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Antipruritic Effect of DA-5018, A Capsaicin Derivative, in Mice

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The antipruritic effect of DA-5018, a capsaicin derivative, was examined in mice. Male ICR mice were topically pretreated with Zostrix-HP (0.075% capsaicin cream), 0.1%, 0.3% DA-5018 cream or cream base (control) twice daily for 4 days. One hour after the last application, itch was induced either by compound 48/80 (50 μg, s.c.) or leukotriene B_4 (0.03 nmol, i.d.) injection into the rostral back of the animals, and the number of scratches made by the animals at the injection site was counted for 60 min post-injection. DA-5018 cream (both 0.1 and 0.3%) significantly inhibited compound 48/80-induced scratching when compared with the cream base control ($p<0.01$), while Zostrix-HP showed minimal inhibition of the scratching behavior. In leukotriene B_4-induced itch model, Zostrix-HP and 0.3% DA-5018 cream significantly inhibited the scratching during the first 10-min period ($p<0.01$). The results suggest that DA-5018 cream can be used as an antipruritic agent and warrant clinical evaluation.

Key words: DA-5018, Antipruritic effect, Itching, Compound 48/80, Leukotriene B_4, ICR mice

INTRODUCTION

Pruritus, or itching, is a sensation that provokes a desire to scratch. It is the most common symptom of cutaneous diseases (e.g., atopic dermatitis, contact dermatitis, urticaria) and accompanies several systemic disorders (e.g., chronic renal failure, cholestasis). Surprisingly, this well-described symptom has not been studied extensively, and its underlying mechanisms are far from being understood. This sensation can be produced experimentally by several endogenous substances, such as histamine, substance P (SP), leukotriene B_4 (LTB_4), vasoactive intestinal peptide and neurotensin (Andoh and Kuraishi, 1998; Hagermark, 1992). SP is one of the most potent endogenous pruritogenic peptides (Hagermark *et al.*, 1978) and speculated to be involved in some pruritic diseases (Farber *et al.*, 1986). Basic peptides, as well as SP, generally degranulate mast cells (Devillier *et al.*, 1989; Ebertz *et al.*, 1987). The itch induced by SP is thought to be mediated by histamine released from mast cells (Hagermark *et al.*, 1978). Capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide), an alkaloid found in many botanical species of the nightshade family (*Solanaceae*), is the chemical substance that makes hot peppers "hot". Capsaicin enhances the release and inhibits the reaccumulation of SP from cell bodies and nerve terminals in the central and peripheral nerve systems (Bernstein *et al.*, 1982). Capsaicin has been used with some success in painful diabetic neuropathy (Basha and Whitehouse, 1991; Scheffler *et al.*, 1991) and postherpetic neuralgia (Bjerring and Arendt-Nielsen, 1990; Peikert *et al.*, 1991). Other studies showed good therapeutic results in pruritic psoriasis (Bernstein *et al.*, 1986; Ellis *et al.*, 1993) and different types of pruritus (Bernstein *et al.*, 1982; Goodless and Eaglstein, 1993; Lotti *et al.*, 1994).

Recently Dong-A Pharmaceutical Company has developed a new capsaicin derivative, DA-5018, to be used as a pain-killer (Kim *et al.*, 1997). DA-5018 exerts its analgesic action through the same mechanism that capsaicin does. In preclinical studies, DA-5018 showed superior analgesic effect in acute pain models as compared to capsaicin (Bae *et al.*, 1997; Kim *et al.*, 1997; Son *et al.*, 1997). Moreover, its irritancy to intact skin was greatly reduced when compared to capsaicin (Kim *et al.*, 1996). These findings suggest that DA-5018 can be used as a therapeutic for various types of pain. However, the antipruritic effect of DA-5018 has not been studied. Therefore, in our study, the effect of DA-5018 on experimental pruritus models was examined.

