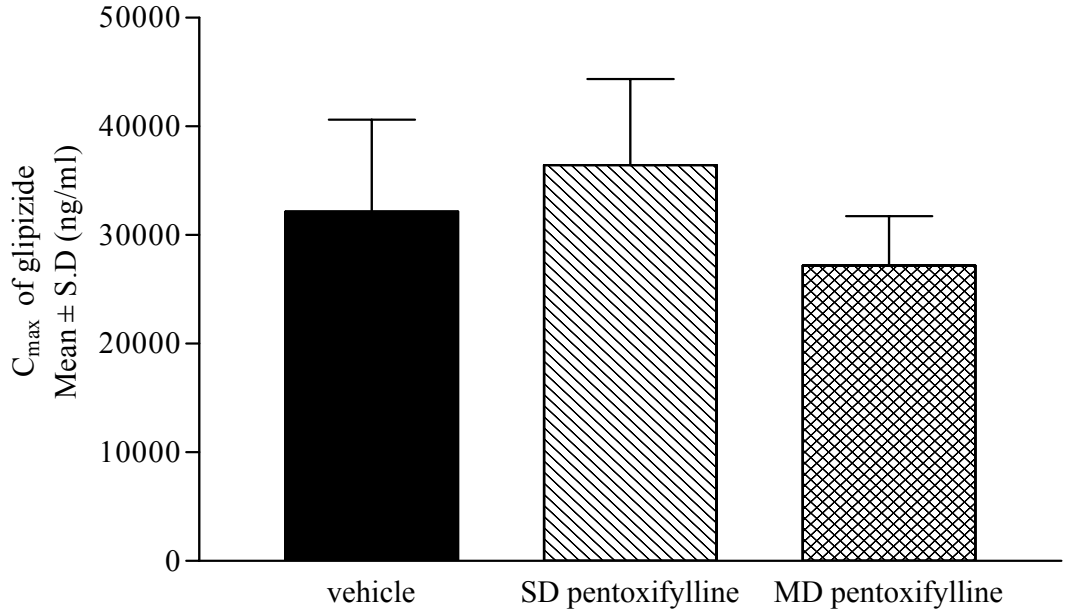



Figure 4.72 *Effect of pentoxifylline on plasma $C_{max}$ of single dose glipizide in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

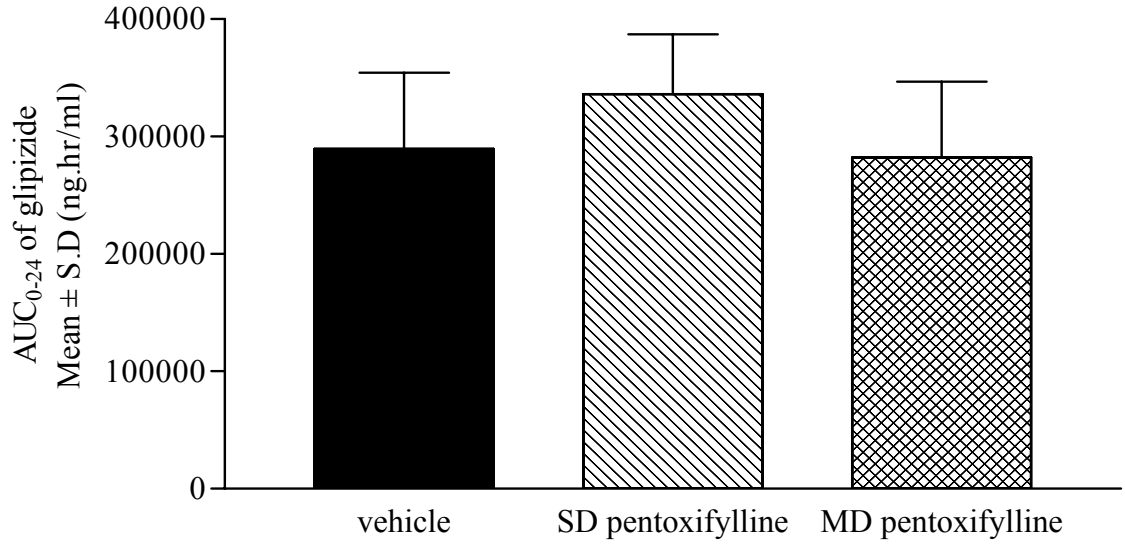



*Figure 4.73 Effect of pentoxifylline on plasma $AUC_{0-24}$ of single dose glipizide in Wistar rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

### 4.4.4 Effect of ADA on cilostazol antiplatelet activity in rats

The ex vivo antiplatelet activity of cilostazol was studied in PRP prepared from pretreatment of vehicle or antidiabetic drug. . The antiplatelet activity of cilostazol was chnaged from 61.60 % (vehicle treated) to 78.93 % (glipizide treated) and was not changed much in presence of other ADAs. Thus glipizide significantly improved ($p<0.001$) the antiplatelet activity of cilostazol.

Table 4.46 Effect of antidiabetic agents on antiplatelet activity of cilostazol in Wistar rats

| S.No. | Group | AUC of % transmittance (mean ± S.D) | % reduction in AUC |
|---|---|---|---|
| 1 | control (w/o cilostazol) | 62.50 ± 2.65 | - |
| 2 | vehicle treated | 24.00 ± 1.50 | 61.60 |
| 3 | Rosiglitazone treated | 24.50 ± 2.78 | 60.80 |
| 4 | Metformin treated | 22.33 ± 0.76 | 64.27 |
| 5 | Nateglinide treated | 19.50 ± 2.29 | 68.80 |
| 6 | Glipizide treated | 13.17 ± 1.76 | 78.93 |

n=3 in each treatment group;

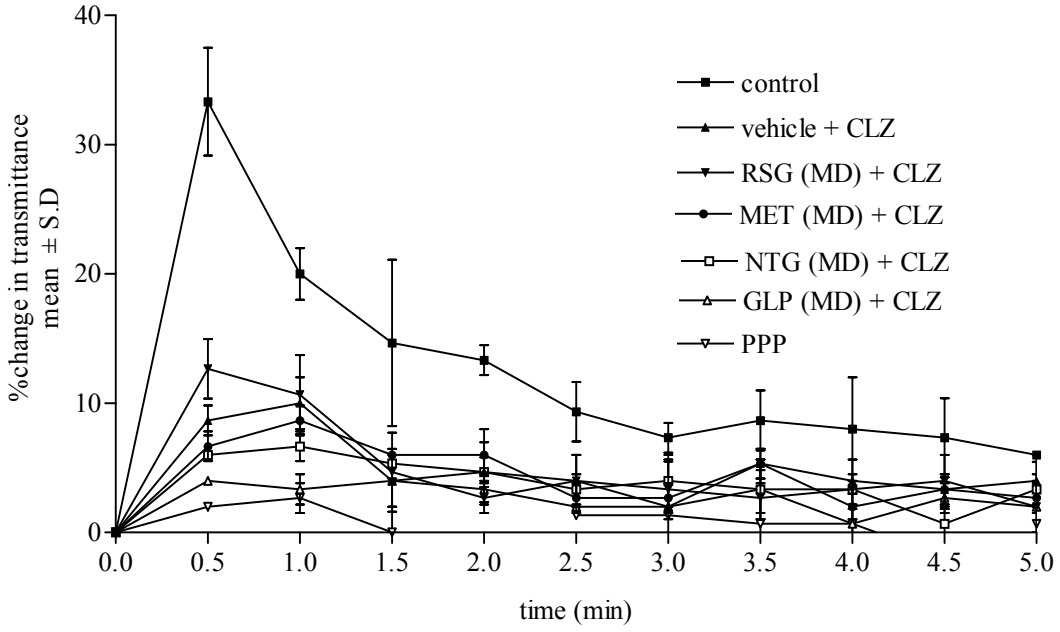



*Figure 4.74 Effect of ADA on platelet activity of cilostazol in rat plasma*
n=3 in each treatment group; PPP-platelet poor plasma; MD-multiple doses

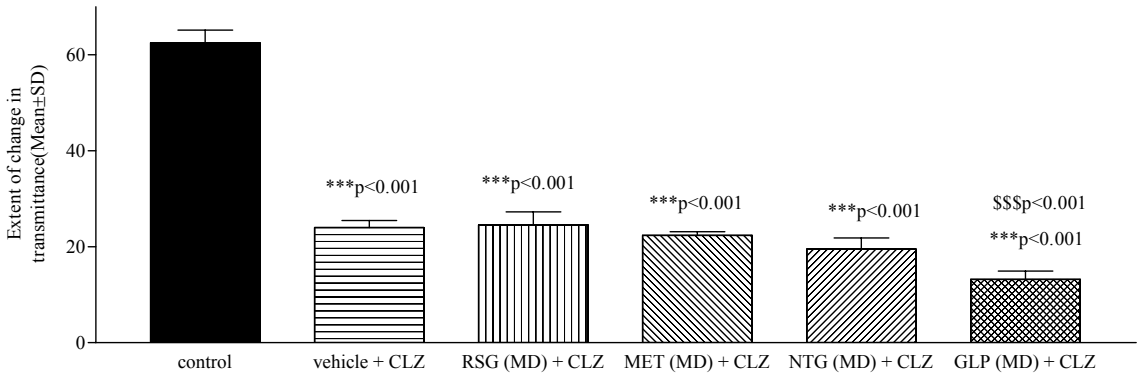



*Figure 4.75 Effect of anti diabetic agents on ex vivo platelet aggregation potential of cilostazol in Wistar rats*

n=3 in each treatment group; MD-multiple doses; * p values compared to that of control using student's unpaired t-test; $ p values compared to that of vehicle + CLZ using student's boniferroni's multiple comparision test

### 4.4.5 Effect of ant diabetic agents on pentoxifylline antiplatelet activity in rats

The ex vivo antiplatelet activity of pentoxifylline was studied in PRP prepared from pretreatment of vehicle or antidiabetic drug. . The antiplatelet activity of pentoxifylline was significantly increased from 44.50 % (vehicle treated) to 58.25 % (glipizide treated) Thus glipizide significantly improved ($p<0.01$) the antiplatelet activity of pentoxifylline.

Table 4.47 Effect of antidiabetic agents on antiplatelet activity of pentoxifylline in Wistar rats

| S.No. | Group | AUC of % transmittance (Mean ± S.D) | % reduction in AUC |
|---|---|---|---|
| 1 | control (w/o pentoxifylline) | 60.67±3.18 | - |
| 2 | Vehicle treated | 33.67±1.76 | 44.50 |
| 3 | Rosiglitazone treated | 38.17±1.04 | 37.09 |
| 4 | Metformin treated | 46.83±1.53 | 22.81 |
| 5 | Nateglinide treated | 30.17±2.84 | 50.27 |
| 6 | Glipizide treated | 25.33±0.76 | 58.25 |

n=3 in each treatment group;

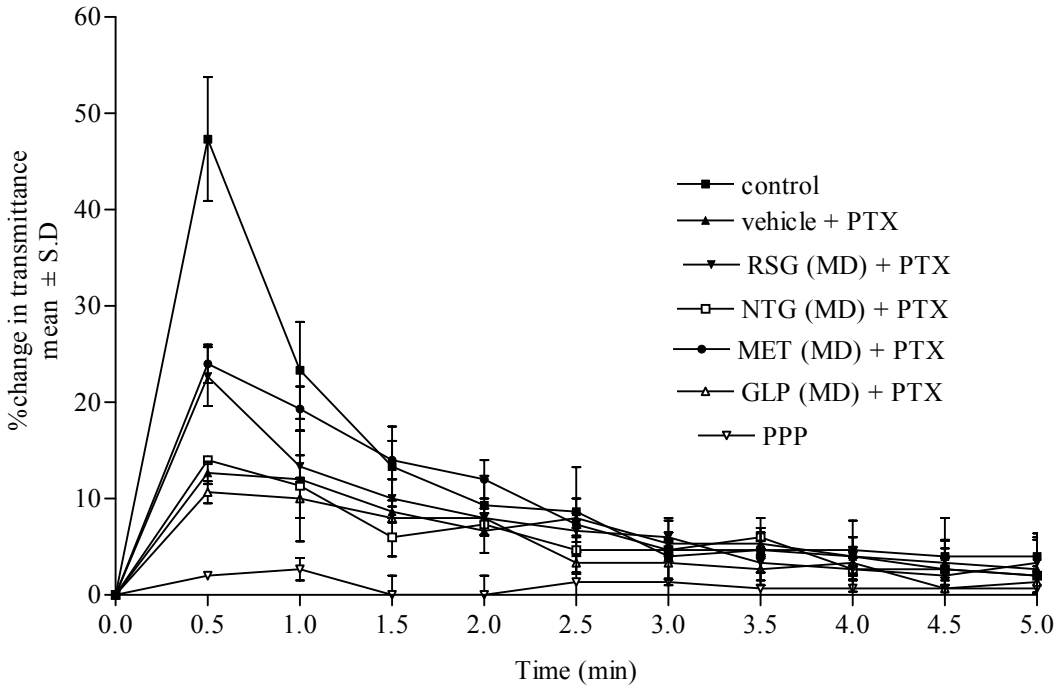



*Figure 4.76 Effect of ADP on platelet activity of pentoxifylline in rat plasma*
n=3 in each treatment group; PPP-platelet poor plasma; MD-multiple doses

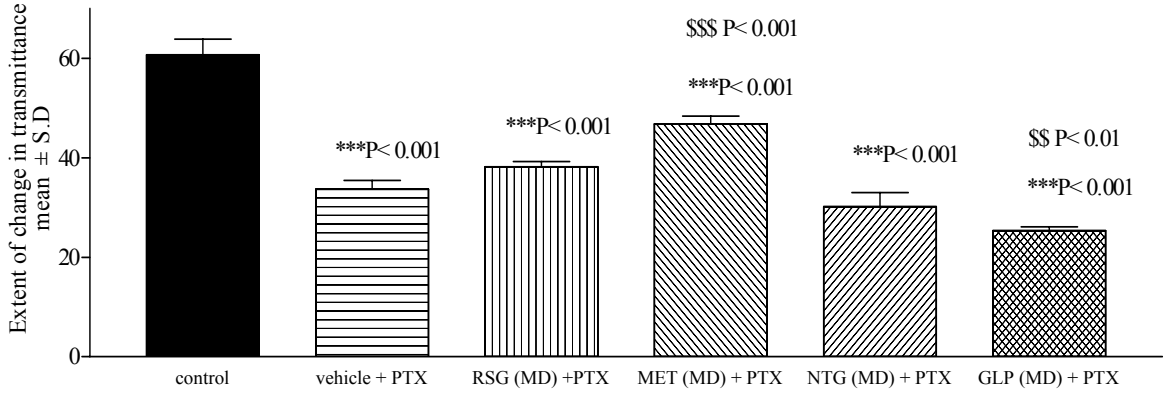



*Figure 4.77 Effect of anti diabetic agents on ex vivo platelet aggregation potential of pentoxifylline in Wistar rats*
n=3 in each treatment group; MD-multiple doses; * p values compared to that of control using student's unpaired t-test; $ p values compared to that of vehicle + PTX using student's boniferroni's multiple comparision test

### 4.4.6 Effect of antiplatelet agents on antidiabetic agent's blood glucose lower activity in rats

The in vivo interacting effect of antiplatelet agents, cilostazol and pentoxifylline, on blood glucose lowering activity of the ADA was studied in rats pretreated with cilostazol or pentoxifylline and the results are presented below.

