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ORIGINAL PAPER

Double blinded, vehicle controlled, crossover study on the efficacy of a topical endocannabinoid membrane transporter inhibitor in atopic Beagles

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Abstract

The endocannabinoid system is important for skin homeostasis and alterations are linked to inflammatory diseases like atopic dermatitis (AD). Importantly, activation of cannabinoid receptor CB2 decreases pruritus and inflammation in mouse models. Reduction of inactivation of endogenous cannabinoids could, therefore, be a therapeutic option for AD. Dogs spontaneously develop AD, which closely mimics the human disease making them suitable to test new therapies. Our study aimed to test the effects of a topical endocannabinoid membrane transporter inhibitor (WOL067-531, 1% gel) on pruritus and dermatitis in a canine model of AD. Nineteen Beagles allergic to dust mites (DM) were randomized to receive either active ingredient or vehicle on inguinal area while challenged epicutaneously with DM twice weekly for 28 days. Treatment was administered twice daily and started after three challenges (day 8). Dermatitis and pruritus were scored weekly by personnel blinded to treatment allocation. Dermatitis was scored using a validated scoring system and pruritus was scored using camera recordings. After a 4-week washout, dogs were crossed over and the study was repeated. On days 15 and 22, dermatitis scores were significantly increased after DM challenge in the vehicle group (16.34, $p=0.0089$ and 7.42, $p=0.04845$, respectively) but not in the active ingredient group ($p=0.3177$ and $p=0.3190$, respectively). Significant decrease on pruritus both on inguinal area and overall ($p=0.048$ and $p=0.032$, respectively) occurred in the active ingredient group. No adverse effects were noted. In conclusion, the newly developed topical endocannabinoid membrane transporter inhibitor (WOL067-531) minimized allergic flares and pruritus in a canine model of AD.

Keywords Atopic dermatitis · Dog model · Topical · Endocannabinoids

Introduction

Atopic dermatitis (AD) is a prevalent, relapsing dermatitis with significant burden and impact on quality of life [3]. Chronic pruritus is the most debilitating sign leading to self-trauma, sleep disturbances, and decreased quality of life. Commonly used topical therapies aim at decreasing inflammation and exhibit adverse effects in the long run (e.g., cutaneous atrophy with topical glucocorticoids, concerns about cancer and immunosuppression with calcineurin inhibitors). Due to major burden and limited treatments, there is still a need of effective and safe topical therapies for AD.

The endocannabinoid system plays an important role in health and disease of the skin acting on receptors present on various cells and sensory nerves to modulate keratinocyte differentiation, skin barrier function, and local immune responses [19]. Disruption of its balance is linked to inflammatory skin diseases such as AD [8, 19, 20]. Agonists of cannabinoid receptors exhibit anti-inflammatory and anti-pruritic activity [5, 9, 10] and have been used to decrease allergic contact dermatitis and pruritus in rodent models [5, 7]. In people, gene expression of CB1, CB2 was downregulated in skin biopsies of atopic patients compared to the healthy controls [13] suggesting a role in pruritus. Concerns

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exist about using exogenous agonists of cannabinoid receptors although augmentation of the cutaneous endocannabinoid tone is beneficial [2, 9, 10, 15]. Thus, strategies to decrease the inactivation of endogenous cannabinoids represent a new approach to treat inflammatory pruritic diseases like AD [2, 17, 18].

Dogs naturally develop a dermatitis that clinically and immunologically mirrors closely human AD [11]. While murine models are good to study the effects of specific mutations, they are not always reliable at predicting clinical efficacy of treatments, as mice models do not replicate the complexity of the human condition. Dogs are very helpful to mimic the intricacy of the human disease. AD spontaneously develop in dogs. Canine AD is multifactorial resulting from the complex interaction between genetic, environmental factors, and skin barrier defects. Like in people, pruritus and recurrent infections are major clinical signs in dogs. Interestingly, in skin samples of dogs suffering from AD, CB_1 and CB_2 immunoreactivity were higher than in skin samples of healthy animals [1] suggesting that pruritus and inflammation are linked to imbalance in the endocannabinoid system of the skin. Additionally, in dogs, palmitoylethanolamide, an endocannabinoid-like molecule, had a beneficial effect in reducing pruritus and skin lesions in dogs with moderate severity of AD [14]. Thus, it is reasonable to consider canine AD to investigate therapies aimed to help people.

