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Superoxide dismutase failed to attenuate allergen-induced nasal congestion in ragweed-sensitized dogs

Natalia Skorohod and Donovan B. Yeates

University of Illinois at Chicago, Chicago, Illinois

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Skorohod, Natalia, and Donovan B. Yeates. Superoxide dismutase failed to attenuate allergen-induced nasal congestion in ragweed-sensitized dogs. *J Appl Physiol* 98: 1478–1486, 2005. First published December 17, 2004; doi:10.1152/jappphysiol.00910.2004.—We hypothesized that augmentation of antioxidant defenses with exogenous superoxide dismutase (SOD), an enzyme that provides an initial defense against oxidative injury, would attenuate allergen-induced nasal congestion in the canine model of allergic rhinitis. Nasal congestion was evaluated by the measurements of nasal resistance and the volume of the nasal passage. In five nonsensitized dogs, 30,000 U of SOD from bovine erythrocytes delivered by aerosol to the nasal passages before histamine challenge reduced the histamine-induced nasal congestion. At 30 min postchallenge, nasal resistance was $1.14 \pm 0.2 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ in the saline pretreatment study vs. $0.36 \pm 0.02 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ in the SOD pretreatment study ($P < 0.05$), and volume of nasal passage was $10.9 \pm 0.5 \text{ cm}^3$ vs. $17.4 \pm 1.3 \text{ cm}^3$ ($P < 0.05$), respectively. In five sensitized dogs, however, neither an analogous pretreatment with SOD nor intranasal aerosolized pretreatment with 30,000 U of SOD conjugated to polyethylene glycol attenuated ragweed-induced nasal congestion. Also, systemic application of SOD did not attenuate responses to challenges with histamine and ragweed in nonsensitized and sensitized dogs, respectively. The antioxidant-induced attenuation of nasal congestion in nonsensitized dogs confirms validity of the model and indicates the involvement of free radical-mediated damage in the genesis of the histamine-induced congestion. In sensitized dogs, the data do not support the hypothesis that oxidative stress is a clinically significant component of acute ragweed-induced nasal congestion. The data do not support the use of SOD for acute protection against allergic rhinitis.

polyethylene glycol-superoxide dismutase; exogenous antioxidants; histamine; nasal resistance; nasal airway volume

HIGHLY OXIDATIVE MOLECULES known as reactive oxygen species (ROS) such as free radicals [e.g., hydroxyl radical ($\text{OH}\cdot$), superoxide anion (O_2^-)] and nonradicals [e.g., hydrogen peroxide (H_2O_2) and hypochlorite (HOCl)] contribute to the pathogenesis of allergic disorders (38). ROS concentrations are controlled by antioxidant scavenger systems. Antioxidants that are found in the epithelial lining fluids and epithelial cells of the airways include superoxide dismutase (SOD), glutathione peroxidase, catalase, thioredoxin, the iron-binding proteins lactoferrin and transferrin, the copper-binding protein ceruloplasmin, and the low-molecular-weight antioxidants glutathione, vitamin C (ascorbate), urate, and vitamin E (α -tocopherol) (7, 33, 38). During homeostasis, a balance exists between the endogenous production of free ROS and their depletion by antioxidant defense mechanisms. Oxidant-antioxidant imbalance

(an excess of oxidants relative to the antioxidant defenses) results in an oxidative stress. Supplementation of the antioxidant defense system, therefore, may limit the severity of allergen-induced physiological responses associated with oxidative stress.

Superoxide dismutases (SODs) provide an initial protection for cells against oxidative insult. They convert extremely reactive superoxide radicals to potentially less toxic H_2O_2 , which in turn is metabolized by catalase and glutathione peroxidase. The cytosolic metalloenzyme copper-zinc superoxide dismutase (Cu,Zn-SOD) comprises 90% of the total SODs (22). Cu,Zn-SOD activity has been shown to be reduced in the epithelial lining fluid (9, 30) and in bronchial epithelial cells (11, 30) of patients with asthma. Otto-Knapp et al. (23) found increased activities of SOD and glutathione peroxidase after ozone exposure in cultured human nasal mucosa. However, SOD levels in upper airways in patients with allergic rhinitis are still undefined.

Considering the similarities of allergic rhinitis and asthma as processes possessing common pathophysiological mechanisms (3, 8, 24), oxidative stress is expected to play an important role in the pathogenesis of both diseases (7). Vitamin C, bromelain, quercetin, and *N*-acetylcysteine have been suggested as therapies in allergic rhinitis (16, 31). Although Cu,Zn-SOD has been studied in the treatment of several ROS-mediated conditions including allergic inflammation (1, 13), to our knowledge, there has been no antioxidant enzyme therapy suggested for the treatment of rhinitis.

We hypothesized that allergen-induced nasal congestion would be alleviated by prior or concurrent administration of SOD. We used canine models of nasal congestion and allergic rhinitis (32) to test 1) the extent to which bovine Cu,Zn-SOD would attenuate histamine-induced nasal congestion, and 2) the potential therapeutic effects of SOD and SOD conjugated with polyethylene glycol (PEG-SOD) on ragweed-induced nasal congestion.

Recent studies indicate that topically administered SOD may be therapeutically superior to other routes of SOD administration (10, 19). Therefore, three delivery strategies (intranasal, intramuscular, and intravenous) were compared in our study. Also, because it is likely that pretreatment with SOD is more protective in the reduction of induced oxidative stress than treatment with SOD administered after the challenge, we administered SOD before the challenge in the experiments employing intramuscular and intranasal routes of SOD delivery and immediately after the challenge in the experiments employing intravenous route of SOD delivery. To prolong the

Address for reprint requests and other correspondence: D. B. Yeates, Dept. of Medicine, M/C 788, Univ. of Illinois at Chicago, Chicago, IL 60612 (E-mail: yeates-d@uic.edu).

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enzyme's half-life, which is less than 10 min after intravenous administration, and to improve its uptake, several modifications of SOD have been proposed, including polyethylene glycol conjugation, liposome encapsulation, and genetic manipulations (reviewed in Ref. 21). We elected to compare the effects of unmodified (native) SOD and PEG-SOD after intranasal route of administration.

