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Rapid communication

Cessation of dexamethasone exacerbates airway responses to methacholine in asthmatic mice

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Abstract

In asthmatic mice, dexamethasone (30.0 mg/kg) was administered orally once daily on Days 24–27. One hour after dexamethasone on Day 25–27, the mice were exposed to ovalbumin aerosols. Twenty-eight days after the initial ovalbumin immunization, we found that dexamethasone reduced methacholine-induced pulmonary gas trapping and inhibited bronchoalveolar lavage eosinophils and neutrophils. However, five days after the last dose of dexamethasone and last ovalbumin aerosol exposure in other asthmatic mice, the airway obstructive response to methacholine was exacerbated in dexamethasone-treated mice compared to vehicle-treated mice on Day 32. Further, eosinophils, but not neutrophils, were still inhibited after cessation of dexamethasone. Thus, discontinuing dexamethasone worsened methacholine-induced pulmonary gas trapping of asthmatic mice in the absence of eosinophilic airway inflammation.

Keywords: Asthma; Oral glucocorticosteroids; Relapse

Although glucocorticosteroids effectively treat asthma, the cessation of inhaled or oral glucocorticosteroids may exacerbate asthmatic symptoms (Henneman et al., 1955; Lederle et al., 1987; Waalkens et al., 1993; Sovijärvi et al., 2003). To our knowledge, this is the first study investigating relapse in asthmatic mice following the cessation of dexamethasone after the primary ovalbumin exposure. Thus, we examined the airway obstructive response to methacholine and bronchoalveolar lavage inflammatory cells one or five days after the last dexamethasone dose in asthmatic mice.

Inbred male BALB/c mice, 7–9 weeks old, were immunized on Days 1 and 14 intraperitoneally with 20 μg of ovalbumin suspended in alum. Ovalbumin-sensitized mice were dosed orally once daily on Days 24–27 with dexamethasone (30.0 mg/kg, Sigma, St Louis, MO) or vehicle (10.0 ml/kg, 1% sodium-carboxymethylcellulose and 0.25% Tween 80). Ovalbumin-sensitized mice were exposed to either 50.0 mg/ml aerosols of ovalbumin (Positive Control and dexamethasone-treated mice) or sodium-chloride (Negative Control mice) for 20 min on Days 25–27. One (Day 28) or five (Day 32) days after the last dexamethasone dose, the mice were exposed to an 8-min methacholine (10.0 mg/ml) aerosol and then killed with a 0.5 ml intraperitoneal injection of FatalPlus (390 mg/ml sodium-pentobarbital) while breathing the exposure chamber atmosphere. After death, the lungs were removed and excised lung gas volume, i.e., pulmonary gas trapping, an indicator of in vivo airway obstruction, was determined by Archimedes' principle (Stengel et al., 1995). The lungs were attached via the tracheal cannula (PE90 tubing) to a brass anchor, placed in a cup, immersed in a beaker of saline sitting on a stationary platform, and suspended from a hook at the top of a Mettler balance. By first taring the brass weight in saline, the lungs plus brass anchor gave a negative weight display in grams that closely approximates the ml of air trapped in the lungs. Lungs were lavaged (with 1.0 ml Dulbecco's phosphate-buffered saline) to determine total and differential cell counts. The experimental protocols and procedures were approved by the Eli Lilly and Company Animal Care and Use Committee.

Exaggerated abdominal breathing, i.e. dyspnea, was apparent in mice exposed to a methacholine aerosol 28 or 32 days after the first ovalbumin immunization. Trachea and mainstem bronchi of the mouse lungs were patent during excised lung gas volume measurements (normalized by body-weight, ml/kg),

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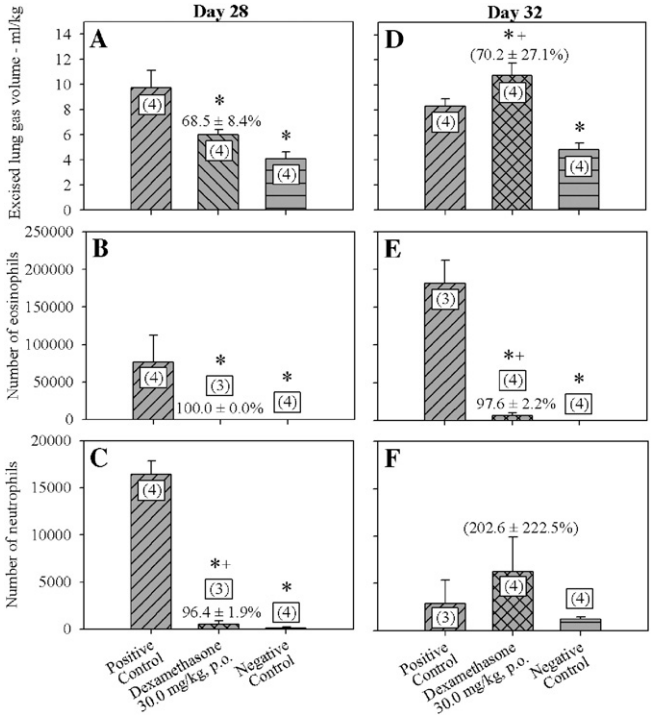

