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Review

Delivery of Antioxidant Enzyme Proteins to the Lung

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ABSTRACT

Protection of alveolar epithelial cells (alveolocytes) and vascular endothelial cells against pulmonary oxidative stress is an important problem. An inadequate delivery to the target cells limits the protective utility of the antioxidant enzymes, superoxide dismutase (SOD) and catalase. SOD and catalase modifications, such as coupling with polyethylene glycol and encapsulation in liposomes, prolong the life span of the active enzymes *in vivo*. The airway administration of SOD and catalase protects alveolocytes against hyperoxic oxidative stress. Although pulmonary endothelium is poorly accessible from the airways, it is accessible from circulation. However, antioxidant enzymes and their derivatives display poor targeting to pulmonary endothelium. To improve the targeting and provide intracellular delivery to endothelium, the enzymes can be conjugated with antibodies against endothelial antigens, such as angiotensin-converting enzyme and adhesion molecules [intercellular adhesion molecule-1 (ICAM-1) or platelet-endothelial cell adhesion molecule-1 (PECAM-1)]. These immunoconjugates accumulate in the pulmonary vasculature in intact animals, enter endothelium, and augment the antioxidant defenses. The immunoconjugates directed against ICAM-1 and PECAM-1 may also provide a secondary therapeutic benefit by blocking of sequestration and infiltration of leukocytes in the lungs. Further investigations are necessary to evaluate the therapeutic effectiveness of the vascular immunotargeting of antioxidant enzymes and solve technical problems associated with production of safe, clinically useful conjugates. Antioxid. Redox Signal. 3, 39–62.

INTRODUCTION

PULMONARY OXIDATIVE STRESS is a pathological condition implicated in many diseases and syndromes. Both alveolar epithelium and vascular endothelium represent important targets for antioxidant therapy. Superoxide dismutases (SOD; enzymes converting superoxide anion to H_2O_2) and catalase (an enzyme converting H_2O_2 to water) represent potential protein drugs for antioxidant therapy. However, the results of animal and clinical studies provide an impression that SOD and catalase afford rather modest (if any) protective effect against oxidative stress. At least in part, the modest benefit can be attributed to an inadequate delivery of the enzymes to the sites of the therapeutic action.

This review discusses the current means for the delivery of SOD and catalase to the lungs. For the sake of brevity, it focuses on protein drugs and does not discuss delivery of genetic material encoding antioxidant proteins. The goal of this article is to analyze critically the potential applicability of antioxidant enzymes for prevention or treatment of pulmonary oxidative stress, outline main problems of this strategy, and reveal avenues for its improvement. The article gives a detailed analysis of the results of the airway administration of antioxidant enzymes and their derivatives and discusses attempts for the intravascular delivery

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of these agents, including a novel means for immunotargeting of catalase and SOD to the pulmonary vascular endothelium.

A BRIEF OVERVIEW OF OXIDATIVE LUNG INJURY; ALVEOLAR EPITHELIUM AND VASCULAR ENDOTHELIUM AS TARGETS FOR ANTIOXIDANT THERAPY

Oxidative injury to the lung that occurs in many diseases is often initiated and propagated by overproduction of the key reactive oxygen species (ROS) superoxide anion and H_2O_2. Superoxide anion produces potent oxidants, hydroxyl radical, and peroxynitrite in reactions with metals or with nitric oxide, whereas H_2O_2 generates hydroxyl radical, hypochlorous acid, and nitrating agents (14, 68, 102). Extracellular ROS first attack components of the extracellular milieu and the outer leaflet of the plasma membrane. Intracellular ROS first attack components of the inner leaflet of the plasma membrane and the cellular interior, including DNA and regulatory proteins. Importantly, intracellular oxidants are less accessible to drugs than the extracellular counterpart.

Oxidative stress in the lung can be initiated and propagated via many scenarios (see Table 1). Inflammation represents the most common mechanism (53). For example, alveolar macrophages activated by pathogens or environmental factors (e.g., smoke) release ROS and pro-inflammatory mediators (chemoattractants and cytokines), which up-regulate adhesion molecules and activate blood leukocytes (184). Activated leukocytes transmigrate into the lung tissue, generate ROS and cytokines, and propagate lung inflammation and oxidative injury (193).

Leukocytes (neutrophils and monocytes) also propagate lung injury caused by mechanical ventilation with high oxygen (hyperoxia). This procedure helps to maintain a sufficient blood oxygenation in the hypoxic patients (e.g., in trauma patients and premature infants). However, mitochondria and other oxygen-utilizing systems are unable to detoxify all ROS formed in the course of oxygen utilization and transport under hyperoxia (27, 75, 180). A direct oxidative injury caused by environmental oxidants is further propagated via the inflammation cascade described above.

Abnormal ventilation in chronic obstructive pulmonary disease leads to a reorganization of the lung tissue and emphysema, a condition associated with elevated protease activity in lung tissue. Excessive proteolysis in lung tissue leads to generation of pro-inflammatory medi-

Table 1. Selected Variants of Pulmonary Oxidative Stress

Pathology	*Mechanism*	*Target compartment and cell*
Inflammation	ROS and cytokines produced by activated alveolar macrophages damage lung tissue, up-regulate endothelial adhesion molecules, attract leukocytes that propagate the injury.	*Injury initiation:* alveolar, epithelium *Injury propagation:* vascular, endothelium
Oxygen toxicity, hyperoxia	Elevated level of O_2 in the lungs leads to overproduction of ROS in the tissue, with some of the above consequences.	*Injury initiation:* alveolar, epithelium *Injury propagation:* vascular, endothelium
Chronic obstructive pulmonary disease	Elevated lung protease activity generates pro-inflammatory cytokines and release of ROS by resident and migrating phagocytes.	*Injury initiation:* interstitial *Injury propagation:* vascular, endothelium
Endotoxemia, sepsis, trauma, ARDS/ALI	Systemic activation of leukocytes and up-regulation of endothelial adhesion molecules lead to vascular injury.	*Initiation and propagation:* vascular, endothelium
Pancreatitis, gastrointestinal ischemia–reperfusion	Leukocytes activated in the abdominal vasculature accumulate in the pulmonary capillaries, damage endothelium, and infiltrate the lung tissue.	*Initiation and propagation:* vascular, endothelium

ators, activation of alveolar macrophages, and recruitment of blood leukocytes, hence oxidative stress and persisting lung injury (147).

Trauma, sepsis, acute pancreatitis, intestinal and hepatic ischemia–reperfusion, hemorrhage, coagulation disorders, and other pathological conditions associated with systemic activation of complement, leukocytes, and generation of inflammatory mediators may cause secondary oxidative stress in the lung, for example, an acute lung injury syndrome [ALI; also known as adult respiratory distress syndrome (ARDS)]. Pulmonary vasculature contains an extended capillary network and serves as a natural filter for activated leukocytes. These aggressive cells attack pulmonary endothelium, infiltrate the lung tissue, and cause oxidative injury (22, 95).

Gas and liquid exchange is the main physiological function of the lung; hence the major portion of the lung volume belongs to the alveolar and vascular compartments. Thus, alveolar epithelium (lining the air surface of the alveoli) and endothelium (lining the capillary network surrounding the alveoli), separated in this compartment by the basal membrane, represent the two major cellular constituents in lung tissue (Table 2). These cell types suffer the most intense oxidative injury and represent important targets for antioxidant therapy.

Two types of alveolar epithelium (alveolocytes) have been described in human lungs. Type I, flat cells that cover the majority of the alveolar surface, are involved mainly in gas and liquid exchange between the air and blood compartments. Type II, cuboidal cells that represent only 5% of the alveolar epithelium, also exercise important functions. First, type II cells are the major source for the repair of alveolar tissue and regrowth of type I cells. Second, type II cells produce and recycle the lung surfactant. This mixture of phospholipids and specific proteins reduces surface tension within the alveoli, prevents alveolar collapse, and thus permits a normal breathing cycle. Surfactant proteins B and C are critically important for this function of the lung surfactant. Third, type II cells are involved in the host defense in the airways, because surfactant protein A (SP-A) is a lectin important for opsonization of pathogens.

Type I and II epithelial cells lining the alveoli are susceptible to oxidant attack by environmental ROS and ROS released from activated macrophages or leukocytes. Oxidative damage to the alveolocytes leads to the surfactant dysfunction, compromises gas and liquid exchange, and may lead to hypoxia, lung edema, and inflammation. Theoretically, antioxidants delivered directly to the alveolocytes via the airways may permit effective protection.

Endothelium is an interface between blood and underlying tissues that transduces chemical and mechanical signals, and regulates levels of vasoactive compounds such as angiotensins, substance P, bradykinin, nitric oxide, oxidants, cytokines, and growth factors. Pulmonary endothelial cells control vascular permeability in the lung, regulate vascular tone, manage the interaction of blood cells with pulmonary tissue, help to maintain the balance between blood coagulation and fibrinolysis in the lung, and contribute to immunological reactions in lung tissue. Endothelium is arguably

Table 2. Pulmonary Epithelial and Endothelial Cells: Targets for Antioxidant Therapy

Cell type	*Type I epithelium*	*Type II epithelium*	*Endothelium*
Compartment	Alveolar, flat cells	Alveolar, cuboidal cells	Vascular, monolayer cells
Main functions	Gas and liquid exchange	Production of surfactant, regeneration of alveolar epithelium	Blood–tissue barrier that regulates vascular pressure, permeability, inflammation
Major target upon	Hyperoxia Inflammation	Hyperoxia Inflammation	ALI/ARDS Inflammation, hyperoxia
Primary/secondary sources of ROS	Macrophages (neutrophils)	Macrophages (neutrophils)	Neutrophils (endothelium, macrophages)
Accessible from	Airways	Airways	Circulation

the most important target for antioxidant therapy in many pathological settings. Pulmonary endothelium is predisposed to injury induced by ROS released from activated leukocytes (68). Endothelial damage is extremely dangerous, because it compromises the vitally important functions of the endothelium and may initiate a cascade of pro-inflammatory events.

In addition to frequently being the target for attack by extracellular oxidants, endothelial cells can generate ROS (e.g., H_2O_2 or O_2^-). Enzymatic pathways that may generate ROS in the endothelium include the following: (i) xanthine oxidase formed from xanthine dehydrogenase upon hypoxia (207); (ii) lipoxygenase and cyclooxygenase activated by hormones, cytokines, or inflammatory mediators (100); (iii) univalent reduction of molecular oxygen by respiratory chain enzymes in the mitochondria (138); (iv) uncoupled superoxide anion generation by nitric oxide synthase (141); and (v) NADPH-oxidase or NADH-oxidase activated upon ischemia or by cytokines (2). ROS up-regulate expression of endothelial surface adhesion molecules used by leukocytes as anchors for rolling, adhesion, and tissue migration. Thus, endothelial cells may provoke neutrophil attack. This mechanism is involved in propagation of pulmonary oxidative stress associated with cardiopulmonary bypass, radiation, and lung transplantation. In these settings, ROS and pro-inflammatory mediators generated in lung tissue initiate the cascade of pro-inflammatory reactions described above, which results in pulmonary sequestration of leukocytes and lung injury. Importantly, a portion of ROS released by the adherent neutrophils diffuse inside the endothelium and become inaccessible to antioxidants in blood (162). At high concentrations, ROS cause irreversible damage and kill vascular endothelium.

Endothelial cells are relatively accessible for drugs circulating in the bloodstream. Ideally, antioxidant therapy of the endothelium would combine (i) intracellular delivery of antioxidant enzymes, to intercept intracellular ROS, and (ii) blockage of endothelial adhesion molecules, to suppress leukocyte attack and inflammation.

GENERAL PROPERTIES OF SOD AND CATALASE AND THEIR DERIVATIVES

Antioxidant enzymes, SOD and catalase, are not consumed by ROS and have high affinities and rates of reaction with ROS. Three forms of SOD, namely CuZnSOD, MnSOD, and extracellular SOD (exSOD), exist in mammals (55, 79). Table 3 gives a brief overview of their properties. All forms of SOD convert superoxide anion to H_2O_2. As superoxide anion may form hydroxyl radical and peroxynitrite, SOD protects against oxidative stress and helps to maintain normal regulatory functions of nitric oxide. In the absence of an adequate detoxification of H_2O_2, however, SOD may aggravate oxidative stress (70, 125, 136, 178). Catalase is a heme-containing tetrameric protein (M_r-240 kDa) that can be found in almost every tissue in many organisms. Each subunit (M_r-60 kDa) consists of a single polypeptide chain associated with a single prosthetic group, ferric protoporphyrin IX. Catalase rapidly decomposes H_2O_2 to water and oxygen.

However, despite the great expectations generated two decades ago, the protective antioxidant potential of SOD and catalase has yet to be translated into effective, reliable, and safe

Table 3. General Properties of SODs and Catalase

Enzyme	*CuZnSOD*	*MnSOD*	*ExSOD*	*Catalase*
General properties	Dimer	Tetramer	Tetramer	Tetramer
Cellular distribution	Cytosol	Mitochondria	Plasma membrane	Peroxisomes
Reaction catalyzed	O_2^- to H_2O_2	O_2^- to H_2O_2	O_2^- to H_2O_2	H_2O_2 to H_2O and O_2
Natural affinity	Not known	Not known	Heparin	Not known
Half-life in blood	4–5 min	4–5 min	3–6 min	10–15 min
Elimination pathway	Renal excretion, hepatic uptake	Renal excretion, hepatic uptake	Hepatic uptake	Hepatic uptake

therapeutic strategies (see 55, 62, 103, 143). Studies in intact animals and in humans reveal that an unfavorable pharmacokinetic profile and an inadequate delivery to the target cells severely restrict therapeutic application of SOD and catalase (62, 143, 194).

Oral administration of SOD and catalase is not effective, because the enzymes are poorly absorbed from and rapidly degraded in the gastrointestinal tract (59, 145). Repeated injections of the enzymes may elicit immune reactions, although recombinant human proteins are likely to be safer in terms of this adverse effect (182). Chemical camouflage (see below) also may help to elude the host defense system and attenuate immune reactions.

