Overexpression of CuZn-SOD Prevents Lipopolysaccharide-Induced Endothelial Dysfunction

Sean P. Didion, PhD; Dale A. Kinzenbaw, BS; Pamela E. Fegan, BS; Lisa A. Didion, BS; Frank M. Faraci, PhD

Background and Purpose—Inflammation is thought to be a major contributor to carotid artery disease. Lipopolysaccharide (LPS) activates inflammatory mechanisms thought to contribute to endothelial dysfunction by mechanisms that are not well defined. The goal of this study was to determine whether overexpression of CuZn-SOD protects against LPS-induced increases in superoxide and endothelial dysfunction.

Methods—Carotid arteries from CuZn-SOD transgenic (SOD-Tg) and nontransgenic (non-Tg) littermates were examined in vitro. Superoxide levels were measured using lucigenin-enhanced chemiluminescence.

Results—In non-Tg mice, LPS (0.5 μg/mL for 22 hours) produced marked impairment of vasorelaxation in response to the endothelium-dependent dilator acetylcholine (ACh). For example, 100 μmol/L ACh relaxed carotid arteries from non-Tg mice by 86±6% and 38±8% after treatment with vehicle and LPS, respectively. In contrast, LPS did not significantly impair responses of carotid artery to ACh in SOD-Tg mice, and LPS had no effect on relaxation responses to the endothelium-independent dilator nitroprusside in carotid artery from non-Tg or SOD-Tg mice. LPS-induced increases in superoxide, as measured using lucigenin-enhanced chemiluminescence, were higher in vessels from non-Tg mice than from SOD-Tg mice.

Conclusions—These results indicate that LPS increases superoxide and impairs endothelium-dependent relaxation. Overexpression of the CuZn isoform of SOD effectively prevents LPS-induced oxidative stress and endothelial dysfunction in the carotid artery. (Stroke. 2004;35:1963-1967.)

Key Words: animal models ▪ carotid arteries ▪ endothelium ▪ inflammation ▪ reactive oxygen species ▪ superoxide dismutase

Impairment of endothelial function is seen in experimental models of inflammation, including exposure to lipopolysaccharide (LPS) and proinflammatory cytokines.1–9 Vascular inflammation is also present, or components of the inflammatory response are activated, within the vessel wall in many cardiovascular diseases, including carotid artery disease.10–15 The mechanisms that account for endothelial dysfunction during vascular inflammation are not clear. Previous studies in this area either have not addressed mechanisms that produce endothelial dysfunction or have provided divergent results and implicated a variety of mechanisms in relation to impairment of endothelium-dependent relaxation. For example, although increases in reactive oxygen species (ROS) including superoxide anion, have been suggested to contribute to endothelial dysfunction, pharmacological scavengers of superoxide and other antioxidants have had inconsistent effects in models using LPS.1,4,7,16,17 In 1 case, scavenging of superoxide actually worsened LPS-induced endothelial dysfunction.1 Thus, the role of superoxide as a mediator of endothelial dysfunction during inflammation is unclear.

LPS is known to activate nicotinamide adenine dinucleotide (phosphate) (NAD[P]H) oxidase and other mechanisms that increase intracellular superoxide.1,18–20 Increased vascular superoxide can exert a variety of effects, including inactivation of nitric oxide and formation of peroxynitrite. Peroxynitrite can cause nitration of protein tyrosine residues, and this effect occurs predominantly intracellularly in vascular cells in response to inflammatory stimuli.21 Based on such findings, we hypothesized that overexpression of the isoform of superoxide dismutase (SOD), which is primarily cytosolic (CuZn-SOD, SOD-1), would be effective in protecting against LPS-induced endothelial dysfunction. Thus, the goal of this study was to use a genetic approach to determine if overexpression of CuZn-SOD protects against LPS-induced increases in superoxide and endothelial dysfunction. LPS is commonly used to activate inflammatory mechanisms in blood vessels, including activation of nuclear factor κB and production of proinflammatory cytokines. We studied carotid arteries, as inflammatory mechanisms are thought to be a major contributor to carotid artery disease, a risk factor for...
stroke. Using CuZn-SOD transgenic (SOD-Tg) and nontransgenic (non-Tg) littermates, we found that LPS increases superoxide and impairs endothelium-dependent relaxation, and that these effects are prevented by overexpression of CuZn-SOD.