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MATERIALS AND METHODS

Animals and housing

All experimental procedures were performed in accordance with the institutional guideline "Standard Procedure for Animal Care and Experiments (SOP-ANC)" of Dong-A pharmaceutical company. Male ICR mice (Daehan Laboratory Animals, Ltd., Korea) of 8 wks old, weighing 30 to 33 g, were used after 6-day acclimation. They were housed in standard polycarbonate cages under controlled temperature (23-25°C) and light (lights on from 08:00 to 20:00). Food (Cheiljedang, Korea) and water were freely available during the experiment.

Drugs

Cream base and DA-5018 [N-(3-(3,4-dimethylphenyl) propyl)-4-(2-aminomethoxyphenyl-acetamide hydrochloride salt] cream formula of 0.1% and 0.3% were obtained from the Research Laboratory of Dong-A pharmaceutical company (Kyunggi, Korea). As a reference drug, Zostrix®-HP (capsaicin 0.075%, GenDerm, U.S.A.) was used. Compound 48/80 (Sigma Chemical Co., St. Louis) and leukotriene B_4 (Sigma) were used as pruritogens.

Experimental procedure

Two experimental models, compound 48/80 model and leukotriene B_4 model, were used to examine the antipruritic effect of DA-5018. After removing the hair from the back of the mouse, 0%, 0.1% or 0.3% DA-5018 cream was locally applied twice a day for 4 consecutive days. Zostrix®-HP (capsaicin 0.075%), as a reference drug, was applied during the same period under the same conditions. At each application, approximately 100 mg of the test cream was loaded onto the marked area of the back and rubbed for at least 2 min. One hr after the last application, an injection of compound 48/80 or leukotriene B_4 was used to induce local pruritus. As described by Rojavin *et al.* (1998), compound 48/80 was subcutaneously injected in the rostral part of the back at a dose of 50 μg per mouse (0.1 ml of 0.5 mg/ml saline solution). Sodium salt of leukotriene B_4 (0.03 nmol per mouse) was dissolved in physiological saline and injected intradermally in a volume of 50 μl into the rostral part of the back for leukotriene B_4 model (Andoh and Kuraishi, 1998). Eight animals per group were used for each test condition.

Before behavioral experiments, the mice (four animals per observation) were put into an acrylic cage (26 × 18 × 30 cm) composed of four cells (13 × 9 × 30 cm) for at least 1 hr for acclimation. Immediately after the pruritogen injection, they were returned to the same cell and their behavior was recorded on video tape using an 8-mm video camera for 1 hr. The video replay showed the number of scratches made by the mouse, using hind paws for about 1 sec. A series of these movements was counted as one bout of scratching (Kuraishi et *al.*, 1995). The severity of itch response was measured by the number of scratches made at the injection site. The results were expressed as absolute numbers of scratches during every 10-min period and during the whole observation period. Also, scratching index (SI) was calculated by dividing the average number of scratches of the DA-5018- or Zostrix-treated group by the same parameter of the corresponding control group (Rojavin et *al.*, 1998). Thus, the smallest value of the SI corresponds to the largest inhibitory effect of the test material on pruritus.

Data analysis

Data were analyzed by one way analysis of variance procedure with post-hoc comparisons using the methods of Dunnett (Dunnett, 1995). A significance condition of $p \leq 0.01$ was used for all comparisons. The SigmaStat® and SAS statistical software were used for analysis. Data were presented as the mean ± S.E.M.

RESULTS

Effects of DA-5018 on compound 48/80-induced itching

Under the current test condition, DA-5018 significantly inhibited the pruritogenic response induced by compound 48/80 ($p < 0.005$). The subcutaneous injection of compound 48/80 (50 μg) into the rostral back of

