Heterozygous CuZn Superoxide Dismutase Deficiency Produces a Vascular Phenotype With Aging

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Abstract—The goal of this study was to test the hypothesis that loss of a single copy of the gene for CuZn superoxide dismutase (CuZnSOD) increases vascular superoxide levels and produces vascular dysfunction with aging. Responses of carotid arteries from young (7 months) and old (22 to 24 months of age) heterozygous CuZnSOD-deficient (CuZnSOD+/−) mice and their wild-type (CuZnSOD+/+) littermates were examined in vitro. Total superoxide dismutase activity in aorta was reduced by ∼30% (P<0.05) in CuZnSOD+/− mice compared with wild-type mice. Responses to acetylcholine (an endothelium-dependent agonist) produced relaxation that was similar (P>0.05) in carotid arteries from young wild-type, young CuZnSOD+/+, and old wild-type mice. In contrast, relaxation to acetylcholine was markedly impaired in old CuZnSOD+/− mice (eg, 100 μmol/L acetylcholine produced 51±5% and 96±5% relaxation in vessels from old CuZnSOD+/− and old wild-type mice, respectively). This effect was selective, because relaxation to nitroprusside (an endothelium-independent agonist) was not affected by either CuZnSOD genotype or aging. The impaired response to acetylcholine in old CuZnSOD+/− mice was restored toward normal with either tempol (a scavenger of superoxide; 1 mmol/L) or PJ34 (an inhibitor of poly-ADP-ribose polymerase; 3 μmol/L). Vascular superoxide levels were increased in aorta in old CuZnSOD+/− mice and increased further in CuZnSOD+/− mice with aging. These findings provide the first direct evidence that normal CuZnSOD expression protects endothelial function and that deficiency in a single copy of the gene that encodes CuZnSOD produces increases in superoxide and marked impairment of endothelial function with aging. (Hypertension. 2006;48:1072-1079.)

Key Words: SOD1 ■ superoxide ■ carotid artery ■ endothelium-dependent responses ■ oxidative stress ■ genetically altered mice

Oxidative stress seems to play a major role in mechanisms related to vascular dysfunction with aging.1–8 Oxidative stress, particularly increases in superoxide, have important implications in terms of vascular function. For example, superoxide reacts with NO more readily than dismutation of superoxide by superoxide dismutase (SOD), thus limiting NO bioavailability.9 Reductions in the bioavailability of NO have been shown to be associated with endothelial dysfunction in several disease states, including aging.1,8,10,11

Although studies have begun to examine the role of superoxide in vascular dysfunction with aging,1–8 little is known regarding mechanisms that protect against vascular dysfunction with aging. One potentially important mechanism in this regard relates to activity of the various SODs.12 Defining the role of SOD in vascular aging is important, because SODs constitute a first line of defense against increases in oxidative stress.

Of the 3 SOD isoforms, CuZnSOD is the most abundant in terms of total SOD expression within the vascular wall.12 Thus, reductions in CuZnSOD activity would be predicted to have profound effects on vascular function. Indeed, we, and others, have shown that homozygous CuZnSOD (CuZnSOD−/−) gene deficiency is associated with increases in superoxide and vascular dysfunction.13,14 In contrast, endothelium-dependent responses are normal in heterozygous CuZnSOD (CuZnSOD+/−) mice under otherwise control conditions.15 Because aging is associated with increases in vascular superoxide and endothelial dysfunction and because the functional importance of CuZnSOD with aging is not known, the first goal of the present study was to test the hypothesis that heterozygous deficiency in CuZnSOD promotes increases in oxidative stress and endothelial dysfunction with aging. To test this hypothesis, we used a genetic approach and examined superoxide levels and vascular responses in young and old wild-type and CuZnSOD+/− mice.

At least 1 important functional consequence of increases in oxidative stress is activation of poly-ADP-ribose polymerase (PARP), an abundant nuclear protein associated with DNA surveillance and repair.16,17 Because superoxide and activation of PARP have been shown to play important roles in endothelial dysfunction in several disease states,18–20 our second goal was to examine the potential role of PARP in...
vascular dysfunction associated with aging and heterozygous CuZnSOD deficiency.

