Acta Derm Venereol 2015; 95: 147–150

INVESTIGATIVE REPORT

Nalfurafine Suppresses Pruritogen- and Touch-evoked Scratching Behavior in Models of Acute and Chronic Itch in Mice

Tasuku AKIYAMA[1], Mirela Iodi CARSTENS[1], Dorothea PIECHA[2], Sonja STEPPAN[2] and Earl CARSTENS[1]
[1]Department of Neurobiology, Physiology and Behavior, University of California, Davis, and [2]Renal Pharmaceuticals International, Fresenius Medical Care Deutschland GmbH, Bad Homburg, Germany

The kappa-opioid agonist, nalfurafine, has been approved in Japan for treatment of itch in patients with chronic kidney disease. We presently investigated if systemic administration of nalfurafine inhibited ongoing or touch-evoked scratching behavior (alloknesis) following acute intradermal injection of histamine or the non-histaminergic itch mediator, chloroquine, in mice. We also investigated if nalfurafine suppressed spontaneous or touch-evoked scratching in an experimental model of chronic dry skin itch. Nalfurafine reduced scratching evoked by histamine and chloroquine. Following acute histamine, but not chloroquine, low-threshold mechanical stimuli reliably elicited directed hindlimb scratching behavior, which was significantly attenuated by nalfurafine. In mice with experimental dry skin, nalfurafine abolished spontaneous scratching but had no effect on alloknesis. Nalfurafine thus appears to be a promising treatment for acute itch as well as ongoing itch of dry skin. *Key words: itch; alloknesis; nalfurafine (TRK-820); mouse; dry skin.*

Accepted Mar 12, 2014; Epub ahead of print Apr 25, 2014

Acta Derm Venereol 2015; 95: 147–150.

Prof. Earl Carstens, Department of Neurobiology, Physiology and Behavior, University of California, 1 Shields Avenue, Davis, CA 95616 USA. E-mail: eecarstens@ucdavis.edu

Chronic itch persisting for more than 6 weeks has a lifetime prevelance of 25% (1–3). Chronic itch has a significant negative impact on the quality of life, creating a great demand for effective treatments (4). A mechanism that potentially underlies chronic itch is the sensitization of itch signaling pathways (2). Itch sensitization is characterized by spontaneous itch, increased itch to a normally pruritic stimulus (hyperknesis), and itch elicited by innocuous touch (alloknesis). Our laboratory has investigated sensitization in rodent models of chronic itch (reviewed in 2), and we have recently developed a model for alloknesis (touch-evoked itch) (5). Innocuous touch stimuli normally do not elicit scratching in naïve C57BL/6 mice. However, following the intradermal injection of certain pruritogens, or in animals treated for chronic dry skin itch, innocuous touch stimuli elicit discrete episodes of hindlimb scratching directed toward the stimulus.

Nalfurafine, a κ-opioid agonist, has been approved in Japan for the treatment for uremic pruritus in hemodialysis patients. The antipruritic efficacy of nalfurafine has been reported for several different animal models of itch (6–12). In the present study, we have extended these findings in two significant ways. First, given recent evidence for histaminergic and non-histaminergic itch-signaling pathways (2), we investigated whether nalfurafine reduced scratching behavior elicited by histamine or by a non-histaminergic mediator, chloroquine. Chloroquine is an antimalarial drug that often elicits severe itch by its action at a molecular receptor, Mas-related G-protein coupled receptor C11 (MrgprC11) (13) that is distinct from the histamine H1 and H4 receptors involved in histamine-evoked itch. We tested if nalfurafine inhibits histamine- or chloroquine-evoked scratching behavior, and touch-evoked scratching, following the acute delivery of these pruritogens.

A common symptom in patients with end-stage renal disease is uremic xerosis (dry skin) with pruritus (14, 15). A second aim of our study was to investigate if nalfurafine suppresses spontaneous scratching behavior and alloknesis in an experimental model of chronic dry skin pruritus in mice.

METHODS

Experiments were conducted using adult male C57BL/6 mice (Harlan, Oxnard CA; 18–21 g) under a protocol approved by the University of California, Davis Institutional Animal Care and Use Committee.

