ENDOTHELIAL DYSFUNCTION: MANY WAYS TO CORRECT- TRENDS THAT PROMISE

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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 specificity. 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 vessel. 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
Table 1. The products of endothelium*.

<table>
<thead>
<tr>
<th>Category</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasoconstrictors:</td>
<td>Endothelins 1, 2 and 3, angiotensin II, thromboxane A₂, superoxide radical (O₂), endothelium-derived constriction factor (EDCF).</td>
</tr>
<tr>
<td>Vasodilators:</td>
<td>Nitric oxide (NO), prostacyclin (PGL₂), PGE₂, endothelium derived hyperpolarization factor (EDHF).</td>
</tr>
<tr>
<td>Agents inducing cell proliferation:</td>
<td>Endothelin 1 and angiotensin II (AT II).</td>
</tr>
<tr>
<td>Growth factors:</td>
<td>Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), transforming growth factor β (TGF β).</td>
</tr>
<tr>
<td>Other proteins:</td>
<td>C-natriuretic peptide, B-natriuretic peptide, adrenomedulin, interleukins, endoadenosine diphosphatase, von Willebrand’s factor, fibrinogen, thrombomodulin, tissue factor, P &amp; E selections, vascular cell adhesive molecules, intracellular adhesive molecules, integrins, nuclear factors α and β, platelet activating factor (PAF), α-urokinase, tissue plasmonogen activator (tPA), plasminogen activator inhibitor (PAI), protein S.</td>
</tr>
</tbody>
</table>

*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 circulation. A plethora of proteins, growth factors, vasoactive substances, matrix supporting proteins are produced by endothelium. During angiogenesis and tissue repair, the endothelial cell is capable of proliferation to provide new cells. Importantly, endothelium regulates the interaction of circulating cells including platelets with the vessel wall. 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 redundant. 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 stress. Generally, endothelial damage is reversible and chronic injury is not observed. 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, microorganisms 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. Furthermore, inflammatory diseases exacerbate endothelial dysfunction and the converse is also true.

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 insults. The presence of oxidized low-density lipoprotein (LDL) and smoking increase superoxide anion production. Nitric oxide combines with superoxide to produce peroxynitrite 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 released 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. Statins also have anti-inflammatory effects and deplete the lipid core in plaques. Presumably, the antioxidant probucol plays a pivotal role with lovastatin or atorvastatin to improve EC function in the same manner as membrane associated vitamin E. 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 statins.

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 release. 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 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. This is further supported by the observations that N-Dimethyl Arginine (L-NMMA) completely blocks the beneficial effect of ACEI in a hypertensive patient. Also, bradykinin B2 receptor blocker icatibant inhibits some of the effects of ACE.

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 stress. 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). 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 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 stress. Shear stress promotes prostaglandin D production by stimulating Lipocalin-type PGD2 synthase (L-PGDS) expression and suggest the possibility that a peroxisome proliferator activated receptor gamma ligand is produced in vascular wall in response to blood flow. 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 growth. Vascular endothelial growth factor is expressed in various types of VEGFs identified which vary in their actions on different organ system, the significance of which remains elusive.

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 antiapoptotic. 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 thrombosponding-1, a negative angiogenesis regulator is downregulated in cancer prone individuals. Angiogenesis has a prognostic value for breast, kidney, prostate, colon, brain and laryngeal cancers. 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. 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-fluororidine 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. Hence, targeting VEGF action have been designed and studied. Warren *et al.*, 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 angiogenesis. 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. 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 profile. P-selectin deficiency has been shown to protect against atherosclerosis. Interestingly, a man without functional estrogen receptors has been reported to lack endothelium based vasodilatation and to have early coronary calcification. Estrogen has a prime function in maintaining the integrity and functional plasticity of the vascular tree. Patients with polycystic ovary syndrome have elevated androgen levels and oligomenorrhea and are typically obese and insulin resistant. This trend is
endothelial cells upregulate cyclooxygenase 2 expression in human vascular injury. Protease activated receptors inflammation. This link is more significant following establishment a cross link between coagulation and are involved in a wide array of processes and said to both vascular contractility and proliferation. Proteases Recently, it has been delineated that PAR 2 mediates 2 in endothelium and vascular smooth muscle. Immunohistochemical techniques have detected PAR receptors activated by proteolytic cleavage. To date, four subtypes of PARs have been identified representing a distinct class of G-protein coupled receptors. Thrombomodulin: Thrombomodulin is a natural anticoagulant protein. Any alteration in the expression of thrombomodulin with or without other anticoagulant proteins is likely to impair endothelial thromboresistance. Wang et al. 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 events. 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 comorbidities.

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 production. In hypercholesterolemic subjects, diet rich in monounsaturated fatty acids greatly increases EC function. Evidently, a low fat diet reverses the endothelial dysfunction in atherosclerosis. It is well recognized that low fat intake reduces the formation of atherogenenic plaques.
of host of endothelial factors including endothelins. 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 thrombosis.

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, 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 endothelins. 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 β-adrenergic receptor blocker nebivolol 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 muscle. 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 flow. The release of EDHF is modulated by a number of factors including agonist stimulation, shear stress, estrogen and disease.

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, 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 dysfunction 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.

**REFERENCES**


\section*{ATTENTION PLEASE!!!}

Abstracts of papers presented at 35th annual conference of IPS, Gwalior will be published in the IJP from April, 2003. The authors who wish to publish their abstracts should send a copy of the same in MS-Word format by email (as an attached file). Abstracts should conform to the IJP format i.e. structured abstract with subheadings (Objective, Methods, Results and Conclusion).

Abstracts should be mailed to \texttt{ijp@jipmer.edu}
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:

```javascript
var PromisePool = require('es6-promise-pool').
var promiseProducer = function () {
    // Your code here
}
```