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Endothelial cell dysfunction: The syndrome in making

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Endothelial cell dysfunction: The syndrome in making. Endothelial cell dysfunction is emerging as the ultimate culprit for diverse cardiovascular diseases and cardiovascular complications in patients with chronic renal diseases, yet the definition of this new syndrome, its pathophysiology and therapy remain poorly defined. Here, we summarize some molecular mechanisms leading from hyperhomocysteinemia, elevated asymmetric dimethylarginine (ADMA) and advanced glycation end products (AGEs)-modified proteins to atherogenic endothelial phenotype and offer a model of endothelial dysfunction based on the interconnectedness of diverse functions.

TOWARD THE DEFINITION OF ENDOTHELIAL DYSFUNCTION

The term was first used to describe a defect in the removal of 5-hydroxytryptamine and norepinephrine in the pulmonary circulation of bleomycin-treated rabbits [1] and a decrease in angiotensin-converting enzyme (ACE) and plasminogen activator activities after monocrotaline-induced lung injury [2]. The modern usage of the term is associated with the study by Ludmer et al [3], who observed that acetylcholine-induced vasorelaxation is impaired, even reversed, early in the course of coronary atherosclerosis and in recipients of cardiac transplants [4]. A host of studies by other investigative groups has confirmed these observations [5–8].

Vascular endothelium, previously viewed as a mere barrier between intravascular and interstitial compartments, is responsible for the regulation of the hemodynamics, angiogenic vascular remodeling, metabolic, synthetic, anti-inflammatory, and antithrombogenic 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 tonicity, proliferative capacity and migratory properties; perturbed synthetic functions and deterrent of neutrophils and monocytes from diapedesis (Fig. 1). The phenotype of endothelial cells characterized by these abnormalities, expressed to various degrees, is emerging as a hallmark of several highly prevalent cardiovascular and renal diseases, including obesity and diabetes, as well as their complications.

PATHOGENETIC MECHANISMS

Convergence of traditional risk factors, genetic predisposition, local and yet unknown factors acting on endothelial cells all contribute to development of ECD, the "ultimate risk of the risk factors" [9]. In addition to traditional cardiovascular risk factors, a host of complementary mechanisms responsible for the high prevalence of cardiovascular complications in patients with chronic kidney disease has been described. Among the most important factors are elevated asymmetric dimethylarginine (ADMA) levels, hyperhomocysteinemia, 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 a 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, hyperhomocysteinemia and nitric oxide synthase (NOS) inhibitor L-arginine analog N-nitro-L-arginine methyl ester (L-NAME) (to mimic the effect of ADMA). The results of these studies into some cellular consequences of several cDNA screens have been published, thus only a brief summary of the essential observations will be presented [10–16].

Functional analysis and retesting of some microarray screen findings using reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting showed that pathophysiologically relevant concentrations of homocysteine result in a profound increase in 3-hydroxy-3-methylglutaryl coenzymeA (HMG-CoA)

Key words: uncoupled eNOS, oxidative stress, vascular wall, atherogenesis.

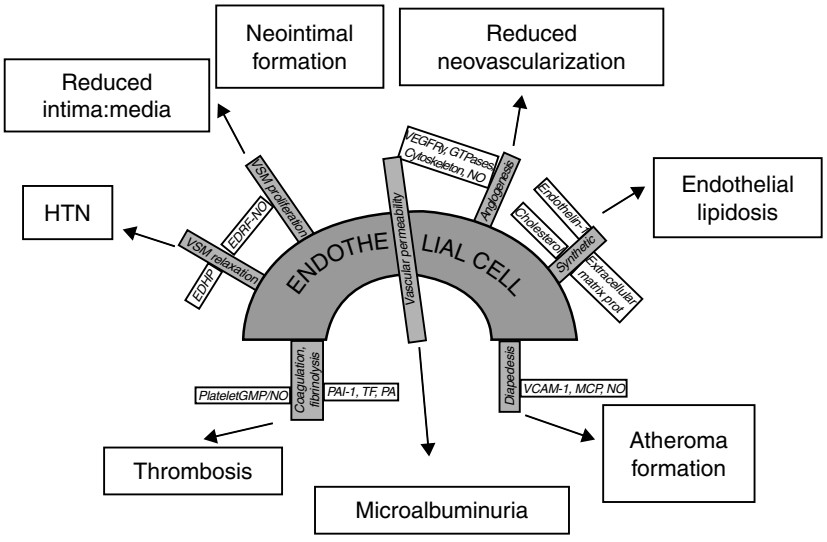

Fig. 1. An "airport hub" model of endothelial functions. In this model all functions are intrinsically interconnected and any impairment of one would affect other functions. Some essential mediators of these functions are shown in brackets. Pathophysiologic consequences of endothelial dysfunction are depicted at the perimeter. HTN is hypertension.

