Extracellular Superoxide Dismutase Deficiency Exacerbates Pressure Overload–Induced Left Ventricular Hypertrophy and Dysfunction

Zhongbing Lu, Xin Xu, Xinli Hu, Guangshuo Zhu, Ping Zhang, Elza D. van Deel, Joel P. French, John T. Fassett, Tim D. Oury, Robert J. Bache, Yingjie Chen

Abstract—Extracellular superoxide dismutase (SOD) contributes only a small fraction to total SOD activity in the normal heart but is strategically located to scavenge free radicals in the extracellular compartment. To examine the physiological significance of extracellular SOD in the response of the heart to hemodynamic stress, we studied the effect of extracellular SOD deficiency on transverse aortic constriction (TAC)–induced left ventricular remodeling. Under unstressed conditions extracellular SOD deficiency had no effect on myocardial total SOD activity, the ratio of glutathione:glutathione disulfide, nitrotyrosine content, or superoxide anion production but resulted in small but significant increases in myocardial fibrosis and ventricular mass. In response to TAC for 6 weeks, extracellular SOD-deficient mice developed more severe left ventricular hypertrophy (heart weight increased 2.56-fold in extracellular SOD-deficient mice as compared with 1.99-fold in wild-type mice) and pulmonary congestion (lung weight increased 2.92-fold in extracellular SOD-deficient mice as compared with 1.84-fold in wild-type mice). Extracellular SOD-deficient mice also had more ventricular fibrosis, dilation, and a greater reduction of left ventricular fractional shortening and rate of pressure development after TAC. TAC resulted in greater increases of ventricular collagen I, collagen III, matrix metalloproteinase-2, matrix metalloproteinase-9, nitrotyrosine, and superoxide anion production. TAC also resulted in a greater decrease of the ratio of glutathione:glutathione disulfide in extracellular SOD-deficient mice. The finding that extracellular SOD deficiency had minimal impact on myocardial overall SOD activity but exacerbated TAC induced myocardial oxidative stress, hypertrophy, fibrosis, and dysfunction indicates that the distribution of extracellular SOD in the extracellular space is critically important in protecting the heart against pressure overload. *(Hypertension. 2008;51:1-2.)*

Key Words: extracellular SOD | hypertrophy | congestive heart failure | oxidative stress | ventricular fibrosis | MMP

Congestive heart failure (CHF) because of a variety of conditions is associated with depressed antioxidant reserves and increased products of oxygen free radical reactions, suggesting that oxidative stress might contribute to contractile dysfunction in the failing heart.1 Superoxide dismutase (SOD) is the first line of defense against free radical attack. Three SOD isozymes have been identified, including a copper/zinc-containing SOD (SOD1), which is primarily cytosolic in location, a mitochondrial manganese SOD (SOD2), and an extracellular SOD (SOD3). SOD3 is a glycoprotein secreted into the extracellular fluid by fibroblasts that bind to sulfated polysaccharides, such as heparin and heparan sulfate.2–3 As well as to other matrix components.4–5 As a result, SOD3 binds to the surface of endothelial cells and the extracellular matrix, which has a high abundance of heparan sulfate.6 Several recent studies have demonstrated that SOD3 expression is decreased in the failing heart, and this was associated with endothelial dysfunction.7–9 In addition, patients in whom SOD3 binding to endothelial cells is decreased as the result of substitution of arginine-213 by glycine (R213G) have an increased incidence of hypertension10 and an increased risk of ischemic heart disease,10,11 suggesting that impaired SOD3 binding can increase the vulnerability to cardiovascular disease. However, because SOD3 has a minimal impact on total myocardial SOD activity, it is uncertain whether SOD3 can influence the response of the heart to hemodynamic overload. To address this question, we examined the effect of SOD3 gene deletion...
SOD3−/− on myocardial oxidative stress and the development of left ventricular (LV) hypertrophy and CHF in hearts exposed to systolic overload produced by transverse aortic constriction (TAC). Here we report that SOD3−/− had no effect on LV function or oxidative stress under normal conditions but resulted in evidence of increased oxidative stress in response to TAC, and this was associated with more severe LV dilation and contractile dysfunction, as well as greater myocardial hypertrophy and fibrosis. The findings imply that the specific distribution of SOD3 is important in protecting the overloaded heart.

