

Effect of a topical corticosteroid on airway hyperresponsiveness and eosinophilic inflammation induced by trimellitic anhydride exposure in sensitized guinea pigs

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Background: Topical corticosteroids are effective in the treatment of asthma by improving bronchial hyperresponsiveness and reducing airway inflammation.

Methods: We assessed the effect of a nebulized corticosteroid, budesonide, on airway hyperresponsiveness and inflammatory response provoked by inhalation of trimellitic anhydride (TMA) dust, a known cause of occupational asthma in human beings, in guinea pigs sensitized to the free hapten. Male Dunkin-Hartley guinea pigs ($n = 24$) were injected intradermally with 0.1 ml of 0.3% TMA in corn oil, followed by exposure 21 to 28 days later to five consecutive doses of budesonide aerosol (0.5 mg/ml) or saline solution, administered for 10 minutes every 12 hours. They were then exposed (noses only) to TMA dust (8 mg/m³) or air for 1 hour (four groups, $n = 6$ in each). Airway responsiveness to acetylcholine, defined as the concentration needed to cause a 200% increase in lung resistance (PC_{200}), was measured 8 hours later.

Results: In saline-treated guinea pigs exposed to TMA, mean PC_{200} was 0.094 mmol/L (geometric SEM, 1.4 mmol/L) compared with 0.31 mmol/L (geometric SEM, 1.3 mmol/L, $p < 0.05$) in those guinea pigs pretreated with budesonide. In sham-exposed sensitized guinea pigs, PC_{200} was 0.35 mmol/L (geometric SEM, 1.2 mmol/L), which was not significantly different from the budesonide-treated group (0.36 mmol/L; geometric SEM, 1.3 mmol/L). There was a significant increase in the number of eosinophils in the subepithelium of guinea pigs further exposed to TMA dust (71.5 ± 6.8 cells/unit area [mean $\pm$ SEM]) compared with those exposed to air (22.7 ± 6.7 , $p < 0.01$). Budesonide did not inhibit the number of subepithelial eosinophils of guinea pigs exposed to TMA dust (54.0 ± 3.7 cells/unit area) or in those exposed to air (24.3 ± 6.7 cells/unit area) and did not affect the increase in eosinophils found in bronchoalveolar fluid.

Conclusions: Budesonide significantly inhibited the increase in airway responsiveness but not the eosinophilic inflammation induced by exposure to TMA dust in sensitized guinea pigs. (J ALLERGY CLIN IMMUNOL 1993;92:450-6.)

Key words: Trimellitic anhydride, airway hyperresponsiveness, sensitization, guinea pig, corticosteroids, budesonide

Trimellitic anhydride (TMA) is a reactive chemical widely used in the paint and plastics industries as a curing agent for epoxy resins. Exposure to TMA in the workplace may result in the development of occupational asthma,¹ which

Abbreviations used

BAL:	Bronchoalveolar lavage
GPISA:	Guinea pig serum albumin
PBS:	Phosphate-buffered saline
PC_{200} :	Concentration of acetylcholine required to induce a 200% increase in R_L
R_L :	Lung resistance

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has most often been associated with a specific IgE antibody response.² Occupational asthma caused by low molecular weight chemicals, including the acid anhydrides, is characterized by airway hyperresponsiveness, and specific inhalation tests in

sensitized individuals can provoke changes in airway calibre and in nonspecific airway responsiveness.³

We have previously shown that intradermal injections of TMA in the guinea pig may induce both IgE and IgG immune responses to TMA conjugated to guinea pig serum albumin (GPSA)⁴ and that intratracheal TMA-GPSA in sensitized guinea pigs induces both bronchoconstriction and airway microvascular leakage suitable for pharmacologic modulation.⁵ We have also shown that further exposure of sensitized guinea pigs to TMA dust for short periods is accompanied by an increase in airway responsiveness and a predominantly eosinophilic infiltrate into the airways 8 hours after exposure.⁶

Topical corticosteroids are the most useful antiinflammatory agents used in the treatment of asthma. They improve airway hyperresponsiveness in asthma and prevent allergen-induced late-phase responses and the associated increase in airway hyperresponsiveness. In addition, topical steroids reduce the number of eosinophils and lymphocytes and mast cells in bronchial biopsy specimens, with restoration of epithelial damage in patients with asthma.⁷⁻⁹ We therefore investigated whether the topical corticosteroid budesonide could inhibit TMA-induced airway hyperresponsiveness and eosinophilic inflammation in a guinea pig model.

