Effect of superoxide dismutase on a rabbit model of chronic allergic asthma

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Background: In bronchial asthma, inflammatory cells infiltrating the airway mucosa release oxygen radicals that cause tissue damage and amplify the airway inflammation. Antioxidant enzymes may have a protective effect on the airways.

Objective: The purpose of this study was to determine whether treatment of a rabbit model of chronic allergic asthma with the antioxidant enzyme superoxide dismutase conjugated to polyethylene glycol will protect the airways from oxygen radical injury, decrease airway inflammation, and attenuate the asthmatic response.

Methods: New Zealand white rabbits were sensitized to ragweed. Baseline histamine PC30, ragweed PD30, and early and late phase asthmatic response to ragweed bronchial challenge were measured. The rabbits were then randomized into two groups that received every 48 hours an intravenous dose of either superoxide dismutase-polyethylene glycol 10,000 U/kg or inactivated superoxide dismutase-polyethylene glycol as control, followed by a 1-hour exposure to aerosolized ragweed extract. After 4 weeks the rabbits had a second bronchial challenge, were sacrificed, and lung histology was studied.

Results: On the posttreatment challenge, the superoxide dismutase-polyethylene glycol group had a rise in ragweed PD30, while the control group had no change in ragweed PD30, and two of five rabbits in the superoxide dismutase-polyethylene glycol group did not have an early or late phase asthmatic response, while all rabbits in the control group had an asthmatic response. There was no significant difference in lung histology between both groups.

Conclusion: A rabbit model of chronic allergic asthma treated with superoxide dismutase-polyethylene glycol showed a trend of improvement in airway responsiveness but no significant effect on airway inflammation.

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INTRODUCTION

Current theories of asthma pathogenesis implicate airway mucosal inflammation as the primary process underlying the clinical manifestations of asthma. The bronchial mucosa in allergic asthma is infiltrated with activated eosinophils,[1] neutrophils, and macrophages.[2] The activated inflammatory cells release oxygen radicals during their oxidative bursts. Oxygen radicals are highly chemically reactive species that induce bronchoconstriction, increase mucous secretion, and cause microvascular leakage leading to edema formation. The antioxidant enzymes, superoxide dismutase, catalase, and glutathione-peroxidase inactivate these oxygen radicals.[3]

Superoxide dismutase, which catalyzes the first reaction in the removal of oxygen radicals, was studied. In chronic allergic asthma, the inflammatory cells infiltrating the airways release oxygen radicals that produce significant tissue damage and amplify the inflammatory response. This manifests clinically as an asthmatic response to antigen challenge which can be measured by a decline in pulmonary functions. Histopathologically, it manifests as an increase in the cellular infiltrate seen on examination of the asthmatic airways. The hypothesis of the study is that repeated treatment of rabbits with chronic allergic asthma with an antioxidant enzyme will ameliorate the inflammatory injury in the airways and will result in an attenuation of the asthmatic response to antigen challenge. This effect can be measured clinically by an improvement in pulmonary functions and histopathologically by a decrease in the inflammatory mucosal damage on lung histology.

A rabbit model of allergic asthma, chronically exposed to the sensitizing allergen, was used to simulate the natural repeated exposures of asthmatics to their sensitizing antigens. The rabbits were treated with repeated doses of superoxide dismutase conjugated with polyethylene glycol, to maintain a steady state level of the enzyme in the airways. The asthmatic response and the histopathology of the superoxide dismutase-polyethylene glycol-treated rabbits were compared with a control group given inactivated superoxide dismutase-polyethylene glycol.

MATERIALS AND METHODS

Sensitization

New Zealand white rabbits were chosen for this study because this strain of rabbit can be sensitized to allergens and induced to develop a bronchospastic response when rechallenged to the sensitizing antigen. Adult rabbits were sensitized to ragweed according to the method described by Metzger.[4] The rabbits were pretreated with 75 mg/kg intraperitoneal injection of a cyclo-

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phosphamide solution prepared by the addition of 1:1:2 parts by volume of ethyl alcohol, normal saline, and cyclophosphamide 100 mg/mL. Two days, 2 weeks, and 6 weeks after the cyclophosphamide dose, the rabbits were injected intraperitoneally with 0.25 mL of a solution of 0.11 allergen units (AU) of ragweed antigen E/mL (Greer Laboratories, Inc., Lenoir, NC), in 5% kaolin/saline. Booster intraperitoneal injections of ragweed antigen E were given every 4 weeks until the rabbits received their first bronchial challenge.

Preparation for Challenges

The rabbits were sedated and anesthetized by the intramuscular injection of 1 mL of xylazine, 20 mg/mL and 1 mL of ketamine hydrochloride, USP, 100 mg/mL. They were then intubated with a cuffed endotracheal tube. The endotracheal tube was connected to an A Fleisch pneumotachograph (Gould Medical Products Division, Oxnard, CA) which was connected to a differential pressure transducer (model DP 45-28, Validyne Engineering Corp., Northridge, CA). The flow signal was integrated to volume. An esophageal catheter was positioned in the lower third of the esophagus so that the change in esophageal pressure equaled the change in airway pressure when the animal breathed against an occluded airway. This validated the esophageal pressure measurement as a reflection of intrapleural pressure. The airway pressure and esophageal pressures were measured using Gould/Statham pressure transducers (Gould Medical Products Division, Oxnard, CA). Proximal airway pressure, esophageal pressure, flow and volume signals were sent through transducer amplifiers, and printed on a 4-channel recorder (Gould 2400 series, Gould Electronics, Centerville, OH).