Methods

Mice and TAC-Induced Systolic Overload

Male C57BL/6 (Taconic, Germantown, NY) and SOD3−/− mice (congenic with the Taconic C57BL/6 strain)3,12 8 to 10 weeks of age were used. This study was approved by the animal care and use committee of the University of Minnesota. The TAC procedure was performed on wild-type (n=19) and SOD3−/− mice (n=25) using the minimally invasive suprasternal approach described by Hu et al.13 Body weight and age-matched wild-type mice (n=12) and SOD3−/− mice (n=8) were used as controls.

Echocardiography and Evaluation of LV Hemodynamics

Mice were anesthetized with 1.5% isoflurane. Echocardiographic images were obtained with a Visualsonics high-resolution Veve 660 system as described previously (n=8 to 13 mice each group).14 For aortic and LV pressure measurement, a 1.2-F pressure catheter (SciSence Inc) was introduced through the right common carotid artery into the ascending aorta and then advanced into the LV for measurement of systolic and end-diastolic pressures and positive and negative LV rate of pressure development (dP/dtmax) as described previously.14

Western Blots, Chemical Analysis, and Histological Analysis

The detailed methods for Western blot, chemical analysis for SOD activity, superoxide anion production, the ratio of glutathione (GSH);glutathione disulfide (GSSG); and thiobarbituric acid reactive substances (TBARS) content are included in the online supplementary data (please see http://hyper.ahajournals.org). Tissue sections (8 μm) from the central portion of the LV were stained with Sirius red (Sigma) for fibrosis and fluorescein isothiocyanate–conjugated wheat germ agglutinin (AF488, Invitrogen) to evaluate myocyte size. For mean myocyte size, the cross-sectional area of ≥120 cells per sample and 4 samples per group was averaged. The percentage of fibrosis was determined as described previously.15

Data and Statistical Analysis

All of the values are expressed as mean±SE. Statistical significance was defined as P<0.05. One-way ANOVA was used to test each variable for differences among the treatment groups with StatView (SAS Institute Inc). If ANOVA demonstrated a significant effect, pairwise posthoc comparisons were made with Fisher’s least significant difference test.

Results

SOD3−/− Exacerbated TAC-Induced Ventricular Hypertrophy and Fibrosis

Under control conditions, ventricular weight and the ratio of ventricular weight:body weight were slightly but significantly increased in SOD3−/− mice as compared with wild-type mice (Figure 1 and Table). Histological staining of LV tissue showed that SOD3−/− mice had a slight but significant increase of ventricular fibrosis and the cardiac myocyte cross-sectional area in comparison with wild-type mice under control conditions (Figure 1). TAC for 6 weeks resulted in a significantly greater increase of ventricular weight and the ratio of ventricular weight:body weight in the SOD3−/− mice.
increase of myocardial matrix metalloproteinase (MMP)-2 wild-type mice (Figure 2A and 2B). In addition, a significant collagen III protein content in the SOD3 in a significantly greater increase of ventricular collagen I and 1D). Consistent with the increased fibrosis, TAC resulted occurred predominantly in a perivascular location (Figure 1B versus the controls (Figure 1A and Table). TAC resulted in a significantly greater increase in the cardiac myocyte cross-sectional area and myocardial fibrosis in the SOD3 mice as compared with the wild-type mice (Figure 1B through1D), indicating that the greater LV hypertrophy in the SOD3 mice in response to TAC resulted from increases of both myocyte size and ventricular fibrosis. The fibrosis after TAC occurred predominantly in a perivascular location (Figure 1B and 1D). Consistent with the increased fibrosis, TAC resulted in a significantly greater increase of ventricular collagen I and collagen III protein content in the SOD3/− than in the wild-type mice (Figure 2A and 2B). In addition, a significant increase of myocardial matrix metalloproteinase (MMP)-2 and MMP-9 protein content was observed in the SOD3/− mice both under control conditions and after TAC (Figure 2A and 2B). Although the mortality rate tended to be higher in the SOD3/− mice in the first week after TAC, this difference was not significant, and total mortality rate during the 6-week period after TAC was not different between SOD3/− (48%) and wild-type mice (47%).