METHODS

Sensitization

Twenty-four male Dunkin-Hartley guinea pigs (which weighed 200 to 250 gm) were injected once intradermally with 0.1 ml of 0.3% TMA (Aldrich, Gillingham, England) suspended in corn oil.

Protocol

The guinea pigs were divided into four groups ($n = 6$ in each group) and were exposed to one of the following: saline pretreatment followed by air exposure (group 1), budesonide pretreatment followed by air exposure (group 2), saline pretreatment followed by TMA dust exposure (group 3), or budesonide pretreatment followed by TMA dust exposure (group 4). The primary aim of the study was to assess the effect of budesonide on TMA-induced hyperresponsiveness and eosinophilic inflammation (i.e., group 3 vs group 4). However, one had to ascertain any potential effect of budesonide on baseline airway responsiveness and eosinophilic inflammation, hence, the comparison of group 1 with group 2.

Preparation of TMA conjugate

TMA was conjugated to GPSA as follows. Four aliquots of 250 mg GPSA (Sigma, Poole, England) were

mixed with 50 ml of 9% NaHCO_3 on ice, and 250 mg of ground TMA dust was added and allowed to stand for 10 minutes. Each aliquot was stirred separately for 1 hour. The aliquots were pooled and spun for 10 minutes at 600 g. The supernatant was separated and dialyzed with 0.02 mol/L NH_4HCO_3 for 48 hours with six changes. This was further dialyzed with distilled water for 24 hours and lyophilized. The degree of substitution was assessed by ultraviolet spectrophotometry. Briefly, 1.0 mg TMA-GPSA was added to 1 ml of 0.1 mol/L borate buffer at pH 9.3. A 0.03 mol/L solution of 2,4,6-trinitrobenzenesulfonic acid (Aldrich) in borate buffer was prepared. Then, 25 μl of 2,4,6-trinitrobenzenesulfonic acid solution was added to 1 ml of the TMA-GPSA solution and incubated for 20 minutes at room temperature. The sample was read on the spectrophotometer at an optical density of 420 nm. The substitution ratio for TMA-GPSA was 21:1.

ELISA for assessment of IgG₁ antibodies

Plates were coated with 5 $\mu\text{g/ml}$ TMA-GPSA in coating buffer and incubated overnight at 4° C. On the next day the plates were washed four times in phosphate-buffered saline (PBS)-Tween. Serum was diluted to 1:50 solution, and plates were coated with PBS-Tween. Serum was added in doubling dilutions across the plate and incubated for 3 hours at 27° C. The plates were again washed four times with PBS-Tween, and rabbit anti-guinea pig IgG₁ (ICN Biomedicals Ltd, High Wycombe, Bucks, England), diluted to 1:2500, was added and incubated for 2 hours at 27° C. The plates were again washed with PBS-Tween. Anti-rabbit IgG-peroxidase (ICN Biomedical Ltd.) was added at 1:5000 dilution and incubated at 27° C for 2 hours. Finally, the plates were washed in PBS-Tween and chromogen was added. The reaction was stopped after 6 minutes with 1 mol/L H_2SO_4 and read at an optical density of 492 nm. The titers of IgG₁ were calculated from the last dilution that was double the control sera.

