ORIGINAL ARTICLE

Crisaborole Inhibits Itch and Pain by Preventing Neutrophil Infiltration in a Mouse Model of Atopic Dermatitis

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Crisaborole, a phosphodiesterase 4 (PDE4) inhibitor, has been approved for the treatment of mild to moderate atopic dermatitis. Atopic dermatitis is often associated with increased pain. Using a mouse model, this study investigated whether crisaborole suppresses pain associated with atopic dermatitis and the potential mechanisms underlying it. The mouse model for atopic dermatitis was developed by repeatedly applying MC903. MC903-treated mice had increased spontaneous scratching (itch-related behaviour) and wiping behaviour (pain-related behaviour). Crisaborole was topically applied to the cheek skin of MC903-treated mice, and it reduced both itch- and pain-related behaviours in these mice. Immunofluorescence staining revealed that crisaborole reduced neutrophil infiltration and interaction of neutrophils with sensory neurones. Intradermal injection of S100A8/A9, proinflammatory neutrophil mediator, enhanced not only itch-related behaviours evoked by histamine or chloroquine, but also pain-related behaviours evoked by capsaicin. Calcium imaging of mouse dorsal root ganglion neurones revealed that pretreatment with S100A8/A9 significantly increased calcium responses to histamine and capsaicin, and the proportion of chloroquine-sensitive neurones. These findings suggest that the PDE4 inhibitor reduces itch and pain, in part by inhibiting infiltration of S100A8/A9-containing neutrophils in a mouse model of MC903-induced atopic dermatitis.

Key words: crisaborole; itch; pain; neutrophil; S100A8/A9.

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SIGNIFICANCE

Atopic dermatitis is often associated with itch as well as increased pain. While the precise mechanisms underlying the effectiveness of crisaborole, a US Food and Drug Administration-approved drug for atopic dermatitis, are not completely understood, this study reveals important insights. It found that crisaborole reduces the infiltration of neutrophils into the epidermis in a mouse model of MC903-induced atopic dermatitis. In addition, the study highlights a potential role of S100A8/A9, proinflammatory neutrophil mediator, in facilitating itch- and pain-related behaviours via direct action on murine sensory neurones. It appears that crisaborole may alleviate itch and pain by inhibiting the neutrophils – S100A8/A9 – sensory neurone axis in the mouse model.

Atopic dermatitis (AD) is a chronic skin condition that affects millions of people worldwide. AD is characterized by inflammation and accompanied by intense itching, which can lead to further irritation and pain if scratched (1). These sensations can significantly reduce the quality of life for those affected (2). The compromised barrier function of the skin in AD allows for easier penetration of allergens and irritants, triggering an immune response that recruits inflammatory cells, such as neutrophils, to the affected area (3, 4). Although recruited neutrophils have been associated with itching sensation in AD (5–7), their role in the pain associated with this condition is currently unknown.

AD can be treated using topical medications, one of which is crisaborole (8). The US Food and Drug Administration (FDA) has approved crisaborole as a non-steroidal topical medication for treating mild to moderate AD in both adults and children aged 2 years and above. Its mechanism of action involves inhibiting the enzyme phosphodiesterase 4 (PDE4), which plays a critical role in the inflammatory response in the skin (8). Our recent research has shown that crisaborole can suppress the expression of chemokines that attract neutrophils and, consequently, inhibit their recruitment to inflamed skin in a mouse model of AD (6).

S100A8/A9 is expressed at high levels in neutrophils and has been demonstrated to possess various functions in these cells, such as the regulation of chemotaxis, activation of pro-inflammatory signalling pathways, and modulation of their antimicrobial activity (9). Moreover, it acts as a damage-associated molecular pattern (DAMP) that triggers the immune system to detect tissue damage and promotes the recruitment of immune cells to the site of injury (10). Recent studies have revealed that immune cells engage in direct interactions with neurones through cytokines, leading to the sensation of itch and pain (11–14). Notably, S100A8/A9 gene expression is upregulated in the skin of patients with AD, particularly

in areas of intense itching (15). However, the precise role of S100A8/A9 in the nociceptive sensations remains to be fully elucidated.

The aim of the current study was to investigate the effectiveness of crisaborole in suppressing itch- and pain-related behaviours in a mouse model of MC903-induced AD. To achieve this, we utilized a cheek model to distinguish between these behaviours (16). A further aim was to determine whether crisaborole could reduce the interaction between neutrophils and nerves in the epidermis of MC903-treated mouse skin. Furthermore, the study explored the potential role of S100A8/A9 in facilitating itch- and pain-related behaviours via direct action on mouse sensory neurons.

METHODS

Animals

Male C57BL/6 mice were purchased from Jackson Labs (Bar Harbor, ME, USA) and were housed in the same colony room with a 12-h light/dark cycle. They were fed *ad libitum* and were given at least 2 weeks to adjust to the colony room after arrival. Experiments were performed under a protocol approved by the University of Miami Institutional Animal Care and Use Committee (21-111).