Table 4.48 Effect of single dose antiplatelet agents on ant diabetic agent's blood glucose lowering activity in rats

| S.No. | Group | $AUC_{0-24}$ of plasma glucose levels in presence of vehicle | $AUC_{0-24}$ of plasma glucose levels in presence of CLZ | $AUC_{0-24}$ of plasma glucose levels) in presence of PTX |
|---|---|---|---|---|
| 1 | rosiglitazone | 2334 ± 136 | 2291± 77 | 2328 ± 137 |
| 2 | metformin | 2554 ± 211 | 2760 ± 63 | 2507 ± 111 |
| 3 | nateglinide | 2645 ± 200 | 2808 ± 230 | 2808 ± 230 |
| 4 | glipizide | 1952 ± 128 | 1622 ± 174 | 1632 ± 115 |

Table 4.49 Effect of multiple dose antiplatelet agents on ant diabetic agent's blood glucose lowering activity in rats

| S.No. | Group | $AUC_{0-24}$ of plasma glucose levels in presence of vehicle | $AUC_{0-24}$ of plasma glucose levels in presence of CLZ | $AUC_{0-24}$ of plasma glucose levels) in presence of PTX |
|---|---|---|---|---|
| 1 | rosiglitazone | 2334 ± 136 | 23451± 134 | 2300 ± 138 |
| 2 | metformin | 2554 ± 211 | 2626 ± 295 | 2475 ± 105 |
| 3 | nateglinide | 2645 ± 200 | 2724 ± 158 | 2422 ± 142 |
| 4 | glipizide | 1952 ± 128 | 1898 ± 210 | 1615 ± 75 |

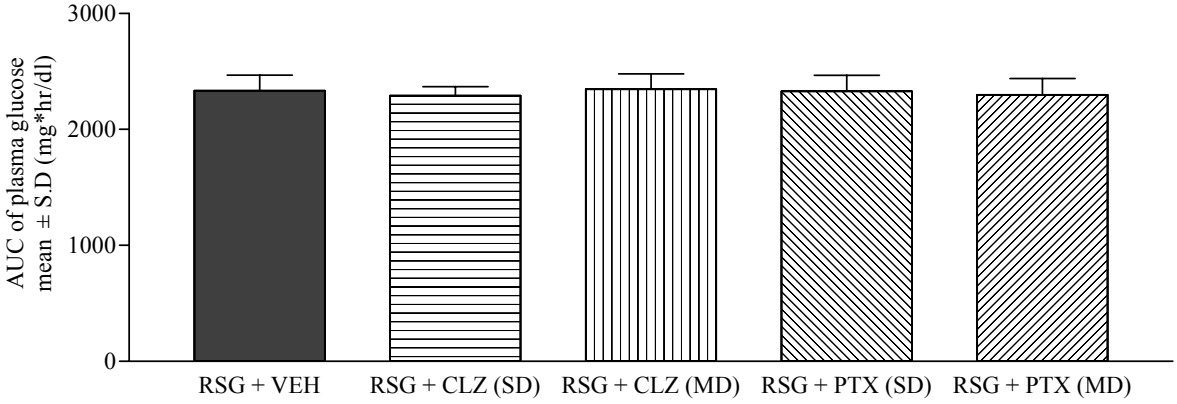



*Figure 4.78 Effect of antiplatelet agents on glucose lowering activity of rosiglitazone in rats*
n=6 in each treatment group; SD-single doses; MD-multiple doses

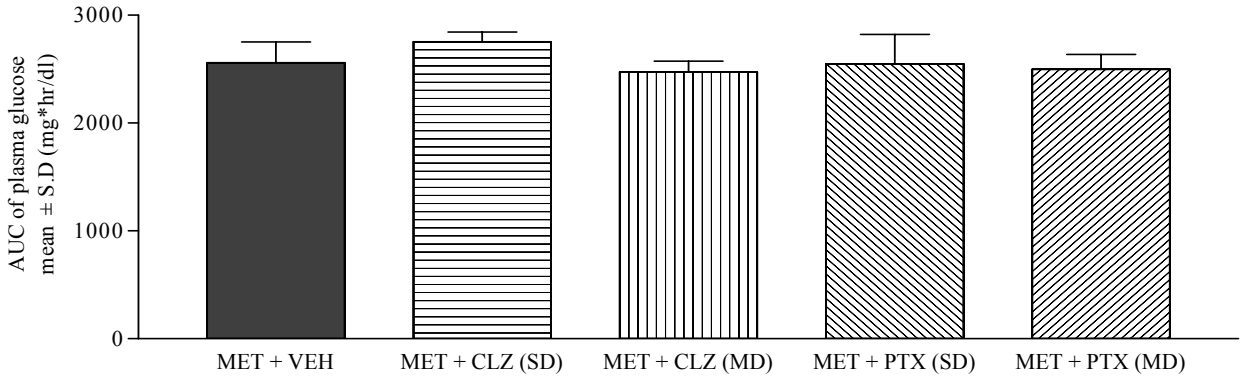



*Figure 4.79 Effect of antiplatelet agents on glucose lowering activity of metformin in rats*
n=6 in each treatment group; SD-single doses; MD-multiple doses

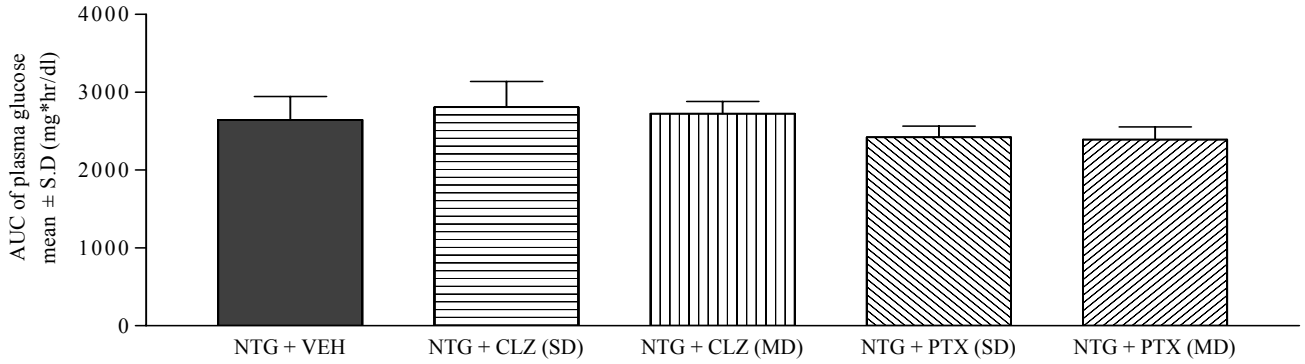



*Figure 4.80 Effect of antiplatelet agents on glucose lowering activity of nateglinide in rats*
n=6 in each treatment group; SD-single doses; MD-multiple doses

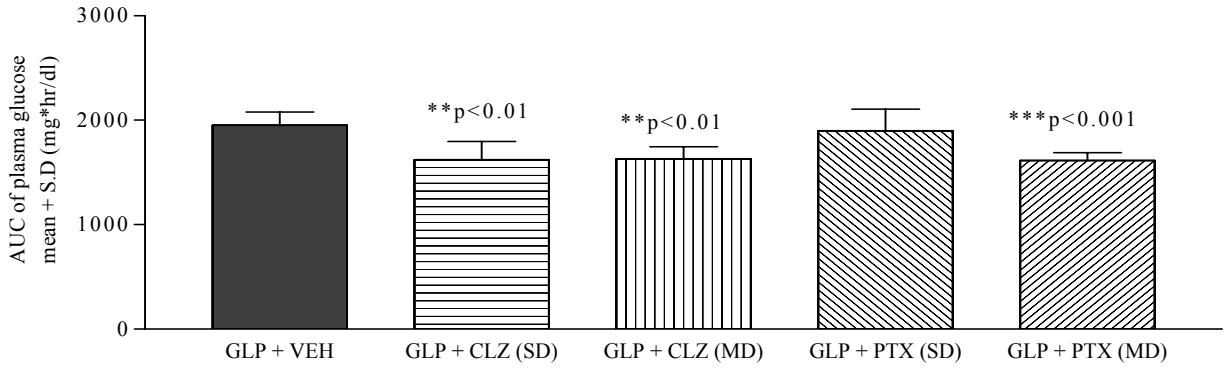



*Figure 4.81 Effect of antiplatelets agent on glucose lowering activity of glipizide in rats*
n=6 in each treatment group; SD-single dose; MD-multiple doses; p values compared to vehicle using student's unpaired t-test

# Chapter 5

# DISCUSSIONS

# 5 DISCUSSIONS

## 5.1 Effect of antidiabetic agents on cilostazol pharmacokinetics in rats

As per literature, information on overdosage with cilostazol in human is limited. The signs and symptoms of an acute overdose can be anticipated to be those of excessive pharmacologic effect: severe headache, diarrhea, hypotension tachycardia, and possibly cardiac arrhythmias. Hence the drug interactions of various drugs need to be studied carefully in those having diabetes and peripheral arterial isorders.

As per literature cilostazol is highly metabolized by hepatic cytochrome p-450 enzymes, mainly 3A4 and to a lesser extent via 2C19 with metabolites largely excreted through urine. The major metabolite, 3, 4-dehydro-cilostazol (DCLZ), was primarily metabolized via CYP3A and inhibition of CYP3A resulted in increased levels of the other active metabolite, 4-'trans-hydroxy-cilostazol (TCLZ), in humans reflected the role of non CYP3A enzymes responsible for this metabolite. In the in vitro metabolic studies using recombinant human CYP enzymes, the reduction in percent remaining of cilostazol has not been observed up to greater extent upon incubation with either CYP3A4 or CYP2C19. However, we observed the formation of DCLZ via CYP3A4 and an unknown metabolite (could not be checked for TCLZ due to lacking of authentic standard) via CYP2C19 and this has been taken as a basis to monitor the effect of other drugs on these metabolite formations in this project.

As discussed in literature review of cilostazol, it has shown a marked sex difference in rats and female after administration of $^{14}$C-cilostazol were shown to have more exposure to the drug. Hence the studies are restricted to male rats in this study to reduce the variation among animals. In man, the plasma concentrations of cilostazol reduced per the same dose were higher than in these animals and the AUC showed a good dose-dependence at doses of 25 to 300 mg/body. Hence 10 mg/kg.b.wt was chosen as the dose in this study which is sufficient to reflect the minimum concentrations of cilostazol get affected during drug interactions.

As per the literature a high fat meal increases absorption, with an approximately 90% in $C_{max}$ and 25% increase in AUC of cilostazol in humans. The animals in these studies have been kept under overnight fasting at least 12 hr prior to dose and continued there after 4 hr post dose of cilostazol single administration. All ADAs investigated in this study have moderately increased plasma

levels of CLZ up on their multiple doses pretreatment. The individual study are being discussed in subsequent sections.

### 5.1.1 Effect of rosiglitazone on cilostazol and its metabolite pharmacokinetic parameters by simultaneous administration in rats

In the present study, we investigated whether a coadministration of rosiglitazone with single dose cilostazol does affect the pharmacokinetics of cilostazol and its active metabolite 3, 4-dehydro-cilostazol. The single dose administration of rosiglitazone with cilostazol in rats resulted in an increase in plasma levels but not significantly changed the $C_{max}$, $AUC_{0-24}$, $AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of cilostazol. However, it has decreased the plasma levels of metabolite and significantly decreased the $C_{max}$, $AUC_{0-24}$ and $AUC_{0-\infty}$ of the active metabolite 3, 4-dehydro-cilostazol. It has significantly delayed the $T_{max}$. The $t_{1/2}$ was also reduced in presence of rosiglitazone but not statistically significant.

The pretreatment of rosiglitazone for seven days prior to concomitant administration of single dose of cilostazol in rats resulted in an increase in plasma levels of cilostazol and also have shown significant increase in the parameters viz. $C_{max}$, $AUC_{0-24}$ and $AUC_{0-\infty}$ without changing the $T_{max}$ and $t_{1/2}$ of cilostazol. It also accompanied by decreased plasma levels of metabolite and significant decrease in the parameters viz. $C_{max}$, $AUC_{0-24}$ and $AUC_{0-\infty}$ of the active metabolite 3, 4-dehydro-cilostazol. It has significantly delayed the $T_{max}$. The $t_{1/2}$ was also reduced in presence of rosiglitazone but not statistically significant.

