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Review

Endothelial dysfunction in heart failure

Johann Bauersachs, Julian D. Widder

Medizinische Klinik und Poliklinik I, Herz-Kreislaufzentrum, Universitätsklinikum, Julius-Maximilians-Universität Würzburg, Josef Schneider 2, D 97080 Würzburg, Germany

Correspondence: Johann Bauersachs, e-mail: j.bauersachs@medizin.uni-wuerzburg.de

Abstract:
Endothelial dysfunction crucially contributes to the development of impaired coronary and systemic perfusion as well as reduced exercise capacity in patients with congestive heart failure, with fundamental impact on morbidity and mortality. Reduced bioavailability of nitric oxide (NO) and abundant formation of reactive oxygen species (ROS) within the vascular wall are the key determinants in endothelial dysfunction. The disbalance between NO and ROS mainly results from neurohumoral activation associated with heart failure. As endothelial derived NO is a major endogenous modulator of platelet function, reduced intravascular bioactivity of NO contributes to platelet activation, adhesion and thromboembolic events in heart failure. Treatment with angiotensin converting enzyme (ACE) inhibitors, angiotensin and aldosterone antagonists, and statins beneficially modulates endothelial dysfunction in heart failure. All these therapies increase NO bioactivity by either modulation of ROS generation, thereby preventing the interaction of superoxide anions with NO, and/or increasing endothelial NO synthase (eNOS) expression/activity. AVE9488, a novel eNOS transcription enhancer, attenuates cardiac remodeling and endothelial dysfunction in rats after large myocardial infarction. Endothelial progenitor cell (EPC) levels and their mobilization are regulated by eNOS. After myocardial infarction in rats, EPC levels and formation of endothelial colony forming units are markedly reduced. AVE 9488, ACE or HMG-CoA reductase inhibition result in significant increases in EPC levels, and beneficial effects on bone marrow molecular alterations after myocardial infarction.

Key words:
endothelial dysfunction, congestive heart failure, nitric oxide, reactive oxygen species

Introduction

Congestive heart failure is a prevalent disease with a broad impact on society and the quality of life of each individual patient [30]. Despite novel treatment options for patients suffering from heart failure, morbidity and mortality rates are still high. With an annual mortality rate of 10%, it is obvious that novel treatment strategies are still necessary. While the heart as the failing "pumping" organ was an initial focus in research and treatment, neurohumoral activation and subsequently the role of a failing endothelium was recognized and investigated in the recent years. Reduced myocardial perfusion and hence impaired ventricular function are at least in part a consequence of reduced endothelium-dependent vasodilator capacity of coronary arteries. Declined peripheral vasodilation causes higher systemic vascular resistance, and together with stiffness of conductance arteries leads to increased afterload. Elevated pre- and afterload further increase cardiac workload and worsen symptoms. The decreased exercise capability is aggravated by vasomotor dysfunction of the skeletal muscle vessels. These changes have been observed in patients with chronic heart failure [15, 32, 35] and experimental

models of cardiac dysfunction [16, 44]. The severity of endothelial dysfunction can even be used as a prognostic factor for the long term outcome of patients suffering from heart failure. Fischer et al. [21] and Katz et al. [33] nicely demonstrated that flow-mediated dilation of the radial and brachial artery is inversely correlated with mortality in patients with heart failure.

Pathophysiology of endothelial dysfunction

As the inner layer of the blood vessel wall, the endothelium plays the major role in the regulation of vascular homeostasis and is the target for a variety of neurotransmitters, hormones, or physiologic stimuli. Vascular tone is controlled by endothelium-derived autacoids such as nitric oxide (NO), prostacyclin, and the endothelium-derived hyperpolarizing factor. A major contributor to endothelial dysfunction is a reduced NO bioavailability [10]. The most important physiological stimulus for endothelial NO synthase (eNOS) gene expression and NO generation is shear stress [26]. As a consequence of impaired left-ventricular function in severe heart failure and reduced blood flow in conductance and peripheral arteries, less shear stress is exerted on the luminal surface of the endothelium resulting in lower production of endothelium-derived NO and reduced endothelium-dependent dilation. Indeed, in two different models of heart failure induced by ventricular pacing or monocrotaline eNOS expression was reduced [13, 56]. However, data on basal NO generation in ischemic heart failure has been controversial: using the amount of constriction in response to a NOS inhibitor as an indirect measure for basal NO release, some investigators found an increase [16, 25] and speculated that enhanced expression of the inducible (i)NOS in the vasculature may be involved in patients with dilated cardiomyopathy [27]. In contrast, other reports found no difference or even a decrease of basal NO-formation in patients with heart failure [36, 40]. During the early stages of developing heart failure even an exaggerated NO formation resulting in an enhanced endothelium-dependent relaxation may occur [42].

