Effects of Repeated Topical Application of a 0.0584% Hydrocortisone Aceponate Spray on Skin Thickness in Beagle Dogs

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ABSTRACT

This study evaluated the long-term cutaneous tolerance of a 0.0584% hydrocortisone aceponate spray (Cortavance®; Virbac) in dogs. Four healthy Beagle bitches were sprayed once daily for 56 days at label dose (260µl/100 cm²) on one-third of the body surface, at a distance of 10 cm from clipped skin areas. The animals were examined daily for general clinical signs and reaction at application sites. Skin biopsies were taken from treated areas before treatment and then every 2 weeks. An histopathologist blinded to date of sampling evaluated skin histology and performed 5 skin thickness micrometer measurements on tissue sections. The general status and behaviour of the animals remained unchanged throughout the trial. No local reactions or histopathological change were detected. No significant changes in skin thickness (epidermis+dermis) were recorded during the trial (median measures [95% confidence interval] at D1: 1386 [1050-1470], D15: 966 [840-1050], D29: 1050 [924-1134], D43: 1134 [1008-1260] and D57: 1260 [1050-1344] µm). The repeated use of 0.0584% hydrocortisone aceponate spray over several weeks does not produce skin atrophy in dogs.

INTRODUCTION

In human dermatology, topical glucocorticoids represent the "standard of care" in the treatment of atopic dermatitis.[1] Concerns about the side effects associated with the use of potent fluorinated topical steroids have stimulated the development of safer derivatives.[2] Molecular modifications of the chemical structure of glucocorticoids have enabled the development of compounds with anti-inflammatory potency comparable to that of earlier potent fluorinated molecules, while minimising the side effects associated with halogenation.[3,4] Hydrocortisone aceponate (hydrocortisone 17-propionate 21-acetate) is one example of a non-halogenated diester topical glucocorticoid with improved benefit-risk ratio.[3,4,5] The specific metabolism of hydrocortisone aceponate in the skin (progressive de-esterification by local esterases) is associated with minimal systemic absorption of the active compound and weak skin atrophogenic potential in man.[6,7] This molecule has been used to treat susceptible or large cutaneous areas in children with good tolerance over several weeks.[3]

In dogs, most available topical glucorticoid products in Europe are lotions, oint-

ments or creams that are impractical to use over widespread areas of skin or in areas covered by hair. Recently a low dose spray formulation of hydrocortisone aceponate (Cortavance®; Virbac), suitable for use over larger areas of hair-covered skin, has been granted approval in the European Union for short-term use (7 days) in the treatment of pruritic and inflammatory dermatitis in dogs. This formulation has been used safely in the field to control clinical signs of atopic dermatitis in dogs when used daily or intermittently for up to 70 days, with no impact on blood parameters or the results of ACTH stimulation tests.[8]

While topical glucocorticoids offer the advantage of direct application of the anti-inflammatory compound to the target skin tissue, reducing systemic side effects, important local adverse effects, such as skin atrophy, comedone formation, subepidermal vesiculation and alopecia, have been associated with their prolonged daily use in dogs. [9,10] The objective of this study was to specifically evaluate at macroscopic and microscopic levels the tolerance by canine skin of the prolonged daily use of the 0.0584% hydrocortisone aceponate spray over two months.

MATERIALS AND METHODS

Ethics

This study was performed in compliance with the animal ethical rules of European Directive 86/609/EEC, in facilities declared in conformity with Good Laboratory Practices by the AFSSAPS (French Health Products Safety Agency). The study plan was agreed by the animal Ethics Committee of the test facility (EVIC, Blanquefort, France).

Animals

Four female Beagle dogs weighing between 7.7 and 9.9 kg and aged between 7 and 9 months were housed individually in units of 1.20 m² (floor) by 1.80 m (height). The premises were maintained under controlled temperature (15-20°C) and relative humidity (50 ± 20%) with renewal of fresh air at a rate of 10 cycles per hour. Artificial light was set to a 12-hour light, 12-hour dark cycle per day. The bitches were fed 400 g of a complete pelleted diet (Croc, Royal Canin, France) and provided with 1500 ml of fresh water every day.