Behavior. The fur on the nape of the neck was shaved and mice were habituated to a Plexiglas recording arena with a transparent cover one week prior to testing. On the test day, the animal received a subcutaneous injection of nalfurafine (10 or 20 μg/kg, Fresenius Medical Care, Bad Homburg, Germany) or saline. Thirty min later, the animal received an intradermal microinjection of 10 μl of either histamine (271 nmol; Sigma-Aldrich, St. Louis MO) or chloroquine (193 nmol; Sigma-Aldrich). Briefly, microinjections were made i.d. in the nape of the neck using a 30-G needle attached to a Hamilton microsyringe by PE-50 tubing. Immediately following the i.d. microinjection, mice were placed into the arena and videotaped from above. Investigators left the room for 30 min and then returned to test for alloknesis, starting 30 min post-injection.

 doi: 10.2340/00015555-1879
 ISSN 0001-5555

Alloknesis testing was performed as in our recent study (5). Briefly, at 5-min intervals, the mouse received 3 separate innocuous mechanical stimuli delivered using a von Frey filament (bending force: 0.7 mN) at separate, randomly-selected sites oriented radially 7 mm away from the injection site. The 0.7 mN von Frey filament was selected because it never elicited scratch bouts in naïve mice, and was the minimum strength to elicit scratch bouts when delivered to skin surrounding the site of histamine injection or dry skin treatment. The presence or absence of a positive response, consisting of a hindlimb scratch bout directed to the site of mechanical stimulation, was noted for each stimulus before the next one was given. The alloknesis score was the total number of positive responses elicited by the 3 stimuli, i.e., 0, 1, 2 or 3. The sequence was repeated out to 60 min post-injection. In many experiments, an overall alloknesis score per 30 min was calculated as the sum of individual alloknesis scores at each 5-min interval over the 30 min period beginning 30 min post-injection.

Videotapes were played back and the number of scratch bouts counted at 5-min intervals over the 30-min post-injection recording period by two independent observers blinded as to the treatment. A scratch bout was defined as one or a series of rapid back-and-forth movements of the hindpaw directed to the site of injection, and ending with licking or biting of the toes and/or placement of the hindpaw on the floor. Between-group comparisons of counts of scratch bouts or alloknesis scores were made by unpaired *t*-test or one way ANOVA followed by Bonferoni-test with $p < 0.05$ considered to be significant.

Dry skin model. To induce chronic dry skin on the nape of the neck, we followed a previously-reported procedure (5, 16). Briefly, a mixture of acetone and diethylether (1:1) was applied to a circumscribed area (approx. 15 x 15 mm spanning the midline) at the nape of the neck for 15 s, followed immediately by distilled water for 30 s, twice-daily for 8 days (designated as acetone–ether–water (AEW) treatment). Application of water only for 45 s (W treatment) serves as a control. Since W treatment does not lead to increased spontaneous scratching behavior (5), it was not done in the present study. Sixteen to 20 h after the second treatment on the 8th treatment day, nalfurafine (20 μg/kg) or saline was administered. After 30 min, alloknesis testing was conducted in a manner similar to that noted above. von Frey stimuli were applied at the border of the AEW treatment area at 5 randomly selected sites. Following the alloknesis test, spontaneous behavior was recorded for another 60 min. Trans-epidermal water loss (TEWL) was measured by applying a Vapo Meter® (Delfin Technologies Ltd., Kuopio, Finland) to the skin for 10 s.

RESULTS

Nalfurafine suppresses histamine- and chloroquine-evoked behaviors. Nalfurafine suppressed scratching behavior elicited by acute i.d. injection of both histamine- and chloroquine (Fig. 1A). The number of histamine-evoked scratch bouts was significantly reduced ($p < 0.05$) by nalfurafine pretreatment in a dose-related manner (Fig. 1A, open, gray and black bars). The number of acute chloroquine-evoked scratch bouts was significantly reduced ($p < 0.05$) following 20 μg/kg nalfurafine (Fig. 1A, vertically striped bar).

Following i.d. histamine, von Frey stimulation reliably elicited hindlimb scratch bouts, confirming our recent report (5). The mean alloknesis score for histamine is shown in Fig. 1B (open bar). Nalfurafine pretreatment significantly reduced mean alloknesis scores for histamine in a dose-related manner (Fig. 1B, gray and black bars). Following i.d. chloroquine, the alloknesis score was very low (Fig. 1B, horizontally striped bar), consistent with our recent report (5). Nalfurafine (20 μg/kg) numerically reduced the alloknesis score further (Fig. 1B, vertically striped bar) although this effect did not reach statistical significance.

