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Effect of diphenhydramine and cetirizine on immediate and late phase cutaneous allergic reactions in healthy dogs: a randomized, double-blinded crossover study

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Background – Currently, there is insufficient evidence to confirm oral diphenhydramine (DPH) efficacy to prevent mast cell degranulation and histamine release in dogs.
Hypothesis/Objective – To determine and compare the effects oral of DPH and cetirizine on the immediate- and late-phase cutaneous allergic reactions in healthy dogs.
Animals – Twelve healthy laboratory beagles.

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Materials and methods – The study was designed as a randomized, double-blinded crossover study in which each dog served as its own control; twice-daily oral DPH (2.2 mg/kg) or cetirizine (2 mg/kg) were given for six days with a two week washout period. Intradermal injections of histamine, compound 48/80 (positive control) and saline (negative control) were performed on the right thorax 10 days before drug administration (baseline), during oral antihistamine administration on day 6 and 10 days after last medication dosage. Global wheal scores (GWS) at 20 min and late-phase reactions (LPR) at 6h post-injection were evaluated by an investigator blinded to the drug and the interventions.

Results –Treatment with cetirizine significantly reduced histamine and compound 48/80 GWS and LPR compared to baseline; there was no significant difference for DPH. Oral DPH and cetirizine reached plasma concentrations considered therapeutic in people in all dogs. No adverse effect or behavioural changes were observed during the study.

Conclusion and clinical significance – In conclusion, oral cetirizine was effective in preventing cutaneous allergic reactions without any obvious adverse effects in dogs. Oral DPH failed to show an inhibitory effect despite attaining plasma drug concentrations that are considered effective in people.

Introduction

In human medicine, the pharmacodynamic aspects relating to antihistamine actions are studied by the experimental test model of wheal and erythema inhibition after oral administration.[1] The antihistamines hydroxyzine and cetirizine have been studied using this model in healthy dogs and both drugs significantly inhibited the histamine-induced wheal and flare reactions after oral administration,[2,3] although the effects of hydroxyzine were attributed to the rapid and almost complete conversion of hydroxyzine to cetirizine in dogs.[2] Oral histamine type-1 (H1) antagonist diphenhydramine (DPH) is frequently administered extra-label to dogs to control mild allergic reactions[4,5] and to prevent mast cell degranulation and histamine release during surgical excision of mast cell tumors.[6,7] However, there is insufficient evidence to confirm oral DPH clinical efficacy in veterinary medicine. A recent uncontrolled pilot study in six healthy research dogs failed to show an inhibitory effect on histamine-induced wheal and erythema reactions after a single oral administration of DPH at 5 mg/kg.[8]

The objective of this study was to determine and compare the effects oral of DPH and cetirizine on the immediate- and late-phase cutaneous allergic reactions in healthy dogs after repeated administration. An additional objective was to determine if oral DPH and cetirizine administration would produce plasma drug concentrations that are considered in the therapeutically-effective range in people, and if the administration would inhibit the histamine-induced reactions.

Methods

Study population

Twelve clinically healthy male castrated research beagle dogs (age 2-3 years) with no previous history of pruritus or skin disease were included in this study. The dogs were housed in the laboratory animal facilities at the university setting under conditions compliant with laboratory animal requirements. The dogs had not received any medications for at least 12 weeks prior to enrolment. The sample size was

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determined to be sufficient to provide an 80% power to detect a 50% difference between two treatment groups (diphenhydramine versus baseline/return to baseline) with a standard deviation of 30% at p=0.05 (http://powerandsamplesize.com/Calculators/). All aspects of the study were approved and conducted in accordance with the Institutional Animal Care and Use Committee (IACUC).

Study protocol

The study was designed as a randomized, double-blinded crossover study in which each dog served as its own control (Figure 1).