Lung compliance was calculated as follows: compliance = $\Delta V/\Delta P$ where V = tidal volume; P = esophageal pressure. For each pulmonary function measurement, three consecutive breaths were measured and the mean value for compliance was calculated.

Throughout the challenges, the rabbits were placed in a restrainer and were kept mildly sedated with injections of xylazine, 20 mg/mL, 0.4 mL intramuscularly, every two hours as needed for comfort.

Aerosols were generated using a Pulmoaide air compressor with a jet nebulizer (Devilbiss 646, Devilbiss, Summerset, PA) and were nebulized through the endotracheal tube.

Pretreatment Challenge

Baseline measurements. Compliance was measured at baseline and immediately following two minutes of inhalation of a normal saline aerosol. The measurements taken after the normal saline aerosol were used as the baseline against which the changes in compliance were compared after the histamine challenge.

Histamine challenge. The rabbits were given an inhalation of solutions of histamine diphosphate (Spectrum Chemical, Gardenia, CA) at concentrations of: 0.3, 0.6, 1.25, 2.5, 5, 10, and 20 mg/mL normal saline nebulized for two minutes. Doses were given at 5-minute intervals until all the doses were given or until a dose caused an immediate drop of 30% in compliance from the postsaline baseline. The concentration resulting in a 30% drop in compliance was recorded as the histamine provocative concentration 30 (PC30). The rabbits that reacted to histamine were allowed to recover. Recovery was marked by a return of compliance to the postsaline baseline or higher. If complete recovery did not occur within an hour, the rabbits were given an intravenous injection of diphenhydramine (50 mg/mL) 0.3 mL. The compliance measured after recovery from the histamine challenge was used as the baseline measurement to which the ragweed challenge was compared.

Ragweed challenge. The rabbits inhaled an aerosolized solution of ragweed antigen E, 20 AU/mL. The nebulization was given in incremental durations as follows: dose 1 given for 30 seconds; doses 2, 3, and 4 for two minutes, and dose 5 for four minutes. Compliance was measured immediately and at five and ten minutes after each dose. When a dose of ragweed caused a 30% or more drop of compliance from the posthistamine baseline, the challenge was considered positive. The cumulative minutes of ragweed nebulization up to the dose that caused the 30% drop in compliance was recorded as the ragweed provocative dose 30 (PD30). Compliance was then measured every 15 minutes for the first hour to measure the early phase asthmatic response, and every 30 minutes for five hours to measure the late phase asthmatic response. Rabbits which received the entire 10.5 minutes of ragweed inhalation without having a 30% drop of compliance from baseline during the entire six hours were considered to have a negative ragweed challenge and were not studied further.

Recovery period and chronic ragweed exposure. Following a positive ragweed challenge, methylprednisolone, USP 40 mg/mL, 0.3 mL, and diphenhydramine 50 mg/mL, 0.3 mL intramuscular, and a nebulization of epinephrine 1:1000 solution 0.25 mL in 2 mL normal saline were given. The rabbits were allowed to recover for 3 weeks. Ragweed exposure was then achieved by inhalation of aerosolized ragweed extract, 15 AU ragweed antigen E in 12 mL of normal saline over a 1-hour period, every 48 hours for 4 weeks.

Preparation of superoxide dismutase-polyethylene glycol and inactivated superoxide dismutase-polyethylene glycol. Bovine erythrocyte copper/zinc superoxide dismutase, activity 3.33×10^5 U/mL (DDI Pharmaceuticals, Inc, Mountain View, CA), and activated polyethylene glycol, 5000 Daltons (Sigma Chemical Company, St Louis, MO) were covalently conjugated by the method described by Pyatak et al.[5] The activity of the conjugated enzyme was 62,210 U/mL.

Inactive superoxide dismutase-polyethylene glycol was prepared by overnight incubation of the active compound in phosphate buffered saline containing 5 mM diethyldithiocarbamate, adjusted to a pH of 3.8.[6] The diethyldithiocar-

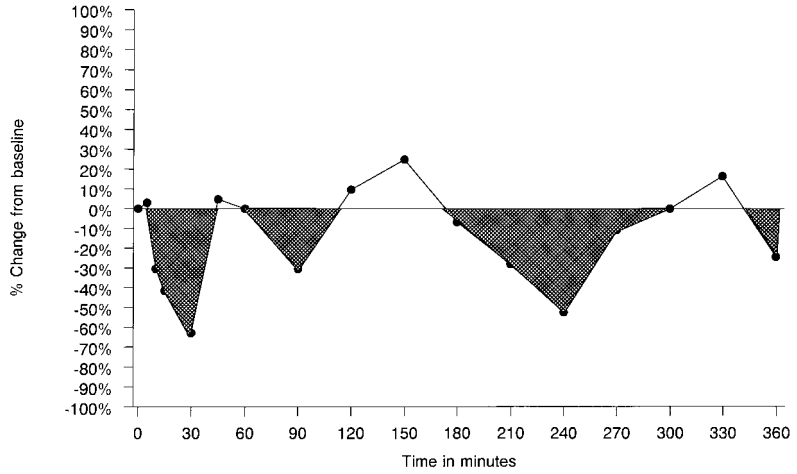

Figure 1. The shaded area is the area measured by the calculation of the area under the curve. The area is the product of the time plotted on the x-axis and the percent change from baseline plotted on the y-axis. The data from the posttreatment challenge of rabbit number 2 are used here for an example.

bamate was then dialyzed out of the solution against a physiologic buffer. The solution was assayed to ensure there was no residual activity and that the pH was at 7.8. The active superoxide dismutase-polyethylene glycol and inactive superoxide dismutase-polyethylene glycol were packaged in two identical sets of vials, labeled "A" and "B," to keep the study investigators blinded, and both were labeled with the potency of the active compound.