Another major cause of reduced NO bioactivity in the vascular wall results from the fact that reactive oxygen species (ROS) and especially superoxide anions (O_2^-) react rapidly with NO, leading to formation of the strong oxidant peroxynitrite. The rate constant for this reaction between NO and O_2^- has been estimated to be $6.7 \times 10^9\ M^{-1} \times sec^{-1}$ [28]. This is higher than the reaction between O_2^- and the antioxidant enzyme superoxide dismutase which is about $2 \times 10^9\ M^{-1} \times sec^{-1}$ [1]. Hence reduction of bioactive NO may occur despite normal or even an increased NO generation if excessive O_2^- levels are present in the vessel wall [3, 9]. Enhanced vascular release of ROS, predominantly of O_2^-, was detected in several experimental heart failure studies [2–4], and patients suffering from heart failure have elevated levels of plasma lipid peroxides providing evidence of an enhanced oxidative stress under this condition [8, 34].

Sources of ROS

Several enzymes are capable of releasing electrons that can reduce molecular oxygen, including the NAD(P)H oxidase, xanthine oxidase, the mitochondrial electron transport chain and NO synthase. Neurohumoral activation in heart failure leads to increased levels of plasma angiotensin II, one of the major stimuli of the NAD(P)H oxidase. Therefore, an enhanced formation of angiotensin II may increase vascular O_2^- formation through higher expression and activity of NAD(P)H-dependent oxidase in the vascular wall (endothelial cells, adventitial, smooth muscle cells) [24, 46]. Increased NADH-dependent O_2^- generation in aortae from rats with chronic myocardial infarction as well as expression of the NADPH oxidase subunit $p47^{phox}$ suggest that this mechanism may be operative in heart failure [3, 62]. Recently, experimental evidence has been presented that this angiotensin II-induced increase of ROS in the vasculature is at least partially mediated by aldosterone [61]. A major contributor to these local aldosterone-mediated effects of angiotensin II is a tissue specific aldosterone system, which produces and releases aldosterone within human vascular cells independently from the adrenal glands [57]. In addition, local angiotensin II formation in the vascular wall has been reported. The presence of angiotensinogen messenger RNA (mRNA) in the adventitial and medial layers of

the rat aorta has been demonstrated and mRNA levels were increased following vascular injury [11, 22, 47].

Another potential important source for increased ROS production is xanthine oxidase, an enzyme catalyzing the oxidation of xanthine and hypoxanthine during purine metabolism. Intravenous allopurinol decreased myocardial oxygen consumption and increased mechanical efficiency in dogs with pacing-induced heart failure [18]. Expression and activity of xanthine oxidase were enhanced in two different rat models of heart failure [14], while allopurinol improved endothelial dysfunction [19]. Landmesser et al. found that increased xanthine oxidase activity induces vascular oxidative stress in heart failure [37]. On the other hand, a decreased activity of antioxidant enzymes such as superoxide dismutase in heart failure leads to enhanced O_2^- levels [37], and gene transfer of extracellular superoxide dismutase improved endothelial function in rats with heart failure [29].

In addition to the interaction of ROS with NO resulting in the reduction of NO bioactivity, ROS can have deleterious other effects by stimulating hypertrophy, fibrosis and inflammation, e.g. by stimulating MAP kinases. p38 MAP kinase activation mediates apoptosis, activation of inflammatory responses, and cell proliferation [64]. We have shown that p38 MAP kinase is activated in the thoracic aorta of rats with experimental heart failure [62]. p38 MAP kinase activation contributes to aggravation of endothelial dysfunction and vascular ROS production as long-term