Behaviour tests

Mice were habituated to a behaviour room, equipment, and handling for a week before experiments began. The fur on the rostral back or the cheek was shaved at least 2 days prior to testing. On the test day, they were habituated to the room for 30 min prior to the start of testing. Then, mice received an intradermal microinjection of 10 µL of 1 of the following: vehicle (phosphate-buffered saline; PBS), S100A8/A9 (0, 1, 10, 100 µg), histamine (54 nmol; Sigma-Aldrich, St Louis, MO, USA), chloroquine (97 nmol; Sigma-Aldrich), S100A8/A9 (0, 1, 10 µg) with histamine (54 nmol), S100A8/A9 (0, 1, 10 µg) with chloroquine (97 nmol), or S100A8/A9 (0, 10 µg) with capsaicin (33 nmol). Microinjections were administered in between the shoulder blades or in the cheek. Immediately after the microinjection, mice were placed in the arena, and their behaviour was recorded for 30 min post-injection. The number of scratch bouts was analysed in 5-min bins by a trained observer blinded to the treatment condition. One scratch bout was defined as 1 or more rapid back-and-forth hindpaw motions directed toward and contacting the injection site, ending with licking or biting of the toes or placement of the hindpaw on the floor. A wipe was defined as a singular motion of the ipsilateral, but not bilateral, forelimb, beginning at the caudal extent of the injected cheek and proceeding in a rostral direction. The inner aspect of the ankle and/or forelimb typically contacted the cheek with the paw closed.

MC903 application and drug treatment

Mice were given a topical application of MC903 (calcipotriol; 20 µL 0.2 mM in ethanol; Tocris Bioscience, Minneapolis, MN, USA)) to the shaved mouse cheek once per day for 8 days. The PDE4 inhibitor crisaborole (2%, 30 µL) was dissolved in acetone/ethanol (1:1 (v/v)) and topically applied to the cheek 1 h prior to MC903 application every day (17). On days 0 and 8, the behaviour was recorded for 60 min.

Immunohistochemistry

On days 0 and 7 of MC903 treatment, animals were euthanized, and the skin was immediately dissected. Skin was fixed in Zamboni Fixative solution (Newcomer Supply, Middleton, WI, USA) followed by 30% sucrose, frozen in optimal cutting temperature (OCT) compound (Tissue-Tek, Sakura Finetek, Torrance, CA, USA), and cut in 40-µm sections on a cryostat. Sections were stored in antifreeze solution at −20°C until staining.

Skin sections were incubated with 5% goat or donkey serum and 0.2% Triton X-100 in PBS, and then immunostained with a primary antibody directed against PGP 9.5 (1:2000; Chemicon (Millipore), Billerica, MA, USA; Cat# AB1761-I, RRID:AB_2868444), to label all epidermal nerve fibres at 4°C overnight, followed by incubation with the corresponding secondary antibody conjugated with AlexaFluor 488 (1:300; Life Technologies Inc., Grand Island, NY, USA) for 2 h. Subsequently, the sections were immunostained with rat Ly6G antibody (1:500; Bio X Cell, Lebanon, NH, USA; Cat# BE0075-1, RRID:AB_1107721), the most commonly used marker for neutrophils at 4°C overnight, followed by incubation with the corresponding secondary antibody conjugated with AlexaFluor 633 (1:300; Life Technologies Inc.) for 2 h. For S100A8 or S100A9 experiments, the sections were incubated with goat S100A9 antibody (1:400; R&D systems, Minneapolis, MN, USA; Cat# AF2065, RRID:AB_2184263) or rat S100A8 antibody (1:50; R&D Systems; Cat# MAB3059, RRID:AB_2184252) followed by goat secondary antibody conjugated with AlexaFluor 555 (1:300; Life Technologies Inc.) or rat secondary antibody conjugated with AlexaFluor 633 (1:300; Life Technologies Inc.) for 2 h, respectively. Then, the sections were immunostained with either rat Ly6G antibody (1:500; Bio X Cell) at 4°C overnight or rat Ly6G-biotin antibody (1:000; Stem-Cell Technologies,Vancouver, British Columbia, Canada; Cat# 60031, RRID:AB_2877150) at 4°C for two overnight, followed by incubation with rat secondary antibody conjugated with Alexa-Fluor 633 (1:300; Life Technologies Inc.) or avidin rhodamine (1:1000; Vector Laboratories) for 2 h, respectively. All sections were counterstained with 4′,6-diamino-2-phenylindole (DAPI) in the mounting medium (Vector Laboratories, Burlingame, CA, USA). Images were captured from 3–4 skin sections from each animal at 20× magnification (5 mice per group) and evaluated by a trained observer blinded to the treatment condition. In the analysis of $Ly6G^+$ cells, 9–10 images were captured from 3 skin sections from each animal at ×20 magnification (5 mice per group). $Ly6G^+$ cells were counted in the randomly selected area (100×100 µm) of epidermis. All analysis was performed by a trained observer blinded to the treatment condition.

