MINIREVIEW

Superoxide Dismutase and Pulmonary Oxygen Toxicity[1] (44076)

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Abstract. **The production of superoxide (O_2^-) under hyperoxic conditions is markedly accentuated leading to the generation of potent oxidants such as hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\bullet$), and peroxynitrite ($ONOO^-$). Superoxide dismutase (SOD), by rapidly removing O_2^-, reduces the tissue concentration of O_2^- and prevents the production of $HO^\bullet$ and $ONOO^-$. Three forms of SOD exist in the lung: CuZnSOD, MnSOD, and extracellular SOD. Considerable supportive, though not all conclusive, evidence suggests that all three forms of SOD are essential for the pulmonary defense against oxygen toxicity, and that enhancement of pulmonary SOD has the potential of protecting against oxygen toxicity.** [P.S.E.B.M. 1997, Vol 214]

Superoxide anion (O_2^-) is a reactive oxygen free radical resulting from the enzymatic or nonenzymatic, univalent reduction of molecular oxygen. Even though O_2^- by itself is only a weak oxidant or a weak reductant, it is capable of producing potent oxidants. These include hydrogen peroxide (H_2O_2) (*via* enzymatic or spontaneous dismutation [Reaction 1]), hydroxyl radical ($OH^\bullet$) (*via* Haber-Weiss reaction [Reaction 2]), or peroxynitrite ($ONOO^-$) (*via* reaction with nitric oxide, $NO^\bullet$ [Reaction 3]) (1, 2):

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 \quad (1)$$

$$O_2^- + H_2O_2 \xrightarrow{Fe^{3+}} OH^- + OH^\bullet + O_2 \quad (2)$$

$$O_2^- + NO^\bullet \longrightarrow ONOO^- \quad (3)$$

The production of these reactive oxygen and nitrogen species (O_2^-, H_2O_2, $OH^\bullet$, and $ONOO^-$) is markedly accentuated under hyperoxic conditions leading to tissue, especially pulmonary, injury (3, 4). Considerable evidence suggests that during the hyperoxic exposure, reactive oxygen species are produced both intracellularly by lung parenchymal cells (5) and extracellularly by lung macrophages (6) and infiltrating neutrophils (7, 8). The relative importance of intracellular versus extracellular reactive oxygen species in the pathogenesis of pulmonary oxygen toxicity is not clear. However, since depletion of alveolar macrophages (6) or neutrophils (7–9), main sources of extracellular reactive oxygen species, results either in no protection or in only mild attenuation of oxygen toxicity, intracellular reactive oxygen species appear to play the major role in the pathogenesis of oxygen toxicity.

Superoxide dismutase (SOD) is a family of enzymes that catalyze the dismutation of O_2^- to H_2O_2 and O_2 (Reaction 1). By rapidly eliminating O_2^-, SOD reduces the tissue concentration of O_2^- and prevents the production of $OH^\bullet$ (Reaction 2) and $ONOO^-$ (Reaction 3). Thus if lung tissue contains sufficient quantities of catalase and glutathione (GSH) peroxidase to dispose H_2O_2, augmentation of pulmonary SOD has the potential of preventing oxygen toxicity (10). This minireview updates our current understanding of the role of pulmonary SOD in the host defense against oxygen toxicity.

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Superoxide Dismutase

Three forms of SOD, with distinct distribution and metal components, exist in mammalian tissues: CuZnSOD, MnSOD, and extracellular SOD (EC-SOD, which, along with CuZnSOD, also contains Cu and Zn) (1, 11). The Cu- and Zn-containing SOD, CuZnSOD and EC-SOD, are sensitive to cyanide and constitute approximately 85%–90% of the total tissue SOD activity, while MnSOD is resistant to cyanide and constitutes approximately 10%–15% of the total tissue SOD activity. In mammalian lungs, the level of EC-SOD is quite variable among different species, ranging from less than 1% (rat, cat, and dog) to about 10% (mouse and human) of the total SOD activity (11).

The intracellular CuZnSOD is usually regarded as a cytosolic enzyme. However, recent evidence suggests that the majority of this enzyme may actually be located in peroxisomes (12). In addition to its O_2^- dismutase activity, CuZnSOD has peroxidase activity (13). Unlike MnSOD, CuZnSOD is inactivated by its enzymatic reaction product H_2O_2. During this inactivation process, $OH^\bullet$ is produced (14). It has also been shown that CuZnSOD is capable of catalyzing the formation of free radicals using anionic scavengers and H_2O_2 as substrates (15).

