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Review Article

EXTRACELLULAR SUPEROXIDE DISMUTASE IN BIOLOGY AND MEDICINE

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Abstract—Accumulated evidence has shown that reactive oxygen species (ROS) are important mediators of cell signaling events such as inflammatory reactions (superoxide) and the maintenance of vascular tone (nitric oxide). However, overproduction of ROS such as superoxide has been associated with the pathogenesis of a variety of diseases including cardiovascular diseases, neurological disorders, and pulmonary diseases. Antioxidant enzymes are, in part, responsible for maintaining low levels of these oxygen metabolites in tissues and may play key roles in controlling or preventing these conditions. One key antioxidant enzyme implicated in the regulation of ROS-mediated tissue damage is extracellular superoxide dismutase (EC-SOD). EC-SOD is found in the extracellular matrix of tissues and is ideally situated to prevent cell and tissue damage initiated by extracellularly produced ROS. In addition, EC-SOD is likely to play an important role in mediating nitric oxide-induced signaling events, since the reaction of superoxide and nitric oxide can interfere with nitric oxide signaling. This review will discuss the regulation of EC-SOD and its role in a variety of oxidant-mediated diseases.

Keywords—Extracellular superoxide dismutase, Cardiovascular disease, Neurological disease, Pulmonary disease, Superoxide, Nitric oxide, Free radicals

INTRODUCTION

With the evolution of aerobic respiration within microbial organisms and the consequential formation of reactive oxygen species (ROS) came the need for antioxidant enzymes to counteract the deleterious effects of these oxygen metabolites. Low levels of ROS are vital for many cell signaling events and are essential for proper cell function. For example, NO is essential for the regulation of vascular tone while superoxide is necessary for proper immune function [1]. Under physiological conditions, a balance exists between the level of ROS produced during normal cellular metabolism and the level of endogenous antioxidants, which serve to protect tissues from oxidative damage. Disruption of this balance, either through increased production of ROS or decreased levels of antioxidants, produces a condition referred to as oxidative stress and leads to variety of pathological conditions including cardiovascular diseases, neurological disorders, lung pathologies, and accelerated aging (reviewed in [2–4]).

Normal cellular metabolism involves the production of reactive oxygen species [5]. Superoxide (O_2^-) produced from a one-electron reduction of oxygen can undergo either spontaneous or enzyme-catalyzed dismutation to hydrogen peroxide (H_2O_2) or can react with nitric oxide (NO) to form the toxic product peroxynitrite ($ONOO^-$, Fig. 1). Either the combination of H_2O_2 with metal ions (iron) or the breakdown of $ONOO^-$ can produce the highly toxic hydroxyl radical ($^{\bullet}OH$). These ROS can react with a variety of cellular macromolecules such as lipids, proteins, DNA, and $[Fe\text{-}S]_4$ centers, leading to the disruption of cell membranes, inappropriate activation or inactivation of enzymes, and genetic mutations. Therefore, persistence of these diverse ROS in and around cells and tissues can have severe pathophysiological consequences.

To protect against oxidative damage, organisms have developed a variety of antioxidant defenses that include metal sequestering proteins, use of compounds such as vitamin C and vitamin E, and specialized antioxidant enzymes. One family of antioxidant en-

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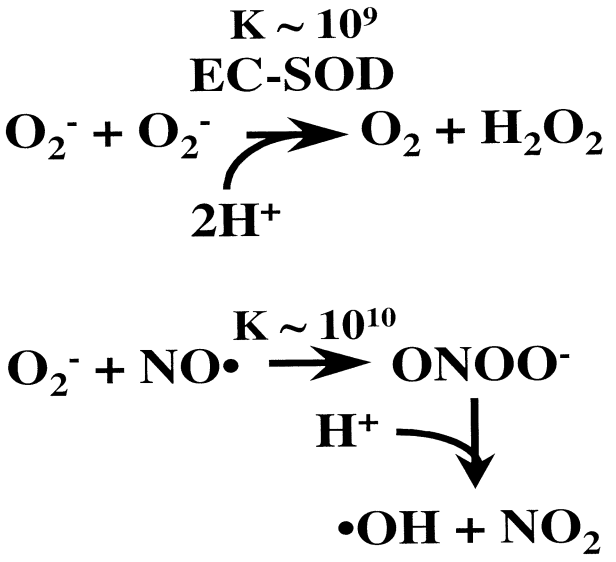

Fig. 1. This illustration shows the enzymatic dismutation of superoxide anion by superoxide dismutases such as EC-SOD (top). In addition, the reaction of superoxide with nitric oxide to produce peroxynitrite anion is depicted along with the products of peroxynitrite decay. (Adapted from [1].)

zymes, the superoxide dismutases (SOD), function to remove damaging ROS from the cellular environment by catalyzing the dismutation of two superoxide radicals to hydrogen peroxide and oxygen (Fig. 1) [6–8]. This reaction displays pseudo first-order kinetics and is diffusion limited ($k = 3 \times 10^9\ M^{-1}\ s^{-1}$) [9]. In eukaryotic cells, two intracellular superoxide dismutases exist: the Cu,ZnSOD [6] and the MnSOD [10]. Cu,ZnSOD is the major intracellular SOD. It exists as a 32 kDa homodimer and is present in the cytoplasm and nucleus of every cell type examined, where it acts as a bulk scavenger of superoxide [11,12]. The MnSOD is a 96 kDa homotetramer and is located primarily in the mitochondrial matrix [10,12,13]. The loss or dysfunction of either Cu,ZnSOD or MnSOD has been associated with ROS-mediated pathologies. For example, mutated Cu,ZnSOD proteins have been linked to instances of amyotrophic lateral sclerosis [14] while loss of MnSOD has been associated with neonatal death [15].

BIOCHEMICAL AND MOLECULAR CHARACTERISTICS OF EC-SOD

In 1982, a third SOD isozyme was discovered by Marklund and coworkers and termed extracellular superoxide dismutase (EC-SOD), as it was shown to be the predominant SOD in extracellular fluids such as lymph, synovial fluid, and plasma [16,17]. EC-SOD is a slightly hydrophobic glycoprotein with an apparent molecular weight of 135,000 kDa [8], although some species-specific differences in molecular weight do exist [18,19]. EC-SOD is present in various organisms as a tetramer [20,21] or, less commonly, as a dimer [22]. Each tetramer is composed of two dimers linked through disulfide bridges formed between carboxyterminal cysteine residues located in each subunit [20,21]. Tetramer formation may also involve interactions between N-terminal domains [23–25]. In addition to dimers and tetramers, larger multimers of EC-SOD can also be formed [20,21]. EC-SOD contains one copper and one zinc atom per subunit [8,26], which are required for enzymatic activity. EC-SOD activity can be inhibited by a variety of agents including azide and cyanide and inactivated by diethyldithiocarbamate (DDC) and hydrogen peroxide [27]. The EC-SOD protein is very stable and displays marked resistance to high temperatures, pH extremes, and high urea concentrations [28].

The location of the EC-SOD gene has been mapped in humans, cows, and mice; it localizes to human chromosome 4q21, bovine chromosome U15, and mouse chromosome 5 [29,30]. The EC-SOD gene is approximately 60% homologous to Cu,ZnSOD [31], but shows minimal homology with MnSOD [32]. The human gene is approximately 5900 bp long, consisting of three exons and two introns [33]. The 720 bp coding region is located entirely within exon 3. The promoter region of the gene contains various regulatory elements including antioxidant response elements (ARE), AP-1 binding sites, xenobiotic response elements, and NF-κB motifs [33,34] (Fig. 2). The human 4.2 kb mRNA is highly expressed in heart, placenta, pancreas, and lung; intermediate amounts are found in kidney, skeletal muscle, and liver; and very little is found in the brain [33]. Similar expression patterns are observed in the mouse [35].

Complementary DNA clones for human [36], rat [37,38], mouse [39], and rabbit [40] have been isolated and sequenced. The human EC-SOD cDNA encodes a 30 kDa [8,26] subunit, which is synthesized as a 240 aa propeptide, containing an 18 aa signal peptide that is removed to yield a 222 aa mature protein [36]. EC-SOD can be divided into roughly three functional domains: (i) the amino terminal residues 1–95 contain one glycosylation site at Asn89 [36,41] and glycosylation at this site primarily contributes to the solubility of the enzyme [42]; (ii) residues 96–193 show strong homology with the final two-thirds of Cu,ZnSOD and contain the active site of EC-SOD; (iii) amino acid residues 194–222 comprise a unique domain in the carboxyterminal end of EC-SOD that contains a cluster of nine amino acids with a positive charge (three lysines and six arginines) [36] (Figs. 3A and 3B).

