

Short communication

The impact of oral versus inhaled glucocorticoids on allergen specific IgE testing in experimentally asthmatic cats[☆]

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ABSTRACT

Glucocorticoids (GCs) are palliative for allergic asthma, but allergen-specific immunotherapy (ASIT), which relies on identification of allergens, represents a potentially curative treatment. The purpose of this study was to determine if oral or inhaled GCs would interfere with identification of sensitizing allergens. The hypothesis was that oral but not inhaled GCs would interfere with accurate allergen-specific IgE identification determined by skin and serum testing in experimentally asthmatic cats.

Asthma was induced in 18 cats using Bermuda grass allergen (BGA). Cats ($n=6/\text{group}$) were randomized to receive oral GCs (10 mg prednisolone q 24 h), inhaled GCs (600 μg budesonide q 24 h) or placebo (q 24 h PO) for one month. Intradermal skin testing (IDST) and serum BGA-specific IgE were measured prior to, during and after treatment. A paired t test was used to compare groups pre- and post-treatment ($P<0.05$ significant).

IDST reactivity was eliminated in 4/6, 3/6, and 1/6 cats receiving oral GCs, inhaled GCs, and placebo respectively. Two weeks after stopping treatment, IDST was again positive in all cats. Serum IgE reactivity to BGA was not significantly diminished by any treatment. In conclusion, a two-week withdrawal from GCs is adequate for IDST, but may not be necessary for serum IgE testing.

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1. Introduction

Allergic asthma is an inflammatory respiratory disease caused by type I hypersensitivity to inhaled allergens and is

a common disorder in cats causing clinical signs including wheezing, coughing, respiratory distress and occasionally death (Dye et al., 1996). Glucocorticoids (GCs) are considered standard therapy for control of airway inflammation in cats with allergic asthma (Moise et al., 1989; Dye et al., 1996). Both inhaled and oral GCs have been shown to be equally effective in reducing eosinophilic inflammation in an experimental model of feline asthma that recapitulates the immunologic, physiologic, and pathologic responses of naturally occurring allergic asthma (Norris Reinero et al., 2004; Reinero et al., 2005). However, GCs only control the clinical signs long after the allergic inflammatory cascade has been triggered without addressing the underlying aberrant immune response. In fact, GC treatment can

Abbreviations: GCs, glucocorticoids; ASIT, allergen-specific immunotherapy; BGA, bermuda grass allergen; IDST, intradermal skin tests.

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aggravate the long term course of allergic diseases and asthma (Stock et al., 2005). Alternatively, allergen specific immunotherapy (ASIT) may offer curative therapy for allergy (Bousquet et al., 2001; Larché et al., 2006).

Allergen specific immunotherapy, a process of desensitization to specific allergen, is thought to induce immunologic tolerance by a variety of different mechanisms, not all well understood. Accurate identification of a specific inciting allergen is important for optimal response to ASIT. Identification of increased allergen specific IgE via either intradermal skin tests (IDST) or serum tests is often used for this purpose (Reinero et al., 2006; Lee-Fowler et al., 2009). Although IDST has a higher sensitivity than serum IgE measurements when using an ELISA to detect allergen specific IgE, both IDST and serum IgE measurements are specific, allowing identification of allergens for ASIT (Lee-Fowler et al., 2009).

Unfortunately, many asthmatic cats are already receiving GCs to control clinical signs when ASIT is first considered and allergen identification becomes necessary. While necessary to control clinical signs, GCs dampen immunologic response and may interfere with allergen identification. Therefore, the purpose of this study was to determine whether oral or inhaled GCs interfere with the identification of the sensitizing allergen in cats with experimentally induced asthma. We hypothesized that oral but not inhaled GCs would interfere with the accurate identification of allergen specific IgE using IDST and serum ELISA tests in cats with experimentally induced asthma.

2. Materials and methods

2.1. Animals

Eighteen intact purpose-bred cats (13 male and 5 female, 5 months to 1 year age) were used. They were cared for according to the NIH Guide for the Care and Use of Laboratory Animals, and all procedures were approved by the University of Missouri Animal Care and Use Committee. Prior to asthma induction, all cats lacked pre-existing eosinophilic airway inflammation in bronchoalveolar lavage fluid (BALF) and were confirmed to be naïve to BGA by a negative IDST.