MnSOD is located in mitochondria. It is an inducible enzyme; induction of MnSOD has been demonstrated following exposure to irradiation (16) or hyperoxia (17), and following treatment with paraquat (18), tumor necrosis factor (TNF) (19), interleukin-1 (IL-1) (20), or endotoxin (lipopolysaccharide [LPS]) (21).

Extracellular SOD is a secreted, CuZn-containing SOD distinct from the intracellular CuZnSOD. It is the dominant SOD isozyme in the plasma and interstitial tissue (11). Three forms of EC-SOD with varying degrees of heparin-binding affinity exist: A, without affinity; B, with intermediate affinity; and C, with high affinity (22). EC-SOD C in the tissue interstitium is almost completely anchored to heparan sulfate proteoglycan in the glycocalyx of cell surface and in the connective tissue matrix *via* its carboxyterminal heparin-binding domains. EC-SOD A and B are present primarily in the extracellular fluid including plasma. Evidence suggests that the A and B forms are derived from the C form by post-translational proteolytic cleavage at the carboxyl terminus (22, 23).

Thus, mammalian tissues are well equipped with SOD to dispose intracellular as well as extracellular O_2^-. However, it should be kept in mind that CuZnSOD has, in addition to the dismutase activity, peroxidase activity (13–15), which may cause deleterious effects. Furthermore, too much SOD may result in an imbalance among antioxidant enzymes (e.g., SOD, catalase, and GSH peroxidase), leading to loss of protective effects or even exacerbation of oxidant damage (24, 25).

In evaluating the role of SOD in the host defense against oxygen toxicity, one looks at the effects of modulating the level of a particular SOD in the lung on hyperoxia-induced pulmonary injury. In this respect, transgenic and gene knock-out (targeted inactivation by homologous recombination) mice, in which a specific gene is enhanced or inactivated (26, 27), provide powerful tools to study the role of a particular gene product such as SOD.

CuZnSOD and Oxygen Toxicity

As the major SOD species in lungs, the role of CuZnSOD in the pulmonary defense against oxygen toxicity has been extensively studied since 1970s. Some of these studies, however, suffer from the shortcoming of measuring the activity of total SOD, instead of CuZnSOD specifically, which leads to inconclusive or even misleading results.

Effect of Reducing CuZnSOD Activity. A boy with partial monosomy 21 and diminished CuZnSOD activity (in humans, the gene encoding for CuZnSOD is located on Chromosome 21) was found to have a markedly increased sensitivity to pulmonary oxygen toxicity (28). This observation suggests that normal levels of CuZnSOD are essential for the pulmonary defense against oxygen toxicity. However, the missing piece of Chromosome 21 contains many genes other than the CuZnSOD gene. Thus, it is possible that the observed increase in oxygen sensitivity may be due to something other than the reduced level of CuZnSOD activity. Recently, mutations of CuZnSOD gene with diminished CuZnSOD activity have been demonstrated in patients with familiar amyotrophic lateral sclerosis (Lou Gehrig disease) (29, 30). Whether these patients have increased sensitivity to oxygen toxicity is not clear. CuZnSOD gene knock-out mice have also been produced. The homozygous mice with complete absence of CuZnSOD activity and the heterozygous mutants with approximately 50% of the wild-type CuZnSOD activity develop normally into adulthood and show no evidence of overt oxidative damage as judged by levels of protein carbonyl content and lipid peroxidation in the brain tissue (31). These results indicate that CuZnSOD is not required for normal development and survival in mice. These mutant mice will provide a useful tool to define the role of CuZnSOD in the host defense against oxygen toxicity.