Methods

Experimental Animals

CuZnSOD-deficient (male and female) mice were derived from breeding pairs of heterozygous CuZnSOD-deficient (B6; 129S-SODtm1Leb) mice from Jackson Laboratories (Bar Harbor, ME). Because homozygous CuZnSOD-deficient (CuZnSOD−/−) mice display impaired endothelium-dependent responses under control conditions,13,14 we studied the effect of aging on responses of carotid arteries from wild-type (littermate controls; CuZnSOD+/+) and heterozygous CuZnSOD-deficient (CuZnSOD−/+ ) mice. C57BL/6 mice from Jackson Laboratories were used in experiments designed to test for the possibility of any potential antioxidant effects of PJ34 (N-[6-oxo-5,6-dihydropyridazin-2-yl]-N,N-dimethylacetamide). Genotypes were obtained from DNA isolated from tail biopsy samples as described previously.13,15

Vascular Preparation

Methods used to measure responses of carotid arteries in mice have been described previously.13,15,22–26 Briefly, wild-type and CuZnSOD−/+ mice were euthanized with pentobarbital (100 mg/kg, IP), followed by removal of both carotid arteries and the thoracic aorta, for studies of vascular function and measurement of SOD activity and superoxide levels (see below), respectively. Arteries were placed in Krebs buffer (95% O2/5% CO2, loose connective tissue was removed, and the vessels were cut into ring ~4 mm in length. Carotid arteries were connected to force transducers for measurement of changes in isometric tension in organ baths containing oxygenated Krebs solution maintained at 37°C. Tension was increased stepwise using a micromanipulator, and resting tension was adjusted to 0.25 g, which was determined previously to be optimal for mouse carotid artery.26 Rings were allowed to equilibrate for 45 minutes before starting the experimental protocols.

Experimental Protocols

Relaxation of carotid arteries in response to the endothelium-dependent dilator, acetylcholine (1 mM to 100 mM), and the endothelium-independent dilator, nitroprusside (1 mM to 100 μM), was measured after submaximal precontraction to the thromboxane analog U46619 (9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin-F2α). We have shown previously using both pharmacological and genetic approaches that relaxation to acetylcholine in mouse carotid artery is mediated by NO and endothelial NO synthase (eNOS).24,26 In addition, because contractile responses may be enhanced with aging,27 we examined responses to serotonin (0.01 to 10 μM) and U46619 (0.03 to 3 μg/mL) in carotid arteries from young and old wild-type and CuZnSOD−/+ mice.

To determine whether endothelial dysfunction with aging was mediated by superoxide, responses to acetylcholine and nitroprusside were examined in carotid arteries from old wild-type and CuZnSOD−/+ mice incubated with vehicle or tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl, a superoxide scavenger, 1 mM) for 1 hour. This concentration of tempol has been shown to be very effective in reducing superoxide levels in blood vessels and in restoring impaired endothelium-dependent responses.4,28

Because oxidative stress has been associated with activation of PARP, we also determined whether PARP contributes to endothelial dysfunction with aging. Thus, in separate groups of mice, responses of carotid arteries to acetylcholine and nitroprusside were examined in vessels incubated with vehicle or PJ34 (3 μM), a selective PARP inhibitor, for 45 minutes.18–20 This concentration of PJ34 has been shown to effectively inhibit activity of PARP and to restore impaired endothelium-dependent responses in other disease models.18–20

In separate studies, we sought to further explore the link between reductions in CuZnSOD activity and activation of PARP. Thus, carotid arteries from young wild-type mice were incubated overnight (24 hours) in 0.5 mM DMEM as described previously,18 with either vehicle or diethyldithiocarbamate (DETC) an inhibitor of copper-containing SODs; 1 μM/L). Inhibition of copper-containing SODs with DETC has been shown previously to reduce SOD activity and produce endothelial dysfunction because of increases in oxidative stress.25,29,30 To test the hypothesis that increases in oxidative stress because of inhibition of SODs are functionally linked to activation of PARP, responses to acetylcholine and nitroprusside were examined in vehicle and DETC-treated vessels in the presence of vehicle or PJ34.

Superoxide Measurement

Relative superoxide levels were measured in aorta from wild-type and CuZnSOD−/− mice using lucigenin (5 μM)-enhanced chemiluminescence as described previously.13,23,25 We have shown previously that the signal obtained using this approach can be markedly inhibited by polyethylene glycol–SOD or Tiron (scavengers of superoxide),13,15,25

In separate studies, we tested whether PJ34 acts as a scavenger of superoxide (ie, has antioxidant effects) using aorta from young C57BL/6 mice. Superoxide was measured under basal conditions and in response to reduced nicotinamide-adenine dinucleotide phosphate ([NADPH] 10 and 100 μmol/L; a stimulus that has been shown previously to increase superoxide levels in blood vessels31) in the presence of vehicle or PJ34 (3 μmol/L). Tiron (1 mmol/L) was added after the final concentration of NADPH to demonstrate that the lucigenin signal was because of superoxide.