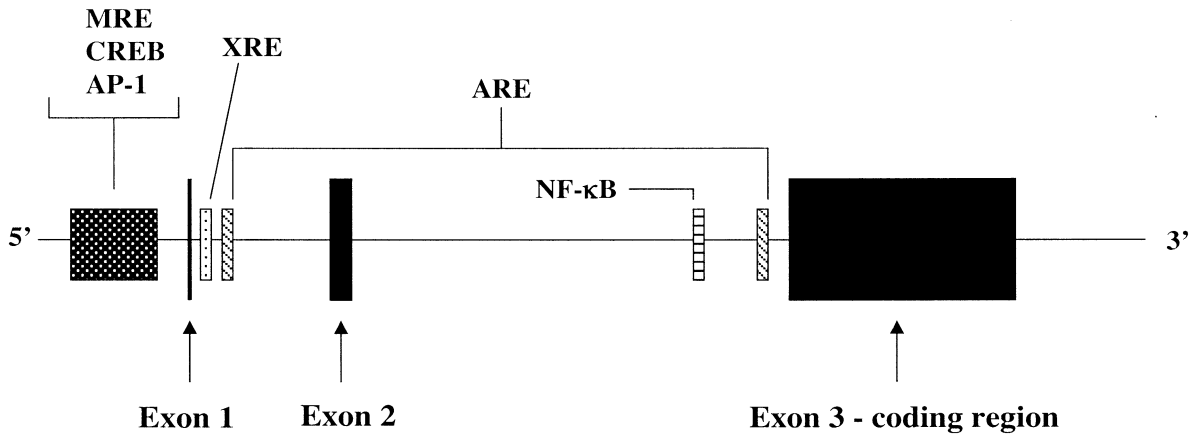

Fig. 2. Schematic diagram of the human EC-SOD gene. The gene contains 3 exons and 2 introns. Exon 3 contains the entire coding sequence for EC-SOD. Several transcription regulatory elements are contained in the promoter region of EC-SOD including a metal regulatory element (MRE, −470 to −464, black dotted box), a cAMP response element (CREB, −438 to −443, black dotted box), AP-1 binding site (AP-1, −398 to −391, black dotted box), a xenobiotic response element (XRE, 525 to 529, white dotted box), two antioxidant response elements (ARE, 93 to 102 and 4464 to 4473, diagonally striped boxes), and an NF-κB binding site (NF-κB, 3696 to 3705, horizontally striped box). (Adapted from [1].)

The primary location of EC-SOD in tissues is in the extracellular matrix and on cell surfaces, where it is found at 20 times the concentration present in plasma. Tissue EC-SOD is thought to account for 90–99% of the EC-SOD in the body [43,44]. Tissue distribution varies among species, but in general is found in highest concentrations in blood vessels, lung, kidney, and uterus, while lower levels are present in the eye, skeletal muscle, liver, and brain [43–46]. EC-SOD is also synthesized and secreted by a variety of fibroblast cell lines, glial cell lines, and endothelial cell lines [44,47]. The species studied to date can be divided into high-expressing (man, pig, sheep, and cow) and low-expressing groups (dog, cat, and rat) [43].

One important characteristic of EC-SOD discovered during the initial purification is that EC-SOD has a strong affinity for heparin [8,48]. Further investigation revealed that the C-terminal domain (amino acids 210–215) is essential for interaction of EC-SOD with heparin and heparan-sulfate [49–52]. These proteoglycans interact in an electrostatic fashion with the positively charged arginines and lysines in the C-terminal region of EC-SOD [53]; chemical modification of these residues abolishes the affinity of EC-SOD to heparin and heparan sulfate [54]. It is through their interaction with heparan sulfate proteoglycans on cell surfaces and in the extracellular matrix that the extracellular localization of EC-SOD is maintained.

When subjected to heparin sepharose chromatography [55], plasma EC-SOD divides into three fractions: one with no heparin affinity (fraction A), one with weak heparin affinity (fraction B), and one with strong heparin affinity (fraction C) [8,51,56,57]. Most tissue EC-SOD exists as type C [44,57], although rat EC-SOD only exists in the A and B forms [56] as it is only found to form dimers secondary to specific sequence alterations in the amino terminal region of rat EC-SOD compared to other species [25]. Fraction C is made up of EC-SOD tetramers containing subunits that have intact C-terminal heparin-binding domains (CCCC). Fraction B is made up of heterogeneous EC-SOD tetramers, some with intact heparin-binding domains and some with the heparin-binding domains removed (i.e., CCCA and CCAA). Fraction A is made up of homotetramers in which all subunits have the heparin-binding domain removed (i.e., AAAA) [49] (Fig. 3C).

Several studies investigating the nature of the interaction of EC-SOD with heparin have shown that intravenous injection of heparin in humans and other species leads to an immediate increase in plasma EC-SOD content, mostly of type C [58]. This suggested that while EC-SOD-A and EC-SOD-B appear to be circulating forms of EC-SOD in the plasma, EC-SOD-C exists in equilibrium between the plasma and endothelial cell surfaces [56,59–61]. Other studies have shown that tissue EC-SOD is manufactured and secreted as type C and can bind heparan sulfate proteoglycans on the surfaces of a variety of cells both in vivo [61] and in culture [62], as well as heparan sulfate located in the extracellular matrix [62].

Removal of the heparin-binding domain of EC-SOD can occur through proteolysis [50,57,63,64] and, thus, proteolysis is thought to control the localization of EC-SOD in tissue. Proteolysis may also account for the heterogeneous nature of EC-SOD in plasma, since C-terminal truncation of the heparin-binding domain would weaken the interaction of EC-SOD with hepa-

