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Frank M. Faraci and Sean P. Didion

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Vascular Protection

Superoxide Dismutase Isoforms in the Vessel Wall

Frank M. Faraci, Sean P. Didion

Abstract—Blood vessels express 3 isoforms of superoxide dismutase (SOD): cytosolic or copper-zinc SOD (CuZn-SOD), manganese SOD (Mn-SOD) localized in mitochondria, and an extracellular form of CuZn-SOD (EC-SOD). Because there are no selective pharmacological inhibitors of individual SOD isoforms, the functional importance of the different SODs has been difficult to define. Recent molecular approaches, primarily the use of genetically-altered mice and viral-mediated gene transfer, have allowed investigators to begin to define the role of specific SOD isoforms in vascular biology. This review will focus mainly on the role of individual SODs in relation to endothelium under normal conditions and in disease states. This area is important because reactive oxygen species and superoxide anion are thought to play major roles in changes in vascular structure and function in pathophysiology. **(*Arterioscler Thromb Vasc Biol.* 2004;24:1367-1373.)**

Key Words: reactive oxygen species ■ endothelium ■ nitric oxide ■ superoxide dismutase ■ peroxynitrite

Superoxide anion (O_2^-) and other reactive oxygen species (ROS) play a major role in vascular biology. In general, relatively low concentrations of ROS are thought to act as mediators or modulators of cell signaling and contribute to other key functions, such as regulation of activity of transcription factors and gene expression.[1–3] In contrast, higher levels of ROS contribute to vascular dysfunction and abnormal cell growth including hypertrophy of vascular muscle. Superoxide levels are increased in blood vessels in many pathophysiological conditions including hypertension, atherosclerosis, diabetes, hyperhomocysteinemia, heart failure, sepsis, subarachnoid hemorrhage, and Alzheimer disease, as well as during aging. Since the initial evidence that superoxide or other ROS inactivate nitric oxide (NO) or endothelium-derived relaxing factor (EDRF),[4] many studies have suggested that inactivation of NO by superoxide contributes to vascular dysfunction under pathophysiological conditions.

Steady-state levels of superoxide are dependent on both its rate of production as well as activity of the various superoxide dismutases (SODs). The goal of this review is to briefly summarize the role of SODs in relation to vascular biology with an emphasis on endothelium. This summary will mainly focus on highlighting recent work from an emerging field, the functional importance of specific SOD isoforms in vascular protection.

Superoxide Dismutases: Basic Characteristics and Functions

In mammals, there are 3 isoforms of SOD,[5] and each are products of distinct genes but catalyze the same reaction:

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From the Departments of Internal Medicine and Pharmacology, Cardiovascular Center, University of Iowa, Carver College of Medicine, Iowa City, Iowa.
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Correspondence to Frank M. Faraci, PhD, Department of Internal Medicine, E318-2 GH, University of Iowa, Carver College of Medicine, Iowa City, IA 52242-1081. E-mail frank-faraci@uiowa.edu
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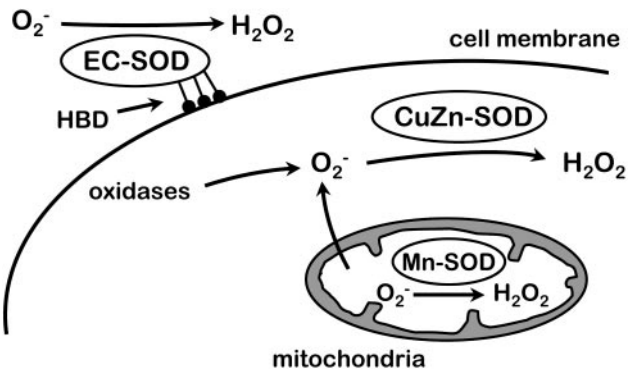

Figure 1. Schematic illustration of the subcellular localization of the 3 SOD isoforms: CuZn-SOD located primarily in cytosol, manganese SOD (Mn-SOD) localized in mitochondria, and extracellular CuZn-SOD (EC-SOD). All 3 isoforms of SOD catalyze the same reaction, producing H_2O_2 from O_2^-. Superoxide is produced by mitochondria, a variety of oxidases (NADPH oxidase, xanthine oxidase, cyclooxygenase, lipoxygenase, etc), as well as autooxidation of some molecules (not shown). HBD indicates heparin binding domain.