TMA dust exposure

On days 21 to 28, guinea pigs were placed in a conical container with their nostrils protruding, fixed in an exposure chamber with an internal volume of 30 L, and exposed to 8 mg/m³ of inhalable dust or air for 60 minutes. The dust was generated by passing air (12 L/min) through a Wright's dust feeder (ADG, Coticote, Herts, U.K.) above the chamber. Dust levels were measured before and during exposure gravimetrically by extracting air samples with a dust sampler (AFC 123, Caselle London Ltd., Bedford, England) onto fiber glass filters (Whatman, Maidstone, England) at 2 L/min for 5 minutes. Particle size was confirmed by a cascade impactor (Marple, Andersen Samples Ltd., Atlanta, Ga.).

Administration of budesonide

Conscious guinea pigs were placed in a Perspex box (6000 cm³) and exposed to nebulized budesonide (2 ml, 0.5 mg/ml) or saline solution for 10 minutes every 12

hours five consecutive times. The aerosol was generated by passing air (6 L/min) through a jet nebulizer (Acorn, Medic-Aid, Sussex, England) and was sampled (flow rate = 120 ± 5 ml/min; mean $\pm$ SEM) with a low flow sampler (Universal flow sample pump, model 224-PCXR3, SKC Inc., Pa.). The amount of budesonide on the filters was analyzed by high-performance liquid chromatography as previously described.¹⁰ The lung burden of budesonide was calculated.¹⁰

Measurement of lung resistance and airway responsiveness

On days 21 to 28, guinea pigs were anesthetized with pentobarbital 60 mg/ml, administered intraperitoneally. A tracheal cannula (10 mm in length with a 2.7 mm internal diameter) was inserted into the lumen of the trachea through a tracheostomy. A polyethylene catheter was inserted into the left carotid artery to monitor blood pressure with a pressure transducer (PDCR 75 S/N 1572, Druck, U.K.). Transpulmonary pressure was measured with a pressure transducer (model FCO 40; ± 1000 mm H₂O, Furness Controls Ltd., Bexhill, Sussex, England) with one side attached to a catheter inserted into the right pleural cavity and the other side attached to a catheter connected to a side port of the intratracheal cannula. The ventilatory circuit had a total volume of 20 ml. Airflow was measured with a pneumotachograph (model F1L; Mercury Electronics Ltd., Glasgow, Scotland) connected to a transducer (model FCO 40; ± 20 mm H₂O; Furness Controls Ltd.). The signals from the transducers were digitalized with a 12-bit analog-digital board (NB-MIO-16, National Instruments, Austin, Texas) connected to a Macintosh II computer (Apple Computer Inc., Cupertino, Calif.) and analyzed with software (Labview, National Instruments), which was programmed to instantaneously calculate lung resistance (R_L) by the method of von Neergaard and Wirz, 1927.¹¹ Transpulmonary pressure and mean blood pressure were also monitored throughout the experiment. Acetylcholine (Sigma) was administered in increasing half log concentrations via an ultrasonic nebulizer (DeVilbiss Health Care, Inc., Somerset, Pa.). The concentration of acetylcholine required to induce a 200% increase in R_L (PC_{200}) was used as an index of airway responsiveness. Blood samples were taken from all guinea pigs for measurement of IgG₁ antibodies to TMA-GPSA.

Bronchoalveolar lavage

At the end of each experiment bronchoalveolar fluid was collected. Guinea pig lungs were instilled with 3 aliquots of 5 ml each of RPMI 1640 solution (Gibco, Grand Island, N.Y.) and placed on ice. The samples were centrifuged at 500 g for 10 minutes at 4° C. The supernatant was drained, and PBS (2 ml) was added. The suspension was shaken gently until cells were fully suspended. Kimura stain (90 μ l) was added to a sample of the suspension (10 μ l). Cells were counted in a

Neubauer chamber (Neubauer GmbH, Neuss, Germany), and suspension was further diluted to produce a count of 2 million cells/ml. Differential cell counts were made from cytopsin preparations stained by May-Grünwald stain. Microscopic fields were chosen randomly, and a total of 500 cells were counted for each slide. The total number of eosinophils, macrophages, neutrophils, and lymphocytes in bronchoalveolar lavage (BAL) was calculated from the percentage found in the sample.

