Pharmacological Interventions to Prevent Vascular Endothelial Dysfunction: Future Directions

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Endothelium forms an innermost lining of blood vessel and it regulates the vascular tone and permeability. A healthy vascular endothelium is antiatherogenic in nature because of its properties such as inhibition of platelet aggregation, adhesion cascades, smooth muscle cell proliferation and leukocyte adhesion. Vascular endothelial dysfunction (VED) is associated with reduced synthesis and release of nitric oxide, proinflammatory and prothrombotic properties followed by diminished vasodilation. VED has been implicated in the pathogenesis of atherosclerosis, hypertension, myocardial infarction, heart failure, renal failure and stroke. Various pharmacological interventions such as angiotensin converting enzyme (ACE) inhibitors, statins, insulin sensitizers, L-arginine as well as agents that target endothelial nitric oxide synthase (eNOS) “coupling” such as folates or tetrahydrobiopterin (BH4) have been noted to improve the function of vascular endothelium. In this review, we discussed various recently developed pharmacological interventions to improve the function of endothelium. Moreover, the novel targets sites involved in the pathogenesis of vascular endothelial dysfunction have been delineated.

Key words — endothelial dysfunction, statin, angiotensin converting enzyme inhibitor, insulin sensitizer, L-arginine, novel target site

INTRODUCTION

Endothelium is the layer of thin specialized epithelium comprising simple squamous cells that line the inner wall of the blood vessel. Endothelium regulates vascular homeostasis through various local mediators that modulate vascular tone, platelet adhesion, inflammation, fibrinolysis and vascular growth.1,2) The vascular homeostasis is dependent on the integrity of the endothelium.3,4) Endothelium maintains the balance between vasodilation and vasoconstriction. Endothelium-dependent vasodilation is mediated by various mediators such as nitric oxide (NO), endothelium derived hyperpolarizing factor (EDHF) and prostacyclin. On the other hand, endothelium dependent vasoconstriction is mediated by endothelin-1 (ET-1), vasoconstrictor prostanoids, angiotensin II (Ang II) and super oxide anion.5) Vascular endothelial dysfunction (VED) has been associated with reduced vasodilation, proinflammatory and prothrombotic properties.6) VED leads to endothelial cell activation.7) The activated endothelium expresses cell-surface adhesion molecules such as vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, endothelial-leukocyte adhesion molecule (ELAM or E-selectin) and von Willebrand factor (vWF),8,9) which all collectively lead to formation of atherosclerotic plaques. A major vasodilator released by the endothelium is NO. Ignarro group had described that endothelium-derived relaxing factor is nothing but NO.10) NO is synthesized from the substrate L-arginine through endothelial nitric oxide synthase (eNOS) in endothelial cell. The cofactors such as nicotinamide adenine din-
nucleotide phosphate (NADP), tetrahydrobiopterin (BH4), flavin-adenine dinucleotide (FAD), cysteine and reduced glutathione are necessary for the production of NO.\textsuperscript{11} NO regulates vascular tone by inhibiting various processes such as vascular smooth muscle cell proliferation, platelet aggregation, platelet and monocyte adhesion to the endothelium, neutrophil activation and adhesion, low density lipoprotein (LDL) oxidation, expression of adhesion molecules, ET-1 production and expression of pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-\(\alpha\)) interleukin-1 (IL-1) and interleukin-6 (IL-6).\textsuperscript{4,12} The hallmark of VED is impairment in endothelium dependent vasodilatation, which is mediated by reduced production of NO and elevated levels of asymmetric dimethylarginine (ADMA).\textsuperscript{2} Further, in VED, the bioavailability of NO is reduced by reactive oxygen species (ROS), which are derived from macrophages, smooth muscle cells and endothelial cells with the help of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH-oxidase).\textsuperscript{13} ROS induce the production VCAM-1, ICAM-1, monocyte chemoattractant protein-1 (MCP-1), IL-6 and oxidized low-density lipoprotein-1 (LOX-1).\textsuperscript{14,15} Impaired endothelial function and decreased NO bioavailability in human endothelium play an important role in the development and progression of atherosclerosis and coronary artery disease.\textsuperscript{16} The risk factors commonly associated with VED are hyperhomocysteinemia,\textsuperscript{17} hypercholesterolemia,\textsuperscript{18} obesity,\textsuperscript{19} hyperuricemia,\textsuperscript{20–22} estrogen deficiency,\textsuperscript{23} aging\textsuperscript{24} and chronic smoking.\textsuperscript{25} The VED has been associated with various cardiovascular disorders such as atherosclerosis,\textsuperscript{18} hypertension,\textsuperscript{26} coronary artery disease,\textsuperscript{27} chronic heart failure,\textsuperscript{28} chronic renal failure,\textsuperscript{6} diabetes\textsuperscript{29} and stroke.\textsuperscript{30} The VED is a major contributing factor for cardiovascular disorders and various interventions are developed to prevent VED and thus the risk of cardiovascular events. The present review delineates various pharmacological interventions to prevent VED and associated cardiovascular complications.

