Increased Superoxide and Vascular Dysfunction in 
CuZnSOD-Deficient Mice

Sean P. Didion, Michael J. Ryan, Lisa A. Didion, Pamela E. Fegan, Curt D. Sigmund, Frank M. Faraci

Abstract—Increased superoxide is thought to play a major role in vascular dysfunction in a variety of disease states. Superoxide dismutase (SOD) limits increases in superoxide; however, the functional significance of selected isoforms of SOD within the vessel wall are unknown. We tested the hypothesis that selective loss of CuZnSOD results in increased superoxide and altered vascular responsiveness in CuZnSOD-deficient (CuZnSOD−/−) mice compared with wild-type (CuZnSOD+/+) littermates. Total SOD activity was reduced (P<0.05) by approximately 60% and CuZnSOD protein was absent in aorta from CuZnSOD−/− as compared with wild-type mice. Vascular superoxide levels, measured using lucigenin (5 μmol/L)-enhanced chemiluminescence and hydroethidine (2 μmol/L)-based confocal microscopy, were increased (approximately 2-fold; P<0.05) in CuZnSOD−/− mice as compared with wild-type mice. Relaxation of the carotid artery in response to acetylcholine and authentic nitric oxide was impaired (P<0.05) in CuZnSOD−/− mice. For example, maximal relaxation to acetylcholine (100 μmol/L) was 50±6% and 69±5% in CuZnSOD−/− and wild-type mice, respectively. Contractile responses of the carotid artery were enhanced (P<0.05) in CuZnSOD−/− mice in response to phenylephrine and serotonin, but not to potassium chloride or U46619. In vivo, dilatation of cerebral arterioles (baseline diameter=31±1 μm) to acetylcholine was reduced by approximately 50% in CuZnSOD−/− mice as compared with wild-type mice (P<0.05). These findings provide the first direct insight into the functional importance of CuZnSOD in blood vessels and indicate that this specific isoform of SOD limits increases in superoxide under basal conditions. CuZnSOD-deficiency results in altered responsiveness in both large arteries and microvessels. (Circ Res. 2002;91:938-944.)

Key Words: carotid artery ■ cerebral arterioles ■ genetically altered mice ■ nitric oxide ■ superoxide dismutase 1

The bioactivity of nitric oxide depends, in part, on its interaction with reactive oxygen species, particularly superoxide.1 Although there has been considerable effort to define the role of nitric oxide in the circulation, the role of superoxide within the vessel wall is poorly understood. Because the initial finding of Kontos and Wei that superoxide inactivates nitric oxide in vivo,2 many studies have suggested that inactivation of nitric oxide by superoxide contributes to impaired vascular function.3–7 Indeed, nitric oxide reacts with superoxide at a rate 3 times faster than dismutation of superoxide by superoxide dismutases (SODs).1 Substances that generate superoxide (such as pyrogallol, tetrahydrobiopterin, amyloid β peptide, and xanthine/xanthine oxidase) impair endothelium-dependent relaxation of blood vessels.5,6,8,9 Under these conditions, impairment of endothelium-dependent relaxation is most likely due to interaction of superoxide with nitric oxide and reduction in nitric oxide bioavailability, because superoxide scavengers are effective in protecting vascular function.5,6,8,9 Thus, local vascular levels of superoxide appear to have profound effects on endothelium-dependent relaxation.

Local vascular levels of superoxide reflect both the rate of superoxide formation and the rate of its removal by endogenous antioxidants (primarily SODs). In blood vessels, potential enzymatic sources of superoxide include cyclooxygenase, nitric oxide synthases, lipoxygenase, NAD(P)H oxidase, xanthine oxidase, and mitochondria.10 Much attention has been focused on sources of superoxide in relation to superoxide levels and vascular dysfunction. However, much less is known regarding the functional importance of alteration in expression or activity of SODs within the vascular wall.

