Published in final edited form as:
Transl Res. 2008 February ; 151(2): 68–78. doi:10.1016/j.trsl.2007.10.003.

Extracellular superoxide dismutase (ecSOD) in vascular biology: an update on exogenous gene transfer and endogenous regulators of ecSOD

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Abstract

Extracellular superoxide dismutase (ecSOD) is the major extracellular scavenger of superoxide ($O_2^{\cdot-}$) and a main regulator of nitric oxide (NO) bioactivity in the blood vessel wall, heart, lungs, kidney, and placenta. Involvement of $O_2^{\cdot-}$ has been implicated in many pathological processes, and removal of extracellular $O_2^{\cdot-}$ by ecSOD gene transfer has emerged as a promising experimental technique to treat vascular disorders associated with increased oxidant stress. In addition, recent studies have clarified mechanisms that regulate ecSOD expression, tissue binding, and activity, and they have provided new insight into how ecSOD interacts with other factors that regulate vascular function. Finally, studies of a common gene variant in humans associated with disruption of ecSOD tissue binding suggest that displacement of the enzyme from the blood vessel wall may contribute to vascular diseases. The purpose of this review is to summarize recent research findings related to ecSOD function and gene transfer and to stimulate other investigations into the role of this unique antioxidant enzyme in vascular pathophysiology and therapeutics.

Oxidative stress induced by superoxide anion ($O_2^{\cdot-}$) produced in vascular cells is involved in the pathogenesis of cardiovascular and metabolic diseases, including atherosclerosis,[1] ischemia–reperfusion injury,[2] diabetes,[3] hyperlipidemia,[4] and hypertension.[5] Moreover, $O_2^{\cdot-}$ may also contribute to pulmonary hypertension,[5] erectile dysfunction,[6] cerebral vasospasm,[7] and other disorders associated with vascular dysfunction. Consequently, strategies to reduce levels of $O_2^{\cdot-}$ have emerged as promising approaches to treating cardiovascular diseases and other conditions associated with enhanced oxidative stress.

Scavenging of $O_2^{\cdot-}$ is performed by a group of anti-oxidant enzymes called superoxide dismutases (SODs), which catalyze the dismutation of $O_2^{\cdot-}$ to H_2O_2 and O_2 efficiently and specifically.

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In mammalian tissues, 3 isoforms of SODs exist: Cu/Zn SOD (SOD1), Mn SOD (SOD2), and extracellular SOD (ecSOD or SOD3). SOD1 is an abundant copper- and zinc-containing cellular protein that is present in the cytosol, nucleus, peroxisomes, and mitochondrial inner membrane. Its primary function is to lower the intracellular steady-state concentration of $O_2^{\cdot-}$. SOD1 mutations are associated with neural diseases such as amyotrophic lateral sclerosis.[8] SOD2 is a mitochondrial enzyme that disposes of $O_2^{\cdot-}$ generated by respiratory chain activity. SOD2 can be induced to protect against prooxidant insults. Conversely, SOD2 activity is decreased in physiologic aging and in diseases such as progeria, cancer, asthma, and transplant rejection.[9] ecSOD, another copper- and zinc-containing dismutase, is a primary antioxidant enzyme secreted to the extracellular space. ecSOD is expressed highly in selected tissues, including blood vessels, heart, lungs, kidney, placenta, and extracellular fluids. ecSOD plays an important role in regulating blood pressure and vascular contraction, at least in part, through modulating the endothelial function by controlling the levels of extracellular $O_2^{\cdot-}$ and nitric oxide bioactivity in the vasculature.[10,11] ecSOD has also been proposed to play an important role in neurologic, pulmonary, and arthritic diseases.[12,13]

The relative expression of SOD isoforms in cells and tissues has been investigated extensively and provides clues as to the sources of $O_2^{\cdot-}$ in pathophysiologic states. Based on our observation, in most tissues, SOD1 is the isoform that is expressed at the highest level. However, many examples exist in which this general pattern of expression differs among tissues and species. For example, ecSOD is expressed highly in vascular tissues, particularly in the arterial wall, and its activity constitutes almost half of the total SOD activity in the human aorta.[14,15] Collectively, these observations suggest that $O_2^{\cdot-}$ in the extracellular space [released from inflammatory and vascular cells, most likely through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity] contributes significantly to oxidant stress in the vascular wall.