METHODS

Description of Animals and Their History

Ten adult beagle dogs weighing 10.5–15.0 kg were used for all experiments. Five dogs were neonatally sensitized to ragweed, and the other five were not sensitized to ragweed (naive). The method used to sensitize the dogs (39) was adapted from the method described by Becker et al. (4). In a previous series of experiments, the ragweed-sensitized dogs exhibited an anaphylactic reaction on inhalation of ragweed extract. They also exhibited nasal congestion on exposure of the nasal passages to ragweed extract. The dogs were not challenged with ragweed extract between the series of experiments conducted in 2001 (32) and the experiments conducted in 2003 described herein. Sensitized dogs had a positive skin prick test response to ragweed extract performed 2 wk before the beginning of experiments. The naive dogs had been previously used for other experiments that would not be expected to influence the responses observed in this study. The protocols for animal use in these experiments were approved by the institutional Animal Care Committees.

Protocols

Two studies were conducted. The first study was conducted in five nonsensitized dogs (four male, one female) and was designed to determine whether SOD, delivered by two different routes, intramuscular or intranasal, would ameliorate histamine-induced nasal congestion. Nasal congestion was induced by delivery of 0.25 ml of 2.5% histamine (81 mM solution in saline) into both nasal passages by a Micro Sprayer catheter (Penn-Century, Philadelphia, PA). The syringe of the Micro Sprayer was loaded with 0.25 ml of solution. The tip of the Micro Sprayer catheter was positioned into the left and right nasal passage in turn to a length of 4 cm to provide aerosol deposition within the nasal passage and anterior nasopharynx. The plunger was completely depressed such that the same mass of aerosol was delivered, independent of the solution with which it was charged.

The second study was conducted in five ragweed-sensitized dogs (one male, four female) and was designed to determine the relative effectiveness of treatment with SOD, delivered by either intramuscular, or intravenous, or intranasal routes. For the intranasal route, we performed two variants of SOD treatment: with unmodified SOD and with PEG-SOD. Nasal congestion was induced by spraying 1.27 units of ragweed extract (Greer Laboratories, Lenoir, NC) dissolved in 0.25 ml of saline into each nasal passage using the technique described for histamine aerosolization. To induce greater responses, the dose of ragweed administered was more than twice the dose previously used (32).

SOD Treatment

Bovine erythrocyte SOD, possessing a specific activity of 3,770 U/mg protein, and SOD from bovine erythrocytes coupled to methoxy-polyethylene glycol, molecular weight 5,000, through secondary amine linkage, PEG-SOD, possessing an activity of 3,390 U/mg protein, were purchased from Sigma-Aldrich (St. Louis, MO). The products were stored at -20°C and dissolved in sterile 0.9% saline directly before use.

The time of SOD administration was chosen with the consideration of the enzyme's pharmacokinetics after intramuscular, intravenous, and intratracheal delivery. For intramuscular administration of native

SOD, the peak of its maximum concentration occurred 2.5 h after administration (34). After intravenous administration, SOD clearance time was less than 10 min (reviewed in Ref. 21). There are no data available regarding SOD concentrations after intranasal route of SOD delivery; however, it has been shown that after intratracheal administration of human recombinant Cu,Zn-SOD, SOD activity in the lung cells was increased by 100% and remained elevated for several hours (26). In our study, doses of SOD were derived from the literature and modified on the basis of our preliminary experiments.

Each of five naive dogs received two forms of SOD treatment: 1) pretreatment with SOD 5,000 U/kg body wt, dissolved in 0.35 ml/kg body wt of saline, administered intramuscularly 2.5 h before histamine aerosolization into nasal passages; and 2) pretreatment with SOD, 15,000 U in 0.25 saline, aerosolized into each nasal passage 10 min before histamine intranasal aerosolization.

Each of five sensitized dogs underwent four forms of SOD treatment: 1) pretreatment with SOD, 5,000 U/kg body wt in saline (0.35 ml/kg), administered intramuscularly 2.5 h before ragweed extract aerosolization into nasal passages; 2) pretreatment with SOD aerosol, 15,000 units in 0.25 ml of saline, delivered into each nostril 10 min before ragweed intranasal aerosolization; 3) pretreatment with PEG-SOD, 15,000 U in 0.25 ml of saline, aerosolized into each nasal passage 10 min before ragweed intranasal aerosolization; and 4) treatment with SOD, 5,000 U/kg in 0.35 ml/kg saline, administered intravenously immediately after ragweed intranasal aerosolization.

Nasal congestion was estimated by continuous measurements of nasal resistance (R_{na}) in combination with intermittent measurements of the volume of nasal passages using acoustic rhinometry (V_{na}) (32).

In each of the two studies, all dogs underwent the analogous experimental protocols with equal volumes of saline replacing each enzyme intervention. Saline rather than inactivated SOD was used because 15,000 U of SOD (4 mg of protein in 0.25 ml saline) would have a negligible effect on the osmotic pressure or the mass of protein in the nasal passages, especially considering wide variations in the normal volume of secretion and much larger changes in the osmotic pressure experienced in the nasal passages during normal breathing.

In each study, the order in which the experiments were conducted was randomized, with the exception of the PEG-SOD treatment and its respective saline treatment, which was performed after the other experiments were completed.

Measurement of R_{na}

R_{na} was determined by measuring the air pressure required to achieve a constant flow through the nasal passage (32). Humidified airflow of 4.9 l/min was applied through a catheter in the left nostril. R_{na} in the left nasal airway, along with ECG and respiratory flow signals, was recorded continuously. Measurements were made at four time points: 1) baseline at 5 min before the first intervention (treatment or provocation); 2) 5 min after the challenge deposition into nasal passages; 3) 15 min after challenge deposition; and 4) 30 min after challenge deposition.

V_{na}

Geometric parameters of the right nasal passage were measured with the Eccovision acoustic rhinometry system (Hood Laboratories, Pembroke, MA). The V_{na} of the right nasal airway was estimated by acoustic measurements that were performed four times during each experiment, as described for R_{na} .