SOD Activity

Total SOD activity of aortic homogenates was determined as described previously.13,15,23

Drugs

Acetylcholine, DETC, lucigenin, NADPH, nitroprusside, serotonin, tempol and tiron were obtained from Sigma, and all were dissolved in saline. PJ34 (Calbiochem) and U46619 (Cayman Chemical) were dissolved in milli-Q water and 100% ethanol, respectively, with subsequent dilutions being made with saline.

Statistical Analysis

All of the data are expressed as mean±SE. Responses to acetylcholine and nitroprusside are expressed as a percentage of relaxation to U46619-induced contraction. Contractile responses to serotonin and U46619 are expressed in grams of tension. Comparisons of relaxation and contraction were made using ANOVA followed by Bonferroni posthoc test. Comparisons of blood pressure, glucose, SOD activity, and superoxide levels were made using unpaired t tests. Statistical significance was accepted at P<0.05.

Results

Characteristics of Young and Old Wild-Type and CuZnSOD−/+ Mice

Young wild-type (n=29) and young CuZnSOD−/+ (n=27) mice, as well as old wild-type (n=25) and old CuZnSOD−/+ (n=31) mice, were of similar age and body weight (young mice: 7±1 g); 36±1 g and old mice: 23±1 month and 34±1 g, respectively). Systolic blood pressure (young wild-type: 116±5 mm Hg; n=6); young CuZnSOD−/+: 118±4 mm Hg [n=6]; old wild-type: 116±7 mm Hg [n=6]; old CuZnSOD−/+: 116±6 mm Hg [n=6]) and blood glucose
Effects of CuZnSOD Deficiency and Aging on Total SOD Activity and Superoxide Levels

SOD activity was similar in aorta from young and old wild-type mice. Total SOD activity was reduced by \( \approx 30\% \) in aortic homogenates from young CuZnSOD\(^{+/+}\) mice compared with wild-type (Figure 1). A further reduction in total SOD activity, of approximately another 30\%, was noted in aortas from CuZnSOD\(^{+/+}\) but not wild-type mice with aging (Figure 1).

Superoxide levels were similar (\( P>0.05 \)) in aorta from young wild-type and CuZnSOD\(^{+/+}\) mice (Figure 1). In contrast, superoxide levels were higher (\( P<0.05 \)) in old wild-type mice (50\( \pm \)7 relative light units (RLU)/s per milligram of protein) as compared with young wild-type mice. Superoxide levels were also higher in old CuZnSOD\(^{+/+}\) mice (67\( \pm \)4 RLU/s per milligram of tissue as compared with young CuZnSOD\(^{+/+}\) mice (Figure 1) and were higher (\( P=0.028 \)) than that observed in old wild-type mice. Tiron eliminated the superoxide signal in all 4 groups of mice, suggesting that the lucigenin signal was due to superoxide (data not shown).

Vascular Responses in Wild-Type and CuZnSOD\(^{+/+}\) Mice With Aging

Acetylcholine produced relaxation that was similar (\( P>0.05 \)) in carotid arteries from young wild-type and CuZnSOD\(^{+/+}\) mice (Figure 2). For example, 100 \( \mu \)mol/L of acetylcholine produced 100\( \pm \)5\% and 103\( \pm \)2\% relaxation in vessels from young wild-type and CuZnSOD\(^{+/+}\) mice, respectively. Relaxation to nitroprusside was similar (\( P>0.05 \)) in young wild-type and CuZnSOD\(^{+/+}\) mice (Figure 2). Taken together, these results suggest that loss of a single gene for CuZnSOD does not alter responses of carotid arteries from young mice.

