

Effect of prednisolone and cetirizine on *D. farinae* and histamine-induced wheal and flare response in healthy dogs

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Key words

Intradermal testing, atopia, antihistamines, canine

Summary

Objective: Prednisolone and antihistamines are highly potent drugs in the treatment of atopic dermatitis and widely used in humans and dogs. In some atopic patients in which antihistamines, corticosteroids or other drugs have already been administered intradermal testing (IDT) may be necessary. The aim of the present study was to compare the effects of cetirizine and prednisolone on IDT results. **Material and methods:** Thirty healthy dogs (average age 5.9 ± 0.6 years) were randomly assigned to three groups. Treatment groups were administered prednisolone (1 mg/kg BW daily, tapering dosage; group I), cetirizine (1 mg/kg BW daily; group II) and placebo (group III) respectively for one week. In the second week, none of the dogs received any medications. IDT was performed prior to drug administration and results obtained were considered as the baseline response. Second and third IDTs were performed at the end of the first and second week, respectively. **Results:** In groups I and II IDT reactivity was reduced at the end of first week ($p < 0.05$). After drug discontinuation the reactivity almost returned to baseline at the end of the 2-week period, with the exception of the prednisolone group for *D. farinae*. **Conclusion:** Prednisolone and cetirizine have significant effects on IDT reactions and must be withdrawn by veterinary practitioners up to 2 weeks prior to IDT.

Schlüsselwörter

Intrakutantest, Atopie, Antihistaminika, Hund

Zusammenfassung

Gegenstand und Ziel: Prednisolon und Antihistaminika stellen bei Menschen und Hunden mit atopischer Dermatitis die wirksamsten Medikamente dar. Bei einigen solcher kaninen Patienten, die bereits Antihistaminika, Glukokortikoide oder andere Medikamente erhalten haben, kann ein Intrakutantest erforderlich werden. Ziel der Studie war, die Auswirkungen von Cetirizin und Prednisolon auf die Resultate des Intrakutantests (IKT) zu vergleichen. **Material und Methoden:** Dreißig gesunde Hunde (mittleres Alter $5,9 \pm 0,6$ Jahre) wurden in drei Gruppen eingeteilt. Die Probanden erhielten eine Woche lang entweder Prednisolon (Initialdosis 1 mg/kg KM einmal täglich per os für 3 Tage, dann graduelle Dosisreduktion; Gruppe I), Cetirizin (1 mg/kg KM einmal täglich per os; Gruppe II) oder ein Plazebo (Gruppe III). In der zweiten Woche erfolgte keine Medikation. Der IKT wurde vor der einwöchigen Medikamentengabe (Ermittlung des Basiswerts) sowie nach 1 und 2 Wochen durchgeführt. **Ergebnisse:** In Gruppe I und II ergab sich am Ende der ersten Woche eine reduzierte Reaktion im IKT ($p < 0,05$). Nach der zweiten Woche hatten die Reaktionen fast wieder die Ausgangswerte erreicht. Dies galt nicht für die Reaktion auf *D. farinae* in Gruppe I. **Schlussfolgerung:** Prednisolon und Cetirizin können die Reaktionen im IKT erheblich beeinflussen. Die Medikation muss mindestens 2 Wochen vor einem IKT abgesetzt werden.

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Auswirkungen von Cetirizin und Prednisolon auf die Resultate des Intrakutantests bei gesunden Hunden

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Introduction

Intradermal testing (IDT) is one of the most useful and specific tests in the diagnosis of immediate hypersensitivity responses, wheal and flare, and often performed in veterinary clinical practice (6, 27, 31). Sometimes IDT has to be performed on patients that have already been administered antihistamines or corticosteroids. It is, however, occasionally uncertain whether the results of IDT are reliable (19).

The beneficial effect of corticosteroids in atopic disease is related to multiple anti-inflammatory mechanisms. These include the repression of many inflammatory cytokines and chemokines that amplify and perpetuate allergic inflammation, inhibit inflammatory stimuli, activate transcription factors, suppress IL-4, IL-5 (critical for eosinophilic inflammation in allergic disease) and IL-13 (important for isotype switching of B lymphocytes to secrete IgE) secreted by T helper lymphocytes and inhibit vascular permeability (4). The effects of glucocorticoids on skin reactivity de-

pend on the formulation and potency of corticosteroids used and also on dosage, frequency of administration, duration of treatment, and patient-related factors (19).