Calcium imaging

The mouse was euthanized under urethane anaesthesia, and upper- to mid-cervical dorsal root ganglions (DRGs) were acutely dissected and enzymatically digested at 37°C for 10 min in Hanks' Buffered Salt Solution (HBSS) (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 20 U/mL papain (Worthington Biochemical, Lakewood, NJ, USA) and 6.7 mg/mL L-cysteine (Sigma, St Louis, MO, USA), followed by 10 min at 37°C in HBSS containing 3 mg/mL collagenase (Worthington Biochemical). The ganglia were then mechanically triturated using fire-polished glass pipettes. DRG cells were pelleted; suspended in Minimum Essential Media (MEM) with Earle's balanced salt solution (Lonza; VWR, Radnor, PA, USA) containing 100 U/mL penicillin, 100 µg/mL streptomycin (Gibco), 1× vitamin (Gibco), and 10% horse serum (Gibco); plated on poly-d-lysine-coated glass-bottom culture dishes; and cultured for 16–24 h.

DRG cells were incubated in Ringer's solution (pH 7.4, 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES,

and 4.54 mM NaOH) with 5 μM Fluo-4 AM and 0.05% Pluronic F-127 (Gibco). S100A8/9 (2, 20, 200 ng/mL) was delivered for 30 s by a perfusion system. This was followed by allyl isothiocyanate (AITC: 100 μM), and capsaicin (1 μM) in the same order. In other experiments, either histamine (100 μM), chloroquine (100 μM), or capsaicin (1 μM) was delivered for 30 s by a perfusion system. Either Ringer's solution (vehicle) or S100A8/A9 (2 ng/mL) was delivered beginning 30 s prior to the chemical application for a duration of 3 min. Potassium chloride (144 mM) was always delivered at the end of each experiment. Images were obtained with an inverted microscope (Eclipse TE2000, Nikon, Melville, NY, USA) at excitation/emission=480/510 nm every 3 s. Fluorescence intensities were normalized to baseline (F/F0). Cells were judged to be sensitive if the fluorescence value increased by 5 SD of the resting level following chemical application. Only cells responsive to high potassium were included for analysis.

Data analysis

Between-group comparisons were made by unpaired *t*-tests (2-tailed), 1-way analysis of variance (ANOVA) followed by the Tukey post hoc test, or 2-way repeated measures ANOVA followed by the Bonferroni post hoc test. In all cases, $p<0.05$ was considered to be significant. The data were graphed in GraphPad Prism software (Boston, MA, USA).

RESULTS

To elicit itch- and pain-related behaviours in the mouse model, MC903 was applied to the cheek to induce AD-like skin lesions (**Fig. 1**A). MC903-treated mice exhibited spontaneous scratching behaviours (itch-related behaviours) as well as wiping behaviours (pain-related behaviours) (Fig.1B). To test the effect of crisaborole on itch- and pain-related behaviours, crisaborole was topically applied to the mouse cheeks once per day for 8 days. There was no discernible difference in skin appearance between crisaborole- and vehicle-treated mouse skin (Fig. 1C). Crisaborole, on the other hand, significantly suppressed both scratching and wiping behaviours in the mice (Fig. 1D).

A previous study found that neutrophils contribute to itch in the mouse model of AD (7). Therefore, we hypothesize that neutrophils infiltrate the epidermis and interact with epidermal nerves to cause itch. To test this hypothesis, MC903-treated mouse skin was stained with anti-Ly6G and anti-PGP9.5 antibodies, which were used to visualize neutrophils and epidermal nerve fibres, respectively. MC903 treatments significantly increased the number of epidermal $Ly6G^+$ neutrophils as well as $Ly6G^+$ neutrophils in contact with nerves (**Fig. 2**A, B, E, F: 1-way ANOVA followed by the post hoc Tukey test, $F(3, 16)=22.10$, $p<0.0001$ for Fig. 2E: one-way ANOVA followed by the post hoc Tukey test, $F(3, 16)=16.51$, $p<0.0001$ for Fig. 2F). Crisaborole treatments significantly inhibited these increases (Fig. 2C, D, E, F). $Ly6G^+$