2.2. Asthma induction

Asthma was induced with Bermuda grass allergen (BGA) as described previously (Norris Reinero et al., 2004) with slight modification. On the first day (D0), subcutaneous (s.c.) injection of 12 µg of BGA in 10 mg of alum and intramuscular injection of 100 ng of *Bordetella pertussis* toxin were given to each cat. On day 14 and 21, cats received intranasal administration with 75 µg of BGA in 0.2 ml of PBS and another s.c. injection of 12 µg of BGA in 10 mg of alum respectively. On day 28, the cats were confirmed to be sensitized to BGA by IDST. Over the next two weeks, sensitized cats received seven aerosol challenges with 500 µg of BGA dissolved in PBS and BALF was collected to confirm development of eosinophilic airway inflammation (>17% eosinophils). Only cats documented to have eosinophilic inflammation were included in this study. These cats were

given weekly maintenance aerosol challenges (500 µg of BGA in PBS) until this study was completed.

2.3. Treatment protocol

Eighteen cats were randomly divided into three groups of six cats. Cats were given oral GCs (10 mg of prednisolone PO q 24 h; 1.8–2.85 mg/kg), inhaled GCs (600 µg budesonide by nebulizer – Omron micro air mesh type nebulizer, Omron Healthcare, Inc. Vernon Hills, IL, 60061, q 24 h; 109–170 µg/kg), or a placebo (empty gelatin capsule, PO q 24 h) for one month.

2.4. BGA-specific IgE test (IDST and serum IgE test)

BGA specific IgE tests including IDST and serum IgE test using ELISA were conducted after the induction of asthma (day 42, baseline); during weeks one (week 1) and four (month 1) of therapy with GCs or placebo; and every two weeks thereafter until both tests were positive.

To perform IDST, reconstituted lyophilized BGA (2.78 mg/ml, Greer Laboratories, Lenoir, NC) was diluted 1:10 with sterile saline. Histamine (Histamine base 0.1 mg/ml, Alk Abello, Port Washington, NY, 1:10 diluted with sterile saline) and sterile saline were used as positive and negative controls, respectively. Twenty minutes after intradermal injection of each agent (0.1 ml), wheals were measured in two dimensions. Intradermal reaction was considered positive when the BGA wheal was equal to or greater than half the difference between the size of the positive and negative control.

For BGA allergen specific ELISA, whole blood was collected into vacutainer tubes without additives using jugular venipuncture. Blood was allowed to clot for at least 15 min at room temperature and spun down at 1720 g × 20 min. Serum was harvested and placed in aliquots into several eppendorf tubes and kept at –20 °C until analysis.

An ELISA was performed using polyclonal chicken anti-feline IgE antisera validated to be specific for feline IgE (data not shown) in a manner similar to our prior validation of polyclonal rabbit anti-feline IgE antisera (Norris, 2003). The positive control was pooled sera from experimentally asthmatic cats with strongly positive IDST results from a different study. The negative control consisted of PBS and pooled sera from non-sensitized kittens. Each ELISA was conducted using a 96 well plate (non-sterile clear flat bottom 96 well plate, Fisher Scientific, Pittsburgh, PA) and each allocated well was sensitized with 1 µg/well of BGA antigen using a carbonate–bicarbonate buffer at pH 9.6 (final volume per each well was 100 µl). The plates were incubated for 2 h at 37 °C or overnight at 4 °C and then 100 µl/well of blocking buffer (1% bovine serum albumin (BSA) in PBS-Tween 0.5%) was added for an additional 2 h to each well of the sensitized plates. After washing with wash buffer (PBS-Tween 0.5%), serum samples diluted 1:5 with diluent buffer (PBS-Tween 0.5%/0.5% BSA) were plated at 100 µl/well in duplicate. Plates were incubated for 1.5 h at 37 °C and then washed. Primary antibody (polyclonal chicken anti-feline IgE) was diluted at 1: 15,000 with diluent buffer and 100 µl/well of the

Table 1

The number of cats in each of the three treatment groups (oral or inhaled glucocorticoids (GCs) or placebo) with positive intradermal skin test results for Bermuda grass allergen.