Superoxide dismutase-polyethylene glycol treatment. The rabbits were randomized into two groups: a group of five rabbits was treated with superoxide dismutase-polyethylene glycol and a group of six rabbits was given inactive superoxide dismutase-polyethylene glycol as control.

In a previous study (unpublished) we observed that the intravenous injection of 10,000 U/kg of superoxide dismutase-polyethylene glycol resulted in a 3.3-fold increase from baseline in the activity of superoxide dismutase in the lung perfusate at two hours. The activity was 2.6-fold the baseline at 24 hours, 1.9 at 48 hours, and 1.4 at 72 hours. The active treatment group of rabbits were injected intravenously with a volume of active superoxide dismutase-polyethylene glycol calculated at 10,000 U/kg, while the control group were injected with an equal volume of inactive superoxide dismutase-polyethylene glycol. The doses were given every 48 hours for the 4-week period during which they underwent the chronic ragweed exposure. All investigators were blinded to the treatment groups.

Post-treatment bronchial challenges. At the end of the 4-week period, each rabbit underwent a second histamine and ragweed challenge as above. This second challenge was done 24 hours after the rabbits received their last dose of either superoxide dismutase-polyethylene glycol or inactive superoxide dismutase polyethylene glycol.

Lung histology. After the challenges were completed, the rabbits were sacrificed by intravenous injection of one mL of sodium pentobarbital, 300 mg/mL (Socumb, Butler).

Bronchoalveolar lavage fluid (BAL) was collected for examination of the cellular composition. The cell density was scored from 1 to 5 based on the approximate number of cells in the preparation, with 1 = very few cells, 3 = moderate number of cells, and 5 = cells clumping together with no separation between cells. On each preparation, 100 cells were counted to determine the percent of eosinophils, macrophages, polymorphonuclear leukocytes, and epithelial cells. The lungs were removed, and one lung was immersed in zinc formol and processed for histologic examination. The pathologist examined the lung histology. The following score was devised to objectively assess the important aspects of lung pathology in asthma: alveolar edema: absent = 0, patchy = 1, confluent = 2; atelectasis: absent = 0, mild = 1, moderate = 2, severe = 3; inflammatory cell infiltrate: absent = 0, mild = 1, moderate = 2, severe = 3; integrity of the bronchial epithelium: intact = 0, denuded = 1. A mean pathology score was assigned for each slide by calculating the mean of the above scores. For each rabbit, one slide specimen, sectioned so as to include bronchi from the hilum to the periphery, was examined by morphometries.

Table 1. Histamine PC30 and Ragweed PD30 Measured During the Pretreatment and Posttreatment Challenges in the Inactivated Superoxide Dismutase-Polyethylene Glycol (InSOD-PEG) and Superoxide Dismutase-Polyethylene Glycol (SOD-PEG) Groups

Challenge	InSOD-PEG Group		SOD-PEG Group	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
Histamine PC30mean mg/mL ± SEM	3.22 ± 1.39	2.18 ± 0.64	7.2* ± 1.16	2.5* ± 0.68
Ragweed PD30mean minutes ± SEM	5.16 ± 1.83	4.5 ± 1.26	4.1 ± 1.72	8.1 ± 1.46

* The difference in the histamine PC30 during the pretreatment and the posttreatment challenges in the SOD-PEG group was statistically significant ($P = .012$).

