Role of extracellular superoxide dismutase in bleomycin-induced pulmonary fibrosis

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Bowler, Russell P., Mike Nicks, Karrie Warnick, and James D. Crapo. Role of extracellular superoxide dismutase in bleomycin-induced pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 282: L719–L726, 2002. First published October 26, 2001; 10.1152/ajplung.00058.2001.—Bleomycin administration results in well-described intracellular oxidative stress that can lead to pulmonary fibrosis. The role of alveolar interstitial antioxidants in this model is unknown. Extracellular superoxide dismutase (EC-SOD) is the primary endogenous extracellular antioxidant enzyme and is abundant in the lung. We hypothesized that EC-SOD plays an important role in attenuating bleomycin-induced lung injury. Two weeks after intratracheal bleomycin administration, we found that wild-type mice induced a 106 ± 25% increase in lung EC-SOD. Immunohistochemical staining revealed that a large increase in EC-SOD occurred in injured lung. Using mice that overexpress EC-SOD specifically in the lung, we found a 53 ± 14% reduction in bleomycin-induced lung injury assessed histologically and a 17 ± 6% reduction in lung collagen content 2 wk after bleomycin administration. We conclude that EC-SOD plays an important role in reducing the magnitude of lung injury from extracellular free radicals after bleomycin administration.

antioxidant; transgenic mouse; immunohistochemistry

BLEOMYCIN ADMINISTRATION RESULTS in increased reactive oxygen species (ROS) that can cause severe organ injury. Pulmonary injury in response to systemic bleomycin treatment is thought to be prominent because of diminished bleomycin inactivating enzymes in the lungs (29). Bleomycin complexes with iron to generate ROS that damage proteins, lipids, and DNA (4, 18). Although intracellular ROS are prominent after bleomycin administration (17), extracellular ROS may also be important mediators of lung injury because bleomycin binds to plasma membranes (33) and is found in the extracellular space several hours after administration (9).

There are several lines of evidence that implicate superoxide as one of the ROS that mediate lung injury. First, bleomycin intercalates with DNA to cause strand breakage via oxygen free radicals (18). Second, cytosolic superoxide dismutase (SOD) can attenuate DNA breakage in vitro (14). Third, bleomycin induces cytosolic SOD activity in the lung (11, 15) and specifically in type II alveolar cells (19). However, additional lines of evidence implicate extracellular superoxide as another source of oxidant stress created by bleomycin. First, plasma levels are detectable several days after bleomycin administration (9). Second, bleomycin does not readily cross the plasma membrane but binds to cell surfaces before being endocytosed (8, 33). Third, bleomycin increases lung leukocytes that subsequently release high levels of extracellular superoxide (39). Fourth, parenterally administered cytosolic SOD, which essentially functions as an extracellular superoxide dismutase (EC-SOD), inhibits bleomycin-induced pulmonary fibrosis (20, 31, 43). Thus many in vitro studies directly implicate intracellular superoxide as mediating bleomycin-induced injury, but the evidence implicating extracellular superoxide is only indirect.

SOD enzymes are the primary defense against excess superoxide. Mammals have three SOD enzymes: in the cytosol, SOD1 (28); in mitochondria, SOD2 (42); and in the extracellular space, SOD3 (24). Of the three known mammalian SODs, only the intracellular SODs have been studied in bleomycin lung injury; however, EC-SOD’s unique extracellular location and its abundance in the lung suggest that it may play a major role in attenuating free radical injury in the lung after bleomycin administration.

In this present study, intratracheal bleomycin was used to test the hypothesis that bleomycin could induce lung EC-SOD, and immunohistochemistry was used to characterize the histological distribution of EC-SOD after bleomycin-induced lung injury. Furthermore, mice overexpressing human EC-SOD in the lung were used to test the hypothesis that high levels of EC-SOD could protect the lung from bleomycin-induced fibrosis.

EXPERIMENTAL PROCEDURES

Reagents. Enhanced chemiluminescence (ECL) +Plus Western blotting detection reagents were from Amersham Pharmacia Biotech (Piscataway, NJ). The protease inhibitors 3,4-dichloroisocoumarin, 1,10-phenanthroline (Boehringer Mannheim), and E-64 (ICN, Costa Mesa, CA) were kept in stock ethanol solution at −20°C. The Vectastain ABC Elite kit and biotinylated anti-rabbit IgG were from Vector Laboratories (Burlingame, CA). Bleomycin sulfate was from Mead

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Johnson Oncology Products. At a dose of 0.2 U/20 g mouse, this preparation was found to induce fibrosis without causing a significant mortality. All other reagents were from Sigma.