SOD and catalase have an extremely short life span in the bloodstream, with a half-life of $<$10 min (79, 80, 163, 182). Renal excretion represents the major pathway for the elimination of CuZnSOD (131, 179). Sinusoidal hepatic phagocytes (Kupffer cells) also display rapid uptake of CuZnSOD *in vivo* (42, 43, 171). ExSOD possesses a significant affinity to heparin (82). Thus, exSOD binding to charged components of glycocalyx leads to rapid hepatic uptake of exSOD; injection of heparin permits the release of a portion of tissue-sequestered exSOD to the bloodstream enabling the elevation of systemic antioxidant potential of circulating exSOD (10, 80). Hepatic uptake is also the major pathway for catalase elimination (118, 202).

Numerous modifications of SOD and catalase have been used to improve their pharmacokinetics. The major goals for the modification of SOD and catalase are as follows: (i) to prolong life span of the enzymes *in vivo*; (ii) to reduce their immunogenicity; (iii) to attain effective delivery to target tissues or cells; and (iv) to facilitate intracellular uptake of the enzymes (see Table 4 for an overview).

The covalent coupling of activated polyethylene glycol (PEG) to amino groups of lysine within the enzymes was introduced by Abuchowski and co-workers in the late 1970s for the optimization of the pharmacokinetics of therapeutic proteins and polymers. Linear PEG molecules form a shell that masks a protein

Table 4. Selected Modifications of SOD and Catalase for Therapeutic Use

Modification, derivative properties	*Properties* in vivo	*Applications (other than lung)*
Coupling of PEG: Protects proteins against recognition by immune system and phagocytes, facilitates intracellular delivery	PEG-SOD and PEG-catalase have a prolonged life span and higher activity *in vivo*.	Myocardial ischemia Cerebral ischemia Gastrointestinal ischemia Renal ischemia Inflammation
Encapsulation in liposomes: Protects proteins against inactivation in blood, promotes cellular uptake	Liposome-encapsulated SOD and catalase have a prolonged life span and higher activity.	Brain injury, ischemia Hepatic necrosis Inflammation Radiation injury
Coupling of SM: Renders enzymes an affinity to albumin	SM-SOD circulates in an albumin-associated form for a prolonged time and accumulates in the sites of acidosis.	Myocardial ischemia Hepatic ischemia
Lecithinized SOD (PC-SOD): Renders SOD an affinity to the plasma membrane components	PC-SOD binds to blood and vascular cells and has a prolonged life span.	Brain injury Vascular injury
Coupling of putrescine: Renders the enzymes an affinity to brain–blood barrier	PUT-catalase and PUT-SOD accumulate in the brain after intravenous injection.	Brain ischemia–reperfusion Brain injury
Coupling of mannose: Renders the enzymes an affinity to mannose receptors	Targeting of mannosylated enzymes to the hepatic macrophages	Liver inflammation
Coupling of gelatin: Increases stability and affinity to extracellular matrix	Gelatin-SOD has a prolonged life span and lower antigenicity.	Peripheral inflammation

from recognition by the immune system and phagocytes (1). Thus, PEG-modified proteins escape rapid elimination by macrophages, and circulate in the bloodstream for a prolonged time without eliciting dangerous immune reactions ("stealth technologies").

Coupling of PEG to SOD and catalase dramatically prolongs the half-lives of the enzymes in the bloodstream, from 5 and 15 min, respectively, to several hours (1, 142, 185). PEG-SOD and PEG-catalase do not cause detectable adverse effects in animal studies (186). The increase in life span *in vivo* and suppression of the immune response depend on the number and length of PEG polymers attached to a protein (154). In most studies, PEG-5000 has been used to modify the therapeutic proteins, including the antioxidant enzymes.

PEG-SOD and PEG-catalase display more effective intracellular delivery than the unmodified enzymes in cell culture (12, 69, 189). The mechanism of the cellular entry and metabolism of PEG-modified enzymes has yet to be fully understood. However, due to a favorable pharmacokinetic profile, PEG-SOD and PEG-catalase afford a more potent protective effect than unmodified enzymes in many animal models and clinical studies.

For example, PEG-modified SOD and catalase are protective in animal models of vascular, renal, hepatic, and cerebral oxidative stress (67, 94, 108). Several reports indicate that PEG-modified SOD and catalase display a superior protective potential over unmodified enzymes in animal models of myocardial ischemia (25, 170). Negative results with PEG-modified enzymes, however, have also been reported (66, 171). Most likely, a prolonged circulation is necessary, but not sufficient for protection. Therapeutic advantages of PEG-modified SOD and catalase remain to be characterized, although some clinical studies imply that these derivatives may have therapeutic merit (115, 177).

Another approach is encapsulation of catalase and SOD in liposomes. Liposomes, synthetic vesicles comprising phospholipids and cholesterol, have gained a prominent profile as a vehicle for delivery of small drugs (antibiotics), genetic material (DNA), and therapeutic proteins. Modulation of the liposome membrane allows production of unilamellar and multilamellar vesicles of defined fluidity, charge, and size (ranging from 50 nm to 50 μm). A liposome-encapsulated drug is protected from inactivation and causes less significant systemic effects. Modification of the outer surface of the liposome with masking and targeting moieties (e.g., PEG and antibodies) allows extension of their life span in the body and accumulation of liposomes in the target sites. Liposome vehicles also may provide the advantage of facilitated intracellular delivery, via fusion with the plasma membrane lipids, receptor-mediated endocytosis, and phagocytosis (140). Encapsulation of SOD and catalase in liposomes prolongs life span of the enzymes in the bloodstream, attenuates immune reactions, and facilitates cellular uptake of the enzymes in cell culture and animal models (26, 52, 123). Encapsulation of SOD in liposomes may improve its bioavailability after oral delivery (144).

Systemic administration of liposomal derivatives of SOD provided protective effect in animal models of toxic hepatic necrosis (122), adjuvant arthritis (26), oxidative injury to the brain (206), and brain ischemia–reperfusion (72). Results of clinical studies imply that liposomal SOD and catalase might be protective against radiation-induced fibrosis (41).

Different forms of SOD–catalase conjugates have been proposed to attain a more safe and complete detoxification of both superoxide anion and H_2O_2. These conjugates show promising protective effect in the models of ischemia–reperfusion injury in isolated rat heart (97) and rat intestine *in vivo* (30).

Natural heparin-binding properties of exSOD allow manipulation of the heparin-mediated cellular binding of SOD. For example, truncation of exSOD reduces its affinity to heparin and thus prolongs the half-life of exSOD in the bloodstream in rabbits from 20 min to 20 h (82, 155). On the other hand, generation of a fusion protein comprising CuZnSOD and a peptide sequence with a high affinity to heparin-like proteoglycans provides a new form of CuZnSOD that possesses an ability to bind to cells via a heparin-dependent mechanism (17).

A covalent coupling of [poly(styrene-*co*-maleic acid)], a hydrophobic organic anion (SM), to the cysteinyl residues of CuZnSOD

generates SM-SOD, a derivative that binds to serum albumin. Binding to albumin prolongs the life span of CuZnSOD (half-life-6 h in rats) and provides an affinity to plasma membrane components, especially when the pH is lower than the physiological level (73). These features may facilitate SM-SOD accumulation in ischemic sites. Intravascular administration of SM-SOD is reported to be protective against tissue oxidative injury in rat models of ischemia–reperfusion in heart (73), liver (86), and brain (168).

A covalent modification of CuZnSOD with a phosphatidylcholine derivative generates lecithinized SOD (PC-SOD), which possesses a high affinity to the plasma membrane components and promiscuously binds to blood and endothelial cells *in vitro* (71). PC-SOD is protective in rat models of oxidative stress associated with ischemia–reperfusion paw injury (71) and brain injury (204, 205). Recently, PC-SOD was reported to cause a vasodilatory effect in the aortic rings, likely due to elimination of superoxide anion and protection of endothelial nitric oxide signaling (124).

Modification of catalase and SOD with *N*,*N*′-dimethyl-1,3-propanediamine ("cationization") renders the enzymes with an affinity to the strongly negatively charged components of some tissues. Cationized catalase is retained at the site of intra-articular or intrajejunal administration and protects against local oxidative injury in these organs in animal models (156).

A covalent coupling of putrescine (PUT; a natural polyamine that binds to a putative receptor in the blood–brain barrier) to carboxylic groups of antioxidant enzymes generates PUT-SOD and PUT-catalase. The ability of PUT-enzymes to permeate the blood–brain barrier (perhaps via a receptor-mediated transcytosis) affords augmentation of SOD and catalase activity in the brain and thus may provide a modality for the treatment of some neurological diseases and syndromes (197). The utility of PUT-catalase and PUT-SOD, however, is likely to be limited to cerebral pathology, because enhanced targeting to the brain is accompanied by a significant reduction of the enzyme levels in the bloodstream (146).

Covalent coupling of sugar moieties alters the affinity and pharmacokinetics of antioxidant enzymes. The general rationale for this approach is that the interaction of sugars with cellular receptors is one of the natural mechanisms of ligand recognition. Thus, coupling of mannose or galactose residues provides derivative protein with an affinity to the corresponding cellular receptors. In contrast, coupling of sialic acid residues masks proteins from recognition by macrophages and immune cells. Sialylation of catalase renders a greater resistance to proteases *in vitro* and retains 70% of enzymatic activity; however, properties of this derivative remain to be characterized *in vivo* (48). SOD modified with carboxymethyl-dextran displays a prolonged life span in mice, whereas diethylaminoethyl-dextran SOD is rapidly cleared from the bloodstream by nonspecific hepatic uptake (56). SOD and catalase conjugated with a dextran-based carrier, Ficoll, have been tested for protection against lung oxidative injury (78, 101).

Mannosylated SOD (Man-SOD) and galactosylated SOD (Gal-SOD) undergo rapid elimination from the bloodstream after intravascular administration, similar to unmodified SOD (56). However, tissue distribution of these derivatives is quite different: unmodified SOD undergoes renal excretion, whereas Man-SOD accumulates in the liver (56). This result can be explained by the fact that Man-SOD, but not Gal-SOD or SOD, binds to macrophage mannose receptors (167). SOD glycosylation also alters the rate of renal filtration and reabsorption (106, 107). Similar to Man-SOD, Man-Catalase accumulates in hepatic nonparenchymal cells (Kuppfer macrophages), whereas Gal-catalase, as well as unmodified catalase, accumulates in hepatocytes (201, 202). Delivery of antioxidant enzymes to hepatic cells may be useful for treatment of oxidative hepatic injury associated with ischemia–reperfusion and inflammation.

Antioxidant enzymes can be conjugated with components of the extracellular matrix. The rationale for this approach is that coupling to these natural products may increase stability of the enzymes and their affinity to the cellular exterior. For example, coupling of succinylated gelatin to CuZnSOD elevates an apparent molecular mass of SOD (from 35 kDa t 98 kDa), prolongs the life span of SOD in the bloodstream (half-life of 29 min versus 4.5 min), and

reduces SOD antigenicity (88). Gelatin-SOD conjugate is more active than unmodified SOD in protection against ischemic foot pad edema and collagen-induced arthritis in mice (88). Conjugation of CuZnSOD with succinylated keratin fragments generates suc-Ker-SOD, which displays greater resistance to inactivation by H_2O_2, lower immunogenicity, slightly prolonged half-life in the bloodstream, and hepatic (instead of renal) clearance in mice (130).

Therefore, a variety of antioxidant enzyme derivatives have been proposed to improve their delivery to different targets in the body. Pharmacological properties and therapeutic advantages of these agents remain to be fully characterized. Some of the SOD and catalase derivatives, however, seem to be suitable for pulmonary delivery and will be discussed in more detail below.

DELIVERY OF ANTIOXIDANT ENZYMES VIA THE AIRWAYS

Airways represent a unique mode of entry that theoretically allows attainment of a high concentration of drugs in lung tissue. This route is widely used in clinical, ambulatory, and home settings for the administration of drugs (e.g., spasmolytics, bronchodilating agents, and agents regulating the production of mucose). These drugs are small molecules, which can be aerosolized easily, thus permitting a more effective and homogeneous distribution in the lung.

More recently, novel applications of the airway route have evolved. Peptides or proteins, which cannot be administered orally (e.g., insulin), can be inhaled to avoid the need for injections. This application requires an effective transfer of protein therapeutics from the alveolar to the vascular compartment. Intratracheal administration has been explored experimentally and clinically in gene therapy for the delivery of viral and nonviral DNA to lung cells. This application requires a sufficient contact of DNA vehicle with the target cells and proper intracellular trafficking of DNA. The tracheal route seems to be well suited for direct delivery of therapeutic proteins to the airway and alveolar cells. Delivery of SOD and catalase to the alveolocytes represents an example of the latter strategy.

There are serious technical issues associated with the delivery of large molecules or microparticles to the alveolar compartment (45). The key requirement is that the drug achieves contact with an extended surface of the airway and alveoli. However, inhaled proteins tend to be retained in the airways and a small proportion of protein reaches the alveoli. The larger a drug is, the more difficult it is to attain its effective and homogeneous delivery to the alveolar compartment. Aerosolization of proteins is technically more complicated than that of small drugs, but this approach increases the efficiency and homogeneity of the alveolar delivery. Proteins in the alveoli, however, can be taken up by macrophages and degraded by proteases released from macrophages. Intracellular delivery of proteins to the alveolocytes represents a challenging goal.

Several studies investigated whether the airway delivery of SOD and catalase affords protection against oxygen toxicity. In hyperoxia, a significant oxidative stress occurs in the alveolar compartment, where epithelial cells suffer a major injury. However, intratracheal administration of CuZnSOD fails to prevent lung injury caused by hyperoxia in rats (28). In more recent studies, CuZnSOD affords some mitigation of hyperoxic injury in piglets (37, 148).

The distribution and duration of active enzymes in the lung tissue are likely to determine the protective effect. For example, even a minute-scale mismatch between the peak of the oxidative insult and the application of catalase compromises the protection in an animal model of the intratracheal coadministration of the ROS-generating enzyme, glucose oxidase, and catalase (57). The kinetics of oxidative stress upon hyperoxia, however, is rather complex: an initial insult caused by mitochondrial overproduction and leakage of ROS (which develops within a few hours) is followed by a secondary insult caused by ROS released from infiltrating neutrophils (which develops several days later). Therefore, active antioxidant enzymes must be present for a prolonged period of time in proper sites of the lung tissue to have a satisfactory therapeutic benefit.