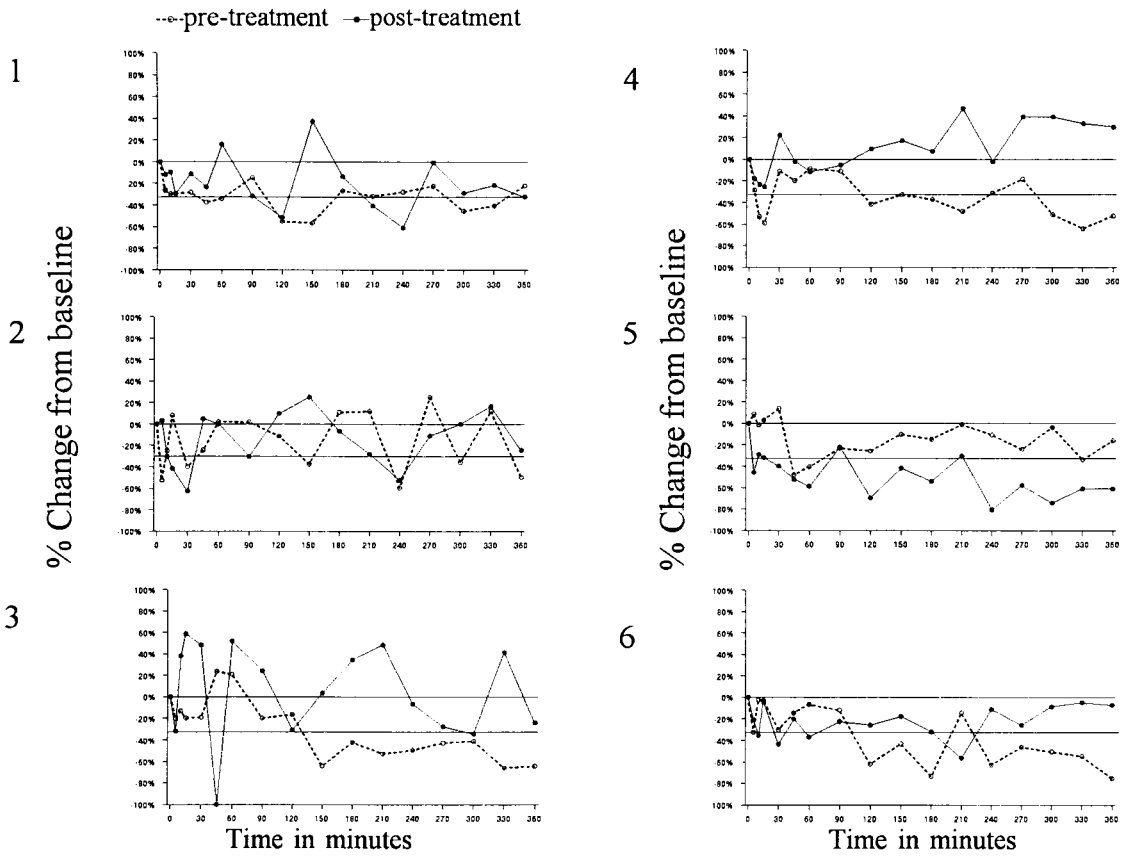

Figure 2. Dynamic compliance shown as percent change from baseline after the pretreatment (–○–), and the posttreatment (—●—) challenge in the rabbits treated with inactivate superoxide dismutase-polyethylene glycol for control.

The internal diameter, and the thickness of the muscularis mucosa of all the bronchi cut in cross section on the specimen were measured.

Assay of superoxide dismutase activity in lung homogenate. Superoxide dismutase activity was assayed on lung tissue that was removed after sacrificing the animal. The lung tissue was rinsed with normal saline, frozen to −70 °C, and later homogenized for assay of superoxide dismutase levels. The assay was done using the nitroblue-tetrazolium assay as described by Spitz and Oberley.[7]

Data analysis. For each rabbit, a curve was constructed by plotting the time points from zero to six hours after the ragweed challenge on the x-axis and the % decrease from baseline measurements of compliance on the y-axis. The area under the curve for compliance was calculated by using the computer program PC!INFO version 2.0 (Retriever Data System, Seattle, WA). The area measured is the product of time plotted on the x-axis and the % change from baseline plotted on the y-axis (Fig 1). The area under the curve and the PC 30% for histamine and ragweed were compared between the two groups, and within each group for the pretreatment and posttreatment challenges. The mean histology score and the mean of the ratio of the muscularis mucosa to the internal diameter of the bronchi were compared between the two groups. The data were analyzed using two-tailed Student's *t* test.

RESULTS

Sensitization

Fifty-two rabbits were sensitized. Thirty-two rabbits expired during the sensitization procedure. Of the remaining 20 rabbits, three had a negative baseline bronchial challenge to ragweed and were therefore not included in the study. Three rabbits expired immediately after a positive baseline bronchial challenge and two more expired during the chronic ragweed exposure. One rabbit expired during the second bronchial challenge. Data for 11 rabbits were available for analysis.

Histamine and Ragweed PC30

At the pretreatment challenge, the histamine PC30 was higher in the group later randomized to the superoxide dismutase-polyethylene glycol than in the group later randomized to the control group, 7.2 ± 1.16 mg/mL versus 3.22 ± 1.39 mg/mL, but the difference was not statistically significant (P = .055). The histamine PC30 decreased during the posttreatment challenge for both groups, indicating that both