Effect of Increasing CuZnSOD Activity. Exposure of rats to a sublethal dose of normobaric O_2 (85% or 90%) for 7 days results in a significant increase in pulmonary levels of CuZnSOD, MnSOD, catalase and GSH peroxidase (32, 33). These antioxidant enzyme-augmented animals become tolerant to subsequent exposure to a lethal dose (>95%) of O_2. Recently Ho *et al.* (34) reported that the sublethal dose of hyperoxia-induced increase in pulmonary CuZnSOD and MnSOD activities as expressed by SOD activity per milligram DNA, was noted as early as 3 days after exposure. However, the increase in MnSOD activity was accompanied by an increase in the steady-state level of MnSOD mRNA, while the increase in CuZnSOD activity was not accompanied by an increase in CuZnSOD mRNA. Likewise, rats preexposed to hypoxia (10% O_2) for 3 days also become tolerant of lethal hyperoxia (35). This hypoxia-

induced oxygen tolerance is associated with increased pulmonary levels of total SOD, catalase, and GSH peroxidase. Neonatal rats, mice, and rabbits are more resistant to lethal doses of O_2 than their adult counterparts (36–38). This is due in part to the ability of these neonatal animals to increase pulmonary levels of SOD, catalase, and GSH peroxidase within 24 hr of O_2 exposure. Since antioxidant enzymes other than CuZnSOD are also increased under the above conditions, the increased level of pulmonary CuZnSOD may not be solely responsible for the increased tolerance of pulmonary oxygen toxicity.

Intraperitoneal (ip) injection of a sublethal dose of LPS (e.g., 500 μg/kg) to rats exposed to lethal hyperoxia results in increased lung total SOD activity, decreased O_2-induced lung damage, and an improvement in survival rate (39). The LPS- and hyperoxia-induced increase in pulmonary SOD activity and the protection against oxygen toxicity were thought to be due to an increase in CuZnSOD, since both the increased pulmonary SOD activity and the protection could be abolished by pretreatment with diethyldithiocarbamate, a Cu-chelating agent and an inhibitor of CuZnSOD (40). In an attempt to elucidate the mechanisms for the increased pulmonary CuZnSOD activity in LPS-treated and O_2-exposed rats, it was initially shown that in these animals the level of pulmonary CuZnSOD mRNA was not increased, but the rate of CuZnSOD protein synthesis was increased (41, 42). A subsequent study revealed that LPS did cause a slight increase in the level of pulmonary CuZnSOD mRNA, but a sustained elevation of CuZnSOD mRNA and its translation into an increased rate of CuZnSOD protein synthesis required O_2 exposure (43). However, there is considerable problem with the above conclusion that LPS-induced protection against oxygen toxicity is due to an increase in pulmonary CuZnSOD. First, diethyldithiocarbamate is a nonspecific inhibitor (44); it may have effects other than inhibition of CuZnSOD to account for the observed protection. Second, levels of pulmonary MnSOD mRNA, specific (immunoreactive) protein, and enzyme activity were not measured in the above studies. In fact, recent studies (45, 46) revealed that LPS-induced oxygen tolerance was not associated with an increase in CuZnSOD. Instead, it was associated with a selective increase in levels of pulmonary MnSOD mRNA and enzyme activity.

Considerable effort has been made in recent years to enhance oxygen resistance by increasing pulmonary SOD activity using exogenous CuZnSOD. Because of the extremely short plasma half-life (a few minutes) and poor cellular uptake of CuZnSOD, administration of bovine CuZnSOD by intravenous (iv) injection or aerosol inhalation does not protect animals against oxygen toxicity (47, 48). Liposome encapsulation or polyethylene glycol (PEG) conjugation of CuZnSOD has been used successfully to prolong its plasma half-life and to increase cellular uptake of the enzyme (48–50). Tracheal insufflation of liposome-encapsulated CuZnSOD (51) or PEG-CuZnSOD (52) protects rats against oxygen toxicity. However, iv or ip administration of liposome-encapsulated CuZnSOD or PEG-CuZnSOD does not protect animals against oxygen toxicity, unless they are co-administered with liposome-encapsulated catalase or PEG-catalase, respectively (50, 53). This may be due in part to the fact that at a similar dosage, tracheal insufflation of PEG-CuZnSOD increases pulmonary SOD activity to a much greater extent than iv injection (52). A recent study (54) demonstrating that tracheal administration of a large dose (5 mg/kg) of recombinant human CuZnSOD protects newborn piglets against oxygen toxicity supports this possibility.

Transgenic mice overexpressing CuZnSOD have also been used to study the protective effect of CuZnSOD. However, the results are not clear-cut. White *et al.* (55) reported that survival advantage was noted in young (2.5-month-old) but not old (5-month-old), female but not male, mice with transgenic overexpression of CuZnSOD (110%–150% over control), exposed to 100% O_2 at a reduced atmospheric pressure (630 torr), but not at the sea level atmospheric pressure (760 torr). It is not clear why the protective effect of increased pulmonary CuZnSOD was noted only in a subgroup of young, female, transgenic mice under reduced atmospheric pressure. In addition, Ho (56) was unable to notice any protection in transgenic mice overexpressing CuZnSOD (80% over control).

