

Superoxide Dismutases: Role in Redox Signaling, Vascular Function, and Diseases

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Abstract

Excessive reactive oxygen species (ROS), especially superoxide anion ($O_2^{\bullet-}$), play important roles in the pathogenesis of many cardiovascular diseases, including hypertension and atherosclerosis. Superoxide dismutases (SODs) are the major antioxidant defense systems against $O_2^{\bullet-}$, which consist of three isoforms of SOD in mammals: the cytoplasmic Cu/ZnSOD (SOD1), the mitochondrial MnSOD (SOD2), and the extracellular Cu/ZnSOD (SOD3), all of which require catalytic metal (Cu or Mn) for their activation. Recent evidence suggests that in each subcellular location, SODs catalyze the conversion of $O_2^{\bullet-}$ to H_2O_2 , which may participate in cell signaling. In addition, SODs play a critical role in inhibiting oxidative inactivation of nitric oxide, thereby preventing peroxynitrite formation and endothelial and mitochondrial dysfunction. The importance of each SOD isoform is further illustrated by studies from the use of genetically altered mice and viral-mediated gene transfer. Given the essential role of SODs in cardiovascular disease, the concept of antioxidant therapies, that is, reinforcement of endogenous antioxidant defenses to more effectively protect against oxidative stress, is of substantial interest. However, the clinical evidence remains controversial. In this review, we will update the role of each SOD in vascular biologies, physiologies, and pathophysiologies such as atherosclerosis, hypertension, and angiogenesis. Because of the importance of metal cofactors in the activity of SODs, we will also discuss how each SOD obtains catalytic metal in the active sites. Finally, we will discuss the development of future SOD-dependent therapeutic strategies. *Antioxid. Redox Signal.* 15, 000–000.

Introduction

AEROBIC ORGANISMS POSSESS antioxidant defense systems that deal with reactive oxygen species (ROS) produced as a consequence of aerobic respiration and substrate oxidation. In the process of normal cellular metabolism, oxygen undergoes a series of univalent reductions, leading sequentially to the production of $O_2^{\bullet-}$, hydrogen peroxide (H_2O_2), and H_2O . Potential enzymatic source of ROS includes components of the mitochondrial electron transport chain, xanthine oxidase, the cytochrome p450 monooxygenases, lipoxygenase, nitric oxide synthase (NOS), and the NADPH oxidase (83) (Fig. 1). Superoxide anion is dismutated by superoxide dismutases (SODs) to H_2O_2 that is catalyzed to H_2O by catalase, peroxidases (Prxs), or glutathione peroxidases (GPx) (Fig. 1). Low levels of either intracellular or extracellular ROS (e.g., superoxide and H_2O_2) are indispensable in many biochemical processes, including intracellular signaling, defense against microorganisms, and cell function (74, 125, 223). In contrast, high dose and/or inadequate removal of ROS, especially superoxide anion, results in oxidative stress, which has been implicated in the pathogenesis of many cardiovascular dis-

eases, including hypercholesterolemia, atherosclerosis, hypertension, diabetes, and heart failure. ROS also represent a component of the innate immune system, and they are not only involved in the respiratory burst of neutrophils, but also signal inflammatory cell chemotaxis into sites of inflammation (20).

Importantly, nitric oxide (NO), which has anti-inflammatory and anticoagulant properties as well as vasodilator effect, can be rapidly inactivated by reaction with $O_2^{\bullet-}$ and leading to the production of the strong oxidant peroxynitrite ($ONOO^-$). This reaction is important in common conditions leading to endothelial and mitochondrial dysfunction, including hypercholesterolemia, hypertension, diabetes, and aging, in which vascular production of $O_2^{\bullet-}$ is increased (83, 146). The major cellular defense against $O_2^{\bullet-}$ and peroxynitrite is a group of oxidoreductases known as SODs, which catalyze the dismutation of $O_2^{\bullet-}$ into oxygen and H_2O_2 . In mammals, there are three isoforms of SOD (SOD1 [CuZnSOD]; SOD2 [MnSOD]; SOD3 [ecSOD]) (Table 1), and each is a product of distinct genes and distinct subcellular localization, but catalyzes the same reaction. This distinct subcellular location of these SOD isoforms is particularly important for

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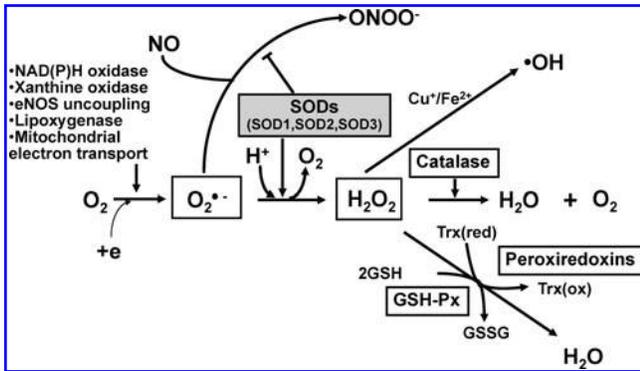


FIG. 1. Generation and metabolism of reactive oxygen species (ROS). Superoxide ($O_2^{\bullet-}$) is produced by NADPH oxidase, xanthine oxidase, nitric oxide synthase (NOS), lipoxygenase, and mitochondrial electron transport. Superoxide is converted by superoxide dismutase (SOD) to H_2O_2 , which, in turn, is reduced to water by catalase, glutathione peroxidases (GPx), and peroxiredoxins (Prx). In the presence of reduced transition metal (Fe^{2+} , Cu^+), H_2O_2 can undergo spontaneous conversion to hydroxyl radical ($OH\bullet$), or related metal-associated reactive species, which is extremely reactive. Importantly, nitric oxide (NO) can be rapidly inactivated by reaction with $O_2^{\bullet-}$ and leading to the production of the strong oxidant peroxynitrite ($ONOO^-$). Thus, SOD is a first line of defense against toxicity of superoxide anion radicals. The enzyme also participates in cell signaling *via* regulating ROS (e.g., $O_2^{\bullet-}$, H_2O_2) and available NO.

compartmentalized redox signaling. The mechanism of dismutation of $O_2^{\bullet-}$ to H_2O_2 by SOD involves alternate reduction and reoxidation of a redox active transition metal, such as copper (Cu) and manganese (Mn) at the active site of the enzyme as shown in Figure 2 (2). This indicates that SOD activity requires a catalytic metal. Thus, this review will discuss how SODs obtain catalytic metal in the active sites and update role of each SOD in vascular diseases, such as atherosclerosis and hypertension. Regarding other mechanisms of regulation of SODs and their role in diseases, please refer to other reviews (63, 156, 243, 251).

Basic Characteristic and Mechanism for Activation of SODs

SOD1 (cytosolic Cu/ZnSOD)

SOD1 is the major intracellular SOD (cytosolic Cu/ZnSOD) (Table 1). It exists as a 32 kDa homodimer and is mainly lo-

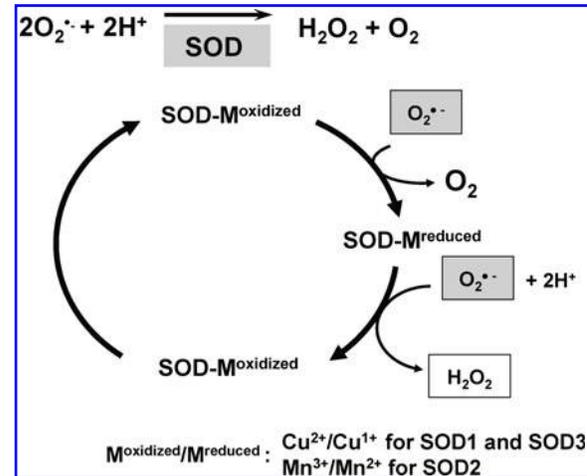


FIG. 2. Common mechanism of scavenging $O_2^{\bullet-}$ by SODs. Enzymatic activity of SOD involves alternate reduction and reoxidation of catalytic metal (*i.e.*, Cu or Mn) at the active site of the enzyme. Thus, Cu or Mn will be a key modulator of SOD activity of SOD1/SOD3 or SOD2, respectively.

calized in the cytosol with a smaller fraction in the inter-membrane space (IMS) of mitochondria (42, 175, 213). It has also been reported that SOD1 is also localized in nuclei, lysosomes, and peroxisomes, using immunocytochemical methods (33), and shows widespread distribution in a variety of cells (42). The enzyme is sensitive to cyanide, which helps to distinguish it from SOD2, which is relatively resistant. The human gene for SOD1 has been localized to the 21q22.1 region of chromosome 21 (136). Thus, it is responsible for Down syndrome (trisomy 21) and these patients have an extra copy of the gene and have SOD1 activity, which is 50% greater than the normal diploid population, in keeping with the gene-dosage effect. Transgenic rats containing an extra copy of the human SOD1 gene displays similar phenotype to Down syndrome, including the neurological defects and premature aging (60). Role of SOD1 in this disease remains unclear, but it is postulated that increased SOD1 activity elevates H_2O_2 levels, which becomes toxic (58). The most widely studied connection between SOD1 and human diseases involves the late onset neurodegenerative disease amyotrophic lateral sclerosis (ALS) (198). Over 100 mutations in the human gene SOD1 are now known to lead to some of the inherited forms of ALS, but its mechanisms remain unclear.

TABLE 1. SUPEROXIDE DISMUTASES IN VASCULAR TISSUE

Isoform	Characteristics	Metal cofactor	Metal delivery-related protein	Location
SOD1 (Cu/ZnSOD)	32 kDa, homodimer	Cu^{2+} (catalytic) Zn^{2+} (stability)	CCS, GSH Unknown	Cytoplasm, mitochondrial IMS, and others (nucleus, lysosomes, peroxisomes)
SOD2 (MnSOD)	96 kDa, homotetramer	Mn^{3+} (catalytic)	Unknown ^a	Mitochondria matrix
SOD3 (ecSOD)	135 kDa, homotetrameric secretory glycoprotein	Cu^{2+} (catalytic) Zn^{2+} (stability)	Atox1, ATP7A (MNK, Menkes ATPase) Unknown	Extracellular matrix, cell surface, extracellular fluids

^aSmf2p and MTM1 in yeast. SOD, superoxide dismutase.

Enzymatic activity of SOD1 depends on the presence of the Cu and Zinc (Zn). Zn participates in proper protein folding and stability. The SOD activity of remetalated derivatives is proportional to the amount of Cu bound in the native Cu site (155, 194). Further, Cu is not replaceable with another metal, whereas Zn is replaceable with cobalt and Cu, and it is not necessary for enzyme activity at low pH (155, 184, 227). More importantly, the mechanism of scavenging $O_2^{\bullet-}$ by SOD involves alternate reduction and reoxidation of the Cu at the active site of the enzyme as shown in Figure 2. Thus, SOD1 activity requires a catalytic Cu to scavenge $O_2^{\bullet-}$ (155). Under physiological conditions, the level of intracellular free Cu is extraordinarily restricted (192). Thus, soluble Cu carrier proteins termed “Cu chaperones” are required to directly transfer Cu to specific cellular targets (Fig. 3). SOD1 has been shown to

obtain catalytic Cu ion through interaction with the cytosolic Cu carrier protein CCS (Cu chaperone for SOD1), a Cu chaperone for SOD1, thereby increasing its activity (44). CCS activates SOD1 not only through Cu insertion, but also through disulfide bond formation. It consists of three functionally distinct protein domains (I, II, III) (172, 202) (Fig. 4). The central domain II resembles SOD1 and serves to dock CCS with SOD1. Once the CCS-SOD1 heterodimer has been formed, Cu insertion and disulfide oxidation may proceed *via* a CXC Cu binding motif at the C-terminal CCS domain III. The N-terminal Domain I of CCS1 including the MXCXXC Cu-binding site. Domain I is required for CCS activity, but its precise role is unclear. Although full activation of SOD1 requires CCS, there is a CCS-independent pathway, which involves GSH (27).