Animal Preparation

Before each experiment, dogs were fasted overnight but allowed water ad libitum. The dog was placed in the upright position in a sling and secured. Each dog was anesthetized with a combination of propofol ($500\text{--}600\text{ }\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, Zeneca Pharmaceuticals, Wilmington, DE) and etomidate ($5\text{ }\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, Abbott Laborato-

ries, North Chicago, IL) administered intravenously. The dog was intubated. A nasal catheter was placed into the left nostril to facilitate the measurements of R_{na} . Physiological monitoring included ECG (Burdick EK/5A interfaced to personal computer), respiratory rate (pneumotachograph Fleisch no. 1, OEM Medical, interfaced to personal computer), arterial blood pressure by use of a pressure cuff on the foreleg, heart rate and hemoglobin oxygen saturation (pulse oximeter Novamatrix 505 with a sensor placed on the dog's tongue), and rectal temperature (DigiTec 5800). Nasal secretions were estimated by visual observations of the open right nostril and posterior oropharynx. After a 10-min stabilization period, baseline measurements of R_{na} and V_{na} values were performed. Animals received either SOD or saline treatment according to the above-mentioned delivery and dosing strategies. Nasal congestion was induced with histamine (nonsensitized dogs) or ragweed extract (sensitized dogs) delivered by aerosol to each nasopharynx. The drugs were well tolerated; no unpredicted side effects were detected. R_{na} in the left nasal airway and V_{na} in the right nasal airway were measured simultaneously. Dogs were allowed to recuperate for at least 1 wk between experiments.

Data Analysis

R_{na} and V_{na} data sets were analyzed with the SigmaStat statistical software package for Windows (Jandel Scientific). Difference between groups was determined by one-way ANOVA with treatment as the independent factor. Data were tested for normal distribution and equal variance. When the normality test failed, a Kruskal-Wallis one-way ANOVA on ranks was used. Additionally, a two-way ANOVA factoring for time and treatment and for dog and treatment was performed. The Student-Newman-Keuls test was employed for post hoc multiple pairwise comparison if statistical significance was indicated by ANOVA.

Differences in changes in respiratory rate, heart rate, mean blood pressure, and hemoglobin oxygen saturation for the studies with intranasal route of SOD administration were evaluated by Student's *t*-test (Excel).

All data are given as means $\pm$ SE. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Nonsensitized Dogs

Treatment with SOD administered intramuscularly before histamine intranasal deposition. Figure 1A shows that histamine delivered to the nasal passages caused a rapid increase in R_{na} values in both saline and SOD intramuscular pretreatment studies: within the first 5 min, R_{na} increased from 0.32 ± 0.03 to 0.79 ± 0.2 $\text{cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ and from 0.34 ± 0.05 to 0.72 ± 0.1 $\text{cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$, respectively. Throughout the remaining observation period, R_{na} values demonstrated a sustained increase. They were 0.85 ± 0.2 $\text{cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ in the saline pretreatment study and 0.65 ± 0.1 in the SOD pretreatment study 30 min after histamine challenge. However, comparison of the R_{na} values in the SOD pretreatment study and those in the respective saline study revealed no significant differences in nasal resistance ($P > 0.05$) (Fig. 1A).

The decreases in V_{na} in the right nasal cavity were reflective of increases in R_{na} in the left nasal passage (Fig. 1). In the saline pretreatment study, histamine administration resulted in a more than twofold decrease in V_{na} within the first 5 min: from 26.1 ± 0.9 cm^3 observed at baseline to 12.2 ± 1.4 cm^3 registered 5 min after histamine challenge. Throughout the remaining observation period, V_{na} values demonstrated a sustained decrease and were 9.2 ± 1.1 cm^3 at 30 min. In the SOD pretreatment study, there was a rapid decrease in V_{na} from

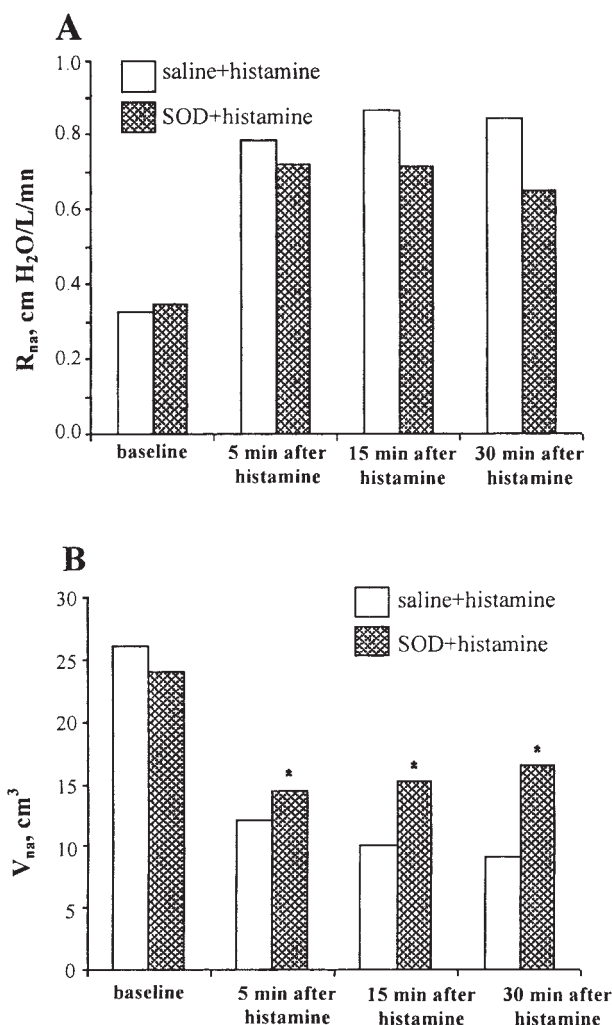


Fig. 1. Effect of intramuscular pretreatment with SOD on histamine-induced increases in nasal resistance (R_{na} ; A) and decreases in volume of the nasal passage (V_{na} ; B) in nonsensitized dogs compared with those obtained with saline pretreatment. * $P < 0.05$ compared with respective saline study.

24.2 ± 1.9 at baseline to 14.5 ± 1.7 cm^3 at 5 min, with a tendency for nasal volume values to recover thereafter. ANOVA indicated less pronounced decreases in V_{na} values in the SOD pretreatment study than in the saline pretreatment study (Fig. 1B).