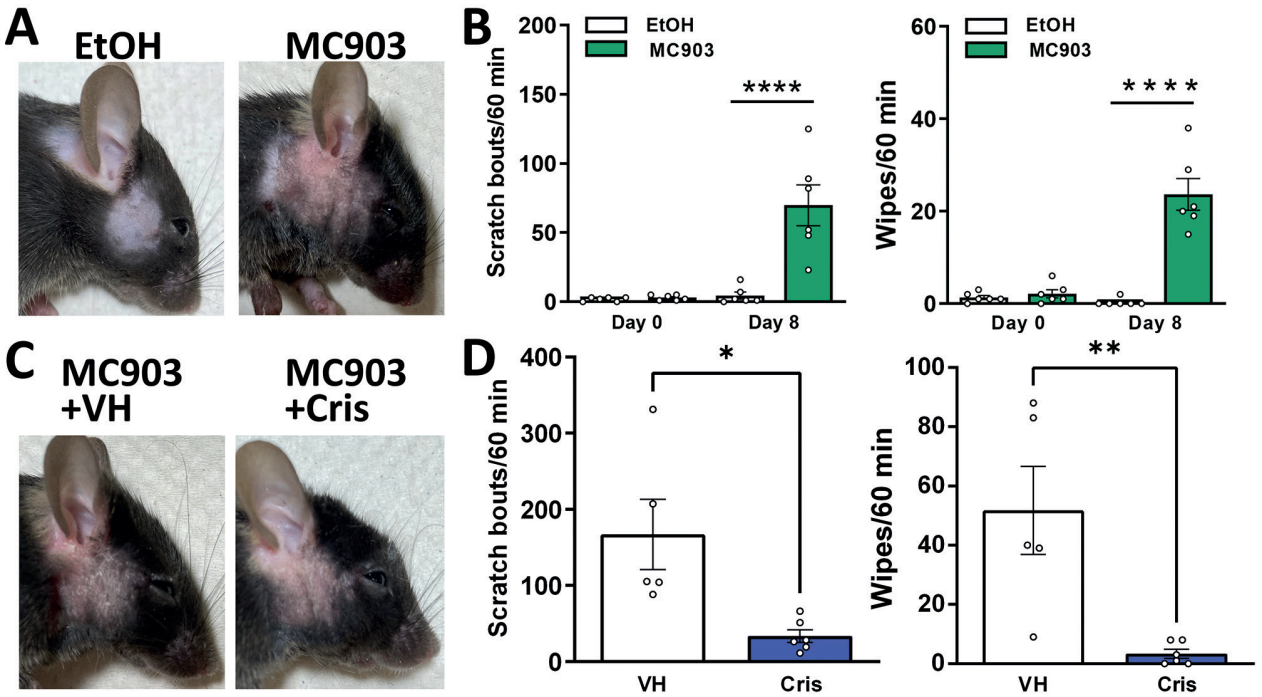

Fig. 1. Effects of crisaborole on spontaneous itch and pain in atopic dermatitis mouse model. (A) Photographs of representative shaved cheek on day 8 treatment with ethanol (EtOH) or MC903. MC903 was topically applied to the cheek once per day for 8 days. (B) Mice were videotaped for 60 min on day 0 and day 8 treatment to assess spontaneous scratching and wiping behaviours. *White and green columns* show, respectively, EtOH- and MC903-treated groups. *Error bars* are standard error of mean (SEM) ($n=6$). ****$p<0.0001$, significant difference from EtOH-treated group. Two-way repeated measures analysis of variance (ANOVA) followed by the post hoc Bonferroni test. $F(1, 10)=18.90$, $p=0.0014$ for scratching. $F(1, 10)=49.19$, $p<0.0001$ for wiping. (C) Photographs of representative shaved cheek on day 8 treatment with MC903 with crisaborole (Cris) or vehicle (VH). Crisaborole or vehicle was applied topically to the cheek 1 h prior to MC903 application every day. (D) Mice were videotaped for 60 min on day 8 of treatment with crisaborole or vehicle to assess spontaneous scratching and wiping behaviours. *White and blue columns* show, respectively, vehicle- and crisaborole-treated groups. *Error bars* are SEM ($n=5$–6). *$p<0.05$, **$p<0.01$, significant difference from vehicle, 2-tailed unpaired *t*-test.