Oral antihistamines are widely used drugs for the treatment of allergic disorders in human medicine and veterinary medicine (10, 20, 28). Their main action is to counter the effects of histamine by H_1 receptor blockade. There are a number of different types of antihistamine medications, classified as either first generation antihistamines or second generation antihistamines. In various studies regarding first generation antihistamines, it has been shown that first generation antihistamines had limited effect on pruritus of dogs that has multiple underlying etiologies (18). First generation antihistamines are moderately to highly potent anticholinergic agents and also commonly act on α -adrenergic receptors and/or 5-HT receptors. It has been shown that receptor selectivity is the basis of the poor tolerability of some of these agents, especially when compared with the second-generation H_1 -antihistamines. However, some authors suggested that sedative effects of first generation antihistamines may in part be responsible for the clinical benefit. Second generation H_1 -antihistamines are more recent drugs which are much more selective for peripheral H_1 receptors in preference to the central nervous system histaminergic and cholinergic receptors. These agents display a selective peripheral H_1 antihistaminic effect, and they usually have lesser anticholinergic effects. Furthermore, they also have a lower affinity for adrenergic and serotonergic receptors and their effects on the central nervous system are limited. The active ingredients apparently do not penetrate the blood brain barrier. Some of the second generation antihistamines, notably terfenadine and astemizole, lead to cardiologic adverse effects (10, 18).

Cetirizine, which is the most important second generation antihistamine, has a greater selectivity for the H_1 -receptors, is long acting with low hepatic metabolism, in contrast to many other second-generation antihistamines (29, 30). It is rapidly absorbed and directly active. In humans, a significant antihistaminic activity of cetirizine has been reported (9, 14). De Vos et al. (11) demonstrated that cetirizine had a more potent activity and longer-acting suppressive effect than other antihistaminic drugs. In the study of Cook et al. (8) cetirizine provided satisfactory control of pruritus in 18% of the pruritic dogs.

The aim of the present study was to evaluate the effects of prednisolone and cetirizine administration on intradermal testing (IDT), and to investigate whether IDT results returned to baseline by 1 or 2 weeks post-administration.

Material and methods

Patient selection

Thirty healthy mixed breed dogs (average age 5.9 ± 0.6 years, 13 males, 17 females) were included in the study. On physical

examination, no abnormalities were found. Before and during the study the animals did not receive any other oral antihistamine, mast cell stabilizer, immunosuppressant or corticosteroid. The dogs of the three groups were housed in different pens and were fed commercial dry food with water ad libitum. Pens were cleaned with water two times daily. The dogs had soft bedding and were walked once a day. The study was approved by the local municipality committee and was carried out in accordance with Animal Welfare Guidelines in the city shelter.

Medication

The dogs were randomly assigned to three groups. In group I, prednisolone (Deltacortril® tablet [tab.], Pfizer, Istanbul, Turkey) was administered once daily with tapering dosage (1 mg/kg BW/day for 3 days, 0.5 mg/kg BW for 2 days, 0.25 mg/kg BW 2 days, PO) for a one week. In group II, cetirizine was administered once daily (1 mg/kg BW orally, Zyrtec® tab., 10 mg/tab., UCB Pharma, Spain). The dose of cetirizine used in our study was adopted from previous studies performed with dogs (6, 8). In group III (placebo), opaque red placebo capsules containing sugar were administered every day orally. The drugs were applied for one week. No drug was given to any group during the second week.

Preparation of *Dermatophagoides farinae* and histamine solution

IDTs were performed before the study and on the 7th and 14th day, using saline (0.09%, Isotonic NaCl®, Dexter, Eczabasi Inc., Istanbul, Turkey), histamine (1:10,000 [0.01 mg/ml], ALK-Abello®, Denmark) and house dust mite (HDM, 1000 PNU/ml, Greer®, Lenoir, NC, USA). The diluted allergens and histamine were stored in glass vials at 4 °C for 2 weeks.

Intradermal testing

All dogs were sedated with xylazine hydrochloride (Alfazyne®, Ege-Vet-Turkey). The skin covering the left or right lateral thorax wall was clipped gently. The skin test sites were marked with a waterproof marker. Histamine solution, antigen and isotonic NaCl (negative control) were injected intradermally with a volume of 0.05 ml. For the injection 0.5 ml insulin syringes and 27 G needles (0.4 mm) were used.