We evaluated the ability of oral DPH and cetirizine administration to suppress reactions from exogenous compounds after six days of administration. The inhibition studies measured the response to histamine, compound 48/80 (positive control) and saline (negative control), immediate wheal and flare reactions as well as late-phase reactions (LPRs) in healthy dogs. Compound 48/80 is a basic secretagogue which causes mast cell degranulation and wheal reactions via activation of the Mas-related G-protein-coupled receptor X2 (MrgprX2).[9-12] Oral DPH and cetirizine drug dosages were selected based on recommendations in pharmacology references,[7,13] and cetirizine pharmacokinetic and pharmacodynamic studies.[3,8]

The first intradermal injections were obtained 10 days prior to drug administration and the results were evaluated as a baseline score (Day 0). All dogs were randomized using statistical computer software (GraphPad Prism 8.0, San Diego, CA, USA) to receive twice-daily oral DPH at 2.2 mg/kg or cetirizine at 2 mg/kg for six days with a two week washout period between treatments. Intradermal injections during oral drug administration were performed after the last drug administration on day 6. A return to baseline intradermal injection score was done 10 days after the last oral medication was completely discontinued (Figure 1, day 46). All dogs were examined by the investigators and confirmed healthy and free of any dermatological or systemic disease five days prior to the first study day.

Intradermal challenge

At least 48 hours prior to each injection, a large (about 15 x 10 cm) area for injection of the test substances was clipped on the right lateral thoracic region. This 48 h delay prevented a skin reaction or irritation from clipping to interfere with the test. Dogs were sedated intravenously using medetomidine (Domitor, Pfizer, Exton, PA, USA) for the testing procedure. It has been established that medetomidine does not interfere with intradermal testing in dogs.[14,15] Testing was performed on days 0 (baseline), after oral DPH and cetirizine administration on day 6 and day 46 (return to baseline). One of the investigators (FB) blinded to oral antihistamine treatment modalities performed intradermal injections of 0.05 mL phosphate-buffered saline (negative control), histamine phosphate (0.1 mg/mL) and compound 48/80 (0.2 mg/ml) on the right lateral side of the thorax. The intradermal injections during oral drug administrations were performed at one and four h post oral administration of DPH and cetirizine, respectively; these time points were selected based on previous pharmacokinetic (tmax) and pharmacodynamic studies.[2,3,8] The order of injections was randomized using a statistical computer software programme (Prism 8.0) and the investigator (FB) was blinded during intradermal injection

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evaluations. The concentrations of the substances were chosen from values reported to induce cutaneous reactions in dogs.[2,8,16]

Clinical evaluation of intradermal injections

Twenty minutes after each injection the extent and severity of the wheals were assessed by the same investigator (FB) who performed the injections. Global wheal score (GWS) was calculated from all measurements as previously reported.[17-19] The GWS = D × E × F (the average diameter (D) in orthogonal directions was measured in millimetres; erythema (E) and firmness (F). Six hours following all intradermal injections, the global late phase reactions (LPR) score was determined as follows: LPR = E x I (erythema (E); the degree of skin induration (I)).[17-19]

Diphenhydramine and cetirizine plasma concentrations

The blood samples were collected at the time of intradermal injections after the oral DPH (1 and 2 hours post dosing) and cetirizine (4 hours post dosing) administration on day 6 to measure the concentration of these antihistamines that coincided with the time of testing. The concentrations were compared to plasma drug concentrations that inhibit the histamine induced wheal and flare reactions in people. The plasma concentrations were measured by high pressure liquid chromatography (HPLC) using a previous method that has been validated for plasma samples from dogs.[2,8]

Behavioural changes

Dogs were housed individually during the study and acclimated to the presence of the observer before administration of the drugs. Sedation scores were obtained by a blinded evaluator before administration of each drug in the morning and at 20 minutes after administration by use of a scoring system (0 = normal behaviour; 1 = mild drowsiness, 2 = moderate drowsiness, 3 = severe drowsiness);[20] day 7 before oral drugs and day 7 after the last oral drug administration served as controls. Activity (sitting, lying or active), excessive lip licking, vomiting and diarrhoea were reported as present or absent during evaluations.[3]

Statistical analysis

All statistical analysis was performed using Prism 8.0 software. Data were evaluated for normality with a D'Agostino and Pearson and Shapiro-Wilk normality test. Depending on the normality test outcome for GWS, LPRs and behavioural data, parametric repeated measures ANOVA or the nonparametric alternative, the Friedman test, was utilized. All tests were considered significant at $p \leq 0.05$; Bonferroni or Dunn's correction test was used for multiple comparisons between all groups of values depending on the parametric versus nonparametric test.