The fate of SOD after intratracheal adminis-

tration has been addressed in some detail. Ex-SOD has the closest association with the epithelial cells and longest life span in the alveoli (>24 h), whereas the life span of CuZnSOD and MnSOD did not exceed several hours (40). Davis and co-workers characterized the fate of fluorescently labeled SOD after intratracheal instillation in piglets (35, 153). In these studies, instillation of human CuZnSOD and MnSOD provides 100% increase in SOD activity in the lung tissue and the activity remains elevated above the basal level for several hours. Within an hour post instillation, CuZnSOD and MnSOD are detectable intracellularly in the alveolar cells and undergo degradation within 12 h post instillation (153).

Intratracheal instillation of SOD and catalase results in a patchy distribution of the enzymes in the lung tissue, mainly in the airways. Aerosolization did not alter specific activity of CuZnSOD, improved distribution of CuZnSOD in the lung, and provided a significant elevation of SOD activity in the epithelial lining fluid (58, 90, 153). A significant proportion of the aerosolized MnSOD is associated with the airway/alveolar surface and with alveolar macrophages (196). Analysis of biochemical, physiological, and morphological parameters showed that aerosolized MnSOD affords a significant protection against hyperoxia in baboons (164, 196).

Modification with PEG and liposome encapsulation protects the enzymes against inactivation and facilitates intracellular delivery. Thus, PEG-modified catalase enters type II alveolocytes in cell culture, augments cellular catalase activity, and protects the cells against exposure to H_2O_2 (69, 189). Intratracheal administration of PEG-modified SOD and catalase provides augmentation of their enzymatic activity in the alveolocytes and affords a significant protective effect in rats exposed to 100% oxygen (172, 176, 191, 198). PEG itself seems to affect the alveolar macrophages and display some protective effect (198); however, the effect of PEG-modified enzymes is significantly stronger. Noteworthy, even preadministration of PEG-SOD in the lungs 24 h prior to exposure to hyperoxia affords a significant protection (172).

Encapsulation of SOD and catalase in liposomes also augments protection. Liposome-encapsulated SOD and catalase undergo internalization by cultured alveolar epithelial cells and protect the cells against oxidative injury by 95% oxygen and H_2O_2 (21, 174). Intratracheal instillation of liposomal SOD and catalase provides intracellular localization of the enzymes in alveolar cells, and elevated SOD and catalase activities in the lung homogenates and type II cell fractions (8). Liposomal SOD and catalase attenuate hyperoxic lung injury and prolong survival of newborn, young, and adult animals (135, 173, 175, 192). As has been noted with PEG, enzyme-free liposomes also provide a significant protective effect, albeit less dramatic than that of the enzyme-containing liposomes (173). Intratracheal instillation of liposomal SOD and catalase affords some protection of rabbit lungs against vascular injury caused by perfusion of ROS-generating enzymes (9) and rat lung injury caused by bleomycin treatment (91).

In the alveolar compartment, drugs may interact with surfactant, which may affect their delivery to epithelial cells. The issue of interaction of the antioxidant enzymes with surfactant components is a subject of controversy. CuZnSOD has been reported to inactivate bovine surfactant *in vitro* (64). The specificity, exact mechanism, and potential clinical significance of this effect need to be addressed in more detail. One group found no effect of either endogenous or exogenous surfactant on the activity and distribution of unmodified CuZnSOD in the lung (35, 38), whereas others report that exogenous surfactant facilitates the uptake of unmodified CuZnSOD and catalase by alveolocytes in cell culture and *in vivo* (129). Two laboratories reported that incorporation of SP-A into liposomes enhances the uptake of encapsulated antioxidant enzymes by cultured type II alveolocytes and augments the antioxidant capacity of the cells (19, 190). SP-A is likely to stimulate the receptor-mediated endocytosis of the enzymes by alveolar cells. Alternative strategies to facilitate catalase endocytosis in the alveoli utilizing Fc-receptor and transferrin receptor have also been tested in cell cultures (65, 149).

Airway administration of antioxidant enzymes causes no significant toxicity and adverse effects in animals (200). Recent clinical

trials in premature infants show that intratracheal administration of recombinant CuZnSOD elevates the enzyme activity in serum and tracheal aspiration fluid and causes no significant adverse effects (39, 152). It is tempting to speculate that intratracheal administration of antioxidant enzymes or their derivatives will evolve as a viable means for the attenuation or prevention of hyperoxic lung injury (36, 151).

INTRAVASCULAR DELIVERY OF ANTIOXIDANT ENZYMES

In contrast to alveolocytes, endothelium is not readily accessible from the airways. The intravascular route may be more suitable for drug delivery to endothelial cells. Numerous experiments in isolated lungs document that perfusion of antioxidant enzymes affords a significant protection against endothelial dysfunction and lung damage caused by ROS generated in the lung upon ischemia–reperfusion, activation of neutrophils, hyperoxia, and other mechanisms (11, 24, 83, 87). Effects of individual enzymes vary; a combination of SOD and catalase affords a more consistent protective effect, whereas SOD may potentiate lung injury (70). The studies in the perfused lungs, however, provide a foundation for numerous attempts to protect lungs against oxidative injury by vascular delivery of SOD and catalase.

Protective effects of SOD and catalase have been tested in a sepsis-like syndrome caused by bacterial endotoxins in animals (endotoxemia). Endothelium is the first target for ROS released from neutrophils activated by pathogens and pro-inflammatory agents formed upon sepsis and other disease conditions. Intravenous infusion of CuZnSOD attenuates pulmonary edema and respiratory disorder in endotoxemic rats (158) and sheep (89). CuZnSOD infusion also protects animals against pulmonary injury caused by inflammatory mediators tumor necrosis factor (TNF) (4), interleukin-1 (92), and immune complexes (104). As in the isolated lungs, SOD occasionally potentiates the lung injury caused by endotoxin, probably due to an excessive formation of H_2O_2 (178). Intravital fluorescence microscopy reveals that intravenous injection of catalase suppresses the generation of H_2O_2 in the lungs of endotoxin-challenged rats (111). In a model of sheep endotoxemia, intravenous injection of catalase suppresses lung lipid peroxidation (159), whereas intraperitoneal injection of catalase attenuates lung injury (110). Infusion of SOD has no significant protective effect on neutrophil-dependent lung injury caused by pneumothorax (44), yet SOD and catalase attenuate lung injury caused by remote activation of neutrophils in acute pancreatitis (63).

Several studies addressed the protective effects of SOD and catalase against lung injury caused by radiation (18, 96) and chemical agents known to generated ROS. Thus, injection of SOD and catalase in rats and dogs is protective against vascular pulmonary injury induced by α-naphthylthiourea (99), alloxan (85), and eugenol (199). MnSOD, but not CuZnSOD, is protective against bleomycin-induced pulmonary fibrosis (139). However, doses of antioxidant enzymes used for injection in this and many other studies exceed the practically acceptable level (see below). Systemic administration of SOD provides no significant protection against paraquat-induced oxidative lung injury (132).

Systemic administration of SOD and catalase was also tested in animal models of environmental oxidative stress, hyperoxia, and smoke inhalation. Injection of megadoses of CuZnSOD attenuates some indices of lung injury in hyperoxic rabbits (109), yet fails to prevent hyperoxic injury in rats despite significant elevation of SOD activity in plasma (160). Combined intravenous bolus and intratracheal instillation of MnSOD attenuate pulmonary fluid balance disorder caused by smoke inhalation in donkeys (128). However, it is difficult to identify the contribution of intravascular and intratracheal fractions of the enzyme in the latter study.

Pulmonary embolism, such as gas embolism causing decompression sickness, has been postulated to be associated with oxidative stress. This notion is supported indirectly by animal experiments in which intravenous infusion of SOD (along with heparin) and intraperitoneal injection of catalase attenuate pulmonary edema caused by air embolism in sheep (49, 50). However, even combined administration

of heparin, SOD, and catalase fails to protect dogs against decompression sickness (23).

In general, protective effects in the animal studies require a prolonged or multiple infusions of megadoses (10–150 mg/kg) of SOD and catalase (18, 50, 85, 89, 109, 110, 199). Even multiple injections (every 12 h for 8 days) of 200 mg/kg CuZnSOD fail to protect neonate rats against hyperoxic lung injury. Use of megadoses of antioxidant enzymes may cause artifacts, for example, a nonintentional administration of trace amounts of endotoxin (60). Endotoxin may cause poorly controlled and paradoxical effects, such as stimulation of endogenous antioxidant defenses.

Some preliminary trials indicate that human subjects tolerate subcutaneous and systemic administration of 0.25 mg/kg SOD (150). However, simple calculations based on effective doses ranging from 10 to 100 mg/kg imply that grams of the enzymes would be required for the therapeutic treatment in humans. This would impose an extraordinary economic burden and a high risk of side effects (e.g., immune reactions). One avenue to address the safety issue is to reduce the dose and number of injections by improving the delivery of protein drugs. Accordingly, PEG-modification, encapsulation in liposomes, and other modifications of SOD and catalase have been explored as means to attain a prolonged life span, reduced immune reactions, better delivery to endothelium, and ultimately, a more effective protection against oxidative vascular stress in the lungs. Some of these studies rather reduced the level of enthusiasm for this strategy. For example, neither Ficoll-catalase nor Ficoll-SOD provides markedly better protection than unmodified enzymes in the animal models of lung injury caused by endotoxin (101) and thrombin (78). Recent studies demonstrated promising protective effects of PC-SOD in animal models of lung injury. In isolated canine lungs, PC-SOD protects against oxidative injury caused by phorbol ester (112). PC-SOD injected in mice attenuates bleomycin-induced pulmonary fibrosis (203) and pulmonary metastasis (169).

PEG-modification facilitates intracellular uptake of CuZnSOD and catalase by the cultured endothelial cells and elevates the enzymes' intracellular activity and cellular resistance to oxidative stress (12, 13). *In vitro*, PEG-SOD decomposes superoxide anion produced by neutrophils (166), yet does not affect neutrophil bactericidal activity, perhaps due to the effect of H_2O_2 (105). Systemic administration of PEG-modified SOD and catalase augments the activity of these enzymes in plasma and, in some studies, in the lung tissue (113, 134, 187, 198). Combined injection of PEG-SOD and PEG-catalase is protective against hyperoxic lung injury in premature lambs (187, 188). In hyperoxic rats, injection of PEG-SOD and PEG-catalase attenuates lung edema and prolongs survival, whereas unmodified SOD and catalase fail to produce protective effects (198). Intraperitoneal injection of PEG-SOD and PEG-catalase protects against hyperoxia in rabbits (77). Intravenous injection of PEG-SOD is protective against endotoxemia in guinea pigs (166), yet fails in pigs (134). Intravenous infusion of PEG-catalase protects against endotoxemic lung injury in pigs (134). Chronic infusion of PEG-catalase with subcutaneously implanted osmotic pumps attenuates lung inflammation and injury caused by asbestos (113, 114). PEG-SOD displays a trend to attenuate some indices of injury in rabbit models of lung reoxygenation (76) and asthma (5). In general, the combination of PEG-modified SOD and catalase affords a more significant effect than individual PEG-modified enzymes (77, 198).

Encapsulation of SOD and catalase in liposomes also facilitates their uptake by cultured endothelial cells, leading to augmentation of the cellular antioxidant defense and resistance to oxidative stress (12, 52). Intravenous injection of liposome-encapsulated CuZnSOD and catalase leads to a significant elevation of the respective enzyme activity in lung tissue and affords some protection in hyperoxic rats (54, 181). One group reported that multiple intramuscular injections of liposome-encapsulated CuZnSOD attenuated radiation-induced pulmonary fibrosis (41).

However, as an attentive reader could already notice, the number of reports on intravascular administration of SOD and catalase has declined in recent years. There are anecdotes of inconsistent, poor, and even worsening effects of PEG-SOD observed in some unpublished studies. Modification with PEG and

liposomal encapsulation indeed prolong life span and activity of SOD and catalase in the bloodstream, but these advantages do not translate into a viable therapeutic strategy. It is likely that prolonging life span of antioxidant enzymes in the circulation is not sufficient for the effective protection of endothelial cells. Blood possesses a tremendous antioxidant potential without injection of PEG-SOD or PEG-catalase. Most likely, one must deliver antioxidants to endothelial cells in a more specific manner, preferably to the intracellular compartment. There is no compelling evidence, however, that the strategies described above deliver active antioxidant enzymes to the pulmonary endothelium *in vivo*. A novel experimental approach to deliver antioxidant enzymes to pulmonary endothelium will be discussed in the next section.

TARGETING OF ANTIOXIDANT ENZYMES TO PULMONARY ENDOTHELIUM

Experiments in isolated perfused lungs and in endothelial cell cultures allow prolonged exposures of endothelium to high doses of SOD and catalase in the absence of blood components. Some studies with cell cultures imply that endothelial cells bind and internalize unmodified SOD under such conditions (98, 133). The specificity and biological/therapeutic significance of binding and uptake of unmodified SOD remain to be fully characterized. However, catalase and SOD possess no significant specific affinity to endothelial cells. PEG-modification and encapsulation in liposomes facilitate internalization in cell cultures, but do not increase the affinity for endothelium.

Some derivatives of CuZnSOD, as well as unmodified exSOD, demonstrate a heparin-dependent binding to endothelial cells in cell culture (17, 74, 81). PC-SOD was also reported to bind to endothelial cells in culture (71). Nevertheless, none of these SOD derivatives accumulate in the lungs after intravenous injection, despite the fact that pulmonary vasculature contains ~30% of the total amount of endothelial cells in the body. From the drug delivery perspective, a lack of pulmonary accumulation implies that these derivatives do not bind to endothelial cells *in vivo*. Thus, the problem of an inadequate delivery of antioxidant enzymes to endothelial cells persists.