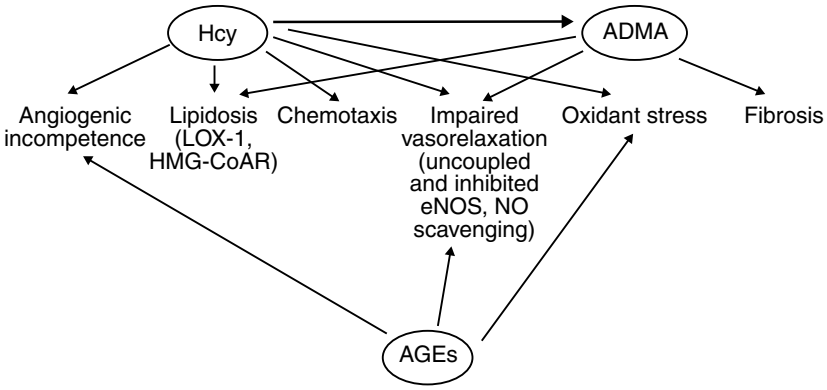

Fig. 2. Synergistic action of homocysteine-, asymmetric dimethylarginine (ADMA)-, and advanced glycation end products (AGEs)-induced molecular pathways predisposing to development of endothelial dysfunction. Abbreviations are: eNOS, endothelial nitric oxide synthase; NO, nitric oxide; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzymeA; Hcy, hyperhomocysteinemia.

reductase, 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) [12]. Up-regulation of connexin-43 expression results in 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 (EDHF) to relax vascular smooth muscle cells (VSMCs) [13]. Together with the uncoupling of eNOS [10] 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 Figure 2.

The functional sequelae of eNOS inhibition (L-NAME mimicking elevated ADMA levels) are presented in Figure 2. Inhibition of nitric oxide synthesis leads not only to the derangement of endothelium-dependent vasorelaxation, but has also proatherogenic sequelae, including enhanced adhesion and transmigration of monocytes and enhanced platelet aggregation. Up-regulation of lectin-like receptor for oxidized low-density lipoprotein (oLDL) (LOX-1), a major receptor for oxidized low-density lipoprotein (oxLDL), results in the increased oxidative stress and lipid accumulation in endothelial cells [15, 16]. Increased synthesis of several chains of collagen and induction of integrin receptors participate in the switch of endothelial cells toward the profibrotic phenotype (unpublished observations). Overexpression of plasminogen activator inhibitor-1 (PAI-1) by endothelial cells presented with advanced glycation end products (AGEs)-modified collagen I is responsible for the early up-regulation 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 [11]. There is a remarkable synergy between these risk factors in inducing oxidative stress, endothelial lipidosis, proinflammatory changes, loss of vasorelaxation, as schematically shown in Figure 2.

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 Asp 298, −786C allele in the promoter and the

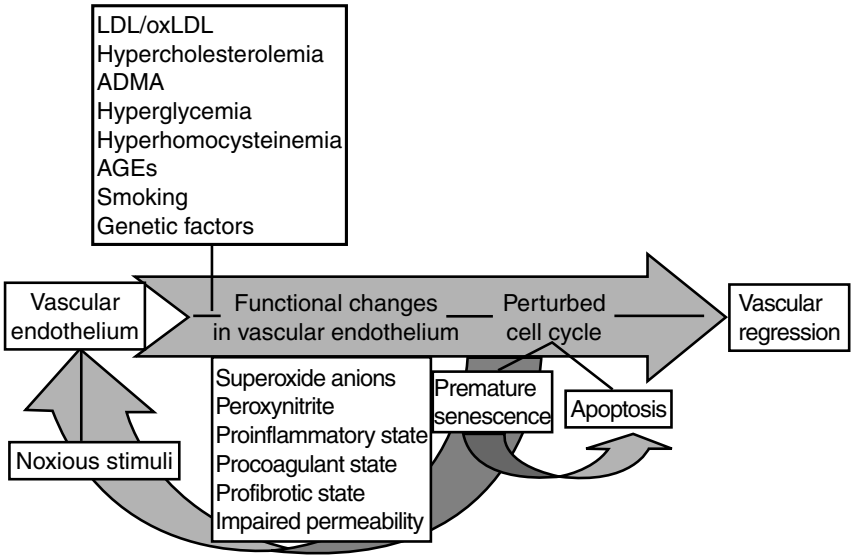

Fig. 3. Hypothetical cartoon of the progression of endothelial cell dysfunction (ECD) to vascular regression. Abbreviations are LDL/oxLDL, low-density lipoprotein/oxidized low-density lipoprotein; ADMA, asymmetric dimethylarginine; AGEs, advanced glycation end products.

intron-4 with the increased risk of cardiovascular complications and its association with ESRD [17]. Recent meta-analysis of 26 studies involving more than 23,000 subjects have demonstrated that homozygosity for Asp298 and intron-4a alleles of eNOS results in a moderately increased risk of ischemic heart disease [18]. 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 relations between perturbed endothelial functions and the ensuing clinical manifestations. These include hypertension, macro- and microvasculopathy due to the endothelial lipidosis and atherogenesis, impaired deterrent of inflammatory cells, increased vascular permeability manifesting as microalbuminuria, and impaired angiogenic competence. Each of these manifestations has been extensively reviewed elsewhere [19–21].