SODs exist as three isoforms (each encoded by separate genes) localized within specific cellular compartments. Copper-zinc SOD (CuZnSOD; SOD1) is located predominately within the cytosol, as well as in the nucleus and is thought to be expressed in all mammalian cells. Manganese SOD (MnSOD; SOD2) is targeted to the mitochondrial matrix and is considered to be the primary SOD isoform in relation to oxidative stress in mitochondria. Extracellular-SOD (EC-SOD; SOD3), also a copper-zinc-containing SOD, is secreted extracellularly and is found primarily bound to
heparan sulfate proteoglycan on cell surfaces. Within blood vessels, the predominant isosform of SOD (when expressed as percent of total SOD activity) is CuZnSOD.11–14 For example, in normal mouse aorta, activity of CuZnSOD accounts for 53% to 80% of total SOD activity, MnSOD accounts for 2% to 7% of total vascular SOD, and EC-SOD accounts for the remainder.11–14 A similar pattern of expression exists in human arteries.11 It has been suggested that release of nitric oxide as it diffuses through the vascular wall.11,15,16 EC-SOD activity is thought to be required for protection of nitric oxide from endothelium is dependent on CuZnSOD, whereas EC-SOD activity is thought to be required for protection of nitric oxide as it diffuses through the vascular wall.11,15,16 Although it is known that the three isoforms of SOD are expressed within the vessel wall, the functional importance of each SOD isosform is unclear.

To more directly examine the role of CuZnSOD and to distinguish it from EC-SOD and MnSOD, we tested the hypothesis that loss of CuZnSOD results in increased superoxide levels and impaired vascular function. To test this hypothesis, we used mice that were selectively deficient in CuZnSOD.17 Although, CuZnSOD-deficient mice have been used previously to examine the effects of cerebral and cardiac cell injury response to ischemia/reperfusion,18–20 we are not aware of any studies that have examined superoxide levels and vascular responses. To this end, we measured superoxide levels and responses of carotid arteries and cerebral arterioles in wild-type mice and mice deficient in the expression of the gene for CuZnSOD.

Materials and Methods

Experimental Animals

Mice used for this study were derived from breeding pairs of heterozygous CuZnSOD-deficient (B6;129S-SOD1tm1Leb) mice obtained from Jackson Laboratory (Bar Harbor, Maine). Two groups of mice were studied: homozygous CuZnSOD-deficient (CuZnSOD−/−) mice and wild-type (CuZnSOD+/+) littermates. All breeding and genotyping was performed in a virus- and pathogen-free barrier-facility at the University of Iowa. The genotype of each mouse was ascertained by polymerase chain reaction of DNA isolated from tail biopsy samples as described on the Jackson Laboratory Web site.21 All experimental protocols were approved by the University of Iowa Animal Care and Use Committee.

SOD Activity

Total SOD activity of aortic homogenates from wild-type and CuZnSOD−/− mice was determined as previously described.22 This assay is based on the competition of SOD and nitroblue-tetrazolium (NBT) for superoxide, thus the percent inhibition of NBT reduction is a measure of the amount of SOD present. The rate of NBT reduction to blue formazan was measured spectrophotometrically for 5 minutes at 560 nm. One unit of SOD activity is defined as the amount of protein that results in 50% inhibition of NBT reduction. SOD activity is expressed as U/mg protein. Total protein content was determined using a Bio-Rad protein assay kit.

Western Blotting for CuZnSOD

CuZnSOD protein expression in aorta of wild-type and CuZnSOD−/− mice was examined by Western blotting as described previously.23,24 Aorta (pool of 3) from wild-type and CuZnSOD−/− mice was homogenized in extraction buffer (1% SDS, 10 mmol/L EDTA in water) and boiled, the protein extract being collected as supernatant after centrifugation. Protein concentrations were determined using a Bio-Rad protein assay kit, and then adjusted to 3 mg/mL with 4 × Laemmli buffer. Protein samples were boiled and 75 μg of protein was loaded onto 4% to 20% gradient Tris-HCl gradient gel (BioRad) and electrophoresed. Proteins were blotted to a nitrocellulose membrane using a semi-wet cell. Proteins were blocked with PBS+0.05% Tween and 5% nonfat milk and then incubated with sheep polyclonal IgG Anti-CuZnSOD primary antibody (2 μL/mL; Upstate Biotechnology) at 4°C overnight. The membranes were washed with PBS+0.05% Tween and 5% nonfat milk and incubated for 1 hour with rabbit anti-sheep IgG-peroxidase-conjugated secondary antibody at a final concentration of 1:200 000 ( Pierce). The membranes were developed with Supersignal (Pierce) for 1 to 3 minutes, exposed to x-ray film, and developed.

