Endothelial cell dysfunction: can’t live with it, how to live without it

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Abstract
Goligorsky, Michael S. Endothelial cell dysfunction: can’t live with it, how to live without it. Am J Physiol Renal Physiol 288: F871–F880, 2005; doi:10.1152/ajprenal.00333.2004.—Endothelial cell dysfunction is emerging as an ultimate culprit for diverse cardiovascular diseases and cardiovascular complications of chronic renal diseases, yet the definition of this new syndrome, its pathophysiology, and therapy remain poorly defined. Here, I summarize some molecular mechanisms leading from hyperhomocysteinemia, elevated asymmetric dimethylarginine, and advanced glycation end product-modified protein level to the proatherogenic, prothrombogenic, and proinflammatory endothelial phenotype and offer a model of endothelial dysfunction based on the interconnectedness of diverse functions. Finally, several therapeutic strategies to prevent and correct endothelial dysfunction are discussed in the light of uncertainty of their action modulated by the endothelial dysfunction per se.

uncoupled endothelial nitric oxide synthase; oxidative stress; vascular wall; atherosclerosis

TOWARD THE DEFINITION OF ENDOTHELIAL DYSFUNCTION: THE SYNDROME IN THE MAKING

A computer search of the international database Ovid shows that since 1983 the number of entries containing the term “endothelial dysfunction” as a keyword has been exponentially increasing. The term was first used to describe a defect in the removal of 5-hydroxytryptamine and norepinephrine in the pulmonary circulation of rabbits intratracheally injected with bleomycin, an agent causing the development of pulmonary fibrosis (20) and, a year later, in reference to the decrease in angiotensin-converting enzyme and plasminogen activator activities, with no change in prostacyclin production by the pulmonary endothelium, after monocrotaline-induced lung injury (70). These investigators concluded that “monocrotaline-induced pulmonary injury is accompanied, and in some cases preceded, by structural and functional abnormalities in the pulmonary endothelium.” The modern usage of the term is associated with the study by Ludmer and co-workers (66), who observed that acetylcholine-induced vasorelaxation is impaired, even reversed, in atherosclerotic coronary arteries and this abnormality “occurs early . . . in the course of coronary atherosclerosis.” The same group of investigators has reported a similar angiographic pattern after coronary injection of acetylcholine in recipients of cardiac transplants (38). They hypothesized that the “impaired response to acetylcholine is a common early finding in heart transplant patients and emphasizes the potential importance of endothelial dysfunction in the development of atherosclerosis.” A host of studies by other investigative groups has confirmed these observations (35, 80, 81, 110). Because the term endothelial dysfunction has not only persevered during the past two decades but is also being used (and sometimes perhaps misused) with increasing frequency, despite the lack of a proper definition, an attempt to summarize the accrued knowledge and define the syndrome would be timely.

The last 20 years have established that the vascular endothelium, rather than being a mere barrier between intravascular and interstitial compartments, is a widely spread organ responsible for the regulation of hemodynamics, angiogenic vascular remodeling, metabolic, synthetic, anti-inflammatory, and anti-thrombogenic processes. Based on the diversity of endothelial functions, it is logical to expect that the definition of the syndrome of “endothelial cell dysfunction” (ECD) should be broad enough to encompass disturbances in the barrier function of the vascular endothelium; its impaired antithrombogenic properties; perturbed angiogenic competence; inappropriate regulation of vascular smooth muscle toxicity, proliferative capacity, and migratory properties; perturbed synthetic functions; and deterrence of neutrophils and monocytes from diapedesis (Fig. 1, a diagrammatic presentation of ECD as a transportation hub harboring diverse functional units). The phenotype of endothelial cells characterized by these abnormalities, expressed in various degrees, is emerging as a hallmark of several highly prevalent cardiovascular and renal diseases, including obesity and diabetes, as well as their complications. I shall discuss this broadened definition of ECD below.