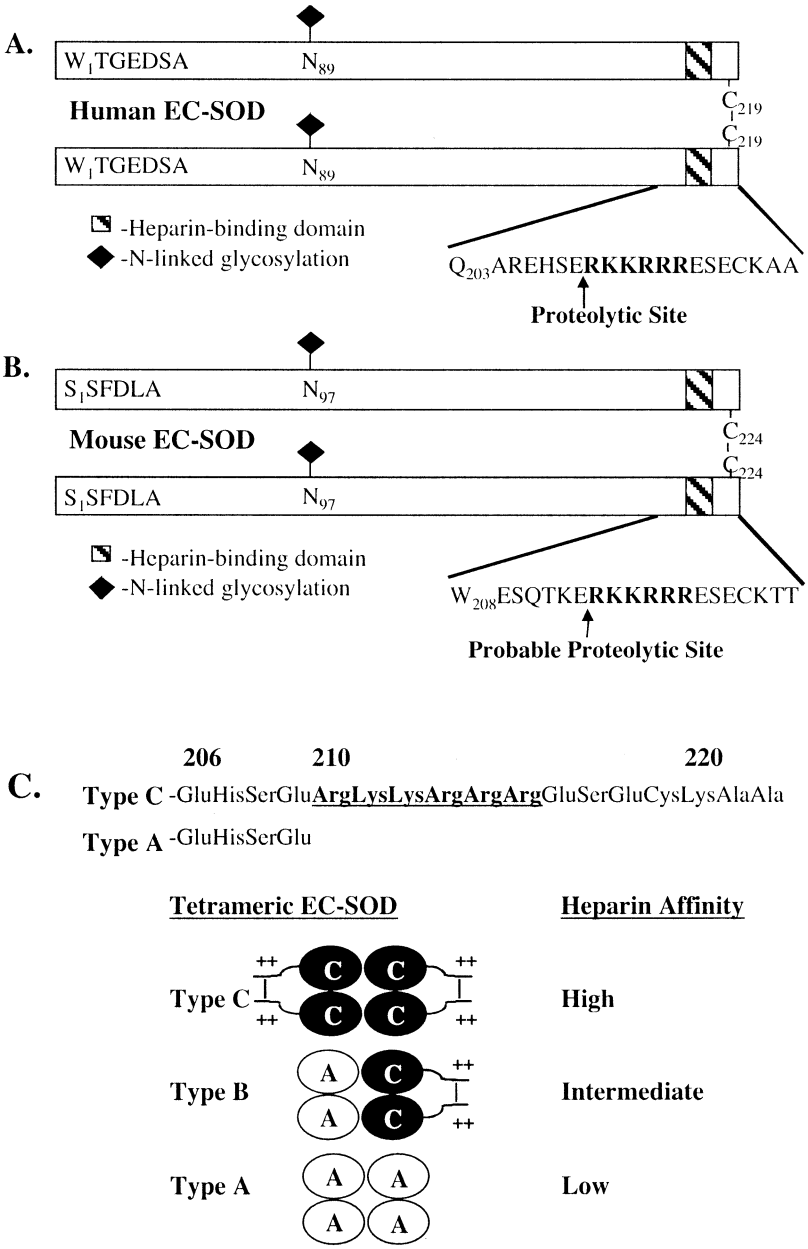

Fig. 3. Schematic illustration of (A) human and (B) mouse EC-SOD. Note that EC-SOD in both species contains an interchain disulfide bond, which links the heparin-binding domains of the two subunits together. Native EC-SOD consists of a tetramer composed of two of these disulfide-linked dimers. (C) Heparin binding affinity patterns of EC-SOD. Tissues contain predominantly type C EC-SOD, which consists of a tetramer in which all four subunits contain the heparin-binding domain (underlined sequence). The intersubunit disulfide bond links the heparin-binding domains of the subunits together. Proteolysis of the heparin-binding domains results in a loss of type C subunit, forming a truncated type A subunit. This proteolysis results in sequential reduction of EC-SOD affinity to heparin and clearance of the protein from the tissue into the serum. (Adapted from [1].)

ran-sulfate proteoglycans on cell surfaces and matrix components and facilitate entrance of cleaved EC-SOD into the vasculature through capillaries and lymph flow [57]. Although the protease responsible for EC-SOD cleavage is not currently known, several candidate enzymes have been identified. Karlsson and coworkers have shown that the heparin-binding domain of EC-SOD can be cleaved by trypsin [63]. Additionally, recent evidence suggests that EC-SOD can be proteolytically processed intracellularly by a furin-like protease immediately prior to its secretion into the extracellular space [65,66].

The proteolysis of EC-SOD-C and its subsequent accumulation in plasma as types A and B may have

important consequences with regard to the continued maintenance of adequate antioxidant protection in tissues. EC-SOD-C has a tissue half-life of approximately 85 h while truncated EC-SOD only has a half-life of 7 h [67]. Thus, with the loss of heparin binding, EC-SOD-mediated antioxidant protection may also be lost from cell surfaces and the extracellular matrix of tissues, which, in turn, may enhance ROS-mediated injury.

Interestingly, a recent study by Ookawara and coworkers suggests that the heparin-binding domain of EC-SOD can act as a nuclear localization signal in certain cell types [68], suggesting that EC-SOD may also provide antioxidant protection to DNA and nuclear proteins. Thus proteolysis of the heparin-binding domain of EC-SOD may have adverse consequences on genome integrity and redox-sensitive gene expression.

Regulation of EC-SOD expression

Heparin and heparan sulfate induced both EC-SOD mRNA and protein expression in cultures of skin fibroblasts. The extent of EC-SOD induction appears to be dependent on the level of sulfation of the glycosaminoglycan. How heparin and heparan sulfate regulate expression is unknown, but it may involve either receptor binding or a direct effect on promoter elements [69]. In vascular smooth muscle cells (VSMC) and lung alveolar type 2 cells, inflammatory cytokines such as IFN-γ and IL-4 can upregulate expression of EC-SOD protein and mRNA. Conversely, EC-SOD is downregulated by TNF-α [70]. Common to many of the cytokine pathways is the stimulation of transcription by NF-κB. It is, therefore, interesting to note that there exists an NF-κB regulatory element in the promoter region of EC-SOD [33,71]. Also, EC-SOD expression can be regulated by certain hormones such as FSH [72,73], cyclic-AMP [74], a wide variety of oxidizing agents such as xanthine oxidase, paraquat, and t-butyl hydroperoxide in fibroblasts [75], and dietary zinc intake [76–78].

Analysis of serum EC-SOD levels in a Japanese population study showed that normal populations can be divided into two distinct groups: those subjects with low plasma EC-SOD levels (93.6% of the studied group) and those with high levels (6.4% of the studied group) [79]. Serum EC-SOD from group 1 patients (low) showed heterogeneity with regard to heparin affinity, while group 2 patients (high) had mostly EC-SOD-C (6.4% of the total population studied) [79]. The high levels of serum EC-SOD in group 2 patients may have had a genetic basis since it was found that one parent of each of the group 2 subjects also had high serum EC-SOD [80]. In a similar Swedish study, analysis of EC-SOD plasma levels in random blood donors revealed a population (2.2% of the total) with a phenotypic variant of EC-SOD with reduced heparin affinity that resulted in 10-fold higher plasma levels of the enzyme [81]. Members of this population were universally heterozygous for a Arg213 to Gly213 substitution mutation in the heparin-binding domain of EC-SOD [81].

The consequences of this mutation are unclear. Patients both heterozygous and homozygous for the Arg213Gly mutation produced EC-SOD with a dose-dependent reduction in heparin affinity, and in vitro studies of this EC-SOD mutant showed reduced endothelial cell binding [82]. The majority of subjects carrying the Arg213Gly mutation show no immediate physical or clinical abnormalities, although this mutation has been shown to be associated with the occurrence of familial amyloidotic polyneuropathy type 1 (see below), poor outcome in diabetic patients requiring hemodialysis [83], and increased levels of cholesterol and serum triglycerides—risk factors associated with cardiovascular disease [84]. The paradox of reduced heparin affinity and higher levels of uncleaved EC-SOD-C in the serum has yet to be resolved, although additional studies have shown that EC-SOD from patients with the Arg213Gly mutation is resistant to proteolysis by trypsin and neutrophil-released proteases [85].

Two additional mutations of the EC-SOD gene have been described in humans. One is a threonine to alanine substitution at position 40 in the amino terminus of EC-SOD. The second is a silent substitution mutation at amino acid 280. Neither of these mutations has been linked with any particular disease [86].

EC-SOD PATHOPHYSIOLOGY

Vascular-related diseases

EC-SOD is highly expressed in blood vessels, particularly arterial walls [87–89], and is the predominant form of SOD in the aortas of baboons and humans, constituting up to 70% of the SOD activity in this tissue [89]. Vascular smooth muscle cells (VSMC) have been shown to secrete large amounts of EC-SOD and it is thought that these cells are the principal source of the enzyme in the vascular wall [88]. The major portion of EC-SOD in the vasculature primarily exists in the extracellular matrix and, to a lesser extent, on endothelial cell surfaces linked to heparan sulfate proteoglycans, but a small fraction of EC-SOD exists in equilibrium between cell surfaces/matrix and plasma [59,87,89].

EC-SOD expression in vascular smooth muscle cells can be controlled by a variety of vasoactive factors. Histamine, vasopressin, oxytocin, endothelin-1, angiotensin-II, and serotonin can upregulate EC-SOD expression in vascular smooth muscle cells through a G-protein coupled mechanism. Heparin and heparan sulfate can

also upregulate EC-SOD through a putative protein kinase C-mediated mechanism. In contrast, PDGF-AA, PDGF-BB, αFGF, βFGF, and EGF all downregulate expression through interactions with tyrosine kinase receptors [90]. Internalization and degradation of EC-SOD by endothelial cells may also represent a regulatory mechanism for EC-SOD expression in the vascular system [91].

Atherosclerosis

Oxidative stress has been implicated in the pathogenesis of atherosclerosis, and several antioxidants have been shown to inhibit atherogenesis [92,93]. The role of superoxide and nitric oxide in this pathological condition has been examined in several models. Studies indicate that superoxide production is increased in atherosclerotic vessels [94,95] and that increased superoxide levels interfere with NO-mediated regulation of vascular tone [96].

Because superoxide, either alone or in combination with nitric oxide, has been associated with the progression of atherosclerosis, the regulation of EC-SOD levels in vessels may be particularly important in the pathogenesis of this disease. For example, EC-SOD expression is substantially reduced in patients with coronary artery disease (CAD), suggesting that reduced EC-SOD activity contributes to endothelial dysfunction in patients with this disease [97]. In addition, proatherosclerotic vasoactive factors such as PDGF, FGF, and EGF downregulate EC-SOD in VSMC [90]. However, in young hypercholesterolemic individuals (pre-CAD), vascular endothelium bound EC-SOD activity is increased, possibly in an early attempt by the body to counteract impairment of endothelial function [97]. This is supported by the fact that overexpression of EC-SOD in vascular endothelial cells can protect against the oxidation of LDL, a major contributing factor to the formation of atherosclerosis [98].

