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Hyperalgesia and Allodynia in Chronic Itch Mouse Model

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Itch (pruritus) is an unpleasant sensation associated with allergy and scratching away insects, plant spicules or invasive parasites from the skin. Acute itch reflects an adaptive mechanism to maintain the integrity of the skin while chronic itch can adversely affect health and quality of life. For the last few years, the role of various transient receptor potential (TRP) and other protein channels have been identified as critical in transducing itchy stimuli into slow local and fast action potentials that are conducted over "pruriceptive" primary afferent fibers into the nervous system. In this work, we studied the effects of thermal hyperalgesia and mechanical allodynia that accompanied itch sensations in the mice chronic itch model. The obtained data showed that daily topical application of squaric acid dibutyl ester (SADBE) for 11 days to the ventral hindpaw glabrous skin of mice resulted in a significant increase in biting and licking behavior. In this group of mice, we revealed consistent reductions in thermal withdrawal latency and mechanical withdrawal threshold on the ipsilateral (SADBE treated) test paw compared to the contralateral(untreated) control paw. These findings confirmed that the SADBE itch model of chronic dermatitis is accompanied by hyperalgesia and allodynia. *© 2024 Bull. Georg. Natl. Acad. Sci.*

antinociception, pain, pruritus, Hargreaves' test, von Frey's test, TRP channels

Itch (Latin *pruritus*) is an unpleasant sensation associated with allergy and an innate reaction to scratch away insects, plant spicules or invasive parasites from the skin epidermis and dermis. Acute itch reflects an adaptive mechanism to maintain the integrity of the skin while chronic itch can adversely affect health and quality of life [1,2].

Chronic pruritus can be categorized by etiology into inflammatory, neuropathic, or a combination of inflammatory and neuropathic pruritus. Chronic pruritus is due to inflammation in approximately 60% of patients and may be caused by eczema, psoriasis, or different types of dermatitis. Chronic itch is associated a neuropathic or mixed etiology in approximately 25% of patients, includes postherpetic neuralgia and *notalgia paresthetica*, and is typically due to localized or generalized nerve dysregulation [3]. Chronic itch is thus a major health issue and the treatment of chronic itch remains challenging and requires the development

of therapeutic approaches and pharmaceuticals targeting the currently known itch transducers and signaling pathways [1].

Most types of chronic itch are resistant to antihistamine drugs, so there is a pressing needto develop novel medications other than antihistamines to treat itch. However, to date themanagement and treatment of itch remains challenging because therapeutic options havefrequently been reported as inadequate [4-6].

For the last few years, the role of various transient receptor potential (TRP) channels and other protein receptors, including Mas-related G-protein-coupled receptors (Mrgprs) and protease-activated receptors (PARs), have been identified as critical in transducing itchy stimuli into slow local and then fast action potentials that are conducted over "pruriceptive" primary afferent fibers into the nervous system [1,7-9].

Given the importance of TRPV1 and TRPA1in mediating histaminergic and nonhistaminergic itch signaling respectively, the investigation of these ion channels has been of considerable interest for their potential roles in contributing to chronic pruritis. Beyond the known expression of TRP channels in thenerve endings of primary afferent neurons, TRP channels have been found in keratinocytes, epidermis, and mast cells and are upregulated in affected skin in several dermatological pathologies associated with atopic dermatitis, psoriasis, and prurigonodularis. To determine the role of TRPA1 and TRPV1 in skin dysfunction, pharmacologicaland genetic knockout experiments have been performed in a variety of murine models of acute and chronic itch as well [10-13].

We have recently found that an intraplantar injection of histamine in mice resulted in significant thermal hyperalgesia and mechanical allodynia ipsilaterally that persisted for 1 h. Pretreatment with the TRPV1 antagonist AMG-517, but not the TRPA1 antagonist HC-030031, significantly attenuated the magnitude and time course of thermal hyperalgesiaand mechanical allodynia elicited by histamine, indicating that these effects are mediatedby TRPV1 [2,14]. Furthermore, we have reported that intraplantar injections of chloroquine, bovine adrenal medulla peptide (BAM8-22), andhexapeptide (Ser-Leu-Ile-Gly-Arg-Leu-NH2) (SLIGRL) elicited thermal hyperalgesia andmechanical allodynia in adult male mice. Pretreatment with the TRPA1 antagonist (HC-030031) significantly reduced thermal hyperalgesia and mechanical allodynia elicited bychloroquine, BAM8-22, and SLIGRL, indicating that hypersensitivity effects developed bythese non-histaminergic itch mediators require TRPA1 channel [14,15]. These data confirmed that acute itch coexists with pain hypersensitivity.