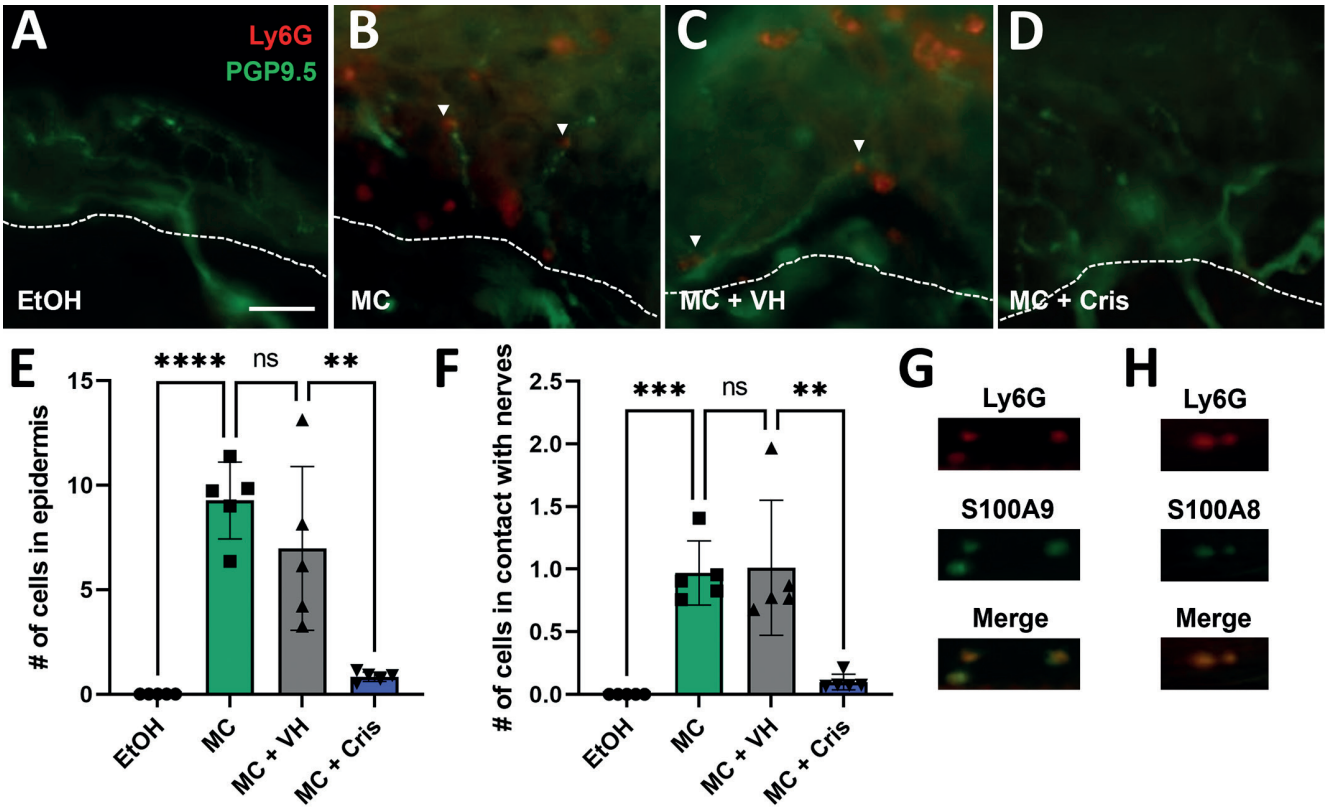

Fig. 2. Effects of crisaborole on neutrophil infiltration in atopic dermatitis mouse model. (A–F) Skin was dissected from mice on day 8 after EtOH (A, E, F), MC903 (MC: B, E, F), MC903+vehicle (MC+VH: C, E, F), or MC903+crisaborole treatment (MC+Cris: D, E, F). Skin sections were immunostained with an antibody for PGP9.5 and Ly6G to visualize nerve fibres (*green*) and neutrophils (red), respectively. *Dotted lines* indicate the dermal-epidermal junction. *White arrows* indicate neutrophils contact with nerve fibres. (E) *Scale bar* indicates 20 µm. Total number of neutrophils in the epidermis was quantified in EtOH (*white bars*), MC (*green bars*), MC+VH (*dark grey bars*), and MC+Cris mice (blue bars). Error bars are SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, significant difference (1-way analysis of variance (ANOVA) followed by Tukey test, $n = 5$/group). (F) As in (E) for epidermal neutrophils in contact with nerves. (G and H) Skin sections were immunostained with an antibody for Ly6G and S100A8 or S100A9.

neutrophils expressed both S100A8 and S100A9, as reported previously (Fig. 2G, 2H).

S100A8/A9 was injected intradermally into the back of the mice, in order to determine if it elicited itch-related behaviour. Scratching behaviour was absent in mice injected with S100A8/A9 (**Fig. 3**A). This suggests that S100A8/A9 alone is insufficient to cause itch in the normal state. Then, S100A8/A9 was co-injected with histamine to test whether S100A8/A9 can facilitate itch-related behaviour. S100A8/A9 increased histamine-evoked scratching dose-dependently (Fig. 3B). Similar to histamine-evoked scratching, co-injecting S100A8/A9 with chloroquine evoked scratching in a dose-dependent manner (Fig. 3C). A mouse cheek model that can distinguish between itch- and pain-related behaviours was used to determine if S100A8/A9 can facilitate both. S100A8/A9 was intradermally co-injected into the mouse cheek with histamine, chloroquine, or capsaicin. S100A8/A9 significantly increased histamine- and chloroquine-evoked scratching behaviour, but had no effect on scratching behaviour after capsaicin injection (Fig. 3D–F). S100A8/A9 significantly increased histamine- and chloroquine-evoked wiping behaviour, as well as capsaicin-evoked wiping behaviour (Fig. 3G–I: $p = 0.021$ for histamine, $p < 0.0001$ for chloroquine, $p = 0.0007$ for capsaicin). Scratching and wiping behaviours were not elicited by intradermal injection of S100A8/A9 alone into the cheek (scratching: 1 ± 0.5/ 30 min; wiping: 0.6 ± 0.4/ 30 min, $n = 5$).

