

ENDOTHELIAL DYSFUNCTION: MANY WAYS TO CORRECT- TRENDS THAT PROMISE

K.V. RAMESH, K. ASHOK SHENOY

Department of Pharmacology, Kasturba Medical College, PB No. 53, Light House Hill Road, Mangalore, Karnataka - 575 001.

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ABSTRACT Endothelium is considered as the largest endocrine organ of the human body. The biological functions of endothelium are numerous and may vary according to the size and distribution of the blood vessel. Endothelium serves and participates in highly active metabolic and regulatory function including control of primary hemostasis, platelet and leukocyte interaction with the vessel wall. Also, it interacts with the lipoprotein metabolism and presentation of histocompatibility antigens. These dynamic and intricate functions of endothelium are extremely vulnerable which forms the basis for many therapeutic goals to be achieved. A plethora of bioactive molecules have been produced by endothelium. Endothelial factors influence vascular tone, blood flow, clot deposition, clot lysis and selective phagocytic activity. Many protein growth factors, matrix supporting proteins and vasoactive substances have been produced by the endothelium. The functions of vascular endothelium are dynamic rather than fixed. Endothelial derived substances can be mutually antagonistic. Injury to the endothelium causes dysfunction. Immune complexes, lipids, angioplasty, germs, hypertension, shear stress, hypoxia, acidosis, smoking, aging, diabetes mellitus and surgery inflict injury to the endothelium.

Endothelial dysfunction is a major cardiovascular factor implicated in the pathogenesis of atherosclerosis, arterial thrombosis, pulmonary hypertension, myocardial infarction, stroke and deep vein thrombosis. New groups of salvaging drugs have been introduced to overcome the consequences of endothelial dysfunction. Yet, the value of existing drug treatment cannot be condoned in conditions of endothelial dysfunction. Time is right to embark on the drugs that modulate endothelial functions to control morbidity and mortality in various cardiovascular diseases. Hopefully, future research will offer us better drugs.

KEY WORDS Endothelins vascular endothelium

Introduction

Over the years, our knowledge about the role of endothelium in health and disease is increasing in rapid pace. In fact, the total mass of endothelium is around 2 kg and it may be considered as the largest endocrine organ of the human body. The endothelium performs a variety of crucial regulatory functions. A drug that modulates endothelial functions can significantly alter morbidity and mortality related to endothelial dysfunction. This review is aimed at highlighting the clinical perspectives of agents that modulate the function of endothelium.

Structure and functions of endothelium: Endothelium is the monolayer of polygonal flat cells

that extend continuously over the luminal surface of the entire vasculature. In different regions, the structural features vary with specificity¹. In brain, cell junctions are mainly tight while intracellular cleft is wide open in liver to facilitate protein biotransport. The endothelial cells of glomerular tuft have small oval windows called fenestrae so that ionic substances can be filtered readily.

The functions of endothelium are numerous and vary according to size and distribution of blood vessel². The endothelium is a potential source of various chemical mediators that influence blood flow, clot deposition, clot lysis and selective phagocytic activity (Table 1). The maintenance of vascular tone, thromboresistant surface, transport of nutrients and

Correspondence: K.V. Ramesh
e-mail: kirugavalramesh@rediffmail.com

Table 1. The products of endothelium*.

Vasoconstrictors:	Endothelins 1, 2 and 3, angiotensin II, thromboxane A ₂ , superoxide radical (O ₂), endothelium-derived constriction factor (EDCF).
Vasodilators:	Nitric oxide (NO), prostacyclin (PGI ₂), PGE ₂ , endothelium derived hyperpolarization factor (EDHF).
Agents inducing cell proliferation:	Endothelin 1 and angiotensin II (AT II).
Growth factors:	Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), transforming growth factor b (TGF b).
Other proteins:	C-natriuretic peptide, B-natriuretic peptide, adrenomedulin, interleukins, endoadenosine diphosphatase, von Willebrand's factor, fibrinogen, thrombomodulin, tissue factor, P & E selections, vascular cell adhesive molecules, intracellular adhesive molecules, integrins, nuclear factors k and b, platelet activating factor (PAF), a-urokinase, tissue plasminogen activator (tPA), plasminogen activator inhibitor (PAI), protein S.

