

# Effects of pentoxifylline on immediate and late-phase cutaneous reactions in response to anti-immunoglobulin E antibodies in clinically normal dogs

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**Objective**—To characterize the effects of pentoxifylline on the gross and microscopic variables associated with immediate and late-phase inflammation following injection of IgE-specific antibodies in the skin of clinically normal dogs.

**Animals**—6 healthy adult mixed-breed dogs.

**Procedures**—Intradermal injections (0.1 mL each) of PBS solution, histamine phosphate, and cross-linking rabbit-origin anti-canine IgE antibodies (3 injections/dog) were administered at 0 hours on day 0; wheal sizes were evaluated at 20 minutes, 6 hours, and 24 hours. Biopsy specimens of injected and noninjected skin were collected 24 hours after injection. On day 2, treatment with pentoxifylline (20 mg/kg, PO, q 8 h) was initiated and continued until day 30. For each dog, injection, measurement, and biopsy procedures were repeated on days 30 to 31 and on days 37 to 38 (ie, after discontinuation of pentoxifylline administration).

**Results**—Pentoxifylline administration was associated with a significant decrease in wheal size at 6 and 24 hours (but not at 20 minutes) after injection of anti-canine IgE. Repeated injections performed 1 week after drug discontinuation revealed partial recovery of the 6-hour cutaneous reaction and complete recovery of the 24-hour cutaneous reaction. Pentoxifylline administration was also associated with inhibition of mast cell degranulation and significant decreases in the total numbers of cutaneous inflammatory cells and eosinophils, compared with pretreatment findings.

**Conclusions and Clinical Relevance**—In clinically normal dogs, pentoxifylline effectively impaired late-phase reactions but not immediate reactions at sites of intradermal injection of IgE-specific antibodies by inhibiting mast cell degranulation and recruitment of cutaneous inflammatory cells, especially eosinophils. (*Am J Vet Res* 2014;75:152–160)

Atopic dermatitis is a common inflammatory skin disorder of humans and several domestic species, including dogs. Atopic dermatitis in dogs has been defined as “a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with immunoglobulin E (IgE) antibodies most commonly directed against environmental allergens.”<sup>1</sup> In affected dogs, onset of pruritus typically

## ABBREVIATIONS

|        |                                                    |
|--------|----------------------------------------------------|
| CADESI | Canine atopic dermatitis extent and severity index |
| ICAM   | Intercellular adhesion molecule                    |
| IL     | Interleukin                                        |
| PDE    | Phosphodiesterase                                  |
| TNF    | Tumor necrosis factor                              |

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occurs at a young age, and the most commonly affected areas are the feet, face, axillae, abdomen, and ears.<sup>2,3</sup> Clinical management of dogs with atopic dermatitis requires a multifaceted diagnostic and therapeutic approach, often including the identification and avoidance of flare factors (eg, flea infestations), treatment of bacterial and fungal infections, allergen avoidance, administration of allergen-specific immunotherapy, and topical or systemic treatment with antipruritic agents.<sup>4</sup> The pruritus associated with canine atopic dermatitis is often initially glucocorticoid responsive.<sup>2</sup> However, the disease tends to increase in severity with time, and progressively higher dosages of glucocorticoids may be required to provide adequate control of pruritus.<sup>2</sup> Although many nonglucocorticoid antipruritic agents

have been identified for use in dogs, no single drug is effective for all patients.<sup>4</sup> For this reason, identification and objective evaluation of new anti-inflammatory treatments to be used as sole or adjunctive treatments for dogs with pruritus remains an active field of study.

Pentoxifylline is a nonselective inhibitor of PDE activity.<sup>5</sup> It inhibits the formation and release of several inflammatory mediators, including TNF- $\alpha$ , IL-1 $\beta$ , and IL-2.<sup>6-8</sup> It has been frequently used in veterinary species for the treatment of dermatologic disorders that are known or suspected to be associated with vascular impairment, including ischemic dermatopathies, such as vasculitis or dermatomyositis, as well as laminitis in horses.<sup>9-13</sup> In addition, it has been reported to be of benefit in the treatment of some nonischemic cutaneous inflammatory disorders, including allergic contact dermatitis.<sup>14</sup> More recently, a small number of studies<sup>15-17</sup> have investigated the use of pentoxifylline in the treatment of atopic dermatitis. Results of 1 retrospective study<sup>15</sup> indicated that pentoxifylline (either administered alone or in addition to other treatments) was able to reduce pruritus to a level considered tolerable in 16 of 37 (43%) dogs with atopic dermatitis. Two blinded prospective studies<sup>16,17</sup> investigating the efficacy of pentoxifylline in treatment of dogs with atopy revealed decreases in owner- and investigator-reported signs of pruritus, cutaneous erythema, or CADESI scores.

Despite these encouraging findings, little has been done to investigate the possible mechanisms by which pentoxifylline may exert anti-inflammatory effects on the cutaneous immune response in dogs. In 1 study<sup>16</sup> in dogs with atopy, administration of pentoxifylline was associated with decreased cutaneous induration following intradermal injection of lipopolysaccharide. Pentoxifylline failed to suppress the dogs' immediate intradermal reactivity to house dust mite antigen.<sup>16</sup> However, late-phase reactions (6 to 48 hours after injection of either lipopolysaccharide or house dust mite antigen) were not evaluated in that study.<sup>16</sup> Furthermore, no attempt was made to evaluate the cutaneous inflammatory cell population in those dogs prior to or following pentoxifylline administration.

In humans and dogs, intradermal injection of cross-linking anti-IgE antibodies induces the development of both immediate and late-phase inflammatory responses.<sup>18-20</sup> These responses both grossly and microscopically resemble those seen after intradermal injection of allergen in patients with spontaneous atopic dermatitis.<sup>20,21</sup> Importantly, both immediate and late-phase responses may be generated in nonsensitized individuals.<sup>19</sup> This phenomenon has been exploited to evaluate the efficacy of a variety of topically and systemically administered anti-inflammatory medications in both dogs and humans.<sup>19,20,22</sup> In those studies, the use of anti-IgE antibodies has allowed the characterization of pharmacological inhibition of IgE-mediated inflammation without requiring the development and maintenance of a dedicated colony of sensitized individuals.

The purpose of the study reported here was to characterize the effects of pentoxifylline on the development of immediate and late-phase inflammation in response to anti-IgE antibodies (equivalent to IgE-mediated responses) in the skin of clinically normal dogs,

and if an anti-inflammatory effect was demonstrated, to determine the duration of inhibition following cessation of drug administration. To this end, we evaluated injection site wheal diameters and the development and magnitude of dermal induration in dogs at 20 minutes, 6 hours, and 24 hours after intradermal injection of PBS solution, histamine phosphate, and cross-linking rabbit-origin anti-canine IgE antibodies. Paired evaluations were conducted prior to and following a 30-day course of pentoxifylline. Further characterization was obtained by evaluating biopsy specimens of injected skin obtained at 24 hours after each intradermal injection for enumeration of total inflammatory cells, neutrophils, and eosinophils, along with total and degranulated mast cells. We hypothesized that administration of pentoxifylline would be associated with inhibition of the inflammatory response generated by the intradermal injection of cross-linking anti-canine IgE in clinically normal dogs.