The current study also investigated whether S100A8/A9 activates primary sensory neurones in cultured DRG neurones. S100A8/A9 activated the recorded neurones in a dose-dependent manner (2 ng/mL: 8.2% ($n = 85$), 20 ng/mL: 16.1% ($n = 155$), 200 ng/mL: 20.2% ($n = 193$)). AITC (mustard oil; TRPA1 agonist) and/or capsaicin TRPV1 agonist activated majority of S100A8/A9 responsive neurones (93.7% ($n = 63$)). Next, the current study tested whether S100A8/A9 enhances the calcium responses evoked by histamine, chloroquine, or capsaicin. **Fig. 4**A, B, or C show a time-course graph of calcium increase evoked by histamine, chloroquine, or capsaicin in DRG neurones. S100A8/A9 significantly increased the calcium responses to histamine and capsaicin, but not to chloroquine (Fig. 4A–F). While the proportions of histamine- or chloroquine-responsive neurones in S100A8/A9- and vehicle-treated DRG neurones were comparable, S100A8/A9 significantly increased the proportion of chloroquine-responsive neurones (Fig. 4G).

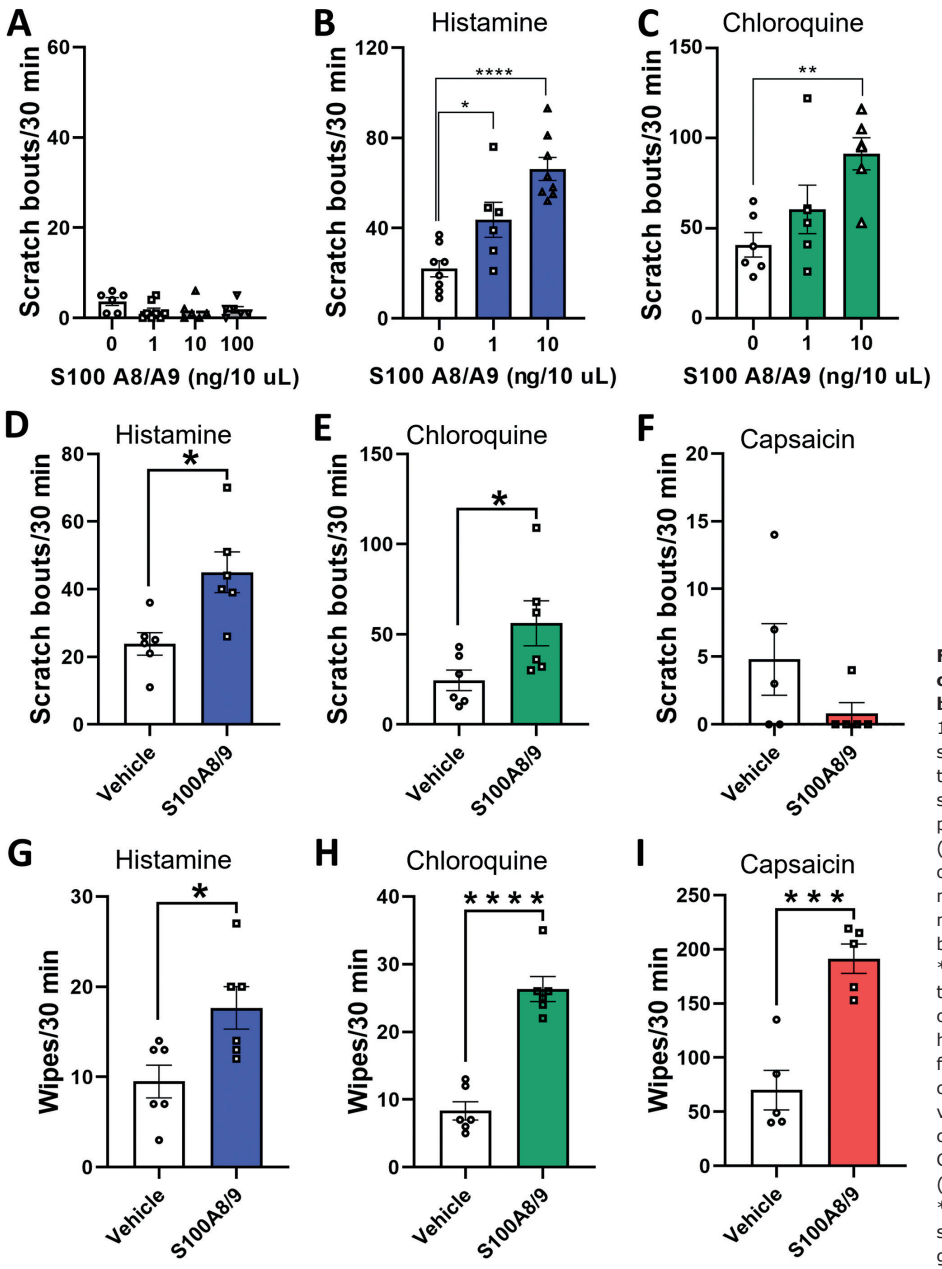

Fig. 3. S100A8/A9 facilitates pruritogen- or algogen-evoked scratching and wiping behaviours in mice. (A) S100A8/A9 (1, 10, 100 ng) or vehicle (phosphate-buffered saline; PBS) was intradermally injected into the nape of the neck. Following the injection, scratch bouts were counted over a 30-min period. Error bars are standard error of mean (SEM) $n = 6–8$. (B, C) S100A8/A9 (1, 10 ng) or vehicle was intradermally injected into the nape of the neck with either histamine (54 nmol: B) or chloroquine (97 nmol: C). Error bars are SEM $n = 6–8$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$, significant difference from the vehicle-treated group. One-way analysis of variance (ANOVA) followed by the post hoc Tukey test. F (2, 19)=18.60, $p < 0.0001$ for histamine. F (2, 15)=6.353, $p = 0.01$ for chloroquine. (D–I) S100A8/A9 (10 ng) or vehicle