**PHARMACOLOGICAL INTERVENTIONS**

The pharmacological agents such as statins,\textsuperscript{31} angiotensin converting-enzyme inhibitors,\textsuperscript{32} ET-1 receptor antagonists,\textsuperscript{33–35} calcium channel blockers,\textsuperscript{36} insulin-sensitizing agents,\textsuperscript{37,38} antioxidants\textsuperscript{39} and various supplements such as arginine,\textsuperscript{40} tetrahydrobiopterin\textsuperscript{41} and folate\textsuperscript{42} have been primarily known to improve the function of vascular endothelium.

**STATINS ON ENDOTHELIAL FUNCTION**

Statins are known to inhibit 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase and thus lower the LDL cholesterol\textsuperscript{43}. Evidences suggest that they are potent agents for improving endothelial function and reducing cardiovascular risk and morbidity and mortality in patients with coronary artery disease.\textsuperscript{44,45} The beneficial effects of statins are not only related to their cholesterol lowering effect; but also their antithrombotic, anti-inflammatory and antioxidant effects.\textsuperscript{46–49} Statins have been shown to induce the expression of eNOS in human endothelial cells\textsuperscript{50} and prevent the expression of caveolin, a negative regulator of eNOS.\textsuperscript{51} Statins activate Akt/protein kinase B, which further activates eNOS.\textsuperscript{52} Moreover, statins appear to inhibit the synthesis of isoprenoids that are required for the posttranslational modification of important signaling molecules such as Rho, Rac and Ras.\textsuperscript{53} Inhibition of Rho activation increases the production of endothelial NO\textsuperscript{50} and reduces the expression of ET-1.\textsuperscript{54} Lovastatin was first shown to inhibit the production of proinflammatory cytokines. The atorvastatin therapy in an animal model of hypertension has improved the function of endothelium and concurrently lowered the expression of p22phox and production of ROS.\textsuperscript{43} In addition, statins reduce chemokine and chemokine receptor expressions in human endothelial cell and macrophages through inhibition of the geranylgeranylpyrophosphate pathway and thus they possess anti-inflammatory effect.\textsuperscript{49} Several studies suggest that improvement in endothelial function is one of the pleotrophic effects of statins independent of their lipid-lowering capabilities.\textsuperscript{55} The mechanisms involved in statins mediated improvement in endothelial function have been shown in Fig. 1.

Various studies have investigated the effect of statins in clinical measures of endothelial function such as flow-mediated vasodilation or forearm blood flow in response to infusion of va-
soactive agents such as acetylcholine. Treatment with lovastatin improved the coronary vasomotor response to acetylcholine in patients with coronary artery disease.\textsuperscript{56} Pravastatin treatment improved endothelium-dependent coronary vasomotion within 24 hr in the absence of significant cholesterol reduction.\textsuperscript{47} The endothelial dysfunction in hypercholesterolemics is due to an oxidative stress and atorvastatin treatment rapidly improved both basal and stimulated endothelium-dependent vasodilation.\textsuperscript{57} Further, treatment with atorvastatin has been noted to improve forearm blood flow and decrease serum markers of oxidative stress and inflammation.\textsuperscript{58} Recent clinical trials of statins show improvement in endothelial function and reduction in cardiovascular risks, morbidity and mortality in patients with coronary artery disease.\textsuperscript{45} Statins enhance endothelial function independent of their lipid-lowering effects.\textsuperscript{44, 59} Short-term improvement in endothelial function has been noted to be superior with pitavastatin as compared to atorvastatin therapy. Pitavastatin could be a potentially better therapeutic choice for lipid-lowering and early alterations in endothelial function.\textsuperscript{55} Short-term lipid-lowering therapy with cerivastatin improved endothelial function and NO bioavailability after two weeks in patients with hypercholesterolemia.\textsuperscript{60} The summary of clinical trials of statins in endothelial function is outlined in Table 1.\textsuperscript{44, 47, 55–65}

**ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORS ON ENDOTHELIAL FUNCTION**

Ang-II plays a major role in mediating vasoconstriction, thrombosis, inflammation and vascular remodeling.\textsuperscript{66} Ang-II has been shown to increase the production of super oxide through activation of membrane-bound NADPH oxidase.\textsuperscript{67} ACE inhibitors may be potentially improving endothelium-dependent vasodilation by preventing the formation of ang-II and increasing the level of bradykinin and consequently NO.\textsuperscript{68} ACE inhibitors protect endothelial cells from oxidative stress-induced apoptosis.\textsuperscript{69} Quinapril has been noted to improve the function of endothelium by decreasing TNF-\(\alpha\) and C-reactive protein (CRP) levels.\textsuperscript{70} Zofenopril has improved the production of NO and consequently reduced oxidative stress in endothelial cells.\textsuperscript{71} Can-desartan, an AT\(_1\) receptor antagonist has been noted to improve endothelium-dependent vasorelaxation.\textsuperscript{72} Irbasartan treatment improves peripheral but not coronary endothelial dysfunction in patients with coronary artery diseases.\textsuperscript{73} It is interesting to note that ACE inhibitor such as enalapril and angiotensin AT\(_1\)-receptor blocker like losartan is equally effective to reverse N(G)-nitro-L-arginine methyl ester (L-NAME)-induced experimental endothelial dysfunction.\textsuperscript{74} Several clinical studies have demonstrated the
Table 1. Overview of Clinical Trials of Statins on Endothelial Function

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Drug</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al., 1995</td>
<td>Coronary artery disease</td>
<td>Lovastatin</td>
<td>Treatment with lovastatin (62 mg/day) improved the coronary vasomotor response to acetylcholine</td>
</tr>
<tr>
<td>Treasure et al., 1995</td>
<td>Coronary artery disease</td>
<td>Lovastatin</td>
<td>Lovastatin (80 mg/day) improved endothelium mediated responses in coronary artery of patients with atherosclerosis</td>
</tr>
<tr>
<td>Dupuis et al., 1999</td>
<td>Hyperlipidemia, Myocardial infarction</td>
<td>Pravastatin</td>
<td>Pravastatin (40 mg/day) rapidly improved endothelial function after 6 weeks of therapy</td>
</tr>
<tr>
<td>Jarvisalo et al., 1999</td>
<td>Coronary artery disease</td>
<td>Statins</td>
<td>HMG CoA reductase inhibitors enhance endothelial function independent of their lipid-lowering effects</td>
</tr>
<tr>
<td>Perticone et al., 2000</td>
<td>Hypercholesterolemic patients</td>
<td>Atorvastatin</td>
<td>Atorvastatin (10 mg/day) rapidly improved endothelium-dependent vasodilation</td>
</tr>
<tr>
<td>Penny et al., 2001</td>
<td>Hypercholesterolemia</td>
<td>Lovastatin</td>
<td>Lovastatin (40 mg/day or greater) treatment reversed endothelial dysfunction</td>
</tr>
<tr>
<td>Laufs et al., 2001</td>
<td>Healthy male</td>
<td>Atorvastatin</td>
<td>In subjects with normal vascular function atorvastatin (80 mg/day) improved endothelial function within 24 hr</td>
</tr>
<tr>
<td>van de Ree et al., 2001</td>
<td>Type 2 diabetes mellitus</td>
<td>Simvastatin</td>
<td>Endothelial function is impaired in type 2 diabetes and is not restored after 6-weeks treatment with simvastatin (40 mg/day)</td>
</tr>
<tr>
<td>John et al., 2001</td>
<td>Hypercholesterolemia</td>
<td>Cerivastatin</td>
<td>Short-term lipid-lowering therapy with cerivastatin improved endothelial function and NO bioavailability after two weeks</td>
</tr>
<tr>
<td>Mercuro et al., 2002</td>
<td>Postmenopausal normocholesterolemic women</td>
<td>Atorvastatin</td>
<td>Atorvastatin (10 mg/day) improved endothelium-dependent vasodilation</td>
</tr>
<tr>
<td>Wassmann et al., 2003</td>
<td>Angina pectoris</td>
<td>Pravastatin</td>
<td>Pravastatin (40 mg) treatment improved coronary endothelial function</td>
</tr>
<tr>
<td>Wassmann et al., 2004</td>
<td>Normotensive patients with known vascular disease or cardiovascular risk factors</td>
<td>Atorvastatin</td>
<td>Atorvastatin (80 mg/day) treatment improved forearm blood flow and decreased serum markers of oxidative stress and inflammation</td>
</tr>
<tr>
<td>Sakabe et al., 2007</td>
<td>Primary hypercholesterolemia</td>
<td>Pitavastatin</td>
<td>Short-term improvement of endothelial function was superior with pitavastatin (2 mg/day) compared to atorvastatin (10 mg/day) therapy</td>
</tr>
</tbody>
</table>