In old wild-type mice, acetylcholine produced relaxation in carotid arteries that was similar (\( P>0.05 \)) to that observed in vessels from young wild-type mice (Figure 2). For example, 100 \( \mu \)mol/L of acetylcholine produced 100\( \pm \)5\% and 96\( \pm \)5\% relation in vessels from young and old wild-type mice, respectively (Figure 2). In contrast, relaxation to acetylcholine was markedly impaired in old CuZnSOD\(^{+/+}\) (\( n=15 \)) mice vs young CuZnSOD\(^{+/+}\) mice. \( P<0.05 \) vs young CuZnSOD\(^{+/+}\) mice. B. Nitroprusside produced similar (\( P>0.05 \)) responses in vessels from wild-type and CuZnSOD\(^{+/+}\) mice irrespective of age, suggesting that the effect of aging in CuZnSOD\(^{+/+}\) mice is selective for endothelium.

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**Figure 1.** A. Total SOD activity is reduced in aorta from young CuZnSOD\(^{+/+}\) mice (\( n=7 \)) vs young wild-type mice (\( n=7 \)). Aging had no effect (\( P>0.05 \)) on total SOD activity in wild-type mice (\( n=7 \)). In contrast, there was an additional reduction in total SOD activity in CuZnSOD\(^{+/+}\) mice with aging (\( n=7 \)). *\( P<0.05 \) vs young wild-type, †\( P<0.05 \) vs young CuZnSOD\(^{+/+}\) mice. B. Superoxide levels in aorta from wild-type and CuZnSOD\(^{+/+}\) mice as detected using lucigenin-enhanced chemiluminescence. Superoxide levels were similar (\( P>0.05 \)) in young wild-type (\( n=11 \)) and young CuZnSOD\(^{+/+}\) (\( n=11 \)) mice. In contrast, vascular superoxide levels were increased in both old wild-type (\( n=14 \)) and CuZnSOD\(^{+/+}\) (\( n=12 \)) mice; however, the increase in superoxide was greater in old CuZnSOD\(^{+/+}\) mice than that in old wild-type mice. *\( P<0.05 \) vs young wild-type, †\( P<0.05 \) vs young CuZnSOD\(^{+/+}\) and old wild-type mice.

**Figure 2.** Cumulative responses to acetylcholine and nitroprusside in carotid arteries from wild-type and CuZnSOD\(^{+/+}\) mice. A. Acetylcholine produced relaxation that was similar (\( P>0.05 \)) in vessels from young (\( n=7 \)) and old wild-type (\( n=8 \)) mice, as well as in young CuZnSOD\(^{+/+}\) (\( n=7 \)) mice. In contrast, relaxation of carotid arteries to acetylcholine was markedly impaired in old CuZnSOD\(^{+/+}\) (\( n=15 \)) mice vs young CuZnSOD\(^{+/+}\) mice. \( P<0.05 \) vs young CuZnSOD\(^{+/+}\) mice. B. Nitroprusside produced similar (\( P>0.05 \)) responses in vessels from wild-type and CuZnSOD\(^{+/+}\) mice irrespective of age, suggesting that the effect of aging in CuZnSOD\(^{+/+}\) mice is selective for endothelium.

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([nonfasting] young wild-type: 143\( \pm \)10 mg/dL \( n=15 \); young CuZnSOD\(^{+/+}\): 139\( \pm \)12 mg/dL \( n=15 \); old wild-type: 154\( \pm \)21 mg/dL \( n=9 \); old CuZnSOD\(^{+/+}\): 160\( \pm \)26 mg/dL \( n=9 \)) were similar (\( P>0.05 \)) in wild-type and CuZnSOD\(^{+/+}\) mice irrespective of age.
Impaired endothelial responses in Old CuZnSOD−/− mice are restored to normal after treatment with tempol (1 mmol/L). Tempol had no effect on vascular responses to acetylcholine in old wild-type mice or to nitroprusside in either old wild-type or CuZnSOD+/− mice. *P<0.05 vs vehicle-treated CuZnSOD+/− mice.

With tempol (Figure 4). This effect was selective, because tempol had no effect (P>0.05) on responses to nitroprusside in old CuZnSOD+/− or in old wild-type mice. These findings provide strong evidence that the impaired response to acetylcholine in old CuZnSOD+/− mice is mediated by superoxide and that it is reversible.