Results

Global wheal scores

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Intradermal injection of histamine and compound 48/80 resulted in a positive wheal and erythema reactions on the right thoracic side in all 12 dogs before (baseline, day 0) and after (return to baseline, day 46) treatment modalities (Figure 2a,b). There were no wheal and flare reactions observed after the intradermal injections of phosphate buffered saline (control; data not shown). Treatment with cetirizine reduced histamine and compound 48/80 GWS compared to baseline (Figure 2a,b; Figure 3a,c,d,f; Friedman test, p=0.0001 and p=0.0006, respectively) and return to baseline (Friedman test, p=0.0041 and p=0.0001, respectively); there was no significant difference for DPH (Figure 2a,b; Figure 3a,b,d,e; Friedman test, histamine; p>0.99 for both timepoints, respectively; and compound 48/80 p>0.99 for both timepoints, respectively).

Late phase reactions

Late phase reactions of histamine and compound 48/80 were significantly inhibited during cetirizine treatment compared to baseline (Figure 2c,d; Friedman test, p=0.02 and p=0.0005, respectively) and return to baseline (Friedman test, p=0.0005 and p=0.003, respectively). Again, oral DPH showed no significant effect for either the histamine (Figure 2c,d; Friedman test, p>0.99 for both timepoints, respectively) or compound 48/80 LPRs (Friedman test, p>0.99 for both timepoints, respectively).

Diphenhydramine and cetirizine plasma concentrations

After oral administration of the antihistamines to 12 healthy dogs, the DPH and cetirizine reached mean plasma concentrations of 114 ng/mL (range 58-179 ng/mL) and 10.9 µg/mL (range 2.92-15.15 µg/mL), respectively (Table S1).

Behavioural changes

There were no severe adverse drug effects observed during the study. Furthermore, there was no development of sedative effects after oral DPH and cetirizine administration during six days in any dogs (scores of "0" at all timepoints). None of the dogs showed signs of excessive lip licking, vomiting or diarrhoea; all dogs were active during the evaluations.

Discussion

The present study confirmed the suppressive effect of cetirizine in an experimental model of wheal and erythema inhibition test in healthy dogs. On the other hand, oral DPH administration for six days failed to reduce the histamine and compound 48/80 induced wheal and flare reactions as well as the late-phase responses. This is the first study comparing two different antihistamines side-by-side in the same dogs.

The findings of this study are consistent with an uncontrolled pilot study that did not show significant differences in the suppression of the histamine-induced responses after single dosing with oral DPH at 5 mg/kg in six dogs.[8] We observed a lack of effect from oral DPH tablets, despite producing plasma concentrations at 1 and 2 hours post last oral administration that were higher than reported in the aforementioned pilot study.[8] Furthermore, DPH administration to the dogs in our study produced plasma

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concentrations in all the treated dogs that are ordinarily considered effective (above 25 ng/mL) in people to reduce histamine induced wheal formation.[21] In people, drowsiness occurs at DPH concentrations from 30-40 ng/mL and mental impairment over 60 ng/mL.[21-24] There were no DPH associated behavioural adverse effects observed in our study during the six day administration. We observed no signs of drowsiness, vomiting, diarrhoea or excessive lip licking which would indicate the dry mouth antihistamine side effect.[3,25]

The present study results confirmed cetirizine's strong inhibitory potential for the histamine induced flare in dogs, as previously reported.[2,3] In a previous study the effective plasma concentration of cetirizine to suppress histamine-induced reactions in dogs was determined to be concentrations greater than 1.5 µg/mL, based on pharmacokinetic-pharmacodynamic analysis.[2] This concentration of cetirizine was easily exceeded in all dogs of our study reported here (Table S1). Cetirizine, similar to a previous study in healthy and allergic humans,[26] significantly suppressed the compound 48/80 induced wheals in healthy dogs. Compound 48/80 induces mast cell degranulation by activating MrgprX2 on mast cells in humans.[9-11] Intradermal injection of compound 48/80 provokes immediate wheal and flare reactions in healthy dogs with a rapid influx of inflammatory cells, predominantly activated eosinophils, mast cells and neutrophils at six hours.[12] Since Mrgpr members are highly homologous to each other and the comparison of the Mrgprs repertoires in dogs revealed that dogs have MrgprX2,[27] it is highly likely that compound 48/80 activates canine MrgprX2.