## Materials and Methods

**Dogs**—Six healthy sexually intact male and female mixed-breed dogs (mean age, 4.6 years) were used for the study. These dogs were chosen on the basis of their lack of clinical or historical evidence of allergic skin disease, cutaneous bacterial infections, or systemic disease. All dogs were given complete physical examinations (including cardiac auscultation) at the beginning of the study and again before every sedation and injection procedure. Dogs were housed in accordance with the National Institute of Health 2011 *Guide for the Care and Use of Laboratory Animals*. All facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Animal use approval was obtained through the university's institutional animal care and use committee.

**Experimental design**—On study day 0, each dog was sedated with dexmedetomidine<sup>a</sup> (4  $\mu$ g/kg, IV). The fur on the left lateral aspect of the thorax was shaved, and intradermal injections (0.1 mL each) of PBS solution, histamine phosphate<sup>b</sup> (0.275 mg/mL), and rabbit-origin IgG polyclonal anti-canine IgE antibodies (0.1 mg/mL) were administered; time of injection was designated as 0 hours. Overall, each dog received 3 injections, modified from the protocol described by Pucheu-Haston et al.<sup>19</sup> Sedation was reversed with atipamezole<sup>c</sup> given IV at a volume equal to dexmedetomidine, if necessary, following the injections. Macroscopic changes at the injection sites were evaluated after 20 minutes, 6 hours, and 24 hours. At the 24-hour time point (day 1), each dog was again sedated with dexmedetomidine; macroscopic changes at the injection sites were evaluated, and biopsy specimens of noninjected skin and skin at the site of injection of anti-canine IgE antibodies were collected for histologic examination. Sedation was reversed with atipamezole, if necessary.

On day 2, oral treatment with pentoxifylline was initiated at a dosage of 20 mg/kg (compounded into a liquid to allow precise dosing) 3 times daily. Treatment continued for 29 days (until day 30). Two hours after discontinuation of drug treatment on day 30, each dog was sedated with dexmedetomidine and again admin-

istered the intradermal injections, as performed on day 0, this time on the right side of the thorax. Macroscopic changes were evaluated at 20 minutes and 6 hours after administration of the injections. Sedation was reversed with atipamezole, if necessary. At 24 hours after these repeated injections (day 31), each dog was again sedated with dexmedetomidine; macroscopic changes at the injection sites were evaluated, and biopsy specimens of noninjected skin and skin at the site of injection of anti-canine IgE antibodies were collected for histologic examination. Sedation was reversed with atipamezole, if necessary.

On day 37, the sedation and intradermal injection procedures were repeated for each dog on the left side of the thorax, ventral to the day 0 injection sites. Macroscopic changes were evaluated at 20 minutes and 6 hours after administration of the injections. Sedation was reversed with atipamezole, if necessary. At 24 hours after these repeated injections (day 38), each dog was again sedated with dexmedetomidine; macroscopic changes at the injection sites were evaluated, but biopsy specimens of skin were not collected. Sedation was reversed with atipamezole, if necessary.

**Grading of macroscopic reactions**—At 20 minutes, 6 hours, and 24 hours after intradermal injections were administered on days 0, 30, and 37, the injection sites were evaluated for the development of cutaneous swelling, erythema, or induration. The width and height of each injected site was measured in millimeters with calipers, with 1 set of measurements obtained per injection site at each time point for each dog. All measurements were performed by the same investigator (CPH). Wheal area ( $\text{mm}^2$ ) was calculated as width  $\times$  length.

**Biopsy specimen collection and processing**—After each of the 24-hour postinjection evaluations (on days 1, 31, and 38) was performed, approximately 0.5 mL of 2% lidocaine was injected SC at the anti-canine IgE injection site as well as at an area of non-injected skin approximately 6 cm distant from the anti-IgE injection site and 3 cm distant from the histamine injection site. Eight-millimeter punch biopsy specimens were then obtained from the noninjected skin as well as skin at the site of injection of IgE antibodies (1 biopsy specimen from the area of non-injected and from the area of anti-IgE injected skin for each dog). The biopsy specimens were placed in 10% formalin for routine histologic processing.

**Treatment with pentoxifylline**—Treatment with pentoxifylline (20 mg/kg, PO, q 8 h) was initiated on day 2 and continued until 2 hours before the dogs were sedated on day 30. This dosage regimen was chosen on the basis of results of previous clinical and pharmacological studies.<sup>11,17,23,24</sup> The dosage of pentoxifylline required to achieve serum drug concentrations of 1  $\mu\text{g}/\text{mL}$  (therapeutic concentrations extrapolated from human data) has been variously reported as 30, 25, and 15 mg/kg, with recommended dosing intervals ranging from every 8 to 12 hours.<sup>11,23–25</sup> In a recent study,<sup>17</sup> there was significant improvement in CADESI and owner-determined pruritus scores for atopic dogs adminis-

tered pentoxifylline orally at a dosage of 20 mg/kg 3 times daily for 30 days. Because this regimen represented a logical and apparently effective compromise between the disparate recommendations, it was selected as the dosage for use in the study.

**Monitoring for adverse reactions**—Adverse reactions to pentoxifylline in humans and dogs are uncommon. In humans, the most common adverse effects are bloating, dyspepsia, nausea, vomiting, abdominal discomfort, dizziness, and headache.<sup>26</sup> Less commonly reported effects ( $< 1\%$ ) include dyspnea, anorexia, seizures, hypotension, cholecystitis, pancytopenia, thrombocytopenia, and leukopenia,<sup>26</sup> although the causal relationship between administration of pentoxifylline and these effects is uncertain. Pentoxifylline-associated adverse reactions appear to be rare in dogs, with either no adverse reactions or mild vomiting or diarrhea detected in most studies.<sup>9,11,14,15,17,23,24</sup> In the present study, dogs were monitored daily for the development of both likely adverse reactions (vomiting and diarrhea) and less likely adverse reactions (anorexia, weight loss, signs of depression, seizures, clinical jaundice, collapse, and petechiation or ecchymoses).

**Histologic examination of biopsy specimens**—Formalin-fixed skin tissue samples were processed and embedded in paraffin. Five-micrometer-thick sections were cut and stained with H&E stain for microscopic examination and cell enumeration (total number of inflammatory cells and number of neutrophils). In skin tissue sections stained with Luna stain, eosinophils were enumerated. In other sections of skin tissue, a low-pH (1.5) toluidine blue stain was used to facilitate evaluation and enumeration of dermal mast cells. Mast cells were identified as mononuclear cells with prominent metachromatically staining cytoplasmic granules.

