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Original article

Anti-pruritic effect of topical capsaicin against histamine-induced pruritus on canine skin

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Abstract

Several human studies have reported that capsaicin has anti-pruritic effects. Moreover, several concentrations of topical capsaicin have been used to alleviate itch. The aim of this study was to investigate the anti-pruritic effect of capsaicin against histamine-induced pruritus compared with that of topical steroid or vehicle in 15 healthy beagles. Fifteen dogs were divided into three groups (n = 5 each), and treated topically with one of the following on the left side of the neck: capsaicin, positive control (steroid), or negative control (vehicle). Each treatment was performed twice daily for 8 days. All dogs were injected with histamine intradermally before treatment and on the 2nd, 4th, 6th, and 8th days of the treatment to evoke itch. Pruritus, wheal, and erythema intensity were assessed at each evaluation; cutaneous temperature was also recorded. On the final day, skin biopsy was conducted for histopathological evaluation for all dogs. The severity of pruritus was lesser in the capsaicin-treated group compared with the negative control group on day 8 ($p<0.05$). In the capsaicin and steroid groups, wheal size, erythema index, and cutaneous temperature also decreased compared with pretreatment. Histopathological evaluation showed that the capsaicin-treated group had a higher number of inflammatory cells in the dermis compared to the vehicle control group; however, the steroid-treated group showed less severe inflammatory reactions than the vehicle control group. These results suggest that capsaicin cannot reduce inflammation but may play a helpful role in reducing pruritus in dogs.

Key words: anti-pruritic effects, capsaicin, dog

Introduction

Pruritus is the most common symptom associated with many skin diseases, and can lead to an increased predisposition to skin infection in dogs (Greaves and Khalifa 2004). In addition, chronic and continuous itching may result in low quality of life with severe discomfort (Hundley et al. 2006, Favrot et al. 2010). To control pruritus, many clinicians use glucocorticoids; however, these are sometimes ineffective and cause unwanted side effects (Wahlgren 1991, Olivry et al. 2010).

Capsaicin is a phytochemical ingredient obtained from the plant genus *Capsicum*. The diverse effects of capsaicin have been reported in various clinical settings (Foreman 1987, Tupker and Coenraads 1992, Ellis et al. 1993, Tarng et al. 1996). The classical view is that capsa-

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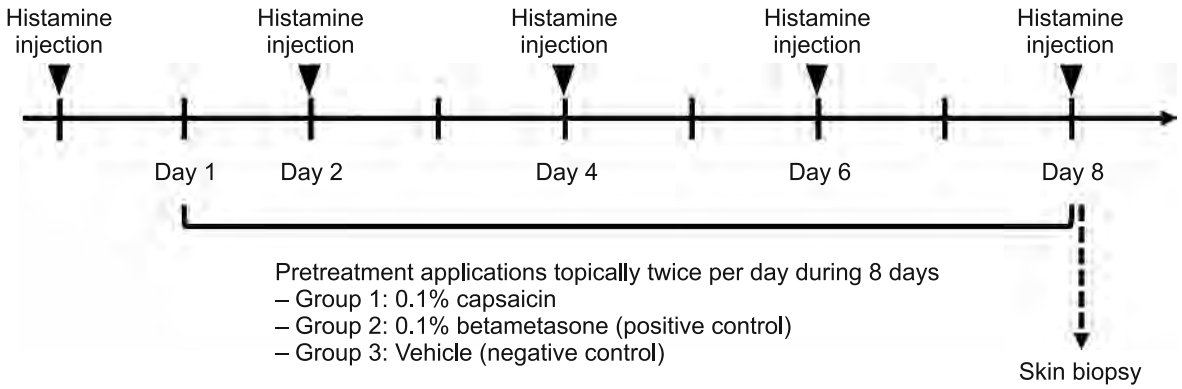

Fig. 1. Experimental design.

icin activates a subset of polymodal nociceptor fibers when it is first applied, increasing membrane permeability to cations by up-regulating cationic receptors. This in turn leads to the release of neuropeptides, including substance P, from nerve terminals and causes burning pain. However, prolonged or repeated application of capsaicin leads to desensitization (Bernstein 1987). In humans, several studies have reported that capsaicin reduces pruritus and pain in several conditions, and topical capsaicin formulations are widely used to relieve pain or itching (Foreman 1987, Tupker and Coenraads 1992, Ellis et al. 1993, Tarng et al. 1996). However, studies investigating the anti-pruritic effects of capsaicin application to canine skin are scarce.