Measurement of Superoxide

Superoxide levels were measured using two approaches. First, basal superoxide levels were measured in aorta using lucigenin (5 μmol/L)-enhanced chemiluminescence as described previously.3,4,25 In some experiments, vessel segments were preincubated for 30 minutes with either 4,5-dihydroxy-1,3-benzene disulfonic acid (Tiron; 10 mmol/L) or polyethylene-glycol-SOD (PEG-SOD) to quench the superoxide signal. Second, superoxide levels were also evaluated in carotid artery using hydroethidine (2 μmol/L)-based confocal microscopy as described previously.3,26–28 Relative increases in ethidium bromide fluorescence were determined using SCION Image software for the PC (version 4.02; Scion Corporation) as previously described.3 Ethidium bromide fluorescence was normalized to the cross-sectional area of the vessel wall for each section.

Vascular Studies

Rings of carotid artery were studied in individual organ chambers as previously described.3,29–31 After equilibration, vessels were contracted submaximally (50% to 60% of maximum) with the thromboxane analogue 9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin F₂α (U46619). After reaching a stable contraction plateau, concentration-response curves were generated for the endothelium-dependent dilator acetylcholine (10 nmol/L to 100 μmol/L), for authentic nitric oxide (0.1 to 10 μmol/L), and for the non–nitric oxide dilator papaverine (0.1 to 100 μmol/L). We have shown previously using pharmacological and genetic approaches that responses of the carotid artery to acetylcholine are mediated by eNOS and nitric oxide.30,31 Because contractile response to vasoconstrictors may be modulated by nitric oxide,32–35 and because mice deficient in the gene that encodes for eNOS demonstrate enhanced sensitivity to serotonin,36 we examined contractile responses in wild-type and CuZnSOD−/− mice. Contractile responses to phenylephrine (10 nmol/L to 100 μmol/L), serotonin (10 nmol/L to 10 μmol/L), and potassium chloride (1 mmol/L to 100 mmol/L) were measured. At the end of each experiment, a concentration-response curve to U46619 (0.03 to 3.0 μg/mL) was generated in order to determine the maximal contractile response of each vessel.

Studies of Cerebral Arterioles

Wild-type and CuZnSOD−/− mice were anesthetized with pentobarbital sodium (75 to 90 mg/kg IP), supplemented regularly at approximately 20 mg/kg per hour. Animals were ventilated mechanically, and arterial blood pressure and blood gasses were monitored continuously.36–37 A cranial window was made over the left parietal cortex, and a segment of a randomly selected pial arteriole per animal was measured under control conditions and during topical application (cumulative administration) of acetylcholine (1 and 10 μmol/L) and nitroprusside (0.1 and 1 μmol/L). Arterial blood gasses were monitored and were similar in the two groups [wild type, PCO₂ = 36±2 mm Hg, PO₂ = 141±16 mm Hg, and pH = 7.30±0.02; CuZnSOD−/−, PCO₂ = 33±2 mm Hg, PO₂ = 116±11 mm Hg, and pH = 7.36±0.02 (mean±SE)].
Blood Pressure Measurements

Because some forms of hypertension have been associated with increases in vascular superoxide and because we hypothesized that vascular superoxide levels would be increased in CuZnSOD-deficient mice. We measured blood pressure in wild-type and CuZnSOD−/− mice using an automated tail-cuff device (BP-2000, Visitech Systems). Because the tail-cuff method typically underestimates blood pressure, we also measured arterial pressure directly through the use of indwelling carotid catheters in freely moving mice as described previously. Blood pressure measurements using both methods were performed in conscious mice because anesthesia in mice lowers blood pressure and tends to normalize differences in blood pressure as compared with the conscious state.

