Current perspectives

Endothelial dysfunction in chronic heart failure: some new basic mechanisms

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Endothelial dysfunction contributes to the maintenance of peripheral vasoconstriction and abnormal vascular compliance in chronic heart failure. Endothelial dysfunction results in an imbalance between vasodilation and vasoconstriction, particularly when adjustments in blood flow are required. Recently, new factors have been recognized to determine endothelial dysfunction: a) disturbances of the L-arginine/nitric oxide pathway, either at the enzymatic or substrate level; b) increased synthesis of endothelin-1; c) microvessel structural remodeling; d) increased adhesive properties to blood cell components; and e) apoptotic cell injury. The understanding of the complex interplay among these factors is the basis for development of new targeted strategies to correct endothelial dysfunction in chronic heart failure.
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Introduction

Chronic heart failure (CHF) is a complex syndrome in which abnormalities of cardiac, hemodynamic, neurohumoral, immunologic, and peripheral functions take place. Endothelial dysfunction is a newly discovered hallmark of CHF. It contributes to increased baseline peripheral vascular tone and accounts for impaired vasodilation during exercise in patients with CHF. In turn, high peripheral vascular resistance increases cardiac afterload and further impairs cardiac function. Since endothelial dysfunction is important in maintaining symptoms of CHF, pharmacological and rehabilitative treatments aimed at normalizing endothelial function have been developed[1-3].

Endothelial dysfunction has been demonstrated in patients with CHF as reduced vasodilation in response to acetylcholine infusion and reactive hyperemia[4,5]. Both these effects are mediated by endothelium-derived nitric oxide (NO). However in CHF, endothelial dysfunction is characterized not only by impaired vasodilation but also by increased synthesis of endothelial vasoconstricting factors, vascular remodeling, increased adhesive properties, and activated apoptotic processes. Several basic mechanisms accounting for endothelial dysfunction in CHF have been identified (Table I). This review focuses on some of the latest findings in this field.

Vasodilation

During CHF there is an imbalance between vasodilating and vasoconstricting factors. Among vasodilating factors, NO is undoubtedly the most important. This gas radical, which is synthesized from the amino acid L-arginine by the enzyme NO synthase (NOS), causes vasodilation by activating soluble guanylate cyclase which leads to reduced intracellular calcium concentration in smooth muscle cells. Baseline NO is reduced in patients with CHF since infusion of N^G-monomethyl-L-arginine (L-NMMA) – a selective NOS inhibitor – into the brachial artery causes a smaller reduction in basal forearm blood flow compared to control subjects[6,7]. Moreover, stimulated NO is reduced since acetylcholine-induced peripheral vasodilation as well as reactive hyperemia is impaired in CHF[8-11]. Finally, exercise-induced peripheral vasodilation is also impaired in CHF[12]. The reduced NO availability may be due to reduced production or increased degradation of NO. Both these events are likely to occur in CHF. A marked reduction in endothelial NOS (eNOS) was observed in the thoracic aorta and in the skeletal muscle microvasculature of rats and

Table I. Endothelial dysfunction in chronic heart failure: basic mechanisms and (patho)physiological effects.

Causes	Effects
↓NO synthesis	↓ Vasodilation
↓eNOS protein	↓ Vessel distensibility
↓Vasodilator prostaglandins	↓ Flow distribution
↓Bradykinin (↑ACE/kininase activity)	↓ Cardiac function
↓Intracellular L-arginine (?)	↓ Exercise tolerance
↓L-arginine delivery to eNOS (?)	↑ Vasoconstriction
↑ NO degradation	↑ VSMC proliferation
↑ Vasoconstricting prostaglandins	↑ Vessel and heart remodeling
↑ Angiotensin II (↑ACE/kininase activity)	↑ Myocyte hypertrophy
↑ Oxidative stress	↑ Myocyte apoptosis (?)
↑ Endothelin-1	↑ Interstitial fibrosis
↑ Adhesion molecules	↑ Blood cells/endothelium interactions
↑ Apoptosis	↑ Skeletal abnormalities
↑ Arginase activity (?)	
↑ Vascular CNP (?)	

