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Spontaneous itch in the absence of hyperalgesia in a mouse hindpaw dry skin model

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Abstract

We presently investigated if chronic dry skin treatment of the mouse hindpaw results in itch-related behavior, with or without accompanying hyperalgesia and allodynia. Following a 10-day period of treatment of one hindpaw with a mixture of acetone-diethylether-water (AEW), mice exhibited a significant increase in spontaneous biting behavior directed to the treated hindpaw compared to control animals in which one hindpaw was similarly treated with water only (W). Biting in the AEW group was significantly attenuated by the μ -opioid antagonist naltrexone but was unaffected by the μ -agonist morphine. There were no significant differences in hindpaw heat withdrawal latency, mechanical withdrawal threshold, or cold-plate latency between W and AEW treatment groups. These results indicate that chronic AEW treatment induces spontaneous itch but does not alter pain sensitivity, supporting the utility of this model for studies of chronic dry skin itch.

Keywords

chronic itch; scratching; allodynia; pain; dry skin (xerosis)

Itch commonly accompanies conditions associated with dry skin, such as xerosis and atopic dermatitis, as well as systemic disorders including chronic renal failure or HIV [20]. A murine model for dry skin has been developed by Miyamoto et al [12]. This model involves repeated topical application to the nape of the neck of a mixture of acetone and diethylether followed by water (AEW) over a 5-day period. This results in an increase in spontaneous hindlimb scratching and transepidermal water loss (TEWL). Similar AEW treatment in rats also resulted in significantly increased spontaneous scratching, accompanied by an increase in the number of c-fos immunoreactive neurons in the cervical superficial dorsal horn [13]. We recently reported that AEW treatment resulted in a significant enhancement of scratching elicited by certain pruritogens, indicative of hyperknesis, greater itch to a normally itchy stimulus [1]. However, it is uncertain if dry skin treatment affects pain sensitivity which is difficult to test in the rostral back treatment area. The present study addressed this question, by testing pain sensitivity following dry skin treatment of the mouse hindpaw. In mice, hindpaw AEW treatment resulted in increased spontaneous biting [14]. We tested if responses to noxious heat or cold, or innocuous mechanical stimuli, changed

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following hindpaw AEW treatment. Furthermore, we tested if spontaneous biting behavior was affected by opioids in a manner consistent with itch (i.e., reduced by μ -opioid antagonists but not agonists).

Methods

Adult male CD-1 mice (Harlan, Oxnard CA) weighing 27–38 g were used. The experimental protocol was approved by the UC Davis Institutional Animal Use and Care Committee. The right hind paw was wrapped up to the ankle by cotton gauze soaked with a mixture of acetone and diethylether (1:1) for 15 s, which was then removed and followed immediately by identical application of gauze soaked with distilled water for 30 s (designated as AEW treatment). A control group was identically treated with distilled water only for 45 s (designated as W treatment). To prevent licking and biting of the treated area, each mouse was fitted with a plastic Elizabethan collar (Kent Scientific Corp, Torrington, CT) placed around the chest. The collar was configured to allow normal movement but to prevent access of the mouth to the hindpaws. AEW or W treatments were performed twice daily for 10 consecutive days with the collars attached at all times. Sixteen to 20 h after the second treatment on day 10, the collars were removed and spontaneous behavior was recorded for up to 40 min, after which pain tests were conducted.

In separate AEW- and W-treated groups, either naltrexone hydrochloride (1 mg/kg, sc; Dupont; Garden, NY), morphine sulfate (1 mg/kg, ip; Mallinckrodt, Hazelwood, MO), or vehicle (isotonic saline) was administered in a volume of 0.1 ml/10 g weight, 20 min prior to recording of spontaneous behavior. The morphine dose was selected because 1 mg/kg morphine was previously shown to significantly attenuate nocifensive behavior including licking elicited by formalin injected in the hindpaw [15], while higher morphine doses elicited circling behavior in the mouse [2] that might interfere with hindpaw biting or licking.

During the recording period, each mouse was placed in an opaque Plexiglas recording arena on a clear glass table and videotaped from below for a maximum duration of 40 min. Up to 4 mice could be videotaped simultaneously. After commencing videotaping, investigators vacated the room for the remainder of the session. Videotapes were reviewed offline by two different investigators blinded as to treatment, to assess biting and licking behavior directed toward the treated paw. A bite was defined by direct contact of the incisors with hindpaw skin within the treatment area distal to the ankle. Biting was accompanied by relatively high-frequency low-excursion head movements which were used as an adjunct measure. The duration of each biting action was timed with a stopwatch, and individual bite durations were summed to provide the cumulative biting time. The cumulative duration of licking, defined as direct contact of the treated hindpaw by tongue protrusion, was also measured. Licks were accompanied by head movements that were of lower frequency and larger excursion compared to those associated with biting, and were also used as an adjunct measure of licking.

