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Nisha Sharma

List of abbreviations

ACE	Angiotensin-converting enzyme
ACE2	Angiotensin-converting enzyme 2
ACEi	Angiotensin-converting enzyme inhibitor
AGT	Angiotensinogen
AKI	Acute kidney injury
ALT	Alanine transaminase
ANF	Atrial natriuretic factor
Ang II	Angiotensin II
Ang III	Angiotensin III
Ang IV	Angiotensin IV
Ang (1-7)	Angiotensin 1-7
ANOVA	One-way analysis of variance
ARB	Ang II receptor blockers
AT1R	Angiotensin II type 1 receptor
AT2R	Angiotensin II type 2 receptor
AST	Aspartate aminotransferase
BAD	BCL2 associated agonist of cell death
BCL-2	B-cell lymphoma 2
BMP-7	Bone Morphogenetic Protein-7
BSA	Bovine serum albumin
BUN	Blood urea nitrogen
C21	Compound 21
CBP	CREB binding protein
cGMP	Cyclic guanosine monophosphate
ChIP	Chromatin-immunoprecipitation
CKD	Chronic kidney diseases
CK-MB	Creatine kinase-MB
Cypro	Cyproheptadine
CRS	Cardiorenal syndrome
CVDs	Cardiovascular diseases

DM	Diabetic Mellitus
Dize	Diminazene Aceturate
DNMT1	DNA methyltransferase 1
ECM	Extracellular matrix
eEOCs	Early endothelial outgrowth cells
EMT	Epithelial-to-mesenchymal transition
ESRD	End-stage renal disease
EZH2	Enhancer of Zeste 2
GCSF	Granulocyte-colony stimulating factor
GFAP	Glial fibrillary acidic protein
GFR	Glomerular filtration rate
GPCR	G protein-coupled receptors
GSH	Reduced glutathione
HATs	Histone acetyltransferases
HDACs	Histone deacetylases
HIF-1α	Hypoxia-inducible factor-1α
HMGCR	3-hydroxy-3-methyl-glutaryl-coenzyme A reductase
HO	Heme-oxygenase
HMT	Histone methyltransferases
IBP	Invasive blood pressure
ICAM-1	Intercellular adhesion molecule 1
IGF-1	Insulin-like growth factor-1
I/R	Ischemia/reperfusion
IRI	Ischemic renal injury
IL-6	Interleukin-6
IP3	Inositol trisphosphate
KC	Keratinocyte chemoattractant
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
LSB	Low salt buffer
MAPKs	Mitogen-activated protein kinases

MCP-1	Monocyte chemoattractant protein 1
MDA	Malondialdehyde
ND	Non-diabetic
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
PAI-1	Plasminogen activator inhibitor 1
Pal	Plasma albumin
PARP	Poly-(ADP-ribose) polymerase
PCr	Plasma creatinine
PGL	Plasma glucose
PGC-1α	Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 α
PKC	Protein kinase C
POC	Postconditioning
PPAR-γ	Peroxisome proliferator-activated receptor-γ
PSR	Picrosirius Red
PTMs	Posttranslational modifications
qRT-PCR	Quantitative real-time polymerase chain reaction
RAS	Renin-angiotensin system
rhACE2	Recombinant human ACE2
ROS	Reactive oxygen species
SAHA	Suberoylanilide hydroxamic acid
SBP	Systolic blood pressure
SNS	Sympathetic nervous system
TGF-β	Transforming growth factor-β
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor-α
TSA	Trichostatin A
ukim-1	Urinary Kidney injury molecule-1
UUO	Unilateral ureteral obstruction
VPA	Valproic acid
VCAM-1	Vascular cell adhesion molecule 1

VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Abstract

Background:

Clinically, acute kidney injury (AKI) is considered a catastrophic condition with incidences allied with high morbidity and mortality. One of the major risk factors for AKI is diabetes mellitus (DM). A lot of research has been carrying out to understand the complex pathogenesis of AKI, but only supportive therapies are available. Growing evidence has demonstrated the vital role of epigenetic regulation in gene expression under the pathogenesis of AKI. SET domain with lysine methyltransferase 7/9 (SET7/9), a histone lysine methyltransferase (HMT), recently suggested exerting a critical role in diabetes-associated kidney disorders. Whereas, the role of SET7/9 in the progression of ischemic renal injury (IRI) remains completely elusive. Hence, the primary objective of the present work was to delineate the role of SET7/9 and histone methylation in the regulation of inflammatory signaling under IRI in DM and non-diabetic (ND) conditions.

In addition, existing reports highlighting the role of the renin-angiotensin system (RAS) in AKI and DM. Adverse renal outcomes in DM and AKI individually are attributed mainly to the renin-angiotensin system (RAS) driven activation of mitogen-active protein kinase (MAPK)-mediated apoptosis, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) mediated inflammation, and redox imbalance promoting oxidative stress. The clinical relevance proposed that all the organs have their own local RAS which remains compartmentalized from the circulation. Following a similar approach, recent reports have demonstrated the activation of local RAS in distant organs i.e. brain, heart and liver. Besides, IRI predisposes distant organ dysfunction including, neurological, cardiac and hepatic dysfunctions, in which the RAS serves as the major contributor. However, in hospital settings, treatment with pressor arm modulators i.e. angiotensin-converting enzyme inhibitor (ACEi) and angiotensin II type 2 receptor blockers (ARBs) claimed to worsens the patient's condition via deteriorating glomerular filtration rate. Away from the conventional approach, in quest of a novel therapy against IRI and its associated distant organ dysfunction, in our research work, we targeted depressor arm of RAS using Angiotensin II type 2 receptor (AT2R) agonist i.e. compound 21 (C21) and Angiotensin-converting enzyme 2 (ACE2) activator i.e. Diminazene aceturate (Dize) alone or combination therapy.