Drugs

Acetylcholine, papaverine, PEG-SOD, phenylephrine, serotonin, and Tiron were obtained from Sigma, and all were dissolved in saline. U46619 was obtained from Cayman Chemical and dissolved in 100% ethanol with subsequent dilution being made with saline. Hydroethidine was obtained from Molecular Probes and dissolved in DMSO at a concentration of 0.1 mol/L. Authentic nitric oxide was prepared as previously described. All other reagents were of standard laboratory grade.

Statistical Analysis

All data are expressed as mean±SE. Relaxation to acetylcholine, nitric oxide, and papaverine is expressed as a percent relaxation to U46619-induced contraction. Comparisons of relaxation and contraction were made using analysis of variance followed by Bonferroni’s multiple comparison test. Comparison of blood pressure, SOD activity, and superoxide levels was made using paired t test. Statistical significance was accepted at P<0.05.

Results

Blood Pressure and Baseline Characteristics

Systolic blood pressure in conscious CuZnSOD−/− mice (102±4 mm Hg; n=6), as measured using tail-cuff, was lower (P<0.05) as compared with that in wild-type mice (115±2 mm Hg; n=6). Measurements using carotid catheters confirmed tail-cuff measurements, in that blood pressure was lower (P<0.05) in CuZnSOD−/− (114±3 mm Hg; n=4) as compared with wild-type littermates (134±5 mm Hg; n=5). Wild-type and CuZnSOD−/− mice were of similar (P>0.05) age (5±1 and 5±1 months, respectively) and body weight (28±3 and 33±3 g, respectively).

SOD Activity

In wild-type mice, total aortic SOD activity was 155±22 U/mg, which is consistent to that reported previously in mouse aorta. Total aortic SOD activity was reduced (P<0.05) in CuZnSOD−/− mice by approximately 60% compared with activity levels in wild-type mice (Figure 1A). Western blotting confirmed that CuZnSOD protein was absent in vascular tissue in CuZnSOD−/− mice (Figure 1B). These results indicate that loss of CuZnSOD through gene targeting results in an absence of CuZnSOD protein and a large reduction in total SOD activity in vessels, consistent with previous findings of CuZnSOD protein and activity levels in other tissues of CuZnSOD-deficient mice.

Superoxide Levels

To determine whether loss of CuZnSOD is associated with increases in superoxide, basal superoxide levels were measured in wild-type and CuZnSOD−/− mice. Basal superoxide levels, as measured using lucigenin (5 μmol/L)-enhanced chemiluminescence, were approximately 2-fold higher (P<0.05) in aorta from CuZnSOD−/− mice compared with levels in aorta of wild-type mice (Figure 1C). Preincubation of aorta with Tiron (10 mmol/L; data not shown) or PEG-SOD (300 U/mL) markedly reduced the lucigenin signal in both groups.

Consistent with results obtained with lucigenin, superoxide levels, as measured using hydroethidine, were higher in carotid artery of CuZnSOD−/− mice compared with wild type (Figure 2A). Quantification of ethidium bromide signal (based on relative difference in fluorescent intensity), revealed higher (P<0.05) fluorescence in carotid artery of CuZnSOD−/− mice as compared with wild-type CuZnSOD−/+ mice (Figure 2B).

Vascular Responses of Carotid Artery

Acetylcholine (Figures 3 and 4) and authentic nitric oxide (Figure 4) produced concentration-dependent relaxation in carotid artery from wild-type mice. In contrast, relaxation to acetylcholine (Figures 3 and 4) and nitric oxide (Figure 4) was impaired (P<0.05) in carotid artery from CuZnSOD−/− mice compared with wild type. For example, maximal relaxation in response to acetylcholine was 50±6% and 69±5% in CuZnSOD−/− and wild-type mice, respectively. To determine if these changes were selective, we examined relaxation responses to the non-endothelium-dependent, non-nitric oxide agonist, papaverine. Papaverine produced relaxation that was impaired (P<0.05) in carotid artery from CuZnSOD−/− mice compared with wild-type mice.
was similar \((P>0.05)\) in wild-type and CuZnSOD \(-/-\) mice (Figure 4), indicating that the impaired response to acetylcholine and nitric oxide observed in CuZnSOD \(-/-\) mice was selective.