OXIDATIVE STRESS LEADING TO ECD, PREMATURE ENDOTHELIAL CELL SENESCENCE, AND APOPTOSIS

Diverse risk factors and pathologic processes targeting the vascular endothelium elicit a default response—oxidative stress, the now well-established centerpiece of the response-to-injury hypothesis of atherosclerosis [22, 23]. This uniformly present component of pathologic 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 to lean controls. Nitric oxide 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 [24, 25]. Development of premature senescence of endothelial cells in vitro could be prevented and reversed by treatments with the peroxynitrite scavenger, ebselen, eNOS intermediate N^{ω}hydroxy-L-arginine (NOHA) or superoxide dismutase (SOD) mimetic Mn, TBAP. Concomitant with the reversal of senescence, ebselen and NOHA each restored nitric oxide production to control levels. Chronic treatment of Zucker diabetic fat rats with ebselen not only prevented, but also partially reversed ECD [25]. These findings indicate that metabolic syndrome with chronic renal insufficiency in vivo elicit premature senescence of the vascular endothelium. Premature senescence of the vascular endothelium is hypothesized to be an important contributor to the vasculopathy and a consquence of reduced nitric oxide availability, peroxynitrite, and/or superoxide excess. These metabolic derangements, together with the oxLDL, tumor necrosis factor-α (TNF-α), and peroxynitrite eventually lead to the increased incidence of apoptosis of endothelial cells, further contributing to the accelerated atherosclerosis (reviewed in [26]) in the macrovasculature and vascular regression in the microcirculatory bed, as depicted in Figure 3.

CLINICAL ASSESSMENT

By detecting the syndrome of ECD at preclinical stages, at present a particularly difficult task, there is a chance of reversing it. Detecting the syndrome with certainty through its ominous clinical manifestations may turn to be too late a stage for any therapy to

meaningfully halt its progression or reverse it. Thus, the efficacy of therapy appears to be inversely proportional to the precision with which the diagnosis can be established early. A variety of surrogate markers of ECD have been proposed, including elevated plasma levels of PAI-1, tissue plasminogen activator (tPA), and von Willebrand factor. Stehouwer [27] suggests that "estimates of different types of ECD may be obtained indirectly by measuring endothelium-dependent vasodilation, plasma levels of endothelium-derived regulatory proteins and, possibly, microalbuminuria." "Men born in 1914" plethysmographic study with more than 20 years' follow-up provided an early indication of the possible role of ECD in increased mortality [28]. However, due to the lack of reliable noninvasive ways of diagnosing ECD, clinical assessment of the patients remains unsatisfactory. 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 was found to be 49% [29]. Several studies have already characterized impaired macrovascular blood flow responses in ESRD patients [30, 31]. Shamim-Uzzaman et al [32] have compared brachial artery flow-mediated dilatation (FMD) with cutaneous microcirculation monitored using laser Doppler flowmetry in patients with coronary artery disease and demonstrated no differences in FMD between coronary artery disease 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 majority of these patients, regardless of the presence of a known coronary artery disease [33]. This implies that microvasculopathy is highly prevalent in this cohort of patients, even when there are no clinical manifestations of the coronary artery disease. We believe that the noninvasive interrogation of the cutaneous microvasculature responses to thermal stimulation may offer a simple screening test for the presence of systemic ECD [33].

CONCLUSIONS AND FUTURE DIRECTIONS

The phenotype of endothelial cells exposed to several highly prevalent risk factors in ESRD, such as ADMA, hyperhomocysteinemia, and AGEs is definitively proatherogenic. These are cells characterized by (*1*) the decreased production of bioavailable nitric oxide, (*2*) increased adhesiveness for monocytes and polymorphonuclear cells, (*3*) accumulation of cholesterol and oxLDL, both leading to endothelial lipidosis, (*4*) defective transmission of EDHF to the smooth muscle cells, (*5*) enhanced expression of profibrotic genes, and (*6*) a 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 VSMCs, and changed patterns of matrix deposition, each just slightly perturbed, collectively are capable of triggering early preclinical forms of generalized ECD. Accordingly, it is important to learn more about noninvasive techniques to assess the functional state of eNOS, the bioavailability of nitric oxide 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*) elevated 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.

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