The first IDT was performed prior to drug administration, and the results were evaluated as a baseline response. The second IDT was performed 6 hours after the last drug administration. At the end of the second week, the third IDT was carried out. The flare and wheal responses were traced on plastic sheets 15 minutes after test challenge. Each skin test site was evaluated objectively at 15 minutes after injection by the same investigators who were blinded to the treatment allocation. Subjective evaluation was not performed.

Table 1

Mean value ($\pm$ SE) of wheal and flare diameter (cm^2) at base line, 7th day and 14th day for *D. farinae*.

<i>D. farinae</i>	Base line	7th day	14th day
Prednisolone (group I)	$0.820 \pm 0.069^{\text{abc}}$	$0.48 \pm 0.071^{\text{bac}}$	$0.63 \pm 0.039^{\text{cab}}$
Cetirizine (group II)	$0.57 \pm 0.073^{\text{ab}}$	$0.29 \pm 0.054^{\text{bac}}$	$0.41 \pm 0.064^{\text{cb}}$
Control (group III)	0.48 ± 0.072	0.47 ± 0.077	0.44 ± 0.072

^{abc} Differences between the values involving different letters in the same row were that are found to be statistically significant at $p < 0.05$.

Table 2

Mean value ($\pm$ SE) of wheal and flare diameter (cm^2) at base line, 7th day and 14th day for histamine.

Histamine	Base line	7th day	14th day
Prednisolone (group I)	$1.340 \pm 0.041^{\text{ab}}$	$0.99 \pm 0.032^{\text{bac}}$	$1.250 \pm 0.062^{\text{cb}}$
Cetirizine (group II)	$1.4 \pm 0.15^{\text{ab}}$	$0.99 \pm 0.073^{\text{bac}}$	$1.3 \pm 0.11^{\text{cb}}$
Control (group III)	1.40 ± 0.022	1.41 ± 0.074	1.440 ± 0.063

^{abc} Differences between the values involving different letters in the same row were that are found to be statistically significant at $p < 0.05$.

Statistical analysis

Data were analyzed using one-way repeated measurements of analysis of variance and a Tukey test was used as Post-Hoc test. P value of less than 0.05 was considered as significant. All statistical analyses were performed using Sigma Stat 3.1 for Windows statistical package (Systat Software, Inc., Point Richmond, CA, USA).

Results

Intradermal injection of histamine caused a wheal and flare response in all of the dogs in all groups. This response was accompanied by the sensation of itching at the injection site. Wheal and flare reactions highly diminished after the first week in all of the groups ($p < 0.05$) (► Table 1, 2). Inhibitions of reactions induced by *D. farinae* in group I and II were 51.2% and 49.1%, respectively at the end of first week. Reactions induced by histamine in groups I and II were 30.8% and 35.7%, respectively.

Dermatophagoides farinae: Skin reactions against *D. farinae* injections were inhibited by prednisolone to a significant degree ($p < 0.05$) on the 7th day. They increased again on the 14th day, although there was still a statistically significant difference to baseline ($p < 0.05$) (► Table 1). As shown in ► Figure 1, cetirizine inhibited skin reaction against *D. farinae* to a significant degree ($p < 0.05$) on the 7th day and the reaction was nearly equivalent to base line on the 14th day. Responses in the placebo group did not differ significantly between base line, 7th and 14th day (► Table 1).

Histamine: The application of prednisolone and cetirizine inhibited skin reactions against histamine to a significant degree ($p < 0.05$) on the 7th day (► Fig. 2). On the 14th day skin reactions were nearly equivalent to base line (► Table 2). In the placebo group, skin reactions did not differ statistically significant between base line, 7th and 14th day (► Table 2).