**Bleomycin administration.** C57/B16 mice overexpressing human EC-SOD using a surfactant protein C (SPC) promoter have been previously described (12). Bleomycin administration was performed on 4 separate days using multiple animals from each group. Transgene-positive (n = 58) or -negative (n = 56) littermates (average age 62 days) were anesthetized with halothane and then given intratracheal bleomycin sulfate 0.2 U/20 g. Control mice, either transgene negative (n = 14) or positive (n = 13), were given vehicle. Mice were weighed daily and given liberal access to food and water and then killed at the end of a fortnight. Lung volumes were calculated by displacement of water after being fixed for 30 min with 4% paraformaldehyde at 20 cmH2O pressure.

**Histology and immunohistochemistry.** Half of the mice had their lungs inflated for 30 min with 4% paraformaldehyde at 20 cmH2O pressure and embedded in paraffin. Multiple 4-μm sections were made of each sample. They were then stained in Hemo-D for 7 min, twice in 100% ethanol for 5 min, twice in 90% ethanol for 5 min, and then placed in distilled H2O for 5 min. One section of each sample was used for hematoxylin and eosin (H&E), Masson’s trichrome, or immunohistochemical staining. For immunohistochemistry, the samples were blocked overnight at 4°C in blocking buffer. The sections were incubated with 20% glycerol in H2O for 30 min and then treated twice in Hemo-D for 7 min, twice in 100% ethanol for 5 min, and then placed in distilled H2O for 5 min. The sections were washed in high-salt buffer and then treated with 20% glycerol in H2O for 30 min and washed with blocking buffer. The sections were incubated with 1/100 affinity purified rabbit polyclonal anti-EC-SOD IgG or 1/2,000 rabbit IgG in blocking buffer for 3 h at room temperature. The sections were washed in high-salt buffer (25 mM Tris pH 7.5, 500 mM NaCl, 1% Tween 20, 10% fish gelatin, and 0.05% NaN3). The samples were then incubated with 20% glycerol in H2O for 30 min and washed with blocking buffer. The sections were incubated with 1/100 affinity purified rabbit polyclonal anti-EC-SOD IgG or 1/2,000 rabbit IgG in blocking buffer for 3 h at room temperature. The sections were washed in high-salt buffer (25 mM Tris pH 7.5, 500 mM NaCl, and 1% Tween 20) three times for 5 min. A biotinylated anti-rabbit IgG, at 1/500 in blocking buffer, was placed on the sections for 30 min at room temperature. The sections were washed one time with high-salt buffer then treated for 3–5 min in 3% H2O2 in water. The samples were then washed two times for 5 min in high-salt buffer without detergent. Vectastain ABC Elite and diaminobenzidine (Vector Laboratories) kit were used as described in the kit instructions. The sections were then stained for 3 min with 1% methyl green and washed with distilled H2O. The sections were then dehydrated twice in 90% ethanol for 5 min, twice in 100% ethanol for 7 min, and twice in Hemo-D for 7 min.

**Hematoxylin and eosin staining.** Hematoxylin and eosin staining was performed according to standard histologic methods. Briefly, each sample was fixed in 10% formaldehyde, dehydrated in a series of alcohol, and clear with xylene. The samples were then embedded in paraffin and sectioned at 4 μm. Specimens were subsequently deparaffinized and stained with hematoxylin and eosin. The slides were then mounted and examined under a microscope.

**Immunohistochemistry.** Immunohistochemistry was performed according to standard methods. Briefly, each sample was fixed in 10% formaldehyde, dehydrated in a series of alcohol, and cleared with xylene. The samples were then embedded in paraffin and sectioned at 4 μm. Specimens were subsequently deparaffinized and stained with hematoxylin and eosin. The slides were then mounted and examined under a microscope.