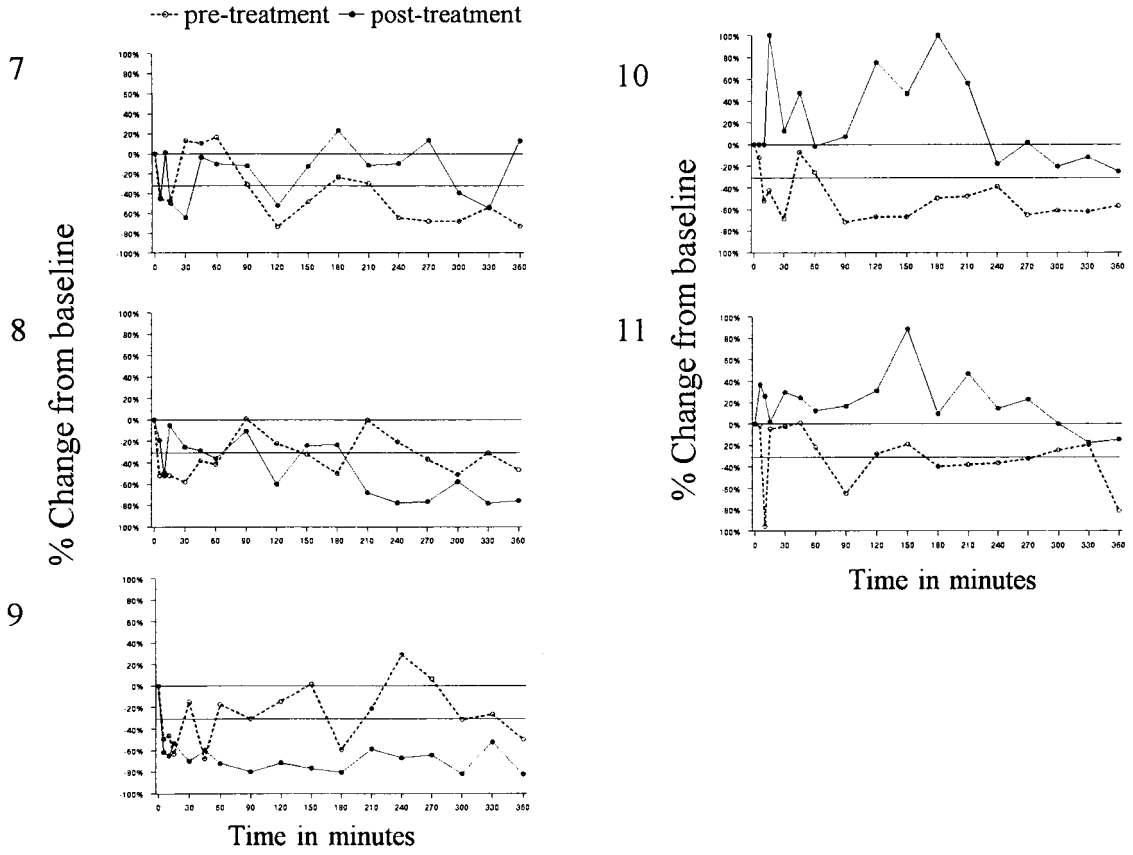

Figure 3. Dynamic compliance shown as percent change from baseline after the pretreatment (–○–), and the posttreatment (—●—) challenges in the rabbits treated with superoxide dismutase-polyethylene glycol. Rabbits number 10 and 11 did not have an early or late asthmatic response on the posttreatment challenge.

groups became more hyperresponsive to histamine. The difference reached statistical significance only for the superoxide dismutase-polyethylene glycol group ($P = .01$) (Table 1). The decrease in compliance after the histamine challenge was not transient as expected. It lasted for up to two hours after the challenge and was completely reversible with diphenhydramine.

There was no difference in the ragweed PD30 at the pretreatment challenge between both groups. In the control group, the ragweed PD30 did not change significantly from the pretreatment to the posttreatment challenge. In the superoxide dismutase-polyethylene glycol group, the ragweed PD30 was higher at the posttreatment challenge than at the pretreatment challenge, 8.1 ± 1.46 versus 4.1 ± 1.72 minutes, indicating that this group became less hyperresponsive to ragweed. The difference did not reach statistical significance ($P = .1$).

Lung Compliance

At the pretreatment challenge, all rabbits had an asthmatic response to ragweed inhalation manifested by a drop of 30% or more in compliance from baseline (Fig 2). All responses occurred within the first 15 minutes except for two rabbits, which had the first drop in compliance below 30% of baseline at the 45 minutes (rabbit number 5, Fig 2) and 120-minute measurements (rabbit number 3, Fig 2). During the randomization procedure, these two rabbits fell in the control group. The area under the curve was not significantly different for both groups at the pretreatment challenge.

At the posttreatment challenge, all six rabbits in the control group had a drop in compliance of 30% or more below baseline within 15 minutes of the ragweed challenge, and five of six rabbits had a late phase asthmatic response (Fig 2). In the superoxide dismutase-polyethylene glycol group, two of five rabbits had neither an early nor late phase asthmatic response to the ragweed bronchial challenge (Fig 3).

As a group, the control group had both an early and late phase asthmatic response on the posttreatment challenge, while the superoxide dismutase-polyethylene glycol group had an isolated delayed asthmatic response (Fig 4).

The area under the curve (mean ± SEM) was for the control group −177.7 ± 28 at the pretreatment challenge and −133.6 ± 41.6 at the posttreatment challenge, and for the superoxide dismutase-polyethylene glycol group −222.1 ± 30.4 at the pretreatment challenge and −180.4 ± 75.7 at the posttreatment challenge. The area

under the curve was thus lower during the posttreatment than the pretreatment challenge for both groups, but the difference did not reach statistical significance. The difference in the area under the curve for compliance between the two groups during the posttreatment challenge was not statistically significant. Individually, four of six rabbits in the control group, and three of five rabbits in the superoxide dismutase-polyethylene glycol group had an improved compliance manifested by a smaller area under the curve during the posttreatment challenge when compared with the pretreatment challenge.

Lung Histology

Examination of section of the lungs for both groups showed areas of atelectasis and areas of hyperaeration typical of findings in asthma (Fig 5). Some sections in both groups showed areas of chronic changes and dense cellular infiltrates (Fig 6). The mean pathology score of the control group was 3.8 ± 0.47 and that of the superoxide dismutase-polyethylene glycol group was 4 ± 0.57. The difference between the two groups was not significant. The morphometries measurements showed that the muscle thickness was 16.3% ± 0.92 of the internal diameter of the small and medium size bronchi for the control group and 16.5% ± 2.1 for the superoxide dismutase-polyethylene glycol group. This difference was not statistically significant. In the BAL (Table 2), the % eosinophils was lower for the superoxide dismutase-polyethylene glycol group than the control group, while the percent of polymorphonuclear leukocytes was higher. None of the differences was statistically significant. Individually, the two rabbits in the superoxide dismutase-polyethylene glycol group who did not have an asthmatic response in the posttreatment challenge had a low eosinophil percent of 2% and 5%.