Counts of neutrophils, eosinophils, mast cells, and total inflammatory cells were determined as the number of cells/ $\text{mm}^2$  of superficial dermis. This was determined by counting the cells of interest in 16 consecutive grid fields ( $0.254 \times 0.254 \text{ mm}$ ) with a 40X objective lens.<sup>4</sup> The first counted field was positioned immediately below the epidermis, and subsequent fields were positioned consecutively and laterally along the dermal-epidermal junction. Fields were counted in a single section from each sample. Endothelial cells and adnexal structures were not counted. For mast cell enumeration, the total number of mast cells as well as the number of visibly degranulated mast cells were counted and used to calculate the percentage of visibly degranulated mast cells.

**Statistical analysis**—The number of study dogs was chosen on the basis of a previous study,<sup>19</sup> in which significant differences in dermal cellularity were obtained following injection of anti-canine IgE antibodies in untreated and prednisolone-treated dogs. Wheal data obtained in the present study were calculated as mean  $\pm$  SE from a single set of measurements (width and height) for each injection site in each dog at each time ( $n = 6$  sets of measurements/injection site/time point). Histologic data were calculated as mean  $\pm$  SE from a single count in a single section of a single biopsy

specimen from each injection site in each dog at each time point ( $n = 6$  counts/injection site/time point). The data in the present study were analyzed by a repeated-measures ANOVA in a mixed-effects model with a commercial software package.<sup>c</sup> Fixed effects included treatment, day, and treatment-by-day interaction. Animal was the random effect in the model. Response variables were log-transformed to stabilize variance terms as necessary. When overall significance was detected, post hoc comparisons were conducted by pairwise  $t$  tests of least-squares means. All comparisons were considered significant at a value of  $P \leq 0.05$ .

## Results

**Effect of pentoxifylline on macroscopic late-phase reactions induced by anti-canine IgE antibodies**—Intradermal injections of rabbit-origin cross-linking anti-canine IgE antibodies and histamine induced the development of subjectively indurated, erythematous wheals at the injection sites within 20 minutes after injection, whereas injection of PBS solution did not (Figure 1). At the 20-minute time point on day 0, the sites injected with anti-IgE antibodies were larger than the PBS solution injection sites, although the difference in wheal area was not significant. By 6 hours after injection, PBS solution injection sites were no longer palpable in 5 of the 6 dogs. In contrast, all dogs had palpable areas of dermal induration and erythema at the sites of injection of anti-IgE antibodies or histamine. At 24 hours after injection (day 1), no PBS solution injection sites were palpable, whereas 4 of 6 dogs had palpable induration at sites of injection of anti-IgE antibodies and all dogs had induration at sites of injection of histamine.

Pentoxifylline administration was well tolerated by all dogs. No serious adverse reactions developed in any dog, although hypersalivation was occasionally seen immediately after medication administration. Treatment with pentoxifylline from day 2 through day 30 had no effects on the subjective features of the macroscopic immediate reaction (erythema and induration), and there was no significant ( $P > 0.05$ ) effect on wheal area, compared with findings prior to the administration of pentoxifylline (Figures 1 and 2). In contrast, the 6-hour late-phase wheal reaction induced by anti-

canine IgE antibodies was significantly ( $P < 0.05$ ) reduced on day 30, relative to wheal area prior to administration of pentoxifylline. This inhibition was even more marked at the 24-hour time point (day 31).

To determine the duration of pentoxifylline-mediated inhibition of the macroscopic cutaneous late-phase reaction, the injection and measurement procedure was repeated on day 37 (1 week after cessation of pentoxifylline administration) with further measurement of wheal area at 24 hours after injection (day 38). On day 38, the 6-hour late-phase reaction was not significantly ( $P < 0.05$ ) different from the pretreatment wheal area (Figure 2), whereas the 24-hour reaction was somewhat larger than the pretreatment wheal area.

**Effect of intradermal injection of anti-canine IgE antibodies on cutaneous inflammatory cells and mast cells**—Specimens of skin injected with anti-canine IgE antibodies were collected 24 hours after injection (ie, on days 1 and 31) and underwent histologic examination, which revealed recruitment of inflammatory cells (Table 1). The cells were located in the superficial dermis, in a vasocentric pattern. The cellular infiltrate consisted primarily of mononuclear cells (histiocytes and lymphocytes), neutrophils, and eosinophils. Sites injected with anti-IgE antibodies had significantly ( $P < 0.05$ ) more total inflammatory cells, eosinophils, and neutrophils, compared with findings in noninjected skin specimens.

Low pH toluidine blue staining of all skin sections revealed numerous oval, mononuclear, granular cells throughout the dermis, particularly scattered around blood vessels and adnexa. Although toluidine blue stain is taken up by both canine mast cells and basophils, basophils are rarely found in either normal or atopic dog skin. For this reason, stained cells were identified as mast cells. Furthermore, the cells found in these skin specimens were mononuclear and not polymorphonuclear, further supporting their identification as mast cells.

Intradermal injection of anti-canine IgE antibodies was associated with a significant ( $P < 0.05$ ) decrease in the total number of mast cells, compared with the number in noninjected skin (Table 1).<sup>18</sup> Many of these cells had faint staining of cytoplasmic granules or visible granule dispersion.

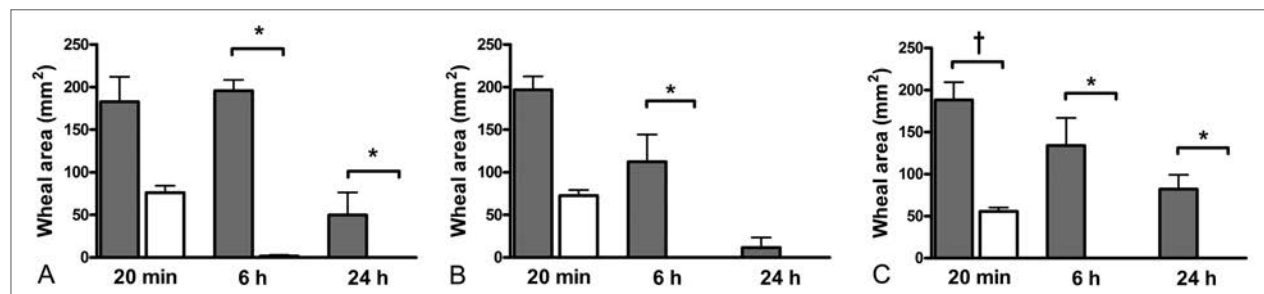


Figure 1—Mean  $\pm$  SE wheal area measurements in 6 healthy adult mixed-breed dogs obtained 20 minutes, 6 hours, and 24 hours after separate intradermal injections of cross-linking rabbit-origin anti-canine IgE antibodies (0.1 mL [gray column]) and PBS solution (0.1 mL [white column]) administered prior to treatment with pentoxifylline (injection procedures on day 0 with measurement procedures on days 0 and 1; A), after oral administration of 20 mg of pentoxifylline/kg 3 times daily beginning day 2 to day 30 (injection procedures on day 30 with measurement procedures on days 30 and 31; B), and 7 days after discontinuation of pentoxifylline administration (injection procedures on day 37 with measurement procedures on days 37 and 38; C). At each time point, the width and height of each injected site was measured in millimeters with calipers to obtain 1 set of measurements per injection site for each dog at each time point; wheal area ( $\text{mm}^2$ ) was calculated as width  $\times$  length. \*Values within a bracket are significantly ( $P < 0.001$ ) different. †Values within a bracket are significantly ( $P < 0.05$ ) different.