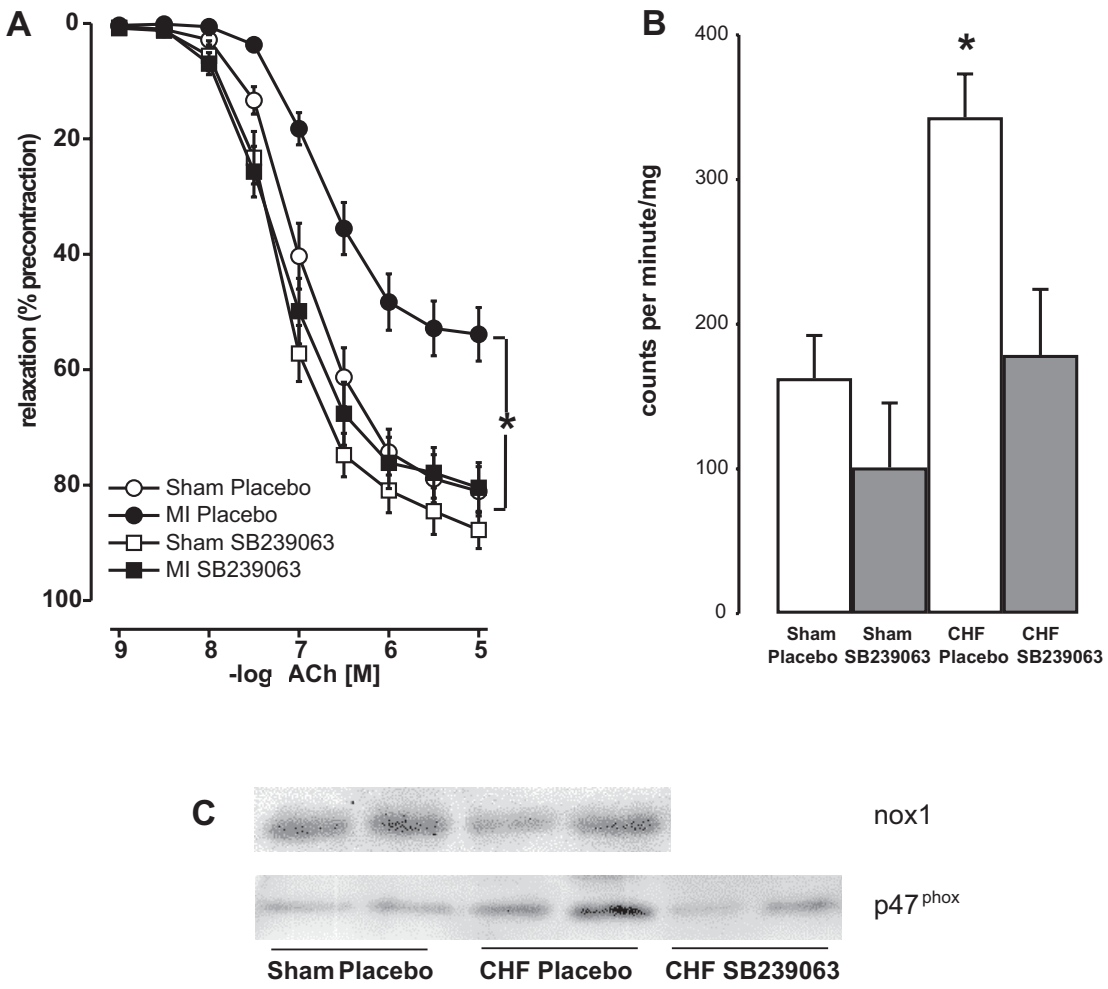

Fig. 1. Long-term treatment with the p38 MAP-kinase inhibitor SB 239063 after experimental myocardial infarction (MI) in rats restores endothelium-dependent relaxation (**A**), normalizes vascular ROS-formation (**B**) and reduces $p47^{phox}$ expression (**C**); * indicates $p < 0.05$ MI Placebo *vs.* Sham Placebo, CHF SB239063 [62]

treatment with a with a specific p38 MAP kinase inhibitor normalized ROS generation, reduced the expression of the NADPH oxidase subunit $p47^{phox}$ and normalized endothelium-dependent relaxation in rats suffering from heart failure (Fig. 1).