$O_2^{\cdot-}$, ecSOD, AND EXPERIMENTAL GENE TRANSFER

Decreasing $O_2^{\cdot-}$-associated oxidative stress by enhancing ecSOD has been applied successfully in various experimental disease models[16–34] (Table I). For example, ecSOD has been shown to restore erectile function in streptozotocin-induced diabetes,[33] protect against vascular dysfunction with aging,[19] blunt ischemia/reperfusion-induced liver injury,[32] reduce systemic vascular resistance and arterial pressure in spontaneously hypertensive rats,[21] improve endothelial dysfunction in hypertension and in heart failure models,[23,35] and ameliorate inflammatory arthritis.[28,29] In most of the aforementioned studies, human ecSOD gene transfer was employed to augment ecSOD activity in animal models. Regarding the behavior and fate of the delivered foreign gene in recipient hosts, most studies showed an increase of ecSOD activity, with a corresponding decrease in $O_2^{\cdot-}$ levels, after gene transfer (Table I). Conversely, in other cases, ecSOD gene therapy failed to protect against cardiovascular diseases (Table I). For example, Laukkanen et al[18] reported that short-term overexpression of ecSOD *in vivo* did not affect atherogenesis in LDL receptor$^{-/-}$ mice. Yamaguchi et al[24] showed that human ecSOD gene transfer failed to prevent cerebral vasospasm in a canine model of subarachnoid hemorrhage. Zimmerman et al[36] reported that adenoviral-mediated delivery of human ecSOD to the subfornical organ failed to prevent the

development of angiotensin II-induced hypertension in mice. The subfornical organ is a region of the brain lying outside the blood– brain barrier and is known to be a primary sensor for blood-borne angiotensin II. The mechanisms associated with the apparent failure of ecSOD gene transfer are still unknown. Note that most successful studies with ecSOD overexpression have been performed in rats, possibly because the level of expression of endogenous vascular ecSOD is lower in rats as compared with many other species of animals.[37] Therefore, the relative degree of enhancement of ecSOD activity after gene transfer is typically higher in rats as compared with other species such as dog,[24] which makes it easier to demonstrate a therapeutic effect.

Please note that in this review, we will not discuss methods or approaches commonly employed to optimize delivery of SOD genes. Such information is provided elegantly in a recent review by Heistad.[38] Also, basic information about the biochemical properties and biology of ecSOD will not be included here but is available from several comprehensive reviews.[39,40] Instead, we will focus on factors known to regulate expression, activity, and function of ecSOD, with special emphasis placed on those relevant to the cardiovascular system.

It is also necessary to point out that in addition to serving as an injurious radical specie,[41] $O_2^{\cdot -}$ can, under some circumstances, serve as a signal transduction molecule that is important to cellular homeostasis.[42,43] Thus, therapies that reduce $O_2^{\cdot -}$ levels below a certain threshold may actually be harmful. In addition, the balance between extracellular and intracellular $O_2^{\cdot -}$ levels may be another important factor. Considering that it is not possible to measure $O_2^{\cdot -}$ levels *in vivo* with sufficient special and temporal resolution, it may be difficult to deliver precisely the appropriate amount of ecSOD to the proper location at the correct time. Moreover, we do not have complete information about how this dismutase is regulated *in vivo* and *in vitro*. Such challenges underlie the field of free radical biology in general and complicate interpretation of basic and clinical research data regarding the role of oxidative stress in vascular diseases.

FACTORS INVOLVED IN THE REGULATION OF ecSOD EXPRESSION AND ACTIVITY

Copper and copper transport pathways

Copper, which is a redox active metal, is an essential element that is required for normal cellular function. During the past decade, our understanding of the factors that regulate cellular copper distribution and transport has improved greatly. First, intracellular copper availability is extraordinarily restricted, as the intracellular milieu has a great capacity to chelate copper.[44] Second, in the cytoplasm, copper is distributed among several proteins, known as copper chaperones, which include antioxidant protein 1 (Atox1) synthesis of cytochrome c oxidase 2, and copper chaperone for superoxide dismutase 1. Copper chaperones compete with chelators for copper and directly insert the cofactor into the target apo-cuproenzymes, such as cytochrome C oxidase, SOD1, and Menkes ATPase, thus converting the latter from an inactive to an active state (holo-cuproenzymes).[45] Third, the metallochaper-one Atox1 directly interacts with the Menkes ATPase and plays a critical role