**Quantification of EC-SOD mRNA.** Mouse lung total RNA was isolated using an RNeasy MIDI kit (Qiagen) and then quantitated using a SmartSpec 3000 (Bio-Rad). A Cepheid SmartCycler was used with the following protocol: RETROscript kit (Ambion, Austin, Texas) was used to generate RT product using the manufacturer’s protocol with a hot start; for mouse (mEC-SOD) PCR, 2 μl of RT product was added to 20 μl of water, 1 μl of EC-SOD forward primer (50 μM TTGTTC-TACGGCTTGCTACTGGC), and 1 μl of reverse primer (50 μM ATTCGATGCTTCGGCAGGC), 1 μl of 0.05× SYBR green (Molecular Probes, Eugene, OR), and one Ready-To-Go PCR bead (Amersham Pharmacia Biotech); the resulting mix was brought to 94°C for 300 s and then cycled 50 times from 94°C to 30 s to 66°C for 30 s. For 18S PCR, an identical protocol was used except that primers were from QuantRNA 18S internal standards (Ambion) and the 50 cycles were 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The ratio of EC-SOD mRNA relative to 18S RNA internal standards was determined using Cepheid SmartCycler software (version 1.01). Total lung 18S RNA was determined using 1% of total RNA for agarose gel electrophoresis followed by ethidium bromide staining and then quantitation (Hitachi Genetic Systems). Total lung EC-SOD mRNA was determined by multiplying total 18S RNA by the ratio of EC-SOD mRNA to 18S RNA.

**Collagen assay.** A complete description of this assay can be found in Lopez-De Leon and Rojkind (22). In brief, paraffin-embedded sections were soaked in Hemo-D to remove the paraffin. Samples were then placed in a (1:1) Hemo-D:ethanol mixture, then boiled, then distilled water followed by 0.5 ml of sirius red and fast green FCF mixture in 0.1% picric acid. After 30 min, the solution was pulled off, and the samples washed three times in distilled water. Next, 0.25 ml of NaOH 0.1 N and 0.25 ml of methanol were added for one min. Samples were aspirated and absorbance measured at 540 and 605 nm using a 96-well plate reader. The amount of noncollagenous protein in milligram per sample was calculated by measuring the absorbance of the eluate at 605 nm divided by 2.22 (the lung fast green FCF color equivalence). The amount of collagenous protein was calculated by measuring the absorbance of the eluate at 540 nm, subtracting 29% of the absorbance at 605 nm, and then dividing by 36.3 (the sirius red color equivalence). The total amount of collagen per lung was calculated by multiplying the total protein of the lung with the ratio of collagenous protein to total protein.

**Statistical analysis.** All mice came from the same C57/B16 background. Wild-type animals were littermates of EC-SOD transgene-positive animals. A one-way analysis of variance (ANOVA) was used to determine if the means were significantly different (P < 0.05). If means were significantly different, a Tukey-Kramer multiple group comparison test was used to compare individual groups. Standard error was indicated for each value by a bar, and significance listed for each comparison. A chi²-test was used to calculate P values for ratios. All values were calculated using GraphPad Prism version 3.00 for Macintosh (GraphPad Software, San Diego, CA).

**RESULTS**

**Physiological changes in the lung after bleomycin treatment.** Overall mortality was 4/141 (3%). The mortality for the bleomycin-treated mice was 2/56 (3%) for...
wild types and 2/58 (3%) for the EC-SOD transgens. For the saline-treated mice, mortality was 0/14 (0%) in the wild types and 0/13 (0%) in the EC-SOD transgens. Although bleomycin-treated wild-type mice lost 17.7 ± 2.6% of their body weight and the saline-treated wild-type mice gained 8.1 ± 1.4% of their body weight (P < 0.001 for saline vs. bleomycin treated), overexpression of EC-SOD did not significantly attenuate this weight loss [17.6 ± 3.9% decrease in wild-type vs. 18.3 ± 3.2% decrease in EC-SOD transgenic mice, P = not significant (NS); see Fig. 1].

Lung fibrosis is characterized by restrictive physiology. After bleomycin-induced fibrosis, lung volumes decreased by 0.19 ± 0.04 ml in wild-type mice. The EC-SOD transgenic mice had less loss of lung volume (0.10 ± 0.08 ml), although this did not reach statistical significance. Because the most significant physiological abnormality of lung fibrosis is reduced lung volume, our results suggest that there may be a trend toward improved lung physiology in the bleomycin-treated EC-SOD transgenic mice compared with controls.