Phenylephrine and serotonin produced concentration-dependent contraction in carotid artery from wild-type mice (Figures 5 and 6). Contractile responses to phenylephrine and serotonin were enhanced \((P<0.05)\) in carotid arteries of CuZnSOD \(-/-\) mice (Figures 5 and 6). Potassium chloride (Figure 6) and U46619 (data not shown) produced similar \((P<0.05)\) levels of contraction in CuZnSOD \(-/-\) and wild-type mice, suggesting that the enhanced response to phenylephrine and serotonin was selective.

**Effects of CuZnSOD-Deficiency on Dilator Responses of Cerebral Arterioles**

Baseline diameter of cerebral arterioles was similar \((P>0.05)\) in CuZnSOD \(-/-\) \((31\pm1\mu m; n=9)\) and wild-type \((33\pm2\mu m; n=9)\) mice under control conditions. Mean arterial pressure (in anesthetized mice) was similar in both groups \((76\pm4\mathrm{mm}\mathrm{Hg} \text{ and } 77\pm4\mathrm{mm}\mathrm{Hg} \text{ in CuZnSOD \(-/-\) and wild-type mice, respectively})\) under control conditions and was not affected by application of agonists into the cranial window.

Acetylcholine and nitroprusside produced concentration-dependent dilatation of cerebral arterioles (Figure 7). Dilatation of cerebral arterioles after topical application of acetylcholine was reduced by about 50% \((P<0.05)\) in CuZnSOD \(-/-\) mice compared with wild type (Figure 7). In contrast, dilatation of cerebral arterioles in response to nitroprusside was similar \((P>0.05)\) in CuZnSOD \(-/-\) and wild-type mice (Figure 7). These findings provide direct evidence that...
CuZnSOD deficiency impaired endothelial function in the microcirculation of a key organ in vivo.

**Discussion**

There are several major new findings of the present study. First, CuZnSOD protein is absent and total SOD activity is markedly reduced in aorta from CuZnSOD−/− mice as compared with wild-type mice. Second, basal superoxide levels in aorta and carotid artery, as measured using two independent methods of superoxide detection, were significantly higher in CuZnSOD−/− mice under control conditions. These findings provide direct evidence that endogenous CuZnSOD limits increases in superoxide within the vessel wall under basal conditions. Third, in addition to increases in superoxide, relaxation of carotid arteries to acetylcysteine and authentic nitric oxide was significantly impaired in CuZnSOD−/− mice as compared with wild-type mice. Fourth, contractile responses to phenylephrine and serotonin were augmented selectively in carotid artery of CuZnSOD−/− mice. Taken together, these findings suggest that increases in basal superoxide in blood vessels due to deficiency in CuZnSOD produces impaired nitric oxide–mediated relaxation and enhanced contraction.

**Selective Deficiency in CuZnSOD Decreases Total SOD Activity and Increases Superoxide Levels in Blood Vessels**

It has been shown previously that deletion of the gene encoding CuZnSOD significantly reduces CuZnSOD protein and activity levels in nonvascular tissue from CuZnSOD-deficient mice. In the present study, we demonstrate for the first time that CuZnSOD protein is absent and total SOD activity is greatly reduced in aorta from CuZnSOD−/− mice as compared with wild-type littermates. The percent reduction in total SOD activity (approximately 60%) in CuZnSOD−/− mice is entirely consistent with what would be expected based on previous measurements, which indicated that 50% to 80% of total SOD activity in blood vessels is due to activity of CuZnSOD.11–14