*Products identified for their biological role are furnished. Supposedly, the list is incomplete.

other solutes, activation and inactivation of various vasoactive hormones fall in the ambit of varied physiological functions of endothelium. The pulmonary endothelium inactivates and removes various polypeptides, biogenic amines, prostaglandins and lipids from circulation³. A plethora of proteins, growth factors, vasoactive substances, matrix supporting proteins are produced by endothelium⁴. During angiogenesis and tissue repair, the endothelial cell is capable of proliferation to provide new cells⁵. Importantly, endothelium regulates the interaction of circulating cells including platelets with the vessel wall^{6,7}. The luminal surface of the endothelial cell is smooth, non-thrombogenic and the albuminal surface is adhesive to inner wall as well as platelets. Further, many functions of vascular endothelium is dynamic than fixed.

Many endothelium-derived substances are functionally active, mutually antagonistic and some are apparently redundant⁸. Injury to the endothelium causes dysfunction. The clinical and pathological manifestations of endothelial injury differ according to the type of insult, blood vessel, blood flow and shear stress⁹. Generally, endothelial damage is reversible and chronic injury is not observed¹⁰. A clear understanding is necessary about the complications of endothelial dysfunction before any drug treatment is begun or proposed.

Endothelium encounters blood borne insults incessantly. The causes of endothelial injury include lipids, immune complexes, angioplasty, micro-organisms and their elaborated toxins. Hypertension,

shear stress, hypoxia, acidosis, smoking, aging, diabetes mellitus, trauma and surgery do inflict injury to endothelium. Reperfusion injury is regarded as a common factor that cause endothelial dysfunction^{11,12}. Furthermore, inflammatory diseases exacerbate endothelial dysfunction and the converse is also true.

The loss of endothelium is coupled with the expression of procoagulant, prothrombotic and pro-inflammatory molecules inducing the release of different growth factors¹³. Thus released growth factors may stimulate or inhibit vascular smooth muscle cell migration and proliferation. Moreover, the disruption of endothelium results in the loss of balance between vasoconstrictor and vasodilator function which may lead to the progression of vascular diseases, myocardial infarction and stroke¹⁴. The hallmark of endothelial dysfunction is enhanced production of EDCF and corresponding decrease in NO and PGI₂ release. Accordingly, therapeutic interventions need to be designed to counteract the early events that occur at the time of endothelial injury. Hopefully, this will effectively prevent and control many disorders of the cardiovascular system.

Atherosclerosis and hypertension: A wide variety of pathophysiological conditions are associated with hypertension and other risk factors. Other risk factors include abnormal lipid metabolism or hypercholesterolemia, insulin resistance or abnormal glucose metabolism and smoking in particular. Lowering of blood pressure and addressing the precipitating factors alone may not reduce the risk of mortality. However, importantly, there is a need to look

at and deal with endothelial dysfunction which is a common phenomenon in an array of cardiovascular disorders. An increased oxidative stress in the blood vessels is a common denominator of various endothelial insults¹⁵. The presence of oxidized low-density lipoprotein (LDL) and smoking increase superoxide anion production¹⁶. Nitric oxide combines with superoxide to produce peroxy nitrite which stimulates adhesion molecules resulting in leukocyte adhesion to endothelium and thus ignites initial inflammatory reaction which culminates in atherogenesis in due course of time. Therefore, the rational approach to treat atherosclerosis lies in the preservation of normal endothelial function. Exercise increases laminar blood flow and hence more NO is released¹⁷ which results in fall of blood pressure, leading to improved endothelial function. It is believed that chronic administration of statins, lipid lowering resins, ACE inhibitors and calcium channel blockers improve the endothelial cell function. Statins with resins seem to normalize endothelial cell (EC) activity^{18,19}. Statins also have anti-inflammatory effects and deplete the lipid core in plaques²⁰. Presumably, the antioxidant probucol plays a pivotal role with lovastatin or atorvastatin to improve EC function in the same manner as membrane associated vitamin E²¹. However, the precise role of the pleiotropic effects (e.g., endothelial function, inflammation & clotting) of statins remain unresolved at present although a number of hypercoagulability markers have been successfully altered by statins²².