A colony of atopic beagles that spontaneously develops AD has been validated as model for the human counterpart and has been successfully used before to test treatment options for AD [11, 12]. In this colony, flares of AD are triggered upon allergen exposure. Dermatitis and response to medication in this colony mimic the behavior of spontaneously occurring disease. This colony was used for the present study.

Question addressed

Our study aimed to investigate the effect of a newly developed topical compound that inhibits endocannabinoid reuptake (leading to prolonged and elevated endocannabinoid concentrations outside of the cell), on severity of pruritus and dermatitis. Endogenous cannabinoids activate cannabinoid receptors; therefore, the hypothesis tested was that topical application of this compound would decrease pruritus and dermatitis in the treated animals. It is important to point out that the compound tested in this study does not directly activate cannabinoid receptors.

Experimental design

This study was randomized, vehicle controlled, double blinded. Nineteen atopic Beagle dogs (age 2.5 years, ten males, nine females) were randomly divided into two groups: one received active ingredient topically applied as a gel (1% gel, WOL067-531, 2 mg/cm^2) on the inguinal area and the other received vehicle for 21 days (Fig. 1). The selection of the dose (2 mg/cm^2) was based on results from preclinical studies using the standard application volume for clinical trials in humans. The selection of concentration was based on pilot studies in rodents, which showed anti-inflammatory effects and no adverse effects (unpublished results, manuscript in preparation). Dogs were challenged epicutaneously with an allergen (*Dermatophagoides farinae*,

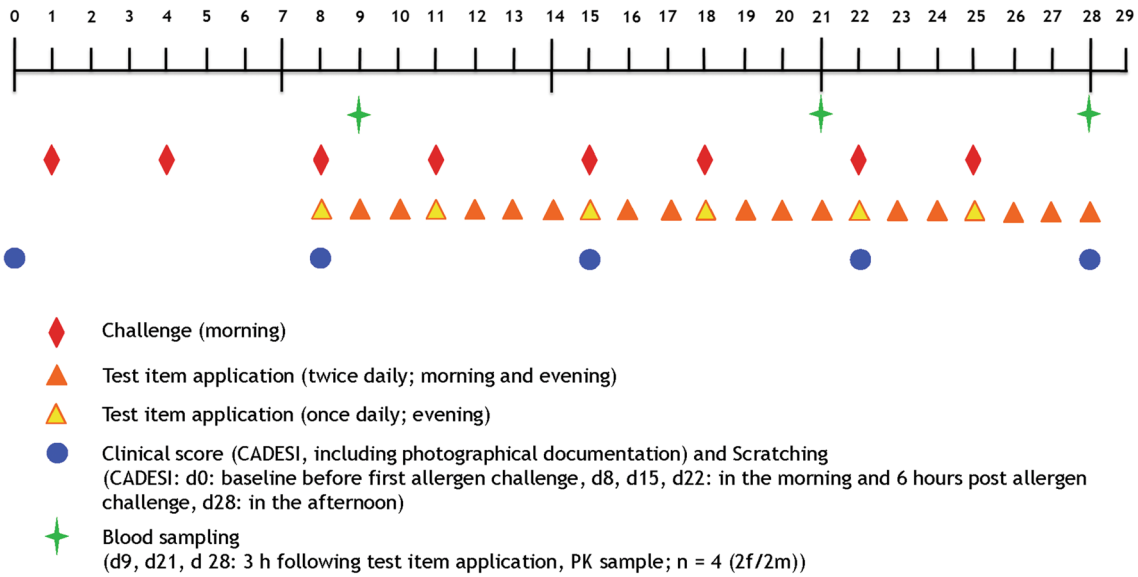

Fig. 1 Timeline of events

Greer Inc, Lenoir, NC, USA) to which they had been sensitized and reacted to [12]. Allergen was applied epicutaneously (1.6 ml, 16.5 mg/ml) to the inguinal area twice weekly for 28 days. Treatment started after third allergen challenge (day 8), twice daily except the day of challenges (once daily, 6 h after challenge). Efficacy of treatment was based on the effect on dermatitis and pruritus.