Methods

Animal Preparation

The animal protocol used in these experiments was reviewed and approved by the University of Iowa Animal Care and Use Committee. Mice for this study were derived from breeding of male hemizygous CuZn-SOD (human)-transgenic (C57BL/6-TgN(SOD-1)110jc) with female C57BL/6J mice to generate SOD-Tg and non-Tg mice within the same litter (Jackson Labs, Bar Harbor, Maine). This approach allowed us to use non-Tg littermates as controls. Mice were fed regular chow and water, which were available ad libitum. The ages of mice in the different groups were similar (non-Tg, 10 ± 1 month; SOD-Tg, 11 ± 1 month), and both male and female mice were studied. Genotyping of mice was performed using PCR of DNA from tail biopsies.

General Preparation

The method used to measure responses of carotid arteries in mice has been described in detail. Briefly, mice were anesthetized with pentobarbital (100 mg/kg, intraperitoneally) followed by removal of both carotid arteries and the thoracic aorta. Arteries were placed in Krebs buffer, loose connective tissue was removed, and vessels were cut into rings (3 to 4 mm in length). Each segment of carotid artery and aorta was placed in individual wells using 48-well cell culture dishes containing 0.5 mL DMEM containing 5 mmol/L glucose, 120 U/mL penicillin, 120 μg/mL streptomycin, and 50 μg/mL polymyxin B. Vessels were then incubated with either vehicle (ddH2O) or LPS (0.5 μg/mL Escherichia coli; serotype 026:B6; Sigma-Aldrich, St. Louis, Missouri) for 22 hours at 37°C. Following incubation, vascular rings were connected to a force transducer to measure isometric tension in an organ bath containing Krebs solution maintained at 37°C. Resting tension was increased stepwise to reach a final tension of 0.25 g, and the rings were allowed to equilibrate for at least 45 minutes. This amount of resting tension is optimal for contraction of murine carotid arteries.

Protocol

Relaxation of carotid arteries in response to acetylcholine (Sigma) and nitroprusside (Sigma) was measured following submaximal precontraction using the thromboxane analog, U46619 (9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin F2α; Biomol Research Laboratories Inc). Using pharmacological approaches and genetargeted mice, we and others have shown previously that responses of the carotid artery to acetylcholine are mediated by endothelial nitric oxide synthase (eNOS). At the end of each experiment, we obtained full dose responses of each carotid artery to U46619 (0.03 to 3 μg/mL) to determine maximal contractile responses.

Measurement of SOD Protein and Activity

Total SOD activity of aortic homogenates from non-Tg and SOD-Tg mice was determined as described. CuZn-SOD protein expression in the aorta of both groups of mice was examined by Western blotting as described.

Measurement of Superoxide

Superoxide levels were measured using aorta and 5 μmol/L lucigenin-enhanced (Sigma) chemiluminescence as described in detail. Basal (control) levels of superoxide are reported as the value of tissue plus lucigenin-containing buffer minus background. Superoxide levels were normalized per dry weight. We have shown previously that the signal obtained using this approach is markedly inhibited by scavengers of superoxide (PEG-SOD or Tiron).

SOD Activity and CuZn-SOD Protein Expression

Western blotting confirmed that CuZn-SOD protein was present in higher levels in vascular tissue in SOD-Tg mice (Figure 1). Two distinct bands were observed in aorta from SOD-Tg mice. The lower band corresponds to endogenous mouse CuZn-SOD, and the slightly higher band corresponds to human CuZn-SOD (human CuZn-SOD is known to be slightly larger than mouse CuZn-SOD). As expected, total SOD activity was higher in aorta from SOD-Tg mice compared with non-Tg mice (Figure 1).

Increases in Vascular Superoxide in Response to LPS Are Prevented in SOD-Tg Mice

Superoxide levels, as measured using lucigenin-enhanced chemiluminescence, were higher in vessels treated with LPS than in those treated with vehicle in non-Tg control mice (Figure 2). In contrast, increases in superoxide in response to LPS were greatly reduced in SOD-Tg mice (Figure 2). These findings indicate that overexpression of CuZn-SOD is effective in preventing large LPS-mediated increases in vascular superoxide.

Vascular Responses in Non-Tg and SOD-Tg Mice

U46619 produced concentration-dependent contraction of carotid arteries. For relaxation studies, arteries were contracted to 60% to 70% of maximum using 0.3 μg/mL U46619. The response to this concentration of U46619 was similar in non-Tg and SOD-Tg mice and was not affected by LPS (P > 0.05). Carotid arteries contracted by...
There are several major findings in this study. First, overexpression of the CuZn isoform of SOD effectively prevents LPS-induced oxidative stress and endothelial dysfunction.

Vascular inflammation is present, or components of the inflammatory response are activated, within blood vessels with aging and in many cardiovascular diseases including atherosclerosis (carotid artery disease), diabetes, hypertension, and hyperhomocysteinemia. This vascular inflammation is thought to contribute significantly to vascular dysfunction, including endothelial dysfunction, in these disease states.