To provide functional evidence that PARP may contribute to endothelial dysfunction in CuZnSOD+/− mice with aging, we examined responses in carotid arteries treated with PJ34. In young CuZnSOD+/− mice, relaxation to acetylcholine was similar in vessels treated with vehicle or PJ34 (Figure 5). In contrast, treatment of vessels from old CuZnSOD+/− mice with PJ34 restored responses to acetylcholine (Figure 5) to that observed in young CuZnSOD+/− mice (Figure 2). For example, 100 μmol/L of acetylcholine produced 42±5% and 100±3% relaxation in vessels from old CuZnSOD+/− mice treated with vehicle and PJ34, respectively. The amount of impairment in this group of old CuZnSOD+/− mice tended to be greater than that in the other groups of old CuZnSOD+/− studied, suggesting that there is some variability in the response to acetylcholine in old CuZnSOD+/− mice. However, this variability was not statistically different among the individual groups studied. Taken together, these data suggest

Figure 3. Cumulative responses to serotonin and U46619 in carotid arteries from wild-type and CuZnSOD+/− mice. A, Serotonin produced contraction that was similar in young wild-type (n=7) and young CuZnSOD−/− (n=7) mice, as well as old wild-type (n=7) mice. In contrast, the response to serotonin was enhanced with aging in old CuZnSOD+/− (n=15) mice. *P<0.05 vs vehicle-treated CuZnSOD+/− mice. B, U46619 produced contraction that was similar in wild-type (n=7 to 8) and CuZnSOD+/− (n=7 to 15) mice irrespective of age, suggesting that the effects of aging on responses to serotonin were selective.

Role of Superoxide and PARP in Impaired Endothelial Responses in Old CuZnSOD+/− Mice

Impairment of endothelial responses in CuZnSOD+/− mice would be predicted to involve superoxide. To test this hypothesis, responses of carotid arteries were examined in old wild-type and old CuZnSOD+/− mice after treatment with 1 mmol/L of tempol. Tempol had no effect (P>0.05) on vascular responses to acetylcholine or nitroprusside in old wild-type mice (Figure 4). In contrast, responses to acetylcholine in old CuZnSOD+/− mice were restored to normal with tempol (Figure 4). This effect was selective, because tempol had no effect (P>0.05) on responses to nitroprusside in old CuZnSOD+/− or in old wild-type mice. These findings provide strong evidence that the impaired response to acetylcholine in old CuZnSOD+/− mice is mediated by superoxide and that it is reversible.

Figure 5. Impaired endothelium-dependent responses to acetylcholine in CuZnSOD+/− (n=6) mice with aging are restored to normal after treatment with PJ34 (3 μmol/L) vs vehicle-treated vessels (right). PJ34 had no effect (P>0.05) on vascular response in old CuZnSOD+/− (n=6) mice (left). *P<0.05 vs vehicle.
that activation of PARP contributes to endothelial dysfunction with aging in CuZnSOD<sup>−/−</sup> mice.

In complimentary studies, inhibition of copper-containing SODs with DETC produced marked inhibition of acetylcholine-induced relaxation in the carotid artery from young wild-type mice (Figure 6). This finding suggests that inhibition of endogenous SODs with DETC produces endothelial dysfunction and is consistent with many previous reports. Responses to acetylcholine in carotid arteries treated with DETC could be normalized by PJ34 (Figure 6). The effects of DETC and PJ34 seem to be selective, because relaxation to nitroprusside was similar in carotid arteries treated with vehicle and DETC, as well as DETC plus PJ34. These findings provide further evidence that endothelial dysfunction resulting from reductions in SOD activity may produce activation of PARP.

To examine whether the effect of PJ34 was attributable simply to antioxidant effects, superoxide levels were examined in aortas from young C57Bl/6 (n=5) mice under basal conditions and in response to NADPH. In vehicle-treated vessels, superoxide levels were 17 ± 3 RLU/s per milligram of tissue under basal conditions and increased to 422 ± 73 and 3091 ± 670 RLU/s per milligram of tissue after the addition of 10 and 100 μmol/L NADPH, respectively. In PJ34-treated vessels, basal superoxide levels (26 ± 10 RLU/s per milligram of tissue) were similar (P>0.05) to those in vehicle-treated vessels. Superoxide levels increased to 480 ± 66 and 3625 ± 1017 RLU/s per milligram of tissue after the addition of 10 and 100 μmol/L of NADPH, respectively, in PJ34-treated vessels and were not different (P>0.05) from that in vehicle-treated vessels. The response to 100 μmol/L of NADPH was markedly reduced (P<0.05) by the addition of Tiron in both vehicle- and PJ34-treated vessels, suggesting that the lucigenin signal in response to NADPH was mediated by superoxide. Taken together, these data strongly suggest that PJ34 does not act as an antioxidant at the concentration used in the present study.