In experiments using the apoE-deficient mouse as a model of atherosclerosis, Fukai and coworkers have shown increased expression and enzymatic activity of a truncated, mutant EC-SOD in macrophages present in atherosclerotic vessels [4,99,100]. The truncated EC-SOD, possibly with reduced heparin affinity, is produced by lipid-laden but not normal macrophages. As the macrophage-derived mutant EC-SOD increases in atherosclerotic lesions, the expression of wild-type EC-SOD in VSMC declines. However, the significance of EC-SOD, mutant or otherwise, in this model is unclear. Genetic deletion of EC-SOD had no significant effect on the size of the aortic lesion generated in response to a proatherosclerotic diet [101]. On the other hand, the EC-SOD/apoE double knockout mice showed significantly smaller diet-induced lesions as compared to apoE knockout mice after 1 month, although this difference disappeared after 3 months on the atherogenic diet [101].

An elevated level of homocysteine in plasma is thought to be a risk factor for increased incidence of coronary vascular disease [102–104]. Homocysteine is formed during the metabolism of methionine. Autoxidation of homocysteine is thought to generate oxidative stress in the form of superoxide and peroxynitrite [105,106]. A recent study of a hospital-based population in Australia showed a positive correlation between levels of total plasma homocysteine and plasma EC-SOD in patients with coronary artery disease [107,108]. Furthermore, treatments that markedly lowered homocysteine levels also lowered EC-SOD levels [108]. Conversely, these same patients showed an inverse correlation between EC-SOD levels with other vascular disease-related conditions such as incidence of myocardial infarction. Other risk factors for coronary artery disease, such as male gender and smoking, also correlated with lower EC-SOD levels [109].

The mechanism by which homocysteine may modulate plasma EC-SOD levels is currently unknown. One possibility is that homocysteine-dependent superoxide production may directly upregulate EC-SOD expression in the vasculature. A second possibility is that homocysteine modulates the binding of EC-SOD to endothelial cells through direct modification of heparan sulfate proteoglycans on the cell surfaces. This mechanism is supported by a study showing that the binding of recombinant EC-SOD to immobilized heparin was markedly decreased by pretreatment of the heparin-bound surface with homocysteine [110]. A third mechanism is that homocysteine interferes with the proper functioning of the endoplasmic reticulum in cells that secrete EC-SOD, resulting in the improper disulfide bond formation or glycosylation of EC-SOD and the incorrect assembly of the protein [111].

Hypertension

Activation of membrane-bound NADPH oxidase by angiotensin II has been shown to be a significant source of ROS in blood vessels [112], and the hypertension caused by angiotensin II can be abrogated by treatment with membrane-targeted forms of SOD [113]. Studies examining the effects of angiotensin II on EC-SOD expression demonstrated that infusion of angiotensin II in mice increased both EC-SOD expression and activity over a 7 d period [114]. Additional treatment of the mice with the angiotensin II receptor 1 (AT1) antagonist losartan completely reversed this effect. Upregulation of EC-SOD by angiotensin II was reproducible both in organ cultures and VSMC cultures [90,114]. This effect

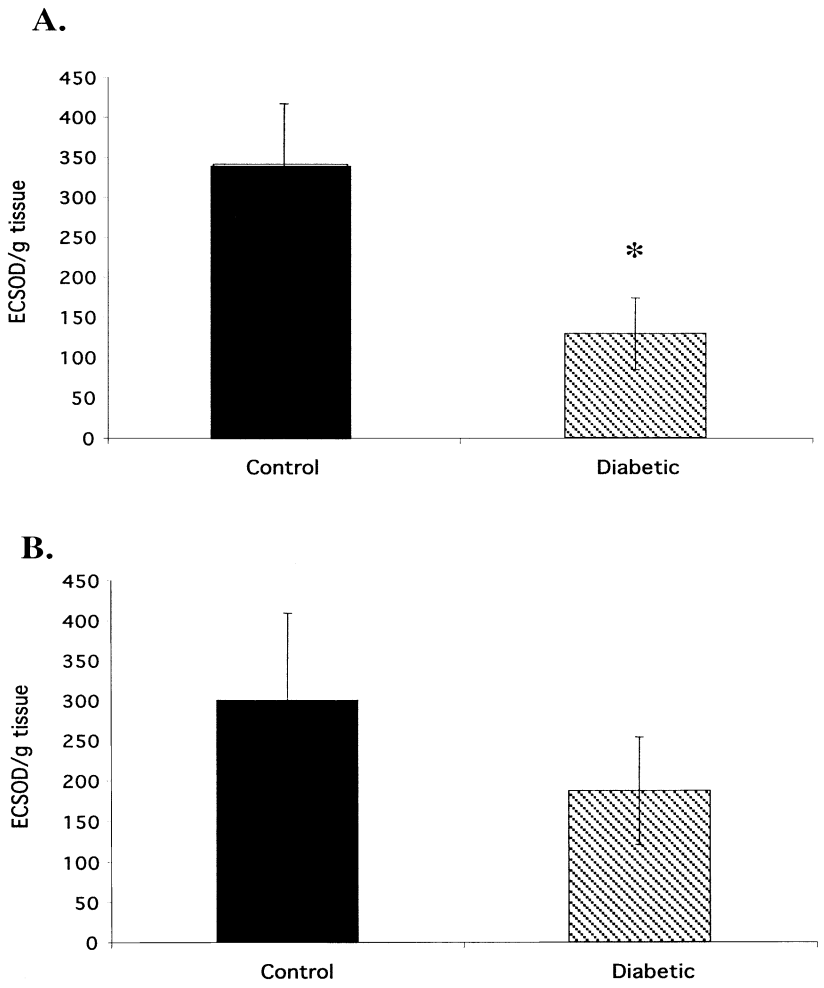

Fig. 4. Arteries from diabetic patients contain less EC-SOD activity than their nondiabetic counterparts. EC-SOD activity was assessed in (A) anterior and (B) posterior tibial arteries obtained from diabetic patients and nondiabetic controls. EC-SOD was markedly decreased in diabetic arteries compared to normal arteries. Asterisk indicates significance by Student's *t*-test, $p < .05$.

of angiotensin II on EC-SOD expression may modulate the oxidative state of the vessel wall in pathological processes mediated by the renin-angiotensin system [114].

However, the effects of angiotensin II on EC-SOD expression may be context specific. In contrast to the data described above, treatment of patients diagnosed with CAD with either losartan or the ACE inhibitor ramipril resulted in increased vessel EC-SOD activity and improved endothelial cell function [115]. In addition, both losartan and ramipril increased NO bioavailability after 4 weeks of therapy. These results suggest that increasing EC-SOD activity in patients with CAD can result in improved endothelial cell function.

Diabetes

The heparin-binding domain of EC-SOD anchors the protein to the endothelial cell surfaces and the extracellular matrix of blood vessels. Heparin affinity can be modulated under conditions of high blood glucose through nonenzymatic glycation of EC-SOD at lysine residues located in the heparin-binding domain [116]. Glycation of these residues results in the loss of heparin affinity but not enzyme activity [117]. Since diabetics have higher steady state concentrations of glucose than nondiabetics, this condition should lead to increased glycation of the heparin-binding domain of EC-SOD. Studies of diabetic patients have revealed higher concentrations of glycated EC-SOD in the serum [117,118], suggesting that the cell surface and matrix-associated EC-SOD may be decreased in diabetes. In fact, recent studies from our laboratory showed that EC-SOD activity is decreased in both anterior and posterior tibial arteries of diabetic patients (Fig. 4). This may result in increased susceptibility of vascular cells to superoxide radicals produced in the extracellular space. Also, pancreatic islet β cell regeneration is delayed in EC-SOD-null mice as compared to wild-type controls in a model