Lung Superoxide Dismutase Level

The mean level of total manganese and copper/zinc superoxide dismutase activity in lung homogenate was higher in the control group than in the superoxide dismutase-polyethylene glycol group (Table 3). The difference in activity between the two groups was not statistically significant.

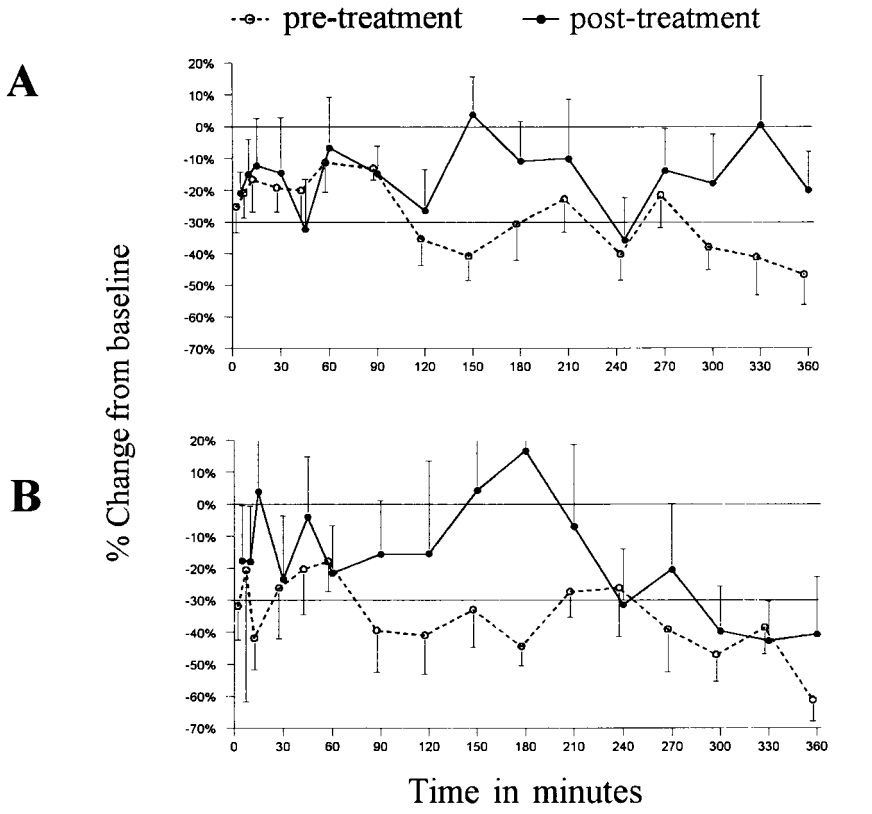

Figure 4. Dynamic compliance of (A) inactivated superoxide dismutase-polyethylene glycol group (n = 6), and (B) superoxide-dismutase-polyethylene glycol group (n = 5) after ragweed challenge. Compliance is shown as mean ± SEM of percent change from baseline during the pretreatment (–○–) and the posttreatment (—●—) challenge. The upward error bars of the pretreatment graph, and the downward error bars of the posttreatment graph have been omitted from the figure for clarity.

DISCUSSION

Oxygen radicals are thought to play a role in inflammation in asthma. Airway cells obtained from BAL of asthmatic subjects had higher spontaneous oxygen radical production than airway cells of normal subjects. The generation of oxygen radicals inversely correlated with the forced expiratory volume in the first second (FEV_1).[8] Airway cells from subjects with nocturnal asthma produced higher amounts of oxygen free radicals at 4 AM than at 4 PM, and the difference correlated with the change in FEV_1.[9] Bronchial alveolar lavage specimens from allergic asthmatic subjects before and 48 to 72 hours after allergen bronchial challenge had increased levels of oxygen radicals in the postchallenge specimens.[10] Also peripheral blood eosinophils,[11] neutrophils,[12,13] and monocytes[14] from asthmatic subjects released more oxygen free radicals than those from nonasthmatic controls. The amount of oxygen free radical generation correlated with the degree of asthma severity.

Superoxide dismutase is found intracellularly in the cytosol and peroxisomes as copper/zinc superoxide dismutase and in the mitochondria as manganese superoxide dismutase.[15,16] The activity of superoxide dismutase and its correlation with asthma has not been well defined. Superoxide dismutase activity was similar in BAL fluid from mildly asthmatic and from