Angiotensin II (AT II), through NADH, NADPH tends to be a pro-oxidant and it predisposes to endothelial dysfunction. Angiotensin converting enzyme is a promiscuous enzyme which has fairly a large variety of peptide substrates including bradykinin (BK). Administration of ACE inhibitors (ACEIs) disrupts the BK degradation, thus accumulated BK acts on the B₂ receptors on the ECs which results in vasodilatation and at the same time other events that occur in vessel wall are also inhibited. In brief, ACE inhibition results in vasorelaxation, decreased hypertrophy, decreased oxidative stress and increased NO release²³. However, this may not be adequate for salvaging endothelial dysfunction. Because, chronic ACE inhibition incompletely suppresses AT II. Eventually, there is at least partial recovery of AT II generation. In addition, an alternate pathway of AT II generation probably operates for

rescue. This is a subject of debate. An enzyme tonin^{24,25} or the clot buster tPA is believed to take part in the conversion of AT I to AT II. It seems that much of the sustained antihypertensive effect of chronic ACEI treatment is due to BK and BK induced NO release^{26,27}. This is further supported by the observations that N-Dimethyl Arginine (L-NMMA) completely blocks the beneficial effect of ACEI in a hypertensive patient²⁸. Also, bradykinin B₂ receptor blocker icatibant inhibits some of the effects of ACEI^{29,30}. Nevertheless, it is important to recognize that reduction of systemic blood pressure improves EC function. An imbalance between vascular relaxing and contracting factors can lead to endothelial dysfunction which, in turn, may lead to cardiovascular morbidities.

Shear stress - A major offender: Obviously, clinical and pathological manifestations of vascular disorders following injury differ according to the types of insult, blood flow and shear stress³¹. Shear stress represents the frictional force that the flow of blood exerts at the endothelial surface of the vessel wall. Shear stress facilitates the opening of K⁺ channels and by the stimulation of acetylcholine receptors releases the endothelial derived hyperpolarizing factor (EDHF)³². It remains to be seen how reduction of shear stress by drug administration is useful in reducing the morbidity of hypertension related vascular diseases. Vascular endothelial cells subjected to fluid shear stress changed their shape and uniformly oriented with the flow. Remuzzi *et al*³³, have revealed that EC exposed to fluid shear stress lose elongated shape and a change in cell direction with time. After 72 h, the original shape of EC may be restored. Shear stress is generated by blood flow. The cytoplasmic free Ca²⁺ acts as an internal signalling system in response to shear stress³⁴. Shear stress promotes prostaglandin D₂ production by stimulating Lipocalin-type PGD₂ synthase (L-PGDS) expression³⁵ and suggest the possibility that a peroxisome proliferator activated receptor gamma ligand is produced in vascular wall in response to blood flow³⁶. In contrast, shear stress did not alter expression level of PGI₂. Thus, fluid shear stress alters both structure and function of vascular endothelium. This is aptly demonstrated both *in vivo* and *in vitro* experiments. Large shear stress gradients can induce morphological and functional changes in the endothelium in regions of disturbed blood flow

in vivo. This may contribute to the formation of atherosclerotic lesions. Therefore, methods to reduce shear stress particularly at the arteriolar branching sites may prove beneficial in the control of atherogenesis.

Endothelial growth factors: Anticancer therapy: Vasculogenesis, the *de novo* formation of new blood vessels and angiogenesis, the formation of blood vessels by sprouting from the preexisting ones are required in many physiological and pathological conditions. Vascular growth is regulated by a wide variety of factors. Vascular endothelial growth factor (VEGF) is unique in that it specifically targets the mitosis of endothelium. Fibroblast growth factor (FGF) and insulin-like growth factor (ILF) stimulate vasculogenesis. Angiostatin and leukemia inhibiting factors have negative control on vascular growth³⁷.

Vascular endothelial growth factor is expressed in the majority of adult and fetal tissues that include endothelial cells, placenta, uterine smooth muscle cells and various cultured cells. The survival factor for newly formed capillaries is VEGF which also induces vasodilatation, hypotension, increase microvascular permeability and enhance coagulation. It is also considered to be antiapoptotic³⁸. The VEGF family of growth factors bind to at least three different tyrosine kinase receptors - VEGFR - 1, 2 and 3, which invariably leads to mitogenesis. However, there are various types of VEGFs identified which vary in their actions on different organ system, the significance of which remains elusive.

With an abnormality in p53 gene, vascular endothelial growth factor has been shown to be upregulated and thrombospondin-1, a negative angiogenesis regulator is downregulated in cancer prone individuals. Angiogenesis has a prognostic value for breast, kidney, prostate, colon, brain and laryngeal cancers³⁹. Endothelial Growth Factors (EGF) are known to be tumor specific. The most potent mitogen among EGF is basic fibroblast growth factor (bFGF) and there may be synergy with vascular endothelial growth factor (VEGF) in tumor growth^{40,41}. Platelet derived endothelial cell growth factor (PD-ECGF) and hepatocyte growth factor (HGF) are believed to take part in microvessel formation, coordinating with bFGF, PD-ECGF and VEGF. Anti-angiogenesis therapy in cancer treatment offers a number of benefits - accessibility, tumor specificity, less possibility of

resistance and possible use in chemoprevention. Tamoxifen, an antiestrogen, medroxyprogesterone acetate, a synthetic progesterone, 5-deoxy-5-fluorouridine are antiangiogenic anticancer drugs. It is believed that at least in part the angiogenic mechanisms of these drugs contribute to their antitumor effect. In view of this, researchers opine that PD-ECGF is destined to become one of the major targets of angiogenesis therapy in future.