Discussion

The primary reason for intradermal testing is the demonstration of IgE mediated allergen hypersensitivity. The presence of a positive reaction in an intradermal test is not always indicative of allergy, as it may sometimes be an indication of subclinical hypersensitivity. In humans, IDT is less frequently performed than prick-puncture testing, primarily due to cost-effectiveness, increased discomfort and increased risk of systemic reactions (12). The discussion of whether to use IDT in addition to (or instead of) skin prick test (SPT) has been the source of significant debate. In humans, intradermal tests are generally used when percutaneous tests are negative despite an adequate history of exposure and clinical signs. There are potential advantages and disadvantages of each testing modality. Generally, IDT is the test of choice to identify offending allergens in canine atopic dermatitis by veterinary practitioners. Although there is some controversy about the evaluation of IDT (19), this test has still been considered as a „gold standard“ for the identification of offending allergens in patients with atopic dermatitis (6, 19).

Contradictory results were reported about glucocorticoid effects on histamine or allergen induced wheal and flare reactions. Oral and topical glucocorticoids have been shown to reduce both immediate and late phase skin reactions to allergens in dogs and humans (1, 2, 23, 26, 33). It was reported that otic betamethasone mildly suppressed IDT reactions to 1:100,000 w/v histamine phosphate dilution and *D. farinae* in dogs. On the other hand, in humans, short- and long-term oral corticosteroid administrations do not appear to have significant effects on IDT (12). In a prospective study (13), which was carried out in human subjects with steroid-dependent asthma treated with oral corticosteroids for a long period, the mean wheal diameters induced by allergens, and more specifically, by *Dermatophagoides pteronyssinus* and *D. farinae* were found to be similar and did not change after treatment. The present study indicated highly significant effects of prednisolone at a

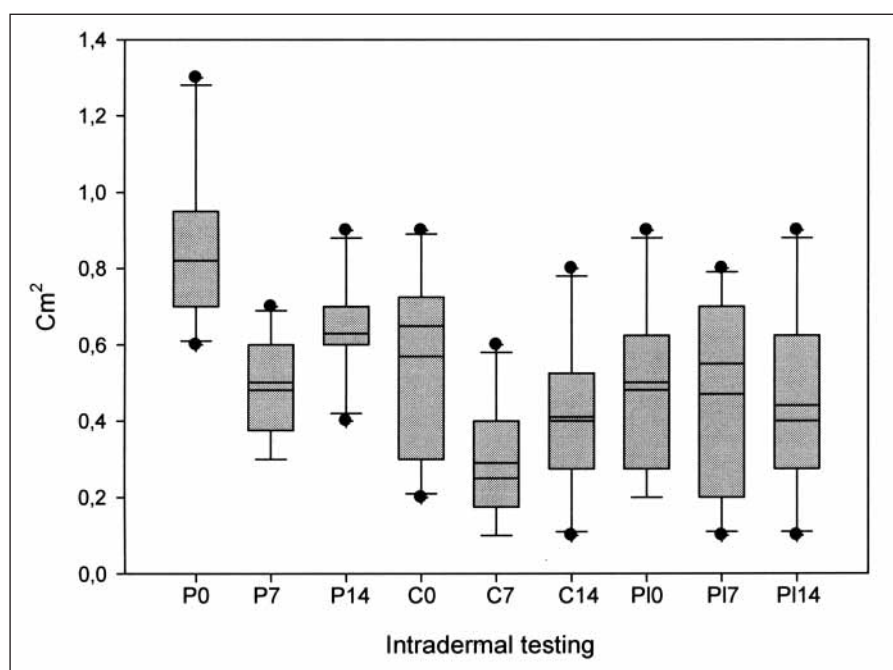


Fig. 1
Wheal and flare diameter after administration of prednisolone (P), cetirizine (C) and placebo (Pl) at day 0, 7th day and 14th day for *D. farinae*.