**Effect of pentoxifylline on cellular influx and mast cell degranulation following intradermal injection of anti-canine IgE antibodies**—Biopsy specimens of skin that had been injected with anti-canine IgE antibodies were collected for histologic examination on day 31 after dogs received pentoxifylline from day 2 through day 30. The total number of inflammatory cells and number of eosinophils were significantly ( $P < 0.05$ ) decreased, compared with findings prior to pentoxifylline administration (Table 1). Despite these decreases, the inflammatory cell count skin injected with anti-IgE antibodies remained significantly ( $P < 0.05$ ) higher than the cell count in noninjected skin. The number of inflammatory cells in noninjected skin specimens did not differ significantly ( $P > 0.05$ ) after pentoxifylline administration, compared with pretreatment findings.

In specimens of skin injected with anti-canine IgE antibodies, administration of pentoxifylline was associated with a significant ( $P < 0.05$ ) increase in the total number of mast cells, compared with the number prior to pentoxifylline administration (Table 1). However, the total number of mast cells in specimens of skin injected with anti-IgE antibodies was decreased, compared with the number in specimens of noninjected skin, despite pentoxifylline treatment. Pentoxifylline was associated with a decrease in the percentage of mast cells with visible granule dispersion in both specimens of skin injected with anti-IgE antibodies and noninjected skin, relative to the pretreatment findings. Interestingly, this decrease was only significant ( $P < 0.05$ ) for noninjected skin specimens.

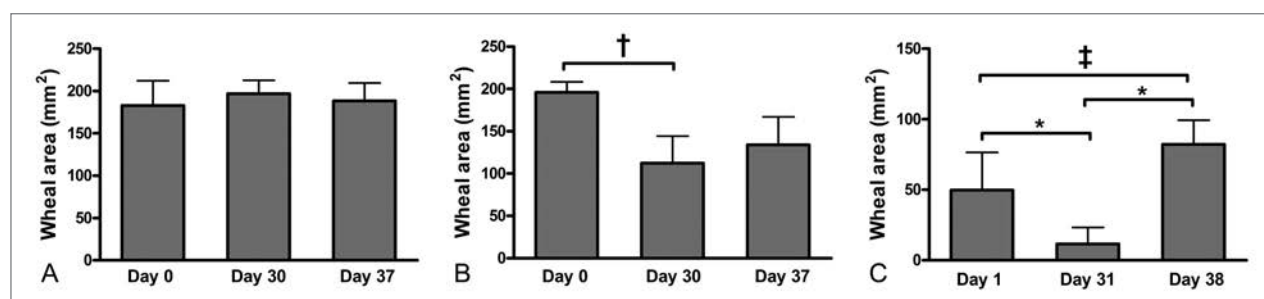


Figure 2—Mean  $\pm$  SE wheal area measurements in 6 healthy adult mixed-breed dogs obtained 20 minutes (A), 6 hours (B), and 24 hours (C) after an intradermal injection of anti-canine IgE antibodies (0.1 mL) administered prior to treatment with pentoxifylline (injection procedure on day 0 with measurement procedures on days 0 and 1), after oral administration of 20 mg of pentoxifylline/kg 3 times daily beginning day 2 to day 30 (injection procedure on day 30 with measurement procedures on days 30 and 31), and 7 days after discontinuation of pentoxifylline administration (injection procedure on day 37 with measurement procedures on days 37 and 38). At each time point, the width and height of each injected site was measured in millimeters with calipers to obtain 1 set of measurements per injection site for each dog at each time point; wheal area ( $\text{mm}^2$ ) was calculated as width  $\times$  length. †Values within a bracket are significantly ( $P = 0.01$ ) different. See Figure 1 for remainder of key.

Table 1—Mean  $\pm$  SE (95% confidence interval) cell counts in biopsy specimens of noninjected skin and skin injected with cross-linking rabbit-origin anti-canine IgE antibodies (0.1 mL) obtained 24 hours after a first intradermal injection (injection procedure on day 0 with biopsy procedure on day 1) and after a second intradermal injection (injection procedure on day 30 with biopsy procedure on day 31) from 6 healthy adult mixed-breed dogs that had been treated orally with 20 mg of pentoxifylline/kg 3 times daily from day 2 through day 30.

| Variable                                              | Day 1                                    |                                      | Day 31                                  |                                       |
|-------------------------------------------------------|------------------------------------------|--------------------------------------|-----------------------------------------|---------------------------------------|
|                                                       | Anti-IgE antibodies                      | No injection                         | Anti-IgE antibodies                     | No injection                          |
| Total inflammatory cell count (cells/ $\text{mm}^2$ ) | 1,819 $\pm$ 275.7<br>(1,110 to 2,528)*†‡ | 297.2 $\pm$ 33.3<br>(211.6 to 382.7) | 810.4 $\pm$ 71.1<br>(627.5 to 993.30)*‡ | 328.5 $\pm$ 32.40<br>(245.2 to 411.8) |
| Neutrophil count (cells/ $\text{mm}^2$ )              | 639.5 $\pm$ 164.1<br>(217.6 to 1,061)*†‡ | 17.03 $\pm$ 4.4<br>(35.1 to 203.3)   | 346.1 $\pm$ 99.2<br>(91.1 to 601.1)*†‡  | 20.97 $\pm$ 4.6<br>(9.1 to 32.9)      |
| Eosinophil count (cells/ $\text{mm}^2$ )              | 425.5 $\pm$ 140.6<br>(64.1 to 786.9)*†‡  | 8.7 $\pm$ 7.7<br>(-11.0 to 28.5)     | 67.07 $\pm$ 21.7<br>(11.3 to 122.8)*†‡  | 1.2 $\pm$ 0.5<br>(0.1 to 2.4)         |
| Total mast cell count (cells/ $\text{mm}^2$ )         | 18.2 $\pm$ 3.7<br>(8.6 to 27.8)*§‡       | 43 $\pm$ 5.4<br>(29.0 to 57.0)       | 32.67 $\pm$ 3.8<br>(22.9 to 42.4) II    | 51.83 $\pm$ 9.4<br>(27.8 to 75.9)     |
| Degranulated mast cells (% of total mast cell count)  | 34.1 $\pm$ 5.7<br>(19.4 to 48.8)¶        | 30.5 $\pm$ 2.5<br>(24.16 to 36.8)¶   | 22.2 $\pm$ 3.6<br>(12.9 to 31.6)        | 13.02 $\pm$ 1.6<br>(8.8 to 17.2)      |

Skin sections stained with H&E stain, Luna stain, and low-pH (1.5) toluidine blue stain were examined microscopically for enumeration of total number of inflammatory cells and number of neutrophils, eosinophils, and dermal mast cells, respectively. Mast cells were identified as mononuclear cells with prominent metachromatically staining cytoplasmic granules. To determine each cell count (number of cells/ $\text{mm}^2$  of superficial dermis), cells of interest were counted in 16 consecutive grid fields (0.254  $\times$  0.254 mm) with a 40 $\times$  objective lens in 1 section from each biopsy specimen for each dog ( $n = 6$ ). The first counted field was positioned immediately below the epidermis, and subsequent fields were positioned consecutively and laterally along the dermal-epidermal junction.