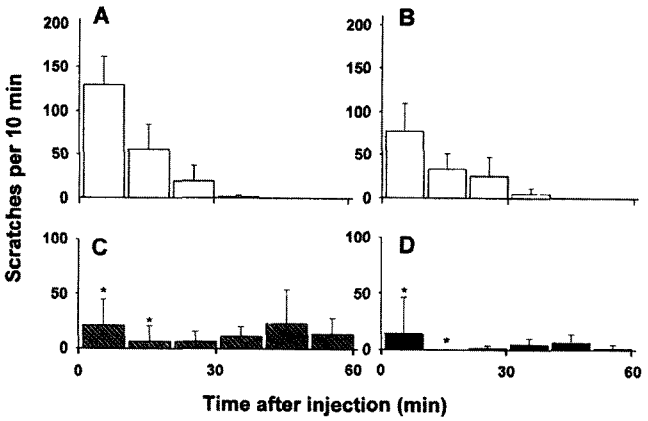

Fig. 1. Inhibitory effect of a capsaicin derivative, DA-5018, on compound 48/80-induced scratching. The ICR mice were topically pretreated with cream base (A), 0.1% (C) and 0.3% DA-5018 creams (D), or Zostrix-HP (B) twice daily for 4 days. One hour after the last treatment, compound 48/80 (50 g per mouse) was injected s.c. into the rostral back of the mice and the scratching of the skin around the injection site was counted for every 10-min periods. Values are the means and S.E.M. for eight animals. * $P < 0.01$ when compared with cream base control (A).

the ICR mouse elicited extensive scratching of the injected site by hind paws, which almost subsided by 30 min after the injection (Fig. 1A). DA-5018 pretreatment apparently reduced initial scratching, induced by compound 48/80 at both concentrations of 0.1% and 0.3% (Fig. 1C and 1D). Fig. 2 shows the average number of scratches of each group for 60 min. The SI values of DA-5018-treated groups were 0.38 and 0.13 for 0.1% and 0.3% formulas, respectively. Zostrix-HP also reduced initial scratching and the cumulative number of scratches without statistical significance ($p = 0.011$ for the first 10 min and $p = 0.065$ for total scratches). The SI of Zostrix-HP was 0.68. The dynamics of scratching activity in control and DA-5018-treated groups were different during the observation period. In the control group, the number of scratches reached a peak during the first 10-min period, and gradually decreased by 30 min after the insult of compound 48/80. In DA-5018 treated mice, however, the scratches were observed during the whole observation period almost evenly (Fig. 1). In contrast, mice pretreated with Zostrix-HP showed a similar scratching dynamics to those of the control mice.

Effects of DA-5018 on leukotriene B_4-induced itching

The intradermal injection of leukotriene B_4 (LTB_4) into the rostral back apparently elicited scratching on the injection site by hind paws. Fig. 3A shows the number of scratches with time for 60 min after the injection of LTB_4 (0.03 nmol). The scratching induced by LTB_4 peaked in the first 10-min period and had almost subsided by 40 min. The scratching during the first 10-min was significantly ($p < 0.01$) inhibited by pretreatment of Zostrix-HP and 0.3% DA-5018 cream (Fig. 3B and 3D), although the average total number of scratching was not significantly different ($p = 0.011$ for Zostrix-HP; $p = 0.040$ for 0.3% DA-5018). Low concentration (0.1%) of DA-5018 pretreatment did not apparently reduce the response within 10-min and total number of scratchings (Fig. 3C). Fig. 4 shows the average number of scratches of each group for 60 min. The calculated scratching indices (SI) for Zostrix-HP, 0.1% and 0.3% DA-5018 were 0.44, 0.64 and 0.50, respectively.

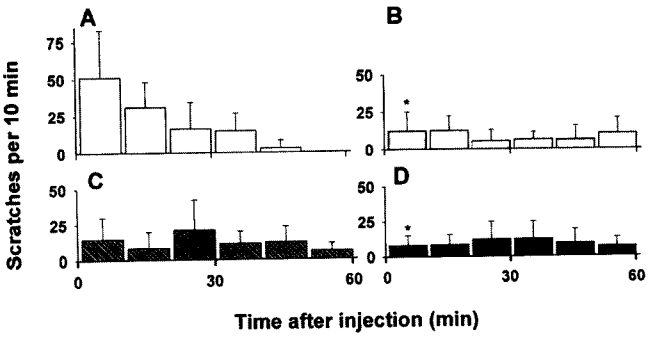

Fig. 3. Inhibitory effect of a capsaicin derivative, DA-5018, on leukotriene B_4 (LTB_4)-induced scratching. The ICR mice were topically pretreated with cream base (A), 0.1% (C) and 0.3% DA-5018 creams(D), or Zostrix-HP (B) twice daily for 4 days. One hour after the last treatment, LTB_4 (0.03 nmol per mouse) was injected i.d. into the rostral back of the mice and the scratching of the skin around the injection site was counted for every 10-min periods. Values are the means and S.E.M. for eight animals. * $P < 0.01$ when compared with cream base control (A)