It is well known that in most established itch behavioral models in mice, assays are performed with pruritogens applied to the hairy skin including the back, nape of the neck, cheek, and hindleg. However, a few examples include palmoplantar pustulosis (or palmar and plantar psoriasis), a chronic skin disease characterized by inflamed scaly skin and intense itch on the palms and soles; dyshidrosis, a skin condition causing itchy blisters to develop only on the palm and soles; and cholestatic itch, an intense itching sensation felt in the limbs, and particularly the palms and soles of feet. Thus, limited studies have been performed to examine itching sensations arising from the hindpaw, especially after applications of pruritogens in glabrous skin [16].

In this paper, we present preliminary findings of pain behavior in a mouse model of chronic itch applicating pruritogen squaric acid dibutyl ester (SADBE) in the glabrous skin of mice. The latter substance is used for the model of chronic contact dermatitis. We studied the effects of thermal hyperalgesia and mechanical allodynia that accompanied itch sensations.

Materials and Methods

Animals. The experiments were conducted in male and female C57BL/6J mice purchased from

Jackson Laboratories (Bar Harbor, ME, USA) < 50 g in body weight. A breeding colony has been established at our laboratory vivarium in Tbilisi. The animals were kept under standard housing conditions (22±2°C, 65% humidity, lights from 6:00 a.m. to 8:00 p.m.), and fed a standard dry diet; water was freely available. All experiments are performed using protocols approved by the local animal care and use committee of Beritashvili Exp BMC. All behavior tests were performed from 10 AM to 2 PM in the light cycle in our animal facility. All mice are acclimated for 30 to 60 min to their testing environment the day before the behavioral tests. In vivo experiments were carried out according to European Union guidelines (EU Directive application 2010/63/EU) and the guideline of the International Association for the Study of Pain (IASP) regarding investigations of experimental pain in conscious animals. Every attempt was made to follow ARRIVE guidelines (arrive-guidelines.org/).

Dermatitis model. Dermatitis was induced on the plantar glabrous skin surface of one hindpaw of mice and itch-related biting and pain-related licking reactions were measured, followed by tests for thermal hyperalgesia and mechanical allodynia in this skin area. Contact dermatitis was produced by treating the mice with the allergen SADBE (Sigma no. 339792; 0.5% in acetone) as described (Steele et al., 2021). The procedure includes 7 days of the elicitation phase and 10 days of the induction phase. During the elicitation phase, 2 μL of SADBEwas applied to the plantar hindpaw glabrous skin once a day for 10 days to initiate dermal inflammation. To limit dermatitis in the glabrous side of the hindpaw, only 2 μL of SADBE will be applied to the plantar skin. Since SADBE is dissolved in acetone, which evaporates quickly after application, the SADBE solution did not flow to the hairy side of the paw.

Signs of chronic itch on the hindpaw were verified as follows. After 10 days of SADBE treatment, a subset of treated mice was videotaped for 30 min to record spontaneous biting and licking behavior directed to the treated hindpaw. Biting is considered to reflect an itch reaction while licking is thought to reflect pain [17].

Behavioral tests. Before formal testing, the baselines were assessed for mice in the experimental and control groups in thermal and mechanical withdrawal tests, averaging multiple (three times) baseline measurements for the left and right hind paws, with 5-minute intervals between tests.

Thermal paw withdrawal (Hargreaves) test: Mice first were habituated to stand on a glass surface heated to 30ºC within a Plexiglas enclosure, over three separate daily sessions. For formal testing, baseline latencies for paw withdrawals evoked by radiant thermal stimulation of each hind paw were measured. A light beam (Plantar Test 390, IITC, Woodland Hills, CA, USA) was focused onto the plantar surface of one hind paw through the glass plate from below, and the latency from the onset of the light to the brisk withdrawal of the stimulated paw was measured. The other hind paw was similarly tested 30-60 sec later.

Electronic-von Frey paw withdrawal test: mice first were habituated to standing on a wire mesh surface. For formal testing, baseline withdrawals were assessed using an Electronic von Frey Aesthesiometer (2390, IITC, CA, USA) filament that was pressed against the ventral paw from below. This device samples and holds force (g) at the moment that the hind paw is withdrawn away from the filament. Each paw was tested for baseline mechanical withdrawals at least three times, with at least 5 min elapsing between successive measurements of a given paw. The same group of mice was used for thermal and mechanical withdrawal tests.