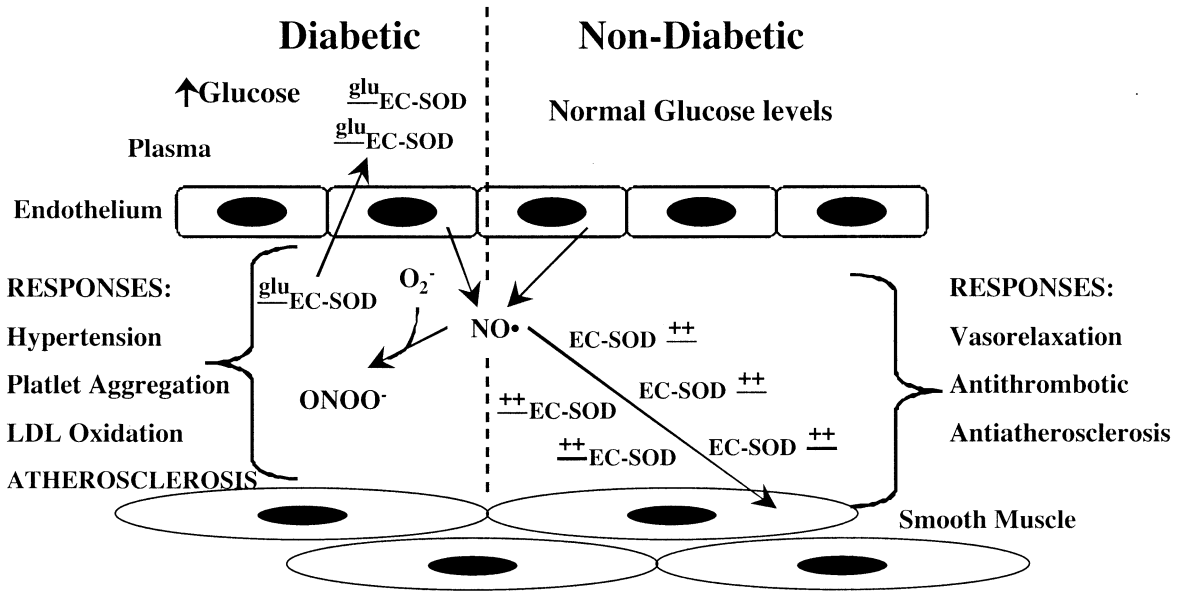

Fig. 5. Schematic diagram illustrating the predicted effect of EC-SOD glycation on vascular disease in diabetics. The nondiabetic artery wall contains high concentrations of EC-SOD. This EC-SOD prevents superoxide (O_2^-) from inactivating nitric oxide (NO•) and, thus, allows normal vascular relaxation to occur, preventing vascular disease. In contrast, elevation of glucose in diabetes results in glycation of EC-SOD (glu-EC-SOD). This disrupts EC-SOD's heparin-binding domain, resulting in decreased concentrations of EC-SOD in the artery due to diffusion into the plasma. Decreased levels of EC-SOD in arteries will allow increased peroxynitrite formation from superoxide-mediated inactivation of nitric oxide, resulting in hypertension and the vascular pathology associated with diabetes mellitus.

of diabetes induced by the superoxide-mediated destruction of islet cells [119]. In addition, diabetic patients expressing the R231G mutation of EC-SOD, which reduces the affinity of EC-SOD for heparin, showed increased progression of renal failure and decreased survival after the initiation of hemodialysis [86].

The loss of EC-SOD from the vasculature of diabetic patients may have implications with regard to the interaction of superoxide and nitric oxide in blood vessels. Although glycation of EC-SOD does not affect enzymatic activity [117], glycation does inhibit the activity of the intracellular Cu,ZnSOD [120–122]. Because nitric oxide species must traverse both intracellular and extracellular domains to signal smooth muscle relaxation, the activities of both EC-SOD and Cu,ZnSOD in the diffusion path of nitric oxide may be important in modulating vascular responses. Furthermore, superoxide production is dramatically increased in the presence of glycated proteins [123]. Therefore, decreased vascular concentrations of EC-SOD, decreased activity of intracellular Cu,ZnSOD, and increased production of superoxide all occur as a direct result of increased glycation of proteins in diabetes. These effects would interfere with the balance of superoxide and nitric oxide species in the vessels of diabetic patients and may play an important role in the pathogenesis of peripheral vascular disease in diabetes (Fig. 5).

However, the role of EC-SOD in the onset and progression of juvenile diabetes may be more complex in younger subjects. For example, Japanese pediatric patients with insulin-dependent diabetes showed a significant decrease in plasma EC-SOD concentration as compared to controls [124]. In contrast, EC-SOD analysis in diabetic children and their normal counterparts showed no difference in plasma EC-SOD levels between groups [125].

Ischemia/reperfusion injury

An important role for reactive oxygen and nitrogen species in the pathogenesis of ischemia/reperfusion injury has been demonstrated by numerous studies. The fact that endogenous antioxidants are essential for the preservation of function following injury has also been shown (reviewed in [126]). The beneficial effects of EC-SOD in reducing myocardial infarct size and preserving cardiac function have been found in numerous laboratories. Initial studies using recombinant EC-SOD demonstrated preserved cardiac function and reduced levels of tissue ROS following ischemia/reperfusion in rat hearts [127–131]. The administration of recombinant EC-SOD was also shown to reduce myocardial infarct size in pigs [132]. EC-SOD-transgenic mice expressing a 5-fold higher level of EC-SOD showed greater preservation of myocardial function after global ischemia/reperfusion than their wild-type counterparts [133,134]. More recently, Li and coworkers have shown that increasing EC-SOD levels in rabbit hearts confers both protection

against myocardial stunning [135] and reduces reperfusion infarct size [136] following ischemia/reperfusion. Similar EC-SOD-mediated protection has been demonstrated in other tissue models of ischemia/reperfusion injury including cerebral ischemia (see below), renal ischemia in the rabbit kidney, and the ischemia induced in the hamster cheek pouch [137,138].

Interactions of superoxide and nitric oxide and the effects on vascular tone

The precise mechanism by which EC-SOD protects against vascular disease has yet to be resolved. Most current theories support the idea that by scavenging superoxide, EC-SOD protects NO bioactivity [1]. NO is exquisitely sensitive to inactivation by superoxide [139,140]. Immunocytochemical localization studies demonstrate that vascular EC-SOD is present in high concentrations between the endothelium and smooth muscle surrounding blood vessels [59,87,89]. This is the same domain that NO must pass through to stimulate smooth muscle relaxation. Therefore, high concentrations of EC-SOD in this region may be important in maintaining low superoxide concentrations and preserving NO function [89].

The regulation of vascular tone by EC-SOD-mediated mechanisms is supported by a study examining EC-SOD effects on arterial relaxation. Using precontracted rabbit aorta rings, Abrahamsson and coworkers demonstrated that preincubation with or continuous infusion of rEC-SOD resulted in a marked reduction of superoxide-mediated inhibition of NO-induced relaxation. Protection of EC-SOD was lost if the rings were also preincubated with heparin. It was concluded that EC-SOD associated with heparan sulfate proteoglycans on endothelial cell surfaces and interstitial structures protects against the detrimental effects of superoxide on NO-derived relaxation [141].

Several recent studies have suggested that regulation of NO production and EC-SOD expression may be tightly associated (reviewed in [142]). For example, findings of one study showed the simultaneous elevation of both iNOS and EC-SOD expression in human and rabbit atherosclerotic lesions [100]. In a second study, EC-SOD expression in eNOS-deficient mice was shown to be markedly decreased as compared to wild-type mice under normal physiological conditions [143]. Additionally, it has been shown that EC-SOD expression in human aortas is upregulated by exogenously administered NO by a cGMP/protein kinase G-dependent or possibly a p38-MAPK-dependent pathway [143]. Also, exercise training of mice induced both eNOS and EC-SOD protein expression in wild-type mice but not in the eNOS-deficient animals. This suggests that NO may be the stimulus for increased EC-SOD expression as a result of increased exercise [143]. Therefore, upregulation of EC-SOD expression by NO may be an important protective mechanism to prevent superoxide-mediated inactivation of nitric oxide as NO travels between endothelial cells and smooth muscle.

However, the correlation between NO levels and EC-SOD expression may not hold true for humans. One study examining Australian patients, with a unique eNOS polymorphism that results in circulating NO levels twice that of normal people, showed that patients homozygous for the ecNOS4a/a polymorphism exhibited much lower plasma EC-SOD levels as control patients, although these patients may be producing primarily EC-SOD-C, which does not usually exist in the plasma [144]. The excessive production of NO, as in inflammatory reactions, may also result in the downregulation of EC-SOD. Exogenously produced NO at a concentration consistent with inflammatory conditions decreased the binding of rEC-SOD to endothelial cell surfaces in vitro [145]. This release of rEC-SOD was inhibited by treatment with nitric oxide synthase inhibitors [145]. NO can degrade heparan sulfate on cell surfaces [146], and this may be a mechanism for this loss of EC-SOD binding [145].

EC-SOD IN NEUROLOGICAL FUNCTION AND DISEASE

Regulation of EC-SOD in brain tissue

Increasing evidence suggests that oxidative stress plays a central role in a variety of neurological disorders including Alzheimer's disease, amyotrophic lateral sclerosis, Huntingdon's disease, and Parkinson's disease (reviewed in [3,147]). Thus, the proper function of brain antioxidant mechanisms may be of considerable importance in the prevention of certain neurological disorders.