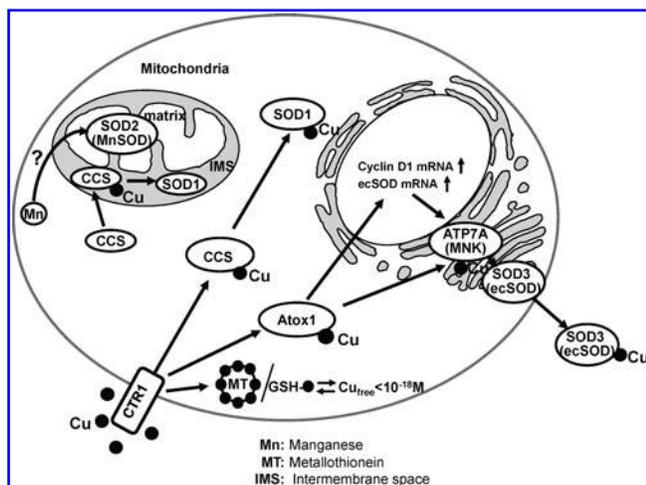


FIG. 3. Metal trafficking pathways to SODs in vascular tissue. Various SOD enzymes employ catalytic metal cofactor such as copper (Cu) and manganese (Mn) to carry out the disproportionation of superoxide. Under physiological conditions, the level of intracellular free Cu is extraordinarily restricted (192). Thus, once transported by Cu uptake transporter hCTR1, soluble cytosolic Cu carrier proteins termed “Cu chaperones” are required for trafficking Cu to specific Cu-containing enzymes through direct protein–protein interaction (116). Three copper chaperones have been characterized thus far: (i) uncharacterized Cu ligands and various Cu chaperones (Cox1, Cox 2, Cox 11, Cox 17, and Sco1), which deliver Cu to cytochrome c oxidase in the mitochondria (not shown); (ii) CCS (Cu chaperone for SOD1), which delivers copper to SOD1 in the cytosol and mitochondrial intermembrane space (IMS); and (iii) Atox1, which delivers copper to some of the secretory copper enzymes such as extracellular superoxide dismutase (ecSOD, SOD3) *via* the copper transporter ATP7A (Menkes ATPase, MNK) in the *trans*-Golgi network. In addition to its chaperone function, Atox1 also function as a Cu-dependent transcription factor for ecSOD and cyclin D1 (102, 103, 105). Thus, full activation of SOD3 requires both Cu chaperone function of Atox1 *via* ATP7A to obtain catalytic Cu as well as Cu-dependent transcription factor function for its transcriptional regulation (103, 105, 189, 190). Cytosolic concentrations of free Cu are typically maintained at exquisitely low levels (10^{-18} M) by metal scavenging systems, including metallothioneins (MT) and GSH (142, 192). Yeast genetic studies show that Smf2 p and Mtm1p are involved in Mn delivery to MnSOD, but role of these proteins in mammals remains unclear (44).

SOD2 (MnSOD)

SOD2 is a mitochondrial manganese (Mn) containing enzyme (MnSOD), which is composed of a 96 kDa homotetramer and localized in the mitochondrial matrix (66, 233) (Table 1). Mn at the active site of SOD2 serves to catalyze the disproportionation of $O_2^{\bullet-}$ to oxygen and H_2O_2 in a similar fashion as SOD1 and SOD3 (Cu/ZnSODs) (95) (Fig. 2). It is synthesized in the cytoplasm and directed to the mitochondria by a signal peptide (Fig. 4), where it is involved in dismutating $O_2^{\bullet-}$ generated by the respiratory chain of enzymes. The active site of SOD2 shows no homology to SOD1, but it is similar to that of the Fe SOD, which is commonly absent from eukaryotes (21). Both SOD2 and Fe-SOD share a common structure with the N-terminal helical hairpin and C-terminal alpha/beta domain (241). Within each subunit, both the N-terminal helical hairpin and C-terminal alpha/beta domains contribute ligands to the catalytic Mn site. Two identical four-helix bundles, symmetrically assembled from the N-terminal helical hairpins, form a novel tetrameric interface that stabilizes the active sites. Unlike SOD1, SOD2 does not exhibit product inhibition by H_2O_2 (10, 17). Also, SOD2 has a half-life in sera of 5–6 hr compared with 6–10 min for the SOD1 (78). The essential role of SOD2 in maintaining mitochondrial function is demonstrated by the neonatal lethality of mice with targeted disruption of the gene for SOD2. Such complete ablation of SOD2 causes dilated cardiomyopathy and neurodegeneration leading to early postnatal death (104, 133, 138).

Yeast genetic studies show that metal insertion process in SOD2 is quite different from that of SOD1, as expected from its distinct genomic and protein structure (44). In addition, it is also different from metal delivery to mitochondrial Cu-dependent enzyme cytochrome c oxidase (CCO), which involves the small ligand and various Cu binding proteins (Cox1, Cox2, Cox11, Cox17, Sco1, *etc.*) (195). For example, the intracellular SOD1 can obtain Cu post-translationally, by way of interaction with the CCS Cu chaperone, which also oxidizes an intrasubunit disulfide in SOD1. In contrast, metal insertion for SOD2 cannot occur post-translationally, but requires new synthesis and mitochondrial import of the SOD2 polypeptide (44). Further, in contrast to SOD1, SOD2 has modest selectivity for metal binding between Mn and Fe (159). In spite of this relatively promiscuous metal binding behavior, only the Mn form is catalytically active, reflecting a strict catalytic specificity for the metal ion. This stark contrast between low selectivity in metal binding and strict specificity for catalysis

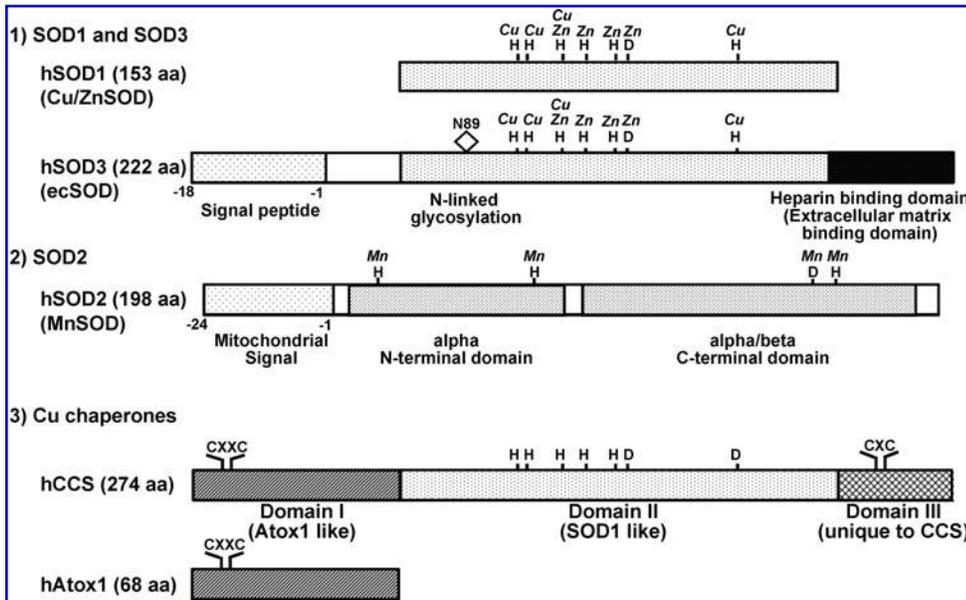


FIG. 4. A schematic alignments of each SOD isoform and Cu chaperones. (1) SOD1 (CuZnSOD) and the SOD3 (ecSOD) active site domain (amino acid residues 96–193) shares about 50% homology, such that all the ligands to Cu and Zn and the arginine in the entrance to the active site in SOD1 can be identified in this domain of SOD3. The distinct region of SOD3, as compared to SOD1, includes (a) an amino-terminal signal peptide, which permits secretion from the cell; (b) an N-linked glycosylation site at Asn-89, which is useful in the separation of SOD3 from SOD1 and greatly increases the solubility of the protein; (c) C-terminal region corresponding to heparin-binding

domain has a cluster of positively charged residues. This region is critical for binding to extracellular matrix, such as heparan sulfate proteoglycan. (2) SOD2 (MnSOD) protein is composed of three domains. First, N-terminal mitochondrial signal peptide directs protein synthesized in the cytoplasm to the mitochondria. Second, the active site of SOD2 contains Mn and has the N-terminal helical hairpin and C-terminal alpha/beta domain (241). They show no homology to SOD1 and SOD3, but it is similar to that of the Fe SOD, which is commonly absent from eukaryotes (21). (3) Cu chaperone CCS folds into three functionally distinct protein domains (123, 202). The N-terminal Domain I of CCS bears striking homology to Atox1, including the MXCXXC copper-binding site. The central domain of CCS (domain II) exhibits significant homology with its target of copper delivery, SOD1. Of note, this domain also share the strong homology with the central domain of SOD3, which is a catalytic site of it, such that all four of the zinc binding ligands of SOD1 and SOD3 and three of four histidine copper binding ligands are present in CCS. The C-terminal Domain III of CCS1 is quite small yet is extremely crucial for activating SOD1 *in vivo* (202). This peptide is highly conserved among CCS molecules from diverse species and includes an invariant CXC motif that can bind copper (202). Two identical four-helix bundles, symmetrically assembled from the N-terminal helical hairpins, form a novel tetrameric interface that stabilizes the active sites.

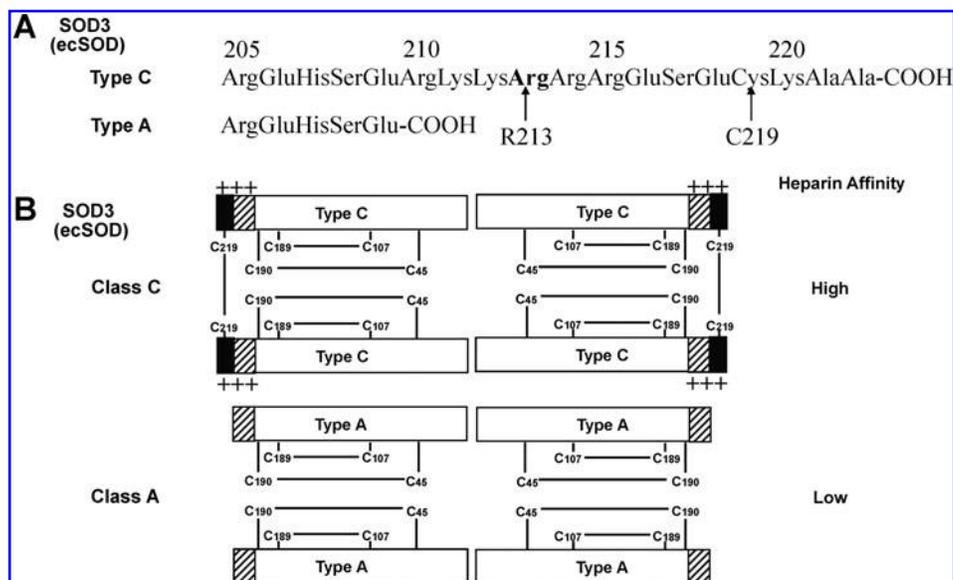
seems to be important for regulating SOD2 function (238, 239). *In vivo* activation of yeast SOD2 by Mn requires the Smf2p manganese transporter and Mtm1p. Smf2p provides Mn to mitochondria from intracellular vesicles, whereas Mtm1p prevents misincorporation of Fe for Mn in SOD2 (44). Role of these proteins in mammals remains unclear.