$$2H^+ + 2O_2^- \rightarrow H_2O_2 + O_2$$

The 3 isoforms of SOD are cytosolic or copper-zinc SOD (CuZn-SOD or SOD-1), manganese SOD (Mn-SOD or SOD-2) localized in mitochondria, and an extracellular form of CuZn-SOD (EC-SOD or SOD-3) (Figure 1). Although the subcellular localization of each isoform of SOD is unique, only very recently have studies begun to focus on the functional importance of individual SOD isoforms within the vessel wall under normal conditions or during vascular disease. Expression and activity of SODs presumably have a profound effect on responses of vascular cells to both acute and chronic oxidative stress. Compartmentalization of various ROS may be very important in relation to overall effects. Recent studies in nonvascular cells suggest the different isoforms of SOD have major but distinctive roles.[5,6]

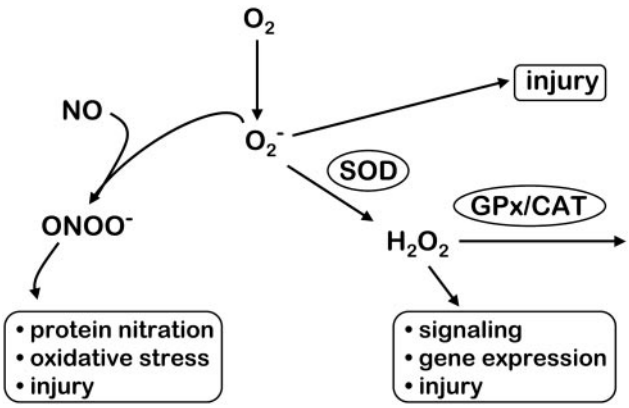

Figure 2. Schematic illustration of the interrelationships of various ROS and NO. O_2^- is produced from molecular oxygen (O_2) by a variety of sources (see Figure 1) and can directly produce injury or can be converted by SOD to H_2O_2. H_2O_2 is an important signaling molecule, but in combination with Fe^{2+}, H_2O_2 can also produce injury by forming hydroxyl radical, a highly reactive ROS (not shown). H_2O_2 can also be degraded by various glutathione peroxidases (GPx) or catalase (CAT). Superoxide can react with NO to form peroxynitrite ($ONOO^-$). This extremely efficient reaction results in a decrease in NO bioavailability and normal NO-mediated signaling. In addition, $ONOO^-$ can indirectly produce additional increases in superoxide and oxidative as a result of effects on Mn-SOD, poly (ADP-ribose) polymerase, iNOS, tetrahydrobiopterin, and the zinc-thiolate complex within eNOS (resulting in eNOS uncoupling) (see text for discussion).

As indicated above, SODs dismute superoxide into hydrogen peroxide plus molecular oxygen. There are several functional consequences of this enzymatic activity. First, SODs protect against superoxide-mediated cytotoxicity, such as inactivation of mitochondrial proteins containing iron–sulfur (Fe–S) centers (eg, aconitase and fumarase) (Figure 2).[5] Such interactions are of potential importance, as damage to such complexes results in release of free iron and subsequent formation for hydroxyl radical (a highly reactive ROS).

NO reacts with superoxide at a rate 3 times faster than dismutation of superoxide by SOD (Figure 2).[7,8] Because of the efficiency of the reaction (superoxide reacts with NO more efficiently than with any other known molecule), the local concentration of SOD is a key determinant of bioactivity (the biological half-life) of NO. Thus, a second major function of SOD is to protect NO and NO-mediated signaling. Multiple lines of evidence have shown that NO signaling plays a major role in vascular biology. In addition to inactivating NO and thus preventing NO-mediated signaling, the reaction of NO with superoxide produces peroxynitrite, a potent oxidant with the potential to produce cytotoxicity.[7] Emerging evidence suggests that formation of peroxynitrite has multiple effects (Figure 2), including (1) selective nitration of tyrosine residues in proteins, such as prostacyclin synthase and Mn-SOD,[9–11] (2) activation of poly (ADP-ribose) polymerase (PARP) and expression of inducible NO synthase (iNOS), potentially important mediators of vascular dysfunction in disease states,[11–15] (3) oxidation of tetrahydrobiopterin,[16] and (4) oxidation of the zinc–thiolate complex in endothelial NOS (eNOS).[17] The latter 2 effects can produce eNOS "uncoupling," a condition in which the normal flow of electrons within the enzyme is diverted such that eNOS produces superoxide rather than NO.