EC-SOD protein expression patterns in the mouse brain suggest that this enzyme is primarily localized in the hippocampus, striatum, suprachiasmatic nuclei, and the habenula [148]. Glial cell lines have also been shown to express EC-SOD [44,149], and EC-SOD was found in the cerebral spinal fluid as well [150]. Although EC-SOD expression is much lower in the brain as compared to other organs [43,44], the extracellular space in the brain is relatively small and, therefore, the actual concentration of EC-SOD in the brain may be considerably higher than initially thought. Investigations into the role of EC-SOD in normal brain function and a variety of neurological disorders have been performed and are described below (see also [151]).

Learning and memory

Recent studies suggest that the regulation of brain extracellular superoxide concentration by EC-SOD may be of functional importance with regard to cognitive function. These studies showed that genetically altered mice that expressed either higher-than- or lower-than-normal levels of EC-SOD displayed impaired learning and memory functions [152–154]. For example, EC-SOD-transgenic mice exhibited a reversible impairment of LTP in hippocampal area CA1 that is related to impaired long-term, but not short-term, memory in contextual fear conditioning [154]. In other studies involving choice accuracy in a radial arm maze, both EC-SOD-transgenic and knockout mice had particular difficulty in making accurate choices under a low motivational state [153]. However, it should be noted that these mice were not incapable of learning; they showed significant progress under the high motivation conditions [152]. These studies demonstrate that, rather than being exclusively a neurotoxic molecule, brain extracellular superoxide may be vital in maintaining adequate learning function [153]. These results also may have important implications for conditions, such as attention deficit hyperactivity disorder (ADHD), that are characterized by cognitive impairments related to motivational states.

Cerebral ischemia

Accumulated evidence suggests that reactive oxygen species may play a role in the events that lead to cellular injury following cerebral ischemia. Numerous studies have shown that ROS directly damage cellular macromolecules such as nucleic acids, proteins, and lipids, which contribute to extensive cell death in ischemic tissues [155]. During the reperfusion of ischemic brain tissue, endogenous antioxidant mechanisms may be overcome by the excessive production of ROS in the reoxygenated tissue.

The EC-SOD-transgenic and knockout mice have been used in both a model of focal ischemia (middle cerebral artery occlusion or MCAO) and global ischemia. In the MCAO model, EC-SOD-transgenic mice subjected to 90 min of ischemia followed by 24 h of reperfusion showed a 27% reduction in cerebral infarct volume as compared to wild-type mice [156]. In contrast, EC-SOD-null mice subjected to a similar model of transient focal cerebral ischemia had an 81% greater infarct size compared to wild-type mice [157]. Also, EC-SOD-transgenic mice subjected to near-complete forebrain ischemia showed reduced cell death of hippocampal neurons, indicating that EC-SOD may play a role in defining the extent of necrosis of selected neuronal populations under conditions of cerebral ischemia [158]. It is also interesting to note that, after 24 h of reperfusion, cortical neurons themselves express EC-SOD, suggesting that the ability of certain cell populations to increase antioxidant levels postischemia may lead to protection of a defined subset of neurons [159]. Overall, these data suggest an important role for both extracellularly produced ROS and their removal by EC-SOD in cerebral ischemic injury.

Studies examining cerebral blood flow responses to changes in PO_2 in EC-SOD-transgenic and -null mice demonstrated that EC-SOD regulates the equilibrium between superoxide and NO in cerebral vascular tissue, thereby controlling vascular tone and reactivity in the brain [160]. EC-SOD opposed NO inactivation as shown by the absence of vasoconstriction in response to increased PO_2 upon exposure to hyperbaric oxygen in EC-SOD-transgenic mice. NO-dependent relaxation is attenuated in EC-SOD knockout mice. Thus, EC-SOD promotes NO vasodilation by scavenging superoxide while hyperoxia opposes NO and promotes constriction by enhancing endogenous superoxide generation and decreasing basal vasodilator effects of NO. These findings have implications in vascular diseases where extracellular superoxide production exceeds EC-SOD function, diminishing responsiveness to NO and allowing production of oxidative/nitrosative stress. The strategic location of vascular EC-SOD in the brain between endothelium and smooth muscle is ideal for preserving and regulating the endogenous dilator function of NO.

Traumatic brain injury

Recent statistics show that traumatic brain injury is the leading cause of death in people under the age of 45 [161]. Superoxide, whether alone or by its combination with NO to produce peroxynitrite, is believed to be a key mediator in the secondary neurological injury that occurs after severe trauma [162–164]. Experiments involving EC-SOD-transgenic mice have found that, after severe cranial impact, EC-SOD-transgenic mice had better neurological outcome and cognitive performance 24 h postinjury, regardless if the injury was severe or moderate [165]. These data implicate extracellular superoxide, possibly produced by infiltrating neutrophils at the site of injury, as a mediator of poor outcome from closed head injury.

EC-SOD may also play a role in regulating vasogenic edema that occurs in brain tissue after traumatic injury. One study investigated the role of EC-SOD in protection against vasogenic edema induced by cold using the EC-SOD-transgenic mice. It was shown that overexpression of EC-SOD is capable of protecting against vasogenic edema and decreasing vascular compromise in the brains of transgenic mice [166]. However, the study by Pineda and coworkers [165] did not show any difference in the

extent of edema between control and transgenic mice, although the authors suggested that the increased mortality in the control group may have obscured any positive results. Taken together, studies performed in models of acute brain injury suggest an important role for EC-SOD in modulating the response of the brain to injury.

Familial amyloidotic polyneuropathy type 1

Familial amyloidotic polyneuropathy type 1 (FAP) is an autosomal dominant, systemic amyloidosis caused by a mutation in transthyretin. Clinically, it is characterized by motor and sensory polyneuropathy, severe autonomic nervous dysfunction, and organ failure. In 1998, Sakashita and coworkers described an autopsy case of a 38 year old that developed FAP characterized by a more rapid onset of symptoms and a more marked amyloid deposition around various organs and tissues, especially blood vessels and interstitial tissues. The disease was accompanied by a 10-fold higher than normal level of plasma EC-SOD. Genetic analysis determined the existence of the Arg213Gly mutation in the gene encoding EC-SOD [167]. As noted above, this particular substitution mutation does not affect EC-SOD activity, but rather its heparin affinity and, thus, EC-SOD may be more readily lost from tissues [79–82]. The excessive amyloid deposition around blood vessels and in interstitial tissues is intriguing since these are the primary locations of EC-SOD in tissues [87,89]. This suggests that oxidative stress, in particular the effects of superoxide, may play a role in regulating the deposition of amyloid protein in FAP and that the proper localization of EC-SOD in tissues can somewhat protect against the organ failure associated with FAP.

Oxygen toxicity in the central nervous system

Unlike the studies of cerebral ischemia and traumatic injury that described a protective role for EC-SOD, an investigation of the effect of EC-SOD in a mouse model of central nervous system (CNS) oxygen toxicity has shown very different results [168]. EC-SOD-transgenic mice exposed to hyperbaric oxygen (6 ATA) displayed increased sensitivity to CNS oxygen toxicity as compared to nontransgenic littermates. Moreover, decreasing EC-SOD activity by the administration of diethyldithiocarbamate (DDC) resulted in increased resistance to CNS oxygen toxicity. These results imply that increased scavenging of superoxide enhances sensitivity to oxidative damage, while inhibition of scavengers affords protection. Explanation of this paradox may lie in the physiological consequences of superoxide/nitric oxide interactions. As mentioned above, nitric oxide secreted from cells can be inactivated by superoxide in the extracellular space. Under hyperoxic conditions, vasoconstriction of cerebral blood vessels may help reduce oxygen delivery to the brain, partially protecting the brain from excess tissue oxygenation. By acting as a vasodilator, NO could antagonize the vasoconstrictor effect of oxygen and, thus, increase brain oxygenation and damage during CNS oxygen toxicity. In fact, studies examining cerebral blood flow in mice demonstrated that NO-mediated vasodilation is promoted in mice that overexpress EC-SOD and attenuated in EC-SOD-null mice. These results show that, under hyperoxic conditions, EC-SOD may actually increase brain tissue damage by increasing cerebral blood flow [160]. In support of this hypothesis, administration of the NO synthase inhibitor N-nitro-L-arginine (L-NAME) dramatically reduced CNS oxygen toxicity in both transgenic and nontransgenic mice [168]. These studies illustrate the importance of maintaining a balance between oxidants and antioxidants to achieve proper physiological function.

