Effects of a 1% Hydrocortisone Conditioner on Hematologic and Biochemical Parameters, Adrenal Function Testing, and Cutaneous Reactivity to Histamine in Normal and Pruritic Dogs*

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ABSTRACT

The purpose of this double-blind study was to examine the effects of a 1% hydrocortisone, leave-on conditioner on hematologic and biochemical parameters, adrenal function tests, and cutaneous reaction to serial dilutions of histamine phosphate in healthy dogs and those with pruritic dermatitis. Groups 1 and 2 each consisted of eight healthy dogs. Seven pruritic dogs comprised Group 3. All dogs were bathed twice weekly for 6 weeks. Groups 1 and 3 had 1% hydrocortisone conditioner applied after each bath. Group 2 had vehicle from the conditioner applied after each bath. The amount of 1% hydrocortisone applied to the treated dogs ranged from 278 to 416 mg/m². Hematologic and biochemical analysis and adrenocorticotrophic hormone (ACTH) stimulation tests were performed on all dogs on days 0, 14, 28, and 42. Mean values for all blood and serum parameters remained within normal limits during the study. Post-ACTH cortisol levels were definitely lower in Group 3 than in Groups 1 and 2 on day 42 (P < 0.05) and when averaged over all days of the study (P < 0.05 and P < 0.01, respectively). Serum alkaline phosphatase levels were significantly lower in Group 3 on day 0 than on days 14 (P < 0.05), 28 (P < 0.01), and 42 (P = 0.05). All dogs received intradermal injections of buffered saline and five serial dilutions of histamine phosphate on days 0, 14, 28, and 42. No significant dif-
ferences were apparent among the groups in subjective and objective evaluation of intradermally injected dilutions of histamine. In this study, the use of a 1% hydrocortisone, leave-on conditioner did not result in clinically evident adverse effects, and only minor changes in blood parameters were detected. Although mean values in all groups remained within reference ranges throughout the study, the finding of statistically significant lower post-ACTH cortisol concentrations in the pruritic dogs (Group 3) suggests that absorption of hydrocortisone may have occurred. The results of this study also show that this product does not significantly suppress cutaneous reactivity to histamine in normal and pruritic dogs.

**INTRODUCTION**

Corticosteroids are used for a variety of canine skin diseases, including flea allergy dermatitis, atopy, food allergy, pyotraumatic dermatitis, autoimmune disorders, and acral lick dermatitis. Oral and parenteral glucocorticoids have been considered the most successful therapy for many dermatologic diseases. These drugs, however, are commonly associated with adverse systemic effects including changes in metabolism, water and electrolyte balance, inflammation, immune responses, and suppression of the hypothalamic-pituitary-adrenal (H PA) axis.

Topical application of corticosteroids has generally been considered a safer route of administration. However, localized use of some potent glucocorticoids, such as prednisolone acetate, dexamethasone, and triamcinolone, in ophthalmic, topical, and otic preparations has been shown to suppress the HPA axis in normal dogs. Generalization of application of topical glucocorticoid products has not been a common treatment modality in veterinary medicine due to a lack of penetration to the skin through the haircoat and concern about ingestion of the product. In addition, it is only recently that such products have become available.

Although potent topical corticosteroid preparations in atopic children have been shown to suppress the HPA axis, conflicting results were found on the effect of topical hydrocortisone preparations on adrenal gland function. Some investigators found that children chronically treated with 1% hydrocortisone did not show adrenal suppression, while others did find evidence of HPA suppression. Although both short-term and long-term use of a 0.01% fluocinolone acetonide shampoo did not result in detectable suppression of the HPA axis in dogs, studies evaluating the systemic effects of application of 1% hydrocortisone to large body surface areas have not been reported.

The purpose of this study was: (1) to examine the effects of a leave-on, 1% hydrocortisone conditioner (HC) for dogs (ResiCort®, Allerderm/Virbac, Ft. Worth, TX) on hematologic and biochemical parameters and on adrenal function tests in healthy dogs and in dogs with pruritic dermatitis; and (2) to examine the effects of this conditioner on cutaneous reactivity to intradermal injections of histamine phosphate in these same dogs.