	Baseline (before treatment)	4 weeks of treatment	2 weeks post-treatment
Oral GCs	6/6	2/6	6/6
Inhaled GCs	6/6	3/6	6/6
Placebo	6/6	5/6	6/6

diluted primary antibody was added to each well. The plates were incubated for 1 h at 37 °C and washed again. To prepare the secondary antibody, biotinylated donkey anti-chicken antibody (Jackson ImmunoResearch Laboratories, Inc. West Grove, PA) was diluted at 1:20,000 with diluent buffer and 100 µl/well of the diluted secondary antibody was added to each well. Again, the plates were incubated for 1 h at 37 °C and washed. Peroxidase-conjugated Streptavidine (Jackson ImmunoResearch Laboratories, Inc. West Grove, PA) was diluted at 1:1000 with diluent buffer and added at a volume of 100 µl/well followed by 1 h incubation at 37 °C. After additional washes, the substrate OPD (o-phenylenediamine dihydrochloride, Sigma, St. Louis, MO), was prepared by adding 10 mg OPD to 10 ml of citrate buffer and 4 µl of hydrogen peroxide. Prepared substrate at a volume of 200 µl/well was added and the plates were incubated at room temperature for 45 min. Finally, the plates were read at dual wavelengths of 450–650 nm on a spectrophotometer (SpectraMax® Plus 384 Microplate Spectrophotometer, Molecular Devices, Sunnyvale, CA). Results from each well were recorded as OD; and the OD of background wells (i.e., PBS-0.5% Tween) was subtracted from the original OD for each sample. To reduce the discrepancy between different plates and different days, the OD value was normalized as follows. The mean OD of positive control from all plates run was divided by the OD of positive control of the plate and that number was multiplied by the OD value of the sample on that plate. Thus the serum BGA specific IgE for each cat was represented as the % of a normalized positive control. For statistical comparison, the difference between pre- and post-treatment normalized OD values for each cat was calculated as: ((% normalized OD post-treatment – % normalized OD pre-treatment)/% normalized OD pre-treatment) × 100.

2.5. Statistical analysis

Statistical analysis was performed using commercially available software (SigmaStat, Systat Software Inc., Chicago, IL). Kolmogorov–Smirnov's test was used to determine whether data were normally distributed. Data that were not normally distributed were transformed using the natural log function to achieve normality. A paired *t* test was used to compare serum BGA-specific IgE among groups between pre- and post-treatment. *P* < 0.05 was considered a significant difference.

3. Results and discussion

3.1. Intradermal skin test

As shown in Table 1, all cats from each group were IDST positive for BGA after allergen sensitization (baseline,

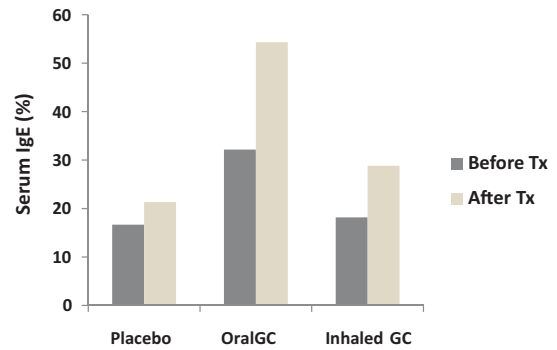


Fig. 1. Average BGA specific serum IgE before and after 4 weeks of treatment with placebo (*n* = 6), oral GC (*n* = 6), or inhaled GC (*n* = 6). Values are expressed as a percentage of pooled serum IgE from cats strongly reactive to BGA in a prior, unrelated study. Serum IgE was not diminished by any treatment.

before treatment), but after 4 weeks of treatment, only two out of six cats in the oral GCs group and three out of six in the inhaled GCs group remained as IDST positive for BGA. One cat in the placebo group became IDST negative. However, within two weeks of discontinuing treatments, all of the cats from each group were again IDST positive for BGA.

3.2. Serum BGA specific IgE test

The average of BGA specific IgE in serum obtained from six asthmatic cats in each group before treatment and after four weeks treatment (placebo, oral GCs or inhaled GCs) is shown in Fig. 1. BGA specific feline IgE reactivity in serum was not reduced by either oral GCs or placebo in any cat. Although significant group change was not detected, two cats in the inhaled GCs group demonstrated decreased BGA specific feline serum IgE reactivity after treatment. GCs treatment did not result in a significant decrease in BGA specific feline IgE reactivity between baseline and month 1 (prednisolone, *P* = 0.072; budesonide, *P* = 0.435).