Currently, the central mediator of tumor angiogenesis is thought to be VEGF^{42,43}. Hence, targeting VEGF action have been designed and studied. Warren *et al.*,⁴⁴ showed a marked reduction in the number and size of liver metastases by administration of anti-VEGF monoclonal antibody in human colon carcinoma bearing nude mice. Many therapeutic strategies have been already documented to inhibit VEGF induced endothelial cell growth. A tyrosine kinase inhibitor Lavendustin - A was also reported to inhibit VEGF induced angiogenesis⁴⁵. These findings clearly evinces that commendable advances have been achieved in the basic understanding of neovascularization. Certainly, this will contribute substantially in changing the nature of cancer chemotherapy in the days to come.

P-selectin and cardiovascular risk: P-selectin (Gmp-ino) a granule membrane protein and a member of immunoglobulin supergene family is expressed in the endothelium^{46,47}. Leukocytes adhere to EC through selectins. P-selectin is stored in platelets as granules and as Weibel palade bodies of EC. Thrombin and histamine activate the release of P-selectin. It has been observed that elevated P-selectin level is associated with increased risk of future myocardial infarction (MI), stroke, coronary revascularization and death due to cardiovascular failure. This association is independent of age, smoking and lipid profile⁴⁸. P-selectin deficiency has been shown to protect against atherosclerosis⁴⁹.

Estrogen and endothelial function: Interestingly, a man without functional estrogen receptors has been reported to lack endothelium based vasodilatation and to have early coronary calcification^{50,51}. Estrogen has a prime function in maintaining the integrity and functional plasticity of the vascular tree^{52,53}. Patients with polycystic ovary syndrome have elevated androgen levels and oligomenorrhoea and are typically obese and insulin resistant⁵⁴. This trend is

supposed to be associated with increased risk of cardiovascular disease preceded by endothelial dysfunction^{55,56}. It remains to be known whether estrogen replacement therapy in postmenopausal women would offer protection from macrovascular diseases in particular.

Septic shock: Activation of endothelial inflammation: The endotoxins released from gram negative bacteria augment endothelial cell production of fibrinolytic inhibitor - plasminogen activator inhibitor - I (PAI-I). Subsequently, this alters endothelial regulatory functions⁵⁷ and frequently leads to clinically well documented disseminated intravascular coagulation. Disseminated intravascular coagulation is a common feature of gram negative sepsis.

Protein C is a natural anticoagulant that inhibits thrombosis, inflammation and promote fibrinolysis. It is argued that protein C reduces the relative risk of mortality in septic shock. The ability of protein C to target inflammatory and procoagulant pathways may be central to its efficacy in septic shock⁵⁸. This view is further strengthened by the fact that reduced levels of activated protein C increases the risk of death in most patients with sepsis. A study on laboratory models of sepsis and a prospective open label study suggest that activated protein C improves the outcome in severe meningococcaemia⁵⁹. Nevertheless, the decision to use activated protein C in septic shock must be taken by experienced intensive care clinicians and should not be used indiscriminately.

Protease activated receptors (PAR) and thrombomodulin: Protease activated receptors represent a distinct class of G-protein coupled receptors activated by proteolytic cleavage. To date, four subtypes of PARs have been identified viz., PAR 1, 2, 3 and 4. PAR 1 is believed to be thrombin receptor and PAR 2 is activated by trypsin. Immunohistochemical techniques have detected PAR 2 in endothelium and vascular smooth muscle⁶⁰. Recently, it has been delineated that PAR 2 mediates both vascular contractility and proliferation. Proteases are involved in a wide array of processes and said to establish a cross link between coagulation and inflammation. This link is more significant following vascular injury. Protease activated receptors upregulate cyclooxygenase 2 expression in human endothelial cells⁶¹ and mediate factor Xa signaling in

endothelial cells⁶². Further, plasmin, elastase and fibrinolytic proteases may regulate PAR 2 signaling in various pathological conditions⁶³.