**MATERIALS AND METHODS**

**Subjects**

Sixteen clinically healthy dogs and seven dogs with pruritic dermatitis were recruited from students and staff of the Veterinary Medical Teaching Hospital at the University of Florida College of Veterinary Medicine. (Information regarding signalment, haircoat length, and amount of product used on individual dogs is available upon request.) All dogs entered into the study had short- to medium-length haircoats and weighed between 5 and 22 kg. The size limitation of the dogs was im-
posed for two reasons: (1) the expense of this product will probably limit its use in larger dogs; and (2) small- to medium-size dogs may be affected by overuse of a topical product due to their increased body surface area (BSA) in relation to body weight. Haircoat length was limited because it is difficult for topical products to penetrate long haircoats or heavy undercoats in therapeutic concentrations.

Criteria for Inclusion
Clinically healthy dogs had no history of dermatologic disease or pruritus and were healthy on physical examination. Pruritic dogs had erythema, alopecia, and/or excoriation in some or all of the following body regions: face, ears, medial forelegs, paws, axillae, ventral abdomen, caudal dorsum. For the purposes of this study, no effort was made to diagnose the cause of the pruritus. Dogs were examined for secondary bacterial or yeast infection via physical examination and cytology prior to entry in the study; infections were resolved before beginning the study. Treatments included topical antibacterial and antifungal therapy and systemic antibiotics as indicated by cytologic examination.

Criteria for Exclusion
Criteria for exclusion from the study included any one of the following: (1) the administration of topical or oral corticosteroids within the previous 4 weeks of beginning the study; (2) administration of injectable, long-acting corticosteroids (e.g., triamcinolone, methylprednisolone) within the 6 weeks previous to beginning the study; (3) diagnosis of naturally occurring hyperadrenocorticism; (4) the use of other systemic or topical corticosteroids during the study; and/or (5) abnormalities at the onset of the study in total white blood cell (WBC), neutrophil, lymphocyte, monocyte, or eosinophil counts or in serum alanine aminotransferase, alkaline phosphatase, glucose, cholesterol, baseline and/or post-ACTH cortisol concentrations. In addition, dogs receiving oral antihistamines or oral essential fatty acid supplements in the 2 weeks prior to the study were excluded due to the potential effect of these agents on histamine response in the dog.15

Treatment Groups
The clinically healthy dogs were assigned to two groups in a double-blind manner. Group 1 \((n = 8)\) was treated with a 1% HC and Group 2 \((n = 8)\) was treated with the product vehicle. The weight and haircoat length of each dog were taken into account by a technician when group assignments were made in order for each group to have similar median weights and haircoat lengths. Seven pruritic dogs comprised Group 3 and were treated with a hydrocortisone-containing product. No control group of pruritic dogs was included in the study because the authors felt it would be unethical to treat pruritic dogs with placebo conditioner.

Shampoo Procedure
All three groups of dogs were shampooed with a hypoallergenic shampoo (Allergroom®, Allerderm/Virbac, Ft. Worth, TX) twice weekly for 6 weeks. Owners were instructed on proper bathing techniques, including appropriate amounts of shampoo and proper rinsing. After each bath, the 1% HC was applied to the dogs in Groups 1 and 3. Application excluded the face, and the dogs were allowed to air-dry (use of a blow-dryer was acceptable, but owners were instructed not to towel-dry the dog after application of the product or vehicle). Group 2 dogs had vehicle from the conditioner applied in a manner similar to the other two groups. The principal investigator applied the product or vehicle on the initial day of the study to determine the appropriate amount to be used on
each dog. The amount of product or vehicle applied was \( \approx 300 \text{ mg of 1\% HC per m}^2 \text{ of BSA} \), but quantities varied slightly between individual dogs due to differences in body composition (size and shape) and length of haircoat (range: 278–416 mg/m\(^2\) of HC; mean: 321.6 mg/m\(^2\)). After bathing and application of the product or vehicle on day 0, the owners were instructed on how to properly apply the product/vehicle after each bath to help ensure consistency between treatments. Additionally, the owner was provided with a clearly marked plastic bottle or syringe so that an identical volume of conditioner or vehicle could be applied for each subsequent treatment.