A third functional consequence of SOD activity is formation of hydrogen peroxide. The importance of this ROS within vascular cells is becoming increasingly apparent. Hydrogen peroxide is relatively stable and diffusible (including through cell membranes), compared with many other ROS. These features make hydrogen peroxide somewhat analogous to NO as a signaling molecule. For example, hydrogen peroxide is a signaling molecule and regulator of gene expression and may be an important mediator of hypertrophy of vascular muscle in response to stimuli such as angiotensin II.[18,19] Hydrogen peroxide can activate select transcription factors and may also function as an endothelium-derived hyperpolarizing factor (EDHF) in some blood vessels,[20–23] but has also been suggested to be an EDRF without functioning as an EDHF.[24] In combination with some transition metals like iron or copper, hydrogen peroxide can react to form hydroxyl radical, a highly reactive ROS, and thus produce cellular injury via the Fenton reaction (Figure 2). Hydrogen peroxide mediated effects and local concentrations are regulated by activity of the various glutathione peroxidases or catalase (Figure 2).

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Thus, SOD plays an important role in vascular biology. Because compartmentalization of superoxide presumably is of fundamental importance in relation to overall effects, the functional importance of individual SOD isoforms has begun to be studied and will now be discussed.

CuZn-SOD (SOD-1)

The CuZn isoform of SOD appears to be expressed at relatively high levels in all cells including blood vessels where it is the predominant isoform of SOD (when expressed as percent of total SOD activity). For example, in normal mouse aorta, both biochemical approaches and results from CuZn-SOD–deficient mice indicate that activity of CuZn-SOD accounts for 50% to 80% of total SOD activity.[25–30] Mn-SOD accounts for approximately 2% to 12% of total vascular SOD, and EC-SOD accounts for the remainder.[25,26,28] A similar pattern of expression was observed in human arteries.[25,31]

Until recently, the functional importance of specific SOD isoforms has been difficult to define. Many pharmacological studies have used diethyldithiocarbamate (DDC or DETC, an inhibitor Cu-containing SODs). These studies support the concept that normal activity of CuZn-SOD or EC-SOD or both are necessary to limit increases in superoxide, allowing release of NO from endothelium and normal endothelium-dependent relaxation. Interpretation of these findings is limited, however, as DDC inhibits both CuZn-SOD and EC-SOD,[32] thus preventing conclusions about the functional importance of individual isoforms of SOD. In addition, because it is a chelator of Cu^{2+}, DDC may exhibit effects unrelated to inhibition of SOD.

Recent findings in gene-targeted mice provided direct evidence regarding the functional importance of CuZn-SOD. Deficiency in CuZn-SOD results in increased levels of vascular superoxide and peroxynitrite, increased myogenic tone, augmented vasoconstrictor responses, and impaired endothelium-dependent (NO-mediated) relaxation in both large arteries and microvessels.[29,33,34] Increases in vascular permeability after ischemia are greatly enhanced in CuZn-SOD–deficient mice.[35] Alterations in expression of CuZn-SOD may also impact vascular structure. For example, preliminary evidence suggests that deficiency in CuZn-SOD produces hypertrophy of cerebral arterioles.[36] These findings provide the first direct evidence that CuZn-SOD normally inhibits vascular hypertrophy, and the findings are conceptually interesting as they raise questions regarding the importance of specific ROS species in mediating hypertrophy of vascular muscle in vivo.

Genetically-altered mice and rats have been generated which overexpress CuZn-SOD.[30,37] Compared with nontransgenic controls, mRNA for CuZn-SOD and SOD activity are increased several-fold in vascular and nonvascular tissue in these models.[30,37–39] Mice that overexpress CuZn-SOD have been shown to be protected against vascular dysfunction in models of subarachnoid hemorrhage[40] and hypoxia with reoxygenation[38] as well as in response to ceramide,[41] lipopolysaccharide (LPS),[39] and overexpression of β-amyloid precursor protein.[42] Angiotensin II–induced expression of monocyte chemoattractant protein (MCP-1) and monocyte infiltration into the vessel wall are inhibited in CuZn-SOD transgenic mice.[43] Adenoviral-mediated gene transfer has also been used to overexpress CuZn-SOD. Using this technology, CuZn-SOD has been shown to decrease vascular superoxide levels in atherosclerosis and diabetes,[44,45] to improve endothelial function in diabetes,[44] and to protect in a model of fluid percussion injury that produces impairment of autoregulation.[46]

