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Experimental Models for Vascular Endothelial Dysfunction

¹Pitchai Balakumar, ¹Seema Jindal,

²Dhvanit Indravadan Shah and ¹Manjeet Singh

¹Department of Pharmaceutical Sciences and Drug Research,
Punjabi University, Patiala-147002, India

²Department of Medicine, Brigham and Women's Hospital and Harvard Medical School,
Boston MA 02115, USA

Abstract: The endothelium is recognized as a physical barrier between blood and vascular wall. It regulates vascular tone, endothelial permeability and vascular growth. Dysfunction of endothelium has been characterized by partial or complete loss of balance between vasorelaxation and vasoconstriction, thrombosis and thrombolysis. Various experimental evidences have shown that endothelial dysfunction mainly occurs due to reduced nitric oxide production and increased oxidative stress. Vascular endothelial dysfunction is associated with pathogenesis of atherosclerosis, hypertension, diabetes mellitus, coronary artery diseases and stroke. In the present review, we have discussed various recently developed animal models for vascular endothelial dysfunction, which may open new vista to develop agents for improving vascular endothelial function.

Key words: Animal models, vascular endothelial dysfunction

INTRODUCTION

Endothelium forms an innermost lining of blood vessels (Lusher and Barton, 1997; Endmann and Schiffrin, 2004). Vascular endothelium has anticoagulant and antithrombotic activities in order to ensure free flow of blood through arteries (Bombeli *et al.*, 1997). It regulates various mediators including nitric oxide (NO), prostanoids, endothelin-1 (ET-1), angiotensin-II (Ang-II), tissue plasminogen activator (tPA), von willbrand factor (vWF), adhesion molecules and cytokines (Quyyumi, 1998; Schalkwijk and Stehouwer, 2005). NO, is also known as endothelium derived relaxing factor (EDRF), which is synthesized from L-arginine by endothelial nitric oxide synthase (eNOS) in endothelium. NO plays a crucial role in regulating wide spectrum of cardiovascular functions such as mediating vasorelaxation, inhibiting leukocytes-endothelial adhesion and preventing platelet aggregation (Kawashima, 2004; Yang and Ming, 2006). Endothelial dysfunction has been characterized by partial or complete loss of balance between vasorelaxation and vasoconstriction (Vane *et al.*, 1990; Masaki, 1995) and thrombosis and thrombolysis (Danon and Skutelsky, 1976). Experimental and clinical evidences suggest that endothelial dysfunction leads to reduced endothelial NO production (Bugiardini *et al.*, 2004; Lerman and Zeiher, 2005). Vascular endothelial dysfunction has been associated with various disorders such as hypertension (Sainani and Maru, 2004), coronary artery diseases (Caramori and Zago, 2000), diabetes mellitus (De Vriese *et al.*, 2000; Nakagami *et al.*, 2005), atherosclerosis (Spieker *et al.*, 2001; Bonetti *et al.*, 2003) and stroke (Cosentino *et al.*, 2001; Faraci and Lentz, 2004). However, the literature for animal models for vascular endothelial dysfunction is inadequately available. The current review has focused on various experimental models to produce vascular endothelial dysfunction.

Corresponding Author: Manjeet Singh, Department of Pharmaceutical Sciences and Drug Research,
Punjabi University, Patiala-147002, India Tel: +91-9815557265 Fax: +91-175-2283073

