Hog barn dust extract increases macromolecular efflux from the hamster cheek pouch

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Departments of ¹Medicine and ²Biopharmaceutical Sciences, Colleges of Medicine and Pharmacy, University of Illinois at Chicago, and ³Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois; and ⁴Department of Internal Medicine, University of Nebraska Medical Center, Omaha, Nebraska

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Rubinstein, Israel, and Susanna G. Von Essen. Hog barn dust extract increases macromolecular efflux from the hamster cheek pouch. J Appl Physiol 101: 128–134, 2006. First published March 30, 2006; doi:10.1152/japplphysiol.01092.2005.—The purpose of this study was to determine whether short-term exposure to an aqueous extract of hog barn dust increases macromolecular efflux from the intact hamster cheek pouch and, if so, to begin to determine the mechanism(s) underlying this response. By using intravital microscopy, we found that suffusion of hog barn dust extract onto the intact hamster cheek pouch for 60 min elicited a significant, concentration-dependent leaky site formation and increase in clearance of FITC-labeled dextran (molecular mass, 70 kDa). This response was significantly attenuated by suffusion of catalase (60 U/ml), but not by heat-inactivated catalase, and by pretreatment with dexamethasone (10 mg/kg iv) (P < 0.05). Catalase had no significant effects on adenosine-induced increase in macromolecular efflux from the cheek pouch. Suffusion of hog barn dust extract had no significant effects on arteriolar diameter in the cheek pouch. Taken together, these data indicate that hog barn dust extract increases macromolecular efflux from the in situ hamster cheek pouch, in part, through local elaboration of reactive oxygen species that are inactivated by catalase. This response is specific and attenuated by corticosteroids. We suggest that plasma exudation plays an important role in the genesis of upper airway dysfunction evoked by short-term exposure to hog barn dust.

A GROWING BODY OF CLINICAL evidence indicates that short-term exposure of workers and healthy volunteers to hog barn dust is associated with an increase in the concentration of α2-macroglobulin, a robust biomarker of plasma exudation, in the bronchoalveolar lavage fluid of these individuals. However, the mechanism(s) underlying the edemagenic effects of hog barn dust in the upper airway were not investigated in these studies. Hence, the purpose of this study was to begin to address this issue by determining whether short-term exposure to an aqueous extract of hog barn dust increases macromolecular efflux from the intact hamster cheek pouch and, if so, to determine the mechanism(s) underlying this response.

METHODS

General Methods

Preparation of animals. Adult male golden Syrian hamsters weighing 120–130 g were used in these studies as previously described in our laboratory and by other investigators (1, 5, 6, 20–22, 28–30). Each animal was anesthetized with pentobarbital sodium (6 mg/100 g body wt ip). A tracheostomy was performed to facilitate spontaneous breathing. The left femoral vein was cannulated to inject the intra-vascular tracer, fluorescein isothiocyanate-labeled dextran (FITC-dextran; molecular mass, 70 kDa), and supplemental anesthesia (2–4 mg·100 g body wt ⁻¹·h⁻¹). The left femoral artery was cannulated to obtain arterial blood samples and to monitor systemic arterial pressure and heart rate during the experiment. Body temperature was kept constant (37–38°C) during the experiment using a heating pad.

To visualize the microcirculation of the cheek pouch, we used a method previously described in our laboratory and by other investigators (1, 5, 6, 20–22, 28–30). Briefly, the left cheek pouch was spread gently over a small plastic baseplate and an incision was made in the skin to expose the cheek pouch membrane. The avascular connective tissue layer was carefully removed, and a plastic chamber was positioned over the baseplate and secured in place by suturing the skin around the upper chamber. This chamber contained the suffusion fluid. This arrangement forms a triple-layered complex: the baseplate, the upper chamber, and the cheek pouch membrane exposed between both plates. The hamster was then transferred to a heated microscope stage. The chamber was connected through thermally insulated tubing to a reservoir containing warmed (37–38°C) bicarbonate buffer [composition (in mM): 131.9 NaCl, 2.95 KCl, 1.48 CaCl₂, 0.76 MgCl₂, and 11.87 NaHCO₃] that enabled continuous suffusion of the cheek pouch. The buffer was bubbled continuously with 95% N₂:5% CO₂ (pH = 7.4). The temperature of the suffusate in the chamber was checked periodically using a thermistor and kept at 37°C throughout the experiment by adjusting the buffer’s temperature in the reservoir.