Test product

Cortavance® (Virbac; Carros, France) is a 76 ml pump spray that delivers 130 µl per stroke of a transparent liquid solution of hydrocortisone aceponate (0.584 mg/ml) in propylene glycol methyl ether. The label regimen is two strokes (260 µl) to treat a surface of 10 x 10 cm on the dog's skin once a day for 7 days, corresponding to a daily dose of 1.52 µg of hydrocortisone aceponate per cm² of affected area, sprayed from a distance of 10 cm from the target area.

Treatment procedure

Twenty-four hours prior to study initiation, the hair coat on both sides of each dog was clipped over an area delimited dorsally by the spine, ventrally by the mammary chain, and laterally by the shoulders and the thighs. This represented about one-third of the total body surface, calculated by the formula (Km x bw0.67)/100, where Km = 10.1 and bw = bodyweight in kg. The test product was sprayed from a distance of 10 cm onto the clipped area at the label dose (two strokes/100cm²) once a day for 56 days. The animals were treated each day at the same time (in the morning between 8:00 and 12:00). Additional clipping was performed regularly over the study period to control hair-regrowth.

Clinical observations, histology and measurement of skin thickness

During the period of the trial, the animals were examined by a qualified veterinarian for the occurrence of any general or local clinical signs twice a day: before treatment and one hour following administration.

Skin biopsies of the clipped area on the flanks were performed with an 8-mm biopsy punch (Stiefel Laboratories; Nanterre, France) before the first treatment (D1) and every 2 weeks during treatment (D15, 29, 43, 57). The bitches were sedated for

skin sampling by intramuscular injection of medetomidine (Domitor®, Pfizer Santé Animale; Paris, France) at 50 µg/kg. Additional local anesthesia was provided with lidocaine. After biopsy the skin was sutured and full recovery from sedation was obtained by intramuscular injection of the antagonist, atipemazole (Antisedan®, Pfizer Santé Animale; Paris, France) at 250 µg/kg. The skin samples were fixed in Bouin's solution. Prior to microscopic examination, the tissue samples were embedded in paraffin wax and 4 µm sections were stained with haematoxylin and eosin. From each skin specimen, one to three sections taken from the center of the block were examined under light microscopy. Any anomaly detected at histology was recorded and graded by the same histopathologist on a 4-point scale as very slight, slight, moderate or severe. The thickness of the epidermis and dermis (distance from the hypodermis to the granular cell layer) was determined by the histopathologist along an axis perpendicular to the skin surface by micrometer measurement. Five skin fold thickness measurements per animal were performed on tissue sections (from the edge to the center) at each time point. For all examinations, the histopathologist was blind to the date of sampling and animal, as all the specimens were identified by a code number.

Statistics

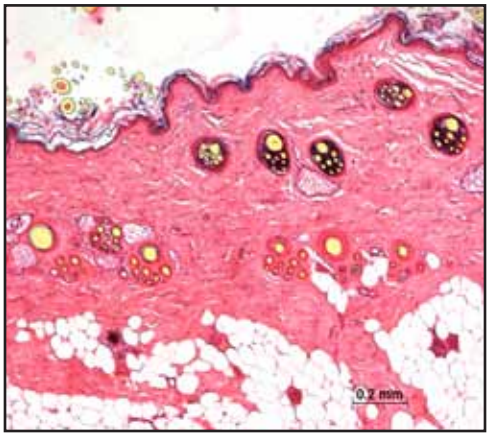
The median of 5 measurements of skin thickness was considered for each animal at each time point. A non-parametric Friedman two-way ANOVA on ranks appropriate for a repeated measure design was used to compare skin thickness between time points. Post-hoc pairwise Wilcoxon signed rank tests were planned to differentiate between time points in case the Friedman test was significant. Statistics were performed using commercial computer software (NCSS® 2004, NCSS, Keysville, Utah, USA). P less than 0.05 were considered statistically significant.