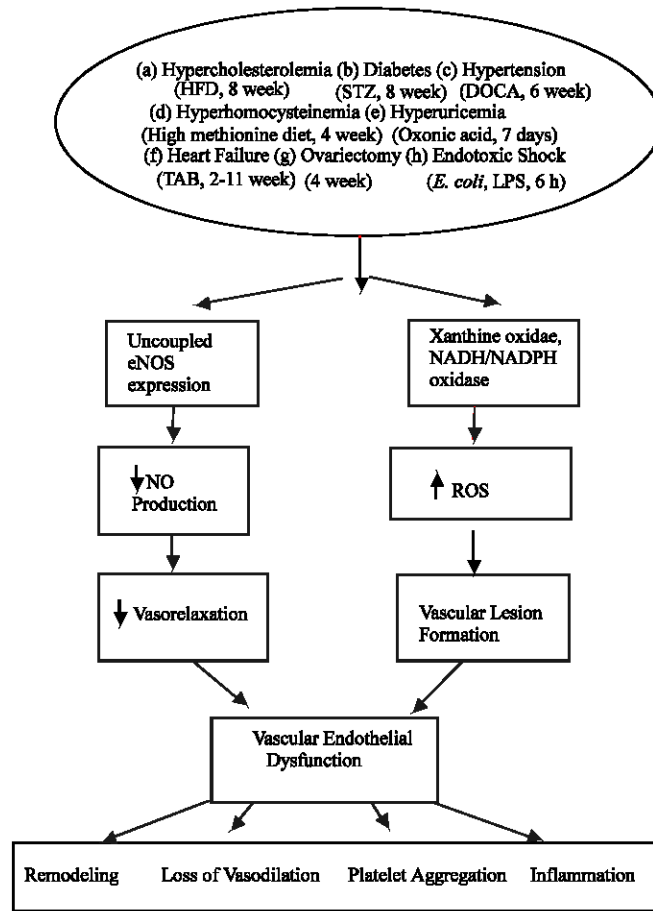


Fig. 1: Pharmacological mechanisms involving in the pathogenesis of vascular endothelial dysfunction. HFD indicates high fat diet, STZ indicates streptozotocin, DOCA indicates deoxycortisone acetate, TAB indicates transverse aortic banding, LPS indicates lipopolysaccharide, eNOS indicates endothelial nitric oxide synthase and ROS indicates reactive oxygen species

Experimental Models to Induce Vascular Endothelial Dysfunction

The animal models for vascular endothelial dysfunction share many features which are universal to human endothelial dysfunction and have been depicted by targeting eNOS, xanthine oxidase and NADH/NADPH oxidase pathways (Fig. 1).

Hypertension-induced Vascular Endothelial Dysfunction

Endothelium plays an important role in regulation of blood pressure (Taddei *et al.*, 2001). Hypertension has been shown to cause vascular endothelial dysfunction (Sainani and Maru, 2004). The method commonly used to induce hypertension in rats is goldblatt techniques such as 2-kidney 1-clip model and 1-kidney 1-clip model, which have been demonstrated to increase arterial blood pressure, total peripheral resistance (TPR) and decrease endothelium dependent relaxation to acetylcholine (Ach) (Share *et al.*, 1982; Sventek *et al.*, 1996). Recently we have shown that uninephrectomy followed by

administration of DOCA salt (40 mg kg^{-1} , s.c.) in olive oil along with 1% NaCl and 0.5% KCl twice weekly for 6 weeks has produced vascular endothelial dysfunction (Shah and Singh, 2006a). Further, treatment with L-NAME (eNOS inhibitor) ($50 \text{ mg kg}^{-1} \text{ day}^{-1}$) for 6 weeks has been shown to increase blood pressure and reduce endothelium dependent relaxation in rats (Kung *et al.*, 1995). Infusion of angiotensin-II ($0.7 \text{ mg kg}^{-1} \text{ day}^{-1}$) for 5 days has been noted to increase systolic blood pressure, generation of superoxide anion and cause impairment of Ach-induced relaxation (Rajagopalan *et al.*, 1996). Moreover, chronic administration of ethinyl estradiol ($1.5 \text{ mg kg}^{-1} \text{ day}^{-1}$) for 3 weeks has been shown to increase blood pressure and consequently reduce endothelium dependent relaxation (Thakre *et al.*, 2000). Furthermore moderately high fat diet administration for 10 weeks has been shown to develop vascular endothelial dysfunction in rats characterized by hypertension, increase in reactive oxygen species (ROS) and lipid peroxidation (Dobrian *et al.*, 2001). Rats treated with single injection of monocrotaline (40 mg kg^{-1} or 100 mg kg^{-1}) has increased mean pulmonary arterial pressure, ventricular hypertrophy and produced injury to endothelium of pulmonary artery (Gout *et al.*, 1999; Leung *et al.*, 2003). Spontaneously Hypertensive Rats (SHR) show cardiovascular responsiveness such as increase in blood pressure, total peripheral resistance and intravascular volume due to fluid retention and have been demonstrated to decrease endothelium dependent relaxation (Sunano *et al.*, 1989).

Diabetes-induced Vascular Endothelial Dysfunction

Diabetes is characterized by hyperglycemia which is an independent risk factor for the development of cardiovascular diseases (Nakagami *et al.*, 2005). Endothelial dysfunction plays an important role in pathogenesis of diabetic vascular diseases. Several mechanisms have been reported in diabetes-induced impairment of endothelium dependent relaxation including impaired signal transduction availability and impaired release of EDRF (De Vriese *et al.*, 2000). Recently, from our laboratory, it has been shown that administration of streptozotocin (55 mg kg^{-1} , i.p. once) in rats produced diabetes and consequently induced vascular endothelial dysfunction (Shah and Singh, 2006b-d).