ACE = angiotensin-converting enzyme; CNP = C-type natriuretic peptide; eNOS = endothelial nitric oxide synthase; NO = nitric oxide; VSMC = vascular smooth muscle cells.

dogs with CHF[13-15]. A specific decrease in synthetic activity of the L-arginine-NO metabolic pathway has been demonstrated in patients with CHF, NYHA functional class II-III[16]. Moreover, we have recently reported a reduction in eNOS protein in human endothelial cells cultured with the serum of patients with CHF, NYHA functional class IV[17]. The reduced peripheral endothelial shear stress, secondary to impairment of left ventricular function, and the activated immune system are likely to be involved in the down-regulation of eNOS in CHF. Indeed laminar shear stress is a mechanical stimulus able to turn on eNOS while the cytokine tumor necrosis factor-α (TNF-α) turns it off[17-19]. Reduced eNOS synthetic activity seems to be related also to reduced availability of intracellular L-arginine since oral supplementation of L-arginine increases exercise-induced blood flow in patients with CHF[20,21]. Indeed in the endothelial cell membrane, the existence of a complex between an L-arginine transporter, the cationic amino acid transporter-1, and eNOS[22] raises the possibility, yet to be tested, that L-arginine delivery to eNOS is altered in the endothelium of patients with CHF. Alternatively, induction by inflammatory molecules of arginase, another L-arginine consuming enzyme, can lower the intracellular pool of L-arginine[23]. Also this hypothesis needs to be tested in patients with CHF. Increased degradation of NO is also possible due to increased oxygen free radical formation[24,25]. The radical NO and superoxide actually react to form peroxynitrite, a strong oxidant with only minimal vasodilating activity. In patients with CHF, increased levels of malondialdehyde, a marker of lipid peroxidation induced by oxygen free radicals, are linked to disease severity[26,27]. In addition, there is a negative relationship between exercise-induced malondialdehyde and exercise capacity in CHF, suggesting that exercise intolerance may be related to oxidative stress in this condition[28]. Indirect evidence of increased free oxygen radicals in CHF is provided by the fact that vitamin C improves endothelial dysfunction[29]. In an experimental model of heart failure secondary to myocardial infarction, vascular NADH oxidase was the likely source of vascular superoxide[30]. Such an oxidase is easily activated by angiotensin II and TNF-α, consistently with the activation of both the renin-angiotensin and the cytokine systems in CHF. Finally, a very recent report on eNOS knockout mice suggests that reduced synthesis of NO and increased production of oxygen free radicals are causally linked[31]. This study indeed shows that endothelium-derived NO is a critical determinant for the expression of vascular superoxide dismutase, the major enzymatic cellular defense against oxygen radicals.

Another isoform of NOS, the inducible NOS (iNOS), is activated in CHF. The iNOS is induced by cytokines which are activated in CHF, such as TNF-α. Since iNOS is a high-output isoform, it is potentially cytotoxic. In fact, in patients with CHF, impaired myocardial responsiveness to β-adrenergic stimulation and exercise intolerance are related to iNOS expression in cardiac and skeletal myocytes, respectively[32,33]. However, although there is an indication of induced iNOS in the vascular endothelium and smooth muscle cells of the failing human heart[34,35], a possible role for iNOS in endothelial dysfunction in CHF is unclear.

Vasoconstriction

Endothelin-1 (ET-1) is the most potent endothelium-derived vasoconstrictor[36]. Plasma levels of this peptide are elevated in patients with CHF and are associated with hemodynamic impairment[37-40]. Endothelial cells

increase ET-1 synthesis in response to angiotensin II, noradrenaline and cytokines, these humoral factors being chronically activated in CHF[41]. Although the failing heart is a producer of ET-1, decreased NO release and reduced endothelial shear stress may also contribute to increased ET-1 synthesis as a consequence of reduced peripheral blood flow in CHF[42,43]. ET-1 induced vasoconstriction depends on activation of the smooth muscle dominant ET_A receptors. ET_B receptors can mediate both vasodilation and vasoconstriction according to endothelial or smooth muscle ET_B activation[44,45]. Chronic treatment of CHF rats with BQ-123, an ET_A receptor antagonist, ameliorates pulmonary hypertension and reduces afterload to the left ventricle resulting in improved survival rate[42,46]. Acute and short-term blockade of endogenous ET-1 with bosentan, a mixed ET_A/ET_B nonpeptide antagonist, significantly improves hemodynamics in patients with CHF reducing systemic and pulmonary vascular resistances with a resulting increase in stroke volume and cardiac output[38,47]. In addition, both BQ-123 and phosphoramidon, an endothelin-converting enzyme inhibitor, cause vasodilation in patients with CHF already receiving an angiotensin-converting enzyme inhibitor[48]. Finally, acute infusion of sitaxsentan, a specific ET_A receptor antagonist, causes selective pulmonary versus systemic vasodilation associated with a reduction in plasma ET-1 in patients with moderate to severe CHF receiving conventional therapy[49].