was intradermally injected into the cheek with either histamine (54 nmol: D, G), chloroquine (97 nmol: E, H), or capsaicin (33 nmol: F, I). Error bars are SEM $n = 5–6$. $^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$, significant difference from the vehicle-treated group. Two-tailed unpaired *t*-test.

DISCUSSION

Crisaborole is a non-steroidal topical medication approved by the FDA for treating mild to moderate AD, which is characterized by the common symptom of itch (18). Itching can lead to painful skin erosion due to scratching (1). While crisaborole is known to alleviate itch (19), its ability to inhibit pain in AD remains unknown. To investigate this, the current study used an MC903-induced AD mouse model and tested the effectiveness of crisaborole in inhibiting itch- and pain-related behaviours. The results showed that crisaborole significantly inhibited both itch- and pain-related behaviours in the mouse model. The results also showed that MC903 treatments increased the number of epidermal neutrophils in contact with epidermal nerves in the mouse model, which crisaborole treatment prevented. In addition, the study found that the proinflammatory neutrophil mediator S100A8/A9 facilitated itch- and pain-related behaviours and directly acted on nociceptive neurones and increased their responsiveness to pruritogen and algogen. Overall, crisaborole demonstrates the potential to relieve itch and pain through the inhibition of the neutrophils-S100A8/A9-sensory neuron axis in the mouse model of AD. This is particularly significant given the substantial elevation of S100A8/A9 levels in both lesional skin and serum of AD patients, which also exhibit a strong correlation with disease severity (20–23). Moving forward, it is crucial for future research to investigate whether crisaborole exerts its pruritus-suppressing effects by targeting the same axis in patients with AD.