Expression of CuZn-SOD in vascular cells may change under a variety of conditions. For example, levels of CuZn-SOD are higher in some arteries in females[47] and may increase with aging.[48] CuZn-SOD expression is increased in endothelium by shear stress in vitro[49] and by exercise,[50] but can be downregulated in vivo in regions of the vasculature with disturbed blood flow.[51] Activation of peroxisome proliferator activated receptors (PPAR) increases expression of CuZn-SOD in endothelium.[52] Thus, increases in CuZn-SOD may contribute to mechanisms by which exercise and activation of PPARγ exert protective effects within the vasculature. Although expression or activity of CuZn-SOD or both are increased within the vessel wall in some models of hypertension,[53,54] the functional importance of these changes are not known. Initial work with transgenic mice suggests that overexpression of CuZn-SOD effectively prevents increases in vascular superoxide and attenuates increases in arterial pressure in response to angiotensin II.[55]

The impact of changes in expression of CuZn-SOD in vascular disease can be more complex than simply affecting NO-bioavailability and signaling. For example, overexpression of CuZn-SOD protects vascular muscle from oxidized LDL–induced DNA fragmentation and caspase activation[30] and attenuates expression of iNOS in endothelium and adventitia of cerebral arteries after subarachnoid hemorrhage.[56] Such effects have broader implications when one considers the implications of DNA protection as well as recent evidence that iNOS may be an important mediator of endothelial dysfunction in some disease states.[14,15,57]

As mentioned above, there is increasing evidence that hydrogen peroxide may function as an EDHF in some blood vessels. Recent experiments in gene-targeted mice suggest that CuZn-SOD specifically may function as an "EDHF-synthase," as it appears to be the major source of hydrogen peroxide in small mesenteric arteries.[23]

Although the use of genetically-altered mice is providing new insight into the functional importance of specific isoforms of SOD, it is important to recall that genetically-altered mice may express compensatory mechanisms that affect their overall phenotype.[58] In this regard, it is noteworthy that deficiency in any single isoform of SOD in blood vessels does not result in detectable compensatory expression by the remaining SOD isoforms.[23,30,59]

Mn-SOD (SOD-2)

Under normal conditions, the mitochondrial electron transport chain is a major source of superoxide, converting up to perhaps 5% of molecular O_2 to superoxide.[5] Because of its subcellular localization, Mn-SOD is considered to be a first line of defense against oxidative stress.[5] This conclusion is supported by the finding that mice completely deficient in

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Mn-SOD die within a few weeks after birth and exhibit a variety of phenotypes (depending on the genetic background) including neurodegeneration, cardiac abnormalities, and extensive mitochondrial damage.[5] On a percentage basis, the overall levels of Mn-SOD in blood vessels are smaller compared with CuZn- and EC-SOD. That only the homozygous Mn-SOD–deficient mouse (of the 3 SOD isoforms) has a lethal phenotype suggests that this simple comparison is misleading. It is important to note that endothelium expresses high levels of Mn-SOD.[60] Thus, when total activity of Mn-SOD is measured in intact vessels, relatively high levels of Mn-SOD in endothelium may be masked by other cell types, which may express lower levels of the protein (but which make up a much greater proportion of vessel wall). In addition to heterogenous expression of Mn-SOD within the vessel wall, there may be regional or segmental differences in expression.[61,62] For example, levels of Mn-SOD are higher in cerebral arteries than in carotid artery or aorta.[61] Finally, there are sex-related differences in expression of Mn-SOD in vessels.[47] Estrogen increases expression of Mn-SOD (and EC-SOD) in vascular muscle. Levels of both Mn-SOD and EC-SOD in aorta are decreased by ovariectomy and restored by estrogen replacement.[63]