\*Value is significantly ( $P < 0.001$ ) different from the value for noninjected skin on day 1. †Value is significantly ( $P < 0.01$ ) different from the value for skin specimens injected with anti-canine IgE antibodies on day 31. ‡Value is significantly ( $P < 0.001$ ) different from the value for noninjected skin on day 31. §Value is significantly different ( $P < 0.001$ ) from the value for skin specimens injected with anti-canine IgE antibodies on day 31. IIValue is significantly different ( $P < 0.05$ ) from the value for noninjected skin on day 31. ¶Value is significantly different ( $P < 0.01$ ) from the value for noninjected skin on day 31.

## Discussion

In the present study, we investigated the effects of orally administered pentoxifylline on the development of immediate and late-phase cutaneous reactions in response to intradermal injections of anti-canine IgE antibodies (equivalent to IgE-mediated responses) in healthy adult dogs. We determined that treatment with pentoxifylline for 29 days did not result in significant inhibition of immediate reactivity to anti-IgE antibodies. However, the macroscopic late-phase response was significantly inhibited at both 6 and 24 hours after injection of anti-IgE antibodies. This inhibitory effect was no longer evident 1 week after cessation of pentoxifylline administration. Furthermore, pentoxifylline administration was associated with significant decreases in the numbers of total inflammatory cells and eosinophils in injected skin specimens.

Pentoxifylline is a nonselective PDE inhibitor in the methylxanthine family.<sup>5</sup> There are currently 11 known PDE isoenzyme families, which differ largely in the degree and specificity with which they hydrolyze diester bonds in cAMP and cyclic guanosine monophosphate.<sup>27</sup> These isoenzymes are differentially expressed in organs and cells. Inhibitors of PDE may be specific (inhibiting a particular isoenzyme) or nonselective (inhibiting multiple isoenzymes).<sup>27</sup> Pentoxifylline has inhibitory effects against several isoenzymes, particularly PDE4.<sup>28</sup> However, inhibitory effects against PDE1, PDE2, PDE3, and PDE5 have also been described.<sup>28,29</sup>

In humans, PDE isoenzymes are expressed by most cells of the immune system, including neutrophils (PDE4), macrophages (PDE1, PDE3, PDE4, and PDE5), monocytes (PDE3 and PDE4), mast cells (PDE3 and PDE4), basophils and dendritic cells (PDE3, PDE4, and PDE5), B cells (PDE3, PDE4, and PDE7), and T cells (PDE1, PDE2, PDE3, PDE4, PDE5, PDE7, and PDE8).<sup>30</sup> Less research has been performed to characterize the distribution of PDE isoenzymes in canine immunocytes. Phosphodiesterase 4 has been demonstrated on canine monocytes and lymphocytes.<sup>31</sup> Furthermore, treatment of dispersed canine mast cells with the PDE4-selective inhibitor rolipram was associated with decreased histamine release.<sup>32</sup>

Pentoxifylline has a variety of immunomodulatory effects, consistent with its potential for inhibition of multiple PDE isoenzymes. It inhibits activation of the nuclear transcription factor  $\kappa$ -B<sup>33,34</sup> and the signaling molecules p38,<sup>35</sup> ERK,<sup>36</sup> and c-jun.<sup>37</sup> Pentoxifylline decreases the production of TNF- $\alpha$  both in vitro<sup>6,7,38–40</sup> and in vivo<sup>41</sup> in a cAMP-dependent manner.<sup>6</sup> It has also been reported to reduce soluble and cell-associated ICAM-1, likely secondary to suppression of TNF- $\alpha$ .<sup>39,42</sup> In addition, the drug reduces the production of a variety of other inflammatory cytokines and mediators, including granzyme A, granulocyte-macrophage colony stimulating factor, IL-1 $\beta$ , IL-2, IL-6, IL-8, IL-13, IL-12, and IL-18 and increases the expression of IL-10.<sup>7,8,38,43–45</sup> Pentoxifylline has also been demonstrated to decrease adhesion of T cells to keratinocytes and dermal endothelial cells<sup>5,46</sup> and to decrease the allostimulatory activity of monocyte-derived dendritic cells.<sup>45</sup>

Pentoxifylline decreases the clinical severity of diseases associated with either helper T cell type 1 or

2 immune responses. Its administration is associated with decreased airway resistance, remodeling, and epithelial thickness after allergen challenge in mice experimentally sensitized to house dust mite antigen or ovalbumin.<sup>33,34,44</sup> Treatment with pentoxifylline prior to allergen challenge results in decreased serum concentrations of IL-13 and interferon  $\gamma$  as well as fewer airway inflammatory cells (as measured by examination of samples collected via bronchoalveolar lavage) and decreased eosinophil exocytosis into tracheal epithelium.<sup>33,44</sup>

For dogs, pentoxifylline has been advocated for use in the management of cutaneous hypersensitivities, such as contact hypersensitivity and atopic dermatitis. In 1 study,<sup>14</sup> successful management of contact allergy in 3 dogs sensitized to plants of the Commelinaceae family was achieved with pentoxifylline (10 mg/kg) administered orally twice daily. In a blinded, crossover study<sup>16</sup> involving dogs that were allergic to house dust mite antigen, oral administration of pentoxifylline at a dose of 10 mg/kg twice daily for 4 weeks significantly decreased erythema as well as both owner- and investigator-determined scores for pruritus. In another study,<sup>15</sup> pentoxifylline (25 mg/kg, PO, q 12 h for 4 weeks) was associated with a good response (defined as decreasing pruritus to a tolerable level) in 16 of 37 dogs with atopic dermatitis, although many of the dogs were also receiving other medications or treatments (eg, allergen-specific immunotherapy and glucocorticoids). More recently, a placebo-controlled study<sup>17</sup> in dogs revealed significant decreases in CADESI scores and visual analog scores for pruritus after pentoxifylline was administered at 20 mg/kg 3 times daily for 30 days, relative to findings following administration of placebo on the same schedule.