Dermatitis was evaluated using a validated scoring system (Canine Atopic Dermatitis and Extent Severity Index, CADESI-03), [16] where the body is divided into regions and scored for erythema, macules, papules, excoriation, and alopecia (0–3, with 3 being more intense). Total score is the sum of the scores of all signs and body sites. For this study, both a total CADESI score and score of the treated area (inguinal region) were calculated. CADESI score was assessed before and 6 h after the first challenge of the week. Pruritus was assessed on the same days as CADESI. After a 4-week washout, dogs were crossed over and the study was repeated.

Scoring of pruritus was done at four time points (baseline, day 8, 15, 22, and 28). Pruritus was always assessed by two people who were unaware of treatment allocation. Two scoring systems were based on 30-min video recording using GoPro cameras. The first scoring was quantitative using the BORIS (Behavioral Observation Research Interactive Software) (https://www.boris.unito.it/). This program allows computer-based review of previously recorded videos or live observations. The behavioral scoring software BORIS was used to score the seconds of licking, biting, and scratching. Videos were played in the software and when the dog performed an action of interest (e.g., licking, biting or scratching), a key was pressed by the observer. For biting or scratching, the observer pressed a key which indicated the start of the behavior and again which indicated the stop. For the licking, each lick was indicated by a key press. This information was exported into an excel spread sheet with the data. The second system for evaluation of pruritus was a subjective global score using a validated Visual Analog Scale (VAS), ranging from 0 to 10 where higher numbers mean the most severe pruritus [6]. The number 0 was described as "no itching is observed" while 10 was described as "severe itching, manifested as interruption of eating, playing or resting in order to itch". In this PVAS, the same two evaluators who reviewed the recordings placed a mark on a 10 cm scale as a global subjective overall global assessment of their perception of the pruritus of the dog.

Blood samples (3 ml) were taken 3 h after the first application of product in the morning of day 9, 21, and end of the study day 28 on four dogs in the active ingredient and four in the vehicle group, evenly mixed between sexes to check for drug absorption in the plasma.

Clinical and pruritus scores were analyzed using ANOVA (SAS System for Windows version 9.0, SAS Institute, Cary, NC, USA). $p < 0.05$ was significant. To compare before and after allergen challenge for each day and each treatment, a paired t test (one tailed) was performed.

Results

Both groups developed dermatitis as result of allergen exposure. The area treated by the products was the area where the allergen was placed. Figure 2 reports the scores of the inguinal area at various time points. Of interest was to specifically see how the two groups reacted after allergen stimulation and considered the mean different in CADESI between pre-allergen scores (AM) and post-allergen scores (PM). In the vehicle group, on days 15 and 22, CADESI-03 scores in the inguinal area were significantly higher after challenge (mean difference of 16.34 points, $p = 0.0089$ on day 15 and 7.42 points, $p = 0.0485$ on day 22). In the active ingredient group, no significant worsening of dermatitis scores was