Histopathologic studies

At the end of the experiment, the guinea pigs were killed. The lungs were inflated with 4% formaldehyde, and the trachea was tied. Lung tissue was fixed in formaldehyde 4%, sectioned (3 μ m sections), and stained with carbol-chromotrope to demonstrate eosinophil granules. The slides were coded and read. In order to quantify the number of eosinophils in the airways, a section of tissue from the right or left main bronchus (chosen randomly) and a similar section diametrically opposite were selected. Eosinophils were counted within a given area with the use of a graticule (area 56 mm²; magnification $\times 400$), which was positioned from the epithelium extending into the subepithelial layers. At the same time mononuclear cells and polymorphonuclear cells were also counted.

Statistical analysis

Data are expressed as means $\pm$ SEM. PC_{200} measurements were log-transformed, and one-way analysis of variance was calculated to determine whether there was any significant effect of any treatment. Unpaired *t* tests were used to determine any significant difference between the groups. Histologic and BAL data were analyzed with Kruskal-Wallis and multiple Mann-Whitney U tests. A *p* value of less than 0.05 was considered significant. Spearman rank correlation coefficient was used to assess the relationship between the numbers of eosinophils in the subepithelium and bronchoalveolar fluid of all groups and the relationship between airway responsiveness and the numbers of cells in the subepithelium and BAL fluid of untreated guinea pigs exposed to TMA or air.

RESULTS

All guinea pigs injected with intradermal TMA had specific IgG₁ antibodies to TMA-GPSA (titer range, 1:800 to 1:12,800). Budesonide-treated animals had levels of specific IgG₁ antibodies that were similar to those of sham-treated animals. The mean lung burden of budesonide randomly sampled from 18 exposures were 2.92 ± 0.12 μ g/kg.

Mean baseline R_L was not significantly different between the groups (group 1: 0.41 ± 0.05 cm H₂O/ml⁻¹/sec⁻¹; group 2: 0.34 ± 0.03 cm H₂O/ml⁻¹/sec⁻¹; group 3: 0.36 ± 0.06 cm

$\text{H}_2\text{O}/\text{ml}^{-1}/\text{sec}^{-1}$ and group 4: $0.31 \pm 0.03 \text{ cm H}_2\text{O}/\text{ml}^{-1}/\text{sec}^{-1}$). There was a significant decrease in PC_{200} to acetylcholine in guinea pigs exposed to TMA dust (mean, 0.094 mmol/L ; geometric SEM 1.4 mmol/L) when compared with guinea pigs exposed to saline solution and air (mean, 0.35 mmol/L ; geometric SEM, 1.2 mmol/L) ($p < 0.05$). In guinea pigs pretreated with nebulized budesonide and exposed to TMA dust, PC_{200} was not significantly different from that of animals exposed to saline solution and air (mean, 0.31 mol/L ; geometric SEM, 1.3 mmol/L) ($p < 0.05$) and from that of guinea pigs treated with budesonide but not exposed to TMA (mean, 0.36 mol/L ; geometric mean, 1.3 mmol/L) (Fig. 1).

There was a significant increase in the number of eosinophils in the subepithelium (71.5 ± 6.8 eosinophils per 56 mm^2) and BAL ($3.5 \pm 0.52 \times 10^6$ eosinophils) ($p < 0.01$) of guinea pigs exposed to TMA compared with guinea pigs exposed to air only. Pretreatment with nebulized budesonide did not significantly affect the increase in the number of eosinophils in the subepithelium (Fig. 2) or BAL (Fig. 3). Budesonide did not affect the number of eosinophils in guinea pigs that were not exposed to TMA dust. There was no significant increase in the numbers of macrophages, lymphocytes, or neutrophils in the BAL fluid and no significant increase of mononuclear or polymorphonuclear cells in the subepithelium of guinea pigs exposed to TMA compared with those exposed to air alone.

There was a significant correlation between PC_{200} measurements and the number of eosinophils in the subepithelium ($r = 0.66$; $p < 0.05$) and BAL fluid ($r = 0.68$; $p < 0.05$) of the non-treated groups exposed to TMA or air. There was a significant correlation ($r = 0.67$; $p < 0.005$) between the numbers of eosinophils in the subepithelium and BAL fluid among all groups.