PATHOGENETIC MECHANISMS

The convergence of traditional risk factors, genetic predisposition, and local and yet unknown factors acting on endothelial cells all contributes to the development of ECD, the “ultimate risk of the risk factors” (10). In addition to traditional cardiovascular risk factors, a host of complementary mecha-
nisms responsible for the high prevalence of cardiovascular complications has been described. Among the nontraditional risk factors that appear to gain in importance are elevated asymmetric dimethylarginine (ADMA) levels, hyperhomocysteinemia (HHCy), and protein modification by nonenzymatic advanced glycation. Several years ago, we elected to study the cellular and molecular derangements induced by the above pathogenic stimuli using nonbiased functional genomic screening. Toward this end, we performed cDNA microarray screening of "cardiovascular relevant" genes modified by two major contributors to ECD in chronic renal disease, homocysteine and the nitric oxide synthase (NOS) inhibitor \(\text{N}^2\)-nitro-L-arginine methyl ester (L-NAME), partially mimicking the effect of ADMA (it is necessary to emphasize, however, that these inhibitors are not equivalent). The results of these studies into some cellular consequences of several cDNA screens have been published; thus I shall describe briefly only the essential observations (23, 59–61, 88, 92, 111).

Functional analysis and retesting of some findings using RT-PCR and Western blotting showed that pathophysiologically relevant concentrations of homocysteine result in a profound induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase, increased synthesis and accumulation of cholesterol in the endothelial cells, and uncoupling of endothelial NOS (eNOS) due to oxidative stress, all manifestations of developing endothelial lipidosis (by analogy with hepatic lipidosis) (60). Upregulation of connexin-43 expression was accompanied by the displacement of this gap junctional protein from the plasma membrane to the mitochondrion, depletion of myoendothelial gap junctional communication, and the failure of the putative endothelium-derived hyperpolarizing factor to traverse myo-endothelial junctions and relax vascular smooth muscle cells (61). Together with the uncoupling of eNOS (111), these findings explain the complex pathogenesis of vasomotor failure. These and other abnormalities and their role in the development of ECD are schematically illustrated in Fig. 2.

The functional sequelae of eNOS inhibition with L-NAME, partially confirmed using ADMA by RT-PCR and Western blot analyses, are presented in Fig. 3. Inhibition of NO synthesis leads to the derangement not only of endothelium-dependent vasorelaxation but also to the proatherogenic sequelae, including enhanced adhesion and transmigration of monocytes and enhanced platelet aggregation. Upregulation of LOX-1, a major receptor for oxidized LDL, results in increased oxidative stress and lipid accumulation in endothelial cells (88, 89). Increased synthesis of several chains of collagen and induction of integrin receptors participate in the switch of endothelial cells toward the profibrotic phenotype (O’Riordan E and Goligorsky MS, unpublished observations). Overexpression of plasminogen activator inhibitor (PAI-1) by endothelial cells presented with advanced glycation end product (AGE)-modified collagen I is responsible for the early upregulation of PAI-1, which is causatively linked to the inappropriate formation of capillary networks during diabetic and/or end-stage renal disease (ESRD) wound healing and eventual vascular dropout (23).

It has been debated whether eNOS gene polymorphism contributes to ECD. Several potential candidate sites have been
identified, including Glu298Asp, -786T>C, and intron 4 polymorphisms and incriminating Asp298, -786C allele in the promoter and intron 4 with the increased risk of cardiovascular complications and its association with ESRD (75). A recent meta-analysis of 26 studies involving more than 23,000 subjects has demonstrated that homozygosity for Asp298 and intron 4a alleles of eNOS results in a moderately increased risk of ischemic heart disease (19). Gene polymorphism of other components of the system is beyond the scope of this review.

CLINICAL SIGNS OF ECD

Manifestations of ECD are many and stem from the aberrations in individual functions of the endothelium. Figure 1 depicts the relationships between perturbed endothelial functions and the ensuing clinical manifestations. These include hypertension, macro- and microvasculopathy due to the endothelial lipidosis and atherogenesis, impaired deterrence of inflammatory cells, increased vascular permeability manifesting as microalbuminuria, and impaired angiogenic competence. Each of these manifestations has been extensively reviewed elsewhere (41, 79, 105).