RHEUMATOID ARTHRITIS

Accumulated evidence suggests that reactive oxygen species play an important role in inflammatory joint disease. Accumulation of inflammatory cells, particularly neutrophils, in the synovial fluid between joints is consistently observed in patients with rheumatoid arthritis. Activation of accumulated neutrophils in the synovial fluid produces superoxide anions, which can react with other cellular components such as iron to produce additional ROS. Early studies by Marklund and coworkers [17] showed that EC-SOD was the major isozyme in synovial fluid and accounted for about 80% of the total superoxide dismutase activity in normal controls. The role EC-SOD in the pathogenesis of rheumatoid arthritis is controversial. Marklund's study showed that patients with rheumatoid arthritis had a 50% reduction in EC-SOD activity compared to normal controls [17], suggesting that a reduction in EC-SOD activity may increase the susceptibility of synovial fluid and joint collagen to damage by oxygen radicals. Conversely, a second study showed that synovial fluid from patients with rheumatoid arthritis had significantly greater SOD activity, possibly due to the release of EC-SOD from neutrophils into the extracellular space [169]. Our immunohistochemical studies of lung inflammation showed that neutrophils accumulated at sites of bleomycin injury stained strongly for EC-SOD [170]; thus, it is possible that the measurement of intracellular EC-SOD in neutrophils may account for differences between the two studies. Further studies are necessary to resolve these conflicting results regarding the role of EC-SOD in rheumatoid arthritis.

EC-SOD IN DEVELOPMENT AND AGING

A recent theory as to why we age suggests that the process of aging may be due, in part, to the gradual overwhelming of endogenous antioxidant defenses by chronic metabolic stress [171]. Many of the diseases already described are associated with oxidative stress and are known to occur more frequently in older populations. Therefore, age-related changes in antioxidant defense mechanisms such as EC-SOD may determine how rapidly we age as well as the number and severity of age-related diseases we experience.

Developmental regulation of antioxidant enzymes is important for the adaptation of the neonatal lung to the relatively high oxygen environment following birth. EC-SOD expression during development in rabbits shows an interesting change in the localization of EC-SOD from intracellular to extracellular compartments within the lung. This relocalization of EC-SOD is also accompanied by an increase in EC-SOD activity. It is interesting to note that nitric oxide synthase and NO levels also increase before birth and mediate the decrease in pulmonary vascular resistance during the transition from fetal to adult circulation [172]. EC-SOD may be important in maintaining NO bioavailability during this time.

Similarly, EC-SOD activity has been described in the human placenta in association with fetal vessels [173] and also undergoes an intracellular to extracellular transition between the second and third trimesters [173,174]. EC-SOD levels are much lower in prenatal children as compared to full-term newborns. The evidence suggests that the expression of EC-SOD is upregulated in the fetal stages for tolerance to the higher oxygen encountered at birth. After birth, plasma EC-SOD levels of infants and children increase in an age-dependent manner until 1 year of age [175,176] and then decrease at a rate of 2% per year until the age of 20 [177]. Age-related changes in adult humans have not been examined, except in relation to various age-associated diseases (see above).

LUNG DISEASES

EC-SOD in the lung

Maintaining the balance between ROS/RNS and antioxidants in the lung is required for proper organ function—maybe even more critical than in many of the organs systems already described—since airways are uniquely exposed to relatively higher levels of oxygen than most other tissues [1]. Highly localized production of low levels of ROS/RNS are essential to normal physiological functions in the lung, such as smooth muscle relaxation in airways and blood vessels and immune responses. Furthermore, disruption of the oxidant/antioxidant balance in the lung is thought to be a key step in the development of many airway pathologies. Therefore, the regulation of antioxidants such as EC-SOD may be important in modulating or preventing the pathogenesis of many pulmonary diseases.

EC-SOD is the primary extracellular antioxidant in lung tissue [39]. Messenger RNA for EC-SOD is selectively expressed in just a few cell types in the lung. Bronchial epithelium, vascular endothelium, alveolar type 2 cells, and alveolar macrophages all produce EC-SOD mRNA [39,178]. Immunohistochemical studies of EC-SOD in lung tissue have revealed that both human and mouse EC-SOD are primarily located in the extracellular matrix of vessels, airways, and alveolar septa [21,87], although some intracellular labeling of EC-SOD is observed in bronchial epithelial cells and in alveolar macrophages [170,179]. In addition, EC-SOD is found associated with type 1 collagen around vessels, airways, and alveolar septa in the lung [87], and we have recently found that it directly binds to this matrix protein (unpublished observation). This binding of EC-SOD to type 1 collagen in the extracellular matrix is thought to play an important role in the regulation of many airway diseases.

Acute lung injury

Numerous studies have shown that oxidants are important mediators of acute lung injury and many investigators have found that the augmentation of antioxidant enzymes is protective when used in animal models of acute lung injury [180–184]. The role of EC-SOD in the pathogenesis of acute lung injury has been investigated in mouse models expressing altered levels of EC-SOD. Mice null for EC-SOD were phenotypically normal until stressed [185]. However, exposure of EC-SOD-null mice to $> 99\%$ oxygen caused significant reduction in viability and an earlier onset of severe lung edema as compared to wild-type mice. This suggests that, under normal physiological conditions, the animals are capable of compensating for the lack of EC-SOD; but, when exposed to oxidative stress, the mice are unable to compensate and show increased sensitivity [185]. Notably, overexpression of EC-SOD in the lungs of mice confers protection against hyperoxia-induced lung injury, further supporting a role for this antioxidant enzyme in protecting the lung against oxidative stress. Furthermore, the EC-SOD-transgenic mice displayed significantly fewer neutrophils in the lungs after exposure to hyperoxia, suggesting that the presence of EC-SOD can attenuate the acute inflammation associated with hyperoxic lung injury [186].

Investigations from our laboratory into potential mechanisms of ROS-mediated lung injury following hyperoxia showed that hyperoxic exposure of wild-type mice significantly decreased EC-SOD levels in both the

lung homogenates and bronchoalveolar lavage fluid (BALF) of mice exposed to 100% oxygen for 72 h. This correlates with a significant depletion of EC-SOD from the alveolar parenchyma as determined by immunofluorescence and immunohistochemistry. As noted above, the heparin-binding domain of EC-SOD can be proteolytically removed, resulting in the loss of heparin affinity. Hyperoxic lung injury is known to lead to elevated levels of activated proteases [187–189]. Analysis of EC-SOD by Western blot showed an increase in the ratio of proteolysed to uncut EC-SOD after hyperoxia, suggesting that hyperoxia depletes EC-SOD from alveolar parenchyma by cutting the heparin-binding domain. This may enhance hyperoxic pulmonary injury by altering the oxidant/antioxidant imbalance in alveolar spaces [190].

In a second model, acute lung injury characteristic of adult respiratory distress syndrome (ARDS) is believed to be mediated by ROS secreted by activated neutrophils [191]. Neutrophil degranulation during the progression of ARDS released a variety of proteolytic enzymes into the extracellular space. A recent study by McCord and coworkers has found that activated neutrophils are capable of causing partial proteolysis of rabbit EC-SOD in vitro, resulting in a diminished affinity of EC-SOD for the endothelial surface [64]. This suggests that reduced affinity of EC-SOD for heparin in ARDS may result in a loss of antioxidant protection and exacerbation of this condition. This is supported by the fact that plasma EC-SOD is elevated in patients with ARDS [64].

Pulmonary fibrosis

The bleomycin-treated mouse is a well-described model system for oxidative stress-induced pulmonary fibrosis. Treatment of mice with bleomycin results in a fibrotic response that occurs in two distinct phases. First, there is an acute phase characterized by an influx of inflammatory cells, in particular macrophages and polymorphonuclear leukocytes (PMN). This is followed by a chronic stage characterized by extracellular matrix remodeling and collagen deposition [192,193]. The role of EC-SOD in the modulation of the progression of bleomycin-induced pulmonary fibrosis has been examined using both the EC-SOD-transgenic and -null mouse models.

