ABSTRACT

Title of PhD Thesis: Possible involvement of Phosphatidylinositol-3-kinase in experimental vascular endothelium dysfunction.

Background

Vascular endothelium plays a role in capillary transport of nutrients and drugs and regulates angiogenesis, homeostasis, as well as vascular tone and permeability. As a major regulator of local vascular homeostasis, the endothelium maintains the balance between vasodilatation and vasoconstriction, procoagulant and anticoagulant, prothrombotic and antithrombotic mechanisms. Diabetes mellitus causes the activation of aldose reductase, polyol pathway and advanced glycation-end-product formation that collectively affect the phosphorylation status and expression of endothelial nitric oxide synthetase (eNOS) and causes vascular endothelium dysfunction. Elevated homocysteine levels have been associated with increase in LDL oxidation, generation of hydrogen peroxides, superoxide anions that increased oxidative degradation of nitric oxide. Hyperhomocysteinemia has been reported to increase the endogenous competitive inhibitors of eNOS viz- L-N-monomethyl arginine (L-NMMA) and asymmetric dimethyl arginine (ADMA) that may contribute to vascular endothelial dysfunction. Hypercholesterolemia stimulates oxidation of LDL-cholesterol, release of endothelins, and generation of ROS. The increased cholesterol and triglyceride level and decreased protective HDL level, decreases the activity and expression of eNOS and disrupts the integrity of vascular endothelium, due to oxidative stress. Hypertension also stimulates release of endothelins, vasoconstrictor prostanoids, angiotensin II, inflammatory cytokines, xanthine oxidase and, thereby, reduces bioavailability of nitric oxide.

Thus, the cellular and molecular mechanisms underlying diabetes mellitus, hyperhomocysteinemia, hypercholesterolemia and hypertension leads to an
imbalance of phosphorylation and dephosphorylation status of lipid and protein kinase that cause modulation of vascular endothelial L-arginine/nitric oxide synthetase (eNOS), to produce vascular endothelium dysfunction.

Phosphatidylinositol -3 kinase is a ubiquitous enzyme involved in plethora of cell signaling including the endothelial cells and it has been reported that signaling through this enzyme and its downstream pathway viz Phosphoinositide-dependent kinase (PDK)/ Protein kinase B (Akt) and eNOS is impaired in diseased conditions . PDK causes phosphorylation and activation of AKT at Kinase domain and Akt has been documented to modulate various sub- cellular effectors such as proapoptotic factors: BAD, Caspase 9, Forkhead transcription factors, NF K-β, mTOR/P70 S6K, eNOS and translocation of Glut-4 to mediate cell growth and cell survival, protein synthesis and glucose metabolism.

Aims and Objectives –

The present study was designed to investigate the role of PI3K and PDK/AKT in vascular endothelial dysfunction produced by diabetes mellitus, hypercholesterolemia, hypertension and hyperhomocysteinemia.

Material and Methods-

Experimental Protocol – Table 1

1) Models employed for experimental vascular endothelium dysfunction

1) Diabetes mellitus was induced by streptozotocin (55 mg/kg, i.v) The animals were used for experimentation 4 weeks later. Assessment was done by serum glucose estimation (GOD/POD) Method (UV Spectrophotometer) (Pieper et al.,1997).

2) Hypercholesterolemia was induced by administering high fat diet to another group of rats for four weeks. High cholesterol diet contained -casein 200 gm, coconut oil – 250 gm, cholesterol – 10 gm, cholic acid – 5 gm, sucrose 484 gm, choline 2 gm, DL-
methionine 4 gm, vitamin mix 10 gm, and mineral mix 35 gm (Zulet et al., 1999). Assessment of Hypercholesterolemia was done by estimation of serum Cholesterol, HDL, Triglyceride (UV Spectrophotometry) (Allian et al., 1974).

3) Hyperhomocysteinemia was induced by L-Methionine (1.7% w/w., p.o x 4 weeks). Assessment was done by High Performance liquid Chromatography (Jacobsen et al., 1994).