**SOD3/− Exacerbated TAC-Induced LV Dysfunction**

Aortic pressure, LV systolic pressure, and LV dP/dt max were not different between wild-type mice and SOD3/− mice under control conditions. TAC caused significant increases of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-Type Controls</th>
<th>SOD3/− Controls</th>
<th>Wild-Type TAC</th>
<th>SOD3/− TAC</th>
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</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>26.0±0.6</td>
<td>25.7±0.6</td>
<td>26.8±1.4</td>
<td>26.7±0.5</td>
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<tr>
<td>Ventricular mass, mg</td>
<td>102±1.6</td>
<td>108±2.1†</td>
<td>199±7.5*</td>
<td>256±9.2†</td>
</tr>
<tr>
<td>Ratio of ventricular mass:body weight, mg/g</td>
<td>3.92±0.04</td>
<td>4.21±0.05†</td>
<td>7.69±0.68*</td>
<td>9.49±0.47†</td>
</tr>
<tr>
<td>Lung mass, mg</td>
<td>134±1.4</td>
<td>135±5.7</td>
<td>238±45*</td>
<td>406±40†</td>
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<tr>
<td>Ratio of lung mass:body weight, mg/g</td>
<td>5.17±0.11</td>
<td>5.23±0.12</td>
<td>9.6±2.4*</td>
<td>15.4±1.6†</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>542±22</td>
<td>534±9.0</td>
<td>506±23*</td>
<td>503±13†</td>
</tr>
<tr>
<td>LV end-systolic diameter, mm</td>
<td>2.11±0.11</td>
<td>2.35±0.12</td>
<td>4.23±0.24*</td>
<td>5.06±0.13†</td>
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<tr>
<td>LV end-diastolic diameter, mm</td>
<td>3.96±0.11</td>
<td>4.15±0.05</td>
<td>5.08±0.12*</td>
<td>5.74±0.11†</td>
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<tr>
<td>LV fractional shortening, %</td>
<td>71.7±1.7</td>
<td>66.7±2.1</td>
<td>30.6±4.9*</td>
<td>22.4±1.5†</td>
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<tr>
<td>LV ejection fraction, %</td>
<td>84.8±1.6</td>
<td>80.5±1.8</td>
<td>41.4±5.6</td>
<td>31.5±2.0</td>
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<tr>
<td>LV posterior wall thickness at end diastole, mm</td>
<td>0.65±0.01</td>
<td>0.68±0.01</td>
<td>0.89±0.04*</td>
<td>0.88±0.02†</td>
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<td>LV posterior wall thickness at end systole, mm</td>
<td>1.04±0.02</td>
<td>1.10±0.01</td>
<td>1.14±0.05</td>
<td>1.08±0.03</td>
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<td>Mean aortic pressure, mm Hg</td>
<td>74.4±3.8</td>
<td>73.9±3.7</td>
<td>112±6.0*</td>
<td>97.7±5.4†</td>
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<tr>
<td>Systolic LV pressure, mm Hg</td>
<td>96.0±2.1</td>
<td>98.8±3.0</td>
<td>168±10.1*</td>
<td>138±8.1†</td>
</tr>
<tr>
<td>LV end systolic pressure, mm Hg</td>
<td>7.4±1.2</td>
<td>6.5±0.9</td>
<td>35.6±2.2*</td>
<td>42.9±1.8†</td>
</tr>
<tr>
<td>LV dP/dt max, mm Hg/s</td>
<td>8341±451</td>
<td>8230±644</td>
<td>5596±536*</td>
<td>4134±536†</td>
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<tr>
<td>LV dP/dt max, mm Hg/s</td>
<td>−6739±768</td>
<td>−7256±315</td>
<td>−5781±444*</td>
<td>−4176±477†</td>
</tr>
</tbody>
</table>