Treatment with SOD administered intranasally before histamine intranasal deposition. Histamine caused an elevation of R_{na} values to their maximum at 5 min postchallenge: from 0.32 ± 0.1 to 1.5 ± 0.4 $\text{cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ in the saline pretreatment study and from 0.26 ± 0.05 to 0.49 ± 0.09 $\text{cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ in the SOD pretreatment study (Fig. 2A). R_{na} values gradually decreased in both studies and at 30 min they were 1.14 ± 0.2 $\text{cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ in the saline pretreatment study and 0.36 ± 0.02 $\text{cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ in the SOD pretreatment study. The data revealed lower nasal resistance in the SOD pretreatment study than in the saline pretreatment study ($P < 0.05$) (Fig. 2A).

The responses indicated by the measurements of V_{na} were physiologically consistent with the responses observed in R_{na} (Fig. 2). Over the period of the first 15 min, V_{na} decreased from 23.4 ± 0.6 to a minimum of 10.7 ± 0.8 cm^3 in the saline

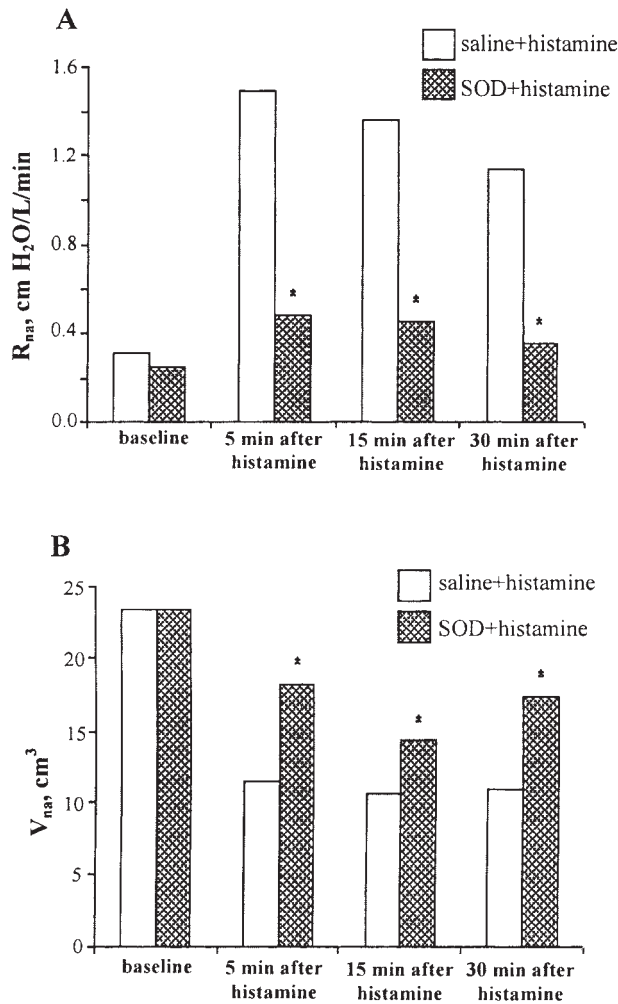


Fig. 2. Effect of intranasal pretreatment with aerosolized SOD on histamine-induced increases in R_{na} (A) and decreases in V_{na} (B) in nonsensitized dogs compared with those obtained with saline pretreatment. * $P < 0.05$ compared with respective saline value.

pretreatment study and from 23.3 ± 1.2 to a minimum of 14.3 ± 1.5 cm³ in the SOD pretreatment study. At 30 min, V_{na} in the saline pretreatment study remained low (10.9 ± 0.5 cm³), whereas in the SOD pretreatment study it recovered to 17.4 ± 1.3 cm³. Analysis of histamine-induced changes in nasal airway volumes revealed that V_{na} was reduced less in the SOD pretreatment study than in the comparable saline study ($P < 0.05$) (Fig. 2B).

Sensitized Dogs

Treatment with SOD administered intramuscularly before ragweed intranasal deposition. Within the first 5 min, ragweed provocation resulted in an increase in R_{na} from 0.24 ± 0.04 to 0.33 ± 0.05 cmH₂O·l⁻¹·min⁻¹ in the saline pretreatment study and from 0.33 ± 0.07 to 0.45 ± 0.07 cmH₂O·l⁻¹·min⁻¹ in the SOD pretreatment study. Surprisingly, R_{na} values in the SOD pretreatment study reflected greater responses to ragweed from the beginning of postchallenge period and, at the end of the experiment, R_{na} values were 0.47 ± 0.06 cmH₂O·l⁻¹·min⁻¹ in the SOD pretreatment study vs. 0.29 ± 0.04 cmH₂O·l⁻¹·min⁻¹ in the saline pretreatment

study. There was a statistically significant difference between the treatment groups ($P < 0.05$) (Fig. 3A).

Ragweed caused V_{na} to decrease during the postchallenge period in both studies. In the saline pretreatment study, V_{na} values decreased from a baseline value of 24.1 ± 0.5 cm³ to 15.7 ± 1.5 cm³ at 5 min and then decreased gradually to 13.1 ± 2.5 cm³ at 30 min. In the SOD pretreatment study, V_{na} decreased from 23.8 ± 0.7 to a minimum of 14.5 ± 1.1 cm³ at 15 min. Despite the tendency toward V_{na} increasing at 30 min in the SOD pretreatment study (17.1 ± 1.5 cm³), no statistically significant differences in V_{na} between the two experimental conditions were found ($P > 0.05$) (Fig. 3B).

Treatment with SOD administered intranasally before ragweed intranasal deposition. Ragweed deposition caused an increase in R_{na} during the postchallenge observation period in both saline and SOD pretreatment studies (Fig. 4A). In the saline pretreatment study, R_{na} increased from 0.29 ± 0.08 to 0.43 ± 0.07 cmH₂O·l⁻¹·min⁻¹ within the first 5 min and remained increased at 30 min (0.45 ± 0.08 cmH₂O·l⁻¹·min⁻¹), whereas in the SOD pretreatment study,