Vascular structure

The endothelium has an important role in the regulation of vascular architecture and chronic alterations of hemodynamic stress can mediate vascular remodeling. This process involves rearrangements of the vascular wall components, i.e. endothelial cells, vascular smooth muscle cells and collagen matrix. The two main endothelial products have striking effects in this setting: NO inhibits mitogenesis and proliferation of vascular smooth muscle cells whereas ET-1 is mitogenic and activates protooncogene expression in vascular smooth muscle cells[50-52]. Chronic inhibition of NO in rats increases wall to lumen ratio and stimulates perivascular fibrosis[53]. The importance of NO in maintaining the vascular architecture is also confirmed by studies on eNOS knockout mice. In these animals the natural remodeling process occurring after reduction in arterial blood flow is absent[53]. In rats with experimentally induced CHF, differential hemodynamic stress induces regional endothelial dysfunction and remodeling (defined as increased medial cross-sectional area, thickness, vascular smooth muscle cell number, elastin, and collagen content) in the pulmonary artery but not in the thoracic aorta[54]. In a renal hypertension-induced rat model of CHF, structural changes in the cardiac microvasculature also occur[55]. In patients with severe CHF, indirect evidence of vascular remodeling, i.e. an abnormal increase in forearm blood flow and vasodilation to nitroprusside, is reported[56]. In the same patients, also direct evidence of structural abnormalities is described such as increased thickness of capillary basement membrane in both cardiac and skeletal muscles and increased hyalinosis in subcutaneous vessels[57-61]. Moreover, a reduction in microvascular density of skeletal muscles is inversely related to peak oxygen consumption, suggesting a possible link with exercise intolerance[62]. These structural abnormalities, as well as dilative function, improve after cardiac transplantation in patients with dilated cardiomyopathy[63].

It is possible that proinflammatory cytokines, angiotensin II and noradrenaline, which are all activated in CHF, contribute to vascular remodeling. These factors indeed stimulate the formation of activated forms of metalloproteases, enzymes involved in the digestion of the vascular extracellular matrix[64,65]. However, the contribution of structural remodeling and decreased distensibility of small vessels to the progression of CHF is still unknown.

Adhesion properties

Increased adhesiveness of the vascular endothelium is another feature of the dysfunctional endothelium in CHF. The phenotypic pattern of endothelial cells is indeed altered in failing hearts and moves toward an increased expression of cell adhesion molecules[66,67]. Adhesion molecules mediate the interaction between leukocytes and endothelial cells and play a key role in the migration of leukocytes through the endothelial layer. The intercellular adhesion molecule (ICAM)-1 is the most sensitive and correlates with the degree of inflammation[66]. The induction of the vascular cell adhesion molecule (VCAM)-1 and selectins, which are not constitutively expressed, also confirm the occurrence of endothelial activation in dilated cardiomyopathy[68]. Such an induction has been related to increased cytokines in CHF. Inflammatory molecules indeed induce the expression of endothelial adhesion molecules both in *in vitro* and *in vivo* experiments. On the contrary, NO selectively reduces endothelial expression of adhesion molecules[69]. Therefore, reduced NO availability occurring in CHF may promote the shift from an anti-adhesive to a pro-adhesive endothelial surface as well. Induction of adhesive molecules is a dynamic process ending with the shedding of the adhesion receptors[70]; in fact, soluble forms of adhesion molecules are increased in the blood of patients with CHF and have a negative prognostic value, suggesting that endothelial activation is relevant to the pathophysiology of CHF[71-73].

Apoptotic processes

Apoptosis is an active gene-directed process of cell suicide which leads to activation of tightly regulated

enzymes (e.g. caspases) and culminates in DNA cleavage and nuclear and cytoplasm shrinkage. Ultimately, the fate of apoptotic bodies is phagocytosis. Apoptosis is a newly discovered determinant of endothelial dysfunction in CHF whereas apoptosis in the myocytes of the failing heart has been an extensively studied topic[74]. Despite the fact that inflammatory cytokines, angiotensin II, catecholamines, bacterial lipopolysaccharides and oxidative stress, all factors activated in CHF[75,76], have adverse effects on both endothelial function and integrity[77-81], only few reports in CHF have been released up to date. Apoptotic endothelial cell damage is evident in the interstitial capillaries of leg skeletal muscles of rats with CHF[82]. In cultured human endothelial cells, the serum of patients with CHF favors apoptosis[17]. Blood levels of the cytokine TNF-α partially accounted for such an effect, once again highlighting the close link between inflammation and endothelial dysfunction in CHF[17]. The possibility that apoptosis contributes to endothelial dysfunction in the peripheral vessels of patients with CHF is interesting but needs further investigation.

Conclusions

The original concept of endothelial dysfunction resulting in reduced NO-dependent vasodilating function in CHF is currently being revised. New aspects of endothelial dysfunction emerge from both animal and human studies, thus leading to new issues to be addressed as well.

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