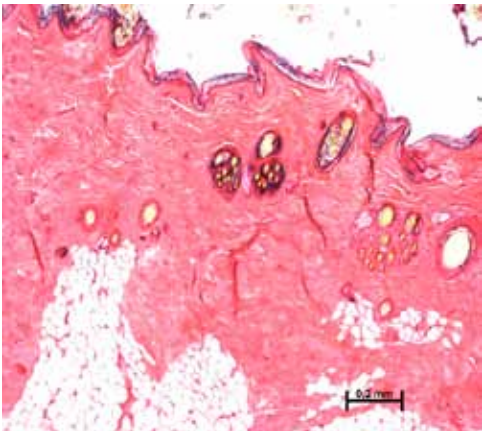
RESULTS

No general or local adverse events were reported during the test period. The general status and behaviour of all 4 bitches remained unchanged throughout the experiment. No obvious sign of cutaneous irritation was perceptible.

No abnormal microscopic findings were recorded by the histopathologist in the biopsies of Beagle dogs skin treated by the hydrocortisone aceponate spray over the study period, except that all subjects exhibited a slight thickening of the normal horny layer at all times, including pre-treatment, and that slight acanthosis was noted in two dogs on

Figure 1. *Photomicrograph of canine skin, dog no. 3 (a) on day 1 before treatment and (b) on day 57 after 8 weeks of daily treatment with 0.0584% hydrocortisone aceponate spray. No significant change from baseline of skin histology or epidermis and dermis thickness is observed after treatment. H&E. Bar = 200 µm.*





D43 only. No flattening of epidermal cells, reduction in the number of epidermal cell layers, or follicular atrophy was observed (Figures 1 a & b). The dermal architecture appeared unaffected by treatment.

No significant change of the total epidermis and dermis thickness was recorded in the skin from the flanks of treated animals at D15, D29, D43 and D57, compared to pre-treatment values in the same areas on D1 (Friedman ANOVA, P=0.4481, Table 1).

DISCUSSION

The results document the good cutaneous tolerance of a 0.0584% hydrocortisone aceponate spray used daily over two months in healthy dogs.

Skin thickness recorded at baseline in this study is in agreement with the reported average thickness of canine skin (500-5000 µm).[11] Variations in measures obtained on each individual at each time point reflect the fact that the upper limit of the hypodermis is not always parallel to the epidermis on biopsy sections. Therefore in the same section there are areas where the skin (epidermis plus dermis) appear thicker and others where it looks thinner (like in figures 1a and 1b). Taking five measurements of skin thickness from the edge to the center of each section therefore was a means to obtain consistent mean values at each evaluation time point. Variations in skin thickness between dogs recorded throughout the study may be related to minor physiologic differences between individuals (eg. the dog hydration status as the dermis contains a third of the interstitial fluid volume) or small differences in the location of biopsy sites on the flanks (skin thickness decreases ventrally on the trunk[11]). An homogeneous population of female Beagle dogs of the same age and size were selected to reduce inter-individual variability, as skin quality is reported to vary among breeds of dogs, and with age and sex.[11,12]

Overall, no significant skin thinning or anomaly at histology was produced by repeated topical use of the 0.0584% hydrocortisone aceponate spray for eight weeks. This contrasts with other reports of epidermal atrophy resulting from prolonged topical use of topical glucocorticoids, such as betamethasone valerate.[13] Skin thinning of the glabrous skin of the ventrum and medial thighs, associated with the histological observation of bullae and clefting in the superficial dermis, hair follicle atrophy and comedones, have also been reported in dogs treated daily with fluorinated corticosteroid creams (0.1% triamcinolone acetonide, 0.025% fluocinolone acetonide) for periods equal to or greater than 4 weeks.[9] In another study, moderately potent (0.025% fluocinolone acetonide) to potent glucocor-

Table 1. *Skin thickness (epidermis + dermis) of Beagle dog skin sprayed daily with 0.0584% hydrocortisone aceponate for 8 weeks*