in perinatal copper homeostasis.[46] Menkes ATPase serves as a copper efflux pump that regulates the amount of copper leaving the cell and supplies copper to secreted cuproenzymes, including ecSOD.[47] These findings provide a basis for understanding how ecSOD biosynthesis could be modulated by copper transport pathways.

The central region of human ecSOD contains the essential amino acid residues involved in the coordination of Cu(II) and Zn(II) ions, which is termed the copper, zinc binding domain. Fukai and colleagues[48,49] recently determined that copper delivery to ecSOD modulates its enzymatic activity. The investigators found that ecSOD-specific activity in the conditioned medium from cultured fibroblasts isolated from Atox1$^{-/-}$ mice or Menkes ATPase mutant mice was decreased markedly and was restored partially by the addition of copper to the conditioned medium.[48,49] Co-immunoprecipitation and *in vitro* pull-down assays demonstrated a direct interaction between ecSOD and Menkes ATPase, and confocal immunoflurescence microscopy showed co-localization of ecSOD with Menkes ATPase in the trans-Golgi network.[49] These observations suggest that Menkes ATPase transports copper to ecSOD in the trans-Golgi network through a direct physical interaction.[49] In keeping with these *in vitro* observations, the aortas of Menkes ATPase mutant mice revealed a decrease in activity of ecSOD in association with a robust increase in $O_2^{\cdot-}$ levels *in vivo*.[49]

Zinc

The essentiality of zinc for human health is well established, and the consequences of severe zinc deficiency have been documented in population studies worldwide.[50,51] Although zinc is not redox active, zinc supplementation has been shown to reduce oxidative damage.[52–54] Zinc has also been observed to have an antiatherosclerotic effect.[55–58] Epidemiologic studies revealed that zinc was associated inversely with cardiovascular disease.[59–61] Olin et al[62] observed that juvenile rats fed zinc-deficient diets exhibited low plasma zinc levels and low ecSOD activity. The enzymatic activity of ecSOD, however, was not restored by *in vitro* addition of zinc to the plasma samples. Furthermore, adolescent rhesus macaques fed diets that contained a marginal amount of zinc also had low plasma zinc levels and low ecSOD activity compared with control animals fed diets containing sufficient zinc.[62] In another study in rats fed a zinc-deficient diet, a trend toward decreased ecSOD activity was also observed.[63,64]

Although positive correlations between zinc levels and ecSOD activities were observed in the animal studies, such correlations have not been observed consistently in humans. Paik et al[65] reported that in healthy adult Koreans, ecSOD activity correlated positively with increasing serum zinc concentrations. Interestingly, SOD1 activity correlated negatively with zinc, which suggests that SOD1 and ecSOD are regulated differentially by this cation. The same group also conducted a clinical trial to evaluate the effects of prenatal zinc supplementation on pregnancy outcome in African-American women.[66] Although a positive effect of zinc supplementation on birth weight was observed, plasma ecSOD activities in these subjects were lower than reported previously for healthy adults,[65] which suggests that plasma ecSOD activity is not a sensitive marker for marginal zinc deficiency. These results are not surprising given the complex interactions of dietary zinc with other factors that regulate ecSOD *in vivo*. For example, dietary zinc deficiency can cause tissue iron

accumulation,[67] which in turn may regulate ecSOD function (as discussed in the next section). In future study, the effect of zinc transport pathway on ecSOD function may also need to be evaluated.