TEWL was measured by applying a Vapo Meter® (Delfin Technologies Ltd, Kuopio, Finland) to the skin for 20 s. TEWL was measured after the behavior study on day 10.

Mice were accommodated to the nociceptive test environments for at least 30 min per day for 3 successive days prior to the day of testing. Accommodation was conducted between daily AEW or W treatments on these days.

Paw withdrawal to mechanical stimulation was evaluated by measuring the threshold of the paw withdrawal evoked by stimulation of the plantar surface of the hindpaw using electronic von Frey (1601C, IITC Life Science Inc., Woodland Hills, CA). Briefly, animals were

placed in a plastic box resting on a wire mesh floor. The electronic von Frey filament was applied to the mid plantar surface of hind paw through the mesh and the force at the time of hindlimb withdrawal was measured 5 times per hindpaw with a minimum of 10 min between successive measurements of a given hindpaw.

Thermal hyperalgesia was evaluated by measuring the latency for hindpaw withdrawal evoked by a heat stimulus. Each mouse was placed in a Plexiglas box resting on a clear glass surface maintained at 30°C $\pm$ 1°C. A radiant heat source (Planter test 390, IITC Life Science Inc) was positioned under the glass surface and focused on the mid-plantar surface of one hindpaw. Withdrawal latencies were measured 5 times for each hindpaw with minimum of 20 min between successive measurements of a given hindpaw. A cutoff of 20 s was set to prevent tissue damage. For both thermal and mechanical paw withdrawals, the middle three values were averaged and the highest and lowest values were discarded.

Cold pain was assessed using a thermoelectric cold plate (AHP-1200DCP, TECA, Chicago, IL). The temperature of the surface was set at either -5 or 0°C ($\pm 0.1^{\circ}\text{C}$) as verified with an electronic thermometer. Mice were placed onto the cold plate and the latency for the appearance of nocifensive behaviors (shaking or licking the hindpaw, or jumping) was recorded. The cut-off latency was set at 150 s to prevent potential tissue damage. Between-group counts were compared using a Student's *t*-test with $p < 0.05$ considered significant.

Results

On day 10 of treatment, TEWL was significantly greater in the AEW group compared to the W group (Fig. 1A). The cumulative duration of spontaneous biting was significantly greater in the AEW than W group, whereas the cumulative duration of licking was not different between treatment groups (Fig. 1B). Licking and biting behaviors occurred throughout the 40-min observation period and did not subside over time. There was considerable within-animal variability in the frequency of occurrence of both behaviors (mean range: 3–16 licks and 2–8 bites per 5-min interval).

In AEW mice, pretreatment with naltrexone resulted in a significant reduction in the cumulative duration of spontaneous biting behavior compared to vehicle controls (Fig. 1C, left-hand gray vs. white bars). In contrast, spontaneous biting was not significantly different between morphine and saline control groups (Fig. 1C, left-hand black vs. white bars). There were no significant differences in the cumulative duration of spontaneous licking in any of the pretreatment groups (Fig. 1C, right-hand bars).

On day 10 of treatment, there was no significant difference in the mean paw withdrawal threshold to mechanical stimulation in the AEW vs. W group (Fig. 2A). Similarly, there was no significant between-group difference in mean paw withdrawal latency to heat (Fig. 2B). Moreover, there were no significant between-group differences for the 0 or -5°C cold plate latencies (Fig. 2C).

Discussion

TEWL increased after 10 days of AEW treatment of the hindpaw, consistent with the original report showing that TEWL increased over a 5-day period of AEW treatment of the rostral back [12]. AEW-treated mice exhibited increased spontaneous hindpaw biting, in confirmation of a previous study [14]. The present hindpaw dry skin model appears to mimic the spontaneous itch and skin dryness associated with senile xerosis [5,10,19,20].

A novel finding of the present study was that spontaneous biting behavior was significantly attenuated by the μ -opioid antagonist naltrexone, but not by the μ -opioid agonist morphine.