The aim of the present study was to investigate the anti-pruritic effect of capsaicin on canine skin. Histamine was injected intradermally to evoke pruritus. Histamine, a well-known mediator in inflammatory diseases has long been known to induce pruritus in dogs (Roßbach et al. 2009). It causes wheal and erythema when injected intradermally. It also causes intense pruritus and is often used as a positive control in studies investigating pruritus. To evaluate the anti-pruritic efficacy of capsaicin, we measured pruritus severity, wheal intensity, erythema index, and cutaneous temperature. On the final day, skin samples were obtained using skin biopsy. In addition, topical betamethasone and vehicle were administered as positive and negative control, respectively.

Materials and Methods

Experimental animals

Fifteen healthy beagles (10 males, 5 females; age 3.7 ± 0.4 years; body weight 9.1 ± 2.4 kg) were used in this study. The dogs were randomly divided into three subgroups: group 1 (treated with capsaicin); group 2 (treated with steroid as a positive control); and group 3 (treated with vehicle as a negative control). Each dog was housed in a separate cage and fed a commercial diet. None of the dogs had a previous history of pruritus or skin disease, and none received any medications for at least 4 weeks before the experiment.

Experimental design

A schematic representation of the study is shown in Fig. 1. Twenty-four hours before the experiment, all dogs were examined by the investigator to confirm the absence of any dermatological or systemic disease. The left dorsal neck region (5 cm from the sixth to the seventh cervical spinous processes) was shaved using a clipper (#10, Oster, USA). Then, throughout the experiment, capsaicin, steroid, or vehicle were topically applied to the same region twice daily. Histamine was injected five times (before treatment, and at days 2, 4, 6, and 8 after test material application). Each test was performed by the same investigator to minimize variability in the application technique. The test site was marked using a marker pen (TLS™ Surgical Skin Markerpen, Portex Surgical Inc., UK). The intradermal histamine injection and measurements were performed in a quiet laboratory with the room temperature maintained at 20 to 25°C and humidity maintained at 30% to 45%. Before each experiment, the animals rested for at least 30 min in the room. On the final day of the experiment, skin specimens from all dogs were obtained for histological evaluation. These study protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Kyunpook National University.

Intradermal histamine injection

Histamine dihydrochloride (assay ≥99%, powder, Sigma-Aldrich Co., USA) was injected intradermally to induce a pruritic response. The histamine was injected into test sites in the neck region. A pilot experiment was performed to determine the optimal concentration of histamine, which was 0.1%. In this study, 0.1 ml of