DISCUSSION

We have confirmed that guinea pigs injected intradermally with TMA in corn oil have specific antibody responses and that further exposure to TMA dust for short periods induces a mean twofold increase in airway responsiveness, associated with an increase in eosinophils in the airway submucosa and BAL fluid. The increase in airway responsiveness is inhibited by pretreatment with repeated doses of nebulized budesonide; however, it did not affect the increase in eosinophils after TMA exposure. Nebulized budesonide also did not affect the number of eosinophils in the sub-

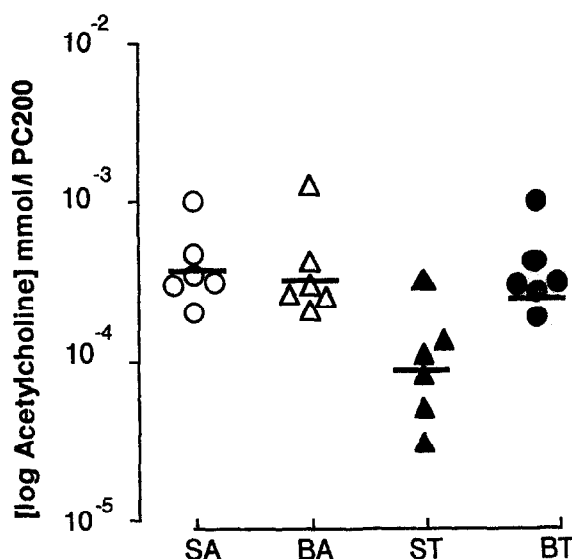


FIG. 1. Effect of TMA dust on airway responsiveness as measured by PC_{200} to acetylcholine in guinea pigs pretreated with budesonide (●) compared with those pretreated with saline solution (Δ). Airway responsiveness in guinea pigs pretreated with budesonide and sham-exposed to air (Δ) did not differ from that of guinea pigs pretreated with saline (○). SA, Saline/air; BA, budesonide/air; ST, saline/TMA; BT, budesonide/TMA. Horizontal bars indicate mean values. * $p < 0.05$ compared with guinea pigs pretreated with budesonide and exposed to TMA.

epithelium or BAL or the baseline airway responsiveness of sensitized guinea pigs.

Guinea pig airways have been considered resistant to the effects of steroids, and high doses of systemic corticosteroids (dexamethasone 4 mg/kg) have been required to attenuate bronchial anaphylactoid responses in sensitized guinea pigs.¹² Andersson and Brattsand¹³ demonstrated that systemic pretreatment with budesonide (5 mg/kg administered intraperitoneally) inhibited the immediate airway response in guinea pigs with demonstrable IgE antibodies but not with IgG antibodies alone. In ovalbumin-sensitized rats, the immediate response to ovalbumin was inhibited by intratracheal or intraperitoneal administration of budesonide at the high dose of 1 mg/kg given 5 hours or more before challenge.¹⁴ Similar results were obtained with budesonide aerosol in these rats, with a lung burden of $950 \text{ } \mu\text{g/kg}$,¹⁰ which far exceeds the aerosol dose used in our study. Although we did not measure the early acute response, it is unlikely that the low dose of budesonide caused inhibition of any acute response to TMA exposure. In addition, a larger dose of budesonide did not inhibit the early acute re-