Iron

Like copper, iron is a redox active metal that is critical to cellular homeostasis.[68] Stralin et al[69] reported that the addition of $FeCl_2$ to smooth muscle cell (SMC) cultures induced a marked dose-dependent increase in ecSOD expression. In addition, low concentrations of iron increased secretion of ecSOD to the medium, whereas higher concentrations inhibited both ecSOD expression and secretion. The mechanisms by which iron may modulate ecSOD are unknown currently. One possibility is that iron-dependent $O_2^{\cdot -}$ production may modulate directly ecSOD secretion in the vasculature. However, removal of external iron by desferal and bathophenanthroline disulfonate did not affect ecSOD expression in smooth muscle cells, and the addition of SOD1 or catalase to suppress Haber–Weiss chemistry did not influence the effects of iron on ecSOD expression.[69] A second possibility is that iron interferes with the proper functioning of the Golgi apparatus and the endoplasmic reticulum involved in secretion of ecSOD. It has been reported that the subcellular distribution (cytosolic vs microsomal) of some proteins is affected by cellular iron status.[70] The third possibility is that iron interferes with the proper functioning of copper and modulates indirectly the ecSOD secretion, because the metabolic fates of copper and iron are linked intimately. For example, systemic copper deficiency is associated with cellular iron deficiency.[71]

TISSUE BINDING OF ecSOD: DISPLACEMENT BY HEPARIN

Heparin is a highly sulfated glycosaminoglycan that is best known for its antithrombotic effects. Heparin also inhibits proliferation and migration of SMCs and regulates the synthesis of proteins in SMCs[72] and fibroblasts.[73,74] Moreover, heparin displaces lipoprotein lipase bound to the endothelial surface, which thus affects lipoprotein metabolism.[72] ecSOD also has a high affinity to heparan sulfate proteoglycans located on endothelial cell surfaces and in the connective tissue matrix.

Intravenous injection of heparin displaces ecSOD from proteoglycans and leads to a prompt increase in plasma ecSOD activity in humans[75] and other mammals.[76,77] For example, 1000 IU of heparin/kg body weight produces a maximal release of ecSOD in pigs. Injection of 50-IU heparin/kg body weight into healthy human volunteers led to an immediate 2.4–2.8-fold increase in serum ecSOD levels. Thus, heparin is highly potent at displacing ecSOD into the circulation. The ecSOD was released from endothelial cells, not from the blood cells.[75] The half-life of ecSOD release into the serum after heparin injection was about 90 min.[78] Furthermore, *in vivo* and *in vitro* experiments suggested that the ecSOD released into the plasma by heparin reestablished its binding to glycocalyx on the vascular endothelial cell surface in proportion to the elimination of heparin from the vascular system. In addition, heparin was shown to induce both ecSOD mRNA and protein expression in cultured skin fibroblasts.[79] The extent of ecSOD induction seems to be dependent on the level of glycosaminoglycan sulfation and may involve either receptor binding or a direct effect on promoter elements.[79] Only a portion of the tissue-bound ecSOD is displaced by heparin

injection,[75] which suggests that other ligands for ecSOD such as collagen type I[80] or fibulin-5[81] may also play an important role in regulating levels of tissue bound ecSOD.

The pathophysiologic significance of heparin-induced ecSOD release has been investigated in a clinical trial, which showed that the levels of ecSOD released by heparin were significantly lower in patients with atherosclerosis as compared with controls. Moreover, the coronary artery disease score was correlated inversely with heparin-induced ecSOD release. As for factors affecting the level of heparin-induced ecSOD release, high-density lipoprotein cholesterol levels and age were correlated positively.[82] Because heparin is a common therapeutic drug for pulmonary embolism and deep vein thrombosis, an interesting question is whether heparin-induced ecSOD release also facilitates the therapeutic effect of heparin.

POTENTIAL INTERACTIONS OF ecSOD WITH OTHER FACTORS THAT MODULATE CARDIOVASCULAR FUNCTION

Estrogen and progesterone

Estrogen and progester-one are 2 steroid hormones that regulate several aspects of cardiovascular function. For example, estrogen acts as an antioxidant[83] by enhancing nitric oxide (NO) bioavailability and by inhibiting the reactive oxygen species (ROS)-generating NADPH oxidase.[84,85] The impact of progesterone on oxidative stress is not as well established as that of estrogens. Strelow et al[86] and Wassman et al[87] studied the effects of estrogen and progesterone on ecSOD expression and activity.[86,87] They reported that in SMCs, estrogen upregulated ec-SOD expression (mRNA and protein level) and enzymatic activity.[86] Conversely, progesterone downregulated basal ecSOD expression and activity and reversed ecSOD induction by estrogen.[87] Moreover, ovariectomy led to a downregulation of ecSOD expression in mice, which was associated with increased levels of vascular ROS. These effects were blunted by estrogen replacement or treatment with pegylated-SOD.[86] Conversely, administration of progesterone to ovariectomized mice abrogated the antioxidant effects of estrogen replacement, including the enhancement of ecSOD expression.[87] In humans, increased estrogen levels correlated with enhanced ecSOD expression in circulating monocytes.[86]