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accordingly. The chamber was also connected via a three-way valve to an infusion pump (Sage Instruments, Cambridge, MA) that allowed for constant administration of drugs into the suffusate.

**Determination of clearance of macromolecules.** The cheek pouch microcirculation was visualized with an Olympus microscope (Olympus America, Melville, NY) coupled to a 100-W mercury light source at a magnification of ×40. Fluorescence microscopy was accomplished with the aid of filters that matched the spectral characteristics of FITC-dextran as previously described (1, 6, 20–22, 28–30). Macromolecular leakage was determined by extravasation of FITC-dextran, which appeared as fluorescent “spots” or leaky sites around postcapillary venules (1, 6, 20–22, 28–30). The number of leaky sites was determined by counting three random microscopic fields every minute for the first 7 min and then at 5-min intervals for 30–60 min after each intervention (see **Experimental Protocols**). The total number of leaky sites was averaged and expressed as the number of leaky sites per 0.11 cm² of cheek pouch, which corresponds to an area of one microscopic field.

In experiments in which clearance of FITC-dextran was calculated, the suffusate was collected at 5-min intervals throughout the experiment by a fraction collector (Microfractionator, Gilson Medical Electronics, Middleton, WI). Samples were collected in glass tube tests, and the concentration of FITC-dextran was determined in each tube. Arterial blood samples were collected in heparinized capillary tubes (70-μl volume; Scientific Products, McGaw Park, IL) beginning 5 min before and 5, 30, 60, 120, 180, and 240 min after intravenous injection of FITC-dextran. The concentration of FITC-dextran was determined in all plasma samples as previously described in our laboratory (1, 6, 20, 28–30).

To quantitate the concentration of FITC-dextran in the plasma and suffusate, a standard curve for FITC-dextran concentrations vs. percent emission was generated on a spectrophotofluorometer (Perkin-Elmer, Norwalk, CT). The standard was FITC-dextran prepared on a weight per volume basis. With bicarbonate buffer as background, a standard curve was generated for each experiment, and each curve was subjected to linear regression analysis. The percent emission for unknown samples (plasma and suffusate) was determined by the spectrophotofluorometer, and the concentration of FITC-dextran was then calculated from the standard curve. In preliminary experiments, minimal fluorescence signal (<2% above background) was detected when drugs were added to the buffer and when plasma and suffusate samples were examined before adding FITC-dextran. Clearance of FITC-dextran was determined by calculating the ratio of suffusate (ng/ml) to plasma (mg/ml) concentration of FITC-dextran and multiplying this ratio by the suffusate flow rate (2 ml/min) as previously described (1, 5, 6, 20–22, 28–30).

**Preparation of hog barn dust extract.** Settled surface dust from a large (~500 animals) hog confinement facility in Nebraska was collected in the winter as previously described by one of us (19, 27). It was prepared in a manner similar to that for grain sorghum dust extract as previously described in our laboratory (1, 6). Briefly, an aqueous extract of hog barn dust was prepared by placing 1 g of dust in 10 ml of Hanks’ balanced salt solution without calcium. The suspension was vortexed and allowed to settle for 1 h at room temperature. The suspension was then centrifuged at 1,800 rpm for 5 min, and the supernatant was removed and centrifuged again. The resulting supernatant, designated as 10% hog barn dust extract, was removed and filtered through a 0.22-μm-pore filter, diluted to the desired concentrations in Hanks’ balanced salt solution without calcium, and used immediately (see **Experimental Protocols**). In preliminary studies, we determined that the concentration of endotoxin in 10% hog barn dust extract was 2.8 endotoxin units/ml (0.28 ng/ml; Limulus Amebocyte Lysate Test, Associates of Cape Cod, East Falmouth, MA). Matsuda et al. (20) and Gao et al. (5) showed that this concentration of endotoxin has no significant effects on macromolecular efflux and vasomotor tone in the intact hamster cheek pouch.