The lung wet/dry ratio was used to assess lung edema (Fig. 1). This ratio was significantly higher after bleomycin treatment in both the wild-type (4.3 ± 0.1 in the saline-treated mice vs. 6.2 ± 0.6 in the bleomycin-treated wild-type mice) and EC-SOD transgenic mouse lung (4.5 ± 0.2 in the saline-treated vs. 5.4 ± 0.2 in the bleomycin-treated). EC-SOD transgene expression led to a 52% reduction in the increase in lung edema as measured by the wet/dry ratio. Thus overexpression of EC-SOD in the lungs led to reduced lung edema but was not sufficient to cause a decrease in mortality or airway function.

EC-SOD increases after bleomycin treatment. Because bleomycin accumulates in the lung and EC-SOD is found in abundance in lung, we hypothesized that the oxidative stress from bleomycin would induce EC-SOD. To test this hypothesis, we measured both EC-SOD protein (Fig. 2A) and mRNA (Fig. 2B). After bleomycin administration, total lung mouse EC-SOD protein increased after bleomycin. At the end of 2 wk there was a 109 ± 25% increase in total lung mouse EC-SOD protein. (*P < 0.05 compared with wild-type day 0; †P < 0.05 compared with transgenic day 0.) B: total lung mouse EC-SOD mRNA increased, but the increase came later than with the protein. At the end of 2 wk, there was a 99 ± 45% increase in total lung mouse EC-SOD mRNA. (*P < 0.05 compared combined wild-type transgenic mice at day 14 compared with day 0.)

Prior work by our laboratory has revealed the EC-SOD is secreted in two different forms: one that is proteolytically processed and one that is not (10). The intracellular processing removes the carboxy terminus and decreases EC-SOD’s affinity toward the extracellular matrix. To investigate whether bleomycin would...
change intracellular proteolytic processing, we used Western blotting to quantitate the ratio of EC-SOD that had been proteolytically processed to the EC-SOD that had not been proteolytically processed. Two weeks after bleomycin, the percentage of lung collagen deposition in the wild-type lung (saline-treated 121 ± 33 μg vs. bleomycin-treated 277 ± 18 μg; \( P < 0.001 \); Fig. 3). Overexpression of EC-SOD still resulted in lung collagen accumulation (saline-treated EC-SOD transgenic mice 141 ± 17 μg vs. bleomycin-treated EC-SOD transgenic mice 228 ± 18 μg, \( P < 0.001 \); Fig. 3). Overexpression of EC-SOD still resulted in lung collagen accumulation (saline-treated EC-SOD transgenic mice 141 ± 17 μg vs. bleomycin-treated EC-SOD transgenic mice 228 ± 18 μg, \( P < 0.001 \); however, the lungs from bleomycin-treated EC-SOD transgenic mice had less collagen than the wild-type (\( P < 0.05 \)). After bleomycin treatment, the EC-SOD transgenic mouse lungs also had a trend toward less total lung weight (EC-SOD transgenic 370 ± 21 mg vs. wild-type 399 ± 30 mg) and less collagen per protein (EC-SOD transgenic 35 ± 3 μg collagen/ mg protein vs. wild-type 41 ± 1.5 μg collagen/mg protein); however, these differences were not statistically significant. Thus after bleomycin treatment, EC-SOD-overexpressing mice had reduced levels of collagen that could be explained only partially by increases in total lung protein.

Effects of overexpression of EC-SOD on histological injury. To further elucidate the differences in collagen content between the EC-SOD transgenic and wild-type mouse lung, we performed histological evaluation (Fig. 4). Histological injury was noted in both the wild-type and EC-SOD transgenic mice treated with bleomycin. There was no injury noted in the control mice. Injury consisted of patchy to confluent fibrosis with a leukocytic cellular infiltration. Severe honeycombing was noted in two specimens (one EC-SOD transgenic and one wild type). We could not discern histological differences between the fibrotic foci of the EC-SOD transgenic and wild-type mouse lungs; however, the injured foci appeared to be smaller in the EC-SOD transgenic lungs compared with the wild-type lungs. To quantitate this difference, we used a grid to quantitate the amount of injury of a histological specimen from each animal.

Two weeks after bleomycin, the percentage of lung with fibrosis was higher in the wild-type mice (21.8 ± 2.3%) compared with the EC-SOD transgenic mice (10.2 ± 1.8%; \( P < 0.01 \); Fig. 5). Two investigators achieved similar scores independently (wild type: 21.8 ± 2.5% and 21.9 ± 1.7%; EC-SOD transgene positive 10.5 ± 2.3% and 9.8 ± 1.4%). Thus EC-SOD overexpression attenuated the severity of bleomycin-induced lung injury by reducing the size of the fibrotic foci.