DISCUSSION

This study was conducted to examine the antipruritic

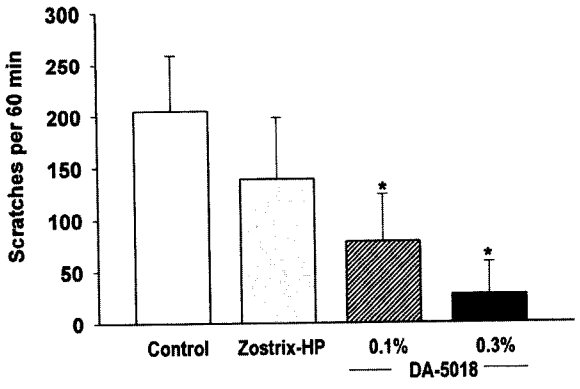

Fig. 2. Inhibitory effect of a capsaicin derivative, DA-5018, on compound 48/80-induced scratching. The ICR mice were topically pretreated with cream base control, 0.1% and 0.3% DA-5018 creams, or Zostrix-HP twice daily for 4 days. One hour after the last treatment, compound 48/80 was injected s.c. into the rostral back of the mice and the scratching of the skin around the injection site was counted for 60 min. Values are the means and S.E.M. for eight animals. * $P < 0.01$ when compared with cream base control

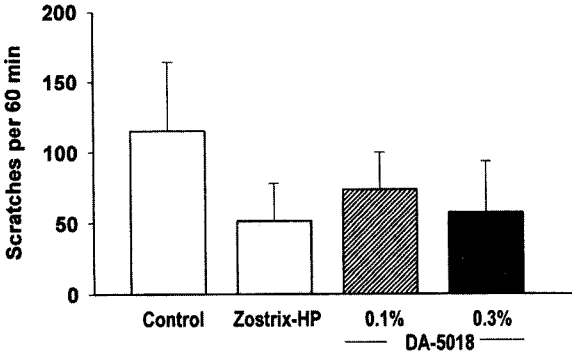

Fig. 4. Inhibitory effect of a capsaicin derivative, DA-5018, on LTB_4-induced scratching. The ICR mice were topically pretreated with cream base control, 0.1% and 0.3% DA-5018 creams, or Zostrix-HP twice daily for 4 days. One hour after the last treatment, LTB_4 (0.03 nmol) was injected i.d. into the rostral back of the mice and the scratching of the skin around the injection site was counted for 60 min. Values are the means and S.E.M. for eight animals

effect of a new capsaicin derivative, DA-5018, in mouse scratching models. DA-5018, under the conditions of our study, significantly inhibited local experimental pruritus induced by compound 48/80 and leukotriene B_4 (LTB_4).

Compound 48/80 itself is pruritogenic (Wahlgren *et al.*, 1990). In mice, compound 48/80 induces scratching (Kuraishi *et al.*, 1995) and acute local edema. As compound 48/80 produces the degranulation of mast cells (Ebertz *et al.*, 1987), itch and edema induced by compound 48/80 are known to be mediated by the compound's histamine-releasing action. In the current study, compound 48/80 produced scratching at the injected site, which almost subsided by 30 min after the injection. Pretreatment with DA-5018 apparently inhibited compound 48/80-induced scratching both in total numbers and response shown within 10 min after the injection. It is interesting that DA-5018-treated mice showed relatively increased numbers of scratching between 30 and 60 min. The absence of scratching in compound 48/80-injected control mice between 30 and 60 min after the injection may be explained by the fatigue of the animals from subsequent scratching within first 30 min or by decreased concentration of compound 48/80 at the injection site by the diluting effect of exudate. In this regard, the local concentration of pruritogen in DA-5018-treated mice may be less diluted during the observation period compared to control because DA-5018 inhibits croton oil-induced ear edema in mice (Kim *et al.*, 1997). However, The present study did not test the anti-inflmmatory action of DA-5018. As DA-5018 inhibited scratching during the first 10-min period, the mice pretreated with DA-5018 showed spontaneous movement and alertness during the second half hr (between 30 and 60 min). However, control and Zostrix-HP-treated mice exhibited calmness and depression during the second half hr.