To increase the specific affinity to endothelium and thus facilitate delivery, antioxidant enzymes can be conjugated with antibodies directed against endothelial surface antigens. This concept, the vascular immunotargeting strategy, has been developed in the last decade (for review, see 120). Endothelium is directly accessible to blood, hence even large immunoconjugates injected in the bloodstream would bind to the target. Pulmonary endothelium receives the entire cardiac blood output and represents the first large vascular area that a drug encounters after intravenous injection. Thus, even carriers that do not discriminate between pulmonary and systemic endothelial cells would accumulate in the lung. In addition, pulmonary endothelium, either normal or engaged in the pathological process, is enriched in some surface antigens (see below). This may provide an additional factor for the improvement of tissue selectivity of the targeting.

There are numerous antigens potentially useful for the immunotargeting of drugs to endothelial cells. At the present time, angiotensin-converting enzyme (ACE) and endothelial adhesion molecules seem to be the most attractive targets for delivery of antioxidant enzymes to the pulmonary endothelium (117, 120) (see Table 5 for an overview).

ACE, a carboxypeptidase localized on the surface of endothelial cells, cleaves vasoactive peptides, converts angiotensin I to angiotensin II, and inactivates bradykinin (47). There are $1–3 \times 10^5$ binding sites for antibodies directed against ACE (anti-ACE) on the luminal surface of a single endothelial cell (119). Pulmonary endothelium is enriched in ACE. As determined by immunohistochemical analysis, 100% of the pulmonary capillaries and 10–20% of nonpulmonary capillaries are ACE-positive (51). Radiolabeled monoclonal anti-ACE accumulates selectively in the lungs after intravenous, intraarterial, and intraperitoneal injections in rats, cats, hamsters, monkeys, healthy human volunteers, and sarcoidosis patients (32, 33, 117). Endothelial cells in culture, perfused rat lung explants, and rat lungs *in vivo* internalize anti-

TABLE 5. SURFACE ANTIGENS POTENTIALLY USEFUL FOR THE IMMUNOTARGETING OF ANTIOXIDANT ENZYMES TO PULMONARY ENDOTHELIUM

Antigen	*Properties and function*	*Advantages of the targeting*	*Potential problems*
ACE	Carboxypeptidase, converts angiotensin I to angiotensin II	High pulmonary specificity	Inflammation inhibits targeting
	Endothelium internalizes and slowly degrades anti-ACE	Intracellular delivery	ACE inhibition
	ROS and cytokines suppress ACE expression	ACE inhibition	
ICAM-1	Promotes firm adhesion of leukocytes to endothelium	Targeting to pulmonary and systemic endothelium	Intracellular targeting is questionable
	Endothelium poorly internalizes anti-ICAM-1	Enhanced targeting to ROS-attacked endothelium	
	ROS and cytokines stimulate ICAM-1 expression	Inhibition of inflammation by blocking of ICAM-1	
PECAM-1	High surface density antigen	Very effective targeting to pulmonary and systemic endothelium	Potential interference with PECAM-mediated endothelial signaling
	Facilitates leukocytes migration through endothelium		
	Endothelium internalizes and slowly degrades modified anti-PECAM	Inhibition of inflammation by blocking of PECAM-1	

ACE, which undergoes relatively slow and modest intracellular degradation (119).

Thus, anti-ACE seems to be a good carrier for the intracellular immunotargeting of antioxidant enzymes to the pulmonary endothelium. Indeed, Fig. 1 shows that almost 5% of ^{125}I-catalase and ^{125}I-CuZnSOD conjugated with anti-ACE accumulate in the lungs after intravenous injection in rats versus 0.5% for IgG-conjugated and <0.2% for nonconjugated enzymes (118). Both SOD and catalase conjugated with anti-ACE are retained in the lung for a prolonged time after injection (118). A recent study documented that catalase, conjugated with anti-ACE, but not nonimmune counterparts, protects isolated rat lungs perfused with

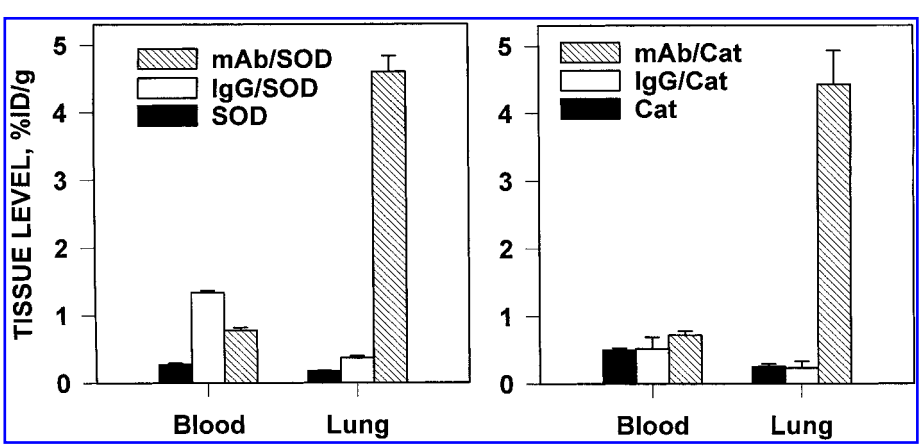

FIG. 1. Pulmonary accumulation of anti-ACE conjugated SOD (left) and catalase (right) after intravenous injection in intact rats. SOD or catalase was labeled with ^{125}I and conjugated with either anti-ACE (hatched bars) or control IgG (open bars). One hour after intravenous injection, the tissue level of the enzymes was determined in the blood and lungs and expressed as percentage of injected dose (ID) per gram of tissue (mean + sem, $n = 4$). Neither IgG/enzyme conjugates nor unconjugated enzymes (filled bars) display significant pulmonary accumulation, whereas anti-ACE conjugated enzymes accumulated in the lungs. Reproduced with modification from (118), with permission.

H_2O_2 against oxidative injury to the vascular endothelium (7).

Importantly, anti-ACE does not fix complement and induces no injury to the endothelium either in cell culture or after administration *in vivo* at doses of 100 mg/kg (33, 34). These data imply that ACE-directed immunotargeting of antioxidant enzymes will not cause harmful side effects mediated by the antibody. However, cytokines and ROS suppress ACE expression in endothelium (195). Therefore, inflammation inhibits immunotargeting to ACE (6, 117).

Surface adhesion molecules [InterCellular, Adhesion Molecule-1 (ICAM-1) or CD54; Platelet-Endothelial Cell Adhesion Molecule-1 (PECAM-1) or CD31; P-selectin and E-selectin) represent attractive targets for the delivery of antioxidant enzymes. Cytokines and ROS upregulate the surface density of these molecules on the endothelial cells (165). Thus, oxidative stress and inflammation may facilitate the immunotargeting. In addition, antibodies to adhesion molecules block leukocyte adhesion to endothelium and their transmigration into the adjacent tissues. Antibodies directed against ICAM-1, PECAM-1, and P-selectin are protective in inflammation-associated animal models of lung injury; nowadays, blocking of the surface adhesion molecules with antibodies has emerged as a novel therapeutic concept (84). Thus, immunotargeting of antioxidant enzymes to endothelial surface adhesion molecules may provide a secondary (anti-inflammatory) therapeutic benefit.

Endothelial cells constitutively express the surface adhesion molecule ICAM-1 (CD54), a transmembrane glycoprotein that is localized on the apical surface of endothelial cells and supports adhesion of leukocytes to endothelium (3). Mediators of inflammation (TNFα, interleukin-1, interferon-γ, and ROS) stimulate ICAM-1 expression in endothelial cells and upregulate the content of ICAM-1 in the lung and other organs (15). Therefore, ICAM-1-directed immunotargeting may provide a more effective delivery of antioxidant enzymes to endothelial cells engaged in inflammation.

Recent results obtained with radiolabeled antibodies directed against ICAM-1 (anti-ICAM) strongly support this hypothesis. ^{125}I-Anti-ICAM accumulates in the lungs of control rats and mice (116, 137). Lung injury induced by endotoxemia, intratracheal deposition of IgG-immune complexes, or TNFα leads to a marked elevation of the vascular binding of ^{125}I-anti-ICAM and stimulates its accumulation in the lung in rats (15, 116, 137). Cytokine-induced up-regulation of the pulmonary uptake of anti-ICAM-1 requires several hours after endotoxin injection and reaches a peak level at 9–12 h (137). Taken together, these results imply that ICAM-1 antibody may serve for the immunotargeting of antioxidant enzymes to the inflammation-engaged endothelium.

Internalization of anti-ICAM-1 has not been systematically studied in the literature. Some reports (3), as well as unpublished results from the author's laboratory, imply that endothelial cells poorly internalize anti-ICAM-1. However, catalase conjugated with anti-ICAM accumulates in the pulmonary vasculature after systemic administration in intact rats and attenuates vascular oxidative stress in the perfused rat lung (7).

PECAM-1 (CD31) is a 130-kDa molecule, constitutively expressed in the endothelial cells, that plays an important role in leukocyte transmigration through the monolayer and contributes to signal transduction in endothelium (126). Endothelial cells possess several million binding sites for anti-PECAM per cell (an order of magnitude more than for anti-ACE or anti-ICAM-1). Cytokines have no marked effect on PECAM-1 vascular expression (183). Although native anti-PECAM is poorly internalizable, chemical modification of anti-PECAM dramatically facilitates intracellular delivery (121). Therefore, reporter enzymes conjugated with anti-PECAM bind to endothelial cells in culture, enter the cells, and display intracellular activity (61, 121, 161). After intravenous injection in intact animals, enzymes and genetic material conjugated with anti-PECAM accumulate in the lungs, enter endothelial cells, and display functional activity in the pulmonary vasculature (29, 93, 121, 161). In cell culture experiments, catalase, conjugated with anti-PECAM, but not with control IgG, enters the endothelial cells and protects them against H_2O_2 (121). Figure 2 shows that in animal experiments, anti-PECAM/catalase conjugate ac-

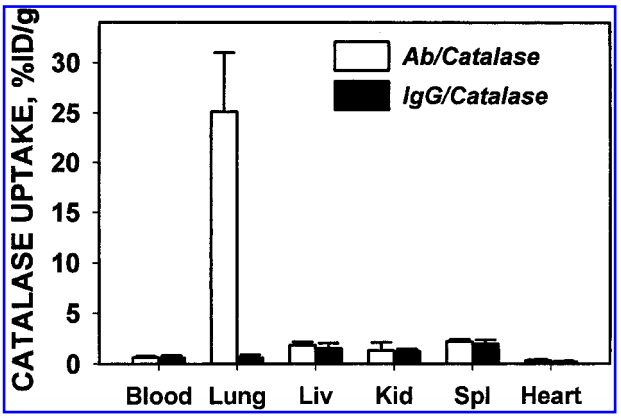

FIG. 2. Organ-selective pulmonary accumulation of anti-PECAM conjugated catalase after intravenous injection in intact rats. The experimental design is similar to that described in the legend to Fig. 1. Open bars, anti-PECAM/catalase; filled bars, IgG/catalase. Reproduced from (121), with permission. Liv, liver; Kid, kidney; Spl, spleen.

cumulates in rat and murine lungs after intravenous injection (121). Preliminary results from our laboratory indicate that anti-PECAM/catalase protects lungs against oxidative injury in animal models *in vivo*. Importantly, anti-PECAM inhibits neutrophil infiltration in the lungs and suppresses pulmonary inflammation (183).

Therefore, antibodies against the endothelial antigens ACE, ICAM-1, and PECAM-1 seem to be good carriers for immunotargeting of antioxidant enzymes to pulmonary vascular endothelium. As with other types of immunotherapy, the safety issues must be rigorously addressed. Antibodies provide the targeting, but they also may cause side effects, such as activation of complement and leukocytes via Fc-fragment. Thus, antibodies, to be useful as carriers, must not fix complement or activate leukocytes. Although some isotypes of immunoglobulins naturally lack these functions, utilization of Fab fragments seems to be obligatory for the targeting. Laboratory animals and patients tolerate injections of large doses of anti-ACE (34, 117) and antibodies against surface adhesion molecules (22, 84, 183). Conceivably, utilization of humanized antigen-binding fragments will minimize the potential adverse effects.

Antibodies and immunoconjugates may mask antigens, inhibit their activity, and alter membrane metabolism of antigens in endothelial cells (20, 34). For example, anti-ACE stimulates ACE shedding from the plasma membrane (34). Cross-linking of antigen molecules by antibodies also may cause significant alterations in endothelial physiology, for example, through stimulation of signal transduction and elevation of intracellular Ca^{2+}.

Vascular immunotargeting is a relatively novel approach for delivery of antioxidant enzymes. Recent results merit further investigation of the strategy, and evaluation of the efficacy of protection by immunotargeting of antioxidant enzymes to endothelium in animal models of pulmonary oxidative stress, such as hyperoxia or endotoxemia.

PERSPECTIVES

Despite a great deal of effort, three decades of intense research has not translated into effective and clinically useful therapeutic strategies for antioxidant therapies using exogenous SOD and catalase. Weak and inconsistent protective effects of the enzymes represent the major problems. Significant problems are associated with poor stability of the enzymes, inadequate delivery to the target sites, and danger of harmful side effects, such as an immune response.

These problems motivated many researchers to seek alternatives in the use of antioxidant scavengers (16), small mimetics of antioxidant enzymes (157), and antioxidant gene therapies (31, 46). These approaches are exciting and possess a potential therapeutic merit. Pulmonary applications of gene therapy are the focus of intense research. Both intratracheal (46) and intravascular (93) routes represent potential avenues for delivery of genetic material.

Therapeutic application of the exogenous enzymes, however, has important and unique advantages. In contrast to genetic material (which requires several hours for protein synthesis and processing), enzymes would be active immediately upon arrival at the therapeutic site. Thus, antioxidant enzymes could be applicable in acute oxidative stress, such as hyperoxia and adult respiratory distress syndrome. Modifications of SOD and catalase may optimize deliv-

ery by prolonging their life span, minimizing immune reactions, and improving targeting to the cells, including alveolar epithelium and vascular endothelium. Future studies will more fully characterize the pharmacological profile and therapeutic value of antioxidant enzyme derivatives.