Despite the demonstration of clinical efficacy, there has been minimal work performed to characterize the anti-inflammatory effect of pentoxifylline on the cutaneous immune response in dogs. In 1 study,<sup>16</sup> pentoxifylline administered orally at a dosage of 10 mg/kg twice daily failed to suppress the immediate intradermal test reactivity to house dust mite antigen, but the immediate and 3-hour postinjection reactions to lipopolysaccharide (administered to increase production of TNF- $\alpha$ ) were both suppressed. In that study,<sup>16</sup> late-phase reactions (6 to 48 hours after intradermal injection of either antigen or lipopolysaccharide) were not evaluated, and no attempt was made to characterize the effects of pentoxifylline on the microscopic inflammatory response.

In the present study, the total number of mast cells in specimens of skin injected with anti-canine IgE antibodies was decreased relative to the total number of mast cells in noninjected skin specimens. This apparent decrease in cell number is consistent with complete degranulation.<sup>18,19</sup> Pentoxifylline administered orally at a dosage of 20 mg/kg 3 times daily for 29 days failed to induce significant suppression of the immediate wheal-and-flare reaction in the study dogs. In contrast, the total number of mast cells in specimens of skin injected with anti-canine IgE antibodies was found to be significantly higher after pentoxifylline treatment, compared with pretreatment findings. This apparent

increase in total mast cell numbers in the face of an identical degranulating stimulus implies that complete degranulation of mast cells was inhibited. This is consistent with the known effects of PDE4 inhibition on canine mast cells.<sup>47</sup> The apparent paradoxical effect on mast cell function (ie, failure to suppress the immediate reaction, despite apparent inhibition of complete mast cell degranulation) is not entirely surprising. Mast cells can release several mediators (including serotonin, IL-6, IL-8, and TNF- $\alpha$ ) in a selective manner, without grossly apparent degranulation.<sup>48</sup> Thus, it is possible that pentoxifylline inhibited grossly visible mast cell degranulation but did not inhibit the immediate release of vasoactive compounds. This putative inhibition of degranulation would be consistent with the results of a previous study,<sup>49</sup> which indicated that pentoxifylline administration inhibited extensive mast cell degranulation following cutaneous wounding in rats. In addition, some other nonspecific PDE inhibitors (eg, theophylline) have been demonstrated to inhibit degranulation of mast cells,<sup>50,51</sup> and pentoxifylline inhibits degranulation of other cell types, such as neutrophils.<sup>52</sup>

In contrast to the lack of effect on either the subjective or objective aspects of the immediate cutaneous reaction against anti-canine IgE antibodies relative to pretreatment findings, pentoxifylline significantly inhibited the dermal induration associated with late-phase cutaneous reactions (as determined at the 6- and 24-hour time points after intradermal injection). This inhibition of macroscopic inflammation was accompanied by significant decreases in the number of total dermal inflammatory cells, especially eosinophils. These results are consistent with previous data demonstrating the ability of pentoxifylline to impair the polarization and migration of various inflammatory cells, including T cells.<sup>53</sup>

It is likely that there are multiple factors underlying the decreased inflammatory cell recruitment seen in skin specimens collected at the 24-hour postinjection time points in the present study. Given the likely inhibition of mast cell degranulation, it is tempting to speculate that at least some of the decreased recruitment was secondary to pentoxifylline's effects on mast cells. Cultured canine mast cells release a number of inflammatory mediators during degranulation, including IL-3, IL-4, IL-13, granulocyte-macrophage colony stimulating factor, CCL5 (RANTES), CCL2 (macrophage chemotactic protein 1), prostaglandin D2, prostaglandin E2, leukotriene B4, and leukotriene C4.<sup>54,55</sup> Expression of several of these mediators (IL-13, CCL2, and CCL5) is also upregulated *in vivo* after cross-linking of mast cell surface IgE.<sup>19</sup> Many of these mediators are known chemotactic agents. Intradermal injection of human CCL5 induces the development of a mixed monocytic-eosinophilic infiltrate at the site of injection in clinically normal dogs, whereas intratracheal administration of prostaglandin D2 induces recruitment of eosinophils in anesthetized dogs.<sup>56,57</sup> In addition, mast cell degranulation has been associated with upregulation of adhesion molecules such as ICAM-1 and P-selectin. Partial or complete inhibition of mast cell degranulation would be expected to inhibit the production and expression of many or all of these factors.

However, it must also be remembered that pentoxifylline has effects on inflammatory cells other than mast cells. Pentoxifylline decreases the release of several inflammatory mediators, including TNF- $\alpha$ , IL-12, and IL-18, from bone marrow-derived dendritic cells.<sup>45</sup> Pentoxifylline also reduces the production of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8 from human peripheral blood mononuclear cells and TNF- $\alpha$  from monocytes and macrophages.<sup>7,8,39,40</sup> These cytokines may induce the chemotaxis and recruitment of leukocytes either directly, indirectly via the induction of other chemotactic factors, or indirectly via increased expression of adhesion molecules such as ICAM-1 and Mac-1.<sup>42,45,53</sup> The suppression of any of these cytokines would be expected to result in decreased cellular recruitment.

An interesting and unexpected finding of the present study was the lack of significant inhibition by pentoxifylline on neutrophil recruitment in skin specimens injected with anti-canine IgE antibodies. In humans, neutrophils (like monocytes, macrophages, lymphocytes, and mast cells) express PDE4, which is known to be inhibited by pentoxifylline.<sup>28,30</sup> Canine monocytes, lymphocytes, and mast cells also express PDE4.<sup>31,32</sup> To the authors' knowledge, no work has yet been performed to determine PDE isoenzyme expression in canine neutrophils. It is possible that canine neutrophils do not express PDE4, unlike their human counterparts. Alternately, they may express an isoform of PDE4 that has poor affinity for pentoxifylline or one of its metabolites. Following administration in humans, 7 metabolites of pentoxifylline have been identified, whereas 4 metabolites (M1, M3, M5, and M7) have been identified in pentoxifylline-treated dogs.<sup>11,23,24</sup> In humans, the M5 metabolite has the highest concentration in the circulation, exceeding even that of the parent compound.<sup>58</sup> Many of the effects of pentoxifylline on neutrophil recruitment and function have been attributed to this metabolite.<sup>59,60</sup> In 2 studies,<sup>11,23</sup> high concentrations of M5 were detected in serum samples obtained from dogs following oral administration of pentoxifylline, but another study<sup>24</sup> failed to detect this metabolite at all. Although this discrepancy may be related to methodological differences between laboratories, it might also suggest the potential for some variability in metabolite production in this species.

The present study did have several limitations. First, histologic samples were only available for a single time point after the first or second intradermal injections. This postinjection time point (24 hours) was chosen on the basis of previous research of IgE-mediated cutaneous inflammation in dogs, in which maximal cell recruitment after injection of anti-canine IgE antibodies was evident at that time.<sup>19</sup> This approach was sufficient to demonstrate inhibition of the (presumably) maximal cellular response following injection of anti-IgE antibodies, but it provides no information with regards to the kinetics of the cellular response. Second, the lack of histologic data following the third injection precluded our ability to confirm the resolution of drug-mediated inhibition on a cellular level. Finally, no attempt was made to acquire samples for evaluation of mRNA or protein expression of inflammatory media-

tors or adhesion molecules. Further studies will be necessary to provide more detailed information.