This screening model is considered a test system to evaluate drug efficacy in an immediate type hypersensitivity; it has additionally been used for studying the anti-inflammatory effect of anti-allergic drugs, such as glucocorticoids.[16] In this study, cetirizine suppressed the late phase of the allergic reaction at 6 h, which is characterized by the recruitment of inflammatory cells (predominantly eosinophils) into the skin reaction site.[16,26] These results are in accordance with previous studies that showed oral cetirizine inhibition of *in vivo* cutaneous LPRs in healthy and allergic human individuals.[28,29]

In conclusion, this study provides guidance for the most appropriate antihistamine drug to consider then it is necessary to suppress histamine induced immediate allergic reactions or potential histamine release from manipulation and excision of the mast cell tumours in dogs. This study in experimental dogs indicates that cetirizine should be preferred over diphenhydramine at the doses used in this study. These findings confirm what has been reported previously on the ability of each of these agents to suppress histamine induced reactions in dogs.[2,8] In this study, oral DPH failed to show an inhibitory effect on the cutaneous allergic reactions despite attaining plasma drug concentrations that are considered effective in people. Oral cetirizine at 2 mg/kg twice daily was effective in preventing wheal formation induced by intradermal histamine injections without any obvious adverse effects and should be preferred over DPH in dogs to prevent mast cell degranulation and histamine release.

This study was performed in healthy research dogs under experimental conditions and it is undetermined if these findings can be extended to clinical cases with naturally occurring diseases. However, these findings offer encouragement to conduct further clinical investigation using cetirizine at the doses cited in our study.

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Figures

Figure 1. Experimental study design.

Figure 2. Global wheal scores (GWS) scores of wheal and flare responses and late phase reactions (LPRs) at baseline, oral antihistamine administration and return to baseline. Oral cetirizine significantly inhibited histamine (a) and compound 48/80 (b) GWS. Histamine (c) and compound 48/80 (d) LPRs were also inhibited by oral cetirizine administration. There was no effect of oral diphenhydramine on histamine and compound 48/80 GWS and LPRs, respectively. Data are expressed as mean ± standard deviation (SD). *$p \leq 0.05$; ***$p < 0.001$

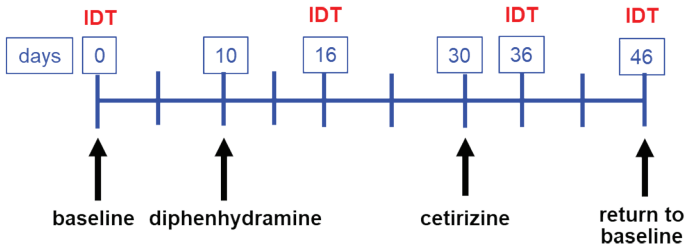
Figure 3. Clinical images of wheal and flare responses in two healthy dogs (dog 8 and 10) at baseline and during oral antihistamine administration. Oral cetirizine suppressed the wheal and erythema reactions (c, f) compared to baseline (a,d); there was no effect of oral diphenhydramine (b, e).

Supporting Information

Table S1. Plasma concentration of diphenhydramine (1 and 2 hours) and cetirizine (4 hours) post administration on day 6 of twice daily oral treatment.