Discussion

There are several major new findings in the present study. First, endothelium-dependent responses to acetylcholine are markedly impaired, and contractile responses to serotonin are selectively enhanced in CuZnSOD<sup>−/−</sup> but not wild-type mice with aging. Thus, a striking vascular phenotype becomes apparent with the combination of aging and heterozygous CuZnSOD deficiency. Second, tempol restored endothelium-dependent responses in CuZnSOD<sup>−/−</sup> mice with aging to normal, providing additional evidence for a role for superoxide in this response. Third, impaired endothelium-dependent response to acetylcholine in CuZnSOD<sup>−/−</sup> mice was normalized using PJ34, suggesting that increases in PARP activity contribute to the vascular phenotype that occurs with aging. Taken together, both our genetic and pharmacological data provide strong support for a role of superoxide in impaired endothelium-dependent responses that occur with the combination of aging and reductions in CuZnSOD.

Vascular SOD Expression and Activity With Aging

To our knowledge, the functional role of CuZnSOD in vascular aging has not been examined previously. In the present study, we found that heterozygous CuZnSOD gene deficiency was associated with an ~30% reduction in total SOD activity in young CuZnSOD<sup>−/−</sup> mice. These results are consistent with previous observations.

Aging has been shown to produce alterations in expression and activity of SOD in several tissues. In blood vessels, SOD activity and/or expression has been shown to be increased, decreased, or unaltered with aging. In agreement with the latter studies, we found that aging had no effect on total SOD activity in aorta from wild-type mice. In contrast, aging was associated with a further reduction in total SOD activity in CuZnSOD<sup>−/−</sup> mice beyond that seen with genetic CuZnSOD deficiency alone. Although the mechanism(s) by which aging reduces SOD activity in CuZnSOD<sup>−/−</sup> mice is not known, we speculate that the reduction in SOD activity in old CuZnSOD<sup>−/−</sup> mice might reflect increased oxidative stress associated with aging. Both CuZnSOD and MnSOD activity can be reduced by increases in oxidative and nitrative stress.

Vascular Responses with Aging

Aging has been shown to be associated with impairment of endothelium-dependent responses and, in some studies, impairment of endothelium-independent responses. Although studies examining vascular response with aging in mice are few, we found that endothelium-dependent and -independent responses were normal in wild-type mice with aging (up to 22 to 24 months of age). Preliminary evidence from our laboratory indicates that endothelial function becomes impaired as the mice continue to age (>25 months; unpublished observation). Thus, carotid arteries of mice, like a variety of blood vessels of other species, exhibit attenuated responses to acetylcholine with age.

A major novel finding of the present study is that responses to acetylcholine are markedly impaired in CuZnSOD<sup>−/−</sup> mice with aging at a time point at when endothelial function is normal in wild-type mice. These findings demonstrate for the
first time that a selective reduction in CuZnSOD is associated with marked vascular dysfunction with aging. Thus, endothelial function may be particularly affected by the combination of aging and heterozygous CuZnSOD deficiency. In contrast, endothelium-dependent responses to acetylcholine were normal in young CuZnSOD+/− mice, which is consistent with previous findings.15

We also observed that contractile responses to serotonin were selectively enhanced with aging in CuZnSOD+/− mice. Previous studies have shown that either removal of endothelial or pharmacological inhibition of eNOS potentiates responses to several vasoconstrictors, including serotonin.45–47 Using a genetic approach, we have shown that contractile responses to serotonin are selectively enhanced in eNOS-deficient mice.48 Thus, enhanced responses to serotonin in the present study may reflect a reduction in NO bioavailability associated with CuZnSOD deficiency and aging.