FINAL COMMON PATHWAY: REACTIVE OXYGEN INTERMEDIATES AND REACTIVE NITROGEN INTERMEDIATES LEADING TO ECD, PREMATURE ENDOTHELIAL CELL SENESCENCE, AND APOPTOSIS

Diverse risk factors and pathological processes targeting the vascular endothelium elicit a default response, excessive generation of reactive oxygen intermediates and reactive nitrogen intermediates, the now well-established centerpiece of the response-to-injury hypothesis of atherosclerosis (46, 82). This uniformly present component of pathological reaction modifies endothelial cell functions and leads to cell demise via premature senescence and apoptosis. We have recently demonstrated an increased frequency of prematurely senescent cells in vivo in aortas of young Zucker diabetic fat rats with chronic renal insufficiency, compared with lean controls. NO production by these senescent endothelial cells was decreased in association with the tissue accumulation of nitrotyrosine-modified proteins, a footprint of oxidative and nitrosative stress (12, 24). Development of premature senescence of endothelial cells in vitro could be prevented and reversed by treatments with the

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**Fig. 2.** Summary of homocysteine-induced molecular pathways predisposing cells to development of endothelial dysfunction. The general strategy used in these experiments was to perform cDNA microarray screening of endothelial cells subjected to pathophysiologically relevant concentrations of homocysteine and to confirm selected findings using Northern blot analysis or RT-PCR and Western blot analysis. Whenever possible, the level of expression of such proteins was manipulated pharmacologically to broaden the study of the phenotype of endothelial cells and establish causality. ET-1, endothelin-1; CYP, cytochrome P; PECAM, CD31; NCK, adaptor protein; eNOS, endothelial nitric oxide synthase; HMGCoAR, 3-hydroxy-3-methylglutaryl coenzyme A reductase.

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**Fig. 3.** Summary of some N⁴-nitro-L-arginine methyl ester (L-NAME)-induced molecular pathways predisposing cells to development of endothelial dysfunction. The general strategy of these experiments was the same as described in the legend to Fig. 2. ADMA, asymmetric dimethylarginine.
peroxynitrite scavenger ebselen, the eNOS intermediate \( \dot{N}^2 \)-hydroxy-L-arginine (NOHA), or the SOD mimetic Mn(III)tetraakis(4-benzoic acid) porphyrin chloride. Concomitant with the reversal of senescence, ebselen and NOHA each restored NO production to control levels. Chronic treatment of Zucker diabetic fat rats with ebselen not only prevented, but also partially reversed, ECD (12). These findings indicate that the metabolic syndrome with chronic renal insufficiency in vivo elicits premature senescence of the vascular endothelium. Premature senescence of the vascular endothelium, a consequence of reduced NO availability and peroxynitrite and/or superoxide excess, is presumed to be an important contributor to vasculopathy. These metabolic derangements, together with the oxidized LDL, TNF-\( \alpha \), and peroxynitrite, eventually lead to the increased incidence of apoptosis of endothelial cells, further contributing to accelerated atherosclerosis (reviewed in Ref. 83). The proposed cascade of events along the pathway of endothelial cell involution triggered by different risk factors is depicted in Fig. 4. It predicts that various traditional and nontraditional risk factors, at times accompanied by an acute event, acting on endothelial cells lead to the excessive generation of reactive oxygen intermediates, reactive nitrogen intermediates, and acquisition of a proinflammatory, prothrombotic, and proatherogenic phenotype, eventually affecting cell cycle arrest and premature senescence. The latter is characterized by an overt vasculopathy, which is reversible in Zucker diabetic fat rats. The predominance of apoptotic death of endothelial cells heralds irreversible damage and may be accompanied by vascular rarefaction.