Vascular expression or activity of Mn-SOD or both may be altered under several physiological and pathophysiological conditions. For example, Mn-SOD is particularly responsive to and upregulated by oxidative stress.[9] The promoter region of the Mn-SOD gene contains response elements for the redox-sensitive transcription factors activator protein-1 (AP-1) and nuclear factor-κB (NF-κB), the latter being critical in regulation of many inflammatory related genes.[9]

Disease states associated with vascular oxidative stress also have altered vascular expression of Mn-SOD. For example, in vascular tissue, LPS and proinflammatory cytokines increase superoxide and expression of Mn-SOD.[9,60,64–66] Vascular expression of CuZn-SOD and Mn-SOD mRNA change in a temporal pattern during atherosclerosis (initially increase, then decrease over time).[67,68] There is a marked increase in Mn-SOD expression in cerebral arteries in bacterial meningitis,[69] and Mn-SOD expression is increased in the vessel wall in models of chronic hypertension.[53,54,70] In nonvascular tissue, Ang II increases levels of Mn-SOD protein but also decreases activity of MnSOD caused by peroxynitrite-induced tyrosine protein nitration.[10] Thus, the functional importance of Mn-SOD in Ang II-induced hypertension is unknown and difficult to predict. Similarly, there was no change in levels of Mn-SOD protein but an increase in tyrosine-nitrated Mn-SOD in aorta (predominantly in endothelium) of old rats.[71] Inactivation of Mn-SOD by peroxynitrite is potentially a key issue (Figure 2), as peroxynitrite increases within blood vessels during a variety of conditions including inflammation, diabetes, hypertension, atherosclerosis, and subarachnoid hemorrhage, as well as aging.

In most cases, the functional significance of these differences in vascular expression or activity of Mn-SOD or both are unknown. Exceptions are recent studies of Mn-SOD heterozygous-deficient mice, which indicate that Mn-SOD normally protects against vascular mitochondrial damage and development of atherosclerosis[72] as well as oxidized LDL–induced DNA fragmentation and caspase activation in vascular muscle.[30]

Overexpression of Mn-SOD using viral and liposomal-mediated gene transfer has produced beneficial effects in several models of vascular disease. Using these approaches, overexpression of Mn-SOD reduces superoxide levels and improves endothelial function in hypercholesterolemia, diabetes, and a low-renin model of hypertension,[44,73,74] while also preventing hyperglycemia-induced formation of reactive oxygen species in endothelium.[75]

EC-SOD (SOD-3)

EC-SOD is the only isoform of SOD that is expressed extracellularly, binding to tissues via its heparin-binding domain that provides affinity of the protein for heparan sulfate proteoglycans on cell surfaces, in basal membranes, and in the extracellular matrix (Figure 2).[76–78] EC-SOD is localized throughout the vessel wall, particularly between endothelium and vascular muscle.[25,77,79] The major source of the protein is thought to be vascular muscle.[25,79,80] Endothelium does not appear to produce EC-SOD.[79]

Unlike some tissue, such as brain,[6] EC-SOD accounts for a large portion of total SOD activity in blood vessels (see discussion related to CuZn-SOD).[25,27,28,31,81] Expression of EC-SOD in vascular cells and within the vessel wall can be altered in response to a variety of stimuli including exercise, growth factors, cytokines, vasoactive stimuli including angiotensin II and NO, and homocysteine as well as during hypertension, atherosclerosis, and diabetes.[26,28,31,79,80,82–85] In contrast to CuZn-SOD, expression of EC-SOD has been reported to increase in regions of the vasculature with disturbed blood flow.[51] In addition to changes in production or excretion of EC-SOD or both, the binding of EC-SOD to tissue can be altered by factors such as NO and homocysteine[86,87] as well as genetic factors such as polymorphisms in the heparin binding domain.[79,80]

Measurements of EC-SOD release into plasma in response to heparin are commonly used as an index of vascular bound EC-SOD. It should be noted that the amount of EC-SOD that is released by heparin using this approach is thought to be only a small fraction of total vascular EC-SOD.[31,88] Although this approach, along with measurements of EC-SOD expression or activity, has been used in numerous studies, there is still relatively little known about the functional importance of this SOD isoform. Based on its extracellular localization, it has been hypothesized that at least 1 major function of EC-SOD is to protect NO as it diffuses from endothelium to its major target (soluble guanylate cyclase) in vascular muscle.[77,89] Although defined as being extracellular, some evidence suggests that EC-SOD may also by expressed intracellularly.[32,90]