In our in vitro plasma protein binding study using non radiolabeled cilostazol compound, the binding was more than 99% to rat plasma. The plasma protein binding of cilostazol was not altered in presence of rosiglitazone at 10 µM when spiked together. Since we knew that rosiglitazone is known to have less binding compared to that of cilostazol and it clearly showed that there is no displacement effect of rosiglitazone on cilostazol to plasma proteins.

The effect of rosiglitazone on cilostazol could be due to decreased expression of cytochrome P-450 enzyme systems. As per literature, rosiglitazone is a weak inhibitor of CYP3A4 with a $k_i$ value of 36 µM and is a competitive inhibitor for CYP3A4 It did not have any effect on CYP2C19 which has ruled out the possibility of affecting the other metabolite of cilostazol. It is also showed that rosiglitazone did not induce the CYP3A4 catalytic activity at 0.5 µM but markedly induced at 50 µM in HL096 preparations (Sahi et al., 2003). The steady state levels reached after seven days

pretreatment with rosiglitazone resulted in concentrations less than 5 μM and it might not have caused any induction of CYP3A4 isozymes in this study.

In the in vitro study using the liver microsomes prepared from rats pretreated with rosiglitazone in the similar fashion has shown that the metabolic stability of cilostazol tested at 10 μM has been significantly increased in presence of rosiglitazone. This clearly showed that rosiglitazone is inhibiting the CYP isozymes in rat and hence the increase in plasma levels of cilostazol in presence of rosiglitazone has been observed accordingly. This was also supported by the increase in metabolic stability of cilostazol observed in CYP3A4 bactosomes. Though the marginal increase in metabolic stability was less in these bactosomes it is significant and accompanied by the reduction in formation of the active metabolite, 3, 4-dehydro-cilostazol. The metabolic stability of 3, 4-dehydro-cilostazol has also shown to be increased in presence of rosiglitazone but not reflected in increasing the in vivo half-life.

This data also in correlation with the other study (Suri et al., 1999) as discussed in literature where erythromycin coadministration increased the $C_{max}$ and AUC by 87% and 73% in healthy humans and this followed by reduction of the formation of 3, 4-dehydro-cilostazol by decreasing its $C_{max}$ by 24% and increased the formation of other metabolite 4'-trans-hydroxy-cilostazol by 29% in $C_{max}$ and 141% in AUC.

As per literature coadministration of omeprazole did not significantly affect the metabolism of cilostazol, but the systemic effect to 3, 4-dehyro-cilostazol was increased by 69%, probably the result of omeprazole's potent inhibition of CYP2C19 in humans (Suri and Brammer et al., 1999). Whereas in this study in rats, the plasma levels of 3, 4-dehydro-cilostazol was decreased greatly and is reflecting no role of rosiglitazone on CYP2C19.

It is evident from literature that the absorption of cilostazol is region dependent, decreasing in the order of duodenum ~ jejunum > ileum and is transported by both secretory and absorptive transporters (Toyobuku, et al., 2003). P-Glycoprotein contributes in part to the intestinal secretion, while the absorptive mechanism has not been identified. However, interactions of these mechanisms, and possibly also metabolism, could cause the large inter individual fluctuation in bioavailability of cilostazol. Effect of rosiglitazone on these mechanisms is not known so far. So if rosiglitazone does have any inhibitory effect of p-glycoprotein or on any efflux transporter in rat, the raise in levels of cilostazol in presence of rosiglitazone could not be denied. But it needs to be

understood clearly. The antiplatelet activity of cilostazol has not been changed in presence of rosiglitazone. It could be due to the compensating mechanism of increased levels of cilostazol and decreased levels of the active metabolite by pretreatment with rosiglitazone.

Based on these results, we also expect a possible role of inhibition of cyctochrome P-450 and P-gp in liver/intestine by rosiglitazone pretreatment which result in the decreased metabolism and increased absorption of cilostazol. However, further studies are needed to confirm the role of P-gp regarding increased absorption of cilostazol in to the intestines.

### 5.1.2 Effect of metformin on cilostazol and its metabolite pharmacokinetic parameters by simultaneous administration in rats

In the present study, it was investigated to check whether a coadministration of metformin with single dose cilostazol does affect the pharmacokinetics of cilostazol and its active metabolite 3, 4-dehydro-cilostazol. The concomitant administration of single dose of metformin with cilostazol in rats resulted in an increase in plasma levels but not significantly changed the $C_{max}$, $AUC_{0-24}$, $AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of cilostazol. However, it increased the plasma levels of metabolite and significantly increased the $AUC_{0-24}$ and $AUC_{0-\infty}$ of the active metabolite 3, 4-dehydro-cilostazol. It has delayed the $T_{max}$. The $t_{1/2}$ was also increased in presence of metformin but not statistically significant.

The pretreatment of metformin in multiple doses prior to administration of single dose of cilostazol in rats resulted in an increase in plasma levels of cilostazol thus showing significant increase in the parameters viz., $AUC_{0-24}$ and $AUC_{0-\infty}$ without changing the $C_{max,}$ $T_{max.}$ The $t_{1/2}$ was increased significantly for cilostazol. It was also accompanied by increased plasma levels of metabolite and significant increase in the parameters viz. $AUC_{0-24}$ and $AUC_{0-\infty}$ of the active metabolite 3, 4-dehydro-cilostazol. It has significantly delayed the $T_{max}$. The $t_{1/2}$ was also increased in presence of metformin and was statistically significant.

Cilostazol is known to be highly bound to plasma proteins. In in vitro plasma protein binding study using non radiolabeled cilostazol compound, the binding was more than 99% to rat plasma. The plasma protein binding of cilostazol was not altered in presence of metformin when spiked together. Since we knew that metformin is known to have less binding compared to that of cilostazol and it clearly showed that there is no displacement effect of metformin on cilostazol to plasma proteins.

Cilostazol is highly metabolized by hepatic cytochrome p-450 enzymes, mainly 3A4 and to a lesser extent via 2C19 with metabolites largely excreted through urine. It is known that metformin does not effect on CYP450 system Hence the effect of metformin on cilostazol could not be due to increased or decreased expression of cytochrome P-450 enzyme systems.

But in in vitro study using the liver microsomes prepared from rats pretreated with metformin in the similar fashion has shown that the metabolic stability of cilostazol has been significantly increased in presence of metformin. The metabolic stability of 3, 4-dehydro-cilostazol has also shown to be increased in presence of metformin for which the enzyme responsible for metabolism in this regard is unknown. This clearly showed that metformin is inhibiting the CYP isozymes in rat and hence an increase in plasma levels of cilostazol in presence of metformin has been observed accordingly. But this was not resulted in influencing the role observed in CYP450 enzymes where no change was observed in presence of metformin as per literature. Hence the effect of metformin in rat liver microsomes is unclear

Whereas in in vivo study in rats, the maximum plasma levels of cilostazol were increased accompanied by its significantly increased systemic exposure of cilostazol as well as its metabolite thus reflecting no or minimal role of metformin on CYP3A4. No inhibition of CYP450 mediated metabolic reactions is expected for metformin based on literature citation. (Glucophage Prescribing information).

As discussed earlier that absorption of cilostazol is region dependent and is transported by both secretory and absorptive transporters (Toyobuku et al., 2003). Metformin is known to interact with PEPT1 and other transporters but unclear about the inhibition of this transporter (Choi et al., 2006). But cilostazol $C_{max}$ levels were not significantly changed in presence of repeat dose administration of metformin and reflecting the no role of metformin on its absorption. But it needs to be understood clearly.

Metformin disposition is mediated through renal OCT2 and hepatic OCT1 transporters and the maximum effect of metformin on these transporters is unknown (Wang et al., 2002). Even metformin is known to get excreted through the renal tubular system and the excretion mechanism of cilostazol and its metabolite is unknown. If these follow the same mechanism and uptake of metformin at renal level might have lead to the competitive elimination of metformin rather than cilostazol and its metabolite at tubular system. Thus the levels of the cilostazol and its metabolite,

3, 4-dehydro-cilostazol, have been maintained for long time and also its half-life got affected by metformin as well leading to the significant increase in its systemic exposure.

The antiplatelet activity of cilostazol in presence of metformin pretreatment was reduced and this could be due to unchanged $C_{max}$ levels of cilostazol and its active metabolite in spite of the increased systemic exposure.

Based on these results, it is expected to have a possible role of modulation of the transporters at hepatic and renal systems by metformin pretreatment that result in the decreased elimination and increased distribution of cilostazol. However, further studies are needed to confirm the role of transporter regarding distribution and elimination of cilostazol.

### 5.1.3 Effect of nateglinide on cilostazol and its metabolite pharmacokinetic parameters by simultaneous administration in rats

The concomitant administration of single dose of nateglinide with cilostazol in rats resulted in an increase in plasma levels with a significant improvement in $AUC_{0-24}$, $AUC_{0-\infty}$ but not significantly changed the $C_{max}$, $T_{max}$ and $t_{1/2}$ of cilostazol. However, it increased the plasma levels of metabolite and significantly increased the $AUC_{0-24}$ and $AUC_{0-\infty}$ of the active metabolite 3, 4-dehydro-cilostazol. It has delayed the $T_{max}$. The $t_{1/2}$ was also increased in presence of nateglinide but not statistically significant.

The pretreatment of nateglinide in multiple dose prior to administration of single dose of cilostazol in rats resulted in an decrease in plasma levels of cilostazol and also showed a decreasing trend in $C_{max}$ but a significant decrease in the parameters viz., $AUC_{0-24}$ and $AUC_{0-\infty}$ without changing the $T_{max.}$ The $t_{1/2}$ was increased but not significant for cilostazol. It was also accompanied by increased plasma levels of metabolite and significant increase in the parameters viz. $AUC_{0-24}$ and $AUC_{0-\infty}$ of the active metabolite 3, 4-dehydro-cilostazol. It has significantly delayed the $T_{max}$ by 0.5 hr. The $t_{1/2}$ was also increased in presence of nateglinide but not statistically significant.

Cilostazol is known to be highly bound to plasma proteins. In our in vitro plasma protein binding study using non radiolabeled cilostazol compound, the binding was more than 99% to rat plasma. The plasma protein binding of cilostazol was not altered in presence of nateglinide at 10 µM when spiked together. Since we knew that nateglinide is known to have less binding compared to that of cilostazol and it clearly showed that there is no displacement effect of nateglinide on cilostazol to plasma proteins.

The effect of nateglinide on cilostazol could be due to decreased expression of cytochrome P-450 enzyme systems. In the in vitro study using the liver microsomes prepared from rats pretreated with nateglinide in the similar fashion has shown that the metabolic stability of cilostazol has been significantly increased in presence of nateglinide. The metabolic stability of 3, 4-dehydro-cilostazol has also shown to be increased in presence of nateglinide for which the enzyme responsible for metabolism in this regard is unknown. This clearly showed that nateglinide is inhibiting the CYP isozymes in rat and hence we observed the increase in plasma levels of cilostazol in presence of nateglinide accordingly. But this was not resulted in metabolic stability of cilostazol observed in CYP3A4 bactosomes where no change was observed in presence of nateglinide. But this was supported by decrease in formation of other metabolite for cilostazol in CYP2C19 bactosomes and thus nateglinide might have inhibited CYP2C19 isozymes.

This data also in correlation with the other study (Suri and Brammer et al., 1999) where coadministration of omeprazole did not significantly affect the metabolism of cilostazol, but the systemic effect to 3, 4-dehyro-cilostazol was increased by 69%, probably the result of omeprazole's potent inhibition of CYP2C19 in humans. Coadministration of erythromycin increased the $C_{max}$ and AUC by 87% and 73% in healthy humans and this followed by reduction of the formation of 3, 4-dehydro-cilostazol by decreasing its $C_{max}$ by 24% and increased the formation of other metabolite 4'-trans-hydroxy-cilostazol by 29% in $C_{max}$ and 141% in AUC (Suri et al., 1999)

Whereas in the current study in rats, the maximum plasma levels of cilostazol were decreased but its systemic exposure was increased significantly and also the systemic exposure of 3, 4-dehydro-cilostazol was increased greatly and is reflecting minimal role of nateglinide on CYP3A4. No inhibition of CYP3A4 metabolic reactions is expected for nateglinide based on literature citation. (Starlix Prescribing information). Hence the repeat administration of nateglinide might have resulted in inhibiting CYP2C19 where by decreasing the formation of other metabolite and leading to the exposure of cilostazol to get more effected by CYP3A4. Thus the levels of the 3, 4-dehydro-cilostazol have been increased and also its half-life got affected by nateglinide as well leading to the significant increase in its systemic exposure.

As discussed earlier that absorption of cilostazol is region dependent and is transported by both secretory and absorptive transporters (Toyobuku et al., 2003). Nateglinide is known to inhibit

PEPT1 and other transporters. Furthermore nateglinide competitively inhibited $H^+$- driven ceftibuten transporter-mediated ceftibuten uptake reflecting that it inhibits ceftibuten/$H^+$ cotransport system (Okumara et al., 2002). So if cilostazol does have any substrate properties for these transporter systems, the decrease in uptake of cilostazol from intestine in presence of nateglinide could not be denied. But it needs to be understood clearly.

The antiplatelet activity of cilostazol by pretreatment of nateglinide was increased but not significant. It could be due to the decrease in $C_{max}$ of cilostazol which might have compensated by the increase in $C_{max}$ of the active metabolite DCLZ.

Based on these results, we also expect a possible role of inhibition of cyctochrome P-450 and transporter in liver/intestine by nateglinide pretreatment which result in the decreased metabolism and increased absorption of cilostazol. However, further studies are needed to confirm the role of transporter regarding increased absorption of cilostazol in to the intestines.

