Oxidative stress contributes to vascular endothelial dysfunction in heart failure

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Indik, Julia H., Steven Goldman, and Mohamed A. Gaballa. Oxidative stress contributes to vascular endothelial dysfunction in heart failure. Am J Physiol Heart Circ Physiol 281: H1767–H1770, 2001.—Congestive heart failure (HF) is characterized by inadequate nitric oxide (NO) production in the vasculature. Because NO is degraded by oxygen radicals, we hypothesized that NO is degraded faster in HF from inadequate peripheral arterial antioxidant reserves. HF was induced in male Sprague-Dawley rats by left coronary artery ligation. Vascular endothelial function was evaluated by measuring the NO-mediated vasorelaxation response to acetylcholine (ACh; 10^{-9}–10^{-4} M) in excised aortas. This was repeated with the free radical generator pyrogallol (20 μM) and again with pyrogallol and superoxide dismutase (SOD; 60 U/ml). Aortic and myocardial SOD activity was also determined. ACh-induced vasorelaxation was reduced in HF (n = 9) compared with normal control rats (n = 11; P < 0.001). Pyrogallol further reduced vasorelaxation in HF: 74 ± 11% at 10^{-4} M ACh versus 58 ± 10% in normal control rats (P < 0.004). There was a trend (P = 0.06) toward reduced SOD activity in HF aortas. In conclusion, altered NO-dependent vasorelaxation in HF is in part due to excessive degradation of NO and is likely related to reduced vascular SOD activity.

Congestive heart failure; superoxide dismutase; nitric oxide; endothelial function

CONGESTIVE HEART FAILURE IS a state characterized by decreased nitric oxide (NO) release in the vasculature with resultant peripheral vasoconstriction. The NO levels are low, partly because of inadequate production by the endothelial enzyme NO synthase (eNOS). However, oxygen free radicals and antioxidants also affect the degradation and regeneration of NO. NO is degraded by combining with superoxide anion to form peroxynitrite, which is toxic to cells and oxidizes lipids, protein, and DNA (16). Superoxide dismutase (SOD), specifically the extracellular (EC) isoform, scavenges superoxide anion to prevent NO degradation.

Vascular endothelium-bound EC-SOD activity is decreased in patients with coronary artery disease, yet increased in patients with hypercholesterolemia (13). Hyperlipidemia also appears to increase antioxidant activity in the rabbit aorta (10). However, whereas oxidative stress has been inferred to increase in heart failure (2, 3, 12, 18, 24), the specific role of oxidative stress and EC-SOD activity in determining endothelial function in heart failure is still unclear.

This study was designed to investigate the role of oxidative stress on NO-mediated vasorelaxation in large conduit arteries in a rat model of ischemic heart failure. We speculate that the inappropriate peripheral vasoconstriction, which is a hallmark of congestive heart failure, is at least in part due to both decreased production of NO as well as increased degradation. We hypothesize that NO bioavailability is determined by a balance between synthesis and catabolism. The increase in NO catabolism was due to an imbalance between oxygen radicals and antioxidant enzyme reserves in the peripheral vasculature. To test these hypotheses, we performed experiments in the rat coronary artery ligation model of heart failure, in which we measured NO-mediated endothelial vasorelaxation in aortic ring segments in response to acetylcholine (ACh) at baseline and after exposure to the oxygen radical generator pyrogallol and then repeated in the presence of pyrogallol and SOD. These functional results were then compared with biochemical measures of SOD activity in aortic segments and myocardial tissue.

METHODS

Myocardial infarction model. Myocardial infarction (MI) and consequent heart failure was induced by coronary artery ligation using standard techniques (6, 21). In brief, Sprague-Dawley rats weighing 250–300 g were anesthetized with ketamine (50 mg/kg ip) and acepromazine (50 mg/kg ip). A left thoracotomy was performed and the heart was expressed from the thorax. The proximal left coronary artery was ligated and the heart was then returned to the chest.

Rats were maintained on standard rat chow and water ad libitum with acetaminophen (67 mg/l) in drinking water for postoperative analgesia. After 3 wk, rats were anesthetized with methoxyflurane and a 9-lead electrocardiogram was performed for screening purposes. The presence of Q waves (>1 mV) in limb lead I or lead aVL and a sum of the R waves in the precordial leads (<10 mV) correlated well with the presence of large left ventricular (LV) MI. However, MI was definitively determined 3 wk later by the use of hemodynam-

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ics (LV end-diastolic pressure >16 mmHg) and by the presence of a large myocardial scar. Rats with hemodynamic and anatomic evidence for large MI were used in the heart failure group. Normal rats that did not undergo thoracotomy constituted the control group. Pilot aortic ring experiments were performed with sham-operated controls to confirm the suitability of using unoperated normal rats for the control group. All rats were ~4–5 mo old at the time of the study.