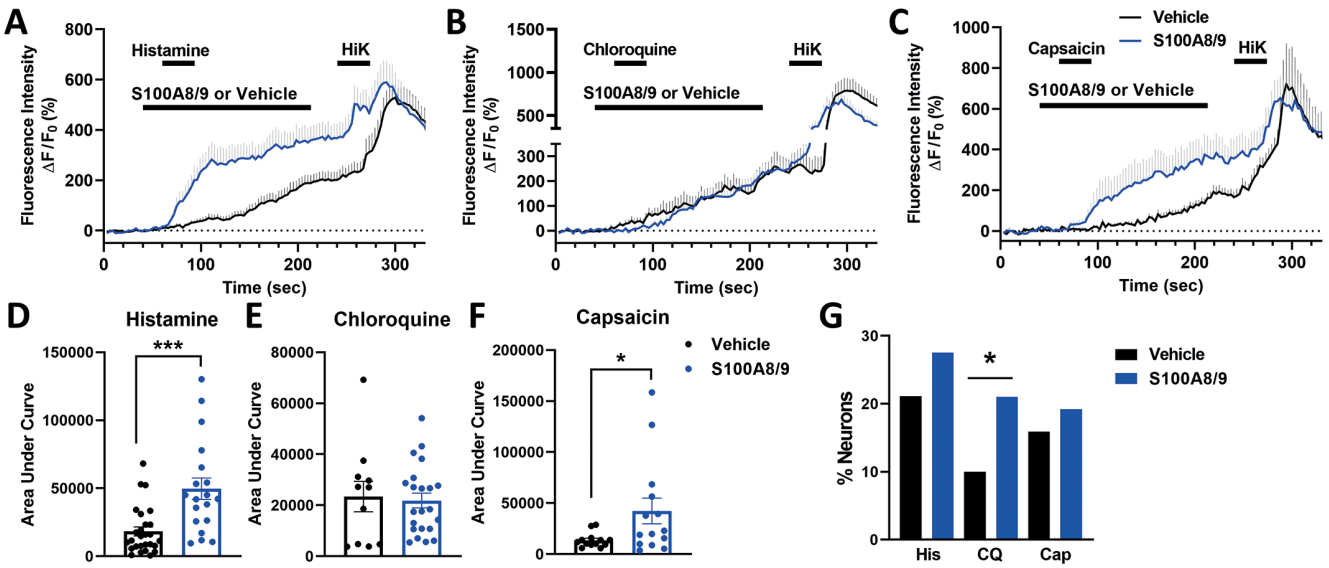

Fig. 4. Effects of S100A8/A9 on pruritogen- or algogen-induced calcium response in mouse dorsal root ganglion (DRG) neurones. (A) Time-course graph of histamine-induced calcium response in DRG neurones. DRG neurones were treated with vehicle (*black line*) or S100A8/A9 (*blue line*) with a subsequent challenge with histamine (100 μM). (B) As in (A) for chloroquine (100 μM). (C) As in (A) for capsaicin (1 μM). (D) Averaged area under the curve of the calcium responses to histamine. DRG neurones were treated with vehicle (*black column*) or S100A8/A9 (*blue column*). Error bars are SEM $n = 19–27$. ***$p < 0.001$, a significant difference from the vehicle group. 2-tailed unpaired *t*-test. (E) As in (D) for chloroquine. $n = 11–22$. (F) As in (D) for capsaicin. $n = 13–14$. (G) Proportions of histamine-, chloroquine-, or capsaicin-responsive DRG neurones pre-treated with vehicle (black column) or S100A8/A9 (blue column). *$p < 0.05$. Fisher exact test. $n = 69–128$.

Studies have previously demonstrated that neutrophils are present in human AD lesions (4, 24–26). Furthermore, the levels of neutrophil chemokines, specifically CXCL1 and CXCL5, are elevated in the skin of patients with AD where neutrophils infiltrate (27). Moreover, higher levels of CXCL1 in the serum are associated with increased severity of AD in the patients (28). Taken together, these findings strongly suggest that the recruitment of neutrophils into the skin plays a crucial role in the pathogenesis of AD.

Several studies have demonstrated that PDE4 inhibitors can reduce neutrophil infiltration in various tissues (29, 30). Recent human proteomic analysis research revealed that the clinical improvement in AD with crisaborole correlated significantly with decreased levels of important protein biomarkers associated with neutrophils (31). In a previous study, we observed that crisaborole reduced the expression of CXCL1 and CXCL2 in a mouse model of AD (6). Consistent with this finding, a recent study showed that crisaborole inhibited the production of CXCL1 and CXCL2 in patients with AD (32). PDE4 inhibitors increase intracellular cAMP to exert their effects, and an activator of adenylyl cyclase can reduce CXCL1 and CXCL2 released from human keratinocytes by synthesizing and elevating intracellular cAMP (33). Thus, PDE4 inhibitors may decrease CXCL1 and CXCL2 through enhanced intracellular cAMP in keratinocytes.

Recent studies have shown that various immune cells, including T-cells, basophils, mast cells, and macrophages, use cytokines or other mediators to communicate directly with epidermal nerves, thereby promoting itch and pain (11–14). In addition to these cells, we found that neutrophils are in close proximity to epidermal nerves in the MC903-treated mouse skin. Human neutrophils express S100A8/A9, which is secreted in response to the production of reactive oxygen species (34). As increased oxidative stress in patients with AD has been reported (35, 36), it is reasonable to hypothesize that neutrophils may communicate directly with epidermal nerves via S100A8/A9. The current study also showed that S100A8/A9 can interact with nociceptive neurones, further supporting this hypothesis. Therefore, neutrophils may use this pathway to directly communicate with epidermal nerves.

The current study did not determine which receptors mediate the activation of nociceptive neurones by S100A8/A9. S100A8/A9 has been shown to bind to the toll-like receptor 4 (TLR4) in murine cell line (37). Previous research has shown that S100A8 can activate murine nociceptive neurones, but this effect was blocked when TLR4 was inhibited (38). Other studies have found that TLR4 is present in both CGRP- and IB4-positive small DRG neurones and is involved in mouse models of chemotherapy- and nerve injury-induced neuropathic pain (39, 40). Taken together, the collected findings imply that S100A8/A9 could potentially activate nociceptive neurons via TLR4. Nevertheless, additional studies are required to substantiate this hypothesis.

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