### 5.1.4 Effect of glipizide on cilostazol and its metabolite pharmacokinetic parameters by simultaneous administration in rats

The concomitant administration of single dose of glipizide with cilostazol in rats resulted in an increase in plasma levels with a significant improvement in $AUC_{0\text{-}24}$, $AUC_{0\text{-}\infty}$ but not significantly changed the $C_{max}$, $T_{max}$ and $t_{1/2}$ of cilostazol. However, it increased the plasma levels of metabolite and significantly increased the $C_{max}$, $AUC_{0\text{-}24}$ and $AUC_{0\text{-}\infty}$ of the active metabolite 3, 4-dehydro-cilostazol. It has delayed the $T_{max}$ and is statistically significant. The $t_{1/2}$ was not changed in presence of glipizide.

The pretreatment of glipizide in multiple doses prior to administration of single dose of cilostazol in rats resulted in an increase in plasma levels of cilostazol with a significant increase in the parameters viz., $C_{max}$, $AUC_{0\text{-}24}$ and $AUC_{0\text{-}\infty}$ without changing the $T_{max.}$ The $t_{1/2}$ was decreased very slightly but not significant for cilostazol. It was also accompanied by increased plasma levels of metabolite and significant increase in the parameters viz. $C_{max}$, $AUC_{0\text{-}24}$ and $AUC_{0\text{-}\infty}$ of the active metabolite 3, 4-dehydro-cilostazol. It has significantly delayed the $T_{max}$. The $t_{1/2}$ was not changed in presence of glipizide.

In our in vitro plasma protein binding study using non radiolabeled cilostazol compound, the binding was more than 99% to rat plasma. The plasma protein binding of cilostazol was not altered

in presence of glipizide when spiked together. Though glipizide is known to have high binding compared to that of cilostazol and it clearly showed that there is no displacement effect of glipizide on cilostazol to plasma proteins.

The effect of glipizide on cilostazol could be due to a minor effect of decreased expression of cytochrome P-450 enzyme systems. It is known that glipizide is a potent substrate of CYP2C9 and does not have any effect on inhibition of CYP3A4 or CYP2C19 as per literature (Kidd et al., 1999).

In the in vitro study using the liver microsomes prepared from rats pretreated with glipizide in the similar fashion has shown that the metabolic stability of cilostazol tested has been significantly increased in presence of glipizide. The metabolic stability of 3, 4-dehydro-cilostazol has also shown to be increased in presence of glipizide for which the enzyme responsible for metabolism in this regard is unknown. This clearly showed that glipizide is inhibiting the CYP isozymes in rat to a minor extent and hence we observed the increase in plasma levels of cilostazol in presence of glipizide accordingly. But this was not resulted in metabolic stability of cilostazol observed in CYP3A4 bactosomes where no change was observed in presence of glipizide. And at the same time this was supported by decrease in formation of other metabolite for cilostazol in CYP2C19 bactosomes and thus glipizide might have inhibited CYP2C19 isozyme to great extent which leads to more levels of cilostazol in presence of glipizide.

This data also in correlation with the other study where coadministration of omeprazole did not significantly affect the metabolism of cilostazol, but the systemic effect to 3, 4-dehyro-cilostazol was increased by 69%, probably the result of omeprazole's potent inhibition of CYP2C19 in humans (Bramer SL, Suri A, et al., 1999).

Coadministration of erythromycin increased the bioavailability of cilostazol followed by reduction of the formation of 3, 4-dehydro-cilostazol and increased the formation of other metabolite 4'-trans-hydroxy-cilostazol (Suri et al., 1999). Whereas in our study in rats, the maximum plasma levels of cilostazol were increased as well as its systemic exposure was increased significantly and also the systemic exposure of 3, 4-dehydro-cilostazol was increased greatly and is reflecting minimal role of glipizide on CYP3A4. No inhibition of CYP3A4 metabolic reactions is expected for glipizide based on literature citation. (Glucotrol$^{TM}$ Prescribing information). Hence the repeat administration of glipizide might have resulted in inhibiting CYP2C19 where by decreasing the

formation of other metabolite and leading to the exposure of cilostazol to get more effected by CYP3A4. Thus the levels of the 3, 4-dehydro-cilostazol have been increased and also its half-life not affected by glipizide as well leading to the significant increase in its systemic exposure. But if we look at the ratio of systemic exposure of metabolite to that of parent drug it decreased by 22% reflects either the saturation of CYP3A4 enzymatic reaction to certain extent.

As discussed earlier that absorption of cilostazol is region dependent and is transported by both secretory and absorptive transporters. Glipizide is known to induce the sulphonylurea – sensitive oraganic anion transporter which extrudes the acidic metabolites of dopamine from PC12 cells (Lamensdorf et al., 2000). So if cilostazol does have any substrate properties for these transporter systems, the increase in extrusion of cilostazol from tissues to extra cellular fluid in presence of glipizide could not be denied. But definitely glipizide looks like to affect on the absorption of cilostazol to a large extent and it needs to be understood clearly.

The antiplatelet activity of cilostazol in presence of glipizide was improved by its pretreatment and this could be due to increase in $C_{max}$ of both cilostazol and its active metabolite by pretreatment of glipizide. However, the increased levels of cilostazol in presence of glipizide in rats are alarming the adverse effects of cilostazol if similar findings are observed in humans.

Based on these results, we also expect a possible minor role of inhibition of cyctochrome P-450 and a major role on any transporter mechanism by glipizide pretreatment that resulted in the decreased metabolism and increased absorption of cilostazol. However, further studies are needed to confirm the role of transporter regarding increased absorption of cilostazol in to the intestines.

## 5.2 Effect of Antidiabetic agents on pentoxifylline pharmacokinetics in rats

Over dosage with pentoxifylline has been reported in pediatric patients and adults (as per literature). Symptoms appear to be dose related; flushing, hypotension, convulsions, somnolence, loss of consciousness, fever, and agitation occurred. All patients recovered. In addition to symptomatic treatment and gastric lavage, special attention must be given to supporting respiration, maintaining systemic blood pressure, and controlling convulsions. Activated charcoal has been used to absorb pentoxifylline in patients who have overdosed. Hence the drug interactions

of pentoxifylline need to be studied carefully in those having diabetes and cardiovascular disorders.

As discussed in literature (Bryce et al., 1989), after the oral administration of $^{14}$C-Pentoxifylline to three healthy male subjects, the radiolabeled drug was rapidly absorbed and also rapidly eliminated. The peak plasma concentrations were reached within 0.25 to 0.75 hr after oral administration. The total recovery of radioactive material was 93% in urine and 3% in feces by 24 hr post dosing out of which 89% of radioactivity was observed within 6 hr post dosing reflecting the rapid elimination of pentoxifylline. The radio activity was decayed in biphasic manner.

As per the innovators information about pentoxifylline (Trental$^{TM}$ prescribing information), after oral administration in aqueous solution to humans, pentoxifylline is almost completely absorbed. It undergoes a first-pass effect and the various metabolites appear in plasma very soon after dosing. Peak plasma levels of the parent compound and its metabolites are reached within 1 hour. The major metabolites are Metabolite l (1-[5-hydroxyhexyl]-3,7-dimethylxanthine) and Metabolite V (1-[3-carboxypropyl]-3,7-dimethylxanthine), and plasma levels of these metabolites are 5 and 8 times greater, respectively, than pentoxifylline. The pharmacokinetics of the parent compound and Metabolite l are dose-related and not proportional (non-linear), with half-life and area under the blood-level time curve (AUC) increasing with dose. The elimination kinetics of Metabolite V are not dose-dependent.

The absorption of pentoxifylline is very rapid and is rapidly eliminated soon in the current rat study. The pharmacokinetic parameters for the metabolites were monitored not in this study due to lack of authentic standards.the ADAs investigated in this study have moderately decreased the plasma levels of pentoxifylline up on their multiple doses pretreatment.

### 5.2.1 Effect of rosiglitazone on pentoxifylline pharmacokinetic parameters by simultaneous administration in rats

In the present study, we investigated whether a coadministration of rosiglitazone with single dose pentoxifylline does affect the pharmacokinetics of pentoxifylline. The concomitant administration of single dose of rosiglitazone with pentoxifylline in rats resulted in a decrease in plasma levels but not significantly changed the $C_{max}$, $AUC_{0-4}$, $AUC_{0-\infty}$ and $t_{1/2}$ of pentoxifylline except in $T_{max}$. However, it showed a decreased trend in all the above parameters except a slight increase in $T_{max}$ and $t_{1/2}$ of pentoxifylline.

The pretreatment of rosiglitazone in multiple doses prior to administration of single dose of pentoxifylline in rats resulted in slight decrease in plasma levels of pentoxifylline and also shown significant decrease in the parameters viz. $AUC_{0-24}$ and $AUC_{0-\infty}$ without changing the $C_{max}$, $T_{max}$ and $t_{1/2}$ of pentoxifylline. It has significantly delayed the $T_{max}$. The $t_{1/2}$ was also reduced in presence of rosiglitazone but not statistically significant.

In the in vitro plasma protein binding study using non radiolabeled pentoxifylline compound, the binding was more than 37 % to rat plasma. The plasma protein binding of pentoxifylline was not altered in presence of rosiglitazone when spiked together. Rosiglitazone is known to have more binding compared to that of pentoxifylline and it did not show displacement effect of rosiglitazone on pentoxifylline to plasma proteins though there was an increase in its own protein binding.

In the in vitro metabolic studies using bactosomes not a great reduction in % remaining of pentoxifylline has been observed upon incubation with either CYP1A2 or CYP2E11. However, it was observed that the metabolism is occurring when the pooled male human liver microsomes were used. In this in vitro study, rosiglitazone has significantly improved the metabolic stability of pentoxifylline upon pre incubation. The effect of rosiglitazone on pentoxifylline could be probably due to decreased expression of cytochrome P-450 enzyme systems. However, it is known that rosiglitazone is a very weak inhibitor of CYP1A2 with a $IC_{50}$ value of 55 μM and it did not have any effect on CYP2E11 (Sahi et al., 2003). The in vitro inhibition potential of PTX on self enzymes is very insignificant as its $IC_{50}$ on CYP1A2 was found to be 60 μM. But the induced effect of rosiglitazone on CYP1A and CYP2E11 is unknown so far.

In the in vitro study using the liver microsomes prepared from rats pretreated with rosiglitazone in the similar fashion has shown that the metabolic stability of pentoxifylline has been significantly decreased in presence of rosiglitazone but it is very negligible effect. This clearly showed that rosiglitazone is not inhibiting the CYP isozymes in rat responsible for pentoxifylline and hence we observed no increase in plasma levels of pentoxifylline in presence of rosiglitazone accordingly. In fact there were reduced plasma levels of pentoxifylline in presence of rosiglitazone. Probably the inducing effect of rosiglitazone on CYP1A2 might have resulted in reduction of pentoxifylline maximum absorption and thus total systemic exposure. But it needs to be studied further to understand this concept.

This data also in correlation with the other study where ciproflaxacin coadministration decreased the $C_{max}$ by 56% and in CD-1 mice and this followed by reduction of the formation of M-1 by decreasing its $C_{max}$ by 47% as discussed in literature. However the ciprofloxacin did not down regulate the CYP1A2 enzyme and the author is of the opinion that role of ciprofloxacin on CYP2E11 need to investigate further (Peterson et al., 2004).

Some in vivo studies in mice (Wyska et al., 2007) showed that the absorption of pentoxifylline is mediated by intestinal transport mechanisms and dose proportionality is non linear due to the saturation of these transport mechanisms. In another study where coadministration of verapamil (P-glycoprotein inhibitor) and ketoconazole (CYP3A) inhibitor with pentoxifylline increases the levels of PTX reflecting the role of transport mechanism across intestinal cells. Effect of rosiglitazone on these mechanisms is not clear. If rosiglitazone does have any inhibitory effect on influx transporter in rat, the decrease in levels of pentoxifylline and there by reduction of AUC in presence of rosiglitazone could not be denied. But it needs to be understood clearly.

Overall it reflects the possible role of rosiglitazone on single dose pentoxifylline by increasing the elimination of pentoxifylline soon. Since the elimination of pentoxifylline is fast and is further enhanced by rosiglitazone, probably this may influence the pharmacodynamics of pentoxifylline in clinical settings if similar findings are being observed in humans. However the antiplatelet activity of pentoxifylline in presence of rosiglitazone was not changed in rats.

Based on these results, we also expect a possible role of induction of CYP1A2 and unknown transport mechanism by rosiglitazone pretreatment which resulted in the decreased absorption and increased metabolism of pentoxifylline. However, further studies are needed to confirm the role of transporters regarding decreased absorption of pentoxifylline in to the intestines.

### 5.2.2 Effect of metformin on pentoxifylline pharmacokinetic parameters by simultaneous administration in rats

In the present study, we investigated whether a coadministration of metformin with single dose pentoxifylline does affect the pharmacokinetics of pentoxifylline. The concomitant administration of single dose of metformint with pentoxifylline in rats resulted in decrease in plasma levels and subsequently $C_{max}$, $AUC_{0-4}$, $AUC0_{-\infty}$, were also decreased but not no significance observed. It also not changed the other parameters like $T_{max}$ and $t_{1/2}$ of pentoxifylline.

The pretreatment of metformin in multiple doses prior to administration of single dose of pentoxifylline in rats resulted in decrease in plasma levels of pentoxifylline through out the study. It also significantly decreased the parameters viz. $C_{max}$, $AUC_{0-4}$, $AUC_{0-\infty}$ but not significantly changed any of the parameters viz., $T_{max}$ and $t_{1/2}$ of pentoxifylline.

In our in vitro plasma protein binding study using non radiolabeled pentoxifylline compound, the binding was more than 37 % to rat plasma. The plasma protein binding of pentoxifylline was not altered in presence of metformin when spiked together. Since we knew that metformin is known to have high binding compared to that of pentoxifylline and it clearly showed that it did not influence on protein binding of pentoxifylline as it is already competitive bound to proteins.