4) Uninephrectomy was done to induce hypertension followed by administration of Deoxycorticosteroneacetate salt (DOCA, 40 mg/kg, s.c. twice x 6 weeks) (Shah and Singh., 2007). Assessment was done by measuring mean arterial blood pressure (Tail cuff method)

II) Parameters employed to assess vascular endothelium dysfunction -

1) Isolated Aortic Ring Preparation (Woodman et al., 2004) a) Acetylcholine-induced, endothelium-dependent relaxation b) Sodium nitroprusside induced endothelium independent vasorelaxtion 2) Serum nitrate/nitrite level (Sastri et al., 2002), as an indirect measure of Nitric Oxide release. 3) mRNA expression of eNOS (Reverse transcriptase Polymerase chain reaction) (Shah and Singh., 2007) 4) Integrity of vascular endothelium (Electron microscopy) (Schiller et al., 1999)

Data for serum levels of glucose, Nitrite/Nitrate, homocysteine, HDL, TG and cholesterol were statistically analyzed using one way ANOVA followed by Tukey’s multiple range test, whereas Data for isolated aortic ring preparation was statistically analyzed using one way ANOVA followed by Newman-Keul’s test. P < 0.05 was considered to be statistically significant.
Results

In the present study, streptozotocin-induced diabetes mellitus, methionine-induced hyperhomocysteinemia, high fat diet-induced hypercholesterolemia and DOCA induced hypertension have been noted to produce vascular endothelium dysfunction. Administration of insulin (0.6 IU/kg/day, s.c), YS 49 (1,2,3,4-Tetrahydro-1-(1-naphthalenylmethyl)-6,7-Isquinolinediol hydrobromide monohydrate) (1.6 mg/kg/day, i.p), DAQ B1(Demethylasteraquinone B1) (5mg/kg/day, i.p) significantly improved acetylcholine-induced endothelium-dependent relaxation, serum nitrate/nitrite level, as well as mRNA expression of eNOS and integrity of vascular endothelium. However, this ameliorative effect of insulin was blocked by Wortmannin, [inhibitor of phosphoinositidyl-3-kinase (PI3K)], UCN-01(Phosphoinositide dependent kinase inhibitor), API-2 (AKT inhibitor) and L-NAME [eNOS inhibitor].

Administration of sodium orthovanadate (24 mg/kg/day, p.o) and atorvastatin (30 mg/kg/day, p.o) (positive control) in diabetes mellitus, hypercholesterolemia, hyperhomocysteinemia and hypertension significantly improved acetylcholine-induced endothelium-dependent relaxation, Serum nitrate/nitrite level, as well as mRNA expression of eNOS and integrity of vascular endothelium. However, this ameliorative effect of SOV was significantly blocked by UCN-01, (PDK inhibitor) and L-NAME (Inhibitor of eNOS).

Summary

Therefore, it may be summarized that

1) Experimental diabetes mellitus, hyperhomocysteinemia, hypercholesterolemia, hypertension downregulated eNOS to produce vascular endothelium dysfunction.
2) Atorvastatin, a standard drug, prevented diabetes mellitus, hyperhomocysteinemia, hypercholesterolemia and hypertension induced vascular endothelium dysfunction.

3) PI3k/PDK/AKT and eNOS pathway is impaired in diabetes mellitus, hypercholesterolemia, hyperhomocysteinemia and hypertension induced vascular endothelium dysfunction.

4) Activation of PI3K with Insulin, YS 49 and AKT with Demthylasteraquinone B1 significantly ameliorates vascular endothelium dysfunction.

5) Sodium orthovanadate, a specific inhibitor of PTPase, stimulated PDK and eNOS and consequently improved vascular endothelium dysfunction.

**CONCLUSION**

Thus it may be concluded that activation of Phosphatidylinositol-3-kinase and its downstream pathways viz Phosphoinositide dependent Kinase/protein kinase B and endothelial Nitric Oxide Synthatase improves vascular endothelium dysfunction and that therapeutic interventions designed for these pathways may provide potential therapeutic strategies to combat vascular complications.