Beneficial effects of ACE inhibitors in improvement of endothelial function in patients of diabetes, hypertension and coronary artery disease (Table 2).\(^70,75–83\) In the Trial on Reversing ENdothelial Dysfunction (TREND) study, normotensive patients with angiographically demonstrated coronary atherosclerosis were treated chronically with quinapril, an ACE inhibitor with high tissue-binding affinity. These patients had restoration of endothelium-dependent vasodilation of coronary vessels in absence of significant reduction in blood pressure.\(^75\) Impairment of endothelial dependent dilation in young subjects with type 1 diabetes has not been improved by treatment with enalapril.\(^77\) On the other hand, enalapril has improved stimulated and basal NO-dependent endothelial function in type 2 diabetic subjects.\(^78\) Further, enalapril has significantly increased the postschismic vasodilator response in patients with coronary hypercholesterolemia.\(^81\) In healthy volunteers, triglyceride-rich lipoproteins-induced endothelial dysfunction was prevented by losartan and quinapril and the preventive effect was more pronounced with quinapril.\(^79\) In mildly hypertensive patients without organ damage, zofenopril, beyond its blood pressure lowering effect and through its sustained antioxidant activity has markedly improved endothelial function.\(^83\) The summary of clinical trials of ACE inhibitors in en-
Table 2. Overview of Clinical Trials of ACE Inhibitor on Endothelial Function

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Drug</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mancini et al., 1996</td>
<td>Normotensive patients with coronary artery disease</td>
<td>Quinapril</td>
<td>Quinapril (40 mg/day) improved endothelial function in normotensive patients</td>
</tr>
<tr>
<td>O’Driscoll et al., 1997</td>
<td>Type 2 diabetic subjects</td>
<td>Enalapril</td>
<td>In type 2 diabetic subjects without evidence of vascular disease, enalapril (20 mg/day) improved NO-dependent endothelial function</td>
</tr>
<tr>
<td>Mullen et al., 1998</td>
<td>Young subjects with type 1 diabetes mellitus</td>
<td>Enalapril</td>
<td>Impairment of endothelial dependent dilation in young subjects with type 1 diabetes has not been improved by treatment with enalapril (20 mg/day)</td>
</tr>
<tr>
<td>O’Driscoll et al., 1999</td>
<td>Type 2 diabetes mellitus</td>
<td>Enalapril</td>
<td>Enalapril (10 mg twice daily) improved NO-dependent endothelial function</td>
</tr>
<tr>
<td>Wilmink et al., 1999</td>
<td>Healthy volunteers</td>
<td>Quinapril</td>
<td>Quinapril (40 mg/day) prevented endothelial dysfunction induced by triglyceride rich lipoproteins</td>
</tr>
<tr>
<td>McFarlane et al., 1999</td>
<td>Type 1 diabetes</td>
<td>Perindopril</td>
<td>Perindopril (4 mg/day) treatment for 3 months did not improve arterial endothelial function</td>
</tr>
<tr>
<td>Esper et al., 2000</td>
<td>Coronary hypercholesterolemia</td>
<td>Enalapril</td>
<td>Enalapril (5 mg/day) increased the post ischemic vasodilator response in patients with coronary hypercholesterolemia</td>
</tr>
<tr>
<td>Bae et al., 2001</td>
<td>Hypertiglyceridemia with coronary artery disease</td>
<td>Lisinopril</td>
<td>No acute beneficial effects of Lisinopril (10 mg/day) on endothelial function</td>
</tr>
<tr>
<td>Kovacs et al., 2006</td>
<td>Post myocardial infarction patients</td>
<td>Quinapril</td>
<td>Low dose of quinapril (10 mg/day) improved endothelial function</td>
</tr>
<tr>
<td>Pasini et al., 2007</td>
<td>Mild hypertensive patients</td>
<td>Zofenopril</td>
<td>Zofenopril (15 to 30 mg/day) beyond its blood pressure lowering effects and through its sustained antioxidant activity, improved the endothelial function</td>
</tr>
</tbody>
</table>

dothelial function is outlined in Table 2.