Methodology:

Streptozotocin (55 mg/kg, *i.p.*) was injected in male Wistar rats to develop a non-genetic DM model, to mimic the early phase of diabetes i.e. hyperglycemic stage. To develop AKI, unilateral/bilateral IRI was performed followed by 48 h and 24 h of reperfusion in DM and ND rats. In the pharmacological intervention study, after completion of two weeks of diabetes induction, the ND and DM rats were administered with different treatments such as i) study 1: cyproheptadine at a low dose (10 mg/kg/day, *i.p.*) and high dose (20 mg/kg/day, *i.p.*) administered 30 min prior to uIRI and after 24 h of reperfusion, ii) study 2: AT2R agonist, C21 (0.3 mg/kg/day, *i.p.*) or ACE2 activator, Dize, (5 mg/kg/day, *p.o.*) either alone or as combination therapy is given 2 days prior to IRI and continued to 48 h of reperfusion, and study iii) C21 (0.3 mg/kg/day, *i.p.*) or Dize (5 mg/kg/day, *p.o.*) either alone or in combination therapy administered two days prior to IRI and continued to next day (24 h of reperfusion time). The effect of these agents on the progression of AKI and its associated distant organ dysfunction was evaluated by various biochemical, hemodynamic and behavioral parameters. The microscopic alterations in kidney, heart, brain and liver architecture were assessed by hematoxylin and eosin (H and E) staining. To study the molecular mechanisms, proximal tubules were isolated from the kidney tissues. Alterations in several RAS components were evaluated by commercially available ELISA kits and immunohistochemistry. Also, the effects of the abovementioned treatments on expressions of mRNA and proteins involved in pathological signaling pathways like oxidative stress, inflammation and apoptosis were studied by quantitative RT-PCR, immunoblotting and immunohistochemistry. For studying epigenetic mechanisms, we extracted histones from isolated proximal tubules and assessed the histone posttranslational modifications (PTMs) by immunoblotting and quantitative RT-PCR. Additionally, the expression of enzymes involved in orchestrating histone H3 lysine 4 (H3K4) methyltransferase SET7/9 was also studied.

Results:

The IRI animal model development was confirmed the elevated blood urea nitrogen (BUN), Plasma Creatinine (PCr), and urinary Kim-1 levels in ND and DM rats. In the epigenetic study, primarily we observed the NF-κB mediated inflammatory cascade like increased p-NF-κB, reduced IκBα levels followed by enhanced leukocyte infiltration which

was shown by increased monocyte chemoattractant protein-1 (MCP-1) expressions. Further, IRI resulted in increased histone H3 methylation at lysine 4 and 36 (H3K4Me2, H3K36Me2), and decreased histone H3 methylation at lysine 9. Additionally, IRI increased the mRNA and protein expression of H3K4Me2 specific histone methyltransferase-SET7/9 in DM and ND rats. The above-mentioned results remain prominent in DM rats compared to ND rats followed by IRI. Further, treatment with a novel SET7/9 inhibitor; cyproheptadine, exerted reno-protective role by significantly improving renal function as shown by reduced BUN level, inflammation via inhibiting SET7/9 expressions in ND and DM rats.

After uIRI, ND and DM rats displayed an increase in plasma ACE, AT1R, AT2R, Ang II, and reduction in ACE2, Ang (1-7) expressions, with augmented renal inflammation and apoptosis. These changes were more prominent in diabetic rats with IRI. Co-administration of C21 and Dize augmented ACE2, Ang (1-7), AT2R and MasR expressions, and effectively attenuated tubular injury in both DM and ND rats which were evidenced by suppressed protein and mRNA expressions of p-NF-κB, MCP-1, interleukin-6 (Il-6) and tumor necrosis factor-α (Tnf-α).

In IRI-induced neurological impairment, we found that IRI drastically reduced the locomotor activity of DM-IRI rats compared to ND-IRI rats. IRI causes increased hippocampal MDA and nitrite levels, augmented inflammatory cytokines (granulocyte-colony stimulating factor, glial fibrillary acidic protein), altered protein levels of Ang II, Ang (1-7) and mRNA expressions of *At1r*, *At2r* and *Masr* in ND and DM rats. In the pharmacological intervention study, treatment with C21 and Dize effectively normalized the aforementioned pathological alterations. Moreover, the protective effect of C21 and Dize combination therapy was better than respective monotherapies, and more likely, exerted via augmentation of protein and mRNA levels of depressor arm components. Further, in IRI-associated cardio-hepatic dysfunction, IRI caused cardio-hepatic injuries via altered oxidant/anti-oxidant levels, elevated inflammatory events, disturbed morphological architecture and altered protein expressions of ACE, ACE2, Ang II, Ang (1-7) and urinary AGT. However, concomitant therapy of AT2R agonist (C21) and ACE2 activator (Dize) exerted protective effect in IRI-associated cardio-hepatic dysfunction as

evidenced by inhibited oxidative stress, downregulated inflammation, and enhanced cardio-hepatic depressor arm of RAS under ND and DM conditions.

Conclusion: Our results clearly indicated the critical role of SET7/9 in mediating active transcription via H3K4Me2, ultimately regulated the NFκB-mediated inflammatory cascade. Moreover, cyproheptadine reverts the SET7/9 expressions promoted by IRI. Therefore, cyproheptadine appears to be a potential therapeutic option to treat IRI which combats renal dysfunction and inflammation and further research needs to be done to propel the molecule, cyproheptadine to further stages of drug development. Additionally, the current studies suggested that targeting the depressor arm of RAS via C21/Dize combination therapy, provides a novel therapeutic approach to combat IRI and its associated distant organ dysfunction under DM and ND conditions.

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