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Short communication

Involvement of substance P in scratching behaviour in an atopic dermatitis model

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Abstract

Substance P is speculated to be a key mediator of itching in atopic dermatitis, possibly acting via the tachykinin NK_1 receptor. Thus, we examined the effect of a tachykinin NK_1 antagonist, BIIF 1149 CL, on scratching behaviour in a picrylchloride-induced dermatitis model in NC/Nga mice. BIIF 1149 CL ((*S*)-*N*-[2-[3,5-bis(trifluoromethyl) phenyl]ethyl]-4-(cyclopropylmethyl)-*N*-methyl-α-phenyl-1-piperazineacetamide, monohydrochloride, monohydrate) at a dose of 100 mg/kg, p.o., significantly inhibited scratching behaviour immediately after administration, and the effect continued up to 6 h. The results suggest that clinical trials of tachykinin NK_1 antagonists for the treatment of itching in atopic dermatitis patients would be warranted.

Keywords: Dermatitis; Animal model; Substance P; Scratching behaviour; NC/Nga mouse

1. Introduction

Itching is the most common symptom of cutaneous diseases (e.g., urticaria, contact dermatitis and atopic dermatitis) and is induced by several chemical mediators, such as histamine, neuropeptide (substance P, calcitonin gene-related peptide, vasoactive intestinal peptide, enkephalins), chymase, and interleukin 2 in humans (Hägermark, 1992; Staender and Steinhoff, 2002). Scratching behaviour is produced by several endogenous substances such as histamine, substance P, serotonin and leukotrienes in mice (Greaves and Wall, 1996; Yamaguchi et al., 1999; Andoh et al., 2001). There are some differences in the cause of itching between mice and humans, because serotonin or leukotrienes do not cause or induce only very weak itching in humans (Hägermark, 1992). Therefore, the mediators of itching remain to be elucidated (Greaves and Wall, 1996).

It has been shown that non-sedative antihistamines without an antiallergic effect have a little or no value in atopic dermatitis (Wahlgren et al., 1990; Wahlgren, 1991). The limited effects of antihistamines suggest that histamine is not the only mediator involved in pruritus. Substance P is one of the most potent endogenous pruritogenic peptides, and the itching induced by substance P is thought to be mediated by histamine released from mast cells (Hägermark et al., 1978). Furthermore, substance P elicits scratching behavior in mice through a histamine-independent mechanism (Andoh et al., 1998). These effects may be mediated via the tachykinin NK_1 receptor. However, it has not been determined whether a tachykinin NK_1 antagonist can inhibit scratching behaviour in a disease-related model.

There are species differences in the affinity of several tachykinin NK_1 antagonists between mice and humans (Aramori et al., 1994; Pradier et al., 1995). Therefore, screening using human tachykinin NK_1 receptor was performed, and a novel tachykinin NK_1 antagonist, BIIF 1149 CL ((*S*)-*N*-[2-[3,5-bis(trifluoromethyl)phenyl]ethyl]-4-(cyclopropylmethyl)-*N*-methyl-α-phenyl-1-piperazineacetamide, monohydrochloride, monohydrate), was discovered. However, neither the affinity of the drug for mouse tachykinin NK_1 receptors nor the effect on tachykinin NK_1 receptors in vivo has yet been established. Thus, we first examined the effect of

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BIIF 1149 CL on substance P-induced scratching behaviour to clarify its tachykinin NK_1 antagonistic effect. Then, we examined the effect of BIIF 1149 CL on scratching behaviour in an atopic dermatitis model.

2. Materials and methods

2.1. Animal care and use

All experimental procedures were approved by the Animal Care Committee of Nippon Boehringer Ingelheim and were based on the ''Guide for the Care and Use of Laboratory Animals'' of the National Institutes of Health (NIH, Bethesda, MD, USA).