Hemodynamics. Rats were anesthetized with inactin (100 mg/kg ip) and constant body temperature was maintained by a specially equipped operating table with a heating pad. After the rats were intubated and placed under a rodent ventilator (Harvard Instruments), a 2-Fr solid-state micro-manometer-tipped catheter with two pressure sensors (Millar) was inserted via the right femoral artery with one sensor located in the LV and the other in the ascending aorta. The zero-pressure baseline was obtained by placing the pressure sensor in 37°C saline before the measurements were taken. A 3-Fr echo-Doppler catheter (Millar Instruments) was introduced via the right carotid artery to measure aortic blood velocity. After a period of stabilization, LV and aortic pressures, heart rate, and aortic blood velocity were recorded and digitized at a rate of 1,000 Hz with the use of a personal computer equipped with an analog-to-digital converter and customized software. The heart was arrested by KCl injection (2 meq/ml) in the LV and then excised, rinsed, and visually examined for infarct size. The aorta was also excised and a portion was used for aortic ring experiments. The remainder was frozen for biochemical studies.

Aortic rings. The measurement of vasorelaxation in an aortic segment has been standard in our laboratory (9). A 3- to 4-mm section of the descending aorta was mounted to a ring apparatus attached to a force transducer. A segment of artery was attached to stainless steel wire stirrups with one wire fixed in place and the other attached to the transducer. Tissue was maintained in Krebs-Henseleit solution bath at a constant temperature (37°C) and bubbled with 95% O2-5% CO2. Rings were stretched to a resting tension of 500 mg and allowed to equilibrate for 45 min. Rings were contracted with continuously infused phenylephrine (3 μM) and reequilibrated. Dose-response relaxation studies were done with increasing doses of ACh (10^{-9}–10^{-4} M). After this experiment, the rings were allowed to equilibrate in the presence of continuously infused phenylephrine and pyrogallol (20 μM), a generator of free radicals. Dose response to ACh was repeated (see above). In a subgroup of aortic rings, a final dose response was done in a bath of continuously infused phenylephrine, pyrogallol, and SOD (60 U/ml). Pilot aortic ring studies were also performed in normal rats and rats with heart failure to confirm that the vasoconstrictive effects of pyrogallol were not likely to be due to toxic irreversible properties. In these studies, the ring was allowed to reequilibrate in normal buffer and was then contracted with phenylephrine alone. An ACh dose response was repeated and showed a return to baseline or near-baseline levels of vasodilation. The presence of an intact endothelium is confirmed by immunohistological staining to factor VIII.

SOD activity. Activity levels of SOD were measured using techniques previously described (11, 17, 20). Tissues were homogenized (1:10) in 50 mmol/l Tris-HCl, pH 8.20, with 1 mmol/l diethylenetriamine pentaacetic acid and were then centrifuged for 20 min at 20,000 g. The supernatant was then assayed for SOD activity by the addition of 300–μg aliquots of protein into a buffer solution containing pyrogallol and catalase. The inhibition of pyrogallol autoxidation was related to absorbance at 420 nm and calibrated to a standard curve with the use of commercially available SOD. SOD activity was measured in both myocardial and aortic tissues. The aortas from three rats were pooled together for each measurement of SOD activity to ensure sufficient protein for analysis.

Statistics. Data are expressed as means ± SE. Two-way analysis of variance was used to assess differences between groups (normal vs. heart failure and baseline vs. pyrogallol). For each rat, the percentage of change in vasorelaxation with pyrogallol compared with baseline was determined and the significance evaluated with Student’s t-test. A paired t-test was used to assess differences in vasorelaxation before and after the addition of SOD. Statistical significance was set at P < 0.05.

RESULTS

Aortic rings. Vasorelaxation in response to escalating doses of ACh (10^{-9}–10^{-4} M) is shown in Fig. 1 for normal rats (n = 11) and rats with heart failure (n = 9). There was a significant impairment in vasorelaxation in response to ACh, P < 0.001, for 10^{-5}–10^{-4} M. Furthermore, pyrogallol, by generating oxygen free radicals, impaired NO-mediated vasorelaxation in both groups of rats (P < 0.001, 10^{-5}–10^{-4} M). Pyrogallol inhibited vasorelaxation to a greater degree in heart failure. Specifically, there was a 74 ± 11% reduction compared with baseline in vasorelaxation in rats with heart failure at 10^{-4} M ACh compared with 58 ± 10% in normal controls (P < 0.004). At 10^{-5} M ACh, there was a 75 ± 12% reduction in heart failure compared with 65 ± 21% in normal rats (P < 0.003). The findings for normal rats were equivalent to those obtained from pilot experiments with sham-operated controls (n = 4, data not shown).