*P<0.05 vs corresponding control conditions.
†P<0.05 vs wild-type mice.

**Figure 2.** Alterations of myocardial atrial natriuretic peptide (ANP), MMP-2, MMP-9 protein, collagen I, and collagen III in SOD3/− mice (KO) and wild-type mice (Wt) under control conditions and after TAC for 6 weeks. **P<0.05 vs the corresponding control; #P<0.05 vs Wt.**
LV systolic pressure in both wild-type and SOD3−/− mice (Table). Six weeks after TAC, LV peak systolic pressure, LV dP/dt max, and LV dP/dt min were significantly less in SOD3−/− mice as compared with wild-type mice, indicating LV dysfunction (Table). The lower LV systolic pressure in the SOD3−/− mice 6 weeks after TAC could not be ascribed to a lesser initial pressure overload in these mice, because the identical TAC procedure was performed on both groups by the same surgeon randomly on the same days, and the SOD3−/− mice developed more LV hypertrophy after TAC than did the wild-type mice.

Echocardiographic imaging of the heart 6 weeks after TAC demonstrated significant increases of LV end-systolic diameter and LV end-diastolic diameter in both SOD3−/− and wild-type mice in comparison with mice of similar body weight without TAC (Table). However, the degree of LV dilatation, assessed as LV end-diastolic diameter, was significantly greater in SOD3−/− mice than in wild-type mice. TAC caused significant increases in LV end-diastolic wall thickness that were similar in SOD3−/− and wild-type mice (Table); the greater increase in LV mass in the SOD3−/− mice was accounted for by the increased LV chamber diameter in these animals. Systolic dysfunction was more severe in the SOD3−/− mice, as demonstrated by greater decreases of LV systolic shortening fraction and ejection fraction after TAC in the SOD3−/− mice (Table), as well as a greater increase in end-systolic diameter, as compared with the wild-type mice (Table).

TAC resulted in significantly greater increases in lung weight and ratio of lung weight:body weight in SOD3−/− versus wild-type mice (Table), suggesting more pulmonary congestion in the SOD3−/− mice. In addition, SOD3−/− also exacerbated the TAC-induced increase of myocardial atrial natriuretic peptide (Figure 2A and 2B). Taken together, these data indicate that the SOD3−/− mice developed more LV dysfunction in response to the sustained pressure overload produced by TAC.

**SOD3−/− Had No Apparent Effect on Oxidative Stress in Normal Hearts**

As anticipated, SOD3 was undetectable in the SOD3−/− mice (Figure 3), and SOD3−/− did not affect myocardial SOD1 or SOD2 protein content under control conditions (Figure 3).

Total myocardial SOD activity and myocardial superoxide anion content were not different between wild-type and SOD3−/− mice under control conditions (Figure 3), consistent with previous reports that SOD3 contributes only a small fraction to overall myocardial SOD activity. In addition, myocardial TBARS and nitrotyrosine were not different between SOD3−/− mice and wild-type mice under control conditions (Figure 4). Both myocardial GSH and GSSG were decreased in the SOD3−/− mice, but the ratio of GSH:GSSG was not different between wild-type and SOD3−/− mice. Myocardial catalase protein content was also not different between wild-type and SOD3−/− mice (data not shown). These findings indicate that SOD3−/− had no detectable effect on oxidative stress in the normal heart.