Immunohistochemistry of EC-SOD after bleomycin-induced lung fibrosis. To investigate the distribution of EC-SOD in the lung after bleomycin treatment, we used immunohistochemical staining with polyclonal antibodies raised against EC-SOD. In our negative controls, we found that neither IgG (Fig. 6A) nor antibody preadsorbed to EC-SOD (not shown) had significant staining. Using an EC-SOD specific antibody, we...
found EC-SOD along alveolar membranes, along large airways, and surrounding blood vessels (Fig. 6B). In the EC-SOD transgenic mice, there was abundant staining associated with type II alveolar cells (Fig. 6E) and bronchial epithelial cells (not shown). In most tissue sections there was an occasional alveolar macrophage that stained positive (Fig. 6E). This staining was consistent with that previously reported by Folz et al. (12) and consistent with the activity of the SPC promoter (6). After bleomycin treatment, both the wild-type and EC-SOD transgenic mice developed patchy fibrosis (Fig. 6, D and F). Within these areas of fibrosis, staining for EC-SOD appeared more intense and occurred in foci that also stained positively for collagen with sirius red. Although the fibrotic foci appeared smaller in the EC-SOD transgenic mice, the staining within these foci was more intense.

DISCUSSION

Bleomycin is a commonly used chemotherapeutic agent that has severe pulmonary side effects. Although bleomycin is a well-known cause of intracellular oxidative stress, several findings in this study suggest that extracellular oxidative stress may also play a role in the pathogenesis of bleomycin-induced lung injury. First, pulmonary overexpression of EC-SOD attenuated both collagen accumulation and histological injury in the lung after bleomycin administration. Second, bleomycin induced pulmonary EC-SOD protein synthesis and accumulation in the lung.

Other investigators have reported that total lung SOD activity increases in rats (11, 19) and rabbits (27) after bleomycin administration, but this is the first study to specifically report changes in EC-SOD. An interesting feature of the rise in lung EC-SOD is that the protein increased 1 wk before the mRNA. Several possible explanations could account for this. First, EC-SOD half-life might increase in the first few days after bleomycin. Second, the efficiency of EC-SOD mRNA translation might be increased. Third, EC-SOD may come from nonpulmonary sources. Although the source of the increase in lung EC-SOD protein is not known, there was prominent EC-SOD immunostaining in lung macrophages and neutrophils, suggesting that the increase may result from the influx of inflammatory cells. Loenders et al. (21) have also reported immunostaining for EC-SOD in inflammatory cells. Later increases in lung EC-SOD may be sustained by increased lung synthesis, as suggested by the late rise in EC-SOD mRNA.

The mediators that induce EC-SOD gene transcription after bleomycin are unknown. Although the mouse EC-SOD promoter has not been described, the human EC-SOD promoter contains two putative antioxidant response elements (13). The complete molecular mechanism of signal transduction by ROS is unknown but is currently under study in our laboratory. Although bleomycin induces a burst of ROS, this occurs immediately after administration. Thus the late increase in EC-SOD gene transcription is probably secondary to other mediators. The cytokines that are induced by bleomycin may be better candidates for regulators of EC-SOD gene transcription (32, 36). Several investigators have found that inflammatory cytokines such as interferon-γ (2) and interleukin-1 (25) can induce EC-SOD in rat and human cell culture lines. One pathway common to both of these pathways is nuclear factor-xB (2). Nuclear factor-xB is also a putative regulatory element in the human EC-SOD gene (13). Thus early increases in EC-SOD may be a result of an influx of inflammatory cells that carry EC-SOD protein, yet, later increases may be secondary to the effects of the cytokine milieu on EC-SOD gene transcription. However, in this model, bleomycin was administered as a single dose; thus the level of lung EC-SOD before bleomycin administration may be more important than any subsequent induction of native mouse EC-SOD protein.

The difference in expression between the endogenous EC-SOD and the transgene EC-SOD is not surprising since the genes are under control of different promoters. The EC-SOD transgene utilized the human SPC promoter and not the mouse EC-SOD promoter. Our findings that bleomycin does not increase the EC-SOD transgenic protein are consistent with previous studies, which report that the SPC promoter is activated only in a sporadic manner after bleomycin injury (6, 7). Additionally, the cytokines that increase EC-SOD in cell culture have not been found to increase SPC message in whole lung preparations (5, 34).