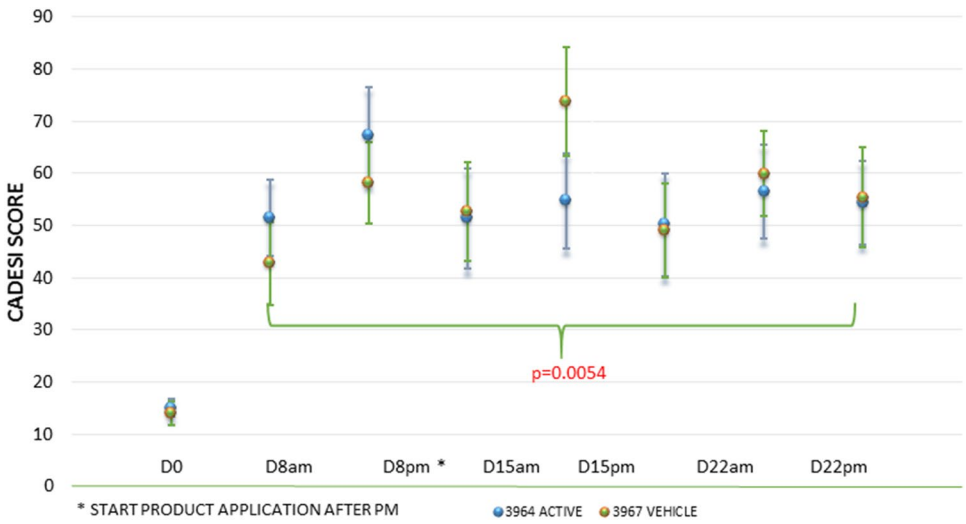

Fig. 2 Dermatitis scores (CADESI) of the area treated by the product. Data are presented as means and standard deviation

detected after allergen exposure on both days (mean difference of 2.42 points, $p=0.317$ and 2.58 points, $p=0.319$, respectively) in the treated area. For total CADESI scores, on day 15 after allergen challenge the active ingredient group had significantly lower scores compared to the vehicle group ($p=0.029$) consistent with a protective effect of the treatment.

In terms of pruritus, increased itching was noticed in both groups after the start of allergen exposure. Figure 3 reports pruritic episodes overall. Interestingly, the mean pruritus of vehicle group was lower on day 22 than active ingredient, although this difference was not statistically significant. A significant decrease on the number of pruritic acts both on inguinal area and overall ($p=0.048$ and $p=0.032$, respectively) at the end of the study was found in the active ingredient group. For the PVAS scores, the mean score at baseline was similar for both groups (2.3 for the active ingredient and 2.2 for the vehicle dogs). After allergen exposure, increase of PVAS was observed for both groups for the remaining of the time ($p=0.0529$) but no significant differences were noted between the groups.

No adverse effects were noted either topically or systemically. The pharmacokinetics data revealed low if any concentration of drug in the plasma. For five out of eight dogs from the active ingredient group for which blood samples were taken, the maximum concentration of WOL067-531 in plasma was determined to be between 0.108 and 0.498 ng/ml. For the other three dogs of this group and all eight dogs from the placebo group, plasma concentration was below 0.100 ng/ml. This confirmed that the observed effects are locally mediated. No correlation existed between CADESI scores and detectable plasma levels ($r=0.06$, $p=0.88$).

Conclusion

In this study, WOL067-531, a newly developed endocannabinoid membrane transporter inhibitor, decreased the severity of flares of dermatitis scores after allergen challenge in atopic dogs. In addition, this active ingredient significantly reduced pruritus, from day 8 to day 28. These results are promising since the dogs used in this model develop severe dermatitis after allergen challenge [11] and support the finding that modulation of the endocannabinoid tone is a beneficial approach to reduce inflammation and pruritus. Due to variability in response and dynamic nature of AD, it is crucial to perform controlled studies. For example, in this study, on day 22, the vehicle-treated dog group had a lower mean than the active ingredient group as several dogs in vehicle group were not very symptomatic on that day. Ways