**SOD3−/− Exacerbated TAC-Induced Evidence of Myocardial Oxidative Stress**

In comparison with wild-type mice, the ratio of myocardial GSH to GSSG was significantly decreased in SOD3−/− mice 6 weeks after TAC (Figure 4). TAC caused increases of myocardial TBARS and nitrotyrosine content both in wild-type mice and in SOD3−/− mice, but these increases were significantly greater in the SOD3−/− mice than in the wild-type mice (Figure 4). Consistent with a greater increase of oxidative stress in SOD3−/− mice after TAC, TAC significantly increased myocardial superoxide production in the SOD3−/− mice as compared with wild-type mice (in vitro assay; Figure 3). After TAC, myocardial SOD activity was significantly decreased both in wild-type mice and in SOD3−/− mice with no difference between the groups (Figure 3). Myocardial catalase protein content was not different between wild-type and SOD3−/− mice after TAC (data not shown). Taken together, the data indicate a greater degree of myocardial oxidative stress in SOD3−/− mice than in control mice after TAC.

**Discussion**

The major new findings of this study are that SOD3−/− had no detectable effect on ventricular SOD activity, indicators of myocardial oxidative stress, or LV function in the unstressed heart but exacerbated LV oxidative stress, hypertrophy, dilatation, fibrosis, and dysfunction in response to pressure overload produced by TAC. These findings imply that the...
specific distribution of SOD3, rather than its contribution to total SOD activity, is critically important in protecting the heart from hemodynamic overload. To our knowledge, these findings provide the first direct evidence that extracellular SOD exerts a critical role in protecting the heart against pressure overload-induced oxidative stress and contractile dysfunction.

In the present study SOD3−/− had no effect on ventricular SOD activity under control conditions. This is consistent with previous reports that SOD3 contributes minimally to overall SOD activity in the heart.2,16 Although myocardial TBARS and nitrotyrosine content, the ratio of GSH:GSSG, and superoxide anion production were unchanged in the SOD3−/− mice under unstressed conditions, the findings of mild but significant increases of ventricular fibrosis, myocyte hypertrophy, and the ratio of ventricular mass:body weight indicate that the absence of SOD3 did have a modest negative impact on the heart under control conditions. Therefore, the inability to detect increased oxidative stress in the hearts of SOD3−/− mice under control conditions likely indicates that the methods were not sensitive enough to detect a small increase of oxidative stress in the SOD3−/− hearts. The increased myocardial fibrosis in the SOD3−/− mice under control conditions is analogous to previous reports indicating that SOD3 has antifibrotic functions in the lung.5,12 An increase of myocardial fibrosis is often associated with a parallel increase of MMP protein content and activity.2,16 The greater increase of collagen I, collagen III, MMP-2, and MMP-9 in the SOD3−/− hearts after TAC is consistent with the greater degree of myocardial fibrosis in this strain.

Although no previous reports have directly examined the effect of SOD3−/− on systolic overload-induced LV hypertrophy and CHF, there is evidence that abnormalities of SOD3 can contribute to cardiovascular disease. In patients with coronary artery disease, both SOD activity in coronary artery segments and endothelium-bound SOD3 released by bolus injection of heparin were decreased.8,9 The lack of SOD3 exacerbates angiotensin-induced hypertension and vascular oxidative stress and attenuates vascular NO bioavailability.17–19 It is consequently not surprising that SOD3 deficiency would have a role in the development of vascular disease or hypertension. The present findings demonstrate that SOD3 also exerts protective effects when the heart is exposed to systolic overload.