SOD3 (extracellular Cu/ZnSOD and ecSOD)

SOD3, a secretory extracellular Cu/Zn-containing SOD (ecSOD), is the major SOD in the vascular extracellular space (Table 1). In most species, SOD3 is a 135 kDa homotetramer composed of two disulfide-linked dimers (Fig. 5). The primary location of SOD3 in tissues is in the extracellular matrix and on cell surfaces with a smaller fraction in the plasma and extracellular fluids. Tissue SOD3 is thought to account for 90%–99% of the SOD3 in the body (152, 153). Tissue distribution varies among species, but in general SOD3 is expressed highly in selected tissues, such as blood vessels, the lung, kidney, uterus, and, to a lesser extent, in heart (65, 152, 178, 211). SOD3 activity is extremely sensitive to cyanide, like SOD1. In vascular tissue, SOD3 is mainly synthesized by vascular smooth muscle cells and fibroblasts (211). In injured tissue and atherosclerosis, SOD3 is also found in inflammatory cells (68, 143, 216). It is secreted and anchored to the extracellular matrix and endothelial cell surface through

binding to the heparan sulfate proteoglycan (HSPGs), collagen, and fibulin-5 (67, 167, 187). Thus, despite the fact that SOD3 is predominantly made by vascular smooth muscle cells rather than endothelial cells (67, 151, 211), it binds to the extracellular matrix such as heparan sulfates on the endothelial cell surface and can be internalized by endothelial cells (36, 174). In addition to signal peptide that permits secretion from the cell, SOD3 protein is composed of three functional domains (Fig. 4) (90). First, the amino terminal residues [1–95] contain an N-linked glycosylation site at Asn-89, which is useful in the separation of SOD3 from cytosolic SOD1 and greatly increases the solubility of the protein (56). Second, the amino terminal residues [96–193] contain active site, showing about 50% homology to SOD1. Indeed, all the ligands to Cu and Zn and the arginine in the entrance to the active site in SOD1 can be identified in this domain of SOD3. Third, the amino terminal residues [194–222] contain a C-terminal region corresponding to heparin-binding domain, which has a cluster of positively charged residues and is critical for binding to heparan sulfate proteoglycan. *In vivo*, both circulating (type A) and tissue bound (type C) are present, with the tissue bound form being about 99% of the total SOD3 (Fig. 5). Nonproteolyzed subunits are classified as type C subunits, whereas the proteolyzed subunits are classified as type A subunits. In some humans, there is a substitution of glycine for arginine at amino acid 213 (R213G) in this heparin binding

FIG. 5. Heparin binding affinity patterns of SOD3 (ecSOD) *in vivo*. (A) The sequence of the heparin-binding domain is present in the full-length type C subunit and confers the heparin-binding affinity to the protein. The truncated type A subunit has no affinity to heparin. Proteolytic processing leading to the appearance of truncated ecSOD *in vivo* can occur both intracellularly and in the extracellular space (59). (B) The subunit compositions of the two classes of ecSOD and their relative affinities to heparin are shown. In most species, ecSOD exists as a tetramer composed of two disulfide-linked dimers (63, 67). Two of these dimers are held together noncovalently. *In vivo*, both circulating (class A) and tissue bound (class C) are present, with the tissue bound (class C) being approximately 99% of the total ecSOD. Class B (not shown in the figure) of ecSOD reveals partial C-terminal truncation and medium heparin affinity. Class C of ecSOD consists of all four C-terminal intact subunits (type C) that contains two disulfide bonds linking two pairs of heparin-binding domains together. In contrast, class A of ecSOD consists of all four C-terminal truncated subunits (type A) that do not contain a disulfide bond to link two heparin-binding domains. Truncation of the C-terminal region does not affect the noncovalent protein-protein interactions stabilizing the tetramer, but affects the heparin binding properties significantly.



domain, which results in reduced heparan sulfate affinity and higher plasma levels (63, 67). Juul *et al.* demonstrated that in a prospective, population-based study of 9188 participants from The Copenhagen City Heart Study, heterozygosity for SOD3 with R213G is associated with increased risk of ischemic heart disease (110). Finally, the importance of SOD3 in the extracellular space is further stressed by the fact that 85% of mice were died within a week of induction of SOD3 ablation, which showed histological changes similar to those observed in adult respiratory distress syndrome, suggesting that SOD3 is essential for survival in the presence of ambient oxygen (75). Together with other studies using genetically altered mice and viral-mediated gene transfer, these findings indicate that SOD3 plays an important role in various oxidative stress-dependent pathophysiologies, including hypertension, heart failure, ischemia-reperfusion injury, and lung injury (63, 67, 191).

SOD3's active site is very similar to that of the cytosolic Cu/Zn SOD (SOD1) (7) (Fig. 4). Using recombinant human SOD3, we demonstrated that the specific activity of SOD3 is linearly related to its Cu content, suggesting that SOD3-specific activity is Cu-dependent (105). Interestingly, the domain II in CCS, which is a copper chaperone for SOD1, shares the strong homology with the central domain of SOD3, which is a catalytic site of it, such that all four of the Zn binding ligands of SOD1 and SOD3 and three of four histidine Cu binding ligands are present in CCS1 (Fig. 4). However, it is important to note that SOD3 is a secretory protein, and thus SOD3 need to get Cu in the secretory pathways instead of cytosol, suggesting that Cu chaperone for SOD3 should be different from CCS (Fig. 3).

Cu delivery pathway to SOD3 involves Cu chaperone Antioxidant-1 (Atox1), which is different from those for SOD1 (CCS) and for mitochondrial cytochrome c oxidase [small li-

gand, Cox 17, and others (38, 39)] (Fig. 3). In addition to Cu chaperone function, Atox1 serves as a transcription factor to increase SOD3 transcription (103). We also identified cyclin D1 gene as another downstream target for transcription factor Atox1, which stimulates Cu-dependent cell proliferation (102). Given that SOD3 is a major Cu-dependent antioxidant enzyme that regulates extracellular levels of $O_2^{\bullet-}$ and that Atox1 functions as a Cu-dependent transcription factor for SOD3 and cyclin D1, it is conceivable that Atox1 serves as a sensor of intracellular Cu concentrations to regulate cell proliferation by controlling SOD3 and cyclin D1 expression.

Given that SOD3 secretes through endoplasmic reticulum-Golgi pathway where Atox1 does not exist, the cytosolic Atox1 itself is required, but not sufficient for delivering Cu to SOD3 protein to regulate its full activity. ATP7A (Menkes ATPase, MNK) has been shown to transport cytosolic Cu to the secretory pathway *via* Atox1. We found that vascular ATP7A is required for full activation of SOD3, but not SOD1, by transporting Cu to SOD3 *via* Cu-dependent interaction with SOD3. Taken together, SOD3 activity requires the Cu chaperone, Atox1, but not CCS, which delivers Cu to SOD3 *via* interacting with the Cu transporter ATP7A at the trans-Golgi network (TGN) in vascular cells and tissue (105, 189, 190). Unlike SOD1, it forms stable tetramers with interchain disulfides that stabilize the quaternary structure (188). However, it remains unknown whether Cu transporters are involved in disulfide formation in a similar fashion to CCS.

Biological Effects of SODs

Vascular $O_2^{\bullet-}$ is produced normally as a byproduct of normal cellular metabolism and serves as a progenitor for a number of other ROS, including H_2O_2 and the $ONOO^-$ (Figs.

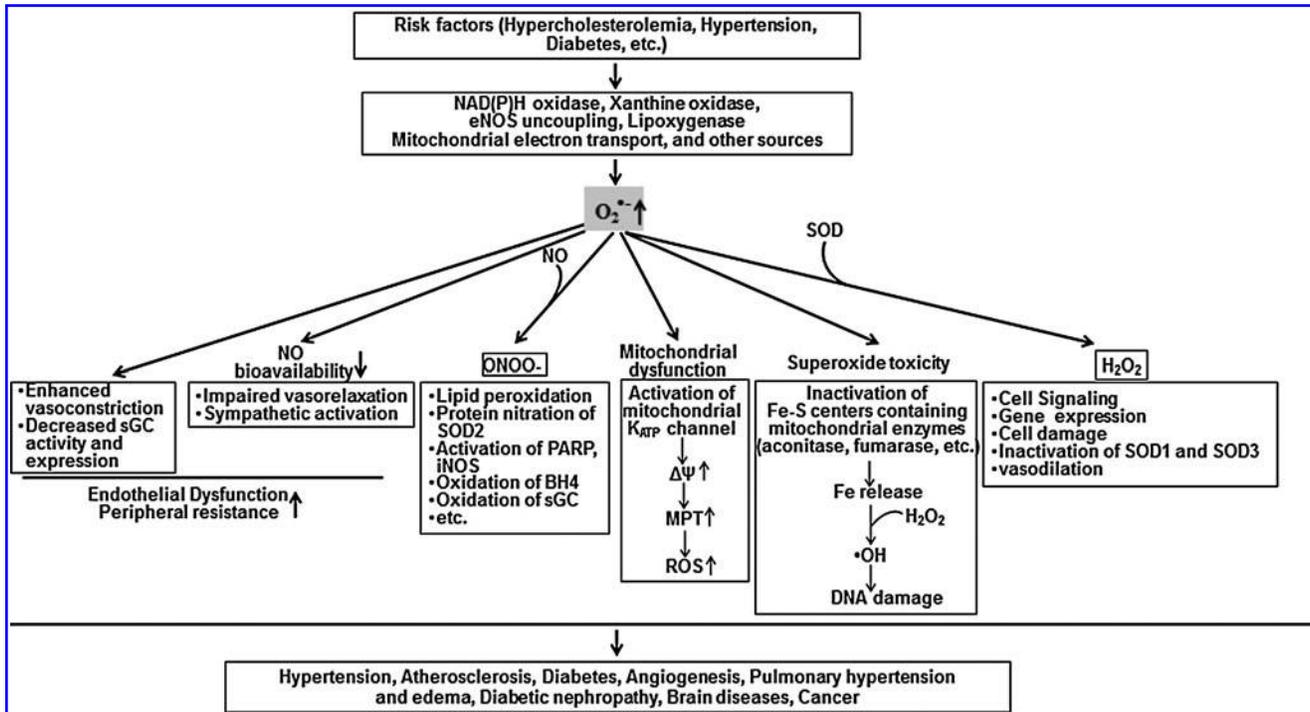


FIG. 6. Role of superoxide in various physiological and pathophysiological functions. SOD has a potential impact on various biological function and pathogenesis by regulating NO signaling, ROS ($O_2^{\bullet-}$, H_2O_2) signaling, and mitochondrial function.

1 and 6). In the vessel, alterations in levels of $O_2^{\bullet-}$ have been shown to modulate vascular tone, gene expression, inflammation, cellular growth, signaling, and apoptosis. SODs serve as the front-line defense against ROS in living cells, catalyzing the redox disproportionation of $O_2^{\bullet-}$ into H_2O_2 and molecular oxygen. There are several consequences of this enzymatic activity (Figs. 6–8).

Role of SOD in redox signaling

As shown in Figure 1, intracellular ROS levels are regulated by the balance between ROS-generating enzymes and antioxidant enzymes, which include SOD, catalase, GPx, and thioredoxin system. All of three SOD enzymes have various biological effects partially through H_2O_2 , the dismutation product of $O_2^{\bullet-}$, since H_2O_2 can function as a signaling molecule (193) (Fig. 7A). These ROS, including $O_2^{\bullet-}$ and H_2O_2 , can stimulate cellular responses (hypertrophy, proliferation, migration, *etc.*) *via* oxidation of signaling molecules (Akt, Src, PLCs, MAPK, *etc.*) or *via* oxidative inactivation of protein tyrosine phosphatases (PTPs), thereby promoting tyrosine phosphorylation-mediated redox signaling or regulating protein function (193, 218, 224). ROS can also activate redox sensitive transcription factors (*e.g.*, NFkB, AP-1, *etc.*) and MMPs, which contributes to vascular inflammation, angiogenesis, and extracellular matrix remodeling. Evidence reveals that the production of ROS by NADPH oxidase (Nox) complex has been implicated in signal transduction after receptor stimulation (125). NADPH oxidase consist of seven isoforms: Nox1, Nox2 (gp91phox), Nox3, Nox4, Nox5, Duox1, and Duox2. It is generally accepted that Nox complexes produce $O_2^{\bullet-}$ on the extracytoplasmic face of cellular membranes (224), which will produce $O_2^{\bullet-}$ outside the cell from the plasma membrane-

bound Noxes and in the lumen of a vesicular compartment from the intracellular Nox complexes (137). It is proposed that $O_2^{\bullet-}$ is dismutated to H_2O_2 , which can diffuse in part through aquaporin channels in the plasma membrane, or that $O_2^{\bullet-}$ is penetrated through the cell membrane anion chloride (ClC-3) channels to initiate intracellular signaling (18, 86, 157, 162). Since ROS are diffusible molecules, compartmentalized production of ROS by NADPH oxidase localized at specific sub-cellular compartments such as caveolae/lipid rafts and endoplasmic reticulum (ER)/endosomes (224) as well as mitochondria (147) plays an important role in activation of specific redox signaling events. Of note, SOD1 localizes in cytosol and mitochondria IMS, SOD2 localizes in mitochondria matrix, and SOD3 is anchored to the extracellular matrix *via* binding to heparan sulfate proteoglycans (HSPGs), collagen, or fibulin-5 (67, 167, 187, 201), as mentioned above. Thus, $O_2^{\bullet-}$ dismutation to H_2O_2 by the three isoforms of SODs may also contribute to activation of many redox signaling events, as described below (Fig. 7B).