As discussed earlier, pentoxifylline metabolism was studied in pooled human liver microsomes but could not be studid the drug interaction potential of metformin on pentoxifylline in CYP1A2 and CYP2E11. The effect of metformin on pentoxifylline metabolism could not be changed due to its lack of inhibitory effect on cytochrome P-450 enzyme systems. However, in the in vitro study using the liver microsomes prepared from rats pretreated with metformin in the similar fashion has shown that the metabolic stability of pentoxifylline has not been changed in presence of metformin This clearly showed that metformin is not inhibiting the CYP isozymes in rat responsible for pentoxifylline and hence that no increase in plasma levels after administration of pentoxifylline in presence of metformin were observed accordingly. In fact, a decrease in plasma levels and half-life was observed thus slightly reduced the systemic exposure for pentoxifylline in presence of metformin.

As discussed earlier (Wyska et al., 2007) that the absorption of pentoxifylline is mediated by intestinal transport mechanisms and non linear dose proportionality occur due to the saturation of these transport mechanisms, the role of drug interaction at gastric absorption could not be denied. Metformin is known to interact with PEPT1 and other transporters but unclear about the inhibition of this transporter. Metformin increases the gastric acid secretion and acts as weak histamine agonist in non diabetic subjects (Molloy et al., 1980). Hence metformin has lowering effect on gastric pH that might have resulted in decrease in $C_{max}$ of pentoxifylline which is supported by another study where elevated levels of pentoxifylline observed in presence of cimetidine (Mauro et al., 1988). Metformin disposition is mediated through renal OCT2 and hepatic OCT1 transporters (Kimura et al., 2005a) and the maximum effect of metformin on these transporters is unknown.

The Vd and $CL_{z\_f}$ of pentoxifylline are drastically changed after metformin repeat administration. Even metformin is known to get excreted through the renal tubular system and the excretion mechanism of pentoxifylline and its metabolites are unknown. If these follow the same mechanism and any interaction at uptake of metformin at renal level then it might have lead to the competitive elimination of pentoxifylline rather than metformin. Hence the effect of metformin on these transporters needs to be understood

The antiplatelet activity of pentoxifylline was decreased in presence of metformin and it could be due to the decrease in $C_{max}$ levels of pentoxifylline by pretreatment with metformin. Overall it reflects the major role of metformin on single dose pentoxifylline accordingly the decrease in maximum absorption and the systemic absorption of pentoxifylline is to be noted. Since the elimination of pentoxifylline is fast and is further enhanced by metformin, probably may influence the pharmacodynamics of pentoxifylline in vivo.

### 5.2.3 Effect of nateglinide on pentoxifylline pharmacokinetic parameters by simultaneous administration in rats

In the present study, we investigated whether a coadministration of nateglinide with single dose pentoxifylline does affect the pharmacokinetics of pentoxifylline. The concomitant administration of single dose of nateglinide with pentoxifylline in rats resulted in no change in plasma levels and not significantly changed the $C_{max}$, $AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of pentoxifylline but decreased the $AUC_{0-4}$. However, it showed a decreased trend in all the above parameters except a slight increase in $T_{max}$ of pentoxifylline. The pretreatment of nateglinide in multiple doses prior to administration of single dose of pentoxifylline in rats resulted in an increase in plasma levels of pentoxifylline through out the study and also significantly changed the parameters viz. $C_{max}$, $AUC_{0-4}$, $AUC_{0-\infty}$ but did not change $T_{max}$ and $t_{1/2}$ of pentoxifylline..

In the in vitro plasma protein binding study using non radiolabeled pentoxifylline compound, the binding was more than 37 % to rat plasma. The plasma protein binding of pentoxifylline was not altered in presence of nateglinide when spiked together. Since we knew that nateglinide is known to have high protein binding compared to that of pentoxifylline and it clearly showed that it did not influence on protein binding of pentoxifylline as it is already competitively bound to proteins.

As discussed earlier, pentoxifylline metabolism was studied in pooled human liver microsomes but could not study the drug interaction potential of nateglinide on pentoxifylline in CYP1A2 and

CYP2E11. It has shown an increase in metabolic stability of pentoxifylline in presence of nateglinide. In an in vitro study using the liver microsomes prepared from rats pretreated with nateglinide in the similar fashion has shown that the metabolic stability of pentoxifylline tested has been very slightly decreased in presence of nateglinide but it is very negligible effect. This clearly showed that nateglinide is not inhibiting the CYP isozymes in rat responsible for pentoxifylline and hence we observed no increase in plasma levels after administration of pentoxifylline in presence of single dose nateglinide accordingly. In fact the decrease in half-life was observed thus slightly reduced the systemic exposure for pentoxifylline in presence of nateglinide. Probably very minor inducing effect of nateglinide on CYP1A2/CYP2E11 might have resulted in reduced half-life of pentoxifylline. But it needs to be studied further to understand this concept. But this effect is overshadowed by the drastic increase in plasma levels in presence of multiple doses of nateglinide and this could be related to interaction at absorption or excretion.

As we discussed that the absorption of pentoxifylline is mediated by intestinal transport mechanisms and non linear dose proportionality occur due to the saturation of these transport mechanisms, the role of drug interaction at gastric absorption could not be denied. Nateglinide is known to inhibit PEPT1 transporters and ceftibuten/[H]+ co-transporter system (Okumara et al., 2002). So if pentoxifylline does have any effect on or by these transporters, it could have result an increase in $C_{max}$ of pentoxifylline in rats. Hence, the effect of nateglinide on these transporters needs to be understood clearly.

The antiplatelet activity of pentoxifylline was slightly increased in presence of nateglinide but was not significant. This trend could be due to the increase in $C_{max}$ of pentoxifylline in presence of nateglinide pretreatment. Overall it reflects a significant role of nateglinide on single dose pentoxifylline by increasing the $C_{max}$ and AUC of pentoxifylline in rats. Since the elimination of pentoxifylline is fast and the increased levels in presence of nateglinide may probably influence the pharmacodynamics of pentoxifylline in vivo if similar findings are being observed in humans.

### 5.2.4 Effect of glipizide on pentoxifylline pharmacokinetic parameters by simultaneous administration in rats

In the present study, we investigated whether a coadministration of glipizide with single dose pentoxifylline does affect the pharmacokinetics of pentoxifylline. The concomitant administration of single dose of glipizide with pentoxifylline in rats resulted in no change in plasma levels and not

significantly changed the $C_{max}$, $AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of pentoxifylline but decreased the $AUC_{0-4}$. However, it showed a decreased trend in all the above parameters except a slight increase in $T_{max}$ of pentoxifylline.The pretreatment of glipizide in multiple doses prior to administration of single dose of pentoxifylline in rats resulted a decrease in plasma levels of pentoxifylline through out the study but not significantly changed any of the parameters viz. $C_{max}$, $AUC_{0-4}$, $AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of pentoxifylline.

In the in vitro plasma protein binding study using non radiolabeled pentoxifylline compound, the binding was more than 37 % to rat plasma. The plasma protein binding of pentoxifylline was not altered in presence of glipizide when spiked together. Since we knew that glipizide is known to have high binding compared to that of pentoxifylline and competitively bound to proteins, it clearly showed that it did not influence on protein binding of pentoxifylline.

As discussed earlier, pentoxifylline metabolism was studied in pooled human liver microsomes but could not study the drug interaction potential of glipizide on pentoxifylline in CYP1A2 and CYP2E11. Glipizide has slightly improved the metabolic stability of pentoxifylline in human liver microsomes. Where as in the in vitro study using the liver microsomes prepared from rats pretreated with glipizide in the similar fashion, the metabolic stability of pentoxifylline has been very slightly changed in presence of glipizide but it is very negligible effect. This clearly showed that glipizide is not inhibiting the CYP isozymes in rat responsible for pentoxifylline and hence we observed no increase in plasma levels after administration of pentoxifylline in presence of glipizide accordingly. In fact the decrease in half-life was observed thus slightly reduced the systemic exposure for pentoxifylline in presence of glipizide. Probably very minor inducing effect of glipizide on CYP1A2/CYP2E11 might have resulted in reduced plasma levels of pentoxifylline. But it needs to be studied further to understand this concept.

As discussed earlier, the absorption of pentoxifylline is mediated by intestinal transport mechanisms and non linear dose proportionality occur due to the saturation of these transport mechanisms, the role of drug interaction at gastric absorption could not be denied. Glipizide is known to cause the gastric delay in rats (Groop et al., 1985). So if glipizide does have any lowering effect on gastric pH and any effect on other transporter mechanism in rat that might have resulted in an increase in $C_{max}$ of pentoxifylline. Hence, the effect of glipizide on transporters needs to be understood.

The antiplatelet activity of pentoxifylline in presence of glipizide is slightly improved and this could be due to an increased $C_{max}$ of pentoxifylline by pretreatment with glipizide. Overall it reflects the a moderate role of glipizide on single dose pentoxifylline however an increase in maximum absorption of pentoxifylline is to be noted thus may influence the pharmacodynamics of pentoxifylline in vivo in clinical settings if similar findings are observed.

## 5.3 Effect of antiplatelet agents on antidiabetic agent's pharmacokinetics in rats

In diabetics having cardiovascular complications, cilostazol and or pentoxifylline are used for PAD chronically in presence of antidiabetic agents. The influence of antiplatelet agent on antidiabetic agent's pharmacokinetics also needs to be understood to make necessary dose adjustments for diabetics. Hence we also investigated the effect of concomitant administration of single dose cilostazol or pentoxifylline as well as pretreatment of cilostazol or pentoxifylline for seven days with single dose of antidiabetic agents in rats.

### 5.3.1 Effect of cilostazol on rosiglitazone pharmacokinetic parameters by simultaneous administration in rats

In this study after the oral administration of single dose rosiglitazone the absorption of rosiglitazone is rapid and is rapidly declined soon in rats. The concomitant administration of single dose of cilostazol with rosiglitazone in rats has shown no change in plasma levels of rosiglitazone but significantly increased $C_{max}$ to a lesser extent. It did not significantly change the parameters viz. $AUC_{0-24}$, $AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of rosiglitazone. The pretreatment of cilostazol in multiple doses prior to administration of single dose of rosiglitazone in rats also resulted in no change in plasma levels of rosiglitazone but significantly increased $C_{max}$ to a lesser extent. It did not significantly change the parameters viz. $AUC_{0-24}$, $AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of rosiglitazone.

This also supported by the earlier studies (Suri et al., 1999) using coadministration of warfarin with twice bath daily administration of cilostazol 100mg did not alter (R)- and (S)-warfarin pharmacokinetics, prothrombin time, partial thromboplastin time, Ivy bleeding times, or cilostazol protein binding. From this it is being observed that cilostazol did not inhibit the CYP3A4, CYP1A2, CYP2C19 (R-warfarin) and also CYP2C9 (S-warfarin).

Rosiglitazone is primarily metabolized to p-hydroxy and N-demethylation products via CYP2C8. Rosiglitazone is metabolized in major through CYP2C8 and to a lesser extent via CYP2C9 (Cox et

al., 2000). Ketoconazole affected the disposition of rosiglitazone in humans, probably by the inhibition of CYP2C8 and CYP2C9, leading to increasing rosiglitazone concentrations that could increase the efficacy of rosiglitazone or its adverse events.

In an in vitro study using CYP2C8 human recombinant enzymes, cilostazol has not shown any influence on the metabolic stability of rosiglitazone. Where as in the in vitro study using the liver microsomes prepared from rats pretreated with cilostazol, the metabolic stability of rosiglitazone has been very slightly increased in presence of cilostazol but it is very negligible. This clearly showed that cilostazol is not inhibiting the CYP isozymes in rat responsible for rosiglitazone and hence we observed no increase in plasma levels after administration of rosiglitazone in presence of cilostazol accordingly. Rosiglitazone is also known to be a potent inhibitor of its self enzyme CYP2C8 with a $k_i$ value of 5.59 μM and is also a weak inhibitor of CYP2C9 with a $k_i$ value of 29.9 μM (Sahi et al., 2003). Hence the self inhibition of its own metabolizing enzymes might occur however it could not resulted the same in this study since the rosiglitazone has been studied as a single dose.

The effect of cilostazol on [$^3$H]daunomycin transport in LLC-GA5-COL150 cells strongly suggested that cilostazol interacts with P-gp (Toyobuku et al., 2003). The effect of P-gp on rosiglitazone permeability is not known so far (Baciewicz et al., 2008) however OATP 1B1 has no role on intestinal uptake of rosiglitazone (Kalliokoski et al., 2008). Hence we could not rule out the possibility of role of cilostazol on rosiglitazone transport mechanism and thus the raise in $C_{max}$ could be due to this factor.

The blood glucose lowering activity of rosiglitazone was not observed in normal rats and the effect of cilostazol on this activity was not altered in spite of slight increased levels of rosiglitazone by pretreatment with cilostazol. Probably this effect may be seen in hyperglycemic model that needs to be studied. Based on these results, we don't expect a possible role of inhibition of cyctochrome P-450 and a possible role on P-gp or any transporters involved in uptake of rosiglitazone in liver/intestine by cilostazol pretreatment which might have resulted in the increased gastric absorption of rosiglitazone. However, further studies are needed to understand the mechanism behind the increased gastric absorption of rosiglitazone.

### 5.3.2 Effect of pentoxifylline on rosiglitazone pharmacokinetic parameters by simultaneous administration in rats

In this study the effect of concomitant administration of single dose and multiple doses pretreatment of pentoxifylline with single dose of rosiglitazone was investigated in rats.

Since pentoxifylline rapidly eliminates soon in rats and also in humans as well, pentoxifylline was administered three times daily for a single or mulitple days to investigate the effect of PTX on rosiglitazone in rats.