Thrombomodulin is a natural anticoagulant protein. Any alteration in the expression of thrombomodulin with or without other anticoagulant proteins is likely to impair endothelial thromboresistance⁶⁴. Wang *et al.*,⁶⁵ have demonstrated deficiency of microvascular thrombomodulin linking endothelial dysfunction to chronic radiation fibrosis. Endothelial thrombomodulin does play a vital role in vascular injury but how interventions at restoring thrombomodulin levels offer therapeutic benefits remain uncertain.

Autoimmune disease and endothelium: Interestingly, repeated endothelial injury caused by responding T cells to an unidentified antigen provokes the cascade of events⁶⁶. Thus damaged endothelium releases factors that accelerates clotting and inflammation and vascular repair. Undoubtedly, in the presence of hyperlipidemia, homocysteine glycation, glycosylated endproduct, toxins and infectious agents, the endothelial systemic function goes into disarray and contributes for major cardiovascular disorders. Therefore, it is pertinent to adopt therapeutic strategies to improve the function of endothelium with the measures to control co-morbidities.

Therapeutics of endothelial dysfunction: It is possible to improve EC dysfunction by several means and methods to achieve benefits in many clinical conditions. Here, clear understanding of the role played by various molecules synthesized by EC is essential to rationalize the drug therapy. Both non-pharmacological and pharmacological treatments are useful to reduce the incidence of EC dysfunction related cardiovascular disorders. The non-pharmacological approach includes regular exercise, low fat diet rich in monounsaturated fatty acids, cessation of smoking and food rich in vegetables and fruits. Physical training and conditioning have been shown to improve EC function in congestive cardiac failure. Exercise induces NO production⁶⁷. In hypercholesterolemic subjects, diet rich in monounsaturated fatty acids greatly increases EC function. Evidently, a low fat diet reverses the endothelial dysfunction in atherosclerosis⁶⁸. It is well recognized that low fat intake reduces the formation of atherogenic plaques.

Table 2. List of drugs known to modulate endothelial cell function which are of therapeutic interest.

1.	Nitric oxide, nitric oxide donors, nitric oxide antagonists
2.	PGI ₂ analogs and PGI ₂ synthesis inhibitors
3.	Angiotensin II synthesis inhibitors and receptor blockers
4.	Adenosine function modulators
5.	Thrombomodulin antagonists
6.	Bradykinin and bradykinin antagonists
7.	Endothelins and their antagonists
8.	tPA substitutes and inhibitors
9.	Lipid lowering agents
10.	K ⁺ channel openers and blockers
Others:	
	Estrogen, mibefrandil, folic acid, nebivolol, NSAIDs, thapsigargin, lebeluzole and antioxidants

In chronic smokers, it has been observed that there is increased oxidative stress within the vessel wall⁶⁹. It is suggested that inactivation of endothelial derived NO by reactive oxygen radicals contribute to EC dysfunction.

A wide variety of drugs (Table 2) modulate endothelial function which are of great therapeutic interest. Obviously, drugs that affect any endothelial protective molecules is likely to perturb thromboresistant, thromboregulatory, vasomotor tone and vascular muscle growth regulatory mechanisms endowed to endothelium⁷⁰. Keeping this in view, the research focus is on the different roles played by endothelium to reduce the morbidity of major cardiovascular disorders including hypertension. Furthermore, the thrust is to embark on one or two groups of drugs that modulate discretely one or more causal endothelial mechanism to achieve therapeutic benefit. However, this is by no means easy. More importantly, pathological endothelial changes that may occur due to cardiovascular diseases are invariably associated with co-morbidities. To counteract these, multiple drug therapy is imminent and the benefit achieved may not be totally attributable to drugs that alter endothelial function. This warrants for an extensive clinical appraisal.

Endothelial dysfunction caused either by shear stress or endotoxin may result in suppression of thrombo-protective effect, expression of procoagulant properties, pro-inflammatory response and release

of host of endothelial factors including endothelins⁷¹. It is rational to address these at endothelial level to stall the cardiovascular morbidity and mortality. It has been overwhelmingly emphasized that endothelial dysfunction is the primary etiological factor for the genesis of hypertension, atherosclerosis, arterial thrombosis, pulmonary hypertension, myocardial infarction, stroke and deep vein thrombosis⁷². Although this argument seems to be logical and sound, it remains largely not clearly elucidated. For example, endothelin released from endothelium is implicated in the genesis of eclampsia^{73,74}, acute myocardial infarction, renal and cerebral vasospasm, heart failure, cardiac hypertrophy, hormone secretion and pulmonary hypertension. Endothelin receptor antagonists-bosentan and sitaxsentan are now being appraised as salvaging drugs in the conditions mentioned above. Nonetheless, administration of already established drug treatment for respective conditions can not be condoned as yet. Hopefully, future clinical studies will offer better prospects.

