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Review

Inflammation and endothelial dysfunction as therapeutic targets in patients with heart failure

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Abstract

Evidence suggests that vascular endothelium plays key role in the regulation of vascular tone, in the process of inflammation and in the thrombotic mechanisms. Recent studies indicate that it is an important component of the pathophysiological mechanisms of heart failure. Heart failure may induce endothelial dysfunction by different mechanisms, such as reduced synthesis and release of nitric oxide (NO), increased degradation of NO or by increased production of endothelin-1. In addition, endothelial dysfunction has been associated with the progression of heart failure. Alterations in neurotransmitters, hormones and also in physiological stimuli are present in heart failure and affect the vascular endothelium. Treatments with beneficial effects on endothelial dysfunction may also improve prognosis in patients with heart failure.

Keywords: Heart failure; Endothelium; Nitric oxide; Inflammation; Thrombosis

1. Introduction

Vascular endothelium plays central role in the regulation of vascular tone, haemostasis and inflammation [1]. Endothelial cells produce vasoactive substances such as nitric oxide, endothelium derived hyperpolarizing factor, prostacyclin and endothelin that modulate the activity of the underlying smooth muscle cell layer [2]. Impaired endothelial function is recognised as the first step in the atherogenic process and has also been described in hypertension, diabetes, hypercholesterolaemia and heart failure [3–8].

Congestive heart failure (CHF) is a common syndrome affecting mostly subjects over the age of 65 years [9]. The failing heart is a condition with diverse aetiology [10]. It is a multisystem disorder which is characterised by signs and symptoms related to abnormal contractility function, fluid accumulation, activation of neurohumoral systems, limitation of peripheral circulation and end organ failure [11]. Recent evidence suggests that immune mechanisms and inflammatory processes play significant role in the pathogenesis and progression of heart failure [12–16].

In this article, we reviewed the interaction between heart failure, inflammation and vascular endothelium. We also focused on the therapeutic interventions that target vascular endothelium in patients with heart failure.

2. Endothelial function in heart failure

There is now considerable evidence that there is abnormal endothelial function in both large conduit and small resistance vessels in patients with CHF [3,4,17–19]. Apart from flow-mediated dilatation [20] and invasive plethysmography [21] which are indirect measures of nitric oxide production several endothelial markers such as von Willebrand factor [22] and E selectin [23] have been used as a surrogate for endothelial activation, endothelial dysfunction and endothelial damage or injury in patients with heart failure.

The development of endothelial dysfunction may not be homogenous in different circulatory beds in patients with heart failure, as suggested by Elkayam et al. [24]. This group of investigators showed that unlike the coronary and

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pulmonary circulation there is preserved endothelial function in renal circulation in these patients. It has been suggested that attenuated endothelial function is observed early in the course of heart failure and may lead to decreased organ perfusion, impaired exercise tolerance and the progression of heart failure [25].

The impaired endothelial function observed in patients with heart failure can be explained by different mechanisms. These include reduced nitric oxide (NO) production, increased NO degradation, increased endothelin-1 production and increased apoptosis.

2.1. NO in heart failure

NO is a soluble gas, which has been shown to have important regulatory functions in the cardiovascular system [1]. NO is formed from the *N*-guanino terminal of the amino acid L-arginine and from molecular oxygen by nitric oxide synthase enzymes [5]. One of these enzymes is Ca^{2+}-dependent and is constitutively produced in various types of cells, including endothelial cells (eNOS) [1]. Another type of nitric oxide synthase (iNOS) is Ca^{2+}-independent and inducible by immunological stimuli [2]. Both forms of NO are produced in human heart and regulate myocardial physiology [2,26]. It has been proposed that NO may protect myocytes from deleterious stimuli [27]. However, when large amounts of NO are produced by the iNOS enzyme, these may have cytotoxic effects and inhibit myocyte contractility [27]. NO induced by cytokines also has negative chronotropic effects [28] and is capable of triggering apoptosis [29]. It is, therefore, clear that a distinction between the eNOS and iNOS produced NO is essential to understand the role that NO plays in heart failure.

Circulating cytokines are increased in chronic heart failure and may contribute to reduced synthesis and release of NO [12,13,16]. It has been shown that TNF-α is able to impair the stability of eNOS mRNA and to down regulate eNOS expression [30]. At the same time cytokines and TNF-α in particular, induce iNOS expression [3].

The main physiological stimulator of endothelial nitric oxide production is shear stress induced by the blood flow in the vessels [31]. While the release of NO via the eNOS is decreased in patients with chronic heart failure, the basal production of NO may be increased via iNOS [3]. The reduced expression of eNOS shown in patients with heart failure may well be the result of reduced blood flow. Shear stress regulates eNOS and ultimately NO [32]. Indeed, chronic reduction of blood flow in patients with heart failure results in impaired flow dependent vasodilation, which can be preserved by physical training [33].