ET-1 RECEPTOR ANTAGONISTS ON ENDOTHELIAL FUNCTION

ET-1 is a potent vasoconstrictor peptide produced by vascular endothelium from big ET-1 via specific cleavage by endothelium converting enzyme. ET-1 produces its effects through the stimulation of ETA and ETB receptors. Numerous studies have shown that plasma concentration of ET-1 is elevated in patients with cardiovascular disorders. Coronary endothelial function has been shown to be preserved with chronic endothelin receptor antagonism in experimental hypercholesterolemia. Endothelin ETA receptor blockade restores NO-mediated endothelial function and inhibits atherosclerosis. ETA receptor antagonism has been demonstrated to have therapeutic potential in the treatment of endothelial dysfunction and atherosclerosis. Dual ETA/ETB receptor blockade improves endothelial function and exerts direct vasodilator effects in atherosclerotic patients who are on treatment with ramipril suggesting that ET receptor blockade may have important therapeutic effects when added to ACE inhibition in atherosclerotic patients. Various studies suggest that endothelin receptor blockade would be an effective therapeutic approach in the management of patients with pulmonary arterial hypertension. Treatment with bosentan, a nonselective ETA/ETB receptor antagonist has been shown to be safe and improve pulmonary haemodynamics in patients with heart disease. Bosentan has been shown to improve vascular endothelial function in patients with systemic sclerosis. Treatment with sitaxsentan, a selective ETA receptor antagonist has been noted to improve clinical status of patients with pulmonary arterial hypertension.

CALCIUM CHANNEL BLOCKERS ON ENDOTHELIAL FUNCTION

Calcium channel blockers are traditionally used as antihypertensive therapy. Amlodipine, a calcium channel blocker enhances the production of
NO. Further, amlodipine activates eNOS, which potentiates the production of NO. Benidipine, a calcium channel blocker has restored endothelial function by promoting the production of NO and accumulating cyclic guanosine monophosphate (cGMP), a second messenger of NO and preventing lysophosphatidylcholine-induced activation of caspase-3. Azelnidipine, a novel calcium channel blocker works as an anti-atherogenic agent by inhibiting ROS dependent expression of VCAM-1 induced by TNF-α in endothelial cells. Nifedipine improves endothelial function in hypercholesterolemia independently of an effect on blood pressure and plasma lipids. Recently, etofedipine, a novel calcium channel blocker, has been shown to improve endothelial function and reduce blood pressure in nondiabetic patients with hypertension. It has been reported that amlodipine showed less effect than olmesartan in the endothelium dependent coronary dilation in hypertensive patients.

**INSULIN-SENSITIZING AGENTS ON ENDOTHELIAL FUNCTION**

An insulin-resistant diabetic state has been associated with obesity and VED. Therapeutic agents that promote the insulin sensitivity may improve endothelial function. Thiazolidinediones group of insulin sensitizers such as troglitazone, pioglitazone and rosiglitazone have been noted to activate peroxisome proliferator activated receptor gamma (PPARγ) and decrease peripheral insulin resistance. Although, troglitazone is the firstly approved Thiazolidinedione as insulin sensitizing agent, it has been withdrawn from the main markets such as U.S.A. and Japan because of its severe hepatotoxicity. Rosiglitazone inhibits proliferation and migration of vascular cells and endothelial cell apoptosis. In endothelial cells, PPARγ activators inhibit the expression of TNF-α, IL-6 and IL-1β and attenuate TNF-α-induced expression of VCAM-1 and ICAM-1 and thereby may protect against the inflammation of vascular endothelium. Moreover, PPARγ activators act as vasorelaxants by enhancing the production of endothelial NO. Recent studies suggest that insulin sensitizers improve endothelial function in type 2 diabetes. Rosiglitazone treatment has been shown to restore the function of endothelium. Pioglitazone has improved both endothelial function and insulin resistance in patients with type 2 diabetes.