Angiotensin II

Angiotensin II is the major effector hormone of the renin-angiotensin system (RAS), which regulates blood volume, arterial pressure, and cardiac and vascular function. Angiotensin II binds to 2 distinct receptors, angiotensin type 1 (AT1) and angiotensin type 2 (AT2). AT1 receptors are distributed widely and mediate most biologic responses of angiotensin II, whereas AT2 receptors antagonize several AT1 receptor-mediated responses; together, these 2 subtypes seem to coregulate blood pressure homeostasis and sodium excretion. Many hypertensive effects of angio-tensin II have been attributed to increased oxidative stress in blood vessels and to the central nervous system. Recently, angiotensin II has been found to upregulate ecSOD expression acutely in murine blood vessels *in vivo*,[88] in cultured human aortic SMCs,[88] and in human uterine arterial SMCs.[89] The upregulation of vascular ecSOD may counterbalance the increased vascular $O_2^{\cdot-}$ production and the decreased total SOD activity[90] stimulated by angiotensin II.

Although angiotensin II upregulated ecSOD expression in vascular tissues *in vivo*, the peptide produced opposite effects on ecSOD expression in the kidney. Chabrashvili et al[91] and Welch et al[92] reported that angiotensin II infusion in rats decreased ecSOD mRNA levels in the kidneys, and this effect was reversed by candesartan, which is an AT1 receptor antagonist,[91] or by Tempol, which is an SOD mimetic.[92] These results suggest that ecSOD may contribute to the protective effects of AT1 receptor blockade on oxidative stress in the kidneys.[93] The reason for the differences between vascular and renal expression of ecSOD in response to angiotensin II is unclear but may relate to differences in redox signaling, vasoactive effects, and/or function of AT2 receptors in kidneys versus blood vessels (for review, see Johren et al[94]).

Modulation of the RAS by angiotensin converting enzyme (ACE) inhibitors and AT1 receptor antagonists in humans produces many beneficial effects on the cardiovascular system. Hornig et al[95] randomized patients with coronary artery disease to 4 weeks of ramipril, which is an ACE inhibitor, or losartan, which is an AT1 receptor antagonist. Flow-dependent, endothelium-mediated vasodilation (FDD) of the radial artery was increased significantly after ramipril or losartan; in particular, the portion of FDD mediated by NO was increased by greater than 75%. Concomitantly, therapy with ramipril or losartan increased ecSOD activity by greater than 200%. These findings suggest that long-term inhibition of the RAS in humans may increase ecSOD activity, thereby reducing oxidative stress in the arterial wall.

Homocysteine

Homocysteine is an intermediate sulfur-containing amino acid that is formed during the metabolism of methionine. An increased level of homocysteine in the plasma has been implicated as a risk factor for cardiovascular disease.[96,97] Autoxidation of homocysteine can generate $O_2^{\cdot-}$ and can lead to peroxynitrite formation, thereby promoting oxidative stress.[98,99] The resultant increase in $O_2^{\cdot-}$ levels may in part be counterbalanced by homocysteine-dependent increases in ecSOD.[100] For example, Wilcken et al[101] reported a positive correlation between plasma levels of homocysteine and ecSOD in patients with coronary artery disease, and therapy that lowered plasma homocysteine also decreased ecSOD levels.[101] ecSOD levels were correlated inversely with the incidence of cardiovascular disease in these patients, and risk factors for coronary artery disease, such as male gender and smoking, were associated with decreased levels of ecSOD in a previous study.[102] The mechanisms by which homocysteine may modulate plasma ecSOD levels are unknown currently. One possibility is that homocysteine modulates the binding of ecSOD to endothelial cells through direct modification of heparan sulfate proteoglycans on cell surfaces. This mechanism is supported by the work of Yamamoto et al,[103] who reported that the binding of recombinant ecSOD to immobilized heparin was decreased markedly by pretreatment of the heparin-bound surface with homocysteine. Also, Nihei et al[104] showed that in patients with coronary artery disease, hyperhomocysteinemia is associated with increased release of ecSOD from the endothelium. Another potential mechanism is that homocysteine may perturb endoplasmic reticulum function, thereby disrupting disulfide bond formation or glycosylation of ecSOD, which is required for normal protein assembly and association with cell membranes.[105]