SOD1 and redox signaling. SOD1 is shown to be actively recruited to redox active endosomal surface after interleukin-1 stimulation (137), thereby facilitating localized production of H_2O_2 and activating redox signaling at endosomes. SOD1 also regulates endosomal Nox2 activity by binding to Rac1 (a coactivator of Nox2) and regulating Rac1 activity (85). Thus, SOD1-Nox2-mediated increase in $O_2^{\bullet-}$ exits endosomes through chloride channels and then SOD1-mediated dismutation of $O_2^{\bullet-}$ at the endosomal surface produces the localized H_2O_2 required for redox activation of NF-kB (162) (Fig. 7B). Morikawa *et al.* reported that SOD1-derived H_2O_2 acts as an endothelium-derived hyperpolarization factor

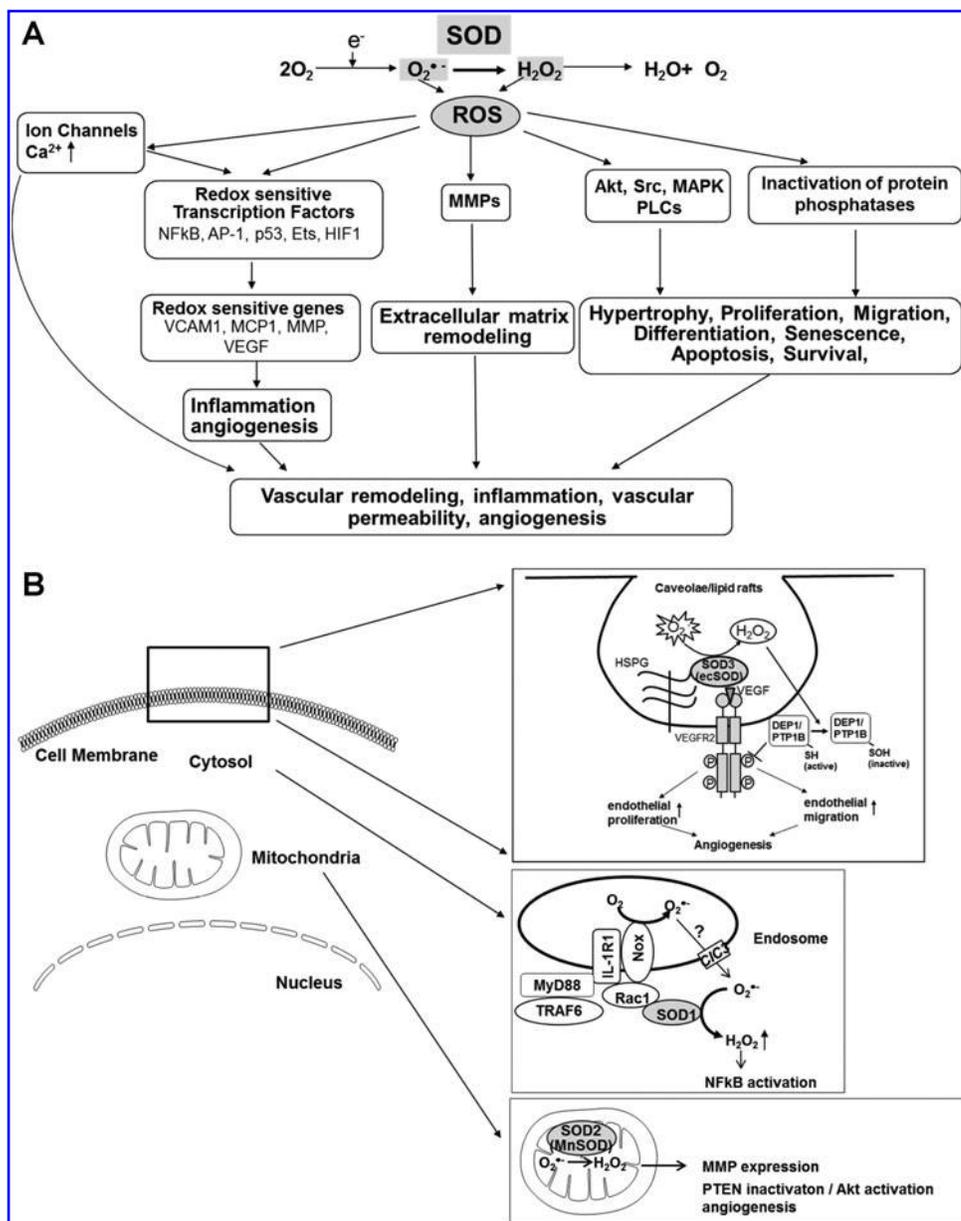
FIG. 7. (A) Role of SOD in redox-sensitive signaling pathways and (B) role of SODs in activation of redox signaling at specific compartments. (A) Production of $O_2^{\bullet-}$ and its metabolite H_2O_2 lead to activation of redox-sensitive kinases and potentially inactivation of specific phosphatases to modulate redox-sensitive signaling, including hypertrophy, proliferation, and migration. Activation of redox-sensitive transcription factors leads to redox-sensitive changes in expression of proinflammatory genes, such as vascular cellular adhesion molecule 1 (VCAM1), monocyte chemoattractant protein 1 (MCP1), and intercellular adhesion molecule 1 (ICAM1). ROS modulate ion channels and, therefore, influence intracellular Ca^{2+} and K^{1+} concentrations. Finally, ROS can activate matrix metalloproteinases (MMPs).

(B) Extracellular H_2O_2 generated by SOD3 (ecSOD) localized at caveolae/lipid rafts *via* binding to heparan sulfate proteoglycans (HSPGs) promotes VEGF receptor type2 (VEGFR2) signaling linked to angiogenesis *via* oxidative inactivation of protein tyrosine phosphatases (PTPs; DEP1 and PTP1B) (180); SOD1 is recruited to redox active endosomal surface where it binds to Rac1 to regulate Nox2 activity. Thus, SOD1-Nox2-mediated increase in $O_2^{\bullet-}$ exits endosomes through chloride channels (ClC3) and SOD1-mediated dismutation of $O_2^{\bullet-}$ at the endosomal surface produces the localized H_2O_2 , thereby promoting redox activation of NF- κ B (162); MnSOD (SOD2) localizes in mitochondria matrix. SOD2 (MnSOD) overexpression-induced H_2O_2 induces the tumor suppressor PTEN oxidation, leading to enhanced formation of phosphatidylinositol 3,4,5-triphosphate, resulting in activation of Akt and angiogenesis *in vivo* (40). Thus, $O_2^{\bullet-}$ dismutation to H_2O_2 by the three isoforms of SODs contributes to activation of specific redox signaling events at distinct compartments.

(EDHF) to regulate endothelial function *in vivo* (160). Overexpression of human SOD1 increases VEGF mRNA and protein expression through generation of intracellular H_2O_2 (81). Inhibition of SOD1 by copper chelator ATN-224 in tumor and endothelial cells prevents the formation of H_2O_2 , resulting in the protection of PTPs from H_2O_2 -mediated oxidation. This, in turn, inhibits growth factor-mediated phosphorylation of ERK1/2 (107). These findings suggest that SOD1-derived H_2O_2 functions as a second messenger to regulate various signal transductions involved in inflammation, angiogenesis, and vascular function.

SOD2 and redox signaling. SOD2 overexpression induces the tumor suppressor PTEN oxidation through H_2O_2

SOD3 and redox signaling. SOD3 is a major secreted extracellular enzyme that catalyzes the dismutation of $O_2^{\bullet-}$ to



increase, leading to enhanced formation of phosphatidylinositol 3,4,5-triphosphate, which results in activation of Akt and blood vessel formation *in vivo* (40) (Fig. 7B). H_2O_2 generated by SOD2 sustains long-term ERK1/2 signaling induced by nerve growth factor, leading to cell differentiation (28). Further, SOD2 converts $O_2^{\bullet-}$ to H_2O_2 in the mitochondria, activating HIF1 α - O_2 -sensitive K^+ channel pathway, resulting in the pathogenesis of pulmonary hypertension (8). Thus, overexpression of SOD2 increases angiogenesis, cell differentiation, and pulmonary hypertension *via* generating signaling molecule H_2O_2 at mitochondria rather than antioxidant function.

SOD3 and redox signaling. SOD3 is a major secreted extracellular enzyme that catalyzes the dismutation of $O_2^{\bullet-}$ to

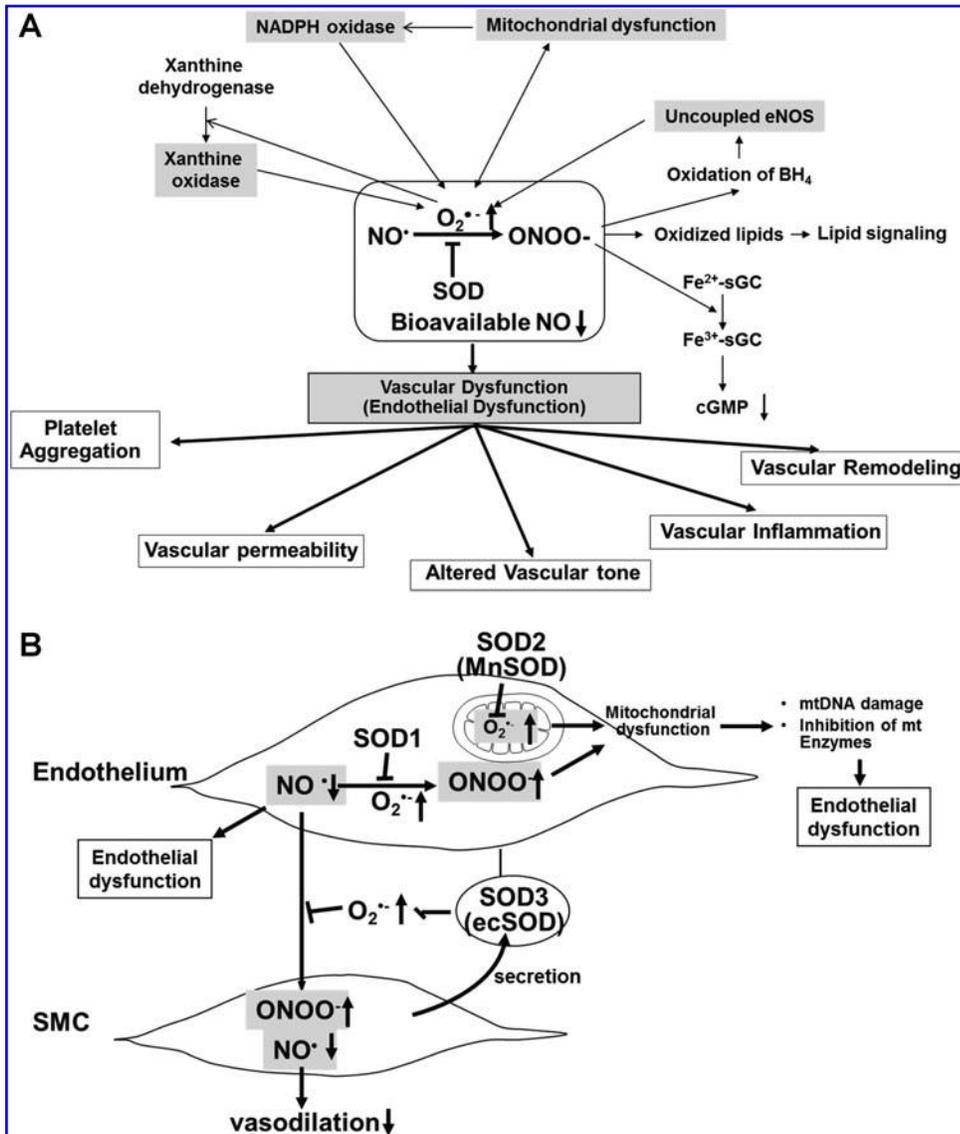


FIG. 8. (A) Role of nitric oxide-superoxide interactions in vascular (endothelial) dysfunction in cardiovascular disease and (B) protective role of SODs in oxidative stress-dependent vascular (endothelial) dysfunction. (A) NO rapidly reacts with O₂•⁻ generated by ROS-generating enzymes, including NADPH oxidase, xanthine oxidase, and mitochondria, to form peroxynitrite anion (ONOO⁻), which in turn oxidizes various molecules, such as the heme of sGC, lipids, and the endothelial NOS (eNOS) cofactor BH₄. This in turn induces uncoupled eNOS to promote further increase in O₂•⁻. These consequences will be further enhanced by interaction of ROS-generating enzymes. Both O₂•⁻ and ONOO⁻ promote mitochondrial dysfunction, thereby increasing mitochondrial ROS production. Mitochondria-derived ROS, which in turn further activates NADPH oxidase, results in increased ROS production and reduced NO bioavailability. Further, either O₂•⁻ or ONOO⁻ can stimulate other ROS-generating enzymes, such as xanthine oxidase. The loss of bioavailable NO and formation of ONOO⁻ can lead to vascular inflammation, vascular remodeling, altered vascular tone, enhanced vascular permeability, and increased platelet aggregation. These responses are

inhibited by SODs. (B) Because of its location, SOD3 (ecSOD) plays a critical role in preventing O₂•⁻-mediated destruction of NO• released from the endothelium at the extracellular space, whereas SOD1 preserves NO levels within the endothelium. Thus, SODs regulate endothelial function and NO mediating signaling by inhibiting O₂•⁻-mediated inactivation of NO•, thereby increasing bioavailable NO•. Because O₂•⁻ and NO• are both radicals and contain unpaired electrons in their outer orbitals, they undergo an extremely rapid, diffusion-limited radical-radical reaction ($6.7 \times 10^9 M^{-1} s^{-1}$, three times faster than the dismutation of O₂•⁻ by SOD). This reaction leads to the formation of nitrite, nitrate, and, very importantly, the peroxynitrite anion (ONOO⁻), which in turn induces endothelial dysfunction, vascular inflammation, vascular remodeling, altered vascular tone, enhanced vascular permeability, and increased platelet aggregation. Both O₂•⁻ and ONOO⁻ promote mitochondrial dysfunction, thereby increasing mitochondrial ROS production, reducing NO bioavailability, mitochondrial (mt)DNA damage, and inhibition of mitochondrial enzymes. These events result in endothelial dysfunction.