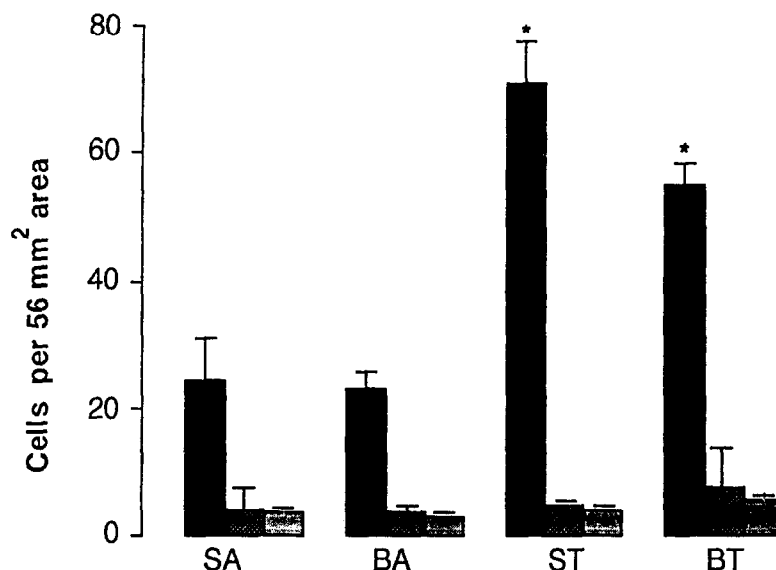


FIG. 2. Mean eosinophil (■), mononuclear (▨) and neutrophil (▧) counts in the subepithelium of guinea pigs sensitized to TMA. Guinea pigs were exposed to either saline solution or budesonide, followed by either sham exposure with air or TMA dust exposure. There was a significant increase in eosinophils alone in the TMA-exposed groups, and budesonide had no effect on eosinophil infiltration. SA, Saline/air; BA, budesonide/air; ST, saline/TMA; BT, budesonide/TMA. * $p < 0.05$ compared with guinea pigs exposed to air.

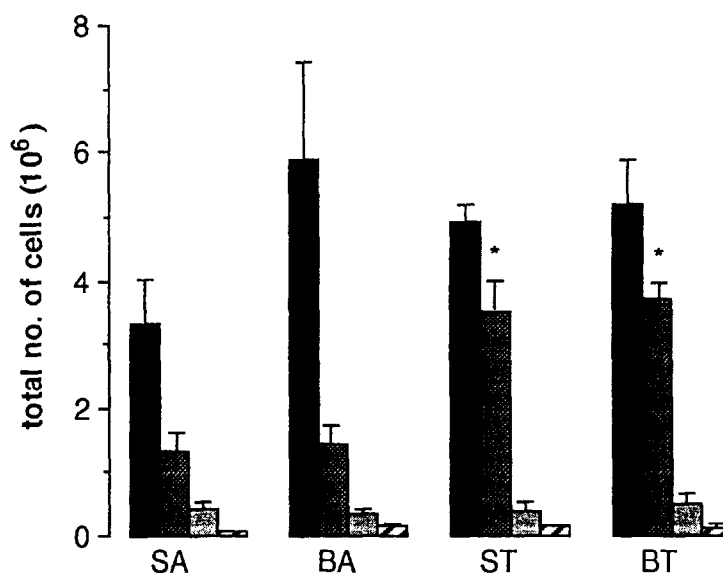


FIG. 3. Mean alveolar macrophage (■), eosinophil (▨), lymphocyte (▧), and neutrophil (▩) counts in BAL fluid from guinea pigs sensitized to TMA. Guinea pigs were exposed to either saline solution or budesonide, followed by either sham exposure with air or TMA dust exposure. There was a significant increase in eosinophils alone in the TMA-exposed groups, and budesonide had no effect on eosinophil infiltration. SA, Saline/air; BA, budesonide/air; ST, saline/TMA; BT, budesonide/TMA. * $p < 0.01$ compared with guinea pigs exposed to air.

sponse to ovalbumin in ovalbumin-sensitized guinea pigs. In our study budesonide did not inhibit IgG₁ antibodies to TMA.