Although IDST and serum IgE tests have been used for detection of allergen specific IgE in cats with experimental asthma (Reinero et al., 2006; Lee-Fowler et al., 2009), the impact of chronic (1 month) GC on detection of IgE in cats has not been evaluated. Oral prednisolone is the most commonly recommended systemically administered GC for the treatment of feline asthma, and the dose chosen in this study is typical of what is used in the clinical setting at the initiation of treatment (Byers and Dhupa, 2005). Although fluticasone delivered as an aerosol by pressurized metered dose inhaler may be used more commonly in the clinical setting than nebulized budesonide, it is approved in humans for twice daily dosing and has only been tested for efficacy twice daily in cats (Cohn et al., 2010; Leemans et al., in press). We chose to use budesonide delivered by nebulization in this study by once daily administration (Scott et al., 2001; Selroos et al., 2004) as this would be matched to the dose frequency of oral GCs.

Several studies in humans report diminished IDST reaction to allergens after topical or systemic GC treatment

of allergic diseases (Anderson and Pipkorn, 1987; Inoue et al., 2002). On the other hand, other studies found opposite results, claiming the absence of GCs' inhibition of IDST reactivity (Slott and Zweiman, 1974; Talbot et al., 1987; Olson et al., 1990; Des Roches et al., 1996). Less information is available about the impact of GCs on IDST results in animals. Recently, the impact of GCs (dexamethasone 20 mg/day) and antihistamine (hydroxyzine 500 mg, BID) treatment on IDST and allergen specific IgE serum testing in horses was reported (Petersen and Schott, 2009). Interestingly, the results in horses treated for 7 days were very similar to our results in cats treated for 4 weeks.

In our study, IDST reaction was inhibited in at least half of the cats receiving oral or inhaled GCs but there was also one placebo-treated cat with a negative IDST response. While IDST response can be negatively impacted by batch-to-batch variation in allergen potency, deterioration of reagents in storage, or lag time between injections (Bond, 1993), these factors were unlikely in our study (i.e., single allergen batch used over a short time frame). All reactions were read by a single investigator.

Immunoglobulin E is believed to play an important role in allergic asthma. Studies in humans have showed inconsistent results in terms of the impact of GCs on serum total or allergen specific IgE level (Zieg et al., 1994; Ohru et al., 1999; Cho et al., 2000; Stelmach et al., 2005). These discordant results in humans might be related to patient populations, the use of different types of GCs, delivery routes, duration of treatment and wash out, and study methodologies.

While IDST has been anecdotally preferred to serum tests for allergy in animals (Scott et al., 2000), identification of sensitizing allergens during GCs therapy appears to be better performed using serum IgE testing. A negative IDST does not mandate that there have been decreases in IgE production, i.e., it is possible that GCs affect other parts of the pathway to wheal formation resulting in a negative IDST when IgE is still present and bound to mast cells. For example, GCs may stabilize mast cell membranes raising the threshold for mast cell degranulation, they may decrease production of other mast cell cytokines/mediators, or they may preserve vascular integrity preventing "leakiness" associated with mediator-induced increases in vascular permeability.

There are several limitations to this study. First, relatively small numbers of cats were used in each treatment group. Second, only one type of oral or inhaled GC was used. Because each GC type has a different potency and half life, more studies are recommended using other GCs in order to ensure that the results of this study are applicable to other types of GCs. Specifically, evaluation of fluticasone, an inhalant GC used commonly to treat asthmatic cats, is warranted. Finally, the duration of GC treatment in this study was only one month, while in a clinical setting asthmatic cats may have been receiving GC treatment for much longer periods, even for years.

In conclusion, both oral and inhaled GCs interfered with the IDST reaction to BGA after four weeks of the treatment, but the reaction was restored when the treatment was discontinued for two weeks. However, neither oral nor inhaled GCs significantly decreased the amount of

serum BGA specific IgE. When the identification of specific allergens becomes necessary in preparation for ASIT in asthmatic cats, a minimum two-week withdrawal from GC treatment is recommended prior to IDST but no withdrawal is needed prior to the use of allergen specific serum IgE testing.

Conflict of interest

None.

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