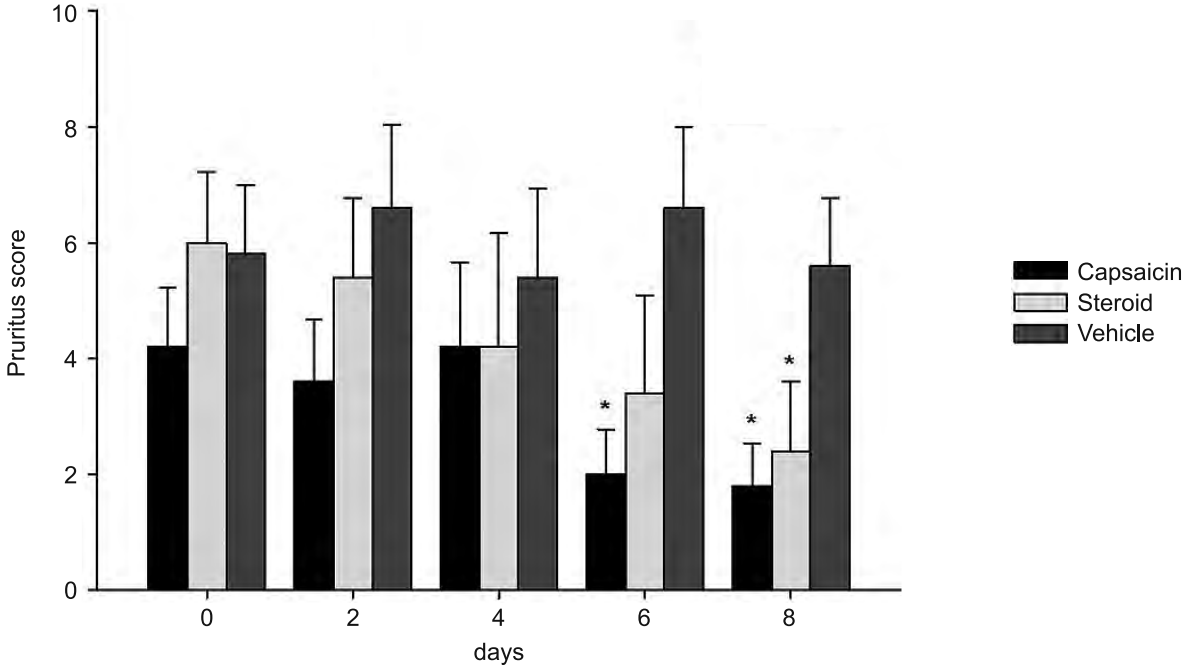

Fig. 2. Pruritus scores following histamine injection in skin treated with capsaicin, steroid and vehicle. Capsaicin showed lower pruritus severity as compared with pretreatment at day 6 and 8. * $p<0.05$ as compared with pretreatment (day 0).

histamine dihydrochloride (0.1%) was injected into the skin. Before histamine injection, the skin was cleaned with alcohol.

Application of treatments

As mentioned earlier, the dogs were divided into three groups and administered their respective treatments. A vial of capsaicin (from *Capsicum*; M2028-50MG, Sigma-Aldrich, USA) was diluted with vehicle containing 2 parts propylene glycol and 1 part 80% ethanol; the final concentration of capsaicin was adjusted to 0.1%. Betamethasone sodium phosphate (Korea Global Pharm., Korea) was used as the positive control, with a final concentration of 0.1%. The vehicle was the solvent used to dilute the capsaicin. 0.3 ml of each treatment solution was applied to the same lesion twice daily for 8 days. Before each treatment, the skin was softly washed with shampoo and water, and then dried.

Pruritus severity

Pruritus severity was assessed according to a visual analogue scale (VAS); pruritic behavior included scratching, chewing, shaking, and licking. The VAS ranged from 0 to 10, with 0 as "no pruritus" and 10 as "worst pruritus". Pruritus score was estimated during the first 10 min observation interval.

Wheal measurement

Wheals were measured using an electronic digital caliper (Digimatric caliper, Mitutoyo Corp., Japan). The wheal diameter was measured at the widest point. The thickness of the wheal was measured by folding the skin.

Erythema index

A Mexameter (MX18, Courage and Khazaka, Germany) was used to evaluate skin redness. This device measures the stimulation of microcirculation before and after histamine application by measuring the hemoglobin value. Skin redness manifests as a numerical value.

Cutaneous temperature

The skin temperature of the injected area was measured using an infrared thermometer (Raytek Co., USA). Temperatures are expressed as the mean of three measurements for each site.

Skin biopsies

To evaluate changes in the skin, 6 mm skin biopsy specimens were taken from all dogs at the end of each treatment. Skin specimens were obtained under local anestheesia (0.5 mL lidocaine hydrochloride 2% per site; Cheil, Korea) using a disposable skin biopsy punch. Sites were sutured in a routine manner.