The doses of budesonide that were used in the current experiment are of a similar order to those

used in patients with asthma and are those that have been reported to significantly reduce airway hyperresponsiveness to histamine and bradykinin.¹⁵ Inhaled corticosteroids, in various animal models and in subjects with allergic asthma have

been shown to be effective in inhibiting allergen-induced airway hyperresponsiveness.¹⁶⁻¹⁸ Andersson et al.¹⁹ demonstrated that pretreatment with a single dose of budesonide (5 mg/kg) instilled intratracheally 6 to 20 hours before ovalbumin challenge in sensitized guinea pigs did not inhibit eosinophil recovery in BAL fluid, although the late-phase response and increased airway microvascular permeability were significantly reduced. These results are in agreement with those of the present study. However, other studies have shown that systemic corticosteroids can inhibit eosinophil influx into the lungs.^{20, 21} In one of these studies,²¹ although eosinophil recruitment was inhibited by systemic corticosteroids, ovalbumin-induced airway hyperresponsiveness was not. In sensitized Brown-Norway rats, Elwood,²² using a similar dose regimen of budesonide, was able to demonstrate both an inhibition of allergen-induced airway hyperresponsiveness and eosinophilia. Our data suggest that in the guinea pig the inhibition of eosinophil recruitment in the airways may be more resistant to the effects of steroids than the induction of airway hyperresponsiveness. The ability of these eosinophils to release mediators may be impaired by corticosteroids. In a recent study, Adelroth et al.²³ showed that although a 4-week treatment with inhaled budesonide did not alter the number of eosinophils in BAL fluid from patients with asthma, the level of eosinophil cationic protein, a specific product of eosinophils, was significantly reduced. Thus we postulate that topical budesonide, although it does not prevent eosinophil recruitment, may be inhibiting eosinophil activation and thereby airway hyperresponsiveness. The persistence of eosinophilia during inhibition of airway hyperresponsiveness has also been described in sensitized and exposed guinea pigs after pretreatment with capsaicin.²⁴

The sensitivity of TMA-induced bronchial hyperresponsiveness to inhaled corticosteroids further reinforces the model as a valid one for occupational asthma. Both orally administered and inhaled corticosteroids have been shown to inhibit the increase in airway responsiveness that occurs after specific bronchial provocation tests in patients with occupational asthma caused by toluene diisocyanate.^{25, 26} Further studies are needed to examine the mechanisms underlying TMA-induced bronchial hyperresponsiveness in our occupational model and those of corticosteroid-induced inhibition of airway hyperresponsiveness.

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REFERENCES

1. Fawcett JW, Newman Taylor AJ, Pepys J. Asthma due to inhaled chemical agents-epoxy resin systems containing phthalic anhydride, trimellitic anhydride and triethylene tetramine. *Clin Allergy* 1977;7:1-14.
2. Zeiss CR, Patterson R, Pruzansky JJ, Miller MM, Rosenberg M, Levitz D. Trimellitic-anhydride induced airway syndromes: clinical and immunologic syndromes. *J ALLERGY CLIN IMMUNOL* 1977;60:96-103.
3. Chan-Yeung M, Lam S. Occupational asthma. *Am Rev Respir Dis* 1986;133:686-703.
4. Hayes JP, Daniel R, Tee RD, Barnes PJ, Chung KF, Newman Taylor AJ. Specific immunological and bronchopulmonary responses to a protein conjugate of trimellitic anhydride in guinea pigs sensitised to the free hapten. *Clin Exp Allergy* 1992;22:694-700.
5. Hayes JP, Lotvall JO, Barnes PJ, Newman Taylor AJ, Chung KF. Involvement of inflammatory mediators in airway responses to trimellitic anhydride in sensitised guinea pigs. *Br J Pharmacol* 1992;106:828-32.
6. Hayes JP, Daniel R, Tee RD, Barnes PJ, Newman Taylor AJ, Chung KF. Bronchial hyperreactivity after inhalation of trimellitic anhydride dust in guinea pigs following intradermal sensitization to the free hapten. *Am Rev Respir Dis* 1992;146:1311-4.
7. Jeffery PK, Godfrey RW, Adelroth E, Nelson F, Rogers A, Johansson S-A. Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma. *Am Rev Respir Dis* 1992;145:890-9.
8. Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a β_2 agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind, parallel-group controlled trial. *J ALLERGY CLIN IMMUNOL* 1992;90:32-41.
9. Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, Howarth PH, Holgate ST. Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. *Am Rev Respir Dis* 1992;145:669-74.
10. Dahlback M, Brattsand R. Antigen-induced bronchial anaphylaxis in actively-sensitized SD rats: effects of local treatment with anti-asthma drugs. *Allergy* 1986;41:594-602.
11. von Neergaard K, Wirz K. Die Messung der Strömungswiderstände in den Atemwegen des Menschen, insbesondere bei Asthma und Emphysem *Z Klin Med* 1927;105: 51-82.
12. Hicks R. The influence of corticosteroid pretreatment on anaphylactic bronchoconstriction in the guinea pig. *J Pharm Pharmacol* 1970;22:170-9.
13. Andersson P, Brattsand R. Protective effects of the glucocorticoid, budesonide on lung anaphylaxis on actively sensitized guinea pigs: inhibition of IgE- but not IgG-mediated anaphylaxis. *Br J Pharmacol* 1982;76:139-47.
14. Dahlback M, Bergstrand H, Brattsand R. Antigen-induced bronchial anaphylaxis in actively-sensitized SD rats: effects of glucocorticoid treatment. *Allergy* 1986;41:210-9.