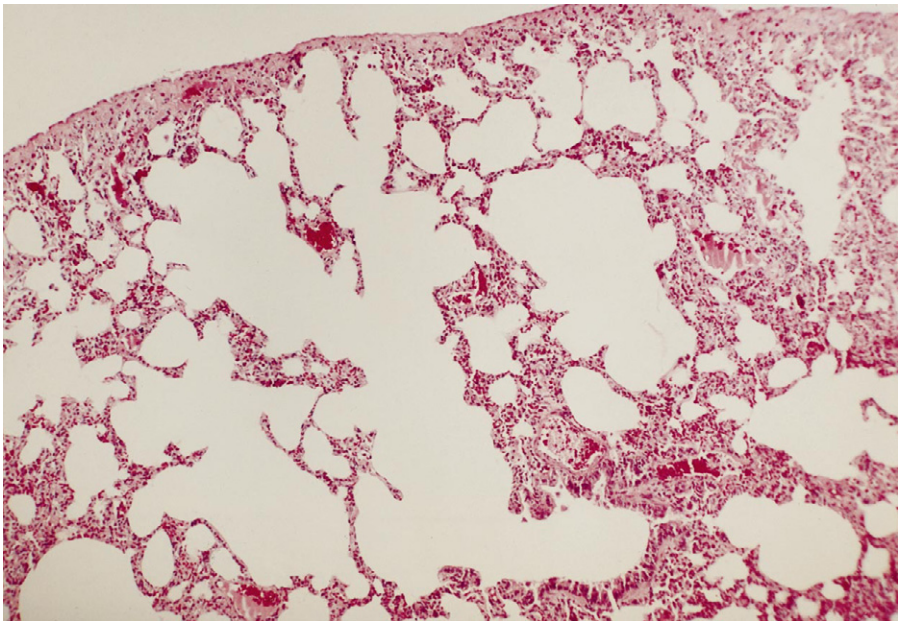

Figure 5. Section from the lung of a rabbit with asthma reveals areas of atelectasis alternating with areas of hyperaeration. (Stain: hematoxylin and eosin, original magnification: ×25)

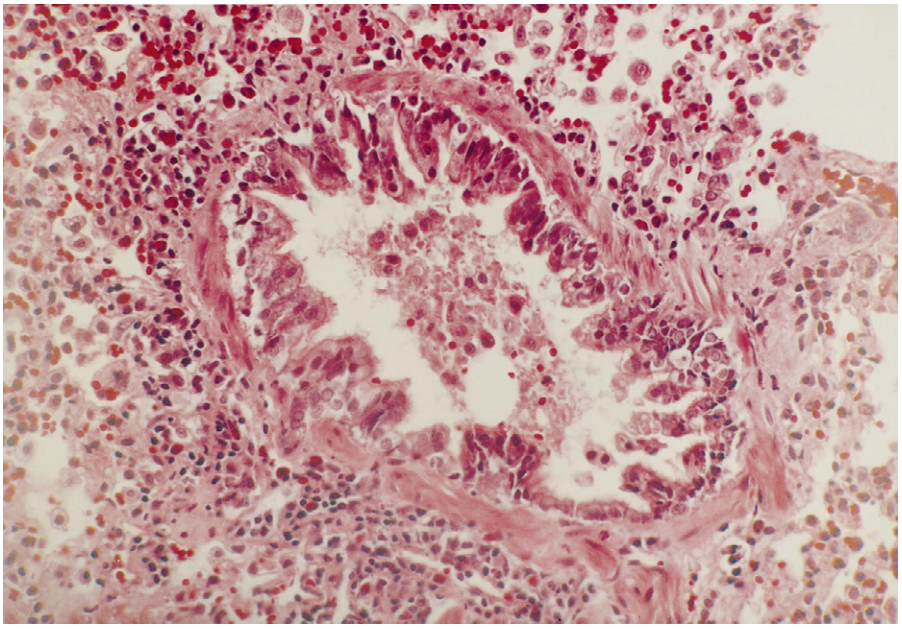

Figure 6. Section from the lung of a rabbit with asthma showing a peribronchiolar inflammatory cell infiltrate with many eosinophils. (Stain: hematoxylin and eosin, original magnification: ×200)

normal subjects.[17] Copper/zinc superoxide dismutase activity in platelets from stable asthmatic patients was significantly higher than that from normal healthy subjects, and higher in atopic than in nonatopic asthmatic patients.[18] Copper/zinc superoxide dismutase activities of neutrophils from mildly asthmatic patients, however, showed no difference from normal controls.[19] In a model of the late asthmatic response in guinea pigs, superoxide dismutase activity in BAL increased in parallel with the increase in inflammatory cells following an antigen challenge.[20]

The role of superoxide dismutase and other antioxidants has been extensively studied in hyperoxic lung damage during the pulmonary response to endotoxin in animal models[21,22] and in children with bronchopulmonary dysplasia.[23] Few studies have addressed their role in asthma. In a model of late asthmatic response in rats, treatment with recombinant human superoxide dismutase almost completely suppressed the late asthmatic response and suppressed the induction of manganese superoxide dismutase in the bronchial epithelial cells of untreated animals after antigen challenge.[24] In asthmatic subjects, a 2-week trial of nimesulide, an oxygen radical scavenger and inhibitor of oxygen radical generation from neutrophils, was not different from placebo in the degree of bronchial reactivity to allergen, both early and late-phase reactions, or to methacholine.[25] Our group found no significant effect of a single treatment of superoxide dismutase given to sensitized rabbits at a dose of 10,000 U/kg intravenously immediately prior to an allergen challenge.[26]

This study examined the effect of superoxidedismutase-polyethyleneglycol on the bronchial responsiveness to histamine and ragweed challenge in chronically sensitized animals. Histamine directly contracts the smooth muscles of isolated rabbit bronchus, an effect blocked by H_1 antagonists.[27] In rabbits immunized with *Alternaria tenuis* up to 3 months of age, there was an increase in airway responsiveness to inhaled histamine that persisted for 12 months. The hyperresponsiveness was unrelated to either a detectable alteration in cellular infiltration within the airway lumen or changes in isolated bronchial smooth muscle responsiveness.[28] The difference in response to histamine between the two groups in this study can be explained similarly by a direct effect of histamine on bronchial smooth muscles in rabbits, and a result of individual variations between

animals in their sensitivity to histamine. The specific hyperreactivity to ragweed decreased in the superoxide dismutase-polyethylene glycol group as shown by a rise in the ragweed PC30. This cannot be explained by tolerization to the sensitizing antigen since there was no change in ragweed PC30 in the control group.