Platelets

In addition to the influence on peripheral vascular resistance and coronary blood flow in heart failure a dysfunctional endothelium has a major impact on platelet function [12, 52]. Platelet activation, adhesion, and aggregation are tightly regulated by NO, and reduced NO bioavailability is associated with arterial thrombosis in animal models and in individuals with endothelial dysfunction. In patients with heart failure as well as in animal models platelets are activated which contributes to the increased risk of stroke [23, 38, 50]. Treatment with ACE-inhibitors and aldosterone antagonists positively influenced endothelial function in heart failure, and in combination normalized the phosphorylation state of the vasodilator-stimulated phosphoprotein (VASP) [50]. VASP phosphorylation is a marker for NO bioavailability in platelets and the vascular wall, and is induced by NO-dependent activation of guanylyl cyclase and subsequent cGMP-mediated stimulation of cGMP-dependent kinases. The NO-cGMP pathway is a key regulator of vascular tone, and vascular wall cGK-I activity can be estimated by the analysis of VASP phosphorylation [43, 55]. In platelets the NO/cGMP-dependent phosphorylation of VASP has a profound inhibitory effect on platelet activation. [17]. We have shown that statin treatment in rats with severe heart failure improved endothelial function and normalized increased platelet activation by enhancing NO bioavailability as measured by the phosphorylation state of VASP [53].

The role of the aldosterone pathway

Targeting the renin-angiotensin-aldosterone-system (RAAS) activation by ACE inhibition and aldosterone antagonists belongs to the standard therapy in heart failure leading to increased survival [45]. We showed that the addition of spironolactone or eplerenone to ACE inhibition in rats with experimental heart failure after myocardial infarction improved endothelial vasomotor dysfunction [5, 51]. This was mediated at least in part by enhancing NO bioavailability by decreasing O_2^- generation. Treatment with spironolactone alone prevented the decrease in eNOS in the left ventricle and aorta and improved NO-dependent vasorelaxation [58], while aldosterone infusion caused acute endothelial dysfunction in humans [20]. Aldosterone reduced NO bioavailability in endothelial cells by enhancing NAD(P)H oxidase activity while decreasing the activity of eNOS [7, 41]. Beneficial effects of mineralcorticoid receptor blockade on endothelial dysfunction are not limited to the chronic heart failure situation. Recently we found that mineralcorticoid receptor antagonism with eplerenone alone improved endothelial dysfunction early post myocardial infarction [48]. This was accompanied by decreased ROS generation during eplerenone treatment at least mediated in part through lowered expression of the NADPH subunit $p22^{phox}$. Phosphorylation of eNOS post myocardial infarction was reduced, and normalized by eplerenone treatment (Fig. 2).

eNOS und LV remodeling

eNOS and NO bioavailability do not only modulate endothelial function in heart failure. Left ventricular remodeling is regulated in large parts by eNOS and NO signaling. Mice lacking eNOS display significantly aggravated left ventricular remodeling after myocardial infarction compared to wildtypes [54]. In line, Jones et al. found that mice with transgenic overexpression of eNOS have improved survival and cardiac function after myocardial infarction [31]. Left ventricular function was improved, and hypertrophy was reduced in animals with selective overexpression of eNOS in cardiomyocytes [39]. Given the importance of NO for vascular and cardiac function in heart failure, increasing its generation by enhancing the expression of the producing enzyme eNOS should have benefical effects. The novel eNOS transcription enhancer AVE9488 increased eNOS transcription as well as the production of NO in cultured human endothelial cells. Pretreatment with AVE9488 improved the functional activity of bone marrow mononuclear