To determine whether the inhibition of NO-mediated vasorelaxation due to oxygen radicals could be reversed, the antioxidant enzyme SOD (60 U/ml) was
added to the pyrogallol bath in a subgroup of aortic rings. The addition of SOD (60 U/ml) in the presence of pyrogallol partially restored vasorelaxation in both groups of rats \((n = 5–6, \text{Figs. 2 and 3})\). There was a significant improvement in vasorelaxation \((P < 0.05)\) for \(10^{-5}–10^{-4}\) M ACh in both groups of rats compared with vasorelaxation in the presence of pyrogallol without SOD. The addition of SOD with pyrogallol restored 53 ± 5% of baseline vasorelaxation in rats with heart failure and 60 ± 5% in normal rats \((P = \text{not significant for } 10^{-4} \text{ M ACh})\). Sham-operated control rats gave similar results. The differences between rats with heart failure and normal rats are likely not statistically significant due to the small sample size in this substudy.

**SOD activity.** We measured SOD activity levels in aortic and myocardial tissues. Myocardial SOD activity was similar in both groups of rats, 4.5 ± 1.0 U/mg protein (heart failure, \(n = 11)\) versus 3.4 ± 0.8 U/mg protein (normal, \(n = 11)\). Similarly, there was no significant difference in SOD activity between both groups but with a trend toward decreased SOD activity in heart failure: 1.6 ± 0.8 U/mg protein (heart failure, \(n = 7)\) versus 4.1 ± 0.9 U/mg protein (normal, \(n = 8, P = 0.06)\).

**DISCUSSION**

Endothelial function in the vasculature is determined by the bioavailability of NO, which promotes vasorelaxation by the activation of guanylate cyclase in smooth muscle cells. The availability of NO is affected by both its rate of production and degradation. Altered NO release has been documented in both patients and animal models of heart failure. In particular, NO release via eNOS, the constitutive NOS, is decreased in rats with heart failure \((4)\). Both basal and \(\beta\)-adrenergic-stimulated release of NO is attenuated after MI in the rat \((7)\). Furthermore, both eNOS protein and ACh-induced vasorelaxation are decreased in the rat hindlimb in heart failure; the transfection of eNOS cDNA corrects protein levels and ACh vasorelaxation \((8)\).

NO availability is also affected by its rate of degradation. The EC isoform of SOD, located between the endothelium and vascular smooth muscle cell layer, is principally responsible for scavenging superoxide anions to prevent the degradation of NO \((22)\). In this report, we have shown that in the rat coronary ligation model of heart failure, endothelial function is further impaired when exposed to oxidative stress. However, endothelial function was improved and vasoconstriction was partially reversed with the addition of SOD. The impaired response to oxidative stress in heart failure may reflect inadequate antioxidant reserves, which is suggested by our finding of a strong trend toward decreased SOD activity in the heart failure aorta.

In normal rats, it has been shown \((15)\) that the deleterious effect of pyrogallol on NO-mediated vasorelaxation in aortic rings can be reversed in part by the administration of carvedilol, presumably due to the antioxidant properties of carvedilol. Vitamin C has also been shown \((5)\) to promote NO-dependent vasorelaxation in aortic rings from normal rats, and furthermore, to preserve vasorelaxation under conditions of oxidative stress.

EC-SOD activity has been measured in patients with coronary artery disease. Landmesser et al. \((13)\) reported that EC-SOD activity was decreased in coronary arteries from patients with coronary artery disease in both segments with and without stenosis. Furthermore, flow-dependent endothelium-mediated dilation of the radial artery was improved by vitamin C administration in patients with heart failure or coronary artery disease but not normal controls \((12, 13)\). In contrast, in hyperlipidemic patients without athero-
sclerotic disease, EC-SOD activity was increased compared with normal controls, suggesting a compensatory response (13).

To repair this imbalance between oxidative stress and antioxidant reserve, gene transfer of SOD to the endothelial layer of large arteries has been successfully accomplished (1, 19). In hyperlipidemic rabbits with induced atherosclerosis, vasorelaxation responses to ACh were not improved after SOD gene transfer, despite decreased endothelial superoxide anion levels (19). Similarly, in stroke-prone hypertensive rats, the transfection of SOD to the endothelial wall of carotid arteries did not improve NO bioavailability (1). It has been suggested that these results are due to the degradation of NO by superoxide anion located in subendothelial layers of the vessel wall. Therefore, it is not enough to scavenge superoxide anion in the vascular endothelium. Rather, SOD must be present within the vascular smooth muscle layer to prevent NO degradation. Our finding that vasorelaxation under oxidative stress was substantially improved with coadministration of SOD suggests that SOD under our experimental conditions probably penetrates through the vessel wall.

It has been suggested that peroxynitrite, formed by the reaction of NO with superoxide anion can initiate lipid peroxidation, including the formation of oxidized low-density lipoprotein (23). Oxidized low-density lipoprotein and other products of lipid peroxidation have in turn been shown to decrease eNOS protein expression in endothelial cells (14), further limiting NO availability. Therefore, oxidant stress may play an even more crucial role by affecting both the degradation of NO and its production by altering eNOS.

In summary, we have shown that oxidative stress has a more pronounced deleterious effect on large artery NO-mediated vasorelaxation in rats with heart failure compared with normal controls. This effect is partially reversed by administration of SOD, suggesting that a systemic state of inadequate antioxidant reserve exists in heart failure.

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