There are several mechanisms by which EC-SOD may protect the lung from injury. Bleomycin administration generates free radicals that injure DNA (26, 40), lipids (15), and proteins (44). Superoxide is one of these free radical mediators and the lung is a major target organ of bleomycin injury. EC-SOD is abundant in lung tissue (24) and could directly attenuate lung injury by reducing superoxide concentrations in the extracellular space. Reduction of superoxide in the extracellular space might have several consequences, including decreased stimulation of fibroblasts and diminished inflammatory cell recruitment. For instance,
bleomycin-induced superoxide production has been shown to stimulate fibroblasts to proliferate and differentiate into myofibroblasts, resulting in a histological appearance that is similar to idiopathic pulmonary fibrosis (41). Superoxide stimulates fibroblasts to secrete collagen (3) and mediates expression of selectins on endothelium, resulting in enhanced recruitment of leukocytes to the lung (1). Thus EC-SOD may protect both directly as an antioxidant and indirectly by blunting the initial inflammation that is induced during bleomycin administration.

The attenuation of bleomycin-induced lung injury with EC-SOD transgene overexpression was similar to that reported by Tamagawa et al. (38) with intravenous lecithinized SOD1. With a dose of either 1 or 10 mg·kg⁻¹·day⁻¹, these investigators found an 11% reduction in lung weight, 16% reduction in lung collagen, and 41% reduction in grade of fibrosis. Other investigators have found similar reductions in lung edema, collagen deposition, and fibrosis score after administration of high levels of SOD2 (31) and other ROS scavengers (30). Although these investigators did not report how much the injections increased lung SOD activity, it is likely that their beneficial effect was secondary to enhanced intravascular SOD activity, since all of these treatments were given parenterally. In the EC-SOD transgenic mice, EC-SOD overexpression occurred primarily in alveolar type II cells and bronchial epithelial cells (12), suggesting that both the airway and alveolar epithelium may be important in defending against free radical injury after bleomycin. Furthermore, it is unlikely that the reduced lung injury in the EC-SOD transgenic mice is due to differences other than an increase in EC-SOD, since cytosolic SOD, mitochondrial SOD, glutathione peroxidase, glutathione, and catalase have been reported to be identical in the lungs of wild-type and EC-SOD transgenic mice (12). Although the protection afforded by EC-SOD overexpression was modest and incomplete, this is the first report to suggest that alveolar and airway extracellular antioxidant enzymes can protect the lung from bleomycin-induced lung fibrosis. This study was not statistically powered to detect differences in secondary outcomes such as weight loss and mortality. The lack of statistically significant improvement in these multifactorial outcomes confirms that mediators besides extracellular superoxide play a large role in the pathogenesis of bleomycin-induced lung injury.

There are several compelling reasons why extracellular antioxidants might be beneficial in clinical treatment or prevention of pulmonary fibrosis. First, patients with pulmonary fibrosis have reduced epithelial lining fluid antioxidant capacity and elevated markers of oxidative stress (35). Second, administration of aerosolized glutathione has been shown to attenuate bleomycin-induced lung fibrosis (16, 23). Third, alveolar inflammatory cells from patients with pulmonary fi-
fibrosis produce excess extracellular superoxide (37). Thus targeting the extracellular space with mimics of EC-SOD may be a potentially beneficial intervention in patients suffering from pulmonary fibrosis or undergoing bleomycin chemotherapy.

In conclusion, bleomycin administration induced EC-SOD in the lung and overexpression of EC-SOD attenuated bleomycin-induced fibrosis. The only difference in the lungs that could explain the decreased fibrosis was a higher level of EC-SOD in the EC-SOD transgenic lungs [0.5 μg EC-SOD/mg lung protein in the wild-type vs. 1.5 μg EC-SOD/mg lung protein in the EC-SOD transgenic mice (12)]. Thus microgram amounts of extracellular SOD activity appear to be biologically effective at reducing bleomycin-induced lung fibrosis. This suggests that small increases in pulmonary extracellular SOD activity, by either pretreatment with EC-SOD or an extracellular SOD mimic, may be of potential therapeutic benefit in reducing bleomycin-induced lung injury.

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