Fig. 1. Effect of dexamethasone on methacholine-induced pulmonary gas trapping, eosinophils, and neutrophils in asthmatic mice on Days 28 (panels A, B, and C) and 32 (panels D, E, and F). Asthmatic mice were dosed orally once daily for 4 days with dexamethasone (30.0 mg/kg) or vehicle on Days 24–27 and then exposed to ovalbumin (50 mg/ml, Positive Control and dexamethasone-treated mice) or sodium-chloride (50 mg/ml, Negative Control mice) aerosols for 20 min on Days 25–27. On Days 28 or 32, mice were exposed to a methacholine aerosol (10.0 mg/ml) for 8 min one or five days after the last dose of dexamethasone or vehicle. Each bar represents the mean±S.E.M. of the number of mice per group in parentheses. One-way analysis of variance was used to compare excised lung gas volume values, eosinophils, and neutrophils among Positive Control, dexamethasone-treated, and Negative Control mice and Fisher's LSD for all pairwise comparisons was performed when appropriate. The relative inhibition of excised lung gas volume responses was determined by subtracting individual dexamethasone-treated mouse excised lung gas volume value from the average excised lung gas volume of Positive Controls and dividing by the difference between the means of Positive Control and Negative Control groups. Normalized values for excised lung gas volumes, eosinophils and, neutrophils were multiplied by 100 to obtain percent inhibition or potentiation (in parentheses). Asterisks indicate significant difference versus Positive Control mice and crosses denote significant difference versus Negative Control mice. Comparisons were considered significant for P values of 0.05 or less.

indicating that the site of airway obstruction must have been distal to the trachea and mainstem bronchi. On Day 28, dexamethasone reduced the airway obstructive response to methacholine (Fig. 1, panel A) and markedly reduced the number of eosinophils and neutrophils recovered in the bronchoalveolar lavage fluid (Fig. 1, panels B and C, respectively). However, after discontinuing dexamethasone (Days 28–32), methacholine-induced excised lung gas volumes of dexamethasone-treated mice were significantly enhanced compared to Positive Controls (Fig. 1, panel D) on Day 32. Eosinophils of dexamethasone-treated mice were still inhibited (Fig. 1, panel E), but not neutrophils (Fig. 1, panel F), compared to Positive Controls.

In this study, dexamethasone inhibited the increase in methacholine-induced pulmonary gas trapping and the number of eosinophils and neutrophils recovered in bronchoalveolar lavage fluid in asthmatic mice 24 h following the final dose of dexamethasone. These results support the notion that reducing airway inflammation plays a role in lessening airway responses to methacholine in asthma. Our findings are consistent with those of Birrell et al. (2003), who demonstrated an inhibition of methacholine-mediated increases in enhanced pause and the number of eosinophils and neutrophils recovered in bronchoalveolar lavage fluid in asthmatic mice dosed twice daily with dexamethasone, 3.0 mg/kg. Although our daily dexamethasone dose, 30.0 mg/kg, was five times their total daily dose of 6.0 mg/kg, one possible explanation for this difference is reflected in the dissimilarity of the two methacholine challenges. Birrell et al. (2003) exposed their mice to a methacholine (3.0 or 10.0 mg/ml) aerosol for 1 min, while we challenged our mice with a methacholine (10.0 mg/ml) aerosol for 8 min.

In contrast to the effectiveness of dexamethasone in reducing the increase in pulmonary gas trapping to methacholine on Day 28, methacholine-induced airway obstruction was exacerbated on Day 32 following cessation of dexamethasone, an effect similar to increased airway responsiveness to histamine after withdrawal of budesonide (Waalkens et al., 1993) or fluticasone proprionate (Sovijärvi et al., 2003) in asthmatics. It is unlikely eosinophils had a role in the worsening of the airway obstructive response to methacholine on Day 32 in our study since the number of eosinophils counted in bronchoalveolar lavage fluid was still inhibited after discontinuing dexamethasone. Yet in the presence of oral corticosteroids, higher eosinophil counts have been measured in asthmatics who relapsed compared to asthmatics who did not relapse (Emerman and Cydulka, 1995). Neutrophils may have played a role in the exacerbation of asthma since recent studies examining either biopsy or sputum samples from severe asthmatics suggest the involvement of neutrophilic inflammation in persistent asthma (Jatakanon et al., 1999; Wenzel et al., 1999). However, due to variability in the number of neutrophils recovered in bronchoalveolar lavage fluid on Day 32, it is unclear what role these neutrophils had in our study. Although additional work is needed to determine what inflammatory events participated in the enhanced methacholine-induced pulmonary gas trapping on Day 32 following cessation of dexamethasone, the relapse we found in the dexamethasone-treated asthmatic mice was similar to that observed in human asthmatics.

In conclusion, our results show that pulmonary gas trapping can be used as an index of methacholine-induced airway obstruction in asthmatic mice. Also, the effect of dexamethasone on the increased airway obstructive response to methacholine and airway inflammation in asthmatic mice is consistent with its therapeutic action in the treatment of human asthma.

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