H₂O₂, and anchors to endothelial cell surface through heparin-binding domain (HBD). Evidence suggests that extracellular redox state regulates intracellular signaling (74) or tumor growth (30) by modulating plasma membrane-associated proteins. Exogenous H₂O₂ induces expression of both VEGF and VEGFR2 (77) and pro-angiogenic responses in ECs (226). We demonstrated that mice lacking SOD3 show impaired postischemic angiogenesis (117), which is associated with decrease in H₂O₂ (180) as well as enhanced O₂•⁻ production and decreased available NO (117) in ischemic muscles in

hindlimb ischemia model. Mechanistically, extracellular H₂O₂ generated by SOD3 localized at caveolae/lipid rafts *via* HBD promotes VEGF receptor type2 signaling *via* oxidative inactivation of protein tyrosine phosphatases (PTPs) in these microdomains (180). This in turn promotes endothelial proliferation and migration, leading to angiogenesis (Fig. 7B). Further, SOD3 overexpression reduces hypoxia-induced erythropoietin gene expression presumably due to a decrease in signaling intermediate O₂•⁻ (250), whereas SOD3 knockout mice show elevation of erythropoietin gene expression in-

duced by hypoxia (214). SOD3 gene transfer in rat hindlimb ischemia model increased glucose metabolism and cell proliferation, thereby promoting recovery of tissue injury (129). This is mediated through the activation of mitogenic Ras-ERK1/2 and PI3kinase-Akt signaling pathways, leading to increased expression of transcription factors AP1 and CRE and VEGF-A and cyclin D1 expression. These reports suggest that SOD3-derived H_2O_2 stimulates various redox signaling pathways to promote angiogenesis and tissue repair.

H_2O_2 derived from SODs may be involved in pathological states by Fenton type reaction and peroxidase activity. In combination with some transition metals like iron or Cu, H_2O_2 can react to form hydroxyl radical or related metal-associated reactive species, a highly reactive ROS, and thus induces cellular injury *via* the Fenton reaction (Fig. 2). In prooxidant pathological states such as atherosclerosis and hypertension, SOD1 and SOD3 are inactivated probably due to its peroxidase activity (89, 109). In this activity, H_2O_2 , the dismutation product of $O_2^{\bullet-}$, can inactivate SOD1 through reacting with the Cu center of this SOD, thereby forming the Cu-OH radical and leading to enzyme inactivation (89). This effect can be prevented by scavenging the Cu-OH radical with small anionic antioxidants, such as urate or nitrite. These reactions appear to be occurred in several disease states, as discussed below. The role of H_2O_2 in SOD function may be consistent with the notion that protective effects of SOD have a dose optimum in several pathophysiological models (166).

Role in NO signaling

NO exerts numerous beneficial antiatherogenic endothelial functions by elevation of cGMP and vasorelaxation *via* activation of soluble guanylate cyclase (sGC) and inhibition of platelet aggregation and inflammatory response. Given that $O_2^{\bullet-}$ reacts with NO• at almost diffusion-limited rates, SOD regulates endothelial function and NO-mediated signaling by inhibiting oxidative inactivation of NO. The role of SOD in vascular function will be discussed later. The reaction between $O_2^{\bullet-}$ and NO results not only in loss of NO bioactivity but also in formation of the ONOO⁻, a potent oxidant, which may contribute to lipid peroxidation and membrane damage. Further, ONOO⁻ has multiple effects (Figs. 6 and 8A), including (i) selective nitration of tyrosine residues in proteins, such as prostacyclin synthase and Mn-SOD (41, 82); (ii) activation of poly (ADP-ribose) polymerase (PARP) and expression of inducible NOS (iNOS), potentially important mediators of vascular dysfunction in disease states (176, 207); (iii) oxidation of the heme of sGC to its ferric NO-insensitive state (208); (iv) oxidation of the endothelial NOS (eNOS) co-factor BH_4 (132); and (v) oxidation of the zinc-thiolate complex in eNOS (257). The latter two effects can produce eNOS "uncoupling," a condition in which the normal flow of electrons within the enzyme is diverted such that eNOS produces $O_2^{\bullet-}$ rather than NO. Further, ONOO⁻ has been shown to inactivate SOD2, but it has no or milder effect on SOD1 *in vitro* (5, 99). Tyrosine nitration and inactivation of SOD2 are observed in chronic rejection of human renal allografts (144), suggesting that inactivation of SOD2 by ONOO⁻ may play a role in their model. The role of ONOO⁻ in regulating activity of SOD1 and SOD3 in oxidative stress-dependent cardiovascular diseases, including vascular injury (135) and atherosclerosis (89), remain unclear.

Role in mitochondria function

SOD regulates mitochondria function by above function (*i.e.*, scavenging $O_2^{\bullet-}$, producing H_2O_2 , inhibition of production of ONOO⁻ by protecting NO) in the mitochondrial matrix (SOD2) and in the intermembrane space (SOD1). Mitochondria are one of the major sources of chronic ROS production under physiological conditions (11). ROS in mitochondria are produced by oxidative phosphorylation pathway involved in energy production. In the mitochondrial electron transport chain, Complex I and III are the primary source of $O_2^{\bullet-}$ production in mitochondria (163). Superoxide is released into the matrix from complex I, whereas it is released into both the matrix and intermembrane space by complex III (163). In addition to being a major site of ROS production, mitochondria are a target for ROS/RNS and compromised by severe and/or prolonged oxidative stress (256). This will represent a vicious cycle to amplify mitochondrial ROS, whereby mitochondrial ROS/RNS causes oxidative damage to mitochondrial DNA (mtDNA), which leads to further mitochondrial dysfunction and oxidant generation. Indeed, mice lacking one of electron transport chain components, such as the adenine nucleotide translocator ANT1, have increased levels of oxidative species and mtDNA damage (62). Importantly, the electron transport chain contains several NO reactive-redox metal centers (24, 61). Thus, mitochondrial NO is an important regulator of $O_2^{\bullet-}$ production (72, 73). Further, NO undergoes radical-radical reaction with $O_2^{\bullet-}$ at near diffusion-limited rates forming ONOO⁻, which causes mitochondrial dysfunction by irreversible nitration of proteins, inactivation of enzymes, DNA damage, and disruption of mitochondrial integrity. At physiological concentrations, NO modulates mitochondrial oxygen consumption by inhibiting cytochrome c oxidase in a reversible process (25, 37), which contributes to the regulation of respiration and exhibits pro- and antiapoptotic responses. Role of mitochondrial NO in vascular function remains unclear.

Prevention of superoxide-induced cytotoxicity

SODs inhibit $O_2^{\bullet-}$ -induced cytotoxicity *via* inactivation of iron-sulfur (Fe-S) centers containing mitochondrial enzymes, such as aconitase and fumarase. This reaction is important, since $O_2^{\bullet-}$ -induced inactivation of Fe-S containing enzymes results in release of iron and subsequent formation for highly toxic hydroxyl radical or related iron-associated reactive species by reacting with H_2O_2 (Figs. 1 and 6), which may contribute to DNA damage. Indeed, mitochondrial SOD2 knockout mice exhibit decreased activities of Fe-S centers containing enzyme aconitase and increased oxidative damage to mitochondrial DNA (240).

SOD and Vascular Function

As a result of rapid reaction of $O_2^{\bullet-}$ and NO to produce ONOO⁻ as well as its membrane impermeability, $O_2^{\bullet-}$ has distinct effects depending on its subcellular location. Thus, in mammals, three isoforms of SODs exist in different subcellular localization, as mentioned before. The role of individual SODs in relation to endothelium under normal conditions and disease states is discussed as shown below (Fig. 8).

SOD1 and vascular function

Because of its cytosolic location, SOD1 plays an important role in endothelial function by protecting NO release from endothelium. Studies from SOD1 knockout mice exhibits increased levels of vascular $O_2^{\bullet-}$ and $ONOO^-$, increased myogenic tone, augmented vasoconstrictor responses (in response to serotonin and phenylephrine), and impaired endothelium-dependent (NO-mediated) relaxation in both large arteries and microvessels (51). Thus, SOD1 not only protects NO-mediated vasorelaxation, but also counteracts vasoconstrictor responses. Increases in vascular permeability after ischemia are greatly enhanced in SOD1-deficient mice (119). Alterations in expression of SOD1 may also impact vascular structure. For example, deficiency in SOD1 produces hypertrophy of cerebral arterioles (13). In contrast, transgenic mice overexpressing SOD1 improves vascular dysfunction in models of subarachnoid hemorrhage (112) and hypoxia with reoxygenation (139) as well as in response to ceramide (47), lipopolysaccharide (LPS) (49), and overexpression of β -amyloid precursor protein (96). Angiotensin II (Ang II)-induced expression of monocyte chemoattractant protein (MCP-1) and monocyte infiltration into the vessel wall are inhibited in SOD1 transgenic mice (100). Adenovirus-mediated gene transfer of SOD1 decreases vascular $O_2^{\bullet-}$ levels in atherosclerosis and diabetes (158, 249) to improve endothelial function in diabetes (249), and protects endothelial function in vascular tissues with aging (50), treated with various oxidative stimulants such as LPS (49), and Ang II (48). There is increasing evidence that H_2O_2 may function as an EDHF in some blood vessels. Experiments in gene-targeted mice suggest that SOD1 specifically may function as an EDHF-synthase, as it appears to be the major source of H_2O_2 in small mesenteric arteries (160).

SOD2 and vascular function

Various pharmacological inhibitors of mitochondrial energy metabolism significantly increase mitochondrial ROS production and impair endothelium-dependent vascular relaxation (22, 43, 53). Rotenone (which inhibits electron transport at flavin mononucleotide) abolished acetylcholine-induced, endothelium-dependent relaxation of rat and mouse carotid arteries (43) and rat and rabbit aortas (79, 196, 232). Similarly, antimycin A (which inhibits electron transport at cytochrome b-c1) and oligomycin (which inhibits mitochondrial ATP synthase) inhibit the production of endothelial NO in rabbit aorta (79). However, rotenone did not affect vascular relaxation induced by NO donors (196), which suggests that intact mitochondrial function plays an important role in the production of NO in endothelial cells. Under normal conditions and during acute oxidative stress such as hypoxia, SOD2 plays a minor role because vasomotor function is similar between WT and SOD2^{+/-} mice (6). Indeed, basal $O_2^{\bullet-}$ production is rather decreased in SOD2^{+/-} mice, which could be due to decreased oxygen consumption.