Airway administration of SOD and catalase for treatment of hyperoxic lung injury is more developed than intravascular administration in terms of potential clinical use. Enzyme aerosolization, derivatization (PEG-modification, encapsulation in liposomes), and inclusion of surfactant components in the formulations offer means to attain more homogeneous alveolar distribution, prolonged retention of active SOD and catalase in the alveolar space, and more effective delivery to the alveolocytes. Clinical trials of antioxidant enzymes are under way and the preliminary results are encouraging (36).

Antioxidant protection of pulmonary endothelium is a more challenging goal. Intravascular administration of SOD and catalase is unlikely to render a significant utility unless their pharmacological profile is markedly improved. Many SOD and catalase derivatives display a more favorable pharmacokinetics; however, the specificity of their binding to endothelial cells and intracellular delivery must be improved. Immunotargeting represents a potential avenue to facilitate the intracellular delivery to endothelium. In addition, immunotargeting may permit a secondary therapeutic benefit of blocking of the target antigens, such as surface adhesion molecules. Biotechnological techniques permit a large-scale production of chimeric proteins combining a humanized antigen-binding domain of a carrier antibody and antioxidant enzyme. Therefore, generation of chimeric, targeted antioxidant enzymes promises a new avenue for antioxidant therapy in the lung and, possibly, other areas in the vasculature.

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ABBREVIATIONS

ACE, angiotensin-converting enzyme; ALI, acute lung injury; ARDS, adult respiratory distress syndrome; exSOD, extracellular SOD; Gal-SOD, galactosylated SOD; ICAM-1, intercellular adhesion molecule-1; Man-SOD, mannosylated SOD; PC-SOD, lecithinized SOD; PECAM-1, platelet-endothelial cell adhesion molecule-1; PEG, polyethylene glycol; PUT-SOD, putrescine-conjugated SOD; ROS, reactive oxygen species; SM-SOD, SOD chemically modified with [poly(styrene-*co*-maleic acid)]; SOD, superoxide dismutase; SP-A, surfactant protein A; TNFα, tumor necrosis factor-α.

REFERENCES

1. Abuchowski A, McCoy JR, Palczuk NC, van Es T, and Davis FF. Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. *J Biol Chem* 252: 3582–3586, 1977.
2. Al-Mehdi A, Zhoa G, Dodia C, Tozawa K, Costa K, Muzykantov V, Ross C, Blecha F, Dinauer M, and Fisher A. Endothelial NADPH oxidase as the source of oxidants with lung ischemia or high K^+. *Circ Res* 83: 730–737, 1998.
3. Almenar-Queralt A, Duperray A, Miles L, Felez J, and Altieri D. Apical topography and modulation of ICAM-1 expression on activated endothelium. *Am J Pathol* 147: 1278–1288, 1995.
4. Amari T, Kubo K, Kobayashi T, and Sekiguchi M. Effects of recombinant human superoxide dismutase on tumor necrosis factor-induced lung injury in awake sheep. *J Appl Physiol* 74: 2641–2648, 1993.
5. Assa'ad AH, Ballard ET, Sebastian KD, Loven DP, Boivin GP, and Lieri MB. Effect of superoxide dismutase on a rabbit model of chronic allergic asthma. *Ann Allergy Asthma Immunol* 80: 215–224, 1998.
6. Atochina E, Hiemish H, Muzykantov V, and Danilov S. Systemic administration of PAF in rat reduces specific pulmonary uptake of circulating monoclonal antibody to ACE. *Lung* 170: 349–358, 1992.
7. Atochina E, Balyasnikova I, Danilov S, Granger D, Fisher A, and Muzykantov V. Immunotargeting of catalase to ACE or ICAM-1 protects perfused rat

lungs against oxidative stress. *Am J Physiol* 275: L806–L817, 1998.
8. Baker RR, Czopf L, Jilling T, Freeman BA, Kirk KL, and Matalon S. Quantitation of alveolar distribution of liposome-entrapped antioxidant enzymes. *Am J Physiol* 263: L585–L594, 1992.
9. Barnard ML, Baker RR, and Matalon S. Mitigation of oxidant injury to lung microvasculature by intratracheal instillation of antioxidant enzymes. *Am J Physiol* 265: L340–L345, 1993.
10. Becker M, Menger MD, and Lehr H. Heparin-released superoxide dismutase inhibits postischemic leukocyte adhesion to venular endothelium. *Am J Physiol* 267: H925–H930, 1994.
11. Becker PM, Sanders SP, Price P, and Christman BW. F_2-isoprostane generation in isolated ferret lungs after oxidant injury or ventilated ischemia. *Free Radic Biol Med* 25: 703–711, 1998.
12. Beckman JS, Minor RL Jr, and Freeman BA. Augmentation of antioxidant enzymes in vascular endothelium. *J Free Radic Biol Med* 2: 359–365, 1986.
13. Beckman JS, Minor RL Jr, White CW, Repine JE, and Freeman BA. Superoxide dismutase and catalase conjugated to polyethylene glycol increases enzyme activity and oxidant resistance. *J Biol Chem* 263: 6884–6892, 1988.
14. Beckman JS, and Koppenol WH. Nitric oxide, superoxide and peroxynitrite: the good, the bad and the ugly. *Am J Physiol* 271: C1424–C1437, 1996.
15. Beck-Schimmer B, Schimmer R, Warner R, Schmal H, Nordblom G, Flory C, Lesch M, Friedl H, Schrier D, and Ward P. Expression of lung vascular and airway ICAM-1 after exposure to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol* 17: 344–352, 1997.
16. Bernard GR. *N*-Acetylcysteine in experimental and clinical acute lung injury. *Am J Med* 91: 54S–59S, 1991.
17. Boissinot M, Kuhn LA, Lee P, Fisher CL, Wang Y, Hallewell RA, and Tainer JA. Rational design and expression of a heparin-targeted human superoxide dismutase. *Biochem Biophys Res Commun* 190: 250–256, 1993.
18. Breuer R, Tochner Z, Conner MW, Nimrod A, Gorecki M, Or R, and Slavin S. Superoxide dismutase inhibits radiation-induced lung injury in hamsters. *Lung* 170: 19–29, 1992.
19. Briscoe P, Caniggia I, Graves A, Benson B, Huang L, Tanswell AK, and Freeman BA. Delivery of superoxide dismutase to pulmonary epithelium via pH-sensitive liposomes. *Am J Physiol* 268: L374–L380, 1995.
20. Brisson G, Archipoff G, Hartman M, Hanau D, Beretz A, Freyssinet T, and Cazenave T. Antibodies to thrombomodulin induce receptor-mediated endocytosis in human saphenous vein endothelial cells. *Thromb Haemost* 68: 737–743, 1992.
21. Buckley BJ, Tanswell AK, and Freeman BA. Liposome-mediated augmentation of catalase in alveolar type II cells protects against H_2O_2 injury. *J Appl Physiol* 63: 359–367, 1987.
22. Carden D, Young JA, and Granger DN. Pulmonary microvascular injury after intestinal ischemia/reperfusion: role of P-selectin. *J Appl Physiol* 75: 2529–2534, 1993.
23. Catron PW, Thomas LB, McDermott JJ, Holt MA, Harabin AL, and Flynn ET. Failure of heparin, superoxide dismutase, and catalase to protect against decompression sickness. *Undersea Biomed Res* 14: 319–330, 1987.
24. Chen X, and Catravas JD. Neutrophil-mediated endothelial angiotensin-converting enzyme dysfunction: role of oxygen-derived free radicals. *Am J Physiol* 265: L243–L249, 1993.
25. Chi LG, Tamura Y, Hoff PT, Macha M, Gallagher KP, Schork MA, and Lucchesi BR. Effect of superoxide dismutase on myocardial infarc size in the canine heart after 6 hours of regional ischemia and reperfusion: a demonstration of myocardial salvage. *Circ Res* 64: 665–675, 1989.
26. Corvo ML, Boerman OC, Oyen WJ, Van Bloois L, Cruz ME, Crommelin DJ, and Storm G. Intravenous administration of superoxide dismutase entrapped in long circulating liposomes. II. In vivo fate in a rat model of adjuvant arthritis. *Biochim Biophys Acta* 1419: 325–334, 1999.
27. Crapo JD, Barry BE, Foscue HA, and Shelburne J. Structural and biochemical changes in rat lungs occurring during exposures to lethal and adapative doses of oxygen. *Am Rev Respir Dis* 122: 123–143, 1980.
28. Crapo JD, DeLong DM, Sjostrom K, Hasler GR, and Drew RT. The failure of aerosolized superoxide dismutase to modify pulmonary oxygen toxicity. *Am Rev Respir Dis* 115: 1027–1033, 1977.
29. Christofidou-Solomidou M, Pietra G, Solomides C, Arguiris E, Harshaw D, Fitzgerald G, Albelda S, and Muzykantov V. Immunotargeting of glucose oxidase to endothelium in vivo causes oxidative vascular injury in the lungs. *Am J Physiol Lung Cell Mol Physiol* 278: L794–L805, 2000.
30. D'Agnillo F, and Chang TM. Reduction of hydroxyl radical generation in a rat hindlimb model of ischemia—reperfusion injury using crosslinked hemoglobin–superoxide dismutase–catalase. *Artif Cells Blood Substit Immobil Biotechnol* 25: 163–180, 1997.
31. Danel C, Erzurum SC, Prayssac P, Eissa NT, Crystal RG, Herve P, Baudet B, Mazmanian M, and Lemarchand P. Gene therapy for oxidant injury-treated diseases: adenovirus-mediated transfer of superoxide dismutase and catalase cDNAs protects against hyperoxia but not against ischemia–reperfusion lung injury. *Hum Gene Ther* 9: 1487–1496, 1998.
32. Danilov S, Martynov A, Klibanov A, Slinkin M, Sakharov IYu, Malov A, Sergienko V, Vedernikov A, Muzykantov V, and Torchilin V. Radioimmunoimaging of lung vessels: an approach using indium-111-labeled monoclonal antibody to ACE. *J Nucl Med* 30: 1686–1692, 1989.
33. Danilov SM, Muzykantov VR, Martynov AV, Atochina EN, Sakharov IYu, Trakht IN, and Smirnov VN. Lung is the target organ for a monoclonal anti-

body to angiotensin-converting enzyme. *Lab Invest* 64: 118–124, 1991.
34. Danilov S, Atochina E, Hiemisch H, Churakova T, Moldobayeva A, Sakharov I, Deichman G, Ryan U, and Muzykantov V. Interaction of monoclonal antibody to angiotensin-converting enzyme (ACE) with antigen *in vitro* and *in vivo*: antibody targeting to the lung induces ACE antigenic modulation. *Int Immunol* 6: 1153–1160, 1994.
35. Das S, Horowitz S, Robbins CG, El-Sabban ME, Sahgal N, and Davis JM. Intracellular uptake of recombinant superoxide dismutase after intratracheal administration. *Am J Physiol* 274: L673–L677, 1998.
36. Davis JM. Superoxide dismutase: a role in the prevention of chronic lung disease. *Biol Neonate* 74: 29–34, 1998.
37. Davis JM, Rosenfeld WN, Sanders RJ, and Gonenne A. Prophylactic effects of recombinant human superoxide dismutase in neonatal lung injury. *J Appl Physiol* 74: 2234–2241, 1993.
38. Davis JM, Rosenfeld WN, Koo MC, and Gonenne A. Pharmacologic interactions of exogenous lung surfactant and recombinant human CuZn superoxide dismutase. *Pediatr Res* 35: 37–40, 1994.
39. Davis JM, Rosenfeld WN, Richter SE, Parad MR, Gewolb IH, Spitzer AR, Carlo WA, Couser RJ, Price A, Flaster E, Kassem N, Edwards L, Tierney J, and Horowitz S. Safety and pharmacokinetics of multiple doses of recombinant human CuZn superoxide dismutase administered intratracheally to premature neonates with respiratory distress syndrome. *Pediatrics* 100: 24–30, 1997.
40. Day BJ, Evans KS, Kang BH, Chang LY, Wagner CD, and Crapo JD. Immunolocalization of inhaled recombinant human manganese superoxide dismutase reveals a prolonged retention in lung surface lining fluids. *Inhalation Toxicol* 7: 1109–1120, 1995.
41. Delanian S, Baillet F, Huart J, Lefaix JL, Maulard C, and Housset M. Successful treatment of radiation-induced fibrosis using liposomal CuZn superoxide dismutase. *Clin Trial Radiother Oncol* 32: 12–20, 1994.
42. Dini L, Rossi L, Lentini A, DeMartino A, and Rotilio G. Immunocytochemical study of binding and internalization of carrier-free Cu,Zn superoxide dismutase by cultured rat hepatocytes. *Cell Mol Biol* 41: 1051–1059, 1995.
43. Dini L, Falasca L, Rossi L, and Rotilio G. In vivo uptake of Cu,Zn superoxide dismutase. Morphological evidence for preferential endocytosis and accumulation by sinusoidal liver cells. *Cell Mol Biol* 42: 269–277, 1996.
44. Doerschuk CM, Allard MF, and Oyarzun MJ. Evaluation of reexpansion pulmonary edema following unilateral pneumothorax in rabbits and the effect of superoxide dismutase. *Exp Lung Res* 16: 355–367, 1990.
45. Edwards DA, Ben-Jebria A, and Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. *J Appl Physiol* 84: 379–385, 1998.
46. Engelhardt JF. Redox-mediated gene therapies for environmental injury: approaches and concepts. *Antioxid Redox Signal* 1: 5–27, 1999.
47. Erdos E. Angiotensin-converting enzyme and the changes of our concept through the years. *Hypertension* 16: 363–370, 1990.
48. Fernandes AI, and Gregoriadis G. Synthesis, characterization and properties of sialylated catalase. *Biochim Biophys Acta* 1293: 90–96, 1996.
49. Flick MR, Hoeffel JM, and Staub NC. Superoxide dismutase with heparin prevents increased lung vascular permeability during air emboli in sheep. *J Appl Physiol Respir Envir Exer Physiol* 55: 1284–1291, 1983.
50. Flick MR, Milligan SA, Hoeffel JM, and Goldstein IM. Catalase prevents increased lung vascular permeability during air emboli in unanesthetized sheep. *J Appl Physiol* 64: 929–935, 1988.
51. Franke F, Metzger R, Bohle R, Kerkman L, Alhenc-Gelas G, and Danilov SM. Angiotesin-I-converting enzyme (CD143) on endothelial cells in normal and in pathological conditions. In: *Leukocyte Typing VI. White Cell Differentiation Antigens*, edited by Kishimoto T, et al. New York: Garland Publishing Inc., 1997, pp. 749–751.
52. Freeman BA, Young SL, and Crapo JD. Liposome-mediated augmentation of superoxide dismutase in endothelial cells prevents oxygen injury. *J Biol Chem* 258: 12534–12542, 1983.
53. Freeman B, and Crapo JD. Biology of disease. Free radicals and tissue injury. *Lab Invest* 47: 412–426, 1987.
54. Freeman BA, Turrens JF, Mirza Z, Crapo JD, and Young SL. Modulation of oxidant lung injury by using liposome-entrapped superoxide dismutase and catalase. *Fed Proc* 44: 2591–2595, 1985.
55. Fridovich I. Superoxide radical and superoxide dismutases. *Annu Rev Biochem* 64: 97–112, 1995.
56. Fujita T, Nishika WAM, Tamaki C, Takakura Y, Hashida M, and Sezaki H. Targeted delivery of human recombinant superoxide dismutase by chemical modification with mono- and polysaccharide derivatives. *J Pharmacol Exp Ther* 263: 971–978, 1992.
57. Gannon DE, He XM, Ward PA, Varani J, and Johnson KJ. Time-dependent inhibition of oxygen radical induced lung injury. *Inflammation* 14: 509–522, 1990.
58. Gillissen A, Roum JH, Hoyt RF, and Crystal RG. Aerosolization of superoxide dismutase. Augmentation of respiratory epithelial lining fluid antioxidant screen by aerosolization of recombinant human Cu^{++}/Zn^{++} superoxide dismutase. *Chest* 104: 811–815, 1993.
59. Giri SN, and Misra HP. Fate of superoxide dismutase in mice following oral route of administration. *Med Biol* 62: 285–289, 1984.
60. Gordon T, Milligan SA, Levin J, Thompson JE, Fine JM, and Sheppard D. Apparent effect of catalase on airway edema in guinea pigs. Role of endotoxin contamination. *Am Rev Respir Dis* 135: 854–859, 1987.
61. Gow A, Branco F, Cristofidou-Solomidou M, Black-