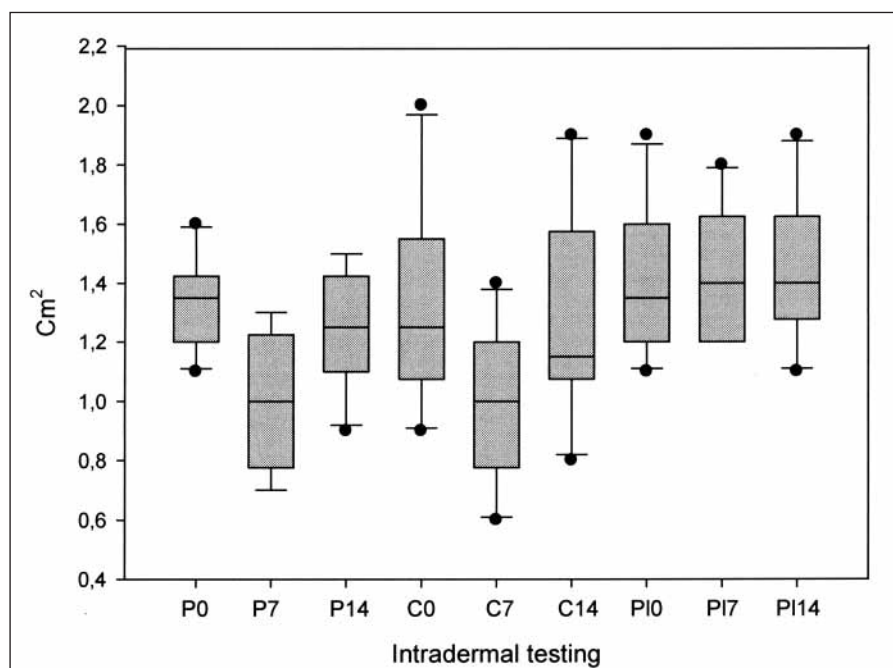


Fig. 2
Wheal and flare diameter after administration of prednisolone (P), cetirizine (C) and placebo (Pl) at day 0, 7th day and 14th day for histamine.

dose of 1 mg/kg BW in inhibiting the skin reactivity against histamine and *D. farinae* after one week, as compared with placebo. It seems that tapering administration of prednisolone over a continuous period suppressed histamine and *D. farinae* induced wheal and flare reactions. In the present study, prednisolone was given for short-term. Longer administrations may suppress the reactions and further studies are needed in veterinary medicine.

Although treatment with glucocorticoid drugs is still the mainstay of management of canine atopic dermatitis (1, 2, 23, 26, 33),

treatment with antihistamines has been reported (8, 18) to improve the control of the disease. Cetirizine hydrochloride is a second generation antihistamine with demonstrated efficacy in the treatment of human disorders (9, 14, 30). In veterinary medicine, studies regarding the use of cetirizine in dogs are limited. In a study by Barbet and Halliwell (3), it was reported that skin reactivity against histamine and flea antigen was significantly suppressed for a maximum of 9 days following oral hydroxyzine dosed at 3 mg/kg BW twice daily (3). In a study performed on dogs by Cook et al. (7)

Conclusion for practice

In the present study it has been shown that cetirizine and prednisolone can significantly alter the cutaneous response to allergens. These medications must be discontinued 2 weeks prior to intradermal tests in case of cetirizine and probably at least 3 weeks prior to the test in case of prednisolone.

cetirizine was administered at 1 mg/kg BW, PO, q24h, and produced an 80% reduction of immediate hypersensitivity reactions against intradermally injected histamine. In another study by De Vos et al. (11) it was reported that cetirizine inhibited the immediate hypersensitivity reaction induced by *Ascaris* extract in dogs and also had a 50% inhibitory effect in comparison to controls on four animal species.

The present study results showed that cetirizine is highly effective in the inhibition of histamine-induced flare in dogs. In the placebo group, no significant difference was observed between first and second week IDT results, whereas cetirizine inhibited both the reactions induced by *D. farinae* (49.1% inhibition) and histamine (35.7% inhibition) at the end of the first week. These results are in accordance with comparative studies in which cetirizine was generally most favorably compared with other non-sedative antihistamines (2, 8, 11, 14, 16, 21, 22, 32). The significantly higher efficacy of cetirizine could be due to high H₁-antihistamine potencies. Moreover, it could also be explained by additional properties of cetirizine, which are related with the late-phase allergic response. The late-phase of the allergic reaction is characterized partially by eosinophil infiltration into the reaction site. This late eosinophil recruitment is associated with the tissue damage and chronicity of the allergic response. At the skin level, cetirizine, as prednisolone (24), has been shown to inhibit the recruitment of inflammatory cells, such as eosinophils, neutrophils, and basophils, to the site of the allergic reactions (7, 15) thus diminishing the late release of histamine in the skin (22).

Reactivity to histamine was back to normal values at the end of the second week in both groups. However, in the *D. farinae* group the inhibition lasted for 2 weeks with prednisolone.

Conflict of interest

The authors confirm that they do not have any conflict of interest.

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