**Laboratory Testing**

On days 0, 14, 28, and 42 of the study, all dogs had blood collected for total WBC, neutrophil, lymphocyte, monocyte, and eosinophil counts and for serum alanine aminotransferase (ALT), serum alkaline phosphatase (SAP), serum glucose, and serum cholesterol concentrations. These values were chosen for analysis because they are most commonly affected by oral or parenteral corticosteroid therapy. Blood and serum were submitted to a commercial laboratory (Antech Laboratories, Farmingdale, NY) in either an EDTA-containing or serum-separator tube, respectively. Samples were submitted for analysis within 24 hours.

**Adrenal Function Testing**

On days 0, 14, 28, and 42, ACTH stimulation tests were performed using synthetic ACTH (Cortrosyn, Organon Inc., West Orange, NJ; 0.25 mg/dog, IV). Serum samples were collected before and 1 hour after ACTH administration and submitted to the same diagnostic laboratory for cortisol determination.

**Histamine Response Testing**

In order to evaluate the effect of the residual hydrocortisone conditioner on the dogs' response to histamine, on days 0, 14, 28, and 42, each dog was intradermally skin-tested with serial dilutions of histamine phosphate (Center Laboratories, Port Washington, NY). Buffered saline with phenol (Greer Laboratories, Lenoir, NC) was used as a negative control. Each dog was intradermally injected with five dilutions of histamine (1/100,000, 1/200,000, 1/400,000, 1/800,000, and 1/1,600,000) as has been performed in previous studies. A volume of 0.05 ml of each dilution was injected intradermally using a 26-gauge, 0.95-cm needle on a tuberculin syringe. Wheal and flare response was evaluated in an objective manner based on wheal diameter measured in millimeters and in a subjective manner using a zero-to-four scale based on erythema, turgidity, and size. To determine wheal diameter in millimeters, the diameter of each wheal was measured at two perpendicular axes, and the average of these measurements was recorded as wheal diameter. Intradermal injections were performed on the lateral thorax after clipping the hair from the area. Testing was not done within 24 hours of receiving a bath and conditioner treatment. Intradermal injections and evaluation of response were performed by one author (R.C.T.) on each day of the study.

**Statistical Analysis**

Hematologic and biochemical analysis, pre- and post-ACTH cortisol levels, and histamine response tests were analyzed with repeated-measures analysis of variance using the MIXED procedure (SAS Institute, Inc., Cary, NC). Effects of treatments and changes within each treatment group with respect to time were analyzed while controlling for the individual differences among dogs. Total WBC, eosinophil, and neutrophil counts and cholesterol concentrations increased in variability as their means increased, and therefore these were ana-
analyzed with a natural logarithmic transformation. Fisher’s least significant difference method was used for multiple comparisons of treatments and changes over time. Differences were considered significant at a $P$-value less than or equal to 0.05.

**RESULTS**

Twenty-three dogs were accepted into and completed the study. No significant differences were found among the three groups with respect to sex, haircoat length, age, weight, BSA, or amount of product used (mg/m$^2$).

Mean baseline cortisol and post-ACTH cortisol concentrations are shown in Figure 1. Mean cortisol concentrations for all groups were within the normal range for the reference laboratory on all study days. With respect to baseline cortisol concentrations, no significant differences were found among the groups at any point during the study or among overall mean values for each group. Within Group 1, baseline cortisol concentrations were significantly lower on day 14 than on day 0 ($P < 0.05$). Overall mean post-ACTH cortisol concentrations were significantly higher in Groups 1 and 2 than in Group 3 ($P < 0.05$ and $P < 0.01$, respectively). In addition, mean post-ACTH cortisol concentrations were significantly higher for Groups 1 and 2 than for Group 3 on day 42 ($P < 0.05$). No significant difference was apparent at any point in the study between Group 1 and Group 2.

Mean values for SAP and ALT are shown in Figures 2 and 3, respectively. Mean values for all hematologic and biochemical parameters remained within normal ranges for the reference laboratory for all groups during the study.