Schultz L, Albelda S, and Muzykantov V. Immunotargeting of glucose oxidase: intracellular production of H_2O_2 and endothelial oxidative stress. *Am J Physiol* 277: L271–L281, 1999.
62. Greenwald RA. Superoxide dismutase and catalase as therapeutic agents for human diseases. *Free Radic Biol Med* 8: 201–209, 1990.
63. Guice KS, Oldham KT, Caty M, Johnson KJ, and Ward PA. Neutrophil-dependent, oxygen-radical mediated lung injury associated with acute pancreatitis. *Ann Surg* 210: 740–747, 1989.
64. Haddad IY, Nieves-Cruz B, and Matalon S. Inhibition of surfactant function by copper-zinc superoxide dismutase (CuZn-SOD). *J Appl Physiol* 83: 1545–1550, 1997.
65. Harrison J, Shi X, Wang L, Ma JK, and Rojanasakul Y. Novel delivery of antioxidant enzyme catalase to alveolar macrophages by Fc receptor-mediated endocytosis. *Pharm Res* 11: 1110–1114, 1994.
66. Haun SE, Kirsch JR, Helfaer MA, Kubos KL, and Traystman RJ. Polyethylene glycol-conjugated superoxide dismutase fails to augment brain superoxide dismutase activity in piglets. *Stroke* 22: 655–659, 1991.
67. He YY, Hsu CY, Ezrin AM, and Miller MS. Polyethylene glycol-conjugated superoxide dismutase in focal cerebral ischemia–reperfusion. *Am J Physiol Heart Circ Physiol* 265: H252–H256, 1993.
68. Heffner JE, and Repine JE. Pulmonary strategies of antioxidant defense. *Am Rev Resp Dir* 140: 531–534, 1989.
69. Holm BA, Hudak BB, Keicher L, Cavanaugh C, Baker RR, Hu P, and Matalon S. Mechanisms of H_2O_2-mediated injury to type II cell surfactant metabolism and protection with PEG-catalase. *Am J Physiol* 261: C751–C757, 1991.
70. Huang YCT, Nozik ES, and Piantadosi CA. Superoxide dismutase potentiates platelet-activating factor-induced injury in perfused lung. *Am J Physiol* 266: L246–L254, 1994.
71. Igarashi R, Hoshino J, Ochiai A, Morizawa Y, and Mizushima Y. Lecithinized superoxide dismutase enhances its pharmacologic potency by increasing its cell membrane affinity. *J Pharmacol Exp Ther* 271: 1672–1677, 1994.
72. Imaizumi S, Woolworth V, Fishman RA, and Chan PH. Liposome-entrapped superoxide dismutase reduces cerebral infarction in cerebral ischemia in rats. *Stroke* 21: 1312–1317, 1990.
73. Inoue M, Ebashi I, Watanabe N, and Morino Y. Synthesis of a superoxide dismutase derivative that circulates bound to albumin and accumulates in tissues whose pH is decreased. *Biochemistry* 28: 6619–6624, 1989.
74. Inoue M, Watanabe N, Morino Y, Tanaka Y, Amachi T, and Sasaki J. Inhibition of oxygen toxicity by targeting superoxide dismutase to endothelial cell surface. *FEBS Lett* 269: 89–92, 1990.
75. Jackson RM. Pulmonary oxygen toxicity. *Chest* 88: 900–905, 1985.
76. Jackson RM, Veal CF, Beckman JS, and Brannen AL. Polyethylene glycol-conjugated superoxide dismutase in unilateral lung injury due to re-expansion (reoxygenation). *Am J Med Sci* 300: 22–28, 1990.
77. Jacobson JM, Michael JR, Hafri MH Jr, and Gurtner GH. Antioxidants and antioxidant enzymes protect against pulmonary oxygen toxicity in the rabbit. *J Appl Physiol* 68: 1252–1259, 1990.
78. Johnson A, Perlman MB, Blumenstock FA, and Malik AB. Superoxide dismutase prevents the thrombin-induced increase in lung vascular permeability: role of superoxide in mediating the alterations in lung fluid balance. *Circ Res* 59: 405–415, 1986.
79. Karlsson K, and Marklund SL. Extracellular superoxide dismutase in the vascular system of mammals. *Biochem J* 255: 223–228, 1988.
80. Karlsson K, and Marklund SL. Plasma clearance of human extracellular-superoxide dismutase C in rabbits. *J Clin Invest* 82: 762–766, 1988.
81. Karlsson K, and Marklund SL. Binding of human extracellular-superoxide dismutase C to cultured cell lines and to blood cells. *Lab Invest* 60: 659–666, 1989.
82. Karlsson K, Sandstrom J, Edlund A, Edlund T, and Marklund SL. Pharmacokinetics of extracellular-superoxide dismutase in the vascular system. *Free Radic Biol Med* 14: 185–190, 1993.
83. Katz SA, Venkatachalam M, Crouch RK, Heffner JE, Halushka PV, Wise WC, and Cook JA. Catalase pretreatment attenuates oleic acid-induced edema in isolated rabbit lung. *J Appl Physiol* 65: 1301–1306, 1988.
84. Kavanaugh A, Davis L, Jain R, Nichols L, Norris S, and Lipsky P. A phase I/II open label study of the safety and efficacy of an anti-ICAM-1 monoclonal antibody in early rheumatoid arthritis. *J Rheumatol* 23: 1338–1344, 1996.
85. Kawada T, Kambara K, Arakawa M, Segawa T, Ando F, Hirakawa S, Emura S, Shoumura S, and Sono H. Pretreatment with catalase or dimethyl sulfoxide protects alloxan-induced acute lung edema in dogs. *J Appl Physiol* 73: 1326–1333, 1992.
86. Kawamoto S, Inoue M, Tashiro S, Morino Y, and Miyauchi Y. Inhibition of ischemia and reflow-induced liver injury by an SOD derivative that circulates bound to albumin. *Arch Biochem Biophys* 277: 160–165, 1990.
87. Kennedy TP, Rao NV, Hopkins C, Pennington L, Tolley E, and Hoidal JR. Role of reactive oxygen species in reperfusion injury of the rabbit lung. *J Clin Invest* 83: 1326–1335, 1989.
88. Kojima Y, Haruta A, Imai T, Otagiri M, and Maeda H. Conjugation of Cu,Zn-superoxide dismutase with succinylated gelatin: pharmacological activity and cell-lubricating function. *Bioconjug Chem* 4: 490–498, 1993.
89. Koyama S, Kobayashi T, Kubo K, Sekiguchi M, and Ueda G. Recombinant-human superoxide dismutase attenuates endotoxin-induced lung injury in awake sheep. *Am Rev Respir Dis* 145: 1404–1409, 1992.
90. Langenback EG, Davis JM, Robbins C, Sahgal N,

Perry RJ, and Simon SR. Improved pulmonary distribution of recombinant human Cu/Zn superoxide dismutase, using a modified ultrasonic nebulizer. *Pediatr Pulmonol* 27: 124–129, 1999.
91. Ledwozyw A. Protective effect of liposome-entrapped superoxide dismutase and catalase on bleomycin-induced lung injury in rats. II. Phospholipids of the lung surfactant. *Acta Physiol Hung* 78: 157–162, 1991.
92. Leff JA, Baer JW, Bodman ME, Kirkman JM, Shanley PF, Patton LM, Beehler CJ, McCord JM, and Repine JE. Interleukin-1 induced lung neutrophil accumulation and oxygen metabolite-mediated lung leak in rats. *Am J Physiol* 266: L2–L8, 1994.
93. Li S, Tan Y, Viroonchatapan E, Pitt BR, and Huang L. Targeted gene delivery to pulmonary endothelium by anti-PECAM antibody. *Am J Physiol Lung Cell Mol Physiol* 278: L504–L511, 2000.
94. Liu TH, Beckman JS, Freeman BA, Hogan EL, and Hsu CY. Polyethylene glycol-conjugated superoxide dismutase and catalase reduce ischemic brain injury. *Am J Physiol* 256: H589–H593, 1989.
95. Louie S, Halliwell B, and Cross C. Adult respiratory distress syndrome: a radical perspective. *Adv Pharmacol* 38: 457–490, 1997.
96. Malaker K, and Das RM. The effect of superoxide dismutase on the pathogenesis of radiation-induced pulmonary damage in the rat. *Pharmacol Ther* 39: 327–330, 1988.
97. Mao GD, Thomas PD, Lopaschuk GD, and Poznansky MJ. Superoxide dismutase (SOD)–catalase conjugates. *J Biol Chem* 265: 416–420, 1993.
98. Markey BA, Phan SH, Varani J, Ryan US, and Ward PA. Inhibition of cytotoxicity by intracellular superoxide dismutase supplementation. *Free Radic Biol Med* 9: 307–314, 1990.
99. Martin D, Korthuis RJ, Perry M, Townsley MI, and Taylor AE. Oxygen radical-mediated lung damage associated with alpha-naphthylthiourea. *Acta Physiol Scand Suppl* 548: 119–125, 1986.
100. Matsubara T, and Ziff M. Increased superoxide anion release from human endothelial cells in response to cytokines. *J Immunol* 137: 3295–3298, 1986.
101. Maunder RJ, Winn RK, Gleisner JM, Hildebrandt J, and Harland JM. Effect of intravenous catalase on the pulmonary vascular response to endotoxemia in goats. *J Appl Physiol* 64: 697–704, 1988.
102. McCord JM. Human disease, free radicals and oxidant/antioxidant balance. *Clin Biochem* 26: 351–357, 1993.
103. McCord JM. Superoxide dismutase: rationale for use in reperfusion injury and inflammation. *J Free Radic Biol Med* 2: 307–310, 1986.
104. McCormick JR, Harkin MM, Johnson KJ, and Ward PA. Suppression by superoxide dismutase of immune-complex-induced pulmonary alveolitis and dermal inflammation. *Am J Pathol* 102: 5–61, 1981.
105. McDonald RJ, Berger EM, White CW, White JG, Freeman BA, and Repine JE. Effect of superoxide dismutase encapsulated in liposomes or conjugated with polyethylene glycol on neutrophil bactericidal activity in vitro and bacterial clearance in vivo. *Am Rev Respir Dis* 131: 633–637, 1985.
106. Mihara K, Hojo T, Fujikawa M, Takakura Y, Sezaki H, and Hashida M. Disposition characteristics of protein drugs in the perfused rat kidney. *Pharm Res* 10: 823–827, 1993.
107. Mihara K, Sawai K, Takakura Y, and Hashida M. Manipulation of renal disposition of human recombinant superoxide dismutase by chemical modification. *Biol Pharm Bull* 17: 296–301, 1994.
108. Mihara K, Oka Y, Sawai K, Takakura Y, and Hashida M. Improvement of therapeutic effect of human recombinant superoxide dismutase on ischemic acute renal failure in the rat via cationization and conjugation with polyethylene glycol. *J Drug Target* 2: 317–321, 1994.
109. Mikawa K, Nishna K, Maekawa N, and Obara H. Attenuation of hyperoxic lung injury in rabbits with superoxide dismutase: effects on inflammatory mediators. *Acta Anesthesiol Scand* 39: 317–322, 1995.
110. Milligan SA, Hoeffel JM, Goldstein IM, and Flick MR. Effect of catalase on endotoxin-induced acute lung injury in unanesthetized sheep. *Am Rev Rspir Dis* 137: 420–428, 1988.
111. Minamiya Y, Abo S, Kitamura M, Izumi K, Kimura Y, Tozawa K, and Saito S. Endotoxin-induced hydrogen peroxide production in intact pulmonary circulation of rat. *Am J Respir Crit Care Med* 152: 348–354, 1995.
112. Miyahara T, Shibamoto T, Wang H-G, Koizumi T, Honda T, Kubo K, Sekiguchi M, and Koyama S. Lecithinized superoxide dismutase attenuates phorbol myristate acetate-induced injury in isolated dog lung. *Eur J Pharmacol* 344: 231–239, 1998.
113. Mossman BT, Marsh JP, Hardwick D, Gilbert R, Hill S, Sesko A, Shatos M, Doherty J, Weller A, and Bergeron M. Approaches to prevention of asbestos-induced lung disease using polyethylene glycol (PEG)-conjugated catalase. *J Free Radic Biol Med* 2: 335–338, 1986.
114. Mossman BT, Marsh JP, Sesko A, Hill S, Shatos MA, Doherty J, Petruska J, Adler KB, Hemenway D, and Mickey R. Inhibition of lung injury, inflammation, and interstitial pulmonary fibrosis by polyethylene glycol-conjugated catalase in a rapid inhalation model of asbestosis. *Am Rev Respir Dis* 141: 1266–1271, 1990.
115. Muizelaar JP, Marmarou A, Young HF, Choi SC, Wolf A, Schneider RL, and Konotos HA. Improving the outcome of severe head injury with the oxygen radical scavenger polyethylene glycol-conjugated superoxide dismutase: a phase II trial. *J Neurosurg* 78: 375–382, 1993.
116. Mulligan M, Vaporciyan A, Warner RL, Jones M, Foreman K, Miyasaka M, Todd RF, and Ward PA. Compartmentalized roles for leukocytic adhesion molecules in lung inflammation injury. *J Immunol* 154: 1350–1363, 1995.
117. Muzykantov VR, and Danilov SM. Targeting of ra-