Targeted overexpression of human EC-SOD in the lungs of mice significantly protected these mice against bleomycin-induced lung injury [194,195]. These mice showed a 53% reduction in histologically evident lung pathology and a 17% decrease in total lung collagen content, indicating reduced fibrosis in these mice [194]. In other related studies, parenteral administration of modified SOD enzymes that are essentially localized to the extracellular spaces also conferred protection against bleomycin-induced pulmonary fibrosis [196–198]. Additionally, treatment of wild-type mice with MnTBAP, an SOD mimetic, resulted in a marked attenuation of bleomycin-induced fibrosis and airway dysfunction [199]. In contrast, EC-SOD-null mice displayed a marked increase in the severity of pulmonary injury after bleomycin treatment (unpublished observation). Therefore, the presence or absence of EC-SOD during the progression of pulmonary fibrosis may be important.

We have recently shown that the induction of pulmonary fibrosis in wild-type C57BL/6 mice resulted in the loss of EC-SOD from the matrix of alveolar septa and the surface of bronchial epithelial cells [170], suggesting that downregulation of native EC-SOD may contribute to injuries leading to fibrosis. We also have shown that the accumulation of proteolysed EC-SOD in BALF of bleomycin-treated mice coincides with the loss of the enzyme from the extracellular matrix of alveolar tissues. Notably, proteolytic cleavage of the heparin-binding domain of EC-SOD does not affect enzymatic activity. Thus, the proteolysis leading to redistribution of EC-SOD may contribute to superoxide-mediated damage to lung tissue induced by bleomycin [170].

EC-SOD and inflammation

Acute inflammation also contributes to the development and progression of all of the lung diseases mentioned above. Uncontrolled ROS production by activated inflammatory cells can lead to parenchymal, epithelial, and endothelial cell injury. Cytokines such as IFN-γ and IL-1 induce superoxide production by inflammatory cells and control their influx and activation. These cytokines also increased EC-SOD expression and secretion in rat alveolar type 2 cells [71] and fibroblasts [200]. Increased expression of EC-SOD in transgenic mice has been associated with decreased inflammation and pulmonary tissue damage in models of LPS-induced inflammation [179], viral pneumonia [201], and oil fly ash [30], as well as acute lung injury and pulmonary fibrosis as described above. It is interesting to note that the same cytokines that control EC-SOD expression also increase NO production in inflammatory reactions. It has been shown that NO release and induction of EC-SOD secretion are linked through NF-κB activation, which simultaneously upregulates transcription of EC-SOD and iNOS. This suggests that EC-SOD expression and iNOS production may occur in the same vicinities of the lung at roughly the same time. Concurrent upregulation of EC-SOD and NO production would inhibit the formation of peroxynitrite, which contributes to toxicity during inflammation [202–205].

However, in many of the disease models described herein, EC-SOD levels in lung tissue are markedly de-

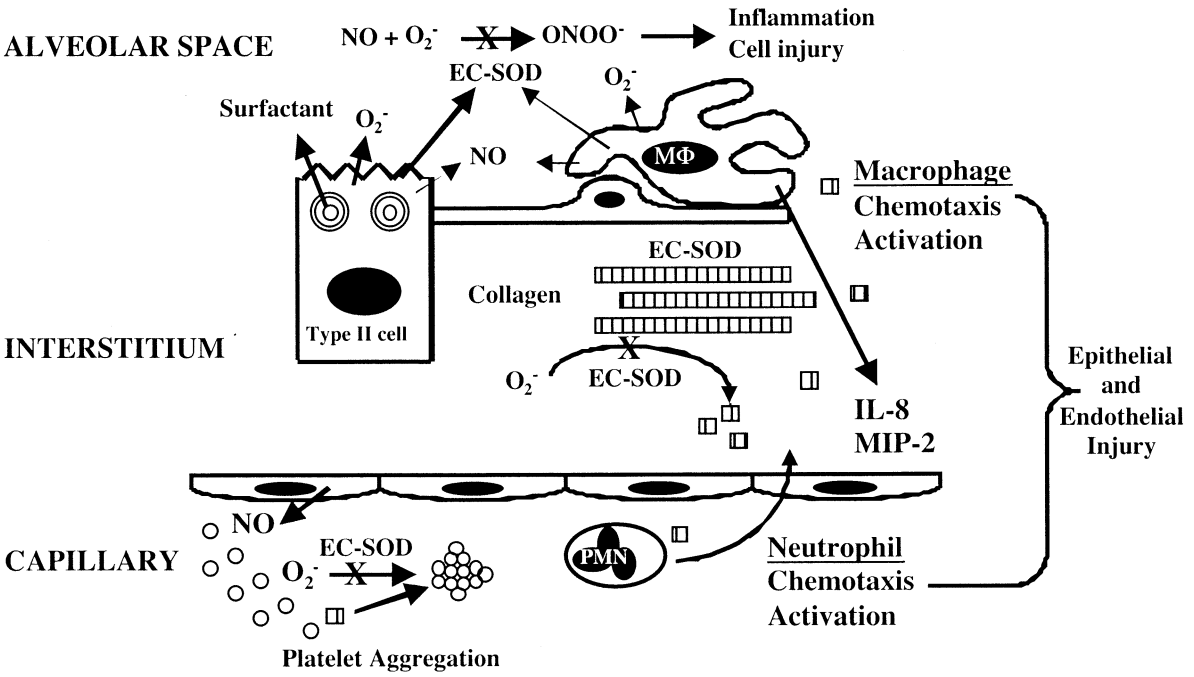

Fig. 6. Schematic illustrating the proposed role of EC-SOD and collagen fragmentation in acute lung injury. EC-SOD released by type 2 cells and macrophages will scavenge extracellular O_2^- and prevent the formation of the proinflammatory peroxynitrite anion ($ONOO^-$) while preserving nitric oxide, an anti-inflammatory molecule and inhibitor of platelet aggregation (○). In addition, EC-SOD protects collagen from superoxide-mediated degradation, thus preventing chemotaxis and activation of neutrophils and macrophages.

creased. This decrease has been attributed to the loss of EC-SOD heparin affinity through proteolysis of the heparin-binding domain of the enzyme. Proteases released from neutrophils or otherwise activated during inflammatory reactions [206] may be responsible for the proteolysis of EC-SOD. The exact mechanism by which loss of EC-SOD from the matrix of lung tissue exacerbates ROS-mediated damage is not entirely clear. Studies by our laboratory suggest that the loss of EC-SOD from the extracellular matrix of lung tissue into the alveolar lining fluid may serve to promote collagen degradation and, perhaps, increase oxidative injury to alveolar epithelial cells. This may result in an increased inflammatory response in these areas and, thus, increased injury. It has been shown that type 1 collagen is sensitive to degradation by the superoxide anion both directly [207,208] and indirectly through the activation of latent collagenases in neutrophils [209,210]. Furthermore, collagen fragments are known to be both chemoattractants and activators of neutrophils [211], and, thus, may enhance inflammatory reactions. In fact, our most recent studies examining bleomycin-induced damage in EC-SOD-null mice demonstrated that the removal of EC-SOD from type 1 collagen in the lung led to increased collagen fragmentation in vivo (unpublished observation). Therefore, increased production or decreased scavenging of superoxide, which results in collagen degradation, may accelerate inflammatory responses and tissue destruction through neutrophil recruitment and activation (Fig. 6).

THERAPEUTIC APPLICATIONS OF EC-SOD

A considerable number of studies have suggested that the administration of a wide variety of enzymatic and nonenzymatic antioxidants can protect against oxidant-induced tissue injury both in animal models and in the human patient. Since EC-SOD levels are decreased in several diseases, it is interesting to entertain the idea of EC-SOD as a potential target for therapy to improve antioxidant capacity and restore the free radical balance. Studies have shown that the half-life of EC-SOD in the vasculature is approximately 20 h. The long vascular half-life and high affinity for heparan-sulfate in the vascular wall of rEC-SOD, coupled with successful attempts at large-scale production [212,213], could make parenterally administered rEC-SOD a particularly efficient therapy against disorders associated with enhanced production of superoxide radicals [214,215]. Several studies have also shown the efficacy of EC-SOD-based gene therapy in models of collagen-induced arthritis [216], paracetamol-induced liver damage [217], and cardiovascular disease [135]; and, EC-SOD may be important for the treatment of cerebral vascular pathologies [218]. Furthermore, several metalloporphyrin-based catalytic

antioxidants [219] with SOD activity have been shown to have beneficial effects in models of asthma [220], airway inflammation in response to cigarette smoke [221], stroke [222,223], cerebral ischemia [224], and lung injury induced by bleomycin [199] or paraquat [225]. A further understanding of the role of EC-SOD in the pathogenesis of oxidant-mediated diseases will provide further insight into the potential therapeutic possibilities of EC-SOD.

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