Under pathological conditions and aging, SOD2 plays an important role in regulating endothelial function. SOD2 deficiency appears to be responsible for endothelial dysfunction by increasing $O_2^{\bullet-}$ and causing chronic mitochondrial damage in ApoE^{-/-} mice (173). However, $O_2^{\bullet-}$ scavenger tiron failed to restore endothelial function, suggesting that chronic mitochondrial damage by deficiency of SOD2 may produce irreversible changes. Aged heterozygous SOD2^{+/-} mice

showed the most pronounced phenotype such as severely impaired vasorelaxation, highest levels of mitochondrial ROS formation and mtDNA damage, suggesting that mitochondrial radical formation significantly contributes to age-dependent endothelial dysfunction (235).

SOD3 and vascular function

Vascular SOD3 is localized in high concentrations between the endothelium and the smooth muscle, where endothelium-derived NO must transverse to stimulate smooth muscle relaxation (181). Because of its extracellular location, SOD3 plays a critical role in preventing destruction of NO• released from the endothelium. Importance of regulation of NO bioavailability by SOD3 has been shown in various experimental disease models. For example, adenovirus-mediated SOD3 gene transfer has been shown to protect against vascular dysfunction with aging (26), reduce systemic vascular resistance and arterial pressure in spontaneously hypertensive rats (35), and improve endothelial dysfunction in hypertension and in heart failure models (64, 97). In addition, SOD3-deficient mice display enhanced impairment of endothelial dysfunction in several models of hypertension and aging, which can be rescued by exogenously added SOD. To support this notion, ATP7A mutant mice lacking SOD3 function with impaired Cu delivery show endothelial dysfunction in a similar phenotype as SOD3^{-/-} mice (189).

Numerous pathophysiologicals, including atherosclerosis, aging, cigarette smoking, and diabetes, are associated with a decline in the production and/or biological activity of endothelium derived NO• (118). In these conditions, the loss of NO leads to a decline in SOD3 expression, since eNOS is a positive regulator for SOD3 expression (69, 179), although there are conflicting results (210). Landmesser *et al.* have shown that in patients with coronary artery disease, activity of endothelium-bound SOD3, released by heparin bolus injection, is positively correlated with flow-dependent, endothelium-mediated dilation (124). It is tempting to speculate that other conditions associated with a long-term loss of NO would also decrease expression of SOD3, adversely impacting the vascular redox state.

Physical exercise has been associated with a reduction in cardiovascular morbidity and mortality (19, 57, 183). One mechanism that may underlie this beneficial effect involves an upregulation of the eNOS, increasing local production of NO• (205). Paradoxically, exercise also increases total body oxygen uptake, increasing production of ROS (106), increasing the susceptibility of plasma LDL to oxidation (206) and increasing conjugated diene formation (200). The manner in which the vasculature adapts to this oxidant stress remains unclear. Since exercise training increases SOD3 expression in eNOS-dependent manner, this SOD3 response may represent an important physiological adaptation that would counteract this increase in oxidant stress in response to exercise training. Thus, the beneficial effect of exercise training on endothelium-dependent vasodilatation observed in several studies (84, 88, 94) may not only be due to an increase in expression of eNOS, but also due to an increase in SOD3 expression, which serves to preserve ambient levels of NO by decreasing its reaction with $O_2^{\bullet-}$. Importantly, unidirectional laminar shear stress dramatically increases expression of the cytosolic SOD1 in human aortic

endothelial cells (98), which would further enhance available NO in response to exercise training.

SOD and Atherosclerosis

Oxidative alterations of lipoproteins and arterial cells are implicated in atherogenesis (209). However, the mechanisms for atherogenic oxidative alterations *in vivo*, such as LDL oxidation, have not been fully revealed. The mechanisms by which $O_2^{\bullet-}$ might modify LDL could involve catalysis by transition metal ions such as Cu and iron (15) or ceruloplasmin (161) or by the reactive ONOO⁻ formed through the reaction between NO and $O_2^{\bullet-}$ (46). In contrast, it has been suggested that the $O_2^{\bullet-}$ can react with the lipid peroxy radical and alkoxy radical formed during lipid peroxidation and that at least the latter reaction might lead to chain termination (166). Such a reaction might balance other pro-oxidant reactions.

SOD catalyzes the dismutation of $O_2^{\bullet-}$ to H_2O_2 , which further reduces to H_2O by catalase, GPX, and Prxs (Fig. 1). Thus, SOD has been proposed to be involved in atherogenesis by inhibition of oxidative alterations caused by $O_2^{\bullet-}$ (29), prevention of $O_2^{\bullet-}$ -mediated removal of NO, thereby facilitating endothelium-dependent vasorelaxation (237) inhibition of leukocyte adhesion to the vascular endothelium (134), and altered vascular cellular responses [vascular smooth muscle cell (VSMC) and endothelial cell (EC) apoptosis, VSMC proliferation, hypertrophy, and migration (145, 185)], as shown in Figures 7 and 8. These findings indicate the potential for a protective role of SODs in atherosclerosis. Of note, in some cases, mice with genetically altered SOD function showed rather proatherogenic effects, as shown in Table 2. In this section, we will summarize and discuss role of SODs in atherosclerosis.

SOD1 and atherosclerosis

Many previous studies have shown that increased expression of SOD1 confers protection against acute or chronic oxidative injury, including atherosclerosis (139, 219). However, pathological role of SOD1 has been reported (58, 220). For example, transgenic mice that overexpress SOD1 develop more extensive fatty-streak deposition than control mice when fed a high cholesterol diet (220). This may be due to the possibility that high SOD activity could enhance oxidative injury by increasing rates of formation of distal oxidants. For example, SOD1 will convert $O_2^{\bullet-}$ to H_2O_2 , which can readily

cross cellular membrane and form hydroxyl radicals or related metal-associated reactive species through its interaction with redox-active transitional metals (248), which may have proatherogenic properties. Consistent with this, overexpression of catalase alone or overexpression of SOD1 and catalase in combination reduced the level of plasma and aortic F2-isoprostane and retarded the development of atherosclerosis in ApoE^{-/-} mice, whereas overexpression of SOD1 alone did not significantly reduce the level of F2-isoprostane and atherosclerosis in these mice (245). Alternatively, SOD also could increase oxidant formation by interacting directly with ONOO⁻ in a reaction that increases highly reactive nitronium ion (NO²⁺) (99). It is possible that effects of SOD are dose dependent, which is characterized by a bell-shaped curve (166, 177). Or localization of SOD may be critical to its protective effects (*i.e.*, extracellular *vs.* intracellular). Indeed, oxidative damage to lipoproteins and the vascular endothelium are attributed primarily to oxygen-derived species that are either generated extracellularly or released into the extracellular space as cellular oxidative waste or in association with the oxidative burst. These processes are more likely to be under the control of SOD3, which is located primarily within the interstitial matrix or anchored to cell-surface HSPGs (67). Finally, the atherogenic role of $O_2^{\bullet-}$ may vary depending on animal models. For example, $O_2^{\bullet-}$ contributes to the development of atherosclerosis in X-ray-exposed mice (219), but not to the nonirradiated models such as fat-fed mice and ApoE^{-/-} mice (220).

SOD2 and atherosclerosis

Superoxide anions in the mitochondrial matrix are quickly dismutated to H_2O_2 by SOD2, whereas those in the mitochondrial IMS are converted by SOD1 (175). Thus, it is conceivable that SOD1 and SOD2 may play a role in atherosclerosis through regulation of mitochondrial $O_2^{\bullet-}$ as well as available mitochondrial NO by preventing oxidative inactivation. Indeed, mitochondrial dysfunction, resulting from SOD2 deficiency, increased mtDNA damage and accelerated atherosclerosis in ApoE^{-/-} mice (12). Of note, mitochondrial DNA damage not only correlated with the extent of atherosclerosis in human specimens and aortas from ApoE^{-/-} mice but also preceded atherogenesis in young ApoE^{-/-} mice. These findings suggest that increased ROS production and DNA damage in mitochondria are early events in the

TABLE 2. ROLE OF SUPEROXIDE DISMUTASES IN ATHEROSCLEROSIS

SODs	Gene altered	Genetic background	Diet and intervention	Fold change of lesion			Reference
				Increase	No change	Decrease	
SOD1 (Cu/ZnSOD)							
1	SOD1 Tg	B6	High fat (add irradiation)			2	219
2	SOD1 Tg	B6	High fat	2.2			220
3	SOD1 Tg	ApoE ^{-/-} /B6	high fat		NS		245
	SOD1 + catalase Tg	ApoE ^{-/-} /B6	High fat			4	
SOD2 (MnSOD)							
4	SOD2 + / -	ApoE ^{-/-} /B6	High fat	2.5			12
SOD3 (ecSOD)							
5	SOD3 - / -	ApoE ^{-/-} /B6	Normal chow High fat		NS (after 3M) NS	1.7 (after 1M)	204

initiation of atherosclerosis (146). In addition, SOD2 regulates endothelial dysfunction/apoptosis and VSMC proliferation/apoptosis, leading to the development of atherosclerosis by controlling mitochondrial ROS and NO (146).

SOD3 and atherosclerosis

Several findings suggest that SOD3 may play a role in atherosclerosis. We and others have demonstrated that SOD3 is abundantly present in the vascular wall and synthesized in atherosclerotic lesions by smooth muscle cells and macrophages (68, 141, 143). Indeed, immunohistochemical studies showed that SOD3 staining colocalizes with lipid-laden macrophages in atherosclerotic vessels of ApoE^{-/-} mice. In contrast, SOD3 activity is decreased in connective tissue-rich human atherosclerotic lesions (124, 143). Thus, in atherosclerosis, SOD3 expression is increased in lipid-laden macrophages, whereas it is decreased in other cellular components such as VSMCs (68). Interestingly, SOD3 is expressed in iNOS-positive, macrophage-rich lesions colocalized with epitopes characteristic of oxidized LDL and peroxynitrite-modified proteins (143). Thus, it is conceivable that high SOD3 expression in the arterial wall may prevent not only deleterious effects of O₂^{•-} but also ONOO⁻ formation. This is supported by the findings that SOD3 attenuates tyrosine nitration and enhances nitrite formation in rabbit balloon injury model (135). In cell culture studies, SOD3 markedly reduced LDL oxidation by endothelial cells (127, 215). Wang *et al.* reported that low plasma SOD3 was independently associated with an increase of history of myocardial infarction (230). Moreover, activity of SOD3, but not that of SOD1 or SOD2, is reduced in coronary artery segments in patients with coronary artery disease compared with normal subjects (143). Adachi reported that the SOD3 affinity to endothelial surface heparan sulfate was decreased in coronary artery disease (3). Note that the R213G polymorphism in the *SOD3* gene, which reduces binding to endothelial surface and increases serum SOD3 levels, has been linked to an increase in cardiovascular risk (110).

In atherosclerotic vessels, SOD3 is regulated in multifaceted ways. First, SOD3 expression is regulated or associated with NO bioavailability, as discussed above (69, 124, 179). Second, the expression level and distribution of SOD3 is regulated by proteolytic removal of heparin binding domain. Level of low heparin affinity forms of SOD3 was increased in atherosclerotic patients, whereas the amount of the high-heparin affinity, C form of SOD3 was decreased (3). It has been shown that proteolytic processing leading to the appearance of truncated SOD3 can occur both intracellularly and in the extracellular space (59). In this context, coronary artery concentration of heparan sulfate, the physiological ligand for SOD3, tends to decrease with the atherosclerotic lesions, whereas the content of chondroitin sulfate increases (247). Third, SOD3 activity is modulated by H₂O₂ produced by lipid-laden macrophages due to its peroxidase activity, because lipid laden macrophage produces a large amount of both O₂^{•-} and SOD3, resulting in the accumulation of H₂O₂. In apolipoprotein E^{-/-} mice, SOD3 activity is inactivated, which can be restored by increasing the plasma concentration of urate (89). This peroxidase-like activity of SOD1 has been shown to inactivate the enzyme (92, 93). Fourth, SOD3 expression is regulated by inflammatory cytokines in VSMCs,

such that TNF α decreases SOD3 expression, whereas interferon γ and interleukin-4 increase its expression (212). Fifth, homocysteine, an independent risk factor for atherosclerosis (169, 244), decreases SOD3 expression and the binding of SOD3 to vascular endothelial surface, resulting in a loss of the ability to protect endothelial surfaces from oxidative stress. Further, homocysteine induces ER stress, and SOD3 activity is regulated by disulfide bridge arrangements (188). Thus, it is conceivable that homocysteine decreases the secretion of SOD3 by disturbing the disulfide bond formation and/or inhibiting the glycosylation, resulting in the incorrect assembly of the protein. Sixth, the methylation status of the 5' flanking region of the *SOD3* gene may be responsible for enhanced expression of SOD3 enzyme in the early stages of atherosclerosis (128, 221). Indeed, reduction in the methylation status of *SOD3* promoters is characteristic of atherosclerotic lesions (128, 221). Finally, SOD3 activity is critically regulated by Cu chaperone/transcription factor Atox1 and Cu transporter ATP7A (105, 190), since the activity of SOD3 requires a catalytic Cu to scavenge O₂^{•-}, as mentioned above (105). Interestingly, Atox1 and ATP7A are highly expressed in atherosclerotic vessels (105, 190), suggesting that they may regulate SOD3 activity in atherosclerotic vessels.