These effects in production of NO are compounded by increased degradation of NO as a result of increased activity of angiotensin converting enzyme and increased production of oxygen free radicals present in patients with heart failure. Angiotensin converting enzyme shares structural homology to kinase II, an enzyme that inactivates bradykinin. It has been suggested that bradykinin regulates eNOS and is associated with decreased NO release [25]. Reactive oxygen species on the other hand may have a direct reaction with NO to form peroxynitrite. In this way, reactive oxygen species may induce endothelial dysfunction in patients with heart failure by depleting the bioavailable NO and also by furthering the oxidative injury to the endothelium [34].

2.2. The role of endothelin-1 in heart failure

Endothelins are a group of peptides, which are produced by various cells including endothelial cells [35]. There are four different isoforms of endothelins (ET). The main isoform ET-1 is primarily produced by endothelial cells and its action is mediated via vascular smooth muscle and endothelial receptors [36]. By acting on vascular smooth muscle receptors ET-A and ET-B, ET-1 causes vasoconstriction while the same isoform, by acting on the ET-B receptors of endothelial cells, induces vasorelaxation [37]. Increased levels of endothelin have been associated with endothelial dysfunction and have also been reported in patients with heart failure [18]. Studies also suggest that ET-1 regulates pulmonary vascular resistance and that high ET-1 levels are associated with increased severity of heart failure and also with increased mortality in patients with CHF [38]. However, randomised clinical trials testing the effect of endothelin receptor antagonists in patients with heart failure have been disappointing. Thus, to date, there is no proven clinical efficacy for using endothelin receptor blockers in patients with CHF [39].

2.3. Apoptosis and heart failure

Apoptosis or programmed cell death is the mechanism by which the cell participates in its own death. The evidence so far suggests that an abnormal apoptotic rate may contribute in the pathogenesis of several cardiovascular diseases including congestive heart failure. In cultured endothelial cells, serum of patients with heart failure has been shown to induce apoptosis [30,40]. In addition, the presence of elevated plasma concentrations of apoptotic membrane microparticles in patients with heart failure further supports the involvement of apoptosis in the pathophysiology of heart failure [40]. Recent findings suggest that oxidative stress regulates apoptosis of endothelial cells, and that increased endothelial cell apoptosis correlates with depressed endothelial vasodilator response to acetylcholine [41]. Furthermore, heart failure is associated with elevated circulatory levels of proinflammatory cytokines [13,42] which can stimulate the generation of reactive oxygen species [43]. Thus, proinflammatory cytokines and oxidative stress may contribute to endothelial dysfunction in patients with heart failure by the induction of endothelial cell apoptosis. Although apoptosis may have an important role in heart failure, its evaluation remains controversial and different methods are needed to evaluate this process.

3. Heart failure and inflammation

3.1. Proinflammatory cytokines in heart failure

Preliminary findings showed that patients with heart failure had increased levels of TNF-α compared to healthy controls [13] and are associated with the severity of heart failure [13,44]. Since 1990, many groups have concentrated on understanding how human inflammatory responses, which are thought to protect the body from infections, become overactive in patients with heart failure and produce harmful effects.

It is well documented that activated macrophages are the main source of TNF-α production; however, monocytes, lymphocytes and neutrophils may also release this molecule [45]. The failing heart can also produce TNF-α, which in excessive levels can promote left ventricular remodelling and can have negative inotropic effects [46]. Overproduction of TNF-α may further aggravate the syndrome of heart failure by inducing peripheral deleterious responses [47]. These responses involve skeletal myopathy, endothelial dysfunction and endothelial apoptosis in patients with CHF [48]. In particular, skeletal muscle atrophy, the ultimate result of catabolic processes in CHF, correlates strongly with exercise capacity [49] in patients with heart failure and seems to be the consequence of the production of proapoptotic cytokines such as TNF-α [50].

TNF-α receptors can now be detected and measured as soluble forms. These measurements give accurate estimates of the activity of TNF-α in CHF [51].

Other cytokines implicated in the pathogenesis of CHF are IL-1 and IL-6. Both have been shown to depress myocardial contractility [12,52,53]. IL-6 in particular, has been associated with the progression of heart failure. However, the benefit of using these inflammatory molecules as prognostic markers in CHF remains controversial. Moreover, recent studies using anti-inflammatory components failed to demonstrate improvement in the mortality or reduction of cardiovascular events [54,55].