Hyperhomocysteinemia-induced Vascular Endothelial Dysfunction

One of the most consistent finding observed in the studies of experimental hyperhomocysteinemia is impairment of NO mediated vasodilation (Lentz *et al.*, 2003). Hyperhomocysteinemia-induced increase in reactive oxygen species may lead to oxidative inactivation of endothelium derived NO (Eberhardt *et al.*, 2000). Further, hyperhomocysteinemia has been shown to elevate asymmetric dimethyl arginine (ADMA) which is an endogenous eNOS inhibitor (Cooke, 2000). Recently, we have shown that hyperhomocysteinemia-induced endothelial dysfunction in rats can be produced by administration of L-methionine (1.7%W/W, p.o., daily) suspension in 0.1% CMC for four weeks (Shah and Singh, 2006b-d).

High Fat Diet-induced Vascular Endothelial Dysfunction

Recently, our laboratory has shown that high fat diet in composition of 5% w/w cholesterol, 10% w/w lard fat, 0.1% w/w sodium cholate and 1% w/w coconut oil mixed with standard chow diet has produced vascular endothelial dysfunction (Shah and Singh, 2006a). Combination of methionine (1%) and cholesterol (0.5%) diet has been noted to abolish endothelium dependent relaxation in rabbits (Zulli *et al.*, 2003). High fat diet comprising of pig chow supplemented with 2% cholesterol, 17.1% coconut oil, 20.3% corn oil and 0.7% sodium cholate has been reported to decrease endothelium dependent relaxation and eNOS level in pigs (Henderson *et al.*, 2004).

Hyperuricemia-induced Vascular Endothelial Dysfunction

It has been suggested that uric acid may induce vascular endothelial dysfunction by inhibiting endothelial NO production and increasing ROS level (Kanellis and Kang, 2005). Mild hyperuricemia can be induced by administering oxonic acid ($750 \text{ mg kg}^{-1} \text{ day}^{-1}$, p.o.) in rats (Khosla *et al.*, 2005). Further, hyperuricemia is induced by administration of yeast extract paste ($20\text{-}30 \text{ mg kg}^{-1} \text{ day}^{-1}$) for 7 days in rats and mice. Yeast would disturb normal purine metabolism by increasing xanthine oxidase activity and generating large quantities of uric acid with ROS and this model has been shown to be similar to human hyperuricemia (Chen *et al.*, 2006).

Heart Failure-induced Vascular Endothelial Dysfunction

Endothelial dysfunction is a newly discovered hallmark of heart failure which is characterized by decreased release of EDRF in vasculature and increased generation of oxygen free radicals. Vascular endothelial dysfunction is induced by left coronary artery ligation model of heart failure in rats (Indik *et al.*, 2001). Partial aortic constriction has been shown to produce pressure overload and consequently ventricular hypertrophy (Balakumar and Singh, 2005, 2006a, b, c). Pressure overloaded ventricular hypertrophy was induced in guinea pig by placing a hemoclip of 0.5 mm in diameter around a sub diaphragmatic aorta just above the renal arteries (Lang *et al.*, 2000; Bell *et al.*, 2001) which has been shown to increase oxidative stress and consequently produce vascular endothelial dysfunction. Further transverse aortic banding (TAB) for 2 to 11 weeks is employed to produce vascular endothelial dysfunction in mice (Ogita *et al.*, 2004).

Oestrogen Deficiency-induced Vascular Endothelial Dysfunction

Oestrogen regulates the endothelial nitric oxide synthase activity either genomically by modulating its expression (Levin, 2005) or nongenomically by regulating its activity (Chambliss *et al.*, 2002). The incidence of cardiovascular disorders such as hypertension, atherosclerosis and coronary artery disease are noted to increase in oestrogen deficiency associated with menopause. The endothelial dysfunction as a result of menopause is characterized by increase in plaque formation and intimal thickening (Squadrito *et al.*, 2000; Beral *et al.*, 2002). Surgical oestrogen deficiency by ovariectomy impairs Ach-induced endothelium dependent relaxation (Walker *et al.*, 2001). To induce endothelial dysfunction by ovariectomy, rats were anaesthetized with chloral hydrate (250 mg kg^{-1} i.p.) and incision was made in right and left dorsal side of flanks. Ovaries along with uterus were pulled out and suture was applied at the end of uterus and beginning of ovary. The ovaries on both sides were removed. The uteri on both sides were pushed back and incisions were sutured in layers. Neosporin antibiotic powder was applied on wounds and animals were allowed for 4 weeks to produce vascular endothelial dysfunction.