An important consideration in studies of vascular function with aging is the presence or absence of disease conditions, which may influence vascular responses independent of the effect of aging per se.3 Both wild-type and CuZnSOD+/− mice in the present study appeared healthy, and on gross visual examination vessels did not contain any evidence of atherosclerotic lesions. In addition, blood pressure and blood glucose levels were not affected by either genotype or age, suggesting that the impairment of vascular responses with aging in heterozygous CuZnSOD deficiency were because of aging and not the presence of potentially confounding disease states. Importantly, it has been shown that mean life span is reduced in CuZnSOD−/− but not in CuZnSOD+/− mice compared with wild-type littermates, suggesting that heterozygous CuZnSOD deficiency is not associated with enhanced mortality.49

Mechanisms of Endothelial Dysfunction With Aging

Previous studies suggest that oxidative stress plays a major role in impairing endothelium-dependent responses in aging.3–5,7,8 In the present study, we found that superoxide levels were higher in old but not young CuZnSOD+/− mice, as compared with wild-type mice, indicating that heterozygous CuZnSOD deficiency is associated with increases in oxidative stress with aging. In support of this concept, tempol, a cell-permeable scavenger of superoxide, which had no effect in wild-type mice, was very effective in restoring vascular responses in CuZnSOD+/− mice. Thus, pharmacological and genetic data provide strong evidence for a role of superoxide in impaired vascular responses that occur with reductions in CuZnSOD activity and aging. Because tempol has been shown previously to enhance NO bioavailability,50 the impaired response in CuZnSOD+/− mice most likely reflects superoxide-mediated reductions in NO bioavailability with aging.

Aging produces increases in oxidative stress, which has been shown to have important functional effects on blood vessels. At least one of the functional effects of increases in oxidative stress is the activation of the nuclear enzyme PARP.16,17,19,51 Increases in peroxynitrite have been shown to be potent inducers of DNA strand breaks, which results in PARP activation.16,17 Under normal conditions, basal activity of PARP is relatively low and is important in maintaining genomic integrity.16,17 However, excessive activation of PARP because of DNA damage results in depletion of cellular oxidized nicotinamide-adenine dinucleotide (NAD+) that required PARP substrate.17,18,20 Reductions in NAD+ concentration have been shown to deplete cellular ATP levels and, hence, cellular energy levels.17,18,20

In the present study, the PARP inhibitor PJ34 was very effective in restoring endothelial responses in old CuZnSOD+/− mice, as well as in carotid arteries from young wild-type mice treated with DETC. Taken together, these data suggest a role for PARP in the impairment of endothelium-dependent responses that occur with reductions in CuZnSOD and aging. PJ34, a competitive and selective inhibitor of PARP activity,16,17 has been shown to compete for the NAD+ binding site resulting in enhanced NAD+ levels.16,17 Thus, a beneficial effect of PARP inhibition is restoration of energy pools necessary for normal cellular activity. Because the effect of PJ34 occurred acutely, these findings suggest that inhibition of PARP may restore cellular energy stores relatively quickly in CuZnSOD+/− mice with aging. Similar acute effects of PARP inhibition have been observed in other disease models using the same or even higher concentrations of PJ34 used in the present study.18–20 Moreover, the effect of PJ34 does not seem to be related to an antioxidant effect, because PJ34 at the concentration used in the present study had no effect on NADPH-induced increases in aortic superoxide levels consistent with previous findings.20

Endothelial function in old CuZnSOD−/− mice could be restored by tempol or PJ34. These results would suggest that formation of superoxide is required for activation of PARP. Most previous studies suggest that peroxynitrite (formed from superoxide and NO) is a potent stimulus for activation of PARP.16–20 Although we are not aware of any studies that implicate superoxide, per se, in PARP activation, we cannot exclude such a possibility. However, numerous studies have implicated peroxynitrite in the activation of PARP. Thus, our results with tempol and PJ34 in old CuZnSOD−/− mice and those with DETC and PJ34 in young wild-type mice suggest that reductions in SOD activity are due to activation of PARP.

Perspectives

Increases in oxidative stress have been linked to impaired endothelium-dependent responses in various disease states, as well as in aging.3–8 However, very little is known regarding mechanisms that limit or protect against increases in oxidative stress and endothelial dysfunction in aging. The present findings suggest for the first time that a selective reduction in CuZnSOD activity greatly enhances vascular dysfunction with aging. Thus, a vascular phenotype with heterozygous CuZnSOD deficiency that was not apparent in young mice was unmasked with aging. A similar effect on endothelial function was noted with heterozygous CuZnSOD deficiency in response to angiotensin II, a stimulus also known to produce vascular oxidative stress.15 Taken together, these findings demonstrate a critical role for CuZnSOD in protecting blood vessels in response to stimuli that produce oxidative stress. The results of the present study also provide
another example of the importance of the use of heterozygous-deficient mice. These findings have important implications for disease states and/or genetic polymorphisms that decrease activity of CuZnSOD within the vessel wall.

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Disclosures

None.

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