MnSOD and Oxygen Toxicity

Despite its strategic location in mitochondria, a major site of O_2^- production under hyperoxic conditions (5), the potential role of MnSOD in the pulmonary defense against oxygen toxicity has not attracted much attention until lately. This is in part due to the fact that MnSOD consitutes a minor fraction of the total pulmonary SOD activity and its activity is more difficult to measure. Most previous studies have only measured total pulmonary SOD activity, which may overlook a significant alteration of MnSOD activity. The above-mentioned increase in pulmonary SOD activity within 24 hr of O_2 exposure in neonatal rats is accounted for solely by an increase in MnSOD activity (38). The recent demonstration that LPS, TNF, and IL-1 selectively induces MnSOD mRNA, leading to increased MnSOD specific protein and enzyme activity, without affecting the levels of other antioxidant enzymes including CuZnSOD, catalase, and GSH peroxidase (19–21), has provided the impetus to study the potential role of pulmonary MnSOD in the protection against oxygen toxicity.

Effect of Reducing MnSOD Activity. Exposure of adult rats to 100% O_2 results in a markedly elevated level of pulmonary MnSOD mRNA within 1 day of O_2 exposure; however, this increase in MnSOD mRNA in adults, in contrast to neonatal rats (38), as stated above, is not associated with an increase in pulmonary MnSOD specific protein or enzyme activity (57–60). Instead, 2–2.3 days (48–55 hr) after O_2 exposure, the levels of pulmonary MnSOD specific protein and enzyme activity are reduced by approximately 30%–50%, and the animals die of oxygen toxicity shortly

thereafter (45, 57–60). Failure of hyperoxia-exposed rats to increase pulmonary MnSOD protein and enzyme activity in the presence of an elevated level of MnSOD mRNA may contribute to the loss of pulmonary defense to oxygen toxicity. It should be pointed out that the effect of a lethal dose of hyperoxia (100%) on pulmonary MnSOD mRNA, protien, and enzyme activity is very different from that of a sublethal dose of hyperoxia (85%), which enhances MnSOD mRNA, protein, and enzyme activity after 3 days of exposure (34).

Pertussis toxin, an inhibitor of a subfamily of heterodimeric guanine-nucleotide-binding regulatory proteins (G proteins) (61), is known to cause lung edema in room air (62). Clerch *et al.* (63) reported that pertussis toxin selectively decreased lung MnSOD enzyme activity (an approximately 50% reduction) without affecting the activities of CuZnSOD, catalase, and GSH peroxidase in young (21- to 35-day-old) rats. The pertussis toxin-induced decrease in pulmonary MnSOD activity occurred within 12 hr when there was no alteration in the steady-state level of pulmonary MnSOD mRNA. Since the pertussis toxin-induced lung edema was attenuated under hypoxic condition (15% O_2) or by LPS treatment which enhanced pulmonary MnSOD activity, and was exacerbated by hyperoxic exposure, it was concluded that reduction of pulmonary MnSOD by pertussis toxin rendered animals sensitive to oxygen toxicity even at the ambient oxygen concentration (20% O_2) (63). However, pertussis toxin and G proteins have a diversity of biological activities (61). Thus, the pertussis toxin-induced lung edema and oxygen sensitivity could be due to something other than its inhibition of pulmonary MnSOD activity.

Recently, MnSOD gene knock-out mice have been produced. The homozygous mutant mice with no detectable MnSOD activity die within the first 10 days after birth with a dilated cardiomyopathy, accumulation of lipid in liver and skeletal muscle, and metabolic acidosis. The mitochondrial ultrastructure is normal; however, the activities of mitochondrial enzymes, succinate dehydrogenase and aconitase, are markedly reduced. There is no increase in lipid peroxidation or lung water content. It was suggested that the animals died of cardiac arrhythmia. The heterozygous mutant mice with approximately 50% MnSOD and normal CuZnSOD activities are phenotypically normal for up to 9 months with no evidence of oxygen toxicity in room air (64). These MnSOD gene knock-out mice will be a powerful tool to define further the role of MnSOD in the host defense against oxygen toxicity.