Nalfurafine suppresses spontaneous scratching but not alloknesis in dry skin. The number of spontaneous scratch bouts increased significantly on day 8 of AEW treatment (Fig. 2A, $p < 0.05$). Systemic pretreatment with nalfurafine completely abolished spontaneous scratching on treatment day 8. The mean alloknesis score increased significantly on day 8 of AEW treatment (Fig. 2B), consistent with our recent report (5). Nalfurafine had no sig-

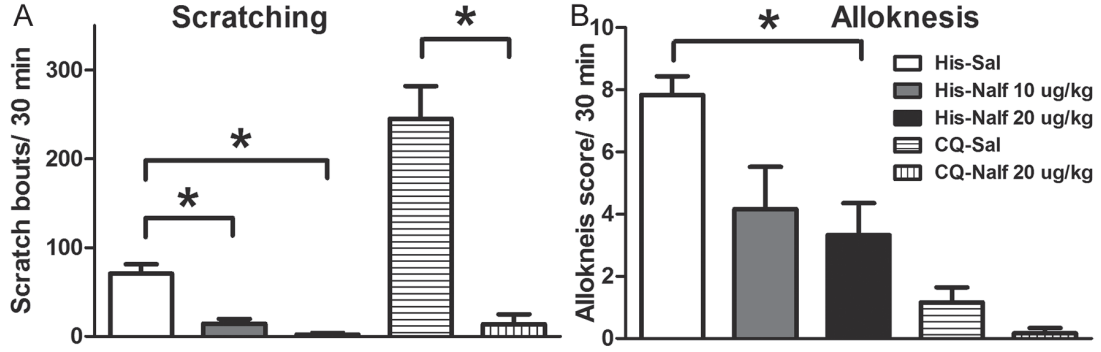

Fig. 1. Nalfurafine inhibits scratching and alloknesis elicited by intradermal (i.d.) histamine and chloroquine. A: Bar graph shows the mean number of scratch bouts/30 min after i.d. injection of histamine (His-Sal) or chloroquine (CQ-Sal) in animals pretreated with saline. Additional groups received i.d. histamine following pretreatment with nalfurafine (His-Nalf, 10 or 20 μg/kg), or i.d .chloroquine following pretreatment with nalfurafine (CQ-Nalf 20 μg/kg). Error bars are SEM ($n = 6$/group). *$p < 0.05$, significantly different from saline-treated group (one way ANOVA followed by Bonferroni test or unpaired *t*-test). B: As in A for the alloknesis scores averaged over 30 min (i.e., maximum score is 21). Error bars are SEM (n=6/group). *$p < 0.05$, significantly difffence from saline-treated group (one way ANOVA followed by Bonferroni test).

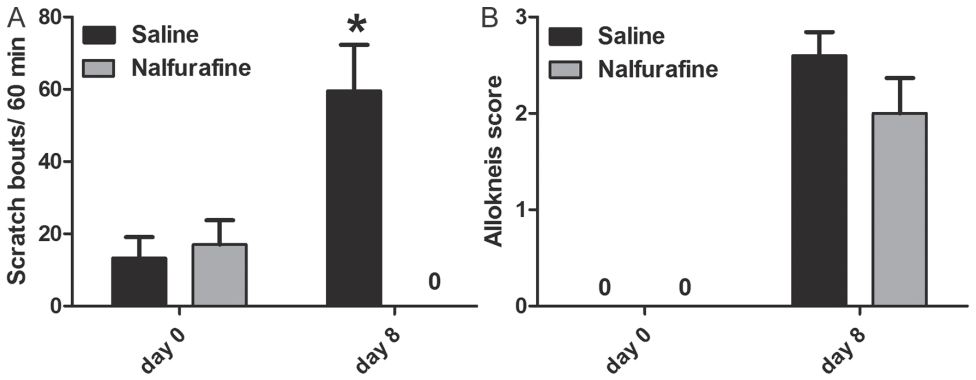

Fig. 2. κ-opioid agonist nalfurafine inhibits spontaneous scratching, but not alloknesis in dry skin-treated mice. A: Dry skin was induced by twice-daily treatment with acetone ether water (AEW) over 8 days. Bar graph shows the mean number of scratch bouts/60 min following pretreatment with saline (black bar) or nalfurafine (gray bar) at day 0 (i.e, prior to AEW treatment) and at AEW treatment day 8. Error bars are SEM ($n = 5–6$/group). *$p < 0.05$, significantly different compared to saline-treated group on day 0 (paired t-test). Nalfurafine completely inhibited spontaneous scratching. B: As in A for the alloknesis score. Error bars are SEM ($n = 5–6$/group). Nalfurafine had no significant effect on alloknesis.

nificant effect on the alloknesis score on treatment day 8 (gray bar). TEWL on day 8 was significantly higher than on day 0 (day 0; 6.7 ± 0.3 g/m^{2}h [SEM], day 8; 25.0 ± 1.1 g/m^{2}h). There was no significant difference in TEWL on day 8 between vehicle- and nalfurafine-treated mice (vehicle; 25.5 ± 1.3 g/m^{2}h, nalfurafine; 24.7 ± 1.7 g/m^{2}h).