**CLINICAL ASSESSMENT**

The principle of uncertainty is best seen in clinical settings. By detecting the syndrome at preclinical stages, at present a particularly difficult task, there is a chance of reversing it. Detecting the syndrome with certainty through its ominous manifestations may turn out to be too late a stage for any meaningful therapy to halt its progression or reverse it. The “men born in 1914” plethysmographic study with a more than 20-year follow-up provided an early indication of the possible role of ECD in increased mortality (48). However, due to the relative novelty of the syndrome and the lack of reliable noninvasive ways of diagnosing it, clinical assessment of patients remains unsatisfactory. These problems underscore the existing lack of well-established criteria for the diagnosis of ECD. A variety of surrogate markers of ECD have been proposed, including elevated plasma levels of PAI-1, tissue plasminogen activator, and von Willebrand factor. Stehouwer (90) suggests that “estimates of different types of ECD may be obtained indirectly by measuring endothelium-dependent vasodilatation, plasma levels of endothelium-derived regulatory proteins and, possibly, microalbuminuria.” Plethysmographic or ultrasonographic measurements of brachial artery responses to flow or acetylcholine have been advocated as promising markers of ECD, but their sensitivity in detecting coronary artery disease (CAD) was found to be 49% (4). Several studies have already characterized impaired macrovascular blood flow responses in ESRD patients (5, 69). Shamim-Uzzaman et al. (86) have compared brachial artery flow-mediated dilatation with cutaneous microcirculation monitored using laser Doppler flowmetry in patients with CAD and demonstrated no differences in flow-mediated dilatation between CAD and control subjects, whereas there were significant abnormalities in the microcirculatory vasodilatory responses. Our studies of ESRD patients using laser Doppler flowmetry and imaging showed that several parameters characterizing reactive and thermal hyperemia are impaired in a majority of these patients, regardless of the presence of known CAD (91). By interrogating the microvasculature with changing shear stress or temperature, it is possible to unmask the states of vasodilatory failure. The available data imply that microvasculopathy is highly prevalent in this cohort of patients, even when there are no clinical manifestations of CAD.

**PROGRESSION OF ECD**

![Fig. 4. Hypothetical sequence of events and triggering mechanisms in the involution of the vascular endothelium. Note that the vasculopathy associated with premature senescence of endothelial cells is partially reversible in experimental animals. When apoptosis of endothelial cells predominates, however, the process tends to become irreversible and vascular rarefaction ensues. AGEs, glycosylated end products; ECD, endothelial cell dysfunction.](http://ajprenal.physiology.org/)

**LDL/oxLDL**

Hypercholesterolemia

ADMA

Hyperglycemia

Hyperhomocysteinemia

AGE’s

Smoking

Genetic factors

**Vascular endothelium**

Vascular regression

Functional changes in vascular endothelium

Perturbed cell cycle

Superoxide anions

Peroxynitrite

Proinflammatory state

Procoagulant state

Profibrinogenic state

Impaired permeability

Premature senescence

Apolpesis

Noxious stimuli

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Attempts at correcting ECD have been mounting, but their success rate remains low to variable, as will be discussed below. Therapeutic interventions, which appear to be pathophysiologically rational, occasionally turned out to produce some paradoxical results. The main point of the forthcoming discussion will be to demonstrate these unexpected results and their possible explanation on the basis of the coincidental expression of “uncoupled” eNOS, which introduces an aberration into the seemingly rational therapy of ECD. The outcomes appear to be largely dependent on the preexisting status of the vascular endothelium.

Correction of ECD by Supplying eNOS Substrate

Both an absolute l-arginine deficiency or a relative deficiency due to the retention of ADMA may result in eNOS uncoupling. Several investigative groups have demonstrated that l-arginine infusion restored the acetylcholine-induced coronary vasorelaxation in hypercholesterolemic patients who, before l-arginine, responded to acetylcholine with paradoxical vasoconstriction and that l-arginine supplementation improved endothelium-dependent vasodilatation in cholesterol-fed rabbits and patients with hypercholesterolemia (29, 35, 39). In addition, l-arginine appears to ameliorate reperfusion injury after myocardial ischemia, inhibit intimal hyperplasia after balloon catheterization-induced endothelial injury, and attenuate endothelial adhesiveness in hypercholesterolemia (64, 96, 105). On the other hand, a randomized, double-blind, placebo-controlled study of l-arginine supplementation in patients with moderate chronic renal failure was deemed ineffective in improving renal function (29). Failure to improve endothelial function was reported in pediatric and adult patients with chronic renal insufficiency (7, 30). In a group of healthy subjects, systemic infusion of l-arginine was found to induce vasodilation and inhibit platelet aggregation, effects that were in part attributable to the stimulation of insulin release (40). In hypercholesterolemic rabbits, supplementation with l-arginine results in a regression of preexisting lesions. This effect is attributed to the induction of apoptosis of macrophages in atheromas (107). Boger and Bode-Boger (9) provided an excellent review of clinical pharmacology of l-arginine, summarizing diseases in which l-arginine beneficially affected cardiovascular end points (exercise tolerance, anginal symptoms, vasospastic attacks): peripheral arterial disease, CAD, congestive heart failure, hypercholesterolemia, and Raynaud syndrome.