Effect of Increasing MnSOD Activity. Tracheal insufflation of a single dose (e.g., 5 μg) of TNF or IL-1 attenuates O_2-induced pulmonary injury and prolongs the survival of rats exposed to 100% O_2 (57, 59). The TNF– and IL-1–induced protection against oxygen toxicity is associated with a selective enhancement of pulmonary MnSOD mRNA, specific protein, and enzyme activity at 2.3 days (55 hr) after O_2 exposure, when control rats start to die of oxygen toxicity (57–60). Immunohistochemistry reveals that the IL-1–induced enhancement of pulmonary MnSOD is manifested in most lung cells, particularly smooth muscle and endothelial cells (65). Protection of mice against oxygen toxicity by TNF is also associated with an induction of pulmonary MnSOD mRNA (66). TNF and IL-1 act synergistically in protecting rats against oxygen toxicity and in enhancing endothelial cell MnSOD, but not CuZnSOD, mRNA levels. Interleukin-6, while providing no protective effect in rats against oxygen toxicity and having no apparent effect on endothelial cell MnSOD mRNA levels, markedly enhances TNF– and IL-1–induced increases in endothelial cell MnSOD mRNA and in oxygen tolerance in rats (67). Similarly, D factor (differentiation inducing factor) and growth hormone enhance the TNF-induced increase in pulmonary MnSOD mRNA and also enhance TNF-induced oxygen tolerance (68).

As stated above, previous studies of LPS-induced protection against oxygen toxicity have focused on the role of CuZnSOD without actually measuring the activities of pulmonary CuZnSOD and MnSOD. Recent studies (45, 46, 69), however, have demonstrated that oxygen tolerance induced by LPS or its lipid component, diphosphoryl lipid A, is associated with a selective enhancement of pulmonary MnSOD mRNA and enzyme activity in O_2-exposed rats without affecting levels of pulmonary CuZnSOD and catalase mRNAs and enzyme activities. Since LPS induces endogenous production of TNF and IL-1, it is possible that the LPS-induced selective induction of pulmonary MnSOD and oxygen tolerance is mediated by these cytokines. However, evidence suggests that the LPS-induced oxygen tolerance is not mediated by endogenously produced cytokines TNF and IL-1 (70).

A number of investigators were unable to detect any changes in pulmonary antioxidant enzymes in LPS- or cytokine-induced oxygen tolerance. Hazinski *et al.* (71) reported that iv injection of LPS protected lambs against O_2-induced pulmonary injury and prolonged their survival. This LPS-induced protection against oxygen toxicity was not associated with any increase in pulmonary antioxidant enzymes when control animals were suffering from severe oxygen toxicity. Similar observations were made by White *et al.* (72, 73), who gave a combination of IL-1 and TNF to rats in split doses by ip and iv injections, and by Berg *et al.* (74), who gave sera containing high levels of TNF and IL-1, and some residual LPS from rats pretreated with LPS, to rats by iv injection. All these investigators have concluded that an increase of pulmonary antioxidant enzymes is not necessary for the protection against oxygen toxicity. However, none of these studies had measured pulmonary MnSOD activity; instead, only total lung SOD activities were measured. Because MnSOD constitutes a minor fraction of the total cellular SOD, it is possible that an enhancement of lung MnSOD activity may have been overlooked if one determines only total SOD activity. In fact, a subsequent study by Lewis-Molock *et al.* (75), which repeated the ex-

periment of White *et al.* (72) but measured pulmonary MnSOD mRNA, specific protein, and enzyme activity, revealed a marked increase in pulmonary MnSOD in TNF– and IL-1–treated and in oxygen-tolerant rats.

The mechanism by which LPS, cytokines, or hyperoxia enhances pulmonary MnSOD mRNA has been studied. Nuclear runoff transcription assay reveals that the increase in pulmonary MnSOD mRNA induced by IL-1 and a sublethal dose of hyperoxia (85% O_2) is associated with an increase in the rate of MnSOD mRNA synthesis (34, 76). Measurements of pulmonary MnSOD mRNA half-life reveal that LPS has no effect, while lethal dose of hyperoxia (100% O_2) increases the stability (half-life) of MnSOD mRNA (45).

The above results suggest that the induction of pulmonary MnSOD may be responsible, at least in part, for the LPS- and cytokine-induced oxygen tolerance. However, since LPS and cytokines have a diversity of biological activities, the protection against oxygen toxicity may be due to their effects other than the induction of pulmonary MnSOD.