As mentioned above, DDC has been used commonly in studies of vascular biology, but DDC does not distinguish between EC-SOD and CuZn-SOD in its effects.[32] Thus, other approaches are needed to define the role of EC-SOD. To date, these approaches have consisted mainly of studies using genetically-altered mice and overexpression of EC-SOD using viral-mediated gene transfer. In aorta of EC-SOD–

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deficient mice, there was increased superoxide, impaired basal activity of NO, and impaired endothelium-dependent relaxation.[59] Responses to an endothelium-dependent agonist (acetylcholine) is that these mice are not altered in small pulmonary arteries[91] and only very modestly attenuated in the cerebral microcirculation.[92] Deficiency in EC-SOD does not alter baseline blood pressure[59,93] but increases arterial pressure in 2 models of hypertension that are greater in EC-SOD–deficient mice than in controls.[59] Vasoconstrictor responses to serotonin are augmented in EC-SOD–deficient mice.[91]

Studies using overexpression strategies have revealed protective effects of EC-SOD on blood vessels. Gene transfer of EC-SOD reduced vascular superoxide levels during atherosclerosis[45] in spontaneously hypertensive rats (SHR)[94] and in an LPS-induced model of inflammation.[95] Effects of overexpression of EC-SOD using this approach on endothelial function have varied. For example, gene transfer of EC-SOD increased basal NO bioactivity in stroke-prone SHR,[96] enhanced endothelium-dependent relaxation in SHR[94] and after LPS,[95] but did not improve endothelial function in atherosclerosis.[45] The presence of the heparin-binding domain was necessary for EC-SOD to exert protective effects.[94,95] Overexpression of EC-SOD with this viral approach or using transgenic mice attenuates vasospasm after subarachnoid hemorrhage.[97,98]

It has been suggested that EC-SOD is the major determinant of NO bioavailability in blood vessels.[79] In this regard, it is noteworthy that effects of deficiency in EC-SOD and CuZn-SOD on endothelial function are similar.[29,34,59] Thus, to protect NO over its entire diffusion route (from site of production within endothelium to its major molecular target in vascular muscle), normal expression of both CuZn-SOD and EC-SOD may be essential.

In relation to EC-SOD, it is important to recall that most previous studies of vascular oxidative stress have been performed in the rat, and there are species differences in relation to vascular EC-SOD content. For example, whereas blood vessels from mice and humans have similar levels of EC-SOD,[25] the rat has very low vascular levels of EC-SOD compared with most other mammalian species that have been studied.[25] This phenotype in the rat is caused by a difference in a key amino acid affecting protein subunit interaction.[32] In contrast to other species, rat EC-SOD has a low affinity for heparin and does not bind to heparan sulfate under physiological conditions. Thus, the rat essentially lacks vascular EC-SOD. Studies of blood vessels in the rat therefore have a potential limitation in that the species may not be particularly representative of other species, including humans, that express normal levels of EC-SOD in the vasculature. Conversely, this phenotype in the rat may be advantageous for experimental studies, as these animals may exhibit increased susceptibility to oxidative stress (ie, greater endothelial dysfunction, larger increases in arterial pressure, etc) than other commonly studied animal models (or humans). In this context, it may seem surprising that rats are very resistant to development of atherosclerosis,[99] as deficiency in EC-SOD might be expected to promote atherosclerosis. However, recent work in gene targeted mice suggests that EC-SOD deficiency has no effect on development of atherosclerosis.[100] Thus, other differences in gene expression or genetic background are more likely to account for resistance to atherosclerosis in rats. Genetic background is known to have a major impact of susceptibility to atherosclerosis in mice.[101,102]

In summary, blood vessels express 3 isoforms of SOD: CuZn-SOD, Mn-SOD, and EC-SOD. Insight into the role of individual SOD isoforms is beginning to emerge, primarily as a result of application of 2 experimental approaches (the use of viral mediated gene transfer and genetically-altered mice). Considering the relative lack of knowledge regarding the role of SOD isoforms in various disease states, these approaches should be insightful for some time. In the future, additional approaches such as the use of RNA interference to produce mRNA knockdown as well as genetic models allowing temporal or spatial control or both of gene expression with the vessel wall should provide further insight into role of SODs in vascular biology.

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