**L-ARGININE ON ENDOTHELIAL FUNCTION**

L-Arginine, a semi-essential amino acid, is the substrate for eNOS and the precursor molecule for synthesis of NO. L-arginine improves the NO-mediated vasodilatory responses and seems to have a direct vasodilator effect on human vasculature. Further, L-arginine improves NO synthesis by antagonizing the deleterious effect of ADMA on eNOS function. L-arginine has additional effects such as stimulating the release of insulin, growth hormone, glucagons, prolactin and inhibiting angiotensin converting enzyme and reducing lipid peroxidation, which may improve the function of endothelium. The dietary supplementation of L-arginine decreased the platelet aggregation and mononuclear cells adhesiveness. Oral supplementation of L-arginine significantly improved endothelial function in patients of peripheral arterial occlusive disease (PAOD) with hyperhomocysteinemia. Further, L-arginine supplementation has partially restored endothelium-dependent vasorelaxation and improved myocardial perfusion in a swine model of chronic myocardial ischemia with hypercholesterolemia-induced endothelial dysfunction. There is currently insufficient evidence to recommend the use of L-arginine in patients with acute stroke.

**BH4 AND FOLATE ON ENDOTHELIAL DYSFUNCTION**

BH4 is one of most potent naturally occurring reducing agents and an essential allosteric factor in coupling of oxidase and reductase domains of eNOS, which regulates the production of NO in endothelial cells. Deficiency of BH4, an essential cofactor for eNOS, decreases the generation of NO and increases the production of reactive oxygen species, which may lead to VED. The infusion of BH4 has been noted to improve acetylcholine-mediated vasodilation. Further, BH4 ameliorates endothelial dysfunction in fructose-fed rats. BH4 may be a promising agent for the treatment of oxidative stress-induced cardiovascular disorders. Elevated plasma total homo-
cysteine (tHcy) concentration is termed as hyperhomocysteinemia, which is a risk factor for cardiovascular disease and thrombotic complications. The exact mechanism by which homocysteine promotes vascular disease remains unclear. However, there is significant support that hyperhomocysteinemia impairs endothelial function via oxidative inactivation of NO. Early clinical trials suggested that lowering tHcy using folic acid may retard the progression of atherosclerosis. However, treatment with high-dose folic acid improves endothelial function in post-acute myocardial infarction patients, independent from homocysteine status. On the other hand, folic acid therapy has been noted to effectively lower plasma homocysteine level and improve total plasma antioxidant capacity in hemodialysis patients. The folic acid supplementation has reduced plasma tHcy level and produced a significantly greater reversal of the endothelial dysfunction.

**ANTIOXIDANTS ON ENDOTHELIAL DYSFUNCTION**

The statement of oxidative stress mediates atherosclerotic endothelial dysfunction implicates a potential for antioxidant therapies to prevent vascular pathology. Several antioxidants such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, BH4, and N-acetylcysteine have been shown to improve endothelial function. Vitamin C, a potent water-soluble scavenger of free radicals, reduces monocyte adhesion to endothelial cells, inhibits LDL oxidation, decreases inactivation of NO and stimulates eNOS activity. Vitamin C has been shown to improve the function of endothelium in patients with cardiovascular diseases. Moreover, vitamin C has been noted to prevent homocysteine-induced impairment of vascular endothelial function. This result supported the adverse effect of homocysteine on vascular endothelial cells are mediated through oxidative stress mechanisms. Furthermore, vitamin C blocks vascular dysfunction and release of IL-6 induced by ET-1. Vitamin E, a fat-soluble inhibitor of lipid peroxidation, inhibits leukocyte adhesion and oxidation of LDL cholesterol. Oral supplementation of vitamin E attenuates transient impairment of endothelial function in smokers.

**HERBAL DRUGS ON ENDOTHELIAL DYSFUNCTION**

Short and long term black tea consumption reverses endothelial dysfunction in patients with coronary artery diseases due to antioxidant and anti-atherosclerotic properties of catechins. Genisten, a phytoestrogen derived from soyabeans, binds to estrogen receptors and produces estrogen like cardiovascular protective effect. Genistein enhances NO mediated relaxation in aortic rings isolated from overiectomized rats. The vasorelaxant response to acetylcholine has been noted to be enhanced in animals supplemented with garlic and turmeric. Concord grape juice (CGJ), a non-alcoholic rich source of grape-derived polyphenols enhanced endothelial formation of NO and endothelium-derived hyperpolarizing factor (EDHF) through redox-sensitive activation of Src kinase with subsequent PI3-kinase/Akt-dependent phosphorylation of eNOS. Astragalus membranaceus and astragalus saponin potently protected endothelium-dependent relaxation against acute injury induced by Hcy through nitric oxide regulatory pathways. Pterospartum tridentatum has been shown to reduce the development of diabetic vascular complications against oxidative injury.