The decrease of the GSH:GSSG ratio and the increases of TBARS, nitrotyrosine, and myocardial superoxide anion production in the SOD3−/− mice exposed to TAC in the present study are consistent with previous reports demonstrating that oxidative stress is increased in the failing heart.1,14,20,21 Thus, in animals with aortic constriction, the development of heart failure was associated with increases of myocardial nitrotyrosine,14,21 TBARS, and superoxide14,21,22 and a decrease of the ratio of GSH:GSSG.22,23 Several sources for increased oxidative stress have been identified in the failing heart, including the mitochondrial respiratory chain,24 uncoupled endothelial NO synthase,14,21 reduced nicotinamide-adenine dinucleotide phosphate oxidase,25 and xanthine oxidase.26 There are several sources of superoxide in the endothelium where SOD3 has its principal site of action. We reported recently that systolic overload produced by TAC in mice caused increased expression of myocardial inducible NO synthase (iNOS) and endothelial NO synthase monomer (a structure that generates superoxide rather than NO), whereas iNOS deletion or selective pharmacological iNOS inhibition with 1400 W decreased markers of oxidative stress and improved LV function, suggesting that either iNOS uncoupling or iNOS-induced endothelial NO synthase uncoupling contributed to the increased oxidative stress and development of CHF in the wild-type mice.2,16 Furthermore, administration of BH4 to prevent NOS uncoupling,14,21 selective inhibition of xanthine oxidase, or reduced nicotinamide-adenine dinucleotide phosphate oxidase has been reported to attenuate oxidative stress and ventricular dysfunction in this model of cardiac overload.

Although there is evidence of increased free radical production in the failing heart, there is also evidence that decreased antioxidant reserves contribute to the increased oxidative stress in several models of myocardial dysfunction. Thus, CHF is associated with decreased SOD3 protein content and activity,8,9,27 and overexpression of SOD3 has been reported to protect the heart against ischemia-reperfusion injury28 and to reduce postinfarct LV remodel-
ing.29 The present study shows that a decrease of SOD3 is not only a consequence of CHF but could also contribute to the development of CHF. The decrease of myocardial SOD activity and SOD1 protein content after TAC is consistent with previous reports in pressure overload–induced heart failure in guinea pigs,20 and myocardial infarct–induced heart failure rats.26 The significant decrease of SOD1 in SOD3−/− mice after TAC may partially contribute to the increased ventricular oxidative stress in SOD3−/− mice after TAC, although the molecular mechanism for the decrease of SOD1 in SOD3−/− mice after TAC is not clear. Oxidative stress has been shown to impair mitochondrial metabolism and contractile function, so it is reasonable that increased oxidative stress could exacerbate the contractile dysfunction in the SOD3−/− mice.

A limitation of the present study is that the effect of SOD3−/− on ventricular structure and function was only studied at baseline and 6 weeks after TAC so that information about changes in kinetics between wild-type and SOD3−/− mice cannot be determined. It should be pointed out that, because SOD3 was knocked out from these mice since conception, the mice have had a lifetime to adapt to the loss of SOD3, which might have allowed them to preserve LV function under basal conditions. Therefore, by using the global SOD3−/− mice, we may underestimate the physiological significance of SOD3 in regulating normal ventricular function.

The finding that SOD3−/− exacerbated TAC-induced LV oxidative stress, hypertrophy, dilation, fibrosis, and contractile dysfunction indicates that SOD3 provides an important protective effect against oxidative stress and contractile dysfunction when the heart is exposed to chronic pressure overload.

Perspectives

SOD3 is strategically located to scavenge free radicals in the extracellular compartment. However, it was not clear whether SOD3 can abrogate oxidative stress or modify ventricular remodeling after pressure overload. The present finding demonstrates for the first time that SOD3−/− exacerbated LV oxidative stress, hypertrophy, dilation, fibrosis, and dysfunction in response to pressure overload produced by TAC, indicating that SOD3 is critically important in protecting the heart from hemodynamic overload. These findings provide the first direct evidence that a reduction of extracellular SOD is not only a consequence of CHF but could also contribute to its development. Therefore, it is anticipated that strategies to decrease extracellular oxidative stress may protect the heart from pressure overload–induced ventricular hypertrophy and CHF.

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Disclosures

None.

References


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