Other factors

Increased oxidative stress is thought to underlie the cardiovascular complications associated with diabetes mellitus. In Japanese patients with diabetes, serum ecSOD levels were correlated positively with the severity of microvascular complications, which may reflect decreased binding of the enzyme to the endothelium and enhanced susceptibility to vascular oxidative stress.[106] Navab et al[107] demonstrated that levels of ecSOD in aorta were decreased in a rat model of diabetes and that treatment with D-4F, which is an apolipoprotein A1 mimetic with anti-inflammatory properties,[107] restored aortic ecSOD protein levels without affecting either Cu, ZnSOD, or MnSOD.[108] The mechanism was suggested to be related indirectly to upregulation of the antioxidant enzyme heme oxygenase.[108] Furthermore, prooxidants such as xanthine oxidase, paraquat, and *tert*-butyl hydroperoxide decreased ecSOD expression in fibroblasts in a dose-dependent manner.[109] In vascular SMCs and lung alveolar type 2 cells, ecSOD expression was upregulated by IFN-γ and IL-4 and downregulated by TNF-α.[110] Finally, growth factors such as TGF-β, PDGF, and FGF depressed ecSOD mRNA levels in fibroblasts and SMCs.[111] Moreover, ecSOD activity is modulated by hydrogen peroxide because of its peroxidase activity.[112,113] The patho-physiologic relevance of *in vitro* alterations in ecSOD expression induced by oxidants, cytokines, and growth factors remains to be determined.

ecSOD GENE VARIANTS

Substitution of arginine-213 with glycine (R213G), which is located in the center of the carboxyl-terminal cluster of positively charged amino acid residues of the heparin binding domain, is a common human gene variant of ecSOD.[114–117] Although this variant does not affect ecSOD enzymatic activity,[115,116] plasma concentrations of ecSOD are increased dramatically in the 2% to 5% of the population that carries the gene variant. The increased concentration of ecSOD in the plasma of R213G carriers is in part caused by impaired heparin and collagen binding affinities, which leads to a 50-fold decrease in binding of the variant gene product to endothelial cells *in vitro*.[115,116,118] In addition, the R213G gene product is resistant to proteolysis by trypsin and neutrophil-derived proteases.[119] In spontaneously hypertensive rats, overexpression of the R213G variant of ecSOD did not improve blood pressure, vascular function, or vascular oxidant stress, which suggests that disruption of the heparin binding domain negates the protective effects of ecSOD in the vasculature.[20]

The clinical significance of ecSOD(R213G) has been investigated in several association studies. Patients with diabetes and end-stage renal disease carrying ecSOD(R213G) had an increased 5-year mortality rate, with significantly higher death rates from ischemic heart disease and cerebrovascular disease than did those of noncarriers.[120] Also, the R213G gene variant was suggested to accelerate the progression of renal failure and atherosclerosis in uremic patients.[121] Finally, a study in Denmark detected a 2.3-fold increase in risk of ischemic heart disease in heterozygotes carrying ecSOD(R213G), with a 9-fold increase for plasma levels of ecSOD,[122] presumably because of increased $O_2^{\cdot -}$ in the vasculature of ecSOD(R213G) carriers.