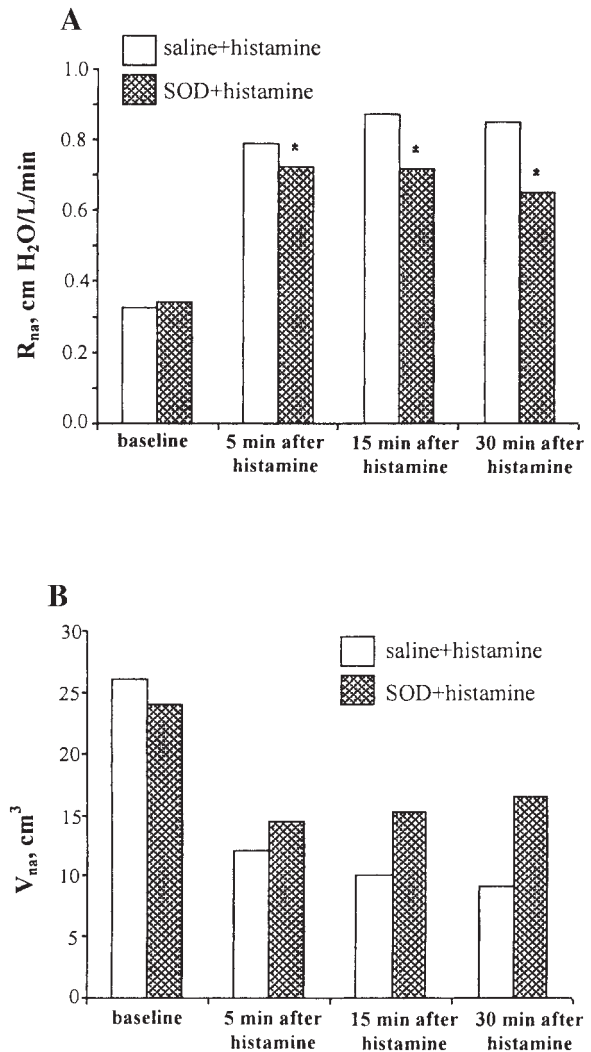


Fig. 3. Effect of intramuscular pretreatment with SOD on ragweed-induced increases in R_{na} (A) and decreases in V_{na} (B) in ragweed-sensitized dogs compared with those obtained with saline. * $P < 0.05$ compared with respective saline study.

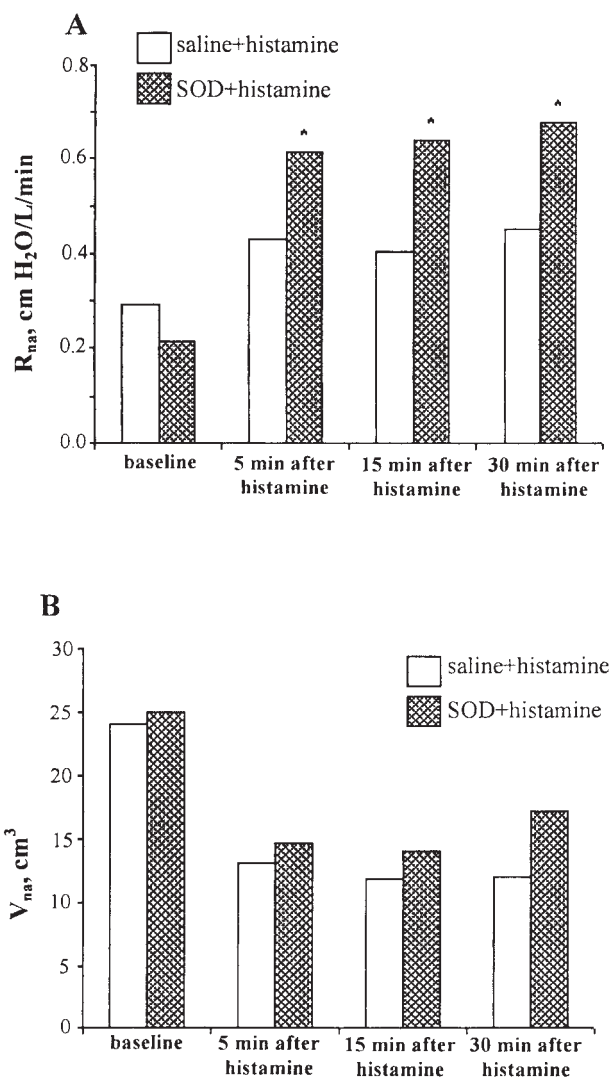


Fig. 4. Effect of intranasal pretreatment with aerosolized SOD on ragweed-induced increases in R_{na} (A) and decreases in V_{na} (B) in ragweed-sensitized dogs compared with those obtained with saline. * $P < 0.05$ compared with respective saline study.

R_{na} increased rapidly to 0.61 ± 0.08 cmH₂O·l⁻¹·min⁻¹ within the first 5 min, and by the end of the 30-min postchallenge observation period it was 0.68 ± 0.10 cmH₂O·l⁻¹·min⁻¹. Thus responses to ragweed were higher in the SOD pretreatment study than those in the saline pretreatment study ($P < 0.05$) (Fig. 4A).

V_{na} values in the saline pretreatment study decreased during the first 5 min after ragweed administration from 24.1 ± 0.8 to 13.0 ± 1.3 cm³ and remained suppressed to the end of the experiment. In the SOD pretreatment study, V_{na} also rapidly decreased within the first 5 min from 25.1 ± 0.4 to 14.7 ± 1.07 cm³, with a tendency to recover at 30 min postchallenge (17.2 ± 1.3 cm³). Nevertheless, the comparison between the two studies revealed no differences in V_{na} values ($P > 0.05$) (Fig. 4B).

Treatment with PEG-SOD administered intranasally before ragweed intranasal deposition. In both the saline and SOD pretreatment studies, ragweed induced increases in nasal resistance (Fig. 5A). In the saline pretreatment study, R_{na} reached a

maximum of 0.49 ± 0.3 cmH₂O·l⁻¹·min⁻¹ at 5 min; the final measurements at 30 min revealed a R_{na} value of 0.48 ± 0.1 cmH₂O·l⁻¹·min⁻¹. In the PEG-SOD pretreatment study, R_{na} values gradually increased during the entire postchallenge period and reached a maximum of 0.72 ± 0.2 cmH₂O·l⁻¹·min⁻¹ at the 30-min time point. Although R_{na} values in the PEG-SOD pretreatment study tended to be higher than those in the saline pretreatment study, the comparison between the studies did not reveal any statistically significant difference ($P > 0.05$) (Fig. 5A).

V_{na} in the saline pretreatment study decreased from 21.9 ± 1.6 cm³ at baseline to 13.5 ± 1.5 cm³ at 30 min. In the PEG-SOD pretreatment study, there were postchallenge decreases in V_{na} values at 5 and 15 min (14.2 ± 1.5 cm³ and 13.76 ± 1.66 cm³, respectively) and a tendency to recover at 30 min (17.7 ± 1.92 cm³ at 30 min postchallenge measurement). However, differences in V_{na} values in the PEG-SOD pretreatment study and saline pretreatment study did not reach statistical significance ($P > 0.05$) (Fig. 5B).

Treatment with SOD administered intravenously immediately after ragweed intranasal deposition. In the saline post-treatment study, R_{na} increased from 0.21 ± 0.03 to 0.48 ± 0.1 cmH₂O·l⁻¹·min⁻¹ at 5 min and continued to increase up to

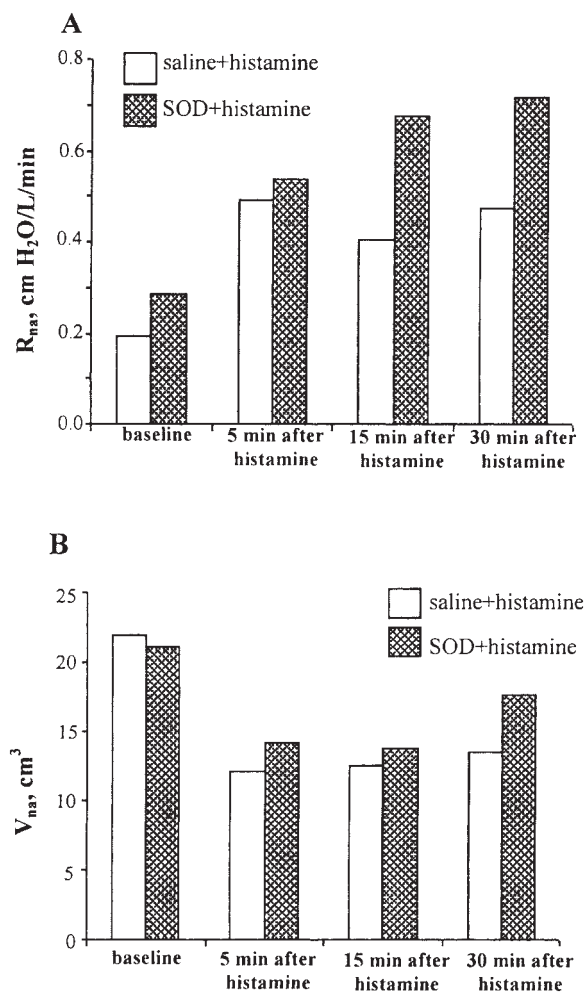


Fig. 5. Effect of intranasal pretreatment with aerosolized PEG-SOD on ragweed-induced increases in R_{na} (A) and decreases in V_{na} (B) in ragweed-sensitized dogs compared with those obtained with saline.

$0.69 \pm 0.2 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ at 30 min. In the SOD posttreatment study, R_{na} increased from 0.22 ± 0.05 to $0.38 \pm 0.08 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ in the first 5 min, after which it tended to recover such that at 30 min R_{na} was $0.31 \pm 0.06 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$. However, the comparison of the data obtained from the SOD posttreatment study and the respective saline study revealed no significant differences in R_{na} values ($P > 0.05$) (Fig. 6A).

V_{na} in the saline posttreatment study gradually decreased throughout the entire observation period from $26.2 \pm 0.6 \text{ cm}^3$ at baseline to $9.7 \pm 0.8 \text{ cm}^3$ at 30 min. In the SOD posttreatment study, V_{na} decreased from $24.7 \pm 0.6 \text{ cm}^3$ to a minimum of $13.9 \pm 0.8 \text{ cm}^3$ at 15 min, and it was $16.8 \pm 0.6 \text{ cm}^3$ at 30 min. Data analysis revealed insignificant differences between the two conditions ($P > 0.05$) (Fig. 6B).

Evaluation of aerosolized saline and SOD pretreatments. While evaluating the direct effect of SOD alone on nasal congestion, we noted that, in the study with intranasal SOD pretreatment in ragweed-sensitized dogs, R_{na} increased from a baseline of 0.22 ± 0.02 to $0.43 \pm 0.1 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ 10 min after SOD intranasal administration (directly before ragweed intranasal challenge), $P < 0.05$. Although to a lesser

degree, there was also an increase in R_{na} 10 min after saline intranasal aerosolization: from 0.29 ± 0.08 to $0.33 \pm 0.07 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$. There was no statistically significant difference in postsaline and post-SOD R_{na} values before ragweed challenge in sensitized dogs (0.43 ± 0.1 vs. $0.33 \pm 0.07 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$, $P > 0.05$). In nonsensitized dogs, there was a more prominent increase after saline alone than after SOD alone; however, no significant difference in post-SOD and postsaline R_{na} values was found. We conclude that pretreatment with aerosolized SOD or saline in the volume of 0.25 ml caused equal changes in nasal congestion.

Respiratory and cardiovascular responses to aerosolized challenges. The respiratory and cardiovascular responses to challenge are presented in the three pairs of studies with aerosolized SOD pretreatment (Table 1). In both sensitized and nonsensitized dogs, no differences were observed in heart rate and blood pressure level after challenge administration. Hemoglobin oxygen saturation after histamine administration was somewhat higher in the study with SOD pretreatment than with saline pretreatment in nonsensitized dogs; however, none of these differences reached statistical significance. In sensitized dogs, hemoglobin oxygen saturation from pulse oximetry did not change. In nonsensitized dogs, respiratory rate increased in 5 min from 17 ± 2 to 66 ± 19 breaths per minute in the saline pretreatment study ($P < 0.05$) and from 13 ± 3 to 20 ± 3 breaths per minute in the SOD pretreatment study ($P > 0.05$). Thus respiratory rate at 5 min after histamine administration was significantly lower in the SOD pretreatment study compared with the saline pretreatment study (20 ± 3 breaths per minute in the SOD study vs. 66 ± 19 breaths per minute in the saline study, $P < 0.05$). In sensitized dogs, respiratory rate varied neither after SOD nor after ragweed deposition. In the series of experiments with intranasal PEG-SOD pretreatment, there was no difference observed in heart rate, blood pressure level, hemoglobin oxygen saturation, or respiratory rate.

DISCUSSION

The data contained herein demonstrate that bovine erythrocyte Cu,Zn-SOD, given in an attempt to attenuate nasal congestion, exerts a protective effect in nonsensitized dogs when administered intranasally before histamine provocation, whereas in sensitized dogs neither an analogous aerosolized SOD pretreatment nor pretreatment with PEG-SOD attenuated ragweed-induced congestion. Intranasal prehistamine treatment with native Cu,Zn-SOD prevented a histamine-induced increase in the respiratory rate in nonsensitized dogs. Thus the effectiveness of intranasal SOD in nonsensitized dogs could serve as an indirect evidence of the importance of superoxide anion production as a cofactor in the mechanisms of histamine-induced nasal congestion. Any potential advantages of PEG-SOD administration over SOD administration in ragweed-challenged sensitized dogs were not apparent. Systemic application of SOD did not attenuate responses to challenges with histamine or ragweed in nonsensitized or sensitized dogs, respectively.

We would like to note that our ex juvantibus approach to investigating the potential efficacy of exogenous Cu,Zn-SOD and PEG-SOD in allergic rhinitis is based solely on the hypothetical implication of oxygen free radicals in the mechanism of the injury. We have evaluated the feasibility of

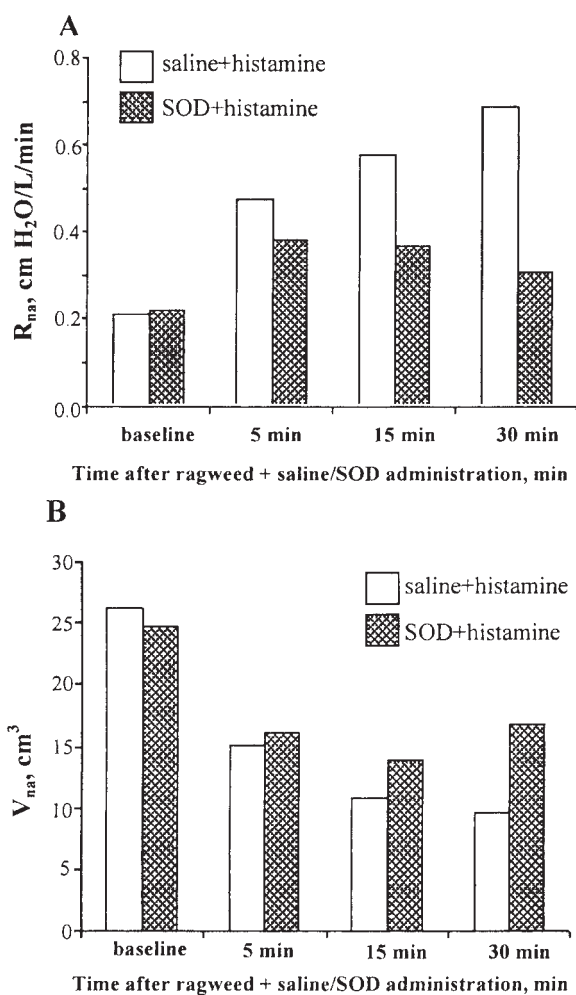


Fig. 6. Effect of the treatment with SOD administered intravenously immediately after ragweed intranasal aerosolization on ragweed-induced increases in R_{na} (A) and decreases in V_{na} (B) in ragweed-sensitized dogs compared with those obtained with saline.

Table 1. *Intranasal pretreatment with aerosolized superoxide dismutase: respiratory and cardiovascular responses in nonsensitized and ragweed-sensitized dogs*

	Nonsensitized Dogs		Ragweed-Sensitized Dogs			
	Saline + histamine	SOD + histamine	Saline + ragweed	SOD + ragweed	Saline + ragweed	PEG-SOD + ragweed
RR, beats/min						
Baseline	17±2	13±3	23±6	28±5	30±8	23±4
5 min	66±19†	20±3*	23±5	24±4	24±6	24±3
15 min	62±26	18±5	20±4	19±4	25±7	24±3
30 min	31±11	14±1	22±4	22±4	28±9	24±3
HR, beats/min						
Baseline	184±11	175±13	150±8	163±9	165±9	145±14
5 min	162±9	175±18	154±2	161±6	168±1	161±4
15 min	169±11	169±16	154±4	164±7	172±9	164±4
30 min	161±10	170±13	152±6	157±8	161±15	173±8
Mean BP, mmHg						
Baseline	115±10	113±10	102±1	101±11	98±5	100±4
5 min	107±11	113±11	99±5	113±9	101±5	97±6
15 min	115±10	110±11	101±4	113±6	99±6	98±6
30 min	105±10	111±13	104±1	111±9	98±4	98±5
SpO ₂ , %						
Baseline	90±1	91±2	92±2	90±1	91±1	92±1
5 min	82±5	86±2†	88±2	88±2	91±1	94±1
15 min	89±1	90±1	90±2	90±2	91±1	93±2
30 min	91±1	92±1	92±1	92±1	92±1	93±2

Values are means ± SE. RR, respiratory rate; HR, heart rate; BP, blood pressure; SpO₂, hemoglobin oxygen saturation. **P* < 0.05 compared with respective saline study; †*P* < 0.05 compared with baseline value.

aerosolizing SOD with a full recognition that the evidence of oxidative stress in the employed canine model of allergic rhinitis has not been documented. Nonetheless, considering 1) a likelihood of oxidative stress in allergic rhinitis (7), 2) decreased SOD levels in the epithelial lining fluid (9, 30) and bronchial epithelial cells (11, 30) of asthmatic patients, and 3) common pathophysiological mechanisms in allergic rhinitis and asthma (3, 8, 24), we hypothesized that augmentation of existing antioxidant defenses with exogenous SOD would attenuate nasal congestion in allergic rhinitis. We considered that, of the three SOD delivery strategies (intranasal, intramuscular, and intravenous), the intranasal route would be the most beneficial. Thus our study was primarily focused on the intranasal delivery of SOD. In the experiments employing the intranasal route of SOD delivery, we observed attenuation of histamine-induced nasal congestion in nonsensitized dogs and no effect on ragweed-induced nasal congestion in sensitized dogs. We exclude methodological explanations for the differences in the results between the two studies on the basis of the following observations. There were no statistically significant differences between baseline levels of R_{na} and V_{na} in sensitized and nonsensitized dogs; the comparable findings were described for healthy subjects and persons with allergic rhinitis (5, 20). The obtained values of nasal airway resistance and nasal airway volume are in agreement with those reported previously (32).

