

SHORT COMMUNICATION

Development of an Allergen-induced Atopic Itch Model in Dogs: A Preliminary Report

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There is currently no properly validated canine model of pruritus to reproduce the itch associated with atopic dermatitis (AD) of dogs and humans. Although a “canine model of atopic pruritus” was published recently, this model (laboratory beagles sensitized to house dust mite [HDM]) showed only inconsistent allergen-induced itch behaviour (1). Similarly, the application of cowhage spicules induces inconsistent itch in Maltese-beagle atopic (MBA) dogs, and this makes such model unsuitable for the evaluation of new therapeutic options (2). Recently, injections of recombinant canine interleukin-31 were found to induce itch in dogs (3). Importantly, modelling itch by stimulating only one pathway is fraught with the risk that interventions tested using such activators might not necessarily translate into clinical efficacy in atopic dogs or humans because of pruritogenic pathway redundancy. There is a model of flea allergy-induced itch, but it is poorly characterized (4).

Our objectives were to validate a reproducible model of atopic itch in HDM-sensitized MBA dogs (5) in response to topically applied HDM. Additionally, we evaluated the effect of prednisolone, a standard-of-care anti-allergic drug.

MATERIALS AND METHODS

Six MBA dogs, sensitized against HDM as described earlier (5), were used for this study. To induce itch and skin lesions, 25 mg of lyophilized *Dermatophagoides farinae* HDM was suspended in 1 ml mineral oil and applied to an area of approximately 200 cm² on the previously clipped right side of the abdomen. Dogs were video-monitored for 24 h before (i.e. “baseline”) and then 24 h after each HDM application. The HDM was applied once to 6 dogs and 3 times, one day apart, to 4 of the same dogs. To determine if the itch induction was reproducible, the 3 daily challenges were repeated 3 times, at least one month apart, in 4 dogs.

Behavior suggesting pruritus (i.e. licking, scratching, biting) at the site of HDM application on the right side of the abdomen was recorded in 5-second epochs, which were added to yield a duration of itch manifestations (DIM) in seconds per 24 h. In parallel, accelerometers (Philips Respironics mini Mitter Acticals, Bend, OR) were used as an alternative to the direct visualization of pruritus-related movements by video. The correlation between data collected with collar-mounted accelerometers and those obtained with concurrent video monitoring was calculated.

Inflammatory skin lesions at the site of HDM administration 24 h after challenge were graded as follows: erythematous macules, oedema, papules/pustules and excoriations were scored as 0 (absent), 1 (faint, mild), 2 (moderate) or 3 (strong, severe). The grades for each lesion were added to yield a skin lesion score (SLS) with a maximal score of 12 (6). To assess

if there was a relationship between pruritus behavior and skin lesions, the correlation between SLS and DIM was calculated.

After another wash-out period of at least 4 weeks, a proof-of-concept therapeutic trial was done in 4 of the 6 dogs used previously. These were treated 3 times with oral prednisolone at 0.5 mg/kg, 12 h, 1 h before and 12 h after HDM challenge. Itch behavior was monitored over the ensuing 24 h and compared with that seen in the same 4 dogs when not receiving this treatment; a SLS was also evaluated as done previously.

Skin lesion scores, accelerometer-detected activity and DIM were analyzed by Friedman test followed by *post-hoc* Dunn’s test. Pearson’s test was used for correlation assessment. The DIM following prednisolone treatment was compared to that without treatment using Wilcoxon test. All analyses were performed with GraphPad Prism 6.01 (Graphpad, San Diego, CA).

RESULTS

All dogs responded to HDM challenge with increasing DIM in the 24 h following each allergen application. In the 4 dogs in which HDM application was done on 3 consecutive days, there was increasing DIM after repeated challenges (Fig. 1a). In each of these 3 days, the DIM was significantly higher than at baseline (Fig. 1a).

Repeating the 3 daily HDM challenges on 3 separate occasions, at least one month apart, led to a reproducible itch induction (Fig. S1¹). The second and third repetitions of the 3-day challenges led to higher first-day average DIM than after the first HDM challenge.

Monitoring activity with accelerometers revealed no significant differences between the total amount of recorded movements before or after administration of HDM (data not shown). However, when only nighttime activity (6 PM to 6 AM) was analyzed, there was a significant increase of accelerometer-recorded movements for the first but not the following nights (data not shown). A weak but significant correlation was observed between nighttime accelerometer and video monitoring data (Pearson r : 0.382, p = 0.0072).

After each daily HDM application, skin lesions were significantly higher than before allergen challenge (baseline mean [95% CI]: 0 [0–0]; Day 1: 1.8 [0.8–2.7], p < 0.01; Day 2: 3.3 [2.2–4.2], p < 0.01; Day 3: 3.8 [2.4–5.3], p < 0.01). There was also a significant correlation between DIM and SLS (Pearson’s correlation; r = 0.71, p < 0.0001, Fig. S2¹); interestingly, several dogs occa-