Intradermal injection of LTB_4 into mice also clearly elicited scratching (Kuraishi *et al.*, 1995). Andoh *et al.* (1998) reported that the dose-response curve of pruritogenic action of LTB_4 was bell-shaped and the most effective dose was 0.03 nmol per site in mice. They also found that LTB_4-induced scratching was inhibited by LTB_4 receptor antagonist, ONO-4057, in a dose-dependent manner, suggesting the mediation of LTB_4 receptors at least in part. However, the exact mechanism of scratch-inducing action of LTB_4 remains to be elucidated. One possible hypothesis is that LTB_4 acts directly on the primary afferent neurons. Itch stimulus is believed to be mediated by C-fibers (Schmelz *et al.*, 1997). LTB_4 also sensitizes cutaneous C-fiber nociceptors of the rat (Martin *et al.*, 1988). Though LTB_4 is well-known as a potent chemoattractant, its action on leukocytes is not thought to be the main cause of scratching, because the onset time of scratching is relatively rapid (within a min in most mice). In the present study, though itching latency of each group was not examined, repeated dermal application of Zostrix-HP or 0.3% DA-5018 cream significantly inhibited LTB_4-induced scratching during the first 10- min period. As in compound 48/80-induced scratching test, the mice pretreated with Zostrix-HP or DA-5018 cream showed a relatively even distribution of scratching throughout the observation period of 60 min while none of control mice showed scratching during the last 10-min period (51 to 60 min after the induction).

Repeated dermal application of DA-5018 cream dose-dependently depletes dermal concentration of substance P (SP) as capsaicin cream does (unpublished data). Andoh *et al.* (1998) reported that SP itself induced scratching in mice and suggested its pruritogenic mechanism is mediated by the direct action on mast cells and cutaneous neurokinin-1. Though it is speculated that DA-5018 exerts antipruritic effect through its action on cutaneous SP, the exact mechanism needs to be elucidated. It should be noted that studies on experimental animals related to itch are not easy to interpret, because their scratching behavior may be stereotypical and non-specific action. Furthermore, there are no flare responses in rodents or cats, and mast cells in rat skin contain little histamine but serotonin, which lacks in human mast cells (Wallengren, 1991). Because of this limitation in animal experiments, clinical studies should be conducted.

Recently itch-specific C-fiber was found (Schmelz *et al.*, 1997). Until recently, because previous research has failed to identify any particular class of primary sensory neurons that would respond preferentially to pruritogenic stimuli, itch has been hypothesized to be induced by the excitation of nociceptors that also mediate pain sensation (von Frey, 1922), or by the activation of polymodal C-fibers (Handwerker, 1992). It is believed that the discovery of itch-specific nerve fiber will facilitate the research related to pruritus and to the development of new antipruritic agents. Although capsaicin cream is clinically useful for the relief of itch from various causes (Bernstein *et al.*, 1982; Bernstein *et al.*, 1986; Goodless *et al.*, 1993; Lotti *et al.*, 1994), the effect of capsaicin on the itch-specific C-receptors is not reported. Therefore, the effects of DA-5018 and capsaicin on these specific nerve fibers should be examined to assess the clinical usefulness of DA-5018 as an antipruritic.

In conclusion, the Present study clearly demonstrate the antipruritic effect of DA-5018 cream in two animal models of itching and warrant clinical evaluation in the prophylaxis or treatment of intractable itch.

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