Many studies have used LPS to activate inflammatory mechanisms, including nuclear factor κB and production of proinflammatory cytokines, in blood vessels. In this experimental model, impairment of endothelium-dependent relaxation is commonly observed. Nitric oxide, which is the major mediator of endothelium-dependent relaxation, reacts with superoxide at a rate 3 times faster than dismutation of superoxide by SOD. LPS and proinflammatory cytokines increase levels of superoxide and peroxynitrite within intact blood vessels and in vascular cells in culture, but the functional importance of these changes has been very difficult to define. For example, treatment with exogenous scavengers of superoxide have had no effect or have worsened endothelial dysfunction in these models. In contrast, high concentrations of vitamin C improved impaired vascular responses to acetylcholine following LPS. An explanation for these divergent results is not clear. There are potential limitations with the use of pharmacological scavengers or exogenous SOD, including cytotoxicity and uncertainties in subcellular access and the degree of scavenging of superoxide. In addition, scavengers of superoxide may have nonspecific effects. Even when an antioxidant has positive effects, as in the case of vitamin C in 1 study, the use of this strictly pharmacological approach provides no insight into subcellu-
lar localization of oxidative stress and site of action of superoxide.

The protection by overexpression of CuZn-SOD observed in this study suggests that increases in superoxide in intracellular or cytosolic compartments following LPS are functionally important. This conclusion is consistent with recent work, which suggests that superoxide contributes to intracellular protein tyrosine nitration (via its reaction with nitric oxide and production of peroxynitrite) in vascular muscle during inflammation. Potential sources of superoxide in inflammation, such as NAD(P)H oxidase, are known to increase superoxide intracellularly. Because superoxide is charged and may not easily diffuse across cell membranes, we hypothesized that overexpression of the isoform of SOD that is expressed in close proximity to these intracellular increases in superoxide would be protective in inflammation. Our results are consistent with this hypothesis in that overexpression of CuZn-SOD was very effective in protecting against LPS-induced increases in superoxide and endothelial dysfunction.

In addition to CuZn-SOD, 2 other isoforms of SOD are expressed within the vessel wall: mitochondrial or Mn-SOD (SOD-2), and extracellular or EC-SOD (SOD-3). Within blood vessels, the predominant isoform of SOD is CuZn-SOD, accounting for approximately 50% to 80% of total SOD activity. Constitutive levels of these SODs were not sufficient to prevent increases in superoxide and endothelial dysfunction in response to a high concentration of LPS in vessels from non-Tg (normal) animals (ie, LPS increased superoxide and produced endothelial dysfunction in this and in previous studies). In SOD-Tg mice, levels of CuZn-SOD protein and SOD activity are increased several-fold (present study). This level of overexpression of CuZn-SOD was sufficient to prevent LPS-induced vascular dysfunction.

It is worth noting that overexpression of any SOD will not always be beneficial or protective, because higher levels of hydrogen peroxide result from increased expression of SOD and hydrogen peroxide can have deleterious effects in some systems. Thus, although it was possible that vascular dysfunction would not be improved by overexpression of CuZn-SOD, we did not find this to be the case. Endothelial function was not altered by overexpression of CuZn-SOD per se (in the absence of LPS) and endothelium was protected from LPS-induced dysfunction.

In summary, overexpression of CuZn-SOD protects against LPS-induced increases in superoxide and vascular dysfunction. These results complement previous work demonstrating that overexpression of this isoform of SOD attenuates/protects against vasospasm after subarachnoid hemorrhage as well as endothelial dysfunction produced by ceramide and overexpression of amyloid precursor protein. SOD-Tg mice are also protected from increases in vascular superoxide, peroxynitrite, and blood pressure in response to angiotensin III. Overall, these studies suggest that overexpression of CuZn-SOD is very effective in attenuating oxidative stress and in attenuating vascular dysfunction in several disease models.

Acknowledgments

This work was supported by National Institutes of Health grants HL-38901, HL-62984, and NS-24621. Dr Sean Didion was funded by DK-25295 and a National Scientist Development Grant from the American Heart Association (0230327N). Genotyping was performed by Norma Sinclair in the University of Iowa Transgenic Facility under the direction of Dr Curt Sigmund. We thank Dr Larry Oberley for providing the CuZn-SOD antibody.

References


Overexpression of CuZn-SOD Prevents Lipopolysaccharide-Induced Endothelial Dysfunction

Sean P. Didion, Dale A. Kinzenbaw, Pamela E. Fegan, Lisa A. Didion and Frank M. Faraci

Stroke. 2004;35:1963-1967; originally published online June 24, 2004; doi: 10.1161/01.STR.0000132764.06878.c5

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/35/8/1963