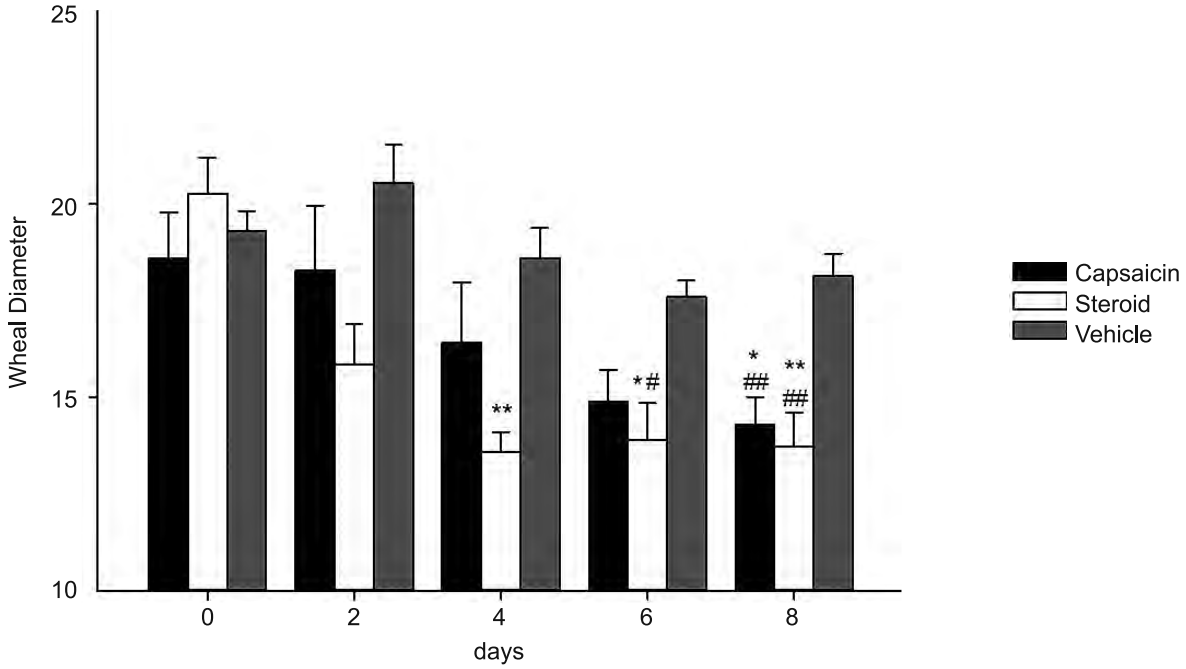

Fig. 3. Wheal diameter following histamine injection in skin treated with capsaicin, steroid and vehicle. Repeated capsaicin and steroid treatment decrease wheal diameter significantly compared with before treatment. *p<0.05, **p<0.01 as compared with pretreatment (day 0). #p<0.05, ##p<0.01 as compared with vehicle control skin.

Prepared sections were stained with hematoxylin and eosin (H&E) for light microscopy examinations. The histological profiles of individual cross-trimmed skin were then observed under light microscopy (E-400, Nikon, Japan).

Statistical analysis

All statistical analyses were performed using commercial statistic software (SigmPlot® 12.0; Systat Software Inc., San Jose, CA, USA). To identify the efficacy of each treatment, the changes in clinical assessments were verified by paired t-tests between the pre-treatment day (day 0) and each post-treatment (day 2, 4, 6, and 8) day. The differences between treatments on the same days were analyzed and compared using one-way ANOVA. All values are expressed as mean ± standard deviation. A p-value < 0.05 was considered to be statistically significant.

Results

Pruritus severity

The changes in pruritus severity are shown in Fig. 2. In most cases, itching began within 1 min of histamine injection and lasted for 10-15 min. Fifteen minutes after the histamine injection, pruritus gradually decreased. Pruritus was severely decreased in the capsaicin-treated groups on days 6 and 8, compared with day 0. Capsaicin treatment significantly decreased pruritus severity by 52.3% on day 6 and 57.1% on day 8 compared to day 0). The steroid-treated groups also exhibited remarkably lower pruritus severity on day 8 compared with day 0 (p<0.05). In this group, the pruritus score had decreased by 60% by day 8 compared to day 0. The vehicle-treated group exhibited stable pruritus severity throughout the experiment.