The degree of airway reactivity was milder during the second challenge for both groups, an observation previously made by our group.[26] Previous animal models of asthma have shown that animals sensitized by the same method segregate into single and dual phase responders.[29] The lack of a significant response to treatment when analyzed by group can be explained by the variability of the time of response of each rabbit. The treatment effect can be more obvious when each animal is examined individually. In that respect, two of five rabbits in the superoxide dismutase-polyethylene glycol group were protected from developing an asthmatic response by the treatment.

The level of activity of both manganese, and copper/zinc superoxide dismutase enzymes in the lung homogenate was not different between the two groups. A similar single dose of 10,000 U/kg superoxide dismutase-polyethylene glycol intravenously was shown to be protective against the accumulation of neutrophils during re-expansion pulmonary edema in rabbits, and resulted in significantly higher activity of superoxide dismutase-polyethylene glycol in lung tissue, lung lavage, and blood.[21] A lower dose, 2,000 U/kg, was shown to attenuate the lung injury in *Escherichia coli*-treated guinea pigs.[30] A single antigen challenge in rat and guinea pig models of late asthmatic response induced superoxide dismutase production, and exogenously administered superoxide dismutase suppressed the endogenous production.[20,24] To date no data were found in the literature regarding the level of activity of superoxide dismutase in chronic asthma in a rabbit model. We, therefore, can only extrapolate from what is known about induction of superoxide dismutase in the lung by the chronic exposure to various stresses and by cytokine stimulation. When exposed for six hours a day for ten days to aerosolized asbestos or silica, manganese superoxide dismutase protein was increased by 1.3-fold and 2.4-fold over control in lungs of rats.[31] Exposure of rat lungs to 7 or 14 days of 85% O_2 increased the concentration of manganese and copper/zinc superoxide dismutase in the mitochondria of interstitial fibroblasts of rat lungs by 197% and 139%.[32] Manganese superoxide dismutase, protein, and activity was shown to be induced by various cytokines including IL-1.[33] Incubation of a human lung fibroblasts cell line for three days with IL-1 led to an increase in copper/zinc superoxide dismutase.[34] Interleukin-1 is known to play a role in asthma, and BAL from patients with symptomatic asthma have significantly higher levels of IL-1 β when compared with BAL from patients with asymptomatic asthma.[35] The level of activity of superoxide dismutase in both groups in this study may reflect a balance between endogenous production stimulated by the repeated antigen challenges, and the accompanying inflammatory cytokine production and the exogenously given treatment that may suppress the endogenous induction. This balance may not have been apparent in single dose experiments but may have become apparent in this chronic exposure experiment.

This study has shown that when superoxide dismutase-polyethylene glycol was given repeatedly at a dose of 10,000 U/kg intravenously to a rabbit model of chronic allergic asthma, there was a trend towards improvement in specific airway responsiveness manifested by a drop in the ragweed PD30, and an amelioration of the asthmatic response as shown by the lack of an asthmatic response on the posttreatment challenge in two of five rabbits. There was no significant effect on airway inflammation since the treatment did not result in a significant difference compared with control in the composition of the inflammatory cells in the

Table 2. Total and Differential Cell Count on the Bronchoalveolar Lavage Fluid from the Inactivated Superoxide Dismutase-Polyethylene Glycol (InSOD-PEG) and the Superoxide Dismutase-Polyethylene Glycol (SOD-PEG) Groups

		InSOD-PEG Group	SOD-PEG Group
Total*	Mean cell density ± SEM	2.4 ± 0.24	2.8 ± 0.6
Eosinophils	Mean % of total ± SEM	37.4% ± 15.2	16.5% ± 7.1
Macrophages	Mean % of total ± SEM	52.8% ± 13.5	51.6% ± 14.2
Polymorphs	Mean % of total ± SEM	2.4% ± 1.9	9.6% ± 5.9
Epithelial cells	Mean % of total ± SEM	7.2% ± 2.2	22% ± 10.7

* The cell density was scored from 1 to 5 based on the approximate number of cells in the preparation, with 1 = very few cells, 3 = moderate number of cells, and 5 = cells clumping together with no separation between cells.

Table 3. Levels of Activity of Total Superoxide Dismutase (SOD), Manganese Superoxide Dismutase (MnSOD), and Copper/Zinc Superoxide Dismutase (CuZnSOD) Measured in Activity per mg of Protein in the Lung Homogenate of the Inactivated Superoxide Dismutase-Polyethylene Glycol (InSOD-PEG) and the Superoxide Dismutase-Polyethylene Glycol (SOD-PEG) Groups

	InSOD-PEG Group		SOD-PEG Group	
	Mean Activity/ mg protein ± SEM	Range of Activity/ mg protein	Mean Activity/ mg protein ± SEM	Range of Activity/ mg protein
Total SOD	172.8 ± 48.8	65–385	86.8 ± 9.7	52–100
MnSOD	15.8 ± 2.3	7–27	11.6 ± 2.1	6–16
CuZnSOD	157 ± 47.6	48–364	75.2 ± 7.9	46–93

BAL or in the lung histology. The study has reported for the first time levels of activity of superoxide dismutase enzyme in lungs of rabbits with chronic allergic asthma. The study describes a rabbit model of chronic allergic asthma that provides the opportunity to study the pathophysiology of chronic allergic asthma, and to study the effects of various drugs.

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