Median (range) of 5 measures (µm)	Day 1	Day 15	Day 29	Day 43	Day 57
Dog 1	1386 (1092-1470)	1302 (1050-1680)	1176 (840-1344)	1260 (1050-1428)	1302 (1134-1848)
Dog 2	1176 (924-1512)	882 (756-1050)	924 (840-1050)	1260 (1050-1470)	1176 (924-1470)
Dog 3	966 (798-1050)	1050 (840-1260)	1134 (1050-1428)	924 (840-1092)	1344 (1050-1386)
Dog 4	1764 (1386-2100)	840 (630-966)	966 (840-1134)	1176 (840-1260)	966 (756-1260)
Median (95% CI) of measures on all four dogs*	1386 (1050-1470)	966 (840-1050)	1050 (924-1134)	1134 (1008-1260)	1260 (1050-1344)

ticoid creams (0.1% diflucortolone valerate, 0.1% momethasone furoate) applied daily for 4 weeks to the skin of Mexican hairless dogs produced marked epidermal thinning associated with flattening of keratinocytes, hair follicle atrophy, and dermal collagen hyalinisation.[14] By contrast a reduced-concentration 0.015% triamcinolone acetonide spray was well tolerated over 4 weeks in a clinical trial on allergic dogs,[15] although no skin thickness measurement or histologic observations were performed to examine cutaneous safety.

Differences in the outcome of studies on topical steroids may reflect differences in dose administered, region of application, glucocorticoid potency, metabolism and carrier vehicle. In the study of Kimura and Doi,[14] fluorinated glucocorticoids at concentrations ranging from 0.025% to 0.1% were applied to the dorsal skin of each dog at the dose of 400µl/100cm². In the present study, the skin of both flanks was sprayed with 0.0584% hydrocortisone aceponate at the dose of 260µl/100cm², ie a dose approximately 4-fold lower. That dose of hydrocortisone aceponate proved effective in markedly reducing pruritus and skin lesions in dogs with various allergic skin conditions.[8, 16] Radioactive 3H-hydrocortisone aceponate formulated in the same vehicle and applied at the same dose penetrates the canine stratum corneum and can be localised in the epidermis and superficial dermis in canine skin biopsies taken 6h after topical application.[17]

The specific metabolism of hydrocortisone aceponate in the skin may account for the lack of cutaneous adverse effects seen in the present study, if similar mechanisms apply in various mammal species. Hydrocortisone aceponate inhibits the proliferation of human skin fibroblasts less than equipotent betamethasone 17-valerate in vitro and also exerts less unfavourable effects on collagen and total protein synthesis.[5,18] Clinical studies performed in human patients show no significant reduction of skin thickness (measured in vivo by high-frequency ultrasound) over periods of 6 weeks to 13 weeks of hydrocortisone aceponate ointment application.[4,6] In the epidermis of rats, the parent compound hydrocortisone aceponate is hydrolysed in the C-21 position by local enzymes naturally present in the skin (esterases) that release the anti-inflammatory hydrocortisone 17-monoester metabolite in the epidermis and upper dermis. Further transformation (acyl migration) and de-esterification in the deeper layers of the skin release unesterified weaker metabolites (mainly hydrocortisone).[2,7] Specific biotransformations of hydrocortisone aceponate to weaker metabolites in the skin layers may thus contribute to reduced antiproliferative effects, particularly on fibroblasts in the dermis.[2]

There are several limitations to this study

The choice of an invasive method for measuring skin thickness (repeated biopsies under sedation every 2 weeks) reduced the number of bitches that could be included in the trial. While diagnosting imaging, and particularly high-frequency ultrasonography, is commonly applied to evaluate the skin in humans, fewer data are available in dogs to validate the use of this non-invasive technique for accurate skin thickness measurement.[19] Moreover, skin samples allowed simultaneous precise evaluation of the consequences of treatment on skin histology. Although the number of subjects was reduced in this study, the repeated measure design (within-subject comparisons) and repetition of measurements at each time point and over time (total of 100 skin thickness assessments) allowed meaningful statistical comparisons. The sites of biopsy could have been more codified on the flanks, evaluating separately for example upper and lower sites, which may have reduced variability in results. In addition, flank skin examined in healthy dogs in the present trial is rarely involved in atopic dermatitis and the effects of long term treatment with the hydrocortisone aceponate spray on the thinner, glabrous skin of the ventral body could be greater. It is not known to what extent the results

of this study performed in healthy female Beagles apply to a variety of dog breeds of both sexes with impaired epidermal barrier in the clinical situation, although no cutaneous atrophy was reported in a 70-day clinical trial on atopic dogs in the field.[8]

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