This finding is also consistent with the original study showing that μ -opioid antagonists significantly reduced spontaneous hindlimb scratching directed to AEW-treated skin on the nape of the neck [12]. Given that μ -opioid antagonists reduce experimentally-evoked itch in humans [7], and μ -opioid agonists reduce pain with or without inducing itch, the naltrexone-sensitive but morphine-insensitive spontaneous biting behavior observed presently in the AEW-treated mice is proposed to reflect itch. This receives further support from studies showing that μ -opioid antagonists significantly reduced hindpaw biting behavior elicited by intradermal hindpaw injection of serotonin [6] as well as spontaneous biting directed toward lumbar lesion sites in NC mice that exhibit symptoms of atopic dermatitis [11]. In contrast, licking behavior is generally considered to reflect pain although it might also reflect itch. Injection of pruritogens into the hindpaw or thigh evoked licking behavior [8]. Bhlhb5 mutant mice which are thought to represent a model of chronic itch exhibited licking behavior directed toward skin lesion sites [17]. Hindpaw injection of serotonin in mice elicited approximately equivalent levels of biting and licking, while formalin elicited almost exclusively licking with very little biting [6]. In the present study, dry skin treatment did not result in an enhancement of spontaneous licking behavior (Fig. 1B), and licking was not significantly affected by naltrexone or morphine (Fig. 1C), suggesting that in the dry skin model licking reflects neither itch nor pain and may instead represent a component of normal grooming behavior.

Another novel finding is that sensitivity to mechanical and noxious thermal stimuli was unchanged in AEW-treated mice. This implies that nociceptive pathways are not sensitized under the present conditions of chronic dry skin. This is consistent with a very recent report showing that Bhlhb5 mutant mice, which lack a subpopulation of spinal inhibitory interneurons, exhibited hyperknesis but not hyperalgesia [17]. Enhanced scratching in the absence of altered pain sensitivity make these models attractive for future studies directed at better understanding how itch-signaling pathways may be altered under conditions of chronic itch.

Our present mechanical and thermal withdrawal data obtained with CD-1 mice are comparable to those of a previous report using same mouse strain [2]. The present cold plate latencies were longer compared to those observed in C57BL/6 mice [4]. We believe that this more likely reflects a strain difference rather than an artifact of our design involving unilateral hindpaw treatment. It was previously reported that significant differences in cold plate sensitivity can be observed in unilaterally treated mice [16].

The mechanism of dry skin itch is still unknown. Increased TEWL indicates a dysfunction of the skin barrier which may result in release of endogenous pruritogens. Using the dry skin model applied to the rostral back of mice, we recently reported that spontaneous hindlimb scratching was significantly reduced by intradermal administration of an antagonist (antibody) of the protease-activated receptor PAR-2 as well as systemic administration of a 5-HT₂ antagonist, suggesting roles for proteases and 5-HT as endogenous pruritogens in dry skin itch [1]. Recent studies have revealed that knockouts of genes encoding phospholipase-C β 3, the gastrin releasing peptide receptor, or MrgprA3, reduced scratching behavior elicited by certain pruritogens [8,9,18]. Future studies can be expected to reveal contributions that these molecules make to itch under normal and pathological conditions.

Finally, c-fos expression was observed in lumbar superficial dorsal horn neurons from hindlimb AEW-treated mice that exhibited spontaneous biting behavior [14]. This implies that under conditions of dry skin, ongoing pruriceptive input excites itch-signaling superficial dorsal horn neurons. It will be interesting to determine in future physiological studies if these neurons exhibit spontaneous firing and sensitized responses to pruritogens or other cutaneous stimuli.

Research Highlights

- Mice exhibited spontaneous biting directed to a hindpaw treated to produce dry skin
- Biting was significantly reduced by naltrexone and unaffected by morphine
- Dry skin treatment did not affect limb withdrawal to von Frey or noxious heat stimuli
- Dry skin is a model for chronic itch without altered pain sensitivity

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Fig. 1. Transepidermal water loss (TEWL) and biting and licking behavior in dry skin treated mice. A: Values for TEWL in W (open bar) and AEW-treated mice (black bar) measured on day 10. Error bars in this and all subsequent figures: SEM. *: significant difference ($p < 0.05$; $n=8$ /group). B: Cumulative duration of biting (left-hand bars) and licking (right-hand bars) in W (open bars) and AEW-treated mice (black bars). *: significant difference ($p < 0.05$; $n=8$ /group). C: Effects of naltrexone and morphine on biting and licking behaviors. Left-hand bars: biting. Right-hand bars: licking. Morphine, naltrexone or saline was systemically administered 20 min before recording. *: significant difference compared with saline group ($p < 0.05$; $n=8$ /group).

Fig. 2.

Lack of effect of AEW treatment on nocifensive behavior. A: Plots mean mechanical paw withdrawal thresholds in W and AEW groups. There was no significant difference between W and AEW groups ($p > 0.05$; $n=8/\text{group}$). B: As in A for mean paw withdrawal latencies to noxious heat. No significant difference between W and AEW groups ($p > 0.05$; $n=8/\text{group}$). C: latencies for nocifensive behavior on -5°C and 0°C cold plates. No significant difference between W and AEW groups ($p > 0.05$; $n=8/\text{group}$).