Wheal measurement

The values of wheal diameter and thickness are shown in Figs. 3 and 4, respectively. In general, the wheal became visible immediately after histamine injection. Wheal formation lasted approximately 20 min, and disappeared approximately 30 min later. The wheal diameter in the vehicle-treated group was stable over a 1-week period. However, over time, wheal diameters in the capsaicin- and steroid-treated groups decreased. The wheal diameter in the capsaicin-treated group was shorter than that in the vehicle-treated group on day 8 (p<0.05). The capsaicin-treated group exhibited reduced thickness on day 8 compared with day 0 (p<0.05). The differences in wheal diameters in the steroid group were even more remarkable. On days 4, 6, and 8, the wheal diameters in the steroid-treated group were significantly lower than those on day 0 (p<0.05). On days 6 and 8, there were significant differences between the steroid-treated and vehicle groups

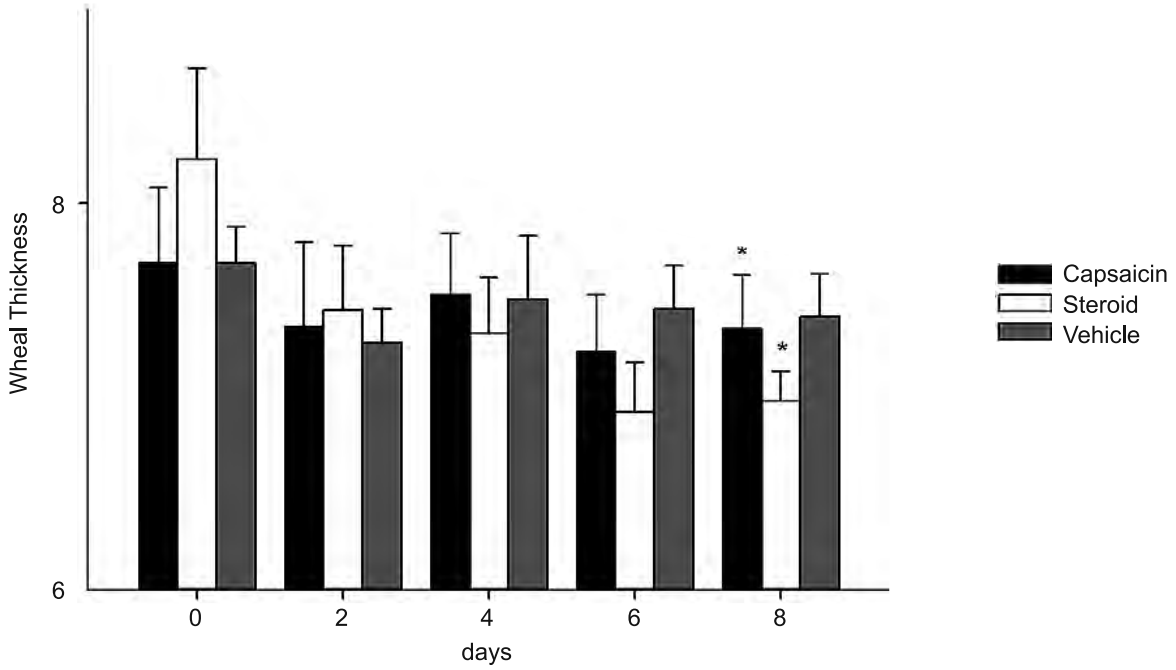

Fig. 4. Wheal thickness following histamine injection in skin treated with capsaicin, steroid and vehicle.
The wheal thickness of capsaicin and steroid treated groups decreased significantly at day 8 compared with pretreatment.
*p<0.05, **p<0.01 as compared with pretreatment (day 0).

($p<0.05$). Wheal thickness in the capsaicin- and steroid- treated groups were significantly lower than those in the vehicle group on day 8 ($p<0.05$).

Erythema index

The vehicle-treated group exhibited no significant changes during the experiment (Fig. 5). The steroid--treated group exhibited a lower erythema index on day 8 compared with day 0. In contrast, the capsaicin-treated group exhibited slightly increased values on day 2 and day 4 compared with day 0 (increased by 8.8% on day 2 and 10.4% on day 4). After that, the erythema index values were lower on days 6 and 8, and the lowest value was estimated on day 8 (decreased by 12.3% compared with day 0)

Cutaneous temperature

Cutaneous temperature is shown in Fig. 6. On day 2, the capsaicin-treated group exhibited lower skin temperatures than the other groups ($p<0.05$). Except for this value, there were no statistically significant differences between groups.