The results of the present study have indicated that administration of 20 mg of pentoxifylline/kg orally 3 times daily was associated with significant inhibition of late-phase (but not immediate) cutaneous reactions against rabbit-origin IgG polyclonal anti-canine IgE antibodies in healthy dogs. This inhibitory effect was essentially resolved 7 days after discontinuation of drug administration. The inhibited macroscopic inflammatory response corresponded to a decrease in the accumulation of total inflammatory cells, especially eosinophils, in areas of injected skin and was associated with decreased IgE-mediated mast cell degranulation. These data have suggested a possible role for pentoxifylline in the management of IgE-mediated cutaneous inflammatory disorders, such as atopic dermatitis.

- a. Dexdomitor, Pfizer Animal Health, Madison, NJ.
- b. Histatrol, ALK-Abello, Port Washington, NY.
- c. Antisedan, Pfizer Animal Health, Madison, NJ.
- d. Olympus BX53 microscope, Olympus America Inc, Center Valley, Pa.
- e. PROC MIXED, SAS, version 9.03, SAS Institute Inc, Cary, NC.

## References

1. Halliwell REW. Revised nomenclature for veterinary allergy. *Vet Immunol Immunopathol* 2006;114:207–208.
2. Favrot C, Steffan J, Seewald W, et al. A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. *Vet Dermatol* 2010;21:23–31.
3. Jaeger K, Linek M, Power HT, et al. Breed and site predispositions of dogs with atopic dermatitis: a comparison of five locations in three continents. *Vet Dermatol* 2010;21:118–122.
4. Olivry T, DeBoer DJ, Favrot C, et al. Treatment of canine atopic dermatitis: 2010 clinical practice guidelines from the International Task Force on Canine Atopic Dermatitis. *Vet Dermatol* 2010;21:233–248.
5. Bruynzeel I, Stoof TJ, Willemze R. Pentoxifylline and skin inflammation. *Clin Exp Dermatol* 1998;23:168–172.
6. Endres S, Fulle HJ, Sinha B, et al. Cyclic nucleotides differentially regulate the synthesis of tumour necrosis factor- $\alpha$  and interleukin-1  $\beta$  by human mononuclear cells. *Immunology* 1991;72:56–60.
7. Neuner P, Klosner G, Schauer E, et al. Pentoxifylline in vivo down-regulates the release of IL-1  $\beta$ , IL-6, IL-8 and tumour necrosis factor- $\alpha$  by human peripheral blood mononuclear cells. *Immunology* 1994;83:262–267.
8. Thanhäuser A, Reiling N, Bohle A, et al. Pentoxifylline: a potent inhibitor of IL-2 and IFN- $\gamma$  biosynthesis and BCG-induced cytotoxicity. *Immunology* 1993;80:151–156.
9. Morris DO. Ischemic dermatopathies. *Vet Clin North Am Small Anim Pract* 2013;43:99–111.
10. Nichols PR, Morris DO, Beale KM. A retrospective study of canine and feline cutaneous vasculitis. *Vet Dermatol* 2001;12:255–264.
11. Rees CA, Boothe DM. Therapeutic response to pentoxifylline and its active metabolites in dogs with familial canine dermatomyositis. *Vet Ther* 2003;4:234–241.
12. Kabbesh N, Gogny M, Chatagnon G, et al. Vasodilatory effect of pentoxifylline in isolated equine digital veins. *Vet J* 2012;192:368–373.
13. Mitchell JD, Elliott J. Towards a new treatment for equine acute laminitis: the importance of signalling pathways. *Vet J* 2012;192:258–259.
14. Marsella R, Kunkle GA, Lewis DT. Use of pentoxifylline in the treatment of allergic contact reactions to plants of the Comelinceae family in dogs. *Vet Dermatol* 1997;8:121–126.
15. Scott DW, Miller WH. Pentoxifylline for the management of pruritus in canine atopic dermatitis: an open clinical trial with 37 dogs. *Jpn J Vet Dermatol* 2007;13:5–11.
16. Marsella R, Nicklin CF. Double-blinded cross-over study on the efficacy of pentoxifylline for canine atopy. *Vet Dermatol* 2000;11:255–260.
17. Singh SK, Dimri U, Saxena SK, et al. Therapeutic management of canine atopic dermatitis by combination of pentoxifylline and PUFA. *J Vet Pharmacol Ther* 2010;33:495–498.
18. Olivry T, Dunston SM, Murphy KM, et al. Characterization of the inflammatory infiltrate during IgE-mediated late phase reactions in the skin of normal and atopic dogs. *Vet Dermatol* 2001;12:49–58.
19. Pucheu-Haston CM, Shuster D, Olivry T, et al. A canine model of cutaneous late-phase reactions: prednisolone inhibition of cellular and cytokine responses. *Immunology* 2006;117:177–187.
20. Roquet A, Raud J, Hallden G, et al. Effects of loratadine on anti-IgE-induced inflammation, histamine release, and leukocyte recruitment in skin of atopics. *Allergy* 1995;50:414–420.
21. Zweiman B, Haralabatos IC, Pham NC, et al. Sequential patterns of inflammatory events during developing and expressed skin late-phase reactions. *J Allergy Clin Immunol* 2000;105:776–781.
22. Bizikova P, Linder KE, Paps J, et al. Effect of a novel topical diester glucocorticoid spray on immediate- and late-phase cutaneous allergic reactions in Maltese-Beagle atopic dogs: a placebo-controlled study. *Vet Dermatol* 2010;21:70–79.
23. Rees CA, Boothe DM, Boeckh A, et al. Dosing regimen and hematologic effects of pentoxifylline and its active metabolites in normal dogs. *Vet Ther* 2003;4:188–196.
24. Marsella R, Nicklin CF, Munson JW, et al. Pharmacokinetics of pentoxifylline in dogs after oral and intravenous administration. *Am J Vet Res* 2000;61:631–637.
25. Papich MG. Pentoxifylline. In: *Saunders handbook of veterinary drugs: small and large animal*. 3rd ed. St Louis: Elsevier Saunders, 2011;596–597.
26. Sanofi-Aventis. Prescribing information: Trental-pentoxifylline tablet, film coated, extended release, 2010;1–4.
27. Boswell-Smith V, Spina D, Page CP. Phosphodiesterase inhibitors. *Br J Pharmacol* 2006;147(suppl 1):S252–S257.
28. Cortijo J, Bou J, Beleta J, et al. Investigation into the role of phosphodiesterase IV in bronchorelaxation, including studies with human bronchus. *Br J Pharmacol* 1993;108:562–568.
29. Meskini N, Nemoz G, Okyayuz-Baklouti I, et al. Phosphodiesterase inhibitory profile of some related xanthine derivatives pharmacologically active on the peripheral microcirculation. *Biochem Pharmacol* 1994;47:781–788.
30. Essayan DM. Cyclic nucleotide phosphodiesterases. *J Allergy Clin Immunol* 2001;108:671–680.
31. Chan SC, Hanifin JM, Holden CA, et al. Elevated leukocyte phosphodiesterase as a basis for depressed cyclic adenosine monophosphate responses in the Basenji Greyhound dog model of asthma. *J Allergy Clin Immunol* 1985;76:148–158.
32. Garcia G, Ferrer L, Demora F, et al. Inhibition of histamine release from dispersed canine skin mast cells by cyclosporin A, rolipram and salbutamol, but not by dexamethasone or sodium cromoglycate. *Vet Dermatol* 1998;9:81–86.
33. Kumar RK, Herbert C, Thomas PS, et al. Inhibition of inflammation and remodeling by roflumilast and dexamethasone in murine chronic asthma. *J Pharmacol Exp Ther* 2003;307:349–355.
34. Fleming CM, He H, Ciota A, et al. Administration of pentoxifylline during allergen sensitization dissociates pulmonary allergic inflammation from airway hyperresponsiveness. *J Immunol* 2001;167:1703–1711.
35. Deree J, Melbostad H, Loomis WH, et al. The effects of a novel resuscitation strategy combining pentoxifylline and hypertonic saline on neutrophil MAPK signaling. *Surgery* 2007;142:276–283.
36. Sandoval J, Escobar J, Pereda J, et al. Pentoxifylline prevents loss of PP2A phosphatase activity and recruitment of histone acetyltransferases to proinflammatory genes in acute pancreatitis. *J Pharmacol Exp Ther* 2009;331:609–617.
37. Peterson TC, Peterson MR, Robertson HA, et al. Selective down-regulation of c-jun gene expression by pentoxifylline and c-jun