![Figure 1](image_url)

*Figure 1.* The graph represents cortisol concentrations. The symbols are as follows: *baseline cortisol levels in Group 1 significantly lower on day 14 compared with day 0 ($P < 0.05$); †post-ACTH cortisol levels in Group 3 significantly lower than Group 1 on day 42 and for overall mean ($P < 0.05$); ‡post-ACTH cortisol levels in Group 3 significantly lower than Group 2 on day 42 ($P < 0.01$) and for overall mean ($P < 0.05$). Reference range: baseline cortisol, 27.6–124.2 nmol/L; post-ACTH cortisol, 151.7–551.8 nmol/L.
With regard to SAP concentrations, no significant difference was found among groups for overall mean or on any day of the study. Within Group 1, SAP concentrations were significantly higher on day 28 than on day 14 (p < 0.05) and day 42 (p < 0.05). Within Group 3, SAP concentrations were significantly lower on day 0 than on day 14 (p < 0.05), day 28 (p < 0.01), and day 42 (p = 0.05).

The overall mean concentrations of serum ALT for the study period were significantly higher for Group 2 than for Groups 1 and 3 (p < 0.05). Additionally, on days 14, 28, and 42, ALT concentrations in Group 2 were significantly higher than in Group 1, and on days 0, 14, 28, and 42, ALT concentrations in Group 2 were significantly higher than in Group 3.

Within Group 1, ALT concentrations were significantly higher on day 0 than on day 14 (p < 0.01), day 28 (p < 0.05), and day 42 (p < 0.01).

On day 42, log converted values for mean cholesterol concentrations were significantly higher in Group 1 than in Group 2 (p < 0.05; data not shown). Otherwise, no significant difference was found in mean cholesterol concentrations among groups.

With respect to log converted mean WBC counts, no significant difference was found among groups for overall mean or on any day of the study. Within all groups, WBC counts were significantly lower on day 42 than on day 0 (p < 0.01), day 14 (p < 0.01), and day 28 (p < 0.01) (data not shown).

There were no significant differences among groups in serum glucose concentrations or neutrophil, lymphocyte, monocyte, and eosinophil counts (data not shown).

No significant difference was found in subjective or objective evaluation of cutaneous reactivity to intradermal injections of histamine at any time during the study (data not shown).
DISCUSSION

This study was designed to determine the effects of generalized topical hydrocortisone conditioner (HC) use in healthy dogs and in dogs with pruritic skin. In humans, absorption of HC through healthy adult skin is minimal, while absorption in injured or diseased skin is increased due to alterations in the skin’s barrier functions. Systemic effects due to absorption of topically applied, fluorinated glucocorticoids have been observed in healthy adult dogs, but the effects of topical HC in healthy dogs are unknown. The authors hypothesized that the absorption of HC would be increased in dogs with pruritic skin, as in humans. Although minimal, there were apparent differences in the absorption of HC in pruritic versus normal canine skin. It is unknown whether differences in cortisol concentrations were due to percutaneous absorption, ingestion, or both. The study was designed to simulate actual use; no effort was made to prevent ingestion of the product.

Although mean baseline and post-ACTH cortisol concentrations remained within normal reference limits at all times during the study, some differences among groups were observed. Mean post-ACTH cortisol concentrations were significantly lower in dogs with pruritic skin (Group 3) when compared with healthy dogs (Group 1) on day 42 and when averaged over the course of the study. Additionally, dogs in Group 1 had significantly lower cortisol concentrations on day 14 than on day 0. While absorption of HC was not measured directly in this study, the results suggest that absorption of HC may occur in sufficient quantity to suppress baseline and/or post-ACTH cortisol concentra-
tion. Because post-ACTH cortisol concentrations in Group 3 were lower than those of the normal dogs treated with 1% H C product, it also suggests that absorption may be increased in dogs with pruritic skin.

Hematologic abnormalities commonly observed with naturally occurring or iatrogenic hyperglucocorticidism include mature leukocytosis, neutrophilia, lymphopenia, monocytosis, and eosinopenia. In this study, none of these changes was observed, as mean values for all hematologic parameters remained within normal limits. The finding of significantly lower WBC counts in all groups on day 42 was unexpected. With significant absorption, increased WBC counts in the two groups receiving 1% H C product would have been expected. Other investigators have seen a decrease in WBC counts in a study evaluating the effect of ophthalmic prednisolone acetate in the canine. The reason for this change is unclear, but it may represent normal variation within WBC parameters.