Consistent with our hypothesis, selective loss of CuZnSOD resulted in increased levels of superoxide in blood vessels. Superoxide was increased approximately 2-fold in both aorta and carotid artery in CuZnSOD−/− mice, as measured using lucigenin-enhanced chemiluminescence and hydroethidine-based confocal microscopy. For both methods, the finding that the superoxide signal was markedly reduced by scavengers of superoxide suggests the assays are selective for superoxide. The present findings are consistent with previous reports, in which pharmacological inhibition of SOD activity with diethyldithiocarbamate (DDC), an inhibitor of copper-containing SODs (CuZnSOD and EC-SOD) resulted in increased superoxide in vessels.19,42

**Deficiency in CuZnSOD Alters Vascular Responses**

Increased superoxide is thought to play a major role in vascular dysfunction (including endothelial dysfunction) in many disease states including atherosclerosis, diabetes, hypertension as well as aging, Alzheimer’s disease, subarachnoid hemorrhage, and ischemia/reperfusion.3,7,26–28 This concept is based in part on the findings that scavengers of superoxide or gene transfer of antioxidant enzymes restores endothelium-dependent relaxation in several of these disease states.3,7,26–28

Because of the association of superoxide levels with vascular dysfunction, many studies have focused on the identification of the source(s) of superoxide during pathophysiological conditions. In addition to changes in the rate of superoxide production, local levels of superoxide are also dependent on the rate of metabolism of superoxide by SODs. Little is known, however, regarding the functional importance of decreased SOD expression and activity in blood vessels. Because basal superoxide levels were increased in CuZnSOD−/− mice and because superoxide interacts readily with nitric oxide (thereby limiting its bioavailability), we examined endothelium-dependent and nitric oxide–mediated relaxation in CuZnSOD−/− mice. Relaxation of carotid artery to the endothelium dependent–dilator acetylcholine and to authentic nitric oxide was reduced in CuZnSOD−/− mice compared with wild type. These findings are consistent with previous studies in which superoxide levels were increased pharmacologically with DDC, resulting in impairment of endothelium-dependent relaxation in several different blood vessels, including cerebral arterioles.4,43,44 However, pharmacological inhibition of endogenous SODs with DDC has at least three major limitations. First, DDC inhibits both CuZnSOD and EC-SOD activity. Thus, DDC does not provide any information regarding the importance of CuZnSOD versus EC-SOD within the vessel wall. This is of importance because CuZn-
SOD and EC-SOD together account for the majority (>90%) of the total SOD activity found in blood vessels.11–14 Thus, DDC-induced increases in superoxide and impairment of vascular function, most likely reflects the loss of activity of both CuZnSOD and EC-SOD. Second, inhibition of SOD activity with DDC may not be complete, as there are uncertainties with regard to the cellular and subcellular access of DDC. Third, DDC may have nonspecific effects including inhibition of other copper-containing proteins. Thus, the use of CuZnSOD-deficient mice allowed us to examine the functional effects of selective CuZnSOD-deficiency on superoxide levels and vascular function.

In addition to impaired relaxation of carotid artery, we also observed impaired dilatation to acetylcholine in cerebral arterioles from CuZnSOD+/− mice, suggesting that CuZnSOD limits increases in superoxide in cerebral blood vessels. These findings also indicate that the influence of CuZnSOD is not only limited to large conduit vessels but also extends to resistance vessels and the microcirculation of a key organ. The findings that relaxation to papaverine and nitroprusside was unaltered in carotid artery and cerebral arterioles in CuZnSOD−/− mice, suggests that the impaired responses to acetylcholine were selective.