Data analysis. All data from behavioral tests were subject to repeated measures of analysis of variance (rMANOVA) and then were compared between

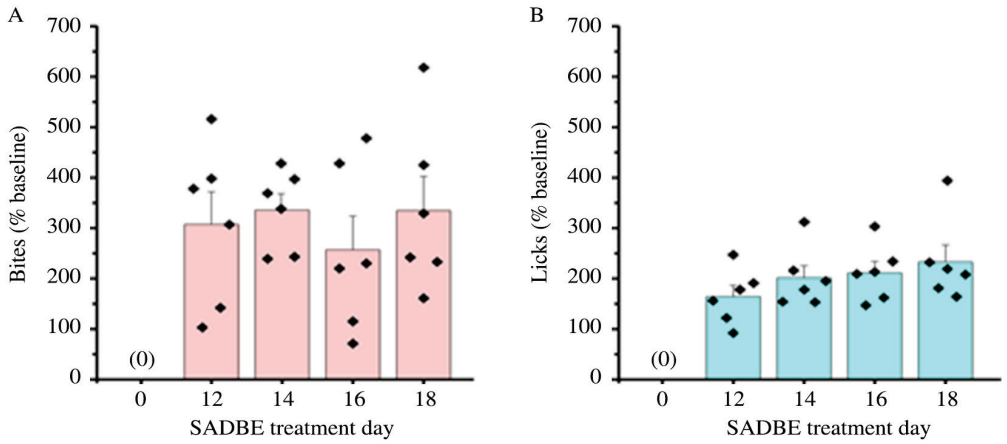

Fig. 1. Percent changes in biting (A; pink bars) and licking (B; teal bars) behavior (% of baseline responses compared to pretreatment day 0) for a glabrous skin allergic dermatitis using topical application of SADBE to the plantar hindpaw. There were significant increases in biting (indicative of itch) and licking (indicative of pain) starting on treatment day 12; n =6 female mice/group; each symbol represents counts for an individual animal.

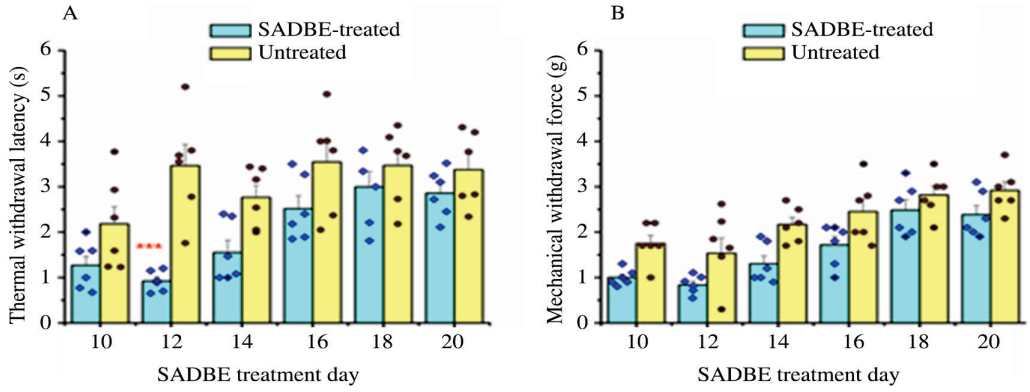

Fig. 2. Changes in thermal withdrawal latency (A) and mechanical withdrawal threshold (B) after SADBE injected in the ipsilateral plantar hindpaw (test paw, teal bars) compared to contralateral paw without injection (control paw, yellow bars); n = 6 female mice/group, (P value - *** $P < 0.001$).

chemicals and vehicle treatment groups by Tukey-Kramer or paired post-hoc t-tests. The data are expressed as Mean ± SEM. The Kolmogorov-Smirnov test was applied to verify normality and equal variance. Statistical significance was acknowledged if $P < 0.05$. The statistical software utilized was InStat 3.05 (GraphPad Software, Inc., San Diego, CA, USA).

Results and Discussion

In the squaric acid dibutyl ester (SADBE) model of chronic contact dermatitis we have found that daily topical application of SADBE (a total of 11 days) to the ventral hindpaw glabrous skin resulted in a significant increase in biting and licking behavior (Fig. 1).