Histopathology

Changes in epidermis thickness, loosening of the dermis, and the number of infiltrated inflammatory cells in the dermis were significantly inhibited by steroid treatment compared with the vehicle control ($p<0.01$), but they were increased by treatment with capsaicin ($p<0.01$). The results of histopathological evaluation are shown in Table 1.

Discussion

The transient receptor potential vanilloid receptor-1 (TRPV1), an important peripheral integrator of pain, was originally identified on C-type nociceptive sensory neurons as a molecular target of capsaicin (Foreman et al. 1983, M Schmelz et al. 1997, Shim et al. 2007). Capsaicin, a TRPV1 agonist, can bind to and activate this receptor, which leads to the release of neuropeptide contents by sensory neurons (Shim et al. 2007, Costa et al. 2008). Via this pathway, an individual may feel pain, itching, or burning sensations (Shim et al. 2007, Costa et al. 2008). However, prolonged stimulation of TRPV1 induces desensitization of the sensory afferents and loss of responsiveness to stimuli (Bernstein 1987, Dray 1992).

Histamine is an endogenous chemical agent known to mediate a variety of inflammatory responses including itching (Magerl et al. 1990, Martin Schmelz et al. 2003). Many studies that investigated the relationship between TRPV1 and histamine have demonstrated that histamine requires the TRPV1 channel for transduction of an itch response, and that activation of TRPV1 by capsaicin has an antipruritic effect (Shim

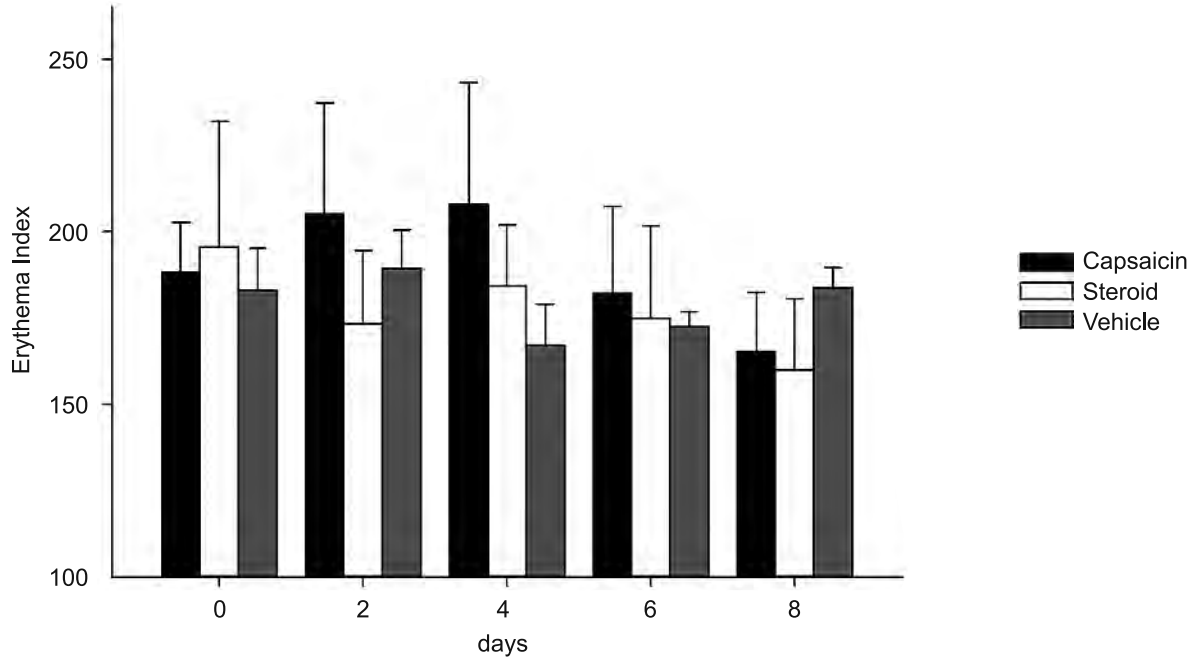

Fig. 5. Erythema index following histamine injection in skin treated with capsaicin, steroid and vehicle. No significant changes were observed in erythema index.

Table 1. Histopathological changes in histamine treated dog skin.