¹<http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2243>

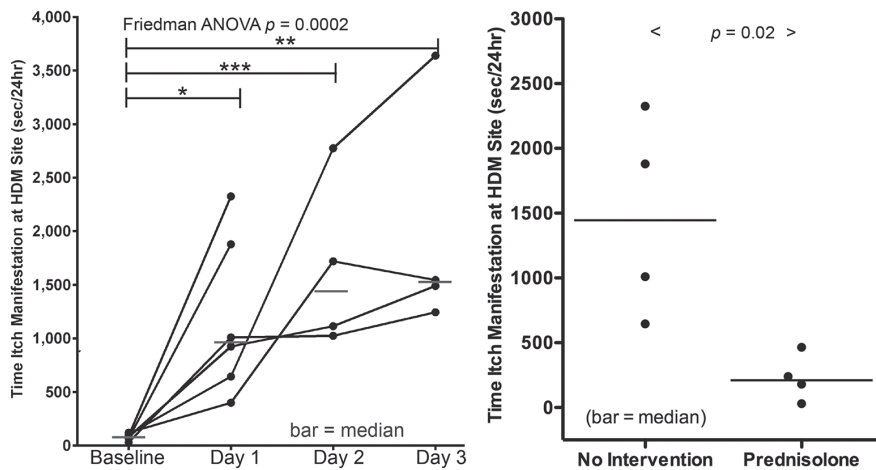


Fig. 1. a) Duration of itch manifestations (licking, scratching biting) after repeated topical application of HDM in sensitized Maltese-beagle atopic dogs. Allergen challenges were performed once in 6 dogs and 3 times, one day apart, in 4 of these 6 dogs; data reported here represent the first challenge done in these dogs (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). b) Duration of itch manifestations after HDM challenge in 4 dogs without treatment or after administration of 3 doses of prednisolone.

sionally exhibited noticeable itch behavior without any visible skin lesions (i.e. SLS = 0) after HDM challenges.

Finally, treatment with prednisolone led to a significant reduction in itch manifestations compared to no treatment (Fig. 1b). Similarly, SLS were also significantly inhibited by prednisolone administration (mean 2.5 (95% CI: 0.9–4.1) versus 0.3 (0.0–1.0); $p = 0.048$).

DISCUSSION

The current study demonstrates that HDM challenges induce a reproducible itch behavior in HDM-sensitized MBA dogs. Most of the time, but not always, itch manifestations were associated with visible skin lesions. This observation mimics a situation that also occurs in spontaneous canine and human AD (7).

In this study, measuring the dog's activity with accelerometers did not adequately reflect pruritic activity. Even when accelerometer data were analyzed for nighttime activity not disturbed by human interaction, there was only significant movement increase during the first night. In this dog model, the correlation between nighttime activity measured with accelerometer and video monitoring is lower than that reported for normal dogs (8). As only video monitoring reliably permitted the assessment of itch-specific behavior, we used this method for assessing the effect of a proof-of-concept antipruritic pharmacological intervention. In this study, treatment with oral prednisolone, a standard therapeutic for canine (9) and human AD (10), led to the expected strong reduction of itch behavior and skin lesions. These results are in contrast with the previously reported dog model of HDM-induced itch behavior in laboratory beagles, in which prednisolone had only a modest effect to reduce itch (1). This difference might

reside in the atopic-predisposing genetic background of our MBA dogs, which should mimic natural AD more closely than HDM-sensitized normal laboratory beagles.

In summary, we reported herein the reproducible itch and skin lesion development after HDM challenges in MBA dogs. The expected reduction of itch by prednisolone also indicates a strong predictive validity. This model will be used to help elucidate possible mechanisms of, and to test the efficacy of novel interventions for atopy-associated itch in both dogs and humans.

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REFERENCES

- Schwab-Richards R, Prost C, Steffan J, Seewald W, Nenci C, Roosje P. Use of activity monitors for assessment of pruritus in an acute model of canine atopic dermatitis. *Vet Dermatol* 2014; 25: 441–446.
- Olivry T, Bizikova P, Paps JS, Dunston S, Lerner EA, Yospovitch G. Cowhage can induce itch in the atopic dog. *Exp Dermatol* 2013; 22: 435–437.
- Gonzales AJ, Humphrey WR, Messamore JE, Fleck TJ, Fici GJ, Shelly JA, et al. Interleukin-31: its role in canine pruritus and naturally occurring canine atopic dermatitis. *Vet Dermatol* 2013; 24: 48–53.
- Bonneau S, Skowronski V, Sanquer A, Maynard L, Eun HM. Therapeutic efficacy of topical hydrocortisone aceponate in experimental flea-allergy dermatitis in dogs. *Aust Vet J* 2009; 87: 287–291.
- Olivry T, Wofford J, Paps JS, Dunston SM. Stratum corneum removal facilitates experimental sensitization to mite allergens in atopic dogs. *Vet Dermatol* 2011; 22: 188–196.
- Bäumer W, Stahl J, Sander K, Petersen LJ, Paps J, Stark H, et al. Lack of preventing effect of systemically and topically administered histamine H(1) or H(4) receptor antagonists in a dog model of acute atopic dermatitis. *Exp Dermatol* 2011; 20: 577–581.
- Hill P, Rybnicek J, Lau-Gillard P. Correlation between pruritus score and grossly visible erythema in dogs. *Vet Dermatol* 2010; 21: 450–455.
- Plant JD. Correlation of observed nocturnal pruritus and actigraphy in dogs. *Vet Rec* 2008; 162: 624–625.
- Olivry T, Foster AP, Mueller RS, McEwan NA, Chesney C, Williams HC. Interventions for atopic dermatitis in dogs: a systematic review of randomized controlled trials. *Vet Dermatol* 2010; 21: 4–22.
- Schmitt J, Schäkel K, Schmitt N, Meurer M. Systemic treatment of severe atopic eczema: a systematic review. *Acta Derm Venereol* 2007; 87: 100–111.