Several other variants of the ecSOD gene have also been described in humans, including a threonine-to-alanine substitution at position 40 in the amino terminus and a silent substitution at amino acid 280. Table II summarizes newly identified variants in the human ecSOD gene.[123,124] The functional significance of these variants, however, remains unknown.[125]

CONCLUSIONS AND FUTURE DIRECTIONS

ecSOD has emerged as an important endogenous regulator of oxidative stress. Moreover, ecSOD gene transfer represents a promising approach to reduce extracellular $O_2^{\cdot-}$ and to protect against oxidative stress, particularly in the cardiovascular system. In this review, we have discussed possible factors affecting endogenous ecSOD expression and activity, tissue-binding, as well as interaction with other factors. Figure 1 illustrates the proposed mechanisms of modulation of ecSOD by endogenous mediators. Understanding the potential therapeutic role of ecSOD in cardiovascular diseases, however, requires fundamental knowledge of how the enzyme is regulated under normal and pathophysiologic conditions. In this regard, several important questions remain to be answered before modulation of ecSOD can be translated into human therapeutics:

- How do alterations in cellular copper, zinc, and iron levels modulate the efficacy of ecSOD gene transfer?
- Do endogenous angiotensin II, estrogen, progesterone, and/or homocysteine influence the activity of ecSOD expressed by gene transfer?
- Is endogenous SOD activity altered by ecSOD gene transfer?
- To what extent does upregulation of ecSOD by pharmacologic agents (ie, estrogens, ACE inhibitors) contribute to the cardiovascular effects of these agents?
- Is displacement of ecSOD from the vasculature by heparin of clinical importance, particularly in the setting of acute coronary syndromes?

Acknowledgments

Supported by NIH grants HL070860, HL076684, and HL62984 (to N.L.W.), and HL70187-01 (to T.F.), and by University of Cincinnati URC faculty development grant (to Z.Q.).

Abbreviations

ACE	angiotensin converting enzyme
AT1	angiotensin type 1
AT2	angiotensin type 2
Atox1	antioxidant protein 1
ecSOD	extracellular superoxide dismutase
FDD	flow-dependent, endothelium-mediated vasodilation
NADPH	nicotinamide adenine dinucleotide phosphate

NO	nitric oxide
$O_2^{\cdot-}$	superoxide anion
RAS	renin–angiotensin system
ROS	reactive oxygen species
R213G	arginine-213 with glycine
SMC	smooth muscle cell
SOD	superoxide dismutase

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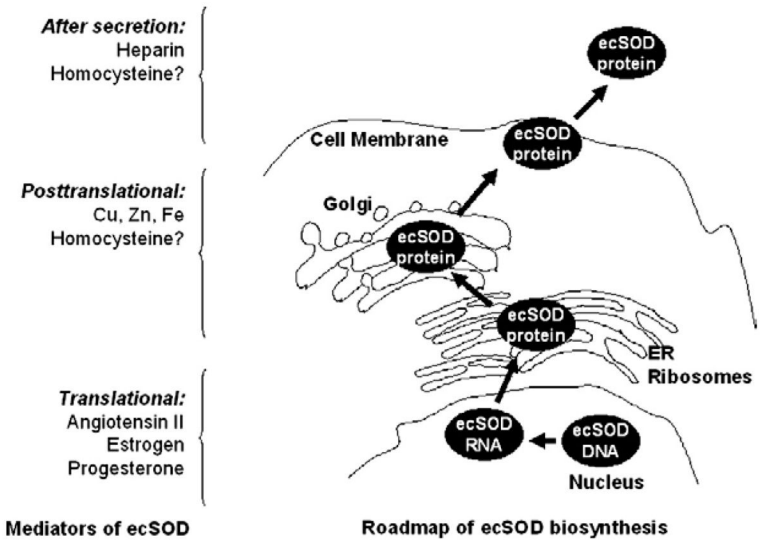

Fig 1.

Proposed mechanisms of modulation of ecSOD by endogenous mediators. Angiotensin II, estrogen, and progesterone modulate ecSOD mRNA levels. Cu and Zn incorporate into the *de novo* protein in the Golgi secretion pathway and are necessary to maintain enzymatic activity. Heparin modulates binding of the secreted ecSOD enzyme. *ER*, endoplasmic reticulum.