diolabeled monoclonal antibody against ACE to the pulmonary endothelium. In: *Targeted Delivery of Imaging Agents*, edited by Torchilin V. Boca Raton, FL: CRC press, 1995, pp. 465–485.
118. Muzykantov V, Atochina E, Ischiropoulos H, Danilov S, and Fisher A. Immunotargeting of antioxidant enzymes to the pulmonary endothelium. *Proc Natl Acad Sci USA* 93: 5213–5218, 1996.
119. Muzykantov V, Atochina E, Kuo A, Barnathan E, Notarfrancesco K, Shuman H, Dodia C, and Fisher A. Endothelial cells internalize monoclonal antibody to angiotensin-converting enzyme. *Am J Physiol* 270: L704–L713, 1996.
120. Muzykantov V. Immunotargeting of drugs to the pulmonary vascular endothelium as a therapeutic strategy. *Pathophysiology* 5: 15–33, 1998.
121. Muzykantov V, Christofidou-Solomidou M, Balyasnikova I, Harshaw D, Schultz L, Fisher A, and Albelda S. Streptavidin facilitates internalization and pulmonary targeting of anti-endothelial antibody (PECAM-1): a strategy for intraendothelial drug delivery. *Proc Natl Acad Sci USA* 96: 2379–2384, 1999.
122. Nakae D, Yamamoto K, Yoshiji H, Kinugasa T, Maruyama H, Farber JL, and Konishi Y. Liposome-encapsulated superoxide dismutase prevents liver necrosis induced by acetaminophen. *Am J Pathol* 136: 787–795, 1990.
123. Nakae D, Yoshiji H, Amanuma T, Kinugasa T, Farber JL, and Konishi Y. Endocytosis-independent uptake of liposome-encapsulated superoxide dismutase prevents the killing of cultured hepatocytes by *tert*-butyl hydroperoxide. *Arch Biochem Biophys* 279: 315–319, 1990.
124. Nakamura T, Igarashi R, Kurashina T, Saito Y, Hoshino J, Sumino H, Sakamoto H, and Nagai R. Lecithinized superoxide dismutase induces vasodilation; evidence of direct contribution of superoxide anions to modulating vascular tone. *Life Sci* 64: 65–70, 1999.
125. Nelson SK, Bose SK, and McCord JM. The toxicity of high-dose superoxide dismutase suggests that superoxide can both initiate and terminate lipid peroxidation in the reperfused heart. *Free Radic Biol Med* 16: 195–200, 1994.
126. Newman PJ. The biology of PECAM-1. *J Clin Invest* 99: 3–7, 1997.
127. Neya K, Takamoto S, Morita K, Matsuda T, Kyo S, Yokote Y, and Omoto R. Reduction of reperfusion injury by recombinant human superoxide dismutase administered intravenously just prior to reperfusion. *Cardiology* 82: 354–364, 1993.
128. Nguyen TT, Cox JR, Herndon CS, Biondo DN, Traber LD, Bush PE, Zophel A, and Traber DL. Effects of manganese superoxide dismutase on lung fluid balance after smoke inhalation. *J Appl Physiol* 78: 2161–2168, 1995.
129. Nieves-Cruz B, Rivera A, Cifuentes J, Pataki G, Matalon S, Carol WA, Tanswell AK, and Freeman B. Clinical surfactant preparations mediate SOD and catalase uptake by type II cells and lung tissue. *Am J Physiol* 270: L659–L667, 1996.
130. Noda J, Otagiri M, Akaike T, and Maeda H. Pharmacological advantages of conjugation of Cu,Zn-superoxide dismutase with succinylated keratin fragment: improvement of biological properties and resistance to oxidative damage. *J Pharmacol Exp Ther* 279: 162–171, 1996.
131. Odlind B, Appelgren LE, Bayati A, and Wolgast M. Tissue distribution of ^{125}I-labelled bovine superoxide dismutase (SOD) in the rat. *Pharmacol Toxicol* 62: 95–100, 1988.
132. Ogata H, Luo XX, and Xu X. Comparison of effects of margarite extract and recombinant human superoxide dismutase on paraquat-induced superoxide anion radicals in rat lung. *Circ Shock* 43: 161–165, 1994.
133. Ohta H, Adachi T, and Hirano K. Internalization of human extracellular-superoxide dismutase by bovine aortic endothelial cells. *Free Radic Biol Med* 16: 501–507, 1994.
134. Olson MC, Grizzle MK, and Anderson DL. Effect of polyethylene glycol-superoxide dismutase and catalase on endotoxemia in pigs. *J Appl Physiol* 63: 1526–1532, 1987.
135. Padmanabhan RV, Gudapaty R, Liener IE, Schwartz BA, and Hoidal JR. Protection against pulmonary oxygen toxicity in rats by the intratracheal administration of liposome-encapsulated superoxide dismutase or catalase. *Am Rev Respir Dis* 132: 164–167, 1985.
136. Paller MS, and Eaton JW. Hazards of antioxidant combination containing superoxide dismutase. *Free Radic Biol Med* 18: 883–890, 1995.
137. Panes J, Perry M, Anderson D, Manning A, Leone B, Cepinskas G, Rosenbloom C, Miyasaka M, Kvietis P, and Granger DN. Regional differences in constitutive and induced ICAM-1 expression in vivo. *Am J Physiol Heart Circ Physiol* 269: H1955–H1964, 1995.
138. Panus PC, Radi R, Chimney PH, Lillard R, and Freeman BA. Detection of H_2O_2 released from vascular endothelial cells. *Free Radic Biol Med* 14: 217–223, 1993.
139. Parizada B, Werber MM, and Nimrod A. Protective effects of human recombinant MnSOD in adjuvant arthritis and bleomycin-induced lung fibrosis. *Free Radic Res Commun* 15: 297–301, 1991.
140. Poznansky M, and Juliano R. Biological approaches to the controlled delivery of drugs. *Pharmacol Rev* 36: 277–335, 1984.
141. Pritchard KA Jr, Groszek L, Smalley DM, Sessa WC, Wu M, Villalon P, Wolin PS, and Stemerman MB. Native low-density lipoprotein increases endothelial cell nitric oxide synthase generation of superoxide anion. *Circ Res* 77: 510–518, 1995.
142. Pyatak PS, Abuchowski A, and Davis FF. Preparation of a polyethylene glycol: superoxide dismutase adduct, and an examination of its blood circulating life and anti-inflammatory activity. *Res Commun Chem Pathol Pharmacol* 29: 113–119, 1980.
143. Rangan U, and Bulkley GB. Prospects for treatment

of free radical-mediated tissue injury. *Br Med Bull* 49: 700–718, 1993.
144. Regnault C, Roch-Arveiller M, Tissot M, Sarfati G, Giroud JP, Postaire E, and Hazebroucq G. Effect of encapsulation in liposomes on the anti-inflammatory properties of superoxide dismutase after oral administration. *Clin Chim Acta* 240: 117–127, 1995.
145. Regnault C, Soursac M, Roch-Arveiller M, Postaire E, and Hazebroucq G. Pharmacokinetics of superoxide dismutase in rats after oral administration. *Biopharm Drug Dispos* 17: 165–174, 1996.
146. Reinholz MM, Haggard JJ, Curran GL, and Poduslo JF. Plasma pharmacokinetics, nervous system biodistribution and biostability, and spinal cord permeability at the blood–brain barrier of putrescine-modified catalase in the adult rat. *Exp Neurol* 159: 91–203, 1999.
147. Repine JE, Bast A, and Lankhorst I. Oxidative stress in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 156: 341–357, 1997.
148. Robbins CG, Horowitz S, Merritt TA, Kheiter A, Tierney J, Narula P, and Davis JM. Recombinant human superoxide dismutase reduces lung injury caused by inhaled nitric oxide and hyperoxia. *Am J Phsyiol Lung Cell Mol Physiol* 16: L903–L907, 1997.
149. Rojanasakul Y, Shi XG, Deshpande D, Liang WW, and Wang LY. Protection against oxidative injury and permeability alteration in cultured alveolar epithelium by transferrin-catalase conjugate. *Biochim Biophys Acta* 1315: 21–28, 1996.
150. Rosenfeld W, Evans H, Jhaveri R, Moainie H, Vohra K, Georgatos E, and Salazar JD. Safety and plasma concentrations of bovine superoxide dismutase administered to human premature infants. *Dev Pharmacol Ther* 5: 151–161, 1982.
151. Rosenfeld W, Evans H, Concepcion L, Jhaveri R, Schaeffer H, and Friedman A. Prevention of bronchopulmonary dysplasia by administration of bovine superoxide dismutase in preterm infants with respiratory distress syndrome. *J Pediatr* 105: 781–785, 1984.
152. Rosenfeld WN, Davis JM, Parton L, Richter SE, Price A, Flaster E, and Kassem N. Safety and pharmacokinetics of recombinant human superoxide dismutase administered intratracheally to premature neonates with respiratory distress syndrome. *Pediatrics* 97: 811–817, 1996.
153. Sahgal N, Davis JM, Robbins C, Horowitz S, Langenback EG, Perry RH, Colflesh, Tierney J, and Simon SR. Localization and activity of recombinant human CuZn superoxide dismutase after intratracheal administration. *Am J Physiol* 271: L230–L235, 1996.
154. Saifer MG, Somack R, and Williams LD. Plasma clearance and immunologic properties of long-acting superoxide dismutase prepared using 35,000 to 120,000 dalton polyethylene glycol. *Adv Exp Med Biol* 366: 377–387, 1994.
155. Sandstrom J, Nilsson P, Karlsson K, and Marklund SL. 10-fold increase in human plasma extracellular superoxide dismutase content caused by a mutation in heparin-binding domain. *J Biol Chem* 269: 19163–19166, 1994.
156. Schalkwijk J, van den Berg WB, van de Putte LB, Joosten LA, and van den Bersselaar L. Cationization of catalase, peroxidase, and superoxide dismutase. Effect of improved intraarticular retention on experimental arthritis in mice. *J Clin Invest* 76: 198–205, 1985.
157. Schnackenberg CG, Welch WJ, and Wilcox CS. Normalization of blood pressure and renal vascular resistance in SHR with a membrane-permeable superoxide dismutase mimetic. Role of nitric oxide. *Hypertension* 32: 59–64, 1998.
158. Schneider J, Friderichs E, Heintze K, and Flohe L. Effects of recombinant human superoxide dismutase on increased lung vascular permeability and respiratory disorder in endotoxemic rats. *Circ Shock* 30: 97–106, 1999.
159. Seekamp A, Lalonde C, Zhu DG, and Demling R. Catalase prevents prostanoid release and lung lipid peroxidation after endotoxemia in sheep. *J Appl Physiol* 65: 1210–1216, 1988.
160. Shaffer SG, O'Neil DH, and Thibeault DW. Administration of bovine superoxide dismutase fails to prevent chronic pulmonary sequelae of neonatal oxygen exposure in the rat. *J Pediatr* 110: 942–946, 1987.
161. Sherpereel A, Wiewrodt R, Christofidou-Solomidou M, Gervais R, Murciano J, Albelda S, and Muzykantov V. Intracellular immunotargeting of active reporter enzyme to endothelium in vivo. (Abstract) *Am J Respir Crit Care Med* 161: A132, 2000.
162. Siflingerbirnboim A, and Malik AB. Neutrophil adhesion to endothelial cells impairs the effects of catalase and glutathione in preventing endothelial injury. *J Cell Physiol* 155:234–239, 1993.
163. Silverman HS, Siddoway LA, Yavin Z, Gonenne A, Weisfeldt ML, and Becker LC. Pharmacokinetics of recombinant human superoxide dismutase. *J Cardiovasc Pharmacol* 16: 107–111, 1990.
164. Simonson SG, Welty-Wolf KE, Huang Y-CT, Taylor DE, Kantrow SP, Carraway MS, Crapo JD, and Piantadosi CA. Aerosolized manganese SOD decreases hyperoxic pulmonary injury in primates. I. Physiology and Biochemistry. *J Appl Physiol* 83: 550–558, 1997.
165. Springer TA. Adhesion receptors of the immune system. *Nature* 346: 425–434, 1990.
166. Suzuki Y, Tanigaki T, Heimer D, Wang WZ, Ross WG, Sussman HH, and Raffin TA. Polyethylene glycol-conjugated superoxide dismutase attenuates septic lung injury in guinea pigs. *Am Rev Respir Dis* 145: 388–393, 1992.
167. Takakura Y, Masuda S, Tokuda H, Nishikawa M, and Hashida M. Targeted delivery of superoxide dismutase to macrophages via mannose receptor-mediated mechanism. *Biochem Pharmacol* 47: 853–858, 1994.
168. Takeda Y, Hashimoto H, Kosaka F, Hirakawa M, and Inoue M. Albumin-binding superoxide dismutase