The concomitant administration of single day dose of pentoxifylline with rosiglitazone in rats resulted reduction in plasma levels of rosiglitazone but did not significantly change the parameters viz. $C_{max}$, $AUC_{0-24}$, $AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of rosiglitazone.

The pretreatment of multiple day doses of pentoxifylline prior to administration of single dose of rosiglitazone in rats also resulted in reduction in plasma levels of rosiglitazone there by significantly decreasing $C_{max}$ to a lesser extent. However it did not significantly change the parameters viz. $AUC_{0-24}$, $AUC_{0-\infty}$. Probably it could be due to the significant increase in $T_{max}$ (delay in absorption) and increase in $t_{1/2}$ of rosiglitazone accordingly.

Rosiglitazone is primarily metabolized to p-hydroxy and N-demethylation products and CYP2C8 is involved in these pathways (Cox et al., 2000). Rosiglitazone is metabolized in major through CYP2C8 and to a lesser extent via CYP2C9. In another study, ketoconazole affected the disposition of rosiglitazone in humans, probably by the inhibition of CYP2C8 and CYP2C9, leading to increasing rosiglitazone concentrations that could increase the efficacy of rosiglitazone or its adverse events. In an in vitro study using CYP2C8 human recombinant enzymes, pentoxifylline has slightly increased the metabolic stability of rosiglitazone. In another in vitro study using the liver microsomes prepared from rats pretreated with pentoxifylline, it slightly increased (signifantly) the metabolic stability of rosiglitazone compared to that of control rats. Hence pentoxifylline could have inhibited the CYP450 enzymes in rats very minimally and thus reflecting a slight increase in half-life of rosiglitazone in presence of pentoxifylline pretreatment.

Rosiglitazone is also known to be a potent inhibitor of its self enzyme CYP2C8 with a $k_i$ value of 5.59 µM and is also a weak inhibitor of CYP2C9 with a $k_i$ value of 29.9 µM and does not have much effect on CYP1A2 andCYP2E11 (Sahi et al., 2003). Hence the self inhibition of its own

metabolizing enzymes may occur however this effect might not been seen in this study because a single dose of rosiglitazone has been used in both control and pentoxifylline pretreated rats.

Pentoxifylline was found to reverse the vincristine resistance of the L1250/VCR mouse leukemic cell subline by overcoming P-gp (Stefankova et al., 1996). In an in vitro study pentoxifylline decreases mdr1 mRNA by 2 fold in presence of 100 mg/l PTX and there by enhanced the doxorubicin (DOX) accumulation in resistant L1210/VCR cell lines (Drobna et al., 2002). Thus PTX is down regulating mdr1 and P-gp protein and can enhance the permeability of drugs are effected by these transporters. The effect of P-gp on rosiglitazone permeability is not known so far (Baciewicz et al., 2008) however OATP 1B1 has no role on intestinal uptake of rosiglitazone (Kalliokoski et al., 2008). But this possibility could be ruled out due to reduced $C_{max}$ values instead of raise in levels.

The blood glucose lowering activity of rosiglitazone was not observed in normal rats and the effect of pentoxifylline on this activity was not altered though the decreased levels of rosiglitazone by pretreatment with cilostazol was observed Probably this effect may be seen in hyperglycemic model that needs to be studied. Based on these results, we expect a possible minimal role of inhibition of cyctochrome P-450 and no role on gastric absorption by pentoxifylline pretreatment which result in the increased systemic exposure of rosiglitazone though the $C_{max}$ reduced slightly. However, further studies are needed to confirm the role of pentoxifylline on rosiglitazone in humans is warranted.

### 5.3.3 Effect of cilostazol on metformin pharmacokinetic parameters by simultaneous administration in rats

As per literature, metformin in humans has 50-60% bioavailability and has lack of dose proportionality. Food decreases the extent of and slightly delays the absorption of metformin.In this study after the oral administration of single dose metformin, the absorption of metformin is rapid and is eliminated slowly in rats. The concomitant administration of single dose of cilostazol with metformin in rats resulted in increase in plasma levels of metformin at later hours. It increased both the parameters $AUC_{0-24}$ and $AUC_{0-\infty}$ significantly but did not significantly change the parameters viz. $C_{max}$, $T_{max}$ and $t_{1/2}$ of metformin. The pretreatment of cilostazol in multiple doses prior to administration of single dose of metformin in rats also increased the plasma levels of metformin and also significantly increased $AUC_{0-24}$, $AUC_{0-\infty}$ to a lesser extent. However, it did not

significantly change the parameters viz., $C_{max,}$ $T_{max}$, $t_{1/2}$ of metformin. It looks like cilostazol has enhanced the early phase of elimination (α) for metformin but not the later phase of elimination (β). Probably the increase in α half-life has maintained the AUC though there was a reduction in terminal half-life. These parameters were not calculated but were predicted from the graphical view of the plasma-time profile plots.

The volume of distribution in humans is high and is negligibly bound to plasma proteins in contrast to cilostazol which is more than 98% protein bound, metformin partitions into erythrocytes, most likely as a function of time. Metformin is excreted unchanged in the urine and does not undergo either hepatic metabolism (no metabolites have been identified in humans) or biliary excretion. Metformin is not metabolized by any of the enzymes studied so far and probably the drugs which influence on CYP does not interact with its pharmacokinetics. Metformin was metabolized mainly via CYP2C11, 2D1, and 3A1/2 in rats (Choi and Lee et al., 2006). This result could contribute to understanding of the possible changes in metformin pharmacokinetics in disease models where CYP2C11 and/or 3A1/2 are altered

In the in vitro studies using rat liver microsomes it was reflected the same as no metabolism of metformin has been observed either in control or cilostazol treated groups.

Renal clearance is approximately 3.5 times greater than creatinine clearance which indicates that tubular secretion is the major route of metformin elimination. Following oral administration in humans, approximately 90% of the absorbed drug is eliminated via the renal route within the first 24 hours, with a plasma elimination half-life of approximately 6.2 hours. In blood, the elimination half-life is approximately 17.6 hours, suggesting that the erythrocyte mass may be a compartment of distribution (Scheen et al., 1996).

Metformin could be absorbed from the whole intestine, with the main absorption site at duodenum. This concentration-dependent permeability behavior in the duodenum indicates that metformin is transported by both passive and active carrier-mediated saturable mechanism (Choi et al., 2006). The P(eff) value can not be increased by co-perfusion with verapamil, indicating that absorption of metformin is not efficiently transported by P-gp in the gut wall. Furthermore metformin is neither a substrate nor an inducer of P-gp (Song et al., 2006). The effect of cilostazol on [$^3$H]daunomycin transport in LLC-GA5-COL150 cells strongly suggested that cilostazol interacts with P-gp (Toyobuku et al., 2003). But the effect of cilostazol on these factors has to be ruled out because

metformin is not a substrate for P-gp. However, Metformin is a substrate of PMAT and its absorption depends upon gastric pH (Zhou et al., 2007) and the effect of cilostazol on PMAT or other transporters need to be understood as it increased the $C_{max}$ of metformin in the current study.

It is being primarily excreted through urine and is a substrate for some of the transporters involved in hepatic and renal distribution. OCT1 is majorly responsible for hepatic and intestinal uptake of metformin while the renal distribution and excretion are governed by other transporter mechanisms (Kumura and Masuda et al., 2005). And also the genotype variants in OCT1 are major determinants of metformin pharmacokinetics (Shu et al., 2008) .

The in vivo distribution of Metformin in rats revealed that the expression level of renal OCT2 was a key factor in the control of the concentrative accumulation of metformin in the kidney. These findings suggest that metformin is superior substrate for renal OCT2 than hepatic OCT1 and renal OCT2 plays a dominant role on its pharmacokinetics (Kimura and Masuda et al., 2005). Hence the renal distribution and accumulation is high compared to that of hepatic accumulation with metformin.

As discussed in literature, a drug interaction study in normal healthy volunteers demonstrated that co-administration of nifedipine increased plasma metformin $C_{max}$ and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. $T_{max}$ and half-life were unaffected. Nifedipine appears to enhance the absorption of metformin. Metformin had minimal effects on nifedipine. Cationic drugs (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, and vancomycin) that are eliminated by renal tubular secretion theoretically have the potential for interaction with metformin by competing for common renal tubular transport systems. Such interaction between metformin and oral cimetidine has been observed in normal healthy volunteers, with a 60% increase in peak metformin plasma and whole blood concentrations and a 40% increase in plasma and whole blood metformin AUC. There was no change in elimination half-life in the single-dose study. Metformin had no effect on cimetidine pharmacokinetics. Although such interactions remain theoretical (except for cimetidine), careful patient monitoring and dose adjustment of metformin and/or the interfering drug is recommended in patients who are taking cationic medications that are excreted via the proximal renal tubular secretory system.

In the current study the multiple doses of cilostazol has decreased the half-life of metformin drastically reflecting that metformin has preferentially being eliminated through kidney thus maintaining the systemic exposure though increase in $C_{max}$ has been observed.

The blood glucose lowering activity of metformin was not observed in normal rats and the effect of cilostazol on this activity was not altered and it could be due to no change in plasma levels of metformin by pretreatment with cilostazol. Probably this effect may or may not be seen in hyperglycemic model that needs to be studied.

Based on these results, we don't expect a possible role of inhibition of cyctochrome P-450 and a possible role on PMAT or any other transporter at liver/renal/intestine by cilostazol pretreatment which result in the increased absorption of metformin. However, further studies are needed to confirm the role of cilostazol on PMAT regarding increased absorption of metformin in to the intestines.

Overall it reflects the a significant role of cilostazol on single dose metformin systemic exposure and probably may not influence the pharmacodynamics of metformin in vivo but needs to be titrated in order to reduce the adverse/toxic effects of metformin.

### 5.3.4 Effect of pentoxifylline on metformin pharmacokinetic parameters by simultaneous administration in rats

Here we have investigated the effect of concomitant administration of single dose and multiple doses of pentoxifylline with single dose of metformin in rats.

Since pentoxifylline rapidly eliminates soon in rats and also in humans as well, we have administered three time a day dose of pentoxifylline either a single day or for seven days to show the effect of PTX on metformin in rats. The concomitant administration of single day dose of pentoxifylline with metformin in rats resulted an increase in plasma levels of metformin and $C_{max}$ but did not significantly changed the parameters viz. $AUC_{0-24}$, $AUC_{0-\infty}$. It has significantly decreased the $T_{max}$ and $t_{1/2}$ of metformin. The pretreatment of multiple doses of pentoxifylline prior to the administration of single dose of metformin in rats also resulted in slight decrease in plasma levels of metformin there by significantly decreasing $AUC_{0-24}$, $AUC_{0-\infty}$ to a moderate extent. However it did not significantly changed the parameters viz. $C_{max}$, Probably it could be due to the significant decrease in $T_{max}$ and decrease in $t_{1/2}$ of metformin accordingly.

In our in vitro study using the liver microsomes prepared from rats pretreated with pentoxifylline, it did not alter the metabolism of metformin greatly.

Since we discussed earlier that pentoxifylline does reduces the expression mdr1 protein and thus P-gp levels. But metformin is neither a substrate nor inducer of P-gp (Choi et al., 2006). However metformin is a substrate for plasma membrane monoamine transporter (PMAT) at intestine and thus affected by pH (Zhou et al., 2007). Probably the raise in $C_{max}$ values of metformin in presence of single dose of pentoxifylline could be due to these transport inhibition mechanisms but it is not changed in presence of repeat dose pentoxifylline. The effects of pentoxifylline on the transporters involved in transport of metformin (viz. OATP-1 and OCT-2) are unclear so far and the possibility of interaction at the hepatic distribution and renal excretion could not be denied but needs to be investigated further.

The blood glucose lowering activity of metformin was not observed in normal rats and the effect of pentoxifylline on this activity was not altered and could be due to decreased levels of metformin by pretreatment with cilostazol. Probably the similar effect may be seen in hyperglycemic model that needs to be studied.

Based on these results, we expect a possible minimal role of role on gastric absorption by pentoxifylline pretreatment and possible major role in distribution or uptake mechanism at liver/renal level which resulted in the decrease of half-life and thus reduced the systemic exposure of metformin accordingly. Hence, further studies are needed to confirm the role of pentoxifylline on metformin in humans is warranted.

### 5.3.5 Effect of cilostazol on nateglinide pharmacokinetic parameters by simultaneous administration in rats

Here we have investigated the effect of concomitant administration of single dose cilostazol as well as pretreatment of cilostazol in multiple doses prior to administration of single dose of nateglinide in rats. In our study after the oral administration of nateglinide at a single dose, the absorption of nateglinide is rapid and is rapidly eliminated soon in rats.

The concomitant administration of single dose of cilostazol with nateglinide in rats resulted in no change in plasma levels of nateglinide and but did not significantly change the parameters viz.

$C_{max}$, $AUC_{0-24}$, $AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of nateglinide. The pretreatment of cilostazol in multiple doses prior to ant administration of single dose of nateglinide in rats also resulted in reduced plasma levels of nateglinide and also significantly decreased $C_{max}$ to a lesser extent. However, it did not significantly change the parameters viz. $AUC_{0-24}$, $AUC_{0-\infty}$, $T_{max}$ but increased significantly $t_{1/2}$ of nateglinide. Probably the increase in half-life has maintained the AUC though there was a reduction in $C_{max}$.