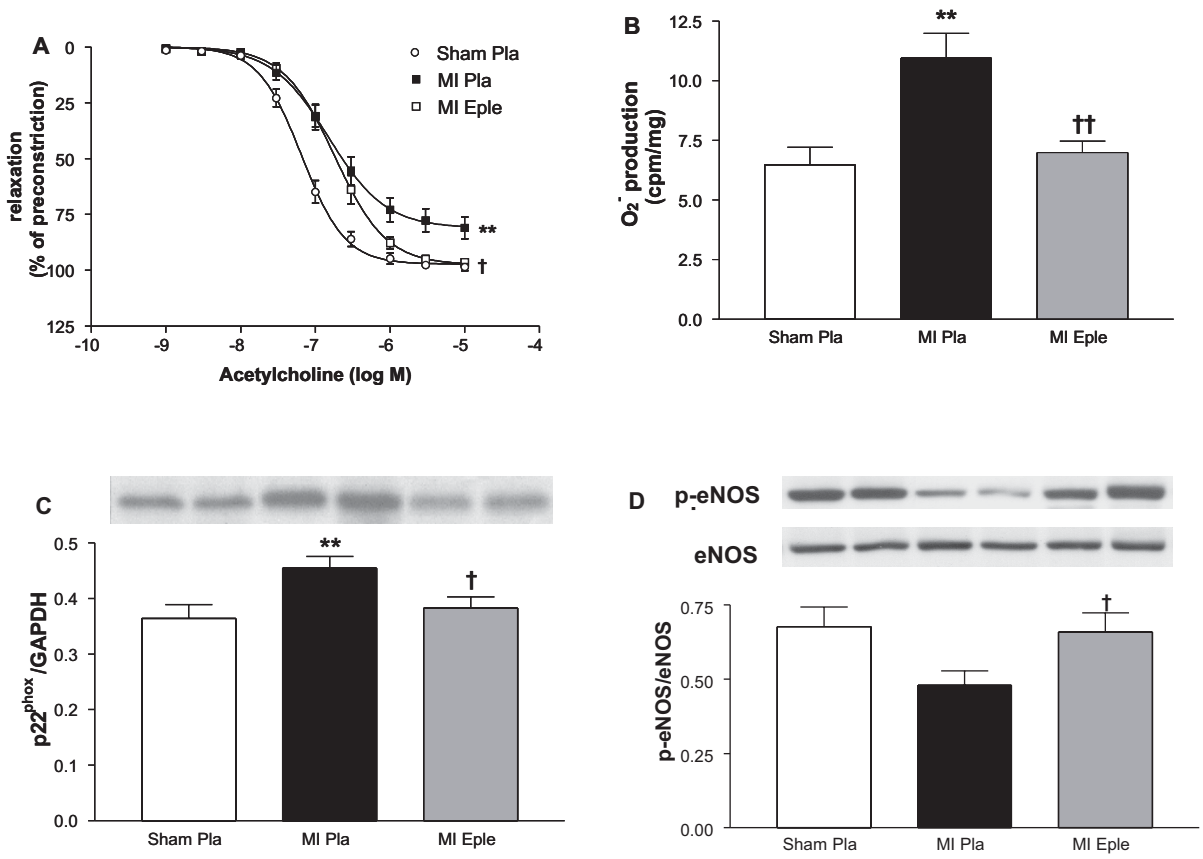

Fig. 2. Treatment with the selective aldosterone antagonist eplerenone (EPLE) improves endothelium-dependent relaxation early post myocardial infarction (MI) (**A**), lowers ROS-generation (**B**) and p22phox expression (**C**), and increases eNOS phosphorylation (**D**); ** indicates $p < 0.01$ *vs.* Sham Placebo (Pla), † indicates $p < 0.05$ *vs.* MI Placebo (Pla), †† indicates $p < 0.01$ *vs.* MI Placebo (Pla) [48]

cells from patients with ischemic cardiomyopathy used for cell therapy of ischemic hind-limb ischemia [49]. In rats with heart failure after myocardial infarction, treatment with the eNOS transcription enhancer AVE9488, former S2431, improved endothelium-dependent relaxation [63]. The elevated ROS production in animals with heart failure was attenuated in the group treated with the eNOS transcription enhancer. Further, AVE9488 treatment versus placebo substantially improved left ventricular function, reduced left ventricular filling pressure, and prevented the rightward shift of the pressure-volume curve [6].

Endothelial progenitor cells

Endothelial dysfunction in heart failure might be in part a consequence of reduced repair mechanisms. Endothelial progenitor cells (EPC) have been identified as potentially important for the regeneration of injured endothelium. We recently found that early after myocardial infarction EPC levels and formation of endothelial colony forming units are reduced [59]. Underlying mechanisms were increased ROS production in the bone marrow and suppressed extracellular signal-regulated kinase phosphorylation and matrix metalloproteinase-9 activity. Enhancing eNOS transcription with AVE9488 resulted in significant increases in EPC levels and improved bone marrow molecular alterations found in rats after myocardial infarction.

Another option to influence NO levels is the application of nitrates, which are powerful NO donors and have been used in the treatment of myocardial ischemia for more than hundred years. We recently demonstrated favorable effects of pentaerythrityl trinitrate, the major metabolite of pentaerythrityl tetranitrate, on EPC function *in vitro*, whereas isosorbide dinitrate in-

duced EPC dysfunction [60]. This was mediated by altered cellular O_2^- production, which was only increased by isosorbide dinitrate but not by pentaerythrityl tetranitrate.

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