Adverse effects of supplemental l-arginine have been reported as well (22). Loscalzo (65) provided a plausible conceptual framework for the balance between benefits vis-à-vis drawbacks of such a therapy by indicating that only a small fraction of this amino acid is utilized as a NOS substrate, whereas a much larger portion of the l-arginine pool is used in the synthesis of creatine. l-Arginine overload may result, therefore, in the increase in homocysteine formation and methylation stress, which could counterbalance any benefits of providing NOS with the substrate. These consideration should discourage any uncontrolled use of l-arginine in clinical settings.

An intriguing observation that short polymers of l-arginine cross plasma membranes independently of the basic amino acid transporter (which serves to carry arginine monomers) has led to preliminary trials of l-arginine heptamers for correction of ECD (100). It was demonstrated that short polymers of arginine inhibit myointimal hyperplasia. Attempts to inhibit the production or accelerate the catabolism of ADMA have not yet produced pharmacologically useful in vivo inhibitors (101). NOHA, an intermediate in the synthesis of NO and a scavenger of superoxide (13, 93, 96), has attracted attention as a potential therapeutic agent. In the recent studies of premature senescence of endothelial cells, Chen et al. (24) showed the ability of NOHA to prevent premature senescence and reverse it with the potency superior to l-arginine.

Correction of Endothelial Dysfunction Due to the Deficiency of eNOS Cofactors

The redox state of the cell and availability of cofactors represent another therapeutic target. Efficacy of ascorbic acid in CAD, congestive heart failure, and vasospastic angina has been reported, in part mediated by regeneration of reduced forms of glutathione and/or tetrahydrobiopterin (49, 52, 53, 97). Improvement of endothelial function with tetrahydrobioterin was documented in familial hypercholesterolemia, as well as in nitroglycerin-tolerant and insulin-resistant rats (44, 87, 92). A cautionary note has been made that an excessive amount of tetrahydrobioterin may be deleterious as it can redox-cycle and generate superoxide (95). Even lesser certainty surrounds the efficacy of vitamin E as assessed in randomized clinical
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studies; except for the Cambridge Heart Antioxidant Study, which showed reduction of nonfatal myocardial infarction, trials have failed to demonstrate a beneficial effect of vitamin E (98).

The third National Health and Nutrition Examination Survey has provided evidence that 50% of the U.S. population had folate intakes below the recommended dietary allowance, and folate deficiency showed a strong correlation with HHcy, a major risk factor for atherosclerosis (85). Supplementation with folic acid (5–10 mg/day) or its active circulating metabolite, 5-methyltetrahydrofolate, restores impaired endothelium-dependent vasodilation in patients with HHcy or hypercholesterolemia (6, 104). Based on these and other findings (reviewed in Ref. 43), it is predicted that supplementation with folic acid would prevent ~4% of deaths due to CAD, thus recommending the need to revise daily folate requirements. Based on the above findings, fortification of cereals with folate was implemented in 1998, already resulting in a 19% decreased incidence of neural tube defect in the United States, reduction of HHcy, but, so far, no detectable reduction of cardiovascular mortality (69).

Another antioxidant, probucol, has been demonstrated to preserve endothelial function in hypercholesterolemic rabbits, thus giving further credence to the therapeutic strategy of limiting oxidant stress and its consequences in patients with ECD (55). Clinical trials of probucol, however, varied from unsuccessful (13) to efficacious in preventing postangioplasty restenosis (94). Halliwell (45) has provided a comprehensive summary of clinical effects of antioxidants.