In the same group of mice, we tested for thermal hyperalgesia and mechanical allodynia between experimental days 10 and 20. There were consistent reductions in thermal withdrawal latency (Fig. 2A) and mechanical withdrawal threshold (Fig. 2B) on the ipsilateral (SADBE treated) test paw compared to the contralateral (untreated) paw. However, these differences were not statistically significant except for day 12 of the Hargreaves test. Here, we used the contralateral untreated paw as a control expressing mirror-image reactions of spinal cord commissural neurons. For formal testing, we also used a group

of mice in which the test paw was first treated with a vehicle as a control group (data not shown).

The obtained data show a significant increase in the number of bites and licks in mice, indicating that itch reactions are accompanied by pain response behavior. The latter effects are confirmed by findings of thermal hyperalgesia and mechanical allodynia in this group of mice.

Recently Steele et al. (2021)[16] have observed dense MrgprC11^{+} nerves in the glabrous skin ofthe mouse plantar hindpaw. MrgprC11 agonist BAM8-22 directlyexcited 14.1% of sensory neurons innervating the glabrous skin.Activation of MrgprC11^{+} nerves in the glabrous skin by eitherspecific agonist BAM8-22 producedrobust biting responses. Moreover, ablation of MrgprC11^{+}neurons almost abolished glabrous skin itch sensation induced byboth BAM8-22 and allergic contact dermatitis. The authors, thus, demonstrated that MrgprC11+ neurons are key mediators forglabrous skin itch [16].

In conclusion, the SADBE model of chronic dermatitis is accompanied by pain behavior reducing thermal withdrawal latency and mechanical threshold. General itch perception should be characterized by some pain sensation in mice and humans. Further investigations are needed to examine physiological differences between hairy and glabrous skin to provide new insights into the thermal and mechanical pain sensations for differentiating itch in hairy and glabrous skin.

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ადამიანისა და ცხოველთა ფიზიოლოგია

ჰიპერალგეზია და ალოდინია თაგვების ქრონიკული ქავილის მოდელში

ი. ნოზაძე*, გ. ღურწკაია*, მ. ცაგარელი*, გ. გედევანიშვილი*, ე. აბზიანიძე*, მ.გ. ცაგარელი*

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(წარმოდგენილია აკადემიის წევრის თ. ზაალიშვილის მიერ)

ქავილი წარმოადგენს უსიამოვნო შეგრძნებას, რომელიც დაკავშირებულია ალერგიასა და კანიდან მწერების, მცენარის სპიკულების ან ინვაზიური პარაზიტების მოცილებასთან. მწვავე ქავილი ასახავს ადაპტაციურ მექანიზმს კანის მთლიანობის შესანარჩუნებლად, ხოლო ქრონიკული ქავილი შეიძლება უარყოფითად მოქმედებდეს ადამიანის ჯანმრთელობასა და ცხოვრების ხარისხზე. ბოლო რამდენიმე წლის განმავლობაში გამოვლინდა სხვადასხვა გარდამავალი რეცეპტორული პოტენციალის (TRP) და სხვა ცილოვანი არხების უაღრესად მნიშვნელოვანი როლი ქავილის შეგრძნებაში. წინამდებარე ნაშრომში შესწავლილია თერმული ჰიპერალგეზიისა და მექანიკური ალოდინიის ეფექტები, რომლებიც თან ახლავს ქავილის შეგრძნებას თაგვების ქრონიკული ქავილის მოდელში. მიღებულმა მონაცემებმა აჩვენა, რომ დიბუტილ ესტერის მჟავას (SADBE) ყოველდღიური აპლიკაცია 10 დღის განმავლობაში თაგვების უკანა თათის ტერფის შიშველ კანზე იწვევდა კბენისა და ლოკვის ქცევის მნიშვნელოვან ზრდას. თაგვების ამ ჯგუფში გამოვლინდა თერმული მოცილების რეფლექსის ფარული პერიოდისა და მექანიკური ზღურბლის თანმიმდევრული შემცირება იპსილატერალურ (SADBE-თი დამუშავებული) სატესტო თათში კონტრალატერალურ (დაუმუშავებელ) თათთან შედარებით. ამ შედეგებმა დაადასტურა, რომ ქრონიკული დერმატიტის ქავილის მოდელს თან ახლავს ჰიპერალგეზიისა და ალოდინიის ეფექტები.

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