3.2. Adhesion molecules in heart failure

Adhesion molecules are responsible for interactions between endothelial cells, leukocytes and platelets and they also play an important role in the biologic activity of cytokines by mediating the cell–cell interactions of the immune response [56]. We [57] and others [23,58] have found that plasma adhesion molecules are increased in patients with heart failure, and high levels of soluble vascular cell adhesion molecule-1 and P selectin have been shown to correlate with the severity of heart failure [57,59].

The appreciation of the role of inflammatory mediators in preclinical and clinical heart failure models led to multicenter clinical trials that used targeted approaches to neutralise or depress these inflammatory responses. However, some of these targeted therapeutic approaches resulted in unfavourable results. These results can be partially explained by the increased toxicity of these agents, or by their specificity, which seems inadequate in a disease as complex as heart failure.

4. Therapeutic targeting of endothelium and inflammation in heart failure

As we have already stated, reduced synthesis or increased degradation of NO play central role in the observed endothelial dysfunction in patients with heart failure. Many groups attempted to treat patients with heart failure by targeting this molecule (Table 1).

In this context, Hornig et al. [60] studied the effect of short and long-term vitamin C administration as an alternative for improving endothelial dysfunction in patients with heart failure. The beneficial effect of antioxidants in restoring impaired endothelial function in patients with heart failure has been further confirmed by other groups [61]. Antioxidant treatment in heart failure aims to neutralise the oxygen free radicals, present in large amounts in the circulation. It was interesting to note that in a study performed by Ellis et al. [61] long-term oral administration of vitamin C was associated with improvement of flow mediated dilation in patients with CHF, without a reduction in oxidative stress. Thus, antioxidant vitamins may exert a beneficial action on endothelial function in other ways apart from depletion of reactive oxygen species. Possible actions could be an increase of the availability of tetrahydrobiopterin [62], the cofactor for eNOS enzyme and also interference with intracellular apoptosis signalling [63].

Other groups tried to improve endothelial function in patients with heart failure by targeting the synthesis of NO. It has been shown that infusion of angiotensin converting enzyme inhibitor quinaprilat is followed by improvement of endothelial function in patients with heart failure [64] by decreasing bradykinin degradation [65]. Thus, inactivation of ACE results in bradykinin mediated endothelial production of NO [66], prostacyclin [67] and endothelium derived hyperpolarizing factor [68]. It has been also suggested that the effect of this treatment in restoring endothelial function is

Table 1
Endothelial dysfunction; therapeutic interventions in heart failure

Stimulating NO synthesis	Protecting deactivation of NO
• Statins	• Antioxidants
• L-Arginine	• Hypolipidemic agents
• Antioxidants (±)	• Folic acid/B12/B6
• ACE inhibitors	• Control of diabetes
• Tetrahydrobiopterin	• Smoking cessation
• Estrogens	• Diet
• etc	• Exercise
	• etc

dependent on the stage of heart failure [69]. Patients with mild to moderate heart failure showed different responses when compared to those with severe heart failure and these responses differ in different vascular beds [64,69].

Other mechanism is to increase the availability of NO synthesis with the modification of L-arginine–NO pathway. Oral supplementation and intravenous infusion of L-arginine in several studies improved endothelial function making plausible the rationale of increased production of NO by supplementation of the substrate L-arginine [70]. The beneficial effect of L-arginine was also noted clinically. Oral administration of L-arginine for 6 weeks improved quality of life and prolonged distances during a 6-min walk test [71].

Some studies tried to bypass the impaired endothelium NO mediated vasorelaxation observed in patients with heart failure by inducing relaxation of smooth muscle layer by administration of a direct NO donor, nitroglycerin [72]. Nitroglycerin has been used to stimulate the cGMP which is the second messenger, and to activate the soluble guanylate cyclase in the vascular smooth muscle. The results from these studies have been controversial [73]. Therefore, it is difficult to suggest that nitrates can be used as a replacement therapy for impaired endothelium.

Diuretics and especially potassium sparing diuretics have been used as an addition to standard therapy in patients with moderate to severe heart failure [74]. These medications apart from their other actions showed an increase in forearm blood flow response to acetylcholine [75]. This possibly suggests their involvement in the regulation of NO bioactivity.

4.1. Regular exercise in heart failure

Regular exercise in patients with heart failure can improve skeletal muscle performance, restore endothelial function and reduce apoptotic and proinflammatory markers.

Skeletal muscle bulk loss and changes in the fiber type with preferential synthesis of fast anaerobic myosin heavy chains and skeletal muscle atrophy have been well documented in CHF [76–78]. Recent experimental and clinical investigations have established the beneficial role of exercise training on skeletal muscle function and its metabolic abnormalities [79–81], and evidence now is provided that regular physical exercise improves both basal endothelial NO formation and agonist-mediated endothelium-dependent vasodilation of the skeletal muscle microvasculature in patients with CHF. This last effect has been attributed to enhanced expression of eNOS synthase and decreased production of vasoconstrictor prostanoids and free radicals [82].