Conflicting results have been obtained using transgenic mice overexpressing MnSOD gene. Wispe *et al.* (77) reported that transgenic mice overexpressing human MnSOD in Type II alveolar epithelial and nonciliated bronchiolar epithelial cells (using human surfactant protein-C, SP-C, promoter) conferred protection against oxygen toxicity. In contrast, Ho (56) was unable to demonstrate protection against oxygen toxicity in transgenic mice overexpressing MnSOD using the human β-actin promoter which caused an increased MnSOD expression in most lung cells including Types I and II alveolar epithelial cells, endothelial cells, and fibroblasts.

EC-SOD and Oxygen Toxicity

Considerable progress has been made in recent years in our understanding of the potential role of EC-SOD in oxygen toxicity. The homozygous EC-SOD gene knock-out mice with no detectable EC-SOD activity are apparently healthy for up to 14 months of age in air. However, when exposed to a lethal dose of O_2 these mutant mice develop severe lung injury earlier and have a shortened survival as compared to wild-type control mice (78). These results suggest that, at least in mice, EC-SOD plays an important role in the host defense against pulmonary oxygen toxicity. Whether these results are applicable to other species is not clear, since mice have the highest lung EC-SOD, constituting approximately 10% of the total lung SOD activity, while in rats, cats, and dogs it constitutes less than 1% of the total lung SOD activity (11).

Transgenic mice overexpressing EC-SOD have also been produced using human β-actin promoter. No increased EC-SOD was noted in the lung, liver, and spleen where baseline EC-SOD levels are high. However, significant increases in EC-SOD activity were noted in brain, heart, and muscle where baseline levels of the enzyme activity were low. Exposure of these transgenic mice to hyperbaric oxygen (100% O_2 at 6 atmospheric pressure for 25 min) paradoxically resulted in more brain toxicity as manifested by faster development of seizure and higher mortality as compared to nontransgenic litter mates. Since inhibition of $NO^\bullet$ synthase attenuated hyperbaric oxygen toxicity, it was suggested that $NO^\bullet$ is an important mediator of oxygen neurotoxicity and EC-SOD increases oxygen neurotoxicity by inhibiting O_2^--mediated scavenging of $NO^\bullet$ (79).

It is not clear whether transgenic mice overexpressing EC-SOD using SP-C promoter will result in an increase in lung EC-SOD activity and protect mice against pulmonary oxygen toxicity. Recombinant human EC-SOD has been produced (80). However, there is no report so far using iv injection of recombinant human EC-SOD to determine whether it can increase lung EC-SOD activity and protect against lung oxygen toxicity.

Conclusion and Directions for Future Studies

Oxygen is an important therapeutic modality for patients with severe hypoxemia. However, prolonged exposure to a high partial pressure of O_2 causes tissue injury which may exacerbate the existing lung pathology (81). At present there is no practical means of preventing oxygen toxicity. Considerable supportive, though not all conclusive, evidence suggests that all three forms of SOD (i.e., CuZnSOD, MnSOD and EC-SOD) are essential for the pulmonary defense against oxygen toxicity, and that enhancement of pulmonary SOD has the potential of protecting against oxygen toxicity. Directions for future investigation include the following: (i) The CuZnSOD and MnSOD gene knock-out mice should be used to conclusively demonstrate whether normal levels of the enzymes are essential for the host defense against oxygen toxicity. (ii) The MnSOD gene knock-out mice or *in vivo* lung gene transfer with antisense MnSOD gene to specifically block LPS- or cytokine-induced induction of pulmonary MnSOD should be used to determine whether LPS- or cytokine-induced protection against oxygen toxicity is in fact due to the induction of pulmonary MnSOD. (iii) Recombinant human EC-SOD, which has a long plasma and tissue half-life (82), should be used to determine whether it can enhance pulmonary SOD activity and protect against oxygen toxicity. (iv) Attempts should be made to develop agents that can selectively induce MnSOD mRNA and enzyme activity without the systemic toxicity of LPS or cytokines (TNF and IL-1). (iv) Studies should now focus on protecting against the toxicity of therapeutic (30%–40%), instead of lethal (>95%), doses of O_2. It is hoped that continued investigation will eventually lead to practical means of preventing oxygen toxicity.

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