with a prolonged half-life reduces reperfusion brain injury. *Am J Physiol* 264: H1708–H1715, 1993.
169. Takenaga M, Igarashi R, Ochiai A, and Mizushima Y. Effects of lecithinized superoxide dismutase (PC-SOD) on experimental pulmonary metastasis in mice. *Free Radic Biol Med* 26:1117–1125, 1999.
170. Tamura Y, Chi LG, Driscoll EM Jr, Hoff PT, Freeman BA, Gallagher KP, and Lucchesi BR. Superoxide dismutase conjugated to polyethylene glycol provides sustained protection against myocardial ischemia/reperfusion injury in canine heart. *Circ Res* 63: 944–959, 1988.
171. Tanaka M, Stoler RC, Fitzharris GP, Jennings RB, and Reimer KA. Evidence against the "early protection-delayed death" hypothesis of superoxide dismutase therapy in experimental myocardial infarction. Polyethylene glycol-superoxide plus catalase does not limit myocardial infarct size in dogs. *Circ Res* 67: 636–644, 1990.
172. Tang G, White JE, Gordon RJ, Lumb PD, and Tsan MF. Polyethylene glycol-conjugated superoxide dismutase protects rats against oxygen toxicity. *J Appl Physiol* 74: 1425–1431, 1993.
173. Tanswell AK, and Freeman BA. Liposome-entrapped antioxidant enzymes prevent lethal O_2 toxicity in the newborn rat. *J Appl Physiol* 63: 347–352, 1987.
174. Tanswell AK, Olson DM, and Freeman BA. Liposome-mediated augmentation of antioxidant defenses in fetal rat pneumocytes. *Am J Physiol* 258: L165–L172, 1990.
175. Thibeault DW, Rezaiekhaligh M, Mabry S, and Beringer T. Prevention of chronic pulmonary oxygen toxicity in young rats with liposome-encapsulated catalase administered intratracheally. *Pediatr Pulmonol* 11: 318–327, 1991.
176. Thibeault DW, Rezaiekhaligh M, and Mabry S. Lung antioxidant enzymes and cardiopulmonary responses in young rats exposed to hyperoxia and treated intratracheally with PEG catalase and superoxide dismutase. *Exp Lung Res* 19: 137–151, 1993.
177. Thomson PD, Till GO, Woolliscroft JO, Smith DJ, and Prasad JK. Superoxide dismutase prevents lipid peroxidation in burned patients. *Burns* 16: 406–408, 1990.
178. Traber DL, Adams T Jr, Sziebert L, Stein M, and Traber L. Potentiation of lung vascular response to endotoxin by superoxide dismutase. *J Appl Physiol* 58: 1005–1009, 1985.
179. Tsao C, Greene P, Odlind B, and Brater DC. Pharmacokinetics of recombinant human superoxide dismutase in healthy volunteers. *Clin Pharmacol Ther* 50: 713–720, 1991.
180. Turrens JF, Freeman BA, and Crapo JD. Hyperoxia increases H_2O_2 release by lung mitochondria and microsomes. *Arch Biochem Biophys* 216: 411–421, 1982.
181. Turrens JF, Crapo JD, and Freeman BA. Protection against oxygen toxicity by intravenous injection of liposome-entrapped catalase and superoxide dismutase. *J Clin Invest* 73: 87–95, 1984.
182. Uematsu T, Nagashima S, Umemura K, Kanamaru M, and Nakashima M. Pharmacokinetics and safety of intravenous recombinant human superoxide dismutase (NK341) in healthy subjects. Internl. *J Clin Pharmacol Ther* 32: 638–641, 1994.
183. Vaporician AA, Delisser HM, Yam HC, Mendiguren I, Thom SR, Jones ML, Ward PA, and Albelda SM. Platelet-endothelial cell adhesion molecule-1 (PECAM-1) is involved in neutrophil recruitment in vivo. *Science* 262: 1580–1582, 1993.
184. Varani J, and Ward P. Mechanisms of endothelial cell injury in acute inflammation. *Stroke* 2: 311–319, 1994.
185. Veronese FM, Boccu E, Schiavon O, Velo GP, Conforti A, Franco L, and Milnino R. Anti-inflammatory and pharmacokinetic properties of superoxide dismutase derivatized with polyethylene glycol via active esters. *J Pharmacol* 35: 757–758, 1983.
186. Viau AT, Abuchowski A, Greenspan S, and Davis FF. Safety evaluation of free radical scavengers PEG-catalase and PEG-superoxide dismutase. *J Free Radic Biol Med* 2: 283–288, 1986.
187. Walther FJ, Gidding CE, Kuipers IM, Willebrand D, Bevers EM, Abuchowski A, and Viau AT. Prevention of oxygen toxicity with superoxide dismutase and catalase in premature lambs. *J Free Radic Biol Med* 2: 289–293, 1986.
188. Walther FJ, Kuipers IM, Pavlova Z, Willebrand D, Abuchowski A, and Viau AT. Mitigation of pulmonary oxygen toxicity in premature lambs with intravenous antioxidants. *Exp Lung Res* 16: 177–189, 1990.
189. Walther FJ, Wade AB, Warburton D, and Forman HJ. Augmentation of superoxide dismutase and catalase activity in alveolar type II cells. *Am J Respir Cell Mol Biol* 4: 364–368, 1991.
190. Walther FJ, David-Cu R, Supnet MC, Longo ML, Fan BR, and Bruni R. Uptake of antioxidants in surfactant liposomes by cultured alveolar type II cells is enhanced by SP-A. *Am J Physiol* 265: L330–L339, 1993.
191. Walther FJ, Nunez FL, David-Cu R, and Hill KE. Mitigation of pulmonary oxygen toxicity in rats by intratracheal instillation of polyethylene glycol-conjugated antioxidant exzymes. *Pediatr Res* 33: 332–335, 1993.
192. Walther FJ, David-Cu R, and Lopez SL. Antioxidant-surfactant liposomes mitigate hyperoxic lung injury in premature rabbits. *Am J Physiol* 269: L613–L617, 1995.
193. Ward PA, and Hunninghake GW. Lung inflammation and fibrosis. *Am J Respir Crit Care Med* 157: S123–S129, 1998.
194. Watanabe BI, Premaratne S, Limm W, Mugiishi MM, and McNamara JJ. High- and low-dose superoxide dismutase plus catalase does not reduce myocardial infarc size in a subhuman primate model. *Am Heart J* 126: 840–846, 1993.
195. Watanabe K, Lam G, Keresztes R, and Jaffe E. Lipopolysaccharides decrease ACE activity expressed by cultured human endothelial cells. *J Cell Physiol* 150: 433–439, 1992.

196. Welty-Wolf KE, Simonson SG, Huang Y-CT, Kantrow SP, Carraway MS, Chang L-Y, Crapo JD, and Piantadosi CA. Aerosolized manganese SOD decreases hyperoxic pulmonary injury in primates. II. Morphometric analysis. *J. Appl Physiol* 83: 559–568, 1997.
197. Wengenack TM, Curran GL, and Poduslo JF. Postischemic, systemic administration of polyamine-modified superoxide dismutase reduces hippocampal CA1 neurodegeneration in rat global cerebral ischemia. *Brain Res* 754: 46–54, 1997.
198. White CW, Jackson JH, Abuchowski A, Kazo GM, Mimmack RF, Berger EM, Freeman BA, McCord JM, and Repine JE. Polyethylene glycol-attached antioxidant enzymes decrease pulmonary oxygen toxicity in rats. *J Appl Physiol* 66: 584–590, 1989.
199. Wright SE, Baron DA, and Heffner JE. Intravenous eugenol causes hemorrhagic lung edema in rats: proposed oxidant mechanisms. *J Lab Clin Med* 125: 257–264, 1995.
200. Wyde PR, Moore DK, Pimentel DM, Gilbert BE, Nimrod R, and Panet A. Recombinant superoxide dismutase administered by aerosol inhibits respiratory syncytial virus infection in cotton rats. *Antiviral Res* 31: 173–184, 1996.
201. Yabe Y, Koyama Y, Nishikawa M, Takakura Y, and Hashida M. Hepatocyte-specific distribution of catalase and its inhibitory effect on hepatic ischemia/reperfusion injury in mice. *Free Radic Res* 30: 265–274, 1998.
202. Yabe Y, Nishikawa M, Tamada A, Takakura Y, and Hashida M. Targeted delivery and improved therapeutic potential of catalase by chemical modification: combination with superoxide dismutase derivatives. *J Pharmacol Exp Ther* 289: 1176–1184, 1999.
203. Yamazaki C, Hoshino J, Hori Y, Sekiguchi T, Miyauchi S, Mizuno S, and Horie K. Effect of lecithinized-superoxide dismutase on the interstitial pneumonia model induced by bleomycin in mice. *Jpn J Pharmacol* 75: 97–100, 1997.
204. Yunoki M, Kawauchi M, Ukita N, Noguchi Y, Nishio S, Ono Y, Asari S, Ohmoto T, Asanuma M, and Ogawa N. Effects of lecithinized superoxide dismutase on traumatic brain injury in rats. *J Neurotrauma* 14: 739–746, 1997.
205. Yunoki M, Kawauchi M, Ukita N, Noguchi Y, Nishio S, Ono Y, Asari S, Ohmoto T, Asanuma M, and Ogawa N. Effects of lecithinized SOD on sequential change in SOD activity after cerebral contusion in rats. *Acta Neurochir Suppl* 71: 142–145, 1998.
206. Yusa T, Crapo JD, and Freeman BA. Liposome-mediated augmentation of brain SOD and catalase inhibits CNS O_2 toxicity. *J Appl Physiol* 57: 1674–1681, 1984.
207. Zweier JL, Kuppusamy P, and Lutty GA. Measurement of endothelial cell free radical generation: evidence for a central mechanism of free radical injury in postischemic tissues. *Proc Natl Acad Sci USA* 85: 4046–4050, 1988.

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2. Yifei Zhang, Jiang Li, Xiang Gao, Song LiTargeted Delivery to the Pulmonary Endothelium 193-233. [CrossRef]
3. J. Ambati, A.M. Lopez, D. Cochran, P. Wattamwar, K. Bean, T.D. Dziubla, S.E. Rankin. 2012. Engineered silica nanocarriers as a high-payload delivery vehicle for antioxidant enzymes. *Acta Biomaterialia* **8**:6, 2096-2103. [CrossRef]
4. Misagh Alipour, Milton G. Smith, Kresimir Pucaj, Zacharias E. Suntres. 2012. Acute toxicity study of liposomal antioxidant formulations containing N-acetylcysteine, #-tocopherol, and #-tocopherol in rats. *Journal of Liposome Research* 1-10. [CrossRef]
5. Elizabeth Hood, Eric Simone, Paritosh Wattamwar, Thomas Dziubla, Vladimir Muzykantov. 2011. Nanocarriers for vascular delivery of antioxidants. *Nanomedicine* **6**:7, 1257-1272. [CrossRef]
6. Vladimir V. Shuvaev, Vladimir R. Muzykantov. 2011. Targeted modulation of reactive oxygen species in the vascular endothelium. *Journal of Controlled Release* **153**:1, 56-63. [CrossRef]
7. Rama Surya Prakash Perepu, Carlos Garcia, David Dostal, Rajat Sethi. 2010. Enhanced death signaling in ozone-exposed ischemic-reperfused hearts. *Molecular and Cellular Biochemistry* **336**:1-2, 55-64. [CrossRef]
8. Kazuyoshi Suga, Yasuhiko Kawakami, Hideyuki Iwanaga, Noriko Hayashi, Akiko Seto, Naofumi Matsunaga. 2008. Assessment of anatomic relation between pulmonary perfusion and morphology in pulmonary emphysema with breath-hold SPECT-CT fusion images. *Annals of Nuclear Medicine* **22**:5, 339-347. [CrossRef]
9. Mohamed Samai, Theresa Hague, Declan P. Naughton, Paul R. Gard, Prabal K. Chatterjee. 2008. Reduction of paraquat-induced renal cytotoxicity by manganese and copper complexes of EGTA and EHPG. *Free Radical Biology and Medicine* **44**:4, 711-721. [CrossRef]
10. Thomas D. Dziubla, Vladimir V. Shuvaev, Nan Kang Hong, Brian J. Hawkins, Muniswamy Madesh, Hajime Takano, Eric Simone, Marian T. Nakada, Aron Fisher, Steven M. Albelda, Vladimir R. Muzykantov. 2008. Endothelial targeting of semi-permeable polymer nanocarriers for enzyme therapies. *Biomaterials* **29**:2, 215-227. [CrossRef]
11. Mitchell Machtay, Arnaud Scherpereel, José Santiago, James Lee, Jim McDonough, Paul Kinniry, Evguenia Arguiri, Vladimir V. Shuvaev, Jing Sun, Keith Cengel, Charalambos C. Solomides, Melpo Christofidou-Solomidou. 2006. Systemic polyethylene glycol-modified (PEGylated) superoxide dismutase and catalase mixture attenuates radiation pulmonary fibrosis in the C57/bl6 mouse. *Radiotherapy and Oncology* **81**:2, 196-205. [CrossRef]
12. Vladimir R Muzykantov. 2005. Biomedical aspects of targeted delivery of drugs to pulmonary endothelium. *Expert Opinion on Drug Delivery* **2**:5, 909-926. [CrossRef]
13. Kazuyoshi Suga, Kawakami Yasuhiko, Hideyuki Iwanaga, Norio Hayashi, Tomio Yamashita, Naofumi Matsunaga. 2005. Enhanced perfusion defect clarity and inhomogeneity in smokers?? lungs with deep-inspiratory breath-hold perfusion SPECT images. *Nuclear Medicine Communications* **26**:9, 801-807. [CrossRef]
14. R RENGEL, J FILIPOVICGRCIC, I CEPELAK, T ZANICGRUBISIC, K BARISIC. 2005. The effect of liposomes with superoxide dismutase on A2182 cells. *European Journal of Pharmaceutics and Biopharmaceutics* **60**:1, 47-51. [CrossRef]

15. J Haddad. 2004. Redox and oxidant-mediated regulation of apoptosis signaling pathways: immuno-pharmaco-redox conception of oxidative siege versus cell death commitment. *International Immunopharmacology* **4**:4, 475-493. [CrossRef]