- antisense interrupts platelet-derived growth factor signaling: pentoxifylline inhibits phosphorylation of c-Jun on serine 73. *Mol Pharmacol* 2002;61:1476–1488.
38. Coward WR, Sagara H, Wilson SJ, et al. Allergen activates peripheral blood eosinophil nuclear factor-kappaB to generate granulocyte macrophage-colony stimulating factor, tumour necrosis factor-alpha and interleukin-8. *Clin Exp Allergy* 2004;34:1071–1078.
  39. Mándi Y, Farkas G, Ocsóvszky I, et al. Inhibition of tumor necrosis factor production and ICAM-1 expression by pentoxifylline: beneficial effects in sepsis syndrome. *Res Exp Med (Berl)* 1995;195:297–307.
  40. Marcinkiewicz J, Grabowska A, Lauterbach R, et al. Differential effects of pentoxifylline, a non-specific phosphodiesterase inhibitor, on the production of IL-10, IL-12 p40 and p35 subunits by murine peritoneal macrophages. *Immunopharmacology* 2000;49:335–343.
  41. van Leenen D, van der Poll T, Levi M, et al. Pentoxifylline attenuates neutrophil activation in experimental endotoxemia in chimpanzees. *J Immunol* 1993;151:2318–2325.
  42. Neuner P, Klosner G, Pourmojib M, et al. Pentoxifylline in vivo and in vitro down-regulates the expression of the intercellular adhesion molecule-1 in monocytes. *Immunology* 1997;90:435–439.
  43. Lazarczyk M, Dziunycz P, Niderla J, et al. Influence of pentoxifylline on natural cytotoxicity and expression of granzymes and PI-9, a specific granzyme B inhibitor. *Int J Mol Med* 2006;17:135–139.
  44. Kim SJ, Kim JW, Kim YH, et al. Effects of tranilast and pentoxifylline in a mouse model of chronic asthma using house dust mite antigen. *J Asthma* 2009;46:884–894.
  45. Vukanic ZS, Colic M, Dimitrijevic M. Effect of pentoxifylline on differentiation and maturation of human monocyte-derived dendritic cells in vitro. *Int Immunopharmacol* 2007;7:167–174.
  46. Bruynzeel I, van der Raaij LM, Willemze R, et al. Pentoxifylline inhibits human T-cell adhesion to dermal endothelial cells. *Arch Dermatol Res* 1997;289:189–193.
  47. Alomar A, Corella F, Garcia-Navarro X. Tacrolimus in diseases other than atopic dermatitis [in Spanish]. *Actas Dermosifiliogr* 2008;99(suppl 2):26–35.
  48. Theoharides TC, Alysandratos KD, Angelidou A, et al. Mast cells and inflammation. *Biochim Biophys Acta* 2012;1822:21–33.
  49. Babaei S, Bayat M. Effect of pentoxifylline administration on mast cell numbers and degranulation in a diabetic and normoglycemic rat model wound healing. *Iran Red Crescent Med J* 2012;14:483–487.
  50. Eleno N, Gajate E, Macias J, et al. Enhancement by reproterol of the ability of disodium cromoglycate to stabilize rat mastocytes. *Pulm Pharmacol Ther* 1999;12:55–60.
  51. Weston MC, Anderson N, Peachell PT. Effects of phosphodiesterase inhibitors on human lung mast cell and basophil function. *Br J Pharmacol* 1997;121:287–295.
  52. Deree J, Lall R, Melbostad H, et al. Neutrophil degranulation and the effects of phosphodiesterase inhibition. *J Surg Res* 2006;133:22–28.
  53. Domínguez-Jiménez C, Sancho D, Nieto M, et al. Effect of pentoxifylline on polarization and migration of human leukocytes. *J Leukoc Biol* 2002;71:588–596.
  54. Lin T-Y, London CA. A functional comparison of canine and murine bone marrow derived cultured mast cells. *Vet Immunol Immunopathol* 2006;114:320–334.
  55. Lin T-Y, London CA. Characterization and modulation of canine mast cell derived eicosanoids. *Vet Immunol Immunopathol* 2010;135:118–127.
  56. Meurer R, Van Riper G, Feeney W, et al. Formation of eosinophilic and monocytic intradermal inflammatory sites in the dog by injection of human RANTES but not human monocyte chemoattractant protein 1, human macrophage inflammatory protein 1 alpha, or human interleukin 8. *J Exp Med* 1993;178:1913–1921.
  57. Emery DL, Djokic TD, Graf PD, et al. Prostaglandin D2 causes accumulation of eosinophils in the lumen of the dog trachea. *J Appl Physiol* 1989;67:959–962.
  58. Nicklasson M, Bjorkman S, Roth B, et al. Stereoselective metabolism of pentoxifylline in vitro and in vivo in humans. *Chirality* 2002;14:643–652.
  59. Fantin M, Quintieri L, Kusz E, et al. Pentoxifylline and its major oxidative metabolites exhibit different pharmacological properties. *Eur J Pharmacol* 2006;535:301–309.
  60. Crouch SP, Fletcher J. Effect of ingested pentoxifylline on neutrophil superoxide anion production. *Infect Immun* 1992;60:4504–4509.