DISCUSSION

Nalfurafine inhibits acute pruritogen-evoked scratching. Our present data showing that nalfurafine suppressed acute histamine- and chloroquine-evoked scratching behavior are consistent with previous reports (6, 10). Our study extends these findings by showing that nalfurafine inhibited spontaneous scratching in an experimental model of chronic dry skin pruritus. This effect cannot be attributed to a generalized suppression of motor activity, since the highest concentration used presently (20 μg/kg) was lower than nalfurafine concentrations reported to reduce locomotor activity (8). Additionally, nalfurafine failed to inhibit touch-evoked scratching in the dry skin model.

The antipruritic effect of nalfurafine is presumably mediated via both peripheral and central mechanisms. A peripherally restricted κ-agonist inhibited chloroquine-evoked scratching, suggesting a role for peripheral κ receptors in mediating the antipruritic effect (6). Recent studies indicate that histamine and chloroquine activate distinct subsets of primary sensory neurons (17–19). Therefore, whether a peripherally restricted κ-agonist inhibits histamine-evoked scratching needs to be addressed. Nalfurafine also inhibited scratching behavior elicited by intracisternal administration of morphine in mice, and by intrathecal injection of morphine in monkeys (11, 12), suggesting that nalfurafine's antipruritic effect is mediated via a central action. Nalfurafine has been prescribed to treat uremic itch in hemodialysis patients. These patients tend to exhibit skin xerosis (14, 15). Although the correlation between pruritus and skin xerosis has not been clearly demonstrated, skin xerosis may contribute to uremic itch by lowering the threshold for itch perception. Elevated epidermal nerve density was observed in chronic experimental dry skin (20). In the present study, nalfurafine completely suppressed spontaneous scratching in the experimental model of chronic dry skin pruritus, consistent with the beneficial effect of nalfurafine to reduce itch in uremic xerosis.

Nalfurafine inhibits alloknesis in acute but not chronic dry skin itch. The present results confirm our previous report of alloknesis following i.d. injection of histamine, but not chloroquine (5). It has been reported that μ-opioid antagonists reduced touch-evoked scratching following i.d. histamine injection in mice (5), as well as histamine-evoked alloknesis in humans (21). We extend these findings by showing that the κ-opioid agonist nalfurafine also reduced touch-evoked scratching following id histamine injection in mice. One potential mechanism underlying alloknesis is the central sensitization of spinal itch signaling pathways to touch. Such a sensitization might be triggered by sustained input from peripheral histamine-sensitive pruriceptors. Nalfurafine may interfere with the synaptic mechanism involved in the induction of central sensitization. In contrast, nalfurafine failed to inhibit alloknesis even though it completely abolished spontaneous scratching in the experimental chronic dry skin model. This implies that the mechanism underlying sensitization to touch is different in animals with chronic dry skin pruritus (i.e., nalfurafine-resistant) compared to normal animals with acute itch (nalfurafine-sensitive). Moreover, in dry skin-treated animals, the mechanism underlying alloknesis (nalfurafine-resistant) is distinct from that underlying spontaneous scratching (nalfurafine-sensitive). In conditions of chronic pain, disinhibition of glycine signaling in the spinal cord was suggested to convert touch into pain by unmasking innocuous

mechanoreceptor input to nociceptive-specific neurons in the superficial dorsal horn of the spinal cord (22, 23). Interestingly, this allodynia (touch-evoked pain) was resistant to opioid modulation (22). Alloknesis in chronic itch may be attributed to a similar mechanism that unmasks innocuous mechanoreceptor input to superficial dorsal horn neurons involved in transmitting itch signals.

ACKNOWLEDGMENTS

This work was supported by a grant from Fresenius Medical Care.

Conflict of interest: Drs. Piecha and Steppan are employed by Fresenius Medical Care who financially supported the research.

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