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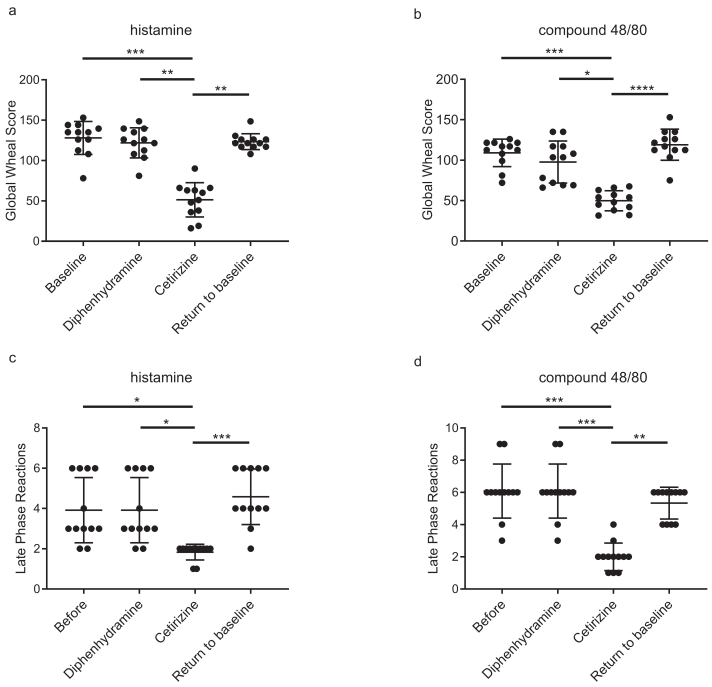
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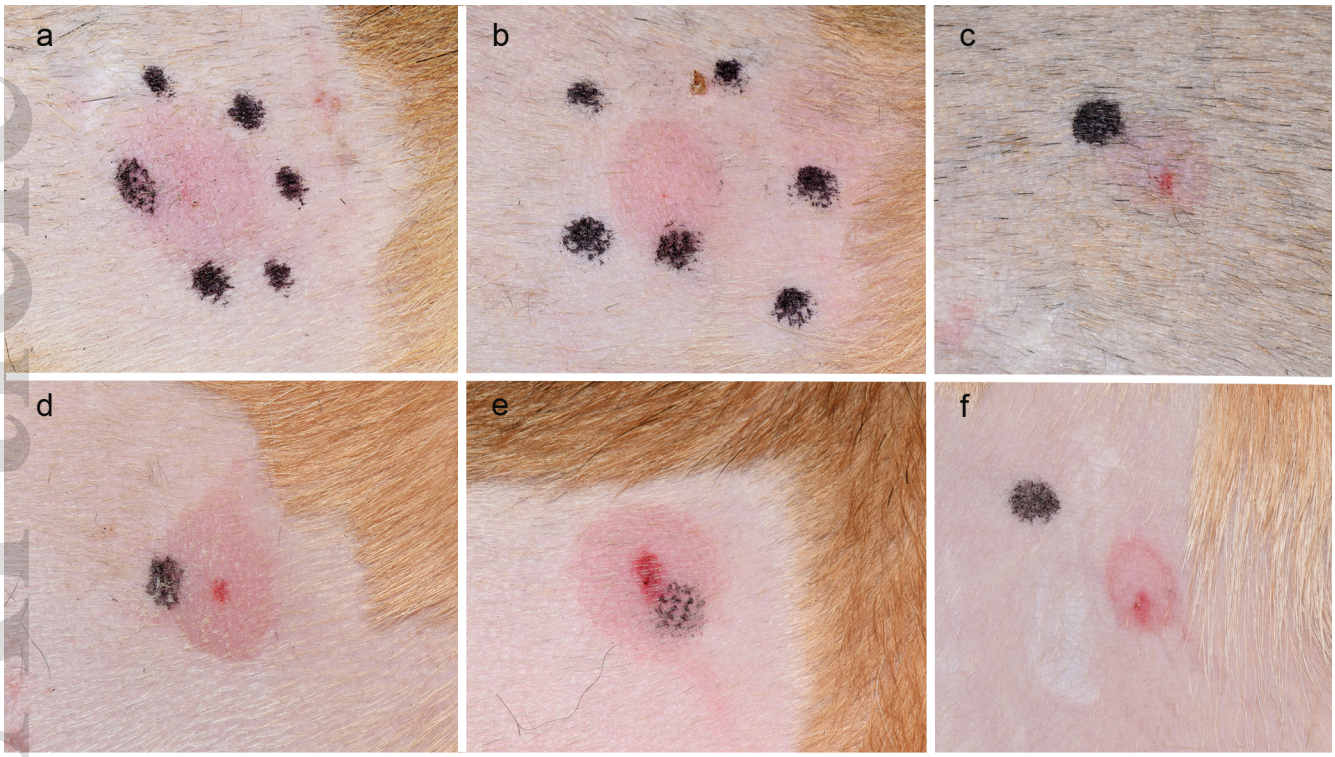


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Supporting Information

Table S1. Plasma concentration of diphenhydramine (1 and 2 hours) and cetirizine (4 hours) post administration on day 6 of twice daily oral treatment in 12 dogs.

	Diphenhydramine (nanograms/mL)			Cetirizine (micrograms/mL)
Dog	1 hour post	2 hour post		4 hour post
1	77	102		9.98
2	152	125		2.92
3	81	58		11.98
4	120	nd		12.09
5	nd	99		12.09
6	79	nd		12.58
7	nd	134		7.52
8	nd	74		9.10
9	104	113		9.87
10	179	93		14.00
11	150	134		14.28
12	122	70		15.15

nd; not determined