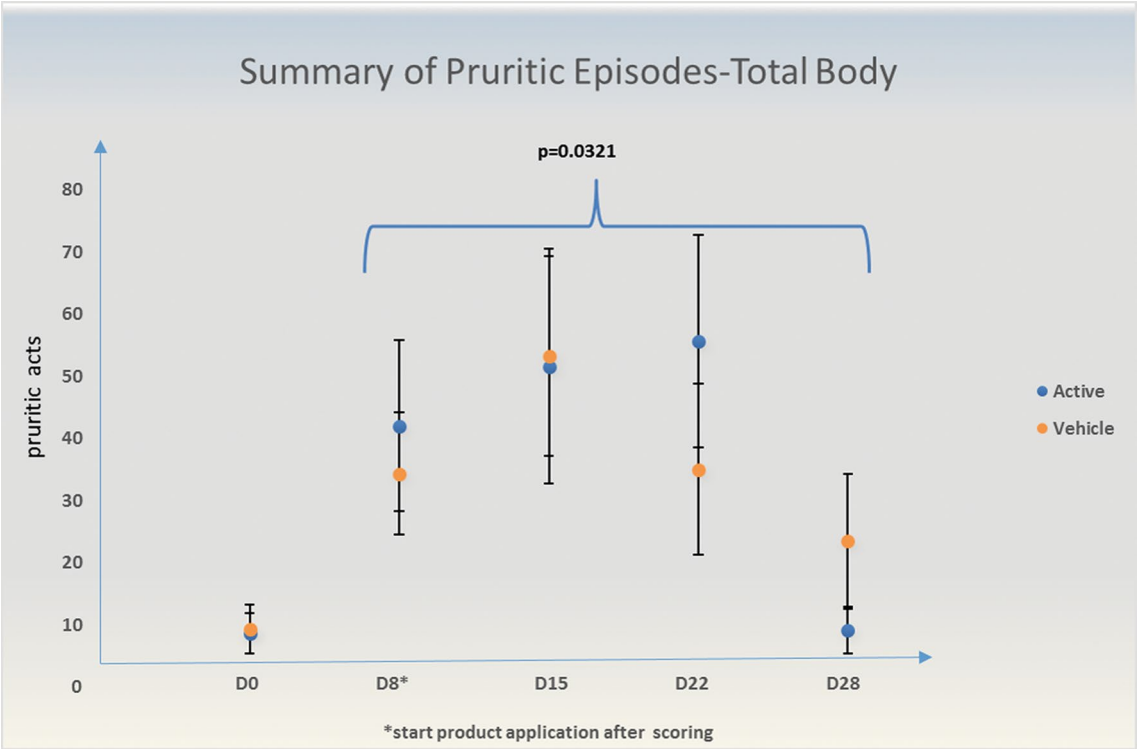

Fig. 3 Mean and standard deviation of pruritic episodes over a course of 30 min. Significant decrease on pruritic acts was found in the active ingredient group ($p=0.0321$)

to minimize the impact of this variability are larger sample size, multiple data points, and always have a control group.

The overall positive response on dermatitis and pruritus observed in this study is consistent with other reports in the literature in both dogs and people. In dogs, a beneficial effect was reported in dogs suffering from AD when treated with palmitoylethanolamide, an endocannabinoid-like molecule [14] which has anti-pruritic and anti-inflammatory properties. Agonists of cannabinoid receptors are some of the promising therapeutic in humans with atopic dermatitis [4]. The beneficial response seen with increased tone of the cannabinoid system is not limited to AD but is observed with other inflammatory skin diseases like allergic contact dermatitis [7] and psoriasis [21]. The effect on psoriasis is believed to be linked to the ability of endocannabinoids to modulate keratinocyte proliferation and differentiation.

In the design of the present study, it is possible that the benefit was underappreciated since the pruritus was evaluated before product application for that day rather than after application. Thus, it is possible that the effect was even stronger that what it was detected had the dogs been evaluated soon after the product application instead. The positive effect on dermatitis is likely a direct effect on inflammation. In this proof-of-concept study, no biopsies were taken; thus, it is not possible to assess the inflammatory infiltrate in atopic skin treated with active ingredient or vehicle. This is a limitation of this study that should be addressed in future studies. Future studies should be aimed at evaluation of the effect of this product on inflammatory cytokines in the skin and whether this approach could lead to improvement of the skin permeability barrier with chronic use.

In conclusion, this newly developed topical endocannabinoid membrane transporter inhibitor reduced AD flares after allergen exposure with negligible penetration into the circulation. Following toxicological studies, a clinical trial should be initiated to investigate the safety and efficacy in humans suffering from AD.

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Compliance with ethical standards

Conflict of interest Dr. Marsella has received Grants from Novartis, Pfizer, Zoetis, Boehringer, Merial, Bioresponse and Artic. Dr. Marsella does not own stock in any of these companies. Dr. Abels and Dr. Soeberdt are employees of Dr. August Wolff GmbH & Co. KG Arzneimittel. Dr. Abels and Dr. Soeberdt are named as inventor on a patent application claiming novel 1,3-benzoxazol-2(3H)-ones and their use as medicaments and cosmetics.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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