In addition to mediating relaxation, endothelium-derived nitric oxide can also exert a profound modulatory influence on the responsiveness of blood vessels to constrictor stimuli. For example, removal of endothelium or pharmacological inhibition of nitric oxide synthase has been shown to potentiate vascular responses to several constrictor stimuli including phenylephrine and serotonin.32–35 We have shown previously contraction of the carotid artery to serotonin is selectively enhanced in eNOS-deficient mice.30 Thus, nitric oxide produced by eNOS can exert a profound influence on vasoconstrictor responses. The present study indicates that responses of carotid artery to phenylephrine and serotonin are enhanced in CuZnSOD-deficient mice, presumably reflecting the loss of nitric oxide bioavailability. The enhanced contractile responses to phenylephrine and serotonin in CuZnSOD−/− mice appear to be selective because contraction to potassium chloride and U46619 were not affected. Although a reduction in bioavailable nitric oxide seems the most likely explanation, we cannot rule out other effects of superoxide that may influence vasoconstrictor mechanisms.

In some experimental models of hypertension, administration of agents (ie, exogenous SODs) that reduce oxidative stress produce reductions in blood pressure.48 The mechanism(s) that produces this effect has not been fully defined but may include vascular effects related to an increased nitric oxide bioavailability. Conversely, reductions in nitric oxide bioavailability, as in the case of eNOS-deficiency (ie, eNOS knockout mice), are associated with increases in blood pressure.49 Thus, vascular levels of nitric oxide and superoxide affect vascular tone and appear to influence blood pressure. Based on this evidence, we hypothesized that increases in superoxide in vessels in CuZnSOD−/− mice may be associated with an elevation in blood pressure. Although, superoxide levels in vessels were elevated, CuZnSOD−/− mice were not hypertensive. Instead, measurements with two independent methods in conscious animals indicated that blood pressure in CuZnSOD−/− mice was approximately 17 mm Hg less than that in wild-type littermates. Although this finding appears to be paradoxical, a recent report found that blood pressure in CuZnSOD transgenic mice was similar to that in nontransgenic mice, despite the fact that basal levels of superoxide were significantly lower in CuZnSOD transgenic mice.50 It is also important to emphasize that increases in vascular superoxide levels, such as in diabetes and atherosclerosis or in response to lipopolysaccharide, are not always associated with increased arterial pressure. Collectively, these findings suggest that oxidative stress produced by CuZnSOD deficiency may influence blood pressure. At this time, we do not know if the mechanism that mediates the reduction in blood pressure in CuZnSOD−/− mice is vascular, central, and/or renal.

Implications of Reduced CuZnSOD Activity to Vascular Dysfunction

The present study suggests that selective loss of CuZnSOD has profound effects on superoxide levels and vascular function. Thus, it seems likely that reduced CuZnSOD activity, due to a disease state or genetic mutation, would also have profound effects on vascular function. For example, impaired endothelial function in diabetic rats is associated with an approximately 50% reduction in total SOD activity.51,52 Additionally, increases in superoxide and impaired vascular responses have been demonstrated in mice deficient in glutathione peroxidase-1, suggesting that other antioxidant enzymes, in addition to CuZnSOD, may also have an important role in regulation of vascular tone.53

In summary, the results of the present study provide the first direct evidence that CuZnSOD protects nitric oxide–mediated vasorelaxation and counteracts vasoconstrictor responses. CuZnSOD normally maintains relatively low levels of vascular superoxide so that the selective loss of CuZnSOD activity results in enhanced superoxide and marked changes in vascular function. Functional effects of CuZnSOD deficiency were observed in vitro and in vivo in both large vessels and the microcirculation.

Acknowledgments

This work was supported by National Institutes of Health grants NS-24621, HL-38901, HL-48058, HL-55006, HL-61446, HL-62984 (to F.M.F. and C.D.S.), and DK-25295 (to S.P.D.). We thank Dale Kinzenbaw, Cynthia Lynch, and Pamela Tompkins for technical assistance. We also thank Jon Andresen for assistance with the SOD activity assay.

References


Increased Superoxide and Vascular Dysfunction in CuZnSOD-Deficient Mice
Sean P. Didion, Michael J. Ryan, Lisa A. Didion, Pamela E. Fegan, Curt D. Sigmund and Frank M. Faraci

Circ Res. 2002;91:938-944; originally published online October 24, 2002;
doi: 10.1161/01.RES.0000043280.65241.04

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/91/10/938

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/