Nicotine-induced Vascular Endothelial Dysfunction

Cigarette smoking is a strong risk factor for vascular diseases and known to cause dysfunction of endothelium (Zhang *et al.*, 2006). Nicotine contributes to smoking-induced endothelial dysfunction because of its ability to impair endothelium dependent vasorelaxation. Administration of nicotine ($2 \text{ mg kg}^{-1} \text{ day}^{-1}$ i.p.) for 4 weeks has been shown to decrease bradykinin mediated endothelium dependent vasodilation in rats (Paganelli *et al.*, 2001; Luo *et al.*, 2006).

Endotoxic Shock-induced Vascular Endothelial Dysfunction

Endothelial dysfunction plays a crucial role in pathophysiology of septic shock due to gram-negative bacteria (Cotran and Pober, 1990). Endothelium derived NO production has been noted to be reduced during endotoxemia (Young *et al.*, 1991; Myers *et al.*, 1995). A single injection of endotoxin (*E. coli*) (15 mg kg^{-1} i.v.) has been shown to produce vascular endothelial dysfunction in rats

within 6 h. Further, in a rabbit model of endotoxic shock, a single lipopolysaccharide (LPS) bolus ($0.5 \text{ mg kg}^{-1} \text{ i.v.}$) *Escherichia coli* endotoxin has produced vascular endothelial dysfunction in about 5 days (Wiel *et al.*, 2000).

Arsenic-induced Vascular Endothelial Dysfunction

Arsenic, a ubiquitous element distributed in the environment and contaminated drinking water is the main source of arsenic (Abernathy *et al.*, 1999). Arsenic has been suggested to inhibit eNOS and produce excessive ROS which contributes to endothelial dysfunction. Chronic arsenic exposure has been associated with diabetes, cardiovascular diseases (Rossman, 2003; Tseng, 2004) and cerebrovascular disorders (Wang *et al.*, 2002; Simeonova *et al.*, 2003). Continuous administration of arsenate (5 mg L^{-1}) in drinking water for 18 weeks has been shown to produce vascular endothelial dysfunction in rabbits (Kumagi and Pi, 2004).

Glutathione Peroxidase Deficiency-induced Vascular Endothelial Dysfunction

Glutathione peroxidase (GPx-1) is an antioxidant enzyme plays an important role in protection of cells against oxidative stress (Raes *et al.*, 1987). GPx-1 deficiency directly induces an increase in vascular oxidative stress and decrease in eNOS mediated NO bioavailability (Schachinger *et al.*, 2000; O'Donnell and Freeman, 2001). Glutathione peroxidase deficiency-induced vascular endothelial dysfunction is shown in murine model of homozygous deficiency of GPx-1 (GPx-1^{-1}). Mesenteric artery of GPx-1^{-1} mice demonstrated paradoxical vasoconstriction to β -methacholine and bradykinin where as wild type (WT) mice showed dose-dependent vasodilation in response to both agonists (Forgione *et al.*, 2002).

Hypochlorite-induced Vascular Endothelial Dysfunction

It has been suggested that blood vessels exposed to hypochlorite (HOCl) exhibit a defect in endothelium derived NO bioavailability manifested as impaired endothelium dependent arterial relaxation (Stocker *et al.*, 2004). HOCl-induced vascular endothelial dysfunction may be due to reduction in NO production by decreasing eNOS level and increasing ROS production (Nuszkowski *et al.*, 2001; Stocker *et al.*, 2004). Pretreatment with HOCl ($400 \mu\text{M}$) for 2 h has been shown to produce impaired Ach-induced relaxation in rabbit aortic ring preparation (Witting *et al.*, 2005).

Excessive Glucocorticoid-induced Vascular Endothelial Dysfunction

Glucocorticoids (GC) have been used widely for the treatment of patients with various disorders including autoimmune disorders. GC such as prednisolone, methylprednisolone and dexamethasone are often limited by several adverse reactions associated with vascular system such as coronary artery diseases, hypertension and atherosclerosis (Ross and Linch, 1982; Saruta, 1996). Various clinical findings suggest that excessive GC causes overproduction of ROS and reduction of NO availability leading to vascular endothelial dysfunction in human subjects (Iuchi *et al.*, 2003). However this has not been well demonstrated in suitable animal models.

CONCLUSIONS

Hypertension, diabetes, hyperhomocysteinemia and hypercholesterolemia-induced vascular endothelial dysfunction are the commonly used experimental models since these models are reflecting clinical similitude of vascular endothelial dysfunction. Developing new models employing recent advances in pathophysiology of vascular endothelial dysfunction can accelerate research in developing novel therapeutic agents to improve vascular endothelial function.

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