Nateglinide is primarily metabolized to hydroxy and dehydrogenated products and CYP2C9 is involved in these pathways (Weaver ML, et al.,). Nateglinide is metabolized in major through CYP2C9 and to a lesser extent via CYP3A4. From warfarin interaction study (Suri et al., 1999) it is being observed that cilostazol did not inhibit the CYP3A4, CYP1A2, CYP2C19 (R-warfarin) and also CYP2C9 (S-warfarin). However in the in vitro study using rat liver microsomes pretreated with cilostazol, the metabolic stability of nateglinide in presence of cilostazol has been observed thus reflecting a minor inhibitory effect of cilostazol on CYP enzymes. It also showed a moderate inhition of CYP2C9 human recombinant enzymes when nateglinide was used as substrate

Cilostazol is not inhibiting CYP3A4 as it showed no interaction potential lovastatin and its metabolite formation as we discussed earlier. In another study., fluconazole affected the disposition of nateglinide in humans, probably by the inhibition of CYP2C9, leading to increasing nateglinide concentrations that could increase the efficacy of nateglinide or its adverse events (Takanohashi et al., 2007).

Nateglinide is also known to be a potent inhibitor of its self enzyme CYP2C9 (Sahi et al., 2004). Hence the self inhibition of its own metabolizing enzymes may occur however it could not have result the same in this current study since the nateglinide has been studied as a single dose in both control and cilostazol pretreated rats. Rifampicin modestly decreased the exposure of nateglinide probably by inducing its oxidative biotransformation (Niemi et al., 2001). The coadministration of gemfibrozil and itraconazole has limited influence on pharmacokinetics of nateglinide in humans (Niemi et al., 2001). In another study sulfinpyrazone, a selective CYP2C9 inhibitor has modestly increased the systemic exposure of nateglinide by 28% and is well tolerated (Sabia et al., 2004).

The effect of cilostazol on [$^3$H]daunomycin transport in LLC-GA5-COL150 cells strongly suggested that cilostazol interacts with P-gp (Tobokuyu et al., 2003). The radiolabeled study showed that P-gp does not influence the permeability of nateglinide and it is a substrate for MCT1

(Okumara et al., 2002). But the effect of cilostazol on MCT-1 is not known so far; this might have resulted in delaying the gastric absorption of nateglinide and hence the decrease in $C_{max}$ in presence of multiple dose of cilostazol could be due to this factor.

Based on these above results, we expect a major role of interaction at absorption and at the same time a possible role of inhibition of cyctochrome P-450 (CYP2C9) by cilostazol pretreatment that resulted in the decreased absorption as well as decreased elimination of nateglinide. However, further studies are needed to confirm the role of MCT-1 and other transporters regarding decreased absorption of nateglinide in to the intestines.

The blood glucose lowering activity of nateglinide was observed in normal rats and the effect of cilostazol on this activity was not altered significantly in spite of decreased levels of nateglinide by pretreatment with cilostazol. Probably this effect may be seen in hyperglycemic model that needs to be studied.

Overall it reflects the no role of cilostazol on single dose nateglinide and probably may not influence the pharmacodynamics of nateglinide in vivo in fact reduce the adverse/toxic events of nateglinide if any.

### 5.3.6 Effect of pentoxifylline on nateglinide pharmacokinetic parameters by simultaneous administration in rats

Here we have investigated the effect of concomitant administration of single and multiple day doses of pentoxifylline with single dose of nateglinide in rats. Since pentoxifylline rapidly eliminates soon in rats and also in humans as well, we have administered three times a day doses of pentoxifylline either a single day or multiple days to show the effect of PTX on nateglinide in rats.

The concomitant administration of single day dose of pentoxifylline with nateglinide in rats resulted decrease in plasma levels of nateglinide and significantly decreased the parameters viz. $C_{max}$, $AUC_{0-24}$, $AUC_{0-\infty}$ but no significant change in $T_{max}$ and $t_{1/2}$ of nateglinide. The pretreatment of multiple day doses of pentoxifylline prior to administration of single dose of nateglinide in rats also resulted in a decrease in plasma levels of nateglinide there by significantly decreasing $C_{max}$, $AUC_{0-24}$, $AUC_{0-\infty}$ to a great extent. However it delayed the $T_{max}$ and slightly increased the $t_{1/2}$ but these were not significant.

As we discussed earlier, nateglinide is metabolized in major through CYP2C9 and to a lesser extent via CYP3A4 (Weaver et al., 2001). In our in vitro study using the liver microsomes prepared from rats pretreated with pentoxifylline, it has reduced the metabolic stability of nateglinide greatly thus enhancing the metabolism. This is in correlation with our in vivo study where the plasma levels of nateglinide were decreased greatly up on multiple dose treatment of pentoxifylline. And pentoxifylline did not show any effect on metabolism of nateglinide in CYP2C9 bactosomes thus reflecting no role of inhibition. But the half life was increased slightly which could be due to the slight increasing tendency of T max. Hence we can not rule out the possibility of pentoxifylline on CYP2C9 induction but we did not investigate in this study.

Since we discussed earlier that pentoxifylline does reduces the expression mdr1 protein and thus P-gp levels (Drobna et al., 2002) . But nateglinide is known to be not a substrate for P-gp (Okumara et al., 2002). However its uptake is mediated by a proton dependent transporter distinct from MCT-1 and the effects of pentoxifylline on these transporters are unclear so far. Probably the decrease in plasma levels and $C_{max}$ of nateglinide could be due to these transport inhibition mechanisms.

The blood glucose lowering activity of nateglinide was observed in normal rats and the effect of pentoxifylline on this activity was not altered significantly in spite of decreased levels of nateglinide by pretreatment with pentoxifylline. Probably this effect may be seen in hyperglycemic model that needs to be studied.

Based on these results, we expect a major role of induction of cyctochrome P-450 and minor role on gastric absorption by pentoxifylline pretreatment which result in the decreased $C_{max}$ and thus systemic exposure of nateglinide accordingly. Hence these findings suggest that the dosage adjustment of nateglinide may be required when given together with pentoxifylline if the similar findings are being observed in humans. Hence further studies are needed to confirm the role of pentoxifylline on nateglinide in humans is warranted.

### 5.3.7 Effect of cilostazol on glipizide pharmacokinetic parameters by simultaneous administration in rats

Here we have investigated the effect of concomitant administration of single dose multiple doses of cilostazol with single dose of glipizide in rats.

As per literature , glipizide is completely absorbed rapid from the gastrointestinal tract and has an oral bioavailability of nearly 100% in humans (Pentikainen et al., 1983). Glipizide is highly bound to plasma protein (92% to 99%) and has a low volume of distribution (Wensing et al., 1989). Glipizide is extensively metabolized in the liver, and its main metabolites are 4-trans-hydroxyglipizide, 3-cis-hydroxyglipizide, and N-(2-acetyl-amino-ethyl-phenyl-sulfonyl) N-cyclohexylurea (DCAA). CYP2C9 is probably the main CYP enzyme involved in the oxidative metabolism of glipizide, because in an individual homozygous for the defective CYP2C9*3 allele, the oral clearance of glipizide was drastically lower than in other subjects (Kidd et al., 1999). The metabolites of glipizide are pharmacologically inactive and are excreted primarily in the urine; its $t_{½}$ is between 2 and 4 hours.

In this study after single dose oral administration of glipizid,e the absorption of glipizide is rapid and is slowly eliminated in rats. The concomitant administration of single dose of cilostazol with glipizide in rats resulted in more plasma levels of glipizide and significantly increased the $C_{max}$ , $AUC_{0-24}$ but did not significantly change the other parameters viz.$AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of glipizide.

The pretreatment of cilostazol in multiple doses prior to the administration of single dose of glipizide in rats also resulted in increased plasma levels of glipizide but eliminated rapidly in such a way that no significant improvement in systemic exposure. It significantly increased $C_{max}$ to a lesser extent compared to that of single dose cilostazol effect on glipizide. However, it did not significantly change the parameters viz. $AUC_{0-24}$, $AUC_{0-\infty}$, $T_{max}$ but decreased significantly $t_{1/2}$ of glipizide. Probably the decrease in half-life has maintained the AUC though there was an increase in $C_{max}$.

The effect of cilostazol on [$^3$H]daunomycin transport in LLC-GA5-COL150 cells strongly suggested that cilostazol interacts with P-gp (Toyobuku et al., 2003). The effect of P-gp on glipizide permeability is not known so far. Hence we could not rule out the any possibility of glipizide as a substrate for Pg-p and other transporters. The cilostazol effect on P -gp and other transporters might have influenced the permeability of glipizide in intestinal region and also any changes in gastric pH caused by cilostazol is unknown.. This might have resulted in delaying the gastric absorption of glipizide and hence the increase in $T_{max}$ about 0.5 hr in presence of multiple dose of cilostazol could be due to this factor. Magnesium hydroxide taken together with glipizide

accelerated the absorption of glipizide and enhanced the early insulin and glucose responses (Kivistro and Neuvonen et al., 1991a). Sodium bicarbonate, but not aluminum hydroxide, may also accelerate glipizide absorption (Kivisto and Neuvonen et al., 1991b) which shows that the alteration in gastric pH effecting on absorption of glipizide. The changes caused by cilostazol on gastric pH are not known. But overall increase in absorption of glipizide with either single dose or multiple dose cilostazol is unclear.

Cilostazol does not effect on warfarin in a drug interaction study (Suri et al., 1999) and from this it is being observed that cilostazol did not inhibit the CYP3A4, CYP1A2, CYP2C19 (R-warfarin) and also CYP2C9 (S-warfarin). In the in vitro study using CYP2C9 human recombinant liver enzymes and rat liver microsomes, cilostazol did not affect the metabolic stability of glipizide. Where as the current in vivo study reflects the possible role of cilostazol on CYP2C9 as it decreases the half-life of glipizide. There were no reports citing the induction potential of cilostazol on any CYP enzymes but it looks like cilostazol induces some of the CYP enzymes in rat liver. This was incorrelation with other study (Niemi et al., 2001), where the 5-day treatment with 600 mg of rifampicin once daily significantly affected the pharmacokinetics of glipizidein humans. Rifampicin reduced the mean AUC $_{0-\infty}$ of glipizide, but elevated the $C_{max}$. The mean $t_{½}$ of glipizide was shortened from 3.0 to 1.9 hours by rifampicin. A reduction in the $t_{½}$, but not in the AUC$_{(0-\infty)}$, was seen in every subject. Statistically non-significant decreases in the decremental AUC $_{(0-7)}$ and maximum decrease in blood glucose were obvious during the rifampicin phase compared to those of the placebo phase. None of the subjects experienced severe hypoglycemic symptoms. In a study in patients with NIDDM, cimetidine (400 mg) and ranitidine (150 mg), taken together with a normal morning dose of glipizide, raised the AUC of glipizide by 23% and 34%, respectively. In that study, the hypoglycemic activity of glipizide was also significantly increased.

Based on these above results, we expect a major role of increase in absorption and at the same time a possible minor role of induction of cyctochrome P-450 by cilostazol pretreatment that resulted in the increased absorption as well as increased elimination of glipizide. However, further studies are needed to confirm the role of P-gp and other transporters regarding decreased absorption of glipizide in to the intestines.

The blood glucose lowering activity of glipizide was observed in normal rats and the effect of cilostazol on this activity was significantly improved and this could be due to the increased levels

of glipizide by pretreatment with cilostazol. Probably this effect may be clinically relevant that needs to be studied.

Overall it reflects the major role of cilostazol on single dose glipizide and probably may influence the pharmacodynamics of glipizide in vivo but in fact it increases the adverse/toxic events (hypoglycemic activity) of glipizide if any.

### 5.3.8 Effect of pentoxifylline on glipizide pharmacokinetic parameters by simultaneous administration in rats

Here we have investigated the effect of concomitant administration of single day and multiple day dose pentoxifylline with single dose of glipizide in rats. The concomitant administration of single day dose of pentoxifylline with glipizide in rats resulted increase in plasma levels of glipizide but did not significantly change the parameters viz. $C_{max}$, $AUC_{0-24}$, $AUC_{0-\infty}$, $T_{max}$ and $t_{1/2}$ of glipizide.

The pretreatment of multiple dose pentoxifylline at 10 mg/kg.b.wt/day/t.i.d for seven days with concomitant administration of single dose of glipizide on day-7 in another group of rats also resulted in decrease in plasma levels of glipizide there by not ignificantly decreasing $C_{max}$ to a moderate extent. However it did not significantly change the parameters viz. $AUC_{0-24}$, $AUC_{0-\infty}$. Probably it could be due to the increase in $C_{max}$ and decrease in $t_{1/2}$ of glipizide accordingly maintaining the AUC.

As we discussed earlier, glipizide is metabolized in major through CYP2C9 and to a lesser extent via CYP2C19 (Kidd et al., 1999). In our in vitro study using the liver microsomes prepared from rats pretreated with pentoxifylline, it has increased the metabolism of glipizide significantly.

Since we discussed earlier that pentoxifylline does reduces the expression mdr1 protein and thus P-gp levels (Drobna et al., 2002). Glipizide effects on P-gp are unclear so far. Probably the raise in $C_{max}$ values of glipizide upon single dose could be due to these transport inhibition mechanisms. But it could have been overshadowed by the induction effect of pentoxifylline on glipizide metabolism thus had not shown an decrease in $C_{max}$ and AUC up on multiple doses pretreatment.

The blood glucose lowering activity of glipizide was observed in normal rats and the effect of pentoxifylline on this activity was significantly improved and this could be due to the increased levels of glipizide by pretreatment with pentoxifylline. Probably this effect may be clinically relevant that needs to be studied.

Based on these results, we expect a possible role of induction of cyctochrome P-450 upon multiple doses and minor role on gastric absorption by pentoxifylline pretreatment upon single dose which result in the slightly decreased $C_{max}$ and thus systemic exposure of glipizide accordingly. However, further studies are needed to confirm the role of pentoxifylline on glipizide in humans is warranted.