2.2. Substance P-induced scratching behaviour in mice

Twenty-four male ddY mice (SPF) aged 8–9 weeks, weighing 34–43 g, were purchased from Japan SLC, and used for the study. Mice were denied food for 4 h prior to the experiment, but had free access to water. One hour after oral administration of vehicle (distilled water) or BIIF 1149 CL 30 mg/kg, p.o., substance P (150 μg/site) was injected intradermally in a volume of 20 μl into the rostral back (around the interscapular level). Immediately after the administration of substance P, the behaviour of the mice was videotaped using a video camera for 60 min; during this period, no one was allowed in the observation room (quiet condition). From the video images, the number of instances of scratching behaviour in the 60-min period was counted by an investigator who was blind to the treatment given. The mice generally started scratching with the hind paws within about 1 s and groups of movements were counted as one instance of scratching.

2.3. Scratching behaviour in picrylchloride-induced NC/Nga mice

Twelve male NC/Nga mice (SPF) aged 6 weeks, weighing 18–23 g, were purchased from Charles River Japan, and used for the study. The mice were kept in an air-controlled barrier system environment during the experimental period. The fur of the thoracic and abdominal regions of ether-anaesthetized animals was shaved off with a hair clipper 1 week before sensitization. Picrylchloride solution (150 μl) was applied to the thoracic and abdominal areas (sensitization), as well as to the soles of the hind paws. Three days after sensitization, induction was performed by applying 200 μl of picrylchloride solution to the back and to the left and right ears. The induction period was 1 to 15 weeks after the start of weekly induction. At 5 weeks, magnets were implanted in the left and right hind paws under ether anaesthesia so that instances of scratching behaviour could be measured automatically. Nine weeks or later, after the start of induction treatment, the frequency of scratching behaviour was counted both after vehicle (distilled water) treatment and drug treatment every 30 min for 12 h after administration at 20:00. The experiment was performed using a cross-over method. A scratching measurement system (MicroAct (NS-SCT16), Neuroscience, Japan) was used to measure the number of instances of scratching behaviour. Furthermore, the area under the curve (AUC; total number of instances of scratching) was calculated. Mice were denied food for 4 h prior to the experiment, but had free access to water. 2,4,6-Trinitrochlorobenzene (Picrylchloride; lot no. FID01) was provided by Tokyo Kasei Chemical (Tokyo, Japan). BIIF 1149 CL was provided by Boehringer Ingelheim Pharma K.G. (Germany) (Fig. 1). Data are presented as the means ± S.E.M. The significance of differences between vehicle and treated groups was tested by means of unpaired *t*-test (Section 2.2) or paired *t*-test (Section 2.3).

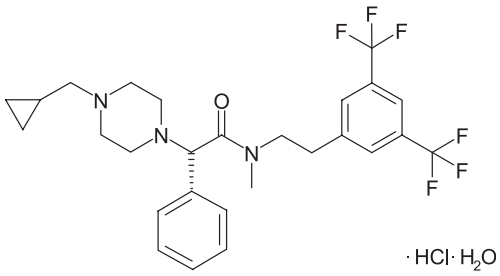

Fig. 1. Chemical structure of BIIF 1149 CL.

3. Results

BIIF 1149 CL at a dose of 30 mg/kg, p.o., significantly reduced substance P-induced scratching behaviour by 50.7 ± 11.2% (Fig. 2A), suggesting that the ED_{50} of BIIF 1149 CL was about 30 mg/kg, p.o. Therefore, we used the doses of 30 and 100 mg/kg, p.o. in this model, in order to clarify the possible role of substance P in scratching behaviour in atopic dermatitis. Scratching behaviour during the night was greater than that during the day (data not shown), presumably because mice are nocturnal, and scratching behaviour may parallel general motility. Thus, we chose to administer the drug or vehicle at 20:00 to examine its anti-scratching action. BIIF 1149 CL (30 and 100 mg/kg, p.o.) significantly inhibited scratching behaviour immediately after treatment, and the effect of 100 mg/kg, p.o., lasted for up to 6 h (Fig. 2B and C).

4. Discussion

First-generation (sedative) antihistamines are effective in reducing itching in patients with atopic dermatitis. However, the therapeutic value of these antihistamines may be principally due to their sedative properties (Wahlgren et al., 1990; Wahlgren, 1991), rather than due to their antihistamine effect on the skin. Furthermore, the application of