Correction of eNOS Function

3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) may exert beneficial effects through reducing the levels of cholesterol and LDL and/or increasing the level of HDL. Liao et al. (62) provided evidence that oxidized LDL decreases eNOS transcription and destabilizes its mRNA. In addition, oxidized LDL may suppress l-arginine uptake by platelets and, by inference, endothelial cells (25). The existing vast literature on atherogenic lipids and endothelial dysfunction has been comprehensively summarized (1). It is also possible that improved levels of HDL may have beneficial effects on endothelial dysfunction (109). It has been appreciated that the effects of 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors on improving endothelial dysfunction transcend their lipid-lowering action (103). Lamas’s group (47) has demonstrated that statins prevent the suppression of eNOS mRNA by oxidized LDL in endothelial cells. Another mechanism whereby statins stimulate eNOS involves activation of protein kinase B/Akt and consequent phosphorylation of eNOS (57). Akt-dependent, shear stress-induced activation of eNOS may also be responsible for the improvement in ECD associated with as simple a maneuver as physical exercise (8). Some of the above-mentioned beneficial effects of statins on ECD could be attributed to their ability to generate and regenerate tetrahydrobiopterin (47) and thus restore the coupled state of eNOS.

Improvement in ECD has been noticed in patients with CAD or congestive heart failure receiving angiotensin-converting enzyme inhibitors (reviewed in Ref. 36). Their acute effects on ECD are due to the inhibition of angiotensin II production with the concomitant reduction in oxidative stress and to the bradykinin-dependent stimulation of eNOS (reviewed in Ref. 10).

Chronic angiotensin-converting enzyme inhibition has been related to the enhanced expression of eNOS (64) and suppression of PAI-1 (102).

17β-Estradiol treatment of hypercholesterolemic rabbits with severe ECD has been found to improve endothelium-dependent vasorelaxation concomitantly with a reduction in the size of atheromas, without altering serum cholesterol level in these animals (34). Similarly, estrogens reduced atherosclerotic lesions in apolipoprotein E-deficient mice (11). One of the potential mechanisms for the observed effects of estrogens has been linked to the estrogen receptor-α-induced activation of eNOS (26). Hisamoto et al. (51) demonstrated activation of Akt and phosphorylation of eNOS mediated via estrogen receptor-α by a nongenomic mechanism. In addition, they showed that activation of eNOS could be mediated by the ERK-dependent cascade, independent of Akt. Clinical trials of estrogens, however, have been terminated. The efficacy of hormone replacement (estrogen and progestin) as well as estrogen replacement therapies to decrease coronary heart disease and incidence of stroke in postmenopausal women “may depend on maintenance of a healthy endothelium,” as discussed in a recent excellent review (58), and their failure to achieve these end points could be attributed to uncoupled eNOS.

Peroxisome proliferator-activated receptor-γ (PPARγ) ligands are emerging as potential therapeutics in ECD. PPARγ is expressed in endothelial cells, and its ligation with 15-deoxy-prostaglandin J2 or ciglitazone has been shown to stimulate NO production (17). Several members of the thiazolidinedione family have been shown to activate eNOS (27), inhibit endothelial-leukocyte interaction (54), and reduce vascular wall inflammation (78). Recently, PPARα agonists (fibrates) have been shown to increase the half-life of eNOS mRNA and the protein abundance (42), although the precise mechanism(s) of this action remains to be established. The previously documented anti-inflammatory action of fibrates and their ability to suppress induction of inducible NO (28) could account for some additional benefits of PPARα agonists via increased bioavailability of NO and reduced formation of peroxynitrite, a product of reaction between NO and superoxide.

Peroxynitrite Scavenging and Prevention of Endothelial Cell Senescence

Recent studies from our laboratory showed that human umbilical vein endothelial cells (HUVEC) cultured on glycolated collagen I or Matrigel lattices develop premature senescence (24). Development of premature senescence of HUVEC on glycolated collagen could be prevented and reversed by treatment with the peroxynitrite scavenger ebselen. Concomitant with the reversal of senescence, a selenoorganic compound, ebselen, restored NO production to levels observed in HUVEC grown on unmodified collagen. Our findings indicate that exposure to glycolated collagen in vitro elicits premature senescence of the vascular endothelium, and ebselen can prevent or reverse it. In vivo testing of these findings is in progress. Chronic treatment of Zucker diabetic fat rats with ebselen prevented and partially reversed ECD (12).