Criteria	Capsaicin	Steroid	Vehicle
Thickness			
Epidermis (μm)	67.78±10.38	62.20±6.09	47.50±5.48
Epidermis to dermis (mm)	2.71±0.27	2.38±0.19	2.59±0.39
Number of inflammatory cells (Cells/mm^2 of dermis)	36.00±5.70	8.20±1.92	20.20±2.59

Values are expressed as mean ± standard deviation of five histological fields
* $p<0.05$, ** $p<0.01$ as compared with vehicle control skin

et al. 2007, Imamachi et al. 2009). Several products containing the active ingredient capsaicin have been approved for treatment of allergies (Foreman 1987, Lundblad et al. 1987), hemodialysis-related pruritus (Tarng et al. 1996), psoriasis (Ellis et al. 1993), and prurigo nodularis (Tupker and Coenraads 1992). As the anti-pruritic effect of capsaicin is dose-dependent, a low concentration of capsaicin (0.025–0.05%) have an increased loading time; relief is usually noted within 14 days and persists for a few weeks.

In the present study, injection of 1% histamine successfully evoked the itch response, whereas repeated administration of 0.1% capsaicin reduced histamine-induced pruritus. The severity of pruritus decreased gradually in the capsaicin-treated group, except on day 4, and lasted until day 8. The most potent reaction to capsaicin treatment was observed after day 6. Pruritus scores also gradually decreased in the steroid-treated group. Compared to day 0, we observed a 60% reduction in pruritus scores in the steroid-treated group on day 8, which was similar to that for the capsaicin-treated group (57.8%). This reduction was not observed in the vehicle-treated group. In the steroid-treated and the capsaicin-treated groups, the wheal diameter and thickness decreased significantly. Previous studies have shown that 1% hydrocortisone conditioner and topical triamcinolone solution adjusted to subjects when injected with intradermal histamine (DeBoer and Cooley 2000, Thomas et al. 2000). Our data contrast with reports by other studies, which did not identify significant differences in histamine reactions. When monitoring for erythema, we found that the vehicle-treated group had more elevated blood flow than the capsaicin- and steroid-treated groups. After the first histamine injection, the dogs began to scratch the histamine-treated site. As a result, the erythema index increased in all the groups, independent of treatment. Capsaicin treatment resulted in an increase in the erythema index until day 4. We observed that capsaicin application probably irritated the skin, although the

pruritic condition was relieved. Cutaneous temperatures also demonstrated no significant changes in the capsaicin-treated group. On histopathological evaluation, marked decreases in epidermal thicknesses with dermal loosening (edema) and mild inflammatory cell infiltration were detected in histamine treated vehicle control skin. However, the dermal edema and mild inflammatory cell infiltrations induced by histamine treatment were decreased by steroid treatment, but increased by capsaicin treatment as compared with the vehicle control. Therefore, treatment with capsaicin had a significant anti-pruritic effect, although it was sufficient to reduce the inflammatory reaction. We found that topical capsaicin was a more potent anti-pruritic agent than the steroid; therefore, capsaicin may be a useful option in patients with contraindications to glucocorticoids such as patients with hyperadrenocorticism.

In humans, capsaicin has remarkable anti-pruritic effects in healthy subjects, but not in patients with atopic eczema (Weisshaar et al. 1998). Similar results have been observed in canines with atopic dermatitis (Marsella et al. 2002). Thus, topical capsaicin can be the choice of drug in patients with allergic dermatitis. A burning sensation or pain has also been noted in patients after administration of capsaicin (Sharma et al. 2013). Therefore, low concentrations (0.025–0.05%) of capsaicin have been investigated as anti-pruritic agents in humans. Pain thresholds are relatively difficult to assess in canines. In addition, canine skin is typically more sensitive than that of humans. Therefore, further investigations are required to adjust capsaicin treatment doses in canines.

In summary, topical capsaicin (0.1%) reduces experimentally induced pruritus. This capsaicin-mediated anti-pruritic effect after repeated treatment is similar to the effect observed with topical steroids. Although there were no anti-inflammatory benefits observed with capsaicin, further studies are warranted to extend its clinical applications to canine patients.

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