The functional significance of SOD3 activity in the development of atherosclerosis remains still unclear. Sentman *et al.* observed that genetic deletion of SOD3 paradoxically caused a slight increase in atherosclerotic lesions in apo(E)-deficient mice after 1-month atherogenic diet, while having no effect after 3 months on the atherogenic diet or after 8 months on standard chow (204). The authors concluded that SOD3 may enhance or have little effect on the development of atherosclerotic lesions. It should be noted that mice with a life-long deficiency in an enzyme may have multiple adaptations. Indeed, mice with the embryonic deletion of SOD3 can tolerate ambient oxygen, whereas those with acute deletion of SOD3 in ambient oxygen can lead to severe lung damage and high mortality (75).

SOD and Hypertension

Hypertension is associated with increased ROS formation, in particular O₂^{•-}, in multiple organs, including the brain, the vasculature, and the kidney, all of which could contribute to hypertension. Further, the adaptive immune system contributes to hypertension by interacting with these organs. A large body of evidence using exogenous SOD support the notion that O₂^{•-} and/or SOD plays a role in hypertension. For example, membrane permeable SOD or SOD mimetic improves hypertension, oxidation markers, endothelium-dependent relaxation, media/lumen ratio, kidney damage, glomerular filtration, and NOx excretion in models such as Ang II infusion (14, 113, 168), SHR (165, 234), endothelin-1 infusion (203), DOCA-salt (16, 252), the one-kidney, one-clip (54), and Dahl salt-sensitive (91). In contrast, SOD has no effect on the response to norepinephrine, which is not accompanied by O₂^{•-} production (14).

Mechanisms by which SOD improves hypertension include modulation of vasodilation, vasoconstriction, vascular remodeling, cardiac hypertrophy, renal sodium handling including tubuloglomerular feedback, and neuronal control of sympathetic activity. As discussed in the previous section, O₂^{•-} induces vascular dysfunction in various models of hy-

pertension by its well described interaction with NO. In addition, SOD mimetic inhibits medial thickening *in vivo*, as well as VSMC hypertrophy and cell proliferation *in vitro*. The Cu chelator DETC, an inhibitor of Cu/ZnSODs (SOD1 and SOD3), increases blood pressure after intravenous administration or infusion into the kidney medulla (148), whereas in Ang II-infused hypertensive rats, acute SOD mimetic tempol injections increased renal blood flow, glomerular filtration rate, and Na excretion (120). Endogenous SOD expression and activity are altered by hypertensive stimuli, such as Ang II (70). These findings suggest that SOD plays an important role in both development and the maintenance of chronic hypertension in various organs. Role of each isoform of SOD in hypertension has been investigated by using mice lacking the enzyme and adenovirus-mediated gene transfer as described below.

SOD1 and hypertension

Transgenic mice overexpressing SOD1 show inhibition of the increase in vascular $O_2^{\bullet-}$ and arterial pressure in response to Ang II without affecting Ang II-induced hypertrophic response (229). In contrast, SOD1 knockout mice exhibit no effect or even lower blood pressure as compared to control mice, in spite of increases in vascular $O_2^{\bullet-}$ and contractile responses to vasoconstrictors, as well as impaired endothelial-dependent relaxation (51). This paradoxical effect may be due to compensatory mechanisms.

SOD1 may play a role in hypertension *via* neuronal mechanism, rather than vascular or renal. Hypertension is also linked to increased brain levels of Ang II, which mediates its effects partially *via* $O_2^{\bullet-}$ and intracellular Ca^{2+} changes. Overexpression of SOD1 attenuates increased voltage-sensitive intracellular Ca^{2+} in response to Ang II in neuroblastoma cells (255). ROS signaling to hypertension is also implicated in the NTS. Compared with Wistar-Kyoto rats, stroke-prone spontaneously hypertensive rats (SHR) exhibit elevated activity of Rac1, a regulator of Nox1 and Nox2, in the NTS, and adenoviral-mediated expression of SOD1 decreases BP, heart rate, and urinary norepinephrine excretion (171). Seminal work by Davisson and coworkers has shown that intracerebroventricular injection of SOD1-expressing adenoviruses inhibits increases in heart rate, BP, and drinking behavior induced by Ang II in mice (254). Interestingly, Chan *et al.* observed that NADPH oxidase stimulated mitochondrial ROS production through an ROS-induced ROS release mechanism (32). Further, they found that overexpression of SOD1 in rostral ventrolateral medulla (RVLM) restored mitochondrial electron transport chain (ETC) activity, reduced mitochondrial ROS production, and prevented hypertension in the spontaneously hypertensive rat. By contrast, intracerebroventricular administration of ETC inhibitors stimulated mitochondrial ROS production and produced hypertension. Thus, neuronal SOD1 in RVLM may play a role in regulating mitochondrial ROS by regulating NADPH oxidase-derived $O_2^{\bullet-}$ in the cytoplasm, which contributes to regulation of sympathetic vasomotor tone and hypertension.

SOD2 and hypertension

In SHRs and Ang II-infused Wistar-Kyoto rats, mitochondrial dysfunction in the rostral ventrolateral medulla and the subsequent production of mitochondrial-derived ROS play a critical role in hypertension (32). Coenzyme Q10 treatment

restores electron transport capacity and reduces blood pressure and sympathetic neurogenic vasomotor tone. Interestingly, p22phox antisense, SOD2, and catalase prevent Ang II-induced ROS generation, suggesting the existence of a feed-forward effect of Nox on mitochondrial function. The importance of neuronal SOD2 in hypertension is also demonstrated by that intracerebroventricular injection of SOD2-expressing adenoviruses inhibits Ang II-induced increase in heart rate, blood pressure, and drinking behavior in mice in a similar fashion to SOD1, as mentioned above (253). Mice lacking SOD2 die of a cardiomyopathy within 10 days of birth and mice lacking one allele of SOD2 (SOD2^{+/-} mice) develop hypertension with aging and in response to a high-salt diet (197). Mitochondria-targeted SOD mimetic mitoTEMPO or overexpression of SOD2 improves endothelial function and reduces hypertension and oxidative stress in mice with Ang II- or DOCA salt-induced hypertension, but not in normotensive mice (52). These findings indicate that mitochondrial $O_2^{\bullet-}$ play an important role in endothelial function and hypertension. In this case, mitochondrion is the initial source of free radicals in response to Ang II, and then NADPH oxidase is secondarily activated by mitochondrial ROS. MitoTEMPO had no effect on blood pressure in normotensive animals. The Framingham Heart Study demonstrated a maternal influence on blood pressure, suggesting that hypertension may be transferred to offspring *via* inheritance of maternal mitochondrial DNA (246). These findings from SOD2 transgenic mice may explain this maternal heritability of blood pressure by linking it to systemic oxidative stress and inappropriate activation of NADPH oxidase in hypertension.

Archer *et al.* recently reported that epigenetic attenuation of SOD2 contribute to pulmonary arterial hypertension (PAH) by promoting cell proliferation and impaired apoptosis of pulmonary artery smooth muscle cells (9). Thus, enhanced mitochondrial H_2O_2 generation by epigenetic SOD2 deficiency plays a role in initiating and/or sustaining PAH, implicating SOD2 as a potential therapeutic target.

SOD3 and hypertension

Because of its extracellular location and $O_2^{\bullet-}$ reacts with NO• at an almost diffusion-controlled rate, SOD3 plays an important role in regulating blood pressure by modulating bioactivity of NO• (67). Indeed, gene transfer of SOD3 reduces $O_2^{\bullet-}$ and restores impairment of endothelium dependent relaxation and renal sodium handling, resulting in the decrease in arterial pressure in a genetic model of hypertension (34, 35). SOD3 deficiency promotes $O_2^{\bullet-}$ increases and impairment of endothelium-dependent relaxation in aortas from mice with the two-kidney and one-clip (2K1C) model (high renin induced hypertension) and in small mesenteric arteries from mice with chronic Ang II infusion, which is associated with an enhanced blood pressure (76, 108). In mice bearing a mutation in the Cu transporter ATP7A, SOD3 activity is reduced because of impaired incorporation of Cu, leading to impaired endothelium-dependent vasorelaxation, further supporting a role for SOD3 in vascular protection (189). In a similar fashion as SOD2, SOD3 deficiency or overexpression had no effect on blood pressure in normotensive animals. This is in keeping with the concept that $O_2^{\bullet-}$ does not affect hemodynamics under normal physiological conditions but begins to play a role in pathophysiological states (131, 165).

In prooxidant pathological states such as atherosclerosis and hypertension, SOD3 is inactivated probably due its peroxidase activity (89, 109, 231). In this case, H_2O_2 , the dismutation product of $O_2^{\bullet-}$, can inactivate SOD3 through reacting with the copper center of SOD3, thereby forming the Cu-OH radical and leading to enzyme inactivation (89). This effect can be prevented by scavenging the Cu-OH radical with small anionic antioxidants, such as urate or nitrite. In the one-kidney and one-clip (1K1C) model of low renin and high-volume hypertension, SOD3 is inactivated by H_2O_2 probably by its peroxidase activity, resulting in promoting the increase in extracellular $O_2^{\bullet-}$ levels, which contribute to enhancing blood pressure and impaired endothelium-dependent vasodilation in high-volume hypertension (109). Indeed, PEG-catalase and the hydroxyl radical scavenger uric acid reduced these responses in this hypertension model. Similarly, in a lamb model of persistent pulmonary hypertension of the newborn (PPHN), Wedgwood *et al.* demonstrated that H_2O_2 generated by PPHN and hyperoxia inactivates SOD3, and intratracheal catalase enhances enzyme function (231).

Lob *et al.* show that subfornical organ (SFO)-targeted ablation of endogenous SOD3 causes a significant elevation in basal blood pressure (140). Note that cells lining the third ventricle highly express SOD3 under normal conditions, which may explain why earlier studies failed to show additional overexpression effect of SOD3 in the SFO to inhibit the pressor effects of Ang II. These regions lack a well-formed blood-brain barrier and are therefore affected by circulating signals like Ang II. In addition, deletion of SOD3 in the SFO increases the sensitivity to systemic low-dose Ang II. More importantly, this central manipulation caused an increase in the percent of T cells with an activated phenotype, and markedly increased the vascular inflammation associated with Ang II infusion. Analysis of heart rate and blood pressure variability indicated that deletion of SOD3 in the circumventricular organ (CVO) enhanced sympathetic outflow (140). These data are in keeping with prior studies by Ganta *et al.*, suggesting that sympathetic outflow can promote T cell activation (71), and a feed-forward loop exists between organ systems. Given that all three SOD isozymes play an important role in neuronal regulation of hypertension, future analysis of the relative expression, distribution, and functional role of these three SOD isozymes in SFO will be important in understanding the mechanisms of central redox signaling and how it regulates hemodynamics and inflammation.

Overexpression of SOD3 ameliorated pulmonary hypertension in monocrotaline-treated rats (111) and attenuated pulmonary vascular remodeling in mice exposed to chronic hypoxia (170). In pulmonary artery smooth muscle cells and lung extracts of fetal PPHN lambs relative to fetal controls, SOD3 specific activity is decreased without altering specific activities of SOD1 and SOD2 (231). In the same model, intratracheal administration of SOD improved oxygenation and reduced $O_2^{\bullet-}$ levels (122). Taken together, these findings further highlight the potential importance of SOD3 in protecting against $O_2^{\bullet-}$ -induced pulmonary hypertension.

SOD and Vascular Remodeling

Vascular repair reaction after injury is characterized by neointimal growth as well as vascular remodeling. Such events contribute not only to restenosis after angioplasty but

also to vascular diseases, such as atherosclerosis, hypertension, and diabetes mellitus. Balloon angioplasty induces an acute increase in systemic oxidative stress, local $O_2^{\bullet-}$ generation, and altered vascular SOD activity (185). Given that only a few reports about role of SOD1 or SOD2 (45, 121), role of SOD3 in vascular remodeling will be discussed here.