Supplementation with NO Donors

This is by far the oldest therapeutic approach, based on the early observations of the efficacy of nitroglycerin. While this
strategy remains one of the cornerstones of treatment in patients with CAD, development of tolerance hinders its efficacy. Novel S-nitrosothiols have been synthesized, which in vitro are devoid of tolerance induction (68). Several new approaches to deliver NO have emerged. Synthesis of NO-aspirin is reported to combine benefits of both parental ingredients, resulting in the improved inhibition of proinflammatory and prothrombotic pathways (33). Recent data demonstrated prevention of pulmonary thromboembolisms by NO-releasing aspirin (71). The same nitroderivative of aspirin, NCX 4016, has been reported to reduce posts ischemic infarct size and ventricular dysfunction in rabbits and rats (84). A NO-releasing naproxen derivative has been shown to possess antihypertensive properties (74). Delivery of NO by inhalation has been investigated and found to exert vasodilatory actions not only in the pulmonary circulation but also systemically (18). cGMP-dependent effects of NO may be enhanced by slowing the degradation of this second messenger. Therefore, a rational strategy to increase the half-life of cGMP in vascular smooth muscle cells and platelets is related to the inhibition of phosphodiesterase type V. In this vein, dipyridamole has been shown to potentiate vasodilatory and antiaggregation effects of NO (14). Caution is required in administering NO donors: the presence of oxidative stress may diminish their efficacy or even enhance formation of peroxynitrite. Moreover, systemic administration of NO donors will result in generalized effects (as opposed to its endogenous generation), which may be undesirable.

CONCLUSIONS AND FUTURE DIRECTIONS

The data and arguments presented above allow me to formulate a provisional functional definition of the syndrome: ECD is a syndrome induced by diverse intrinsic and extrinsic factors that lead to disturbances in the barrier function of the vascular endothelium; its impaired antithrombogenic properties; perturbed angiogenic competence; inappropriate regulation of vascular smooth muscle tone, proliferative capacity, and migratory properties; and perturbed synthetic functions and deterrence of neutrophils and monocytes from diapedesis. ECD may be reversible within a certain time frame.

The phenotype of endothelial cells exposed to several highly prevalent risk factors in ESRD, such as ADMA, HHCy, and AGE, is definitively proatherogenic. These are cells characterised by 1) decreased production of bioavailable NO; 2) increased adhesiveness for monocytes and polymorphonuclear cells; 3) accumulation of cholesterol and oxidized LDL, both leading to endothelial lipodosis; 4) defective transmission of endothelium-derived hyperpolarizing factor to the smooth muscle cells; 5) enhanced expression of profibrotic genes; and 6) tendency toward premature senescence and apoptosis.

It is quite possible that individual manifestations of vascular dysfunction such as inadequate angiogenesis at ischemic foci, increased adhesion of leukocytes, loss of antithrombogenic properties, inappropriate proliferation of vascular smooth muscle cells, and changed patterns of matrix deposition, each just slightly perturbed, collectively are capable of triggering early preclinical forms of generalized ECD. This would imply that therapeutic intervention cannot be based on a single agent; rather their combination would have improved chances of preventing and reversing ECD. The data linking eNOS function and NO generation or availability to vascular remodeling and signaling, hyperlipidemia, AGE, and hyperglycemia, as presented above, should be viewed within the broader framework of ECD. Accordingly, it is important to learn more about noninvasive techniques to assess the functional state of eNOS, the bioavailability of NO, and expression of other surrogate markers of ECD in the clinical settings. Our pharmacopeia needs to be enriched with agents to 1) correct elevated ADMA levels, 2) elevate homocysteine levels, 3) effectively suppress oxidative stress in the vascular wall, 4) correct increased vascular permeability, 5) improve impaired angiogenesis, and 6) curtail adhesion and diapedesis of monocytes. Future research in this area is needed to define early preclinical markers of ECD. Given accumulating evidence that disturbances of NO production or availability are major determinants of ECD, an intensification of therapeutic efforts toward correction of eNOS activity and NO bioavailability in blood vessels is warranted.

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