**FUTURE DIRECTIONS**

Rho-kinase, a serine threonine kinase is expressed in vascular smooth muscle cells and endothelial cells. Activation of Rho-kinase inhibits myosin light chain phosphatase and consequently increases vascular tone that involves in the pathogenesis of hypertension and coronary/cerebral vasospasms. Our laboratory has recently shown the ameliorative effect of fasudil, a selective inhibitor of Rho-kinase in hypertension, diabetes mellitus and hyperhomocysteinemia-induced VED. The oxidant mediated activation of poly (ADP-ribose) polymerase (PARP) plays a pivotal role in the development of endothelial dysfunction. The PARP overactivation results in rapid depletion of intracellular NAD and ATP pools, slows the rate of glycolysis and mitochondrial respiration and eventually leads to endothelial dysfunction. PARP inhibitors such as PJ-34 and INO 1001 exert beneficial effects against diabetes, hyperhomocysteinemia, hypertension, aging and en-
dotoxic shock-induced VED.\textsuperscript{141,143–146} Activation of Akt stimulates phosphorylation of eNOS, increases the production of NO and reduces oxidative stress.\textsuperscript{147–149} Demethylasterriquinone B1 (DAQ B1), an activator of Akt has reduced oxidative stress and prevented hypertension, diabetes mellitus and hyperhomocysteinemia associated VED.\textsuperscript{149,150} The inhibition of protein tyrosine phosphatase (PTPase) has been documented to activate Akt.\textsuperscript{151,152} We have recently shown that bis(maltolato)oxovanadium (BMOV), a PTPase inhibitor activates eNOS by opening of ATP-sensitive K\textsuperscript{+} channels and consequently decreases oxidative stress to prevent VED.\textsuperscript{22,155} GGTY-298, an inhibitor of geranylgeranyltransferase-I, inhibits the activation of certain Rho family GTPase such as Rho A and Rac 1, which in turn results in increased eNOS activity and hence NO production with diminished release of ROS.\textsuperscript{154} Benfotiamine, a lipophilic derivative of thiamine is a transketolase activator, which prevents vascular accumulation of advanced glycation end products (AGE) and induction of pro-apoptotic caspase-3.\textsuperscript{155} Moreover, benfotiamine has been shown to reduce endogenous AGE production and oxidative stress and prevent micro and macrovascular dysfunction induced by an AGE-rich meal in patients with type 2 diabetes.\textsuperscript{156} Activation of protein kinase A (PKA) stimulates eNOS phosphorylation and increases the generation of NO.\textsuperscript{157} Further, PKA activation inhibits RhoA activation in endothelial cells and prevents increased endothelial permeability induced by inflammatory mediators.\textsuperscript{158} Our laboratory has reported that 8-Br-cAMP, an activator of PKA markedly prevented the dysfunction of vascular endothelium.\textsuperscript{159,160}

**CONCLUSION**

It is well-known fact that vascular endothelial dysfunction plays a pivotal role in the pathogenesis of cardiovascular disorders. Hence, finding the most promising pharmacological intervention to prevent vascular endothelial dysfunction in humans is the target of current interest. Therapeutic strategies selectively targeting in the improvement of vascular endothelial function may directly promote clinical outcome in patients with cardiovascular diseases. Growing lists of therapeutic agents such as statins, ACE inhibitors, calcium channel blockers, L-arginine and BH4 have been shown to prevent endothelial dysfunction and reduce the risk of cardiovascular complications. The protective role of insulin sensitizers and antioxidant therapy in endothelial dysfunction remains to be further explored. Various experimental studies have demonstrated the endothelial protective role of inhibitors of Rho-kinase, PARP, PTPase, geranylgeranyl transferase and transketolase, and activators of Akt and PKA, which would be novel future candidates for treating cardiovascular disorders. However, further studies are warranted to explore the novel role of these signaling systems in the modulation of cardiovascular functions.

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