Among commonly employed antihypertensive drugs ACEI and calcium channel blockers appear to have more effects on endothelium to improve its function. ACEIs reduce angiotensin II levels which in turn, decrease endothelin activation, interrupts degradation of NO by superoxide anions and inhibits bradykinin breakdown which augments NO release. These actions of ACEI undoubtedly offer benefit in many clinical settings by improving endothelial function. However, much remains to be known about long term benefit.

Dihydropyridine calcium channel blocker nifedipine enhances the availability of endothelial NO, attenuating vascular effects of endothelins⁷⁵. Nifedipine is also known to restore endothelial cell permeability. Thus calcium channel blockers possess anti-atherosclerotic effect. Yet, what remains to be answered is *does this have therapeutic accountability?* Especially, new b-adrenergic receptor blocker nebivolol⁷⁶ activates L-arginine - NO pathway and carvedilol has a strong antioxidant activity. Do these restore endothelial dysfunction, which can be related to the clinical efficacy? Time alone can answer.

Endothelial derived hyperpolarizing factor - A drug target: Endothelial derived hyperpolarizing factor is a novel, non-nitric oxide, non-prostanoid endothelial product. The possibility that EDHF is a

cannabinoid agonist is being investigated. This factor mediates cellular effects by either directly or indirectly opening K⁺ channels on vascular smooth muscles or via hyperpolarisation of EC by facilitating electrical coupling between endothelium and vascular smooth muscle⁷⁷. The functional characterisation of EDHF varies depending on vascular size, vascular bed and species. The vascular resistance is more specifically mediated by resistance sized arteries that require EDHF. This in turn, regulates tissue blood flow⁷⁸. The release of EDHF is modulated by a number of factors including agonist stimulation, shear stress, estrogen and disease⁷⁹.

As already mentioned opening of K⁺ channels releases EDHF. Drugs like iberiotoxin, glibenclamide, charbodotoxin, apamin and 4-aminopyridine block K⁺ channels and impair EDHF release. The impact of these agents on EC function requires to be examined. It is prudent to have a fresh look on long-term use of glibenclamide especially with regard to EC function related vascular pathological features that occur in diabetic patients.

Patients with homocysteinuria may have advantages from folic acid treatment. Folic acid has been shown to reverse the endothelial dysfunction. Thapsigargin induces sustained Ca²⁺ levels in EC. Consequently, EC releases NO, EDHF and prostacyclin, which relax vascular smooth muscle and counteract the action of various endogenous vasoconstrictors. Nebivolol, a beta blocker, causes vasodilatation by endothelium dependent mechanism, thus pave a way for the development of new generation beta blockers which may become useful in hypertensive heart failure patients. Lubeluzole, a NO inhibitor^{80,81} is now being evaluated as a neuroprotective agent and may be beneficial in ischemic stroke. It is known to act by blocking NO mediated pathway of glutamate toxicity. Lubeluzole is administered within 6 h of ischemic stroke in a dose of 10 mg/kg for 5 days. However, the efficacy of lubeluzole is not yet proved convincingly.

Concluding remarks: The endothelium is a biological barrier between the blood and the vascular smooth muscle with diverse functions. Endothelium is a source of host of active endogenous substances including growth factors and enzymes. Endothelial dysfunction is a hallmark of various cardiovascular disorders. Drugs that modulate endothelial dys-

function have been used to treat many clinical conditions including hypertension, atherosclerosis and cardiac hypertrophy. Further understanding of the physiological role of endothelial products and the response of endothelium to noxious stimuli may alter the approaches of drug therapy in future.

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Note that `Promise.all()` doesn't trigger the promises to start their work, creating the promise itself does. With that in mind, one solution would be to check whenever a promise is resolved whether a new promise should be started or whether you're already at the limit. However, there is really no need to reinvent the wheel here. One library that you could use for this purpose is `es6-promise-pool`. From their examples: // On the Web, leave out this line and use the script tag above instead. `var PromisePool = require('es6-promise-pool').var promiseProducer = function () { // Y`