Several studies using SOD3 gene transfer show its protective effect on balloon-induced or cuff-induced neointima formation (23, 126, 182) and constrictive remodeling (135) as well as cardioprotective properties (4) such as recovery of the endothelial layer in arterial wall. These SOD3-induced protective effects are associated with reduced inflammatory cell accumulation, decreased VSMC proliferation, and altered NO levels through iNOS or eNOS expression (23, 126, 135, 182). They may be by either regulating extracellular $O_2^{\bullet-}$ level or by preserving NO level by protecting its oxidative inactivation. Interestingly, SOD3 reduced collagen content in response to vascular injury (182), which may contribute to prevention of constrictive remodeling. Consistent with this, liposomal SOD delivery reduces the redox-dependent expression of TGF beta1 and collagen in dermal myofibroblasts (228).

Role of SOD in Angiogenesis and Stem/Progenitor Function

Neovascularization is involved in physiological process such as development and wound repair as well as pathophysiology such as ischemic heart/limb diseases, and atherosclerosis. Postnatal new blood vessel formation involves not only angiogenesis but also vasculogenesis, which is mediated through mobilization of bone marrow (BM)-derived angiogenic stem/progenitor cells as well as their homing to the ischemic tissues (101). Angiogenesis is dependent on cell proliferation, migration, and capillary tube formation in endothelial cells (ECs). BM-derived stem/progenitor cells or mesenchymal stem cells (MSCs) have been used for cell-based therapy to promote revascularization after peripheral or myocardial ischemia. ROS play an important role in redox signaling linked to angiogenesis in ECs as well as stem/progenitor cell mobilization, homing, and differentiation, thereby promoting neovascularization (222, 225). Although physiological levels of ROS are required for normal function, excess amounts of ROS produced in various pathophysiology contribute to dysfunction of endothelial progenitor cell (EPC) and stem/progenitor cells (225). Number and functional capacity of EPCs are reduced in oxidative stress-dependent cardiovascular diseases, including hypertension, atherosclerosis, diabetes, coronary artery disease, and heart failure as well as comorbid risk factors such as aging, hypercholesterolemia, and cigarette smoking (225). Role of SODs in postnatal angiogenesis and stem/progenitor function has been demonstrated, as shown below.

SODs and postnatal angiogenesis

Studies using SOD3-deficient mice show that SOD3 plays an essential role in reparative neovascularization in response to ischemic injury by protecting ischemic tissues from overproduction of $O_2^{\bullet-}$ (117) or generating H_2O_2 (180). Gene transfer of SOD3 promotes neovascularization with an increase in H_2O_2 (180) as well as tissue injury recovery by activating mitogenic Ras-ERK and PI3kinase-Akt pathways

leading to increased VEGF and cyclin D1 expression (129). These responses are associated with reduced adhesion molecule and cytokine expression and inflammatory cell recruitment (130) in ischemic tissues in hindlimb ischemia model. Consistent with $SOD3^{-/-}$ mice, $SOD1$ -deficient mice show impaired neovascularization, as assessed by reduction of blood flow recovery and capillary density in ischemic muscle (80). Overexpression of human $SOD1$ in mouse NIH 3T3 fibroblasts increased SOD activity, enhanced intracellular generation of H_2O_2 , and significantly stimulated VEGF production (81). FGF-induced angiogenesis and tumor development are enhanced in $SOD1$ transgenic mice (150). Connor *et al.* (40) reported that overexpression of $SOD2$ promotes mitochondrial H_2O_2 production, thereby stimulating EC sprouting and neovascularization in the angiogenesis assay. Further, VEGF-induced ROS *via* activation of Rac1 upregulate $SOD2$ expression in ECs (1), which could represent a feed-forward mechanism by which ROS-induced H_2O_2 enhances angiogenesis. Thus, all three SOD isoforms serve as not only $O_2^{\bullet-}$ scavenger but also H_2O_2 -generating angiogenic enzymes. Thus, $SODs$ are required for postnatal angiogenesis and $SODs$ treatment is essential therapeutic approach for treatment of angiogenesis-dependent cardiovascular diseases.

SODs and stem cells/progenitor cells function

In $SOD3^{-/-}$ mice, EPCs in both peripheral blood and BM are reduced, which is associated with decreased differentiation of BM cells into EC-like cells as well as levels of NO in the BM (117). Moreover, defective neovascularization in $SOD3^{-/-}$ mice was rescued by SOD mimetic infusion as well as transplantation of BM from WT mice (117). In human mesenchymal stem cells, secreted $SOD3$ play an important role in their neuroprotective effect by promoting cell survival (114, 115). These results strongly suggest that $SOD3$ in BM-derived cells plays an important in protecting overproduction of $O_2^{\bullet-}$ or generating signaling molecule H_2O_2 to maintain the normal function of BM in mediating stem/progenitor cells mobilization and function. $SOD1$ -deficient mice also show a reduction in the number of EPCs in the BM and spleen. The impaired neovascularization in these mice is rescued by wild type, but not $SOD1^{-/-}$, EPC supplementation (80). Functionally, $SOD1^{-/-}$ EPCs show increased $O_2^{\bullet-}$ levels, decreased NO production, and a reduced ability to migrate and integrate into capillary-like networks *in vitro*. Marrotte recently reported that EPCs-mediated augmentation of angiogenesis and wound repair are functionally impaired in diabetes due to decreased $SOD2$ expression in EPCs, which is restored by $SOD2$ gene therapy (154). Dihydropyridine calcium antagonist, which reduces cardiovascular events, improves migratory ability of circulating EPCs *via* upregulation of $SOD2$ (186). Taken together, $SODs$ in BM-derived stem/progenitor cells and EPCs play an important role in protecting against oxidative stress, which appears to be an important for their normal function.

Conclusions and Future Directions

$SODs$ play a critical role in endothelial and mitochondrial function by inhibiting oxidative inactivation of bioavailable NO, and thus preventing ONOO formation or OH radical formation *via* inhibition of oxidation of Fe-S cluster containing

enzymes. Since $SODs$ catalyze dismutation of $O_2^{\bullet-}$ to H_2O_2 and localize at distinct compartments (cytosol (for $SOD1$), mitochondria (for $SOD2$), and extracellular matrix (for $SOD3$)), they participate in compartmentalized redox signaling to regulate many vascular function. Dysregulation of these signaling pathways leads to endothelial dysfunction, altered vascular tone, vascular inflammation, vascular remodeling, enhanced vascular permeability, and increased platelet aggregation, which contribute to impaired angiogenesis as well as various vascular diseases such as atherosclerosis and hypertension. Lack of $SOD1$ contributes to vascular abnormality (increased vasoconstriction and endothelial dysfunction) and impaired angiogenesis (13, 51, 80, 119). The role of $SOD2$ in maintaining mitochondrial function is demonstrated by the neonatal lethality of mice lacking $SOD2$ (104, 133, 138). The importance of $SOD3$ is shown by 85% mortality of mice within a week of induction of $SOD3$ ablation (75). A prospective, population-based study from the Copenhagen City Heart Study showed that heterozygosity for $SOD3$ with R213G is associated with increased risk of ischemic heart disease (110). Given the importance of $SODs$ in cardiovascular disease, the concept of antioxidant therapies, that is, reinforcement of endogenous antioxidant defenses to protect more effectively against oxidative stress, is of substantial interest.

Abundant data from epidemiological observational studies have shown an inverse association between antioxidant intake or body status and the risk of cardiovascular diseases (149, 242). By contrast, clinical trials, such as the GISSI and HOPE trials, have found no benefits of vitamin E supplementation on cardiovascular disease risk (149, 242). Given the compartmentalized redox signaling, this may be because agents such as vitamin E and vitamin C are not targeted to sites of ROS generation that are most important in pathological conditions. Further, effect of SOD may depend on cell type, context specific, and disease states. For example, we found that $SOD3$ is essential for angiogenesis either by preserving NO bioactivity or by dismutating $O_2^{\bullet-}$ to H_2O_2 (117, 180). In contrast, $SOD3$ overexpression inhibits, instead of increasing, tumor angiogenesis and tumor invasion (31, 236). In diabetic mice, Nox2 deficiency improved neovascularization (55), whereas it impairs angiogenesis in wild-type mice (217). This may be explained by the concept that optimal levels of ROS are required but excess or lower amounts of ROS are inhibitory for biological responses. Note that in hypertensive rats or mice, SOD reduces blood pressure, but in normal rats or mice, SOD had no pressure effect (35, 52, 131, 165). Thus, therapeutic efficacy of SOD may be varied depending on the diseases states. Finally, in pro-oxidant pathological conditions such as atherosclerosis and hypertension, $SOD1$ and $SOD3$ seem to be inactivated by H_2O_2 derived from ecSOD due to its peroxidase activity (89, 109, 231). Thus, further detailed studies of $SODs$ and other antioxidant defense systems are needed.

The evidence discussed in this review supports the notion that an increase in $O_2^{\bullet-}$, H_2O_2 , and ONOO⁻, which critically regulated by $SODs$, contributes to modulation of cell signals related to vascular physiologies and pathophysiologies. Thus, antioxidant enzymes such as $SODs$ are potential candidate drugs (199). However, effective protection against vascular oxidative stress by antioxidant enzymes requires prolonged half-life and specific recognition of cell types such

as endothelium and targets to specific subcellular compartments. In this regard, vascular immunotargeting and gene transfer would be a promising approach (87, 164). Given the importance of Cu importers, transporters, and chaperones in regulating activity of SODs, understanding the processes and effects of metal ion insertion on SOD function is essential and subject of further investigation. Because of the compartmental localization of each SOD, approaches to target their site-specific expression will be very important and could be used to aid the development of novel SOD-dependent therapeutic strategies.

Acknowledgments

This research was supported by NIH R01 HL070187 (T.F.) and NIH R01 HL077524 (to M.U.-F.).

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Date of first submission to ARS Central, March 19, 2011; date of final revised submission, April 6, 2011; date of acceptance, April 7, 2011.

Abbreviations Used

1K1C = 1-kidney-1-clip
 2K1C = two-kidney and one-clip
 ALS = amyotrophic lateral sclerosis
 Ang II = angiotensin II
 Atox1 = antioxidant-1
 ATP7A = Menkes ATPase, MNK
 BM = bone marrow
 CCO = cytochrome c oxidase
 CCS = Cu chaperone for SOD1
 ClC-3 = chloride channels-3
 Cu = copper
 CVO = circumventricular organ
 EC = endothelial cell
 EDHF = endothelium-derived hyperpolarization factor
 eNOS = endothelial nitric oxide synthase
 EPC = endothelial progenitor cell
 ER = endoplasmic reticulum
 ETC = electron transport chain
 Fe-S = iron-sulfur
 GPx = glutathione peroxidases
 H₂O₂ = hydrogen peroxide
 HBD = heparin-binding domain
 HSPGs = heparan sulfate proteoglycans
 ICAM1 = intercellular adhesion molecule 1
 IMS = intermembrane space
 iNOS = inducible NOS
 LPS = lipopolysaccharide
 MCP-1 = monocyte chemoattractant protein-1
 MMPs = matrix metalloproteinases
 Mn = manganese
 MSCs = mesenchymal stem cells
 MT = metallothioneins
 mtDNA = mitochondrial DNA
 NO = nitric oxide
 NO₂⁺ = nitronium ion
 Nox = NADPH oxidase
 Nox2 = gp91phox
 O₂^{•-} = superoxide anion
 OH• = hydroxyl radical
 ONOO⁻ = peroxynitrite
 PAH = pulmonary arterial hypertension
 PARP = poly (ADP-ribose) polymerase
 PPHN = persistent pulmonary hypertension of the newborn
 Prxs = peroxiredoxins
 PTPs = protein tyrosine phosphatases
 ROS = reactive oxygen species
 RVLM = rostral ventrolateral medulla
 SFO = subfornical organ
 sGC = soluble guanylate cyclase
 SHR = spontaneously hypertensive rats
 SOD1 = cytoplasmic Cu/Zn SOD
 SOD2 = mitochondrial MnSOD
 SOD3 = extracellular Cu/ZnSOD, ecSOD
 SODs = superoxide dismutases
 TGN = trans-Golgi network
 VCAM1 = vascular cellular adhesion molecule 1
 VSMC = vascular smooth muscle cell
 Zn = zinc