Table I

Studies of experimental gene transfer with ecSOD

Target tissue + condition	Species	Model	Vector	End points	Reference
Aorta + heart failure	rat	*in vivo* IV	Ad	Restored NO levels and responses to acetylcholine; decreased levels of $O_2^{\cdot-}$ and peroxynitrite	16
+ hypercholesterolemia	mouse	*in vivo* IV	Ad	Did not affect atherogenesis	18
+ aging	rat	*in vivo* IV	Ad	Decreased vascular $O_2^{\cdot-}$	19
+ spontaneous hypertension	rat	*in vivo* IV	Ad	Decreased levels of $O_2^{\cdot-}$ and nitrotyrosine	20
+ LPS treatment	rat	*in vivo* IV	Ad	Decreased $O_2^{\cdot-}$ and LPS-induced adhesion of leukocytes to aortic endothelium	22
+ balloon denudation	rabbit	*in vivo* local delivery	Ad	Decreased $O_2^{\cdot-}$, neointimal formation, and macrophage infiltration; IV gene transfer did not decrease restenosis	17
Carotid artery + spontaneous hypertension	rat	*in vivo* IV	Ad	Increased basal NO levels and responses to acetylcholine	20
+ spontaneous hypertension	rat	*in vivo* IV	Ad	Increased sodium balance and relaxation to acetylcholine; decreased mean arterial pressure	21
+ stroke-prone, spontaneous hypertension	rat	*in vivo* IV	Ad	Increased NO availability; improved endothelial function	23
Basilar artery + subarachnoid hemorrhage	dog	*in vivo* cisterna magna	Ad	Failed to prevent delayed cerebral vasospasm	24
Brain	rabbit	*in vivo* cisterna magna	Ad	Increased SOD activity in cerebrospinal fluid	25
Basilar artery + subarachnoid hemorrhage	rabbit	*in vivo* cisterna magna	Ad	Decreased cerebral vasospasm	26
Portal vein + acute liver injury	mouse	*in vivo* IV	ecSOD lipoplexes	Increased serum SOD activity and liver glutathione levels; decreased lipid peroxidation	27
Knee joint + antigen-induced arthritis	rat	*ex vivo*	ecSOD-expressing synoviocytes	Decreased joint swelling and gelatinase activity; decreased infiltration of inflammatory cells into the synovial membrane	28
+ collagen-induced arthritis	mouse	*ex vivo*	ecSOD-expressing fibroblasts	Decreased mononuclear cell infiltration and synovial proliferation; improved histologic abnormalities, including destruction of cartilage and bone	29
Heart + myocardial infarction	rabbit	*in vivo* IV	Ad	Markedly decreased infarct size	30
+ myocardial stunning	rabbit	*in vivo* IV	Ad	Increased ecSOD levels in the liver; improved left ventricular wall motion	31
Liver + paracetamol-induced damage	mouse	*in vivo* IV	Ad	Significantly attenuated release of liver enzymes and inhibited necrosis and apoptosis	18

Target tissue + condition	Species	Model	Vector	End points	Reference
+ ischemia/reperfusion- induced liver injury	mouse	*in vivo* IV	polylipid nanoparticles	Increased SOD activity and decreased $O_2^{\cdot -}$ levels and oxidative stress in liver; decreased enzyme release and improved liver histology	32
Cavernosum + diabetes	rat	*in vivo* IV	Ad	Decreased $O_2^{\cdot -}$ levels	33
Bowel + colitis	mouse	*ex vivo*	ecSOD-expressing fibroblasts	Increased chance of survival	34

Abbreviations: Ad, adenovirus; IV, intravenous; LPS, lipopolysaccharide.

Table II

Newly identified variants of the human ecSOD gene

Mutation	Type	Country	Findings	Reference
Ala40Thr	Missense mutation	Japan	Frequency of Thr allele, and the number of subjects with Thr allele (Ala/Thr + Thr/Thr), was higher in type 2 diabetic patients; patients with Thr allele also exhibited earlier age of onset of diabetes and decreased insulin sensitivity	123
Leu53Leu	Silent mutation	Japan	No differences in allele frequencies were found between diabetic and nondiabetic subjects	123
T40A	Missense mutation	Italy	Allele frequency 43%	124
F131C	Missense mutation	Italy	Allele frequency 5%	124
V160L	Missense mutation	Italy	Allele frequency 0.25%	124
R202L	Missense mutation	Italy	Allele frequency 0.84%	124
32 (GCG > GCT)*	Silent mutation	Italy	Allele frequency 0.34%	124

* Based on codon position.