Experimental and clinical data have shown that regular exercise can not only improve skeletal muscle performance of patients with CHF [33], but can also restore abnormal endothelial function [83,84]. Cardiac output is increased during exercise and subsequently there is an increase in endothelial shear stress. The increased shear stress is then the stimulus for enhanced NO production [33]. Regular physical training can also exert its beneficial action on the vascular endothelium by modulating peripheral immune responses in patients with heart failure [85]. Different groups have confirmed that regular physical training in CHF reduces cytokines such as TNF-α and adhesion molecules that are known to be elevated in patients with heart failure. In CHF patients undertaking a physical exercise training programme a significant improvement has been found in the levels of soluble apoptotic markers [86].

4.2. Adjunctive treatments in heart failure

In adjunction to regular therapy, other treatments have shown to decrease inflammation and endothelial cell death in experimental models. However, there is still controversy over their clinical benefit in patients with heart failure. These treatments include growth hormone (GH) therapy, micronutritients such as carnitine, and ATII receptor blockers (Table 1).

Experimental data and early clinical reports have suggested that GH as a therapeutic adjunct may benefit patients with chronic heart failure. GH can increase the contractility of the heart, restore or augment ventricular wall mass [87,88], reduce skeletal mass atrophy [89], restore oxidative fiber pattern [89], enhance skeletal muscle strength, restore peripheral vascular endothelial dysfunction [90] and also reduce circulating levels of proinflammatory cytokines and inflammatory markers [91,92]. Despite these beneficial actions of GH, randomised studies have failed to confirm functional improvement in patients with heart failure after GH treatment [93]. Large randomised well-powered placebo controlled clinical trials are needed to elucidate the exact role of GH in patients with heart failure.

L-carnitine is a crucial component of activated fatty acid transport mechanism across the mitochondrial membrane [94]. It is a free radical scavenger, participates in the metabolism of branched chain amino acids and stabilises cellular membranes. In addition, it is likely to have key role in nuclear transcription. Carnitine is released from the ischemic myocardium and its concentration in the coronary sinus is proportional to the concentration of lactate [95]. Systemic carnitine deficiency manifests mainly as dysfunction of skeletal muscles and myocardium. Relative myocardial deficiency in carnitine is observed during heart failure and many experimental data suggest that some negative metabolic and biologic effects of heart failure are alleviated by carnitine or its derivative propionyl-L-carnitine supplementation [96,97]. Although oral propionyl-L-carnitine has been associated with improved exercise tolerance in patients with CHF [98], other studies have failed to show the same benefit [99]. Additional studies are needed to further elucidate the rationale for carnitine supplementation in patients with CHF.

The angiotensin receptor blockers were synthesized to block specifically the attachment of angiotensin II to its receptor. In animal models, administration of angiotensin

receptor blocker has reduced the generation of reactive oxygen species and prevented the impairment of vascular relaxation to acetylcholine [100]. In addition, angiotensin receptor blockers may affect the fibrinolytic system and have been shown to have a beneficial effect on inflammatory markers including TNF-α and soluble vascular cell adhesion molecule-1 [101,102]. Despite the fact that angiotensin receptor blockers appear to have beneficial effect on endothelial function and inflammatory markers, there is not enough evidence to suggest their use in the treatment of patients with CHF. Several ongoing studies will further define their role in the treatment of heart failure [103].

In our institution, we have examined the effects of short term atorvastatin treatment (4 weeks) on endothelial function and inflammatory process in patients with heart failure. We found that administration of atorvastatin, 10 mg daily, improved endothelial function by reducing the expression of proinflammatory cytokines and adhesion molecules [104].

5. Conclusions

In patients with heart failure, impairment of endothelial function has been demonstrated following the enhanced production of cytokines, reduced peripheral blood flow, increased production of oxygen free radicals and increased ACE activity. Endothelial dysfunction contributes to the progression of the disease by increasing the cardiac afterload, deteriorating myocardial ischemia and having a direct detrimental effect on the heart and it has been associated with the severity of heart failure.

The process of inflammation with the involvement of vascular endothelium plays a key role in heart failure and may have important clinical implications. Multiple clinical trials have shown that traditional treatments for heart failure, among their other actions, have also a positive effect on vascular endothelium. At the same time, more specific approaches to reduce inflammation gave contradictory and mostly disappointing results. As our knowledge is expanding on the pathophysiology of this complex disease, new opportunities for medical treatment to improve the outcome and prognosis of this syndrome seem possible. In this effort, further study of the vascular endothelium and its responsiveness in the context of heart failure is warranted. This knowledge widens the area for further research to identify effective drugs in addition to conventional treatment in chronic heart failure.

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