# Chapter 6

# CONCLUSIONS AND FUTURE SCOPE OF WORK

# 6 CONCLUSIONS AND FUTURE SCOPE OF WORK

**Conclusions**

- The single and multiple dose administration of cilostazol has slightly influenced the pharmacokinetics of single dose rosiglitazone however, rosiglitazone has significantly changed the pharmacokinetics of single dose cilostazol in rats. The effect of cilostazol on rosiglitazone resulted in an increase its maximum levels but not change in systemic exposure. The effect of rosiglitazone on cilostazol resulted in increased bioavailability of cilostazol along with reduction in the formation of major active metabolite of cilostazol (3, 4-dehydro-cilostazol). Hence this could not influence the pharmacodynamics of cilostazol in our study. However, the increased levels of cilostazol might result in cardiovascular problems and other side effects. Hence the effect of rosiglitazone on cilostazol pharmacokinetics in humans needs to be studied further.
- The multiple dose administration of pentoxifylline has slightly influenced the pharmacokinetics of single dose rosiglitazone however; rosiglitazone has significantly changed the pharmacokinetics of single dose pentoxifylline in rats. The effect of pentoxifylline on rosiglitazone resulted in decrease in maximum levels of rosiglitazone and increase in the elimination half life leading to no change in the systemic exposure. The effect of rosiglitazone on pentoxifylline resulted in decreased bioavailability of pentoxifylline and is probably due to increased metabolism of pentoxifylline in liver and or interaction with transporter mechanism in intestine. This resulted in slight decrease in the pharmacodynamics of pentoxifylline but not significant in our study. However the effect of rosiglitazone on pentoxifylline pharmacokinetics in humans needs to be studied further to monitor dosage adjustment.
- The single and multiple dose administration of cilostazol has significantly influenced on pharmacokinetics of single dose metformin at the same time, metformin also has significantly changed the pharmacokinetics of single dose cilostazol in rats. The effect of cilostazol on metformin resulted in increased bioavailability of metformin and this could be due to its decreased volume of distribution and clearance. The effect of metformin on

cilostazol resulted in increased bioavailability of cilostazol and is probably due to inhibition of any uptake transporters in hepatic/renal systems and or decreased excretion of cilostazol and its metabolite in urine. Since it increased the formation of major active metabolite as well and this may probably influence the pharmacodynamics of cilostazol in vivo. However, the increased levels of cilostazol might result in cardiovascular problems and other side effects. Hence the effect of metformin on cilostazol pharmacokinetics in humans needs to be studied further. The increase in levels of metformin in presence of repeat dose cilostazol also needs to be investigated in humans to avoid adverse/toxic effects of metformin.

- The multiple dose administration of pentoxifylline has significantly influenced the pharmacokinetics of single dose metformin and at the same time metformin also has shown influence on the pharmacokinetics of single dose pentoxifylline in rats. The effect of pentoxifylline on metformin resulted in decreased bioavailability of metformin and is probably due to its decreased volume of distribution. And it may probably influence the pharmacodynamics of metformin in vivo. Hence the effect of pentoxifylline on metformin pharmacokinetics in humans needs to be studied further. At the same time effect of metformin on pentoxifylline in humans is also warranted as it reduced the bioavailability up on repeated dose administration and thus reflected in decreased the antiplatelet activity of pentoxifylline significantly. Over all it is suggested that a dosage adjustment may be required in case both these drugs are given together.
- The multiple dose administration of cilostazol has not influenced the pharmacokinetics of single dose nateglinide where as nateglinide has significantly changed the pharmacokinetics of single dose of cilostazol in rats. The effect of cilostazol on nateglinide resulted in decreasing of maximum plasma concentration but not in systemic exposure but this was not reflected in change of blood glucose lowering activity. The effect of nateglinide on cilostazol resulted in increased bioavailability of cilostazol and is probably due to decreased metabolism of cilostazol in liver and or other transporter mechanisms. But it also moderately increased the formation of major active metabolite of cilostazol by decreasing its elimination and this was not reflected in improving the antiplatelet activity of cilostazol in our study. However, the increased levels of cilostazol might result in cardiovascular problems and other side effects. Hence the effect of nateglinide on cilostazol

pharmacokinetics in humans needs to be studied further. The drop in $C_{max}$ of nateglinide in presence of cilostazol needs to be investigated further.

- The single and multiple dose administration of pentoxifylline has significantly influenced the pharmacokinetics of single dose nateglinide where as nateglinide has shown slight influence on single dose of pentoxifylline in rats. The effect of pentoxifylline on nateglinide resulted in drastic decrease in bioavailability of nateglinide and also its maximum levels and is probably due to induction of CYP2C9. Hence his may greatly influence the pharmacodynamics of nateglinide in vivo by decreasing its hypoglycemic activity however this could not resulted the same in our study. Hence the effect of pentoxifylline on nateglinide pharmacokinetics in humans needs to be studied further. At the same time effect of nateglinide on pentoxifylline in humans is also warranted as it increased the bioavailability slightly up on multiple dose administration.
- The multiple dose administration of cilostazol has significantly influenced the pharmacokinetics of single dose glipizide and at the same time glipizide also has changed the pharmacokinetics of single dose cilostazol in rats. The effect of cilostazol on glipizide resulted in an increase of its maximum plasma levels and reduced elimination half life leading to no change in the systemic exposure. This also has resulted in improving the hypoglycemic activity of glipizide. The effect of glipizide on cilostazol resulted in drastic increase in bioavailability of cilostazol and is probably due to decreased metabolism of cilostazol to its other metabolite in liver and or inhibition of P-gp/efflux transporter in intestine. But it also increased the formation of major active metabolite of cilostazol and this may probably influence the pharmacodynamics of cilostazol in vivo and also reflected the same in improving the antiplatelet activity of CLZ in our study. However, the increased levels of cilostazol might result in cardiovascular problems and other side effects. Hence the effect of glipizide on cilostazol pharmacokinetics in humans needs to be studied further. Hence the dose titration needs to be considered for those using both cilostazol and glipizide chronically to avoid hypoglycemic effects as well as cardiovascular toxicity.
- The multiple dose administration of pentoxifylline has not shown any influence on the pharmacokinetics of single dose glipizide where as glipizide has shown slight influence on

pharmacokinetics of single dose pentoxifylline. The effect of pentoxifylline on glipizide resulted in no change in maximum levels and bioavailability of glipizide but delayed the gastric absorption. However, it has increased the hypoglycemic activity of glipizide in normal rats in our study. Hence the effect of pentoxifylline on glipizide pharmacokinetics in humans needs to be studied further. At the same time effect of glipizide on pentoxifylline in humans is also warranted as it increased the maximum plasma levels of pentoxifylline and thus improved the antiplatelet activity of pentoxifylline up on single dose administration in rats.

**Future Scope of Work**

- The mechanism were tried to identify the interaction at metabolism wherever possible using liver microsomal systems however, the mechanisms of interaction at the absorption, distribution and excretion need to be studied.
- The transport mechanism need to be investigated further
- The pharmacodynamic effects have been studied using normal healthy rats to understand preliminary information of drug interaction effect wherever possible but these significant pharmacokinetic interactions needs to be evaluated for its effect on pharmacodynamic activity using proper animal models viz. Euglycaemic models for insulin sensitizers and biguanide derivatives.
- Further pharmacodynamic models of antiplatelet agents can be studied to understand the effect on cilostazol and pentoxifylline in detail.
- The significant drug interactions in this study may predict the interaction in human but these needs to be investigated properly in clinical environment.

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# REFERENCES


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# LIST OF ORIGINAL PUBLICATIONS

This original research articles pertaining to this thesis work either communicated or presented in conferences/symposiums are as follows.

Research papers in peer reviewed journals

1. **Varanasi VS Kanthi Kiran**, Veerraghavan Shridhar, Potharaju Suresh, Satheesh TRS, Rashmi Raghavan, and Vakkalanka VS Swaroop Kumar. "**Validated High Performance Liquid Chromatographic Method for Simultaneous Determination of Rosiglitazone, Cilostazol, 3, 4-dehydro-cilostazol in Rat Plasma and its application to pharmacokinetics**", *Arzneimittelforschung*, AF 0608-02 (Article in press).
2. **Kanthi Kiran VS Varanasi**, Suresh Potharaju, Shraddha Rajak, Sridhar Veerraghavan, Pankajini Mallick and Swaroop Kumar VS Vakkalanka. "**Effect of Pentoxifylline on the Pharmacokinetics of Rosiglitazone in Wistar rats**". *Methods Find Exp Clin Pharmacol* manuscript accepted with revision.
3. **Kanthi Kiran VS Varanasi***, Sridhar V, Suresh Potharaju, Shraddha R, Sivakumar SPN, Kanaga Sabapathi S, Satheeshmanikandan TRS, Swaroop Kumar VVS, "**Development and validation of a liquid chromatography /tandem mass spectrometry assay for the simultaneous determination of nateglinide, cilostazol and its active metabolite 3, 4-dehydro-cilostazol in wistar rat plasma and its application to pharmacokinetic study**". *Journal of Chromatography B* (2008), doi; 10.1016/j.jchromb.2008.02.013 (article in press)
4. **Kanthikiran VVS** *, Potharaju S, Shraddha R, Sridhar V, Pankajini Mallick, Sivakumar SPN and Swaroop Kumar VVS, "**Effect of Pentoxifylline on the Pharmacokinetics of Nateglinide in Wistar Rat**", *Ind J Pharm Sci* manuscript communicated

Presentations in conferences/symposiums

5. **Kanthi Kiran V S Varanasi**, Shridhar V, Potharaju S, Shraddha R, Anand G Patil, Pankajini Mallick, and. Swaroop Kumar Vakkalanka "**Drug-Drug Interaction: Effect of Thiazolidinedione Analogues, Rosiglitazone and Pioglitazone, on pharmacokinetics of Cilostazol in Rats**" *12-14$^{th}$ September 2006, 9$^{th}$ Annual Land-O'-Lake conference, Merrimac, Wisconsin, U.S.A*

# BRIEF BIOGRAPHY OF SUPERVISOR


Name : **Swaroop Kumar V.S. Vakkalanka**

Present Designation : President – Drug Discovery & Clinical Development,
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Dr. Swaroop Kumar is a Pharmaceutical Scientist, with special expertise in Drug Metabolism, Pharmacokinetics and Clinical Pharmacology. He has been intensely involved in pharmaceutical research from last 15 years, working in several capacities in the Indian Pharmaceutical Industry.

He is PhD in Pharmaceutical Sciences from the Andhra University, India from which he acquired his B.Pharm as well as M.Pharma with distinction and won gold medals during his academic career. Subsequent to acquiring his doctorate in pharmaceutical Sciences, he continued his research in pharmaceutical industry.

He has started his career as a Research Scientist in Drug Metabolism and Pharmacokinetic division in Dr.Reddy's Research Foundation (Hyderabad, India). He served as the core member of Drug Discovery Programs of Dr.Reddy's Research Foundation in the areas of Cancer and Diabetes. Later on he moved to Sun Pharma Advanced Research Centre (Vadodara, India) as Head – Pharmacokinetics and Clinical Pharmacology. He initiated, planned, designed and established an Integrated Pre-clinical and Clinical Pharmacokinetic Department as well as Clinical Pharmacology Section to support in-house R & D efforts in the areas of Drug Discovery and Product Development. The departments were built as per the global quality system following GLP & GCP.

Currently he is working as President – Drug Discovery & Clinical Development for Glenmark Pharmaceuticals Ltd, leading a team of 150 scientists across scientific disciplines that include Medicinal Chemistry, Pharmacology, Pharmacokinetics, Molecular Biology, Toxicology and Clinical Research. The current areas of research in Glenmark Pharmaceuticals Ltd include Asthma, Diabetes, anti–inflammatory and pain disorders. He has to his credit the development of 3 NCE's to Phase – II stage and has guided several Discovery / Pre-Clinical Programs.

He has various peer reviewed paper publications and international poster presentation on various topics. Dr.Swaroop is frequently invited to lecture around the world on his research to both academic and industrial audiences. He has also been speaker at many International Meetings. Dr.Swaroop guided 15 post graduate students to date and is currently guiding two PhD students. He is also a member of the several scientific associations which includes American Diabetes Association, European Respiratory Society, Drug Information Association, American Association of Pharmaceutical Scientist, Indian Pharmacological Society etc.

# BRIEF BIOGRAPHY OF CANDIDATE


| | | |
|---|---|---|
| **Name** | : | **Varanasi Venkata Sesha Kanthi Kiran** |
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Mr. Kanthi Kiran is a post graduate (M.Pharm) in Pharmaceutical sciences from the Andhra University, India from which he acquired his B.Pharm as well with first class during his academic career. He was also awarded MBA (Marketing Research) from Indira Gandhi National Open University. Subsequent to acquiring his post graduation in pharmaceutical Sciences, he continued his research in pharmaceutical industry.

His career started with Dr. Reddy's Research Foundation [Hyderabad, India] in 1997 as senior pharmacologist. Where he has worked in the areas of diabetes, hyperlipidemia, cancer and anti-inflammatory research to screen and develop the new chemical entities using various pharmacological assays. He left Dr. Reddy's in 2000 to join Sun Pharma Advanced Research Centre [Vadodara, India] as a Sr. Executive in Pharmacokinetics and clinical pharmacology department. At Sun Pharma he played major role in development of clinical pharmacology unit and established international regulatory environment. From 2000 to 2003 his work was mainly in the area of clinical research and pharmacokinetics. Then he moved to Glenmark Pharmaceuticals Limited [Navi Mumbai, India] in 2003 as Sr. Research Scientist and later promoted to Principal Scientist. Currently he is working as Head- Pharmacokinetics and Drug metabolism where he established several in vitro and in vivo ADME techniques. He supported Pharmacokinetic, toxicokinetic and drug metabolism needs of in-house R&D of Drug Discovery as well as product development. He has contributed his knowledge in screening and development of the new chemical entities for asthma & COPD, diabetes and pain disorders. These are currently in phase II clinical trials and procured the outstanding milestone payments to the organization.

He has 3 peer reviewed paper publications and several international poster presentations. He has guided 8 post graduate students to date and currently guiding 2 more students. He is also a member of the several scientific associations which includes Chromatography Society of India, DSE and etc.