

Biochemical Mechanisms of Homocysteine and its Role in RETINAL VASCULAR DISEASES

THESIS

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By

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CHAPTER 6: CONCLUSION

Homocysteine metabolism is reported in literature. The role of trace elements iron and GSH synthesis and its turnover in homocysteine metabolism was studied in this work.

Developed a method for simultaneous determination of amino acids involved in Homocysteine pathway. The amino acids cysteine, glutamic acid and glycine were significantly decreased whereas Hcy was significantly increased in ED and ARMD. Hcy estimation was done by this in-house developed HPLC method and compared with gold standard ELISA are comparable, thus validating the HPLC method is reliable one. Due to the decreased level of above mentioned amino acids, the level of rate limiting enzyme GCL also decreased and leads to the decreased synthesis of GSH in ED. In PBMC, the mRNA expression of both GCLC and GCLM expression of GCL found to be decreased in ED. It was proved by in-silico approach that homocysteine and cysteine are binding to GCL enzyme. The studies further show that the cofactors Mg^{2+} and ATP interact with cysteine to form a GCL complex, for glutathione synthesis, whereas in hyperhomocysteinemia instead of cysteine, homocysteine binds to cofactors to prevent the glutathione synthesis.

Homocysteine and its metabolite Homocysteine-thiolactone play an important role in atherothrombosis. Thus Hcy and HcyTL were increased in ED and Age related macular degeneration. The Protein-Cys bound Homocysteine and Protein-Lys bound Homocysteine were increased in ED and Age related macular degeneration. Thus Hcy and HcyTL alter the protein structure and function in ED and ARMD.

Iron is playing an important role in homocysteine pathway. The levels of total iron binding capacity, haemoglobin in serum were increased. The level of heme and ALAS in serum were increased, thus synthesis of iron and its breakdown (heme oxygenase) were increased in ED. The level of ferritin is increased and transferrin is decreased in serum and PBMC. The VEGF level in serum and PBMC were increased in ED. Iron sensor hepcidin was increased and ferroportin level was decreased in Eales' disease. Collectively the results indicate the dysregulation of iron homeostasis in ED may be due to the infection. Both iron and homocysteine are synergistically act as risk factors for vascular diseases.

Further, the homocysteine metabolism and GSH synthesis was studied in *invitro* cell culture model namely retinal pigment epithelial cells and human umbilical vein endothelial cells. In these cells, the mRNA expression of both the catalytic unit (GCLC) and modifier unit (GCLM) of GCL were altered in increased concentration of Homocysteine through nuclear erythroid related factor 2 (Nrf2).

FUTURE SCOPE:

1. Simultaneous determination of GSH, GSSG and GCL in large number of samples by LC-MS/MS.
2. To study the Homocysteine metabolism related to ocular vascular diseases in an animal model.
3. *In vitro* cell culture of iron along with the Homocysteine to see the GSH synthesis and its turnover.
4. Using iron chelators to see the GSH synthesis and its turnover after Homocysteine treatment to the cells.
5. The effects of Taurine in Homocysteine need to be studied.
6. To design the small molecules to inhibit the Homocysteine and see the GSH synthesis in cell culture experiment.
7. To study the S-adenosyl methionine (SAM) involvement in Homocysteine metabolism related to ocular vascular diseases.