Mean values for biochemical tests remained within normal limits for all groups during this study. Serum biochemical abnormalities commonly associated with hyperadrenocorticism in the dog include increases in SAP, ALT, cholesterol, and fasting glucose concentrations. Although no differences were observed among the three groups with regard to SAP concentrations, the pruritic dogs had significantly higher SAP concentrations on days 14, 28, and 42 than on day 0, suggesting absorption (either percutaneous or via ingestion). However, as mean SAP concentrations were never out of the normal reference range, the significance of these differences is unknown.

Curiously, ALT levels in Group 2 were significantly higher than in either Groups 1 or 3. This finding is opposite of what would be expected had there been significant absorption of the H C. There is no known explanation for this finding but, once again, all mean values remained within normal limits.

Although mean values for hematologic and biochemical parameters and cortisol concentrations remained within normal limits throughout the study, individual dogs within Groups 1 and 3 had abnormalities in these tests that suggested H C absorption. Also, a single dog in Group 3 had a lack of response to exogenous ACTH administration at the end of the study, which returned to normal within 4 weeks of stopping treatment. Therefore, because of individual variation in response to corticosteroids, the product should be used with care. It may be useful for future studies to evaluate the effects of more chronic use of this product and how quickly any changes in adrenal function return to normal following its discontinuation.

In this study the results of both the subjective and objective evaluations of intradermal injections with dilutions of histamine phosphate suggest that topical 1% H C does not suppress cutaneous reactivity to histamine phosphate. This was not unexpected, as H C is the least potent of the available topical glucocorticoids. However, these results should be interpreted with some caution. Although the mean values for each group were not significantly different, some individuals within Groups 1 and 3 had wheal reactions that were erythematos but appeared to be lacking induration and turgidity (i.e., they were flat). Therefore, it appears, as with all corticosteroids, that there is variability in individual responses to topical H C. In addition, as histamine directly causes degranulation of mast cells, it may be more difficult to suppress cutaneous reactivity to histamine than to other allergens.

Serial dilutions of histamine were chosen to be smaller dog with generalized erythroderma but few excoriations. It had a longer haircoat than most of the other dogs, so it received a higher quantity of H C/m2 than the other dogs.
evaluate the effect of HC on intradermal skin testing because it is an established model. In previous studies, skin reactivity to histamine was similar between atopic and healthy dogs. This was important because of the desire to compare skin test results between both groups of healthy dogs and between healthy and pruritic dogs in this study. Based on the assumption that cutaneous response to histamine may be difficult to suppress, five doubling dilutions of histamine were included to see if HC had subtle effects on histamine reactivity that would be inapparent at the most concentrated histamine dilutions.

Although used as a common experimental model, it is possible that histamine is not the ideal way in which to evaluate the mild effects of the less potent corticosteroids on intradermal skin testing. Cutaneous reactivity to histamine may be strong enough to override the effects of hydrocortisone, even at the most dilute histamine concentrations used in the study. In previous studies, dilutions as low as 1:25,600,000 weight per volume have been used to evaluate histamine reactivity. It is possible that using more dilute concentrations of histamine in this study would have demonstrated significant differences between groups. Further studies will be necessary to determine whether this topical HC administration has an effect on diagnostic intradermal skin testing.

In the study reported here, the indirect effects of absorbed HC were examined because there is no validated technique to measure directly HC absorption in the dog. Recently, a technique for quantifying systemic absorption of topical HC in human patients with erythroderma has been reported. Adaptation and validation of this technique for the dog would allow direct measurement of systemic absorption of topical HC. Studies in humans have shown that the skin's barrier function improves over the course of treatment for atopic dermatitis.

Direct quantification techniques would allow the direct measurement of absorption in dogs with dermatitis and how it changes over the course of treatment. However, because of the problems with potential ingestion through self-grooming, studies of this kind would require use of an Elizabethan collar or other device to allow differentiation of percutaneous from oral absorption.

CONCLUSION
The use of a 1% hydrocortisone, leave-on conditioner twice weekly for 6 weeks was found not to significantly alter mean biochemical, hematologic, baseline, and post-ACTH cortisol concentrations. Some systemic absorption appeared to occur in dogs with pruritic skin, resulting in significantly lower post-ACTH cortisol concentrations compared with dogs with healthy skin. Hydrocortisone did not suppress cutaneous reactivity to intradermally injected histamine in this study. Use of the product was well tolerated by the dogs and was not associated with any clinically evident adverse reactions.

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