15. Fuller RW, Choudry NB, Eriksson G. Action of budesonide on asthmatic bronchial hyperresponsiveness. *Chest* 1991;100:670-4.
16. Abraham WM, Lanes S, Stevensen JS, Yerger LD. Effect of an inhaled glucocorticoid (budesonide) on post-antigen induced increases in airway responsiveness. *Clin Respir Physiol* 1986;22:387-92.
17. Elwood W, Lotvall JO, Barnes PJ, Chung KF. Effect of dexamethasone and cyclosporin A on allergen-induced airway hyperresponsiveness and inflammatory cell responses in sensitized Brown-Norway rats. *Am Rev Respir Dis* 1992;145:1289-94.
18. Cockcroft DW, Murdoch DY. Comparative effects of inhaled salbutamol, sodium chromoglycate, and beclomethasone dipropionate on allergen-induced early responses, late responses and increased bronchial hyperresponsiveness to histamine. *J ALLERGY CLIN IMMUNOL* 1987;79:734-40.
19. Andersson P, Brange C, von Kogeler B, Sonmark B, Stahne G. Effect of glucocorticosteroid treatment on ovalbumin-induced IgE mediated immediate and late allergic response in guinea pigs. *Int Arch Allergy Appl Immunol* 1988;87:32-9.
20. Gulbenkian AR, Fernandez X, Kreutner W, et al. Anaphylactic challenge causes eosinophil accumulation in bronchoalveolar lavage fluid of guinea pigs. *Am Rev Respir Dis* 1990;142:680-5.
21. Sanjar S, Aoki S, Kristersson A, Smith D, Morley J. Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitised guinea pigs: the effect of anti-asthma drugs. *Br J Pharmacol* 1991;99:679-86.
22. Elwood W. Allergen-induced airway hyperresponsiveness in the Brown-Norway rat. London, England: University of London; 1992. Thesis.
23. Adelroth E, Rosenhall L, Johansson S, Linden M, Venge P. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage. *Am Rev Respir Dis* 1990;142:91-9.
24. Matsuse T, Thompson RJ, Cheng X-R, Salari H, Schellenberg RR. Capsaicin inhibits airway hyperresponsiveness but not lipoxygenase activity or eosinophilia after repeated aerosolized antigen in guinea pigs. *Am Rev Respir Dis* 1991;144:368-72.
25. Fabbri LM, Chiesura-Corona P, DalVecchio L, et al. Prednisone inhibits late asthmatic reactions and the associated increase in airway hyperresponsiveness induced by toluene-diisocyanate in sensitized subjects. *Am Rev Respir Dis* 1985;132:1010-4.
26. DeMarzo N, Fabbri LM, Crescioli S, Plebani M, Testi R, Mapp CE. Dose-dependent inhibiting effect of inhaled beclomethasone on late asthmatic reactions and increased responsiveness to methacholine induced by toluene diisocyanate in sensitised subjects. *Pulm Pharmacol* 1988;1:15-20.