The dosages selected for this study were chosen with consideration for the well-known bell-shaped dose-response curve described for the efficacy of SOD, whereby SOD was effective only in moderate doses (reviewed in Ref. 17). In our preliminary experiments, both unmodified SOD and PEG-SOD at the dose of 2,000, 3,600, 4,000, 10,000, or 15,000 U aerosolized intranasally did not affect histamine- or ragweed-induced changes in R_{na} and V_{na} values, whereas 30,000 U (15,000 U aerosolized into each nasal passage) of SOD or PEG-SOD

appeared to improve them. We considered the dosage of 30,000 U of SOD or PEG-SOD, delivered intranasally, to be optimal to attenuate nasal congestion. Clearly this dose was appropriate in nonsensitized dogs. Given the kinetics of SOD, it is considered unlikely that the time of the peak physiological responses, 5 min postchallenge for histamine and 15 min postchallenge for ragweed, could explain the marked attenuation of the histamine-induced congestion but not ragweed extract-induced congestion. There were no significant differences between postsaline and post-SOD nasal resistance values measured 10 min after their intranasal administration. Thus, given that pretreatment with 0.25 ml of aerosolized SOD or saline caused equal changes in nasal congestion, the use of saline as a control is appropriate.

In allergic rhinitis, even when the symptoms of inflammation are absent, there exists a low level of persistent inflammation of the epithelium in the upper airways (2, 18, 25). In our experiments, ragweed-sensitized dogs underwent exposure to the relevant antigen every 1–2 wk. Thus we presume a latent inflammation of the epithelium of the upper respiratory tract in ragweed-sensitized dogs. Also, inflammation has been associated with epithelial damage. Several investigators (6, 27, 36) reported a marked epithelial loss in the upper airways in allergic rhinitis. Glück and Gebbers (14) provide evidence that the altered epithelium may lead to an impairment of the local secretory IgA defense system and thereby to an increased allergen uptake. These pathophysiological conditions lead to two possible reasons why SOD did not attenuate the ragweed-induced nasal congestion.

First, there are allergen-induced activated pathways that induce nasal congestion, which involve mediators and receptors independent of any oxidative inflammatory mechanisms. Allergen-induced anaphylactic responses only partially may be attributed to the action of histamine (15, 37, 40). Generalized airway eosinophilia is a characteristic feature of inflammation

in allergic rhinitis. Eosinophils are a source of leukotrienes, prostaglandins, platelet activating factor, cytokines, and cytotoxic proteins that can be released on stimulation (8). In our study, ragweed provocation in sensitized dogs caused responses that, in addition to histamine, were likely mediated by arachidonic acid derivatives (leukotrienes and prostaglandins), vasoactive peptides (kinins), phospholipid mediators (platelet activating factor), and cytokines (interleukins and other bioreponse modifiers). Parenthetically it should be mentioned that the contribution of multiple mediators to the stimulation of nasal secretion in allergic rhinitis (15) explains the copious rhinorrhea we observed after ragweed provocation in sensitized dogs. Contrariwise, histamine-induced reduction in nasal patency was not associated with overt rhinorrhea, an observation consistent with those of other investigators (32). Thus, in the multifactorial mechanisms of ragweed-induced nasal congestion in sensitized dogs, an inability of SOD to attenuate the allergen-induced congestion might be consistent with these additional mediators and secretory responses overriding any free radical-mediated damage.

Second, the oxidative stress within the nasal passages may be so considerable that it overwhelms the antioxidant enzyme administered. The presence of chronic inflammation in the epithelium of upper airways in allergic rhinitis, mentioned above, could contribute to the development of the considerable persistent oxidative stress. Airway inflammatory cells are the likely source of an increase in reactive oxygen species production (7, 38). In allergic inflammatory conditions, reactive oxygen species from eosinophils have been implicated in promoting oxidative tissue damage (30). In addition, epithelial loss, which accompanies inflammatory injury of the epithelium in the upper respiratory tract in allergic rhinitis (6, 27, 36), could also conceivably cause a decrease in the generation of antioxidants, thereby intensifying oxidative stress. It is possible, therefore, that the treatment that was appropriate in nonsensitized dogs, in ragweed-sensitized dogs turned to be to be clinically ineffective owing to the high-level oxidative processes or/and insufficient level of antioxidant defense.

Because a product of the dismutation of SOD is H_2O_2 , we considered the possibility, however not a likely one, that our treatment with SOD resulted in an increase in H_2O_2 concentration and, thus, in oxidative stress aggravation. H_2O_2 has recently been reported to be increased in the exhaled breath condensate of nonasthmatic subjects with allergic rhinitis (28, 41) and has been associated with airway hyperresponsiveness (41). Moreover, ciliary activity, a determining factor in the clearance of secretions in the nose, has been shown to be suppressed by H_2O_2 (12, 35, 42). In human respiratory epithelium, H_2O_2 -mediated ciliary dyskinesia was observed within the first hour, often as early as 15 min after exposure of the cells to H_2O_2 (12). We cannot exclude that, in sensitized dogs, after intranasal (Fig. 4) and intramuscular (Fig. 3) SOD pretreatment and after intranasal PEG-SOD pretreatment (Fig. 5), H_2O_2 contributed to the higher responses to ragweed in the SOD pretreatment studies than those in the respective saline studies.

In conclusion, our findings do not uphold our original hypothesis. In sensitized dogs, the attempts to mitigate allergen-induced nasal congestion by means of influencing superoxide radical production were of no clinical benefit. However, the antioxidant-induced attenuation of nasal congestion in

nonsensitized dogs confirms the validity of the model and indicates the involvement of free radical-mediated damage in the pathogenesis of histamine-induced congestion. The results could stem from the differences in the mechanisms of histamine- and ragweed-induced nasal congestion. Another factor, the preexistent oxidative stress, aggravated by the ragweed challenge, could be so intense that the SOD administered was inadequate. It would have been tempting to explain attenuation of histamine-induced congestion by a histamine H_1 -antagonist properties of SOD; however, no data in the literature support this speculation. Regardless of the mechanism, the data did not prove that oxidative stress is a clinically significant ingredient in the complex pathogenesis of ragweed-induced nasal congestion. The data do not support the use of SOD in allergic rhinitis.

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