**Experimental Protocols**

**Effects of hog barn dust extract on macromolecular efflux.** The purpose of these studies was to determine whether hog barn dust extract increases macromolecular efflux from the intact hamster cheek pouch. After suffusing buffer for 30 min (equilibration period), FITC-dextran was injected intravenously, and the number of leaky sites and clearance of FITC-dextran were determined for 60 min. Then, increasing concentrations of hog barn dust extract (0.1 and 1.0%) were suffused onto the cheek pouch in a nonsystematic fashion. Each concentration was suffused for 60 min. The number of leaky sites was determined before and every minute for 7 min and at 5-min intervals for 60 min thereafter. Clearance of FITC-dextran was determined before and every 5 min thereafter for 60 min. The time interval between subsequent suffusions of hog barn dust extract was at least 45 min (1, 5, 6, 20, 28–30). In preliminary studies, we determined that repeated suffusions of hog barn dust extract (0.1 and 1.0%) were associated with reproducible results. In addition, suffusion of saline (vehicle) for the entire duration of the experiments was not associated with visible leaky site formation or significant increase in clearance of FITC-dextran. The concentrations of hog barn dust extract used in these experiments were based on preliminary studies.

**Effects of catalase on hog barn dust extract-induced responses.** The purpose of these experiments was to determine whether reactive oxygen species mediate, in part, hog barn dust extract-induced increase in macromolecular efflux from the intact hamster cheek pouch (2, 3, 9, 15, 20–22). The experimental design was similar to that outlined above except that catalase (60 U/ml) was now suffused onto the cheek pouch 30 min before and during suffusion of hog barn dust extract (1.0) for 60 min. The number of leaky sites and clearance of FITC-dextran were determined during each intervention as outlined above. In preliminary studies, we determined that suffusion of catalase (60 U/ml) for 90 min was not associated with visible leaky site formation or significant increase in clearance of FITC-dextran (9). The concentrations of catalase used in these experiments were based on preliminary studies.

**Specificity of catalase-induced responses.** To determine the specificity of catalase-induced responses, we utilized two experimental approaches. In the first series of experiments, catalase (60 U/ml) was incubated in saline at 60°C for 15 min before being suffused onto the cheek pouch 30 min before and during suffusion of hog barn dust extract (0.1%) for 60 min as outlined above. In the second series of experiments, catalase (60 U/ml) was suffused 30 min before during and for 30 min after adenosine (10 μM) was suffused for 10 min (1, 7, 10, 11, 24, 30). We chose adenosine because it modulates microvascular responses through a reactive oxygen species-independent mechanism(s) (24, 30, 38).

The number of leaky sites and clearance of FITC-dextran were determined during each intervention as outlined above. In preliminary studies, we found that the concentration of adenosine used in these studies evoked leaky site formation and increased clearance of FITC-dextran from the cheek pouch to a similar extent to that observed with suffusion of 0.1% hog barn dust extract (see **RESULTS**).

**Effects of dexamethasone on hog barn dust extract-induced responses.** The purpose of these studies was to determine whether dexamethasone attenuates hog barn dust extract-induced increase in macromolecular efflux from the intact cheek pouch (1, 4, 32). The experimental design was similar to that outlined above except that dexamethasone (10 mg/kg) was infused intravenously for 30 min before suffusing hog barn dust extract (1.0%) onto the cheek pouch for 60 min. The number of leaky sites and clearance of FITC-dextran were determined during each intervention as outlined above. In previous studies, we found that intravenous administration of dexamethasone (10 mg/kg) alone for 30 min was not associated with a significant decrease in clearance of FITC-dextran (1). In addition, intravenous administration of dexamethasone (10 mg/kg) alone for 30 min had no significant effects on arteriolar diameter (1). The concen-
tration of dexamethasone used in these experiments was based on a previous study in our laboratory (1).

**Effects of hog barn dust extract on arteriolar diameter.** The purpose of these studies was to determine whether suffusion of hog barn dust extract modulates arteriolar diameter in the intact cheek pouch microcirculation. To accomplish this goal, we utilized a technique previously described in our laboratory (1, 5, 10, 11). Briefly, the cheek pouch microcirculation was visualized with an intravital microscope (Nikon, Tokyo, Japan) coupled to a 100-W mercury light source at a magnification of ×40. The microscope image was projected through a low-light television camera (Panasonic TR-124 MA, Matsushita Communication Industrial, Yokohama, Japan) onto a video screen (Panasonic). The inner diameter of second-order arterioles (baseline diameter 42–51 μm) was determined during the experiment from the video display of the microscope image using a video micrometer (model VIA 100, Boeckler Instruments, Tucson, AZ). In each animal, the same arteriolar segment was used to measure vessel diameter during the experiment.

**Drugs and Chemicals**

FITC-dextran, bovine catalase and adenosine were purchased from Sigma-Aldrich (St. Louis, MO). Dexamethasone was obtained from American Regent Laboratories (Shirley, NY). Hanks’ balanced salt solution without calcium was purchased from Biosource International (Camarillo, CA). All drugs were prepared and diluted in saline to the desired concentrations on the day of each experiment.

**Data and Statistical Analyses**

Data are expressed as means ± SE. Because the number of leaky sites returned to baseline (nil) between successive applications of test compounds, all vehicle (saline) control data are expressed as a single value for each experimental condition. When a test compound was suffused onto the cheek pouch, the maximal change in arteriolar diameter was measured and compared with baseline diameter as previously described in our laboratory (1, 5, 10, 11). Statistical analysis was performed on actual values using repeated-measures analysis of variance with Newman-Keuls multiple range post hoc test to detect values that were different from control values. *P < 0.05 was considered statistically significant; n is given as the number of experiments, with each experiment representing a separate animal.

**RESULTS**

Mean arterial pressure was 98 ± 4 mmHg at the beginning and 95 ± 3 mmHg at the conclusion of the experiments (n = 42 animals; P > 0.5). Heart rate was 311 ± 5 beats/min at the beginning and 306 ± 7 beats/min at the conclusion of the experiments (n = 42 animals; P > 0.5).

**Effects of Hog Barn Dust Extract on Macromolecular Efflux**

Suffusion of hog barn dust extract induced a significant concentration-dependent increase in leaky site formation and clearance of FITC-dextran (Fig. 1; each group, n = 4 animals; *P < 0.05). The number of leaky sites increased significantly from nil during suffusion of saline (vehicle) to 8 ± 2/0.11 and 26 ± 3/0.11 cm² during suffusion of 0.1 and 1.0% hog barn dust extract, respectively (Fig. 1, top, each group, n = 4 animals; *P < 0.05). Similarly, clearance of FITC-dextran increased significantly from 11 ± 2 ml/min × 10^-6 during suffusion of saline (vehicle) to 34 ± 9 ml/min × 10^-6 and 84 ± 16 ml/min × 10^-6 during suffusion of 0.1 and 1.0% hog barn dust extract, respectively (Fig. 1, bottom; each group, n = 4 animals; *P < 0.05). Leaky sites were visible within 15 min of initiating hog barn dust extract suffusion, reached a maxi-

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Fig. 1. Effects of suffusion of hog barn dust extract (HBDE) for 60 min on leaky site formation (top) and clearance of FITC-dextran (bottom) from the hamster cheek pouch. Values are means ± SE; each group, n = 4 animals. *P < 0.05 compared with control. #P < 0.05 compared with 0.1% HBDE. Maximum 35–40 min thereafter, and were no longer visible 15 min after suffusion was stopped.

**Effects of Catalase on Hog Barn Dust Extract-Induced Responses**

Suffusion of catalase (60 U/ml) significantly attenuated hog barn dust extract (1.0%)-induced leaky site formation and increase in clearance of FITC-dextran from the cheek pouch (Fig. 2; each group, n = 4 animals; *P < 0.05). The number of leaky sites decreased significantly from 26 ± 3/0.11 cm² during suffusion of hog barn dust extract (1%) to 9 ± 1/0.11 cm² during suffusion of hog barn dust extract and catalase (Fig. 2, top; each group, n = 4 animals; *P < 0.05). Similarly, clearance of FITC-dextran decreased significantly from 84 ± 16 ml/min × 10^-6 during suffusion of hog barn dust extract (1.0%) to 45 ± 12 ml/min × 10^-6 during suffusion of hog barn dust extract and catalase (Fig. 2, bottom; each group, n = 4 animals; *P < 0.05).

**Specificity of Catalase-Induced Responses**

Suffusion of heated catalase (60 U/ml) had no significant effects of hog barn dust extract (0.1%)-induced leaky site

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formation and increase in clearance of FITC-dextran from the cheek pouch (Fig. 3; each group, \( n = 4 \) animals; \( P < 0.05 \)).

**Effects of Hog Barn Dust Extract on Arteriolar Diameter**

Suffusion of hog barn dust extract (1.0%) had no significant effects of arteriolar diameter throughout the 90-min suffusion period (1 ± 1% change from arteriolar diameter at baseline; \( n = 4 \) animals; \( P > 0.5 \)).

**DISCUSSION**

There are three new findings of this study. First, we found that an aqueous extract hog barn dust elicits a significant concentration-dependent increase in macromolecular efflux from the intact hamster cheek pouch (Fig. 1). These effects were not related to nonspecific damage to postcapillary venular endothelium because FITC-dextran efflux returned to baseline once suffusion of hog barn dust extract was stopped. Second,
Hog barn dust extract-induced increase in macromolecular efflux was mediated, in part, by local elaboration of reactive oxygen species because catalase, an enzyme that catalyzes conversion of hydrogen peroxide to water and oxygen (2, 3, 9, 15, 20), significantly attenuated this response (Fig. 2). The salutary effects of catalase were specific because heat-inactivated catalase had no significant effects on hog barn dust extract-induced responses, and because catalase had no significant effects of adenosine-induced increase in macromolecular efflux from the cheek pouch (Figs. 3 and 4). We chose adenosine because it modulates microvascular responses through a reactive oxygen species-independent mechanism(s) (7, 24, 30, 38).

Last, dexamethasone, a potent anti-inflammatory drug (1, 4, 32), attenuated hog barn dust extract-induced increase in macromolecular efflux (Fig. 5). These data are consistent with the study of Akhter and his colleagues (1), who showed that dexamethasone attenuates grain sorghum dust extract-induced increase in macromolecular efflux from the cheek pouch.

In addition, they showed that dexamethasone had no significant effects on adenosine-induced responses and on arteriolar diameter in the cheek pouch (1). Collectively, these data indicate that an aqueous extract of hog barn dust increases macromolecular efflux from the intact hamster cheek pouch, in part, through local elaboration of reactive oxygen species in a specific fashion and that this response is attenuated by corticosteroids. We suggest that plasma exudation elicited by reactive oxygen species plays an important role in the genesis of upper airway dysfunction evoked by short-term exposure to hog barn dust (12–14, 17, 18, 25, 26, 35).

Our laboratory has previously shown that grain sorghum dust extract elicits neurogenic plasma exudation in the intact hamster cheek pouch and that dexamethasone attenuates this response (6). These data coupled with the results of this study suggest that different organic dusts present in the agricultural environment activate distinct dexamethasone-responsive inflammatory pathways in the upper airway, leading to plasma...
exudation, interstitial edema, and tissue dysfunction (1, 4, 6, 25, 32). The mechanism(s) underlying the antiedemogenic effects of dexamethasone during short-term exposure to hog barn and grain sorghum dust extracts remains to be determined.

The results of this study support and extend those reported by Ek et al. (4) and Vesterberg et al. (33). They showed that short-term exposure of healthy volunteers to hog dust is associated with plasma exudation in the upper and lower respiratory tract, respectively. However, the mechanisms underlying this response were not elucidated in these studies. We found that local elaboration of reactive oxygen species inactivated by catalase mediates, in part, the edemogenic effects of hog barn dust extract in the intact hamster cheek pouch. We did not attempt to quantify overall production of reactive oxygen species during exposure to hog barn dust extract nor to identify their cellular origin(s) in the cheek pouch (2, 3, 9, 20–22, 38). Rather, our goal was to probe their proinflammatory effects in the microcirculation during this intervention. Whether reactive oxygen species play a role in the host inflammatory response to hog barn dust in the human upper airway and, if so, which reactive species are involved merits further investigation.

The above notwithstanding, the salutary effects of dexamethasone on hog barn dust extract-induced responses in the intact cheek pouch are consistent with those reported by Ek et al. (4), who showed that intranasal administration of fluticasone, a potent corticosteroid, attenuates plasma exudation evoked by topical application of hog barn dust to the nose of healthy individuals. Clearly, further studies to determine the effects of corticosteroids on elaboration of reactive oxygen species in the upper airway of humans during short-term exposure to hog barn dust are warranted.

The hamster cheek pouch is a well-established animal model used in our laboratory and by other investigators to study the effects of environmental toxicants and inflammatory mediators, such as grain sorghum dust and reactive oxygen species, on macromolecular efflux from the microcirculation in situ and mechanisms underlying these phenomena (1–3, 6–9, 20–22, 28–31). Solute efflux emanates from postcapillary venules and is determined by two reproducible parameters, leaky site formation and clearance of FITC-dextran, thereby providing quantitative appraisal of macromolecular transport across postcapillary venules in the cheek pouch during experimental interventions. Importantly, successive suffusions of test compounds, such as aqueous extracts of grain sorghum and hog barn dust (1, 6), at appropriate time intervals are associated with reproducible formation of leaky sites and increases in clearance of FITC-dextran in the absence of tachyphylaxis. Consequently, changes in macromolecular efflux can be tested repeatedly in the same cheek pouch so that each animal serves as its own control. This, in turn, reduces the overall number of animals required to conduct the study and facilitates data analysis.

Conceivably, the increase in macromolecular efflux elicited by hog barn dust extract may have been mediated, in part, by changes in vasomotor tone and/or increase in venular driving pressure in the cheek pouch (1, 6–8, 20–23, 28–31, 37). However, this possibility seems unlikely because we found that suffusion of the extract had no significant effects of arteriolar diameter in the cheek pouch throughout the observation period. In addition, other investigators showed that agonist-induced increases in macromolecular efflux from postcapillary pressure in the hamster cheek pouch and other microvascular beds are independent of changes in vasomotor tone and increase in venular driving pressure (8, 23, 31, 37).

The component(s) in hog barn dust extract that stimulates resident and/or migrant cells in the cheek pouch to elaborate reactive oxygen species was not identified in this study. Dissecting this component(s) would require intensive analysis of this complex organic material. Nonetheless, current concepts suggest that endotoxin is an important inflammatory stimulus in organic dusts, including hog barn dust (12–14, 16–18, 25, 35). To this end, Matsuda et al. (20) showed that antioxidants attenuate endotoxin-induced increase in macromolecular efflux from the intact hamster cheek pouch. In addition, Gao et al. (5) showed that allopurinol, a scavenger of reactive oxygen species, attenuates vasodilation evoked by endotoxin in this preparation. However, the concentration of endotoxin suffused onto the cheek pouch in these studies was in the submilligram range, whereas the concentration of endotoxin detected in hog barn dust extract used in this study was very low, 0.28 ng/ml. The latter has no effects on macromolecular efflux from the cheek pouch, implying that endotoxin is an unlikely candidate to mediate hog barn dust extract-induced increase in macromolecular efflux in the upper airway mucosa. Separation of materials present in the aqueous extract of hog barn by molecular weight distribution and testing them in the cheek pouch could represent an attractive experimental approach to address this issue in future experiments planned for this research project. Irrespective of the offending component(s) in hog barn dust, these data suggest that antioxidants may be beneficial in the treatment of plasma extravasation evoked by hog barn dust in the upper airway mucosa. Additional studies are indicated to support or refute this hypothesis.

In summary, we found that hog barn dust extract increases macromolecular efflux from the in situ hamster cheek pouch, in part, through local elaboration of reactive oxygen species that are inactivated by catalase. This response is specific and attenuated by corticosteroids. We suggest that plasma exudation plays an important role in the genesis of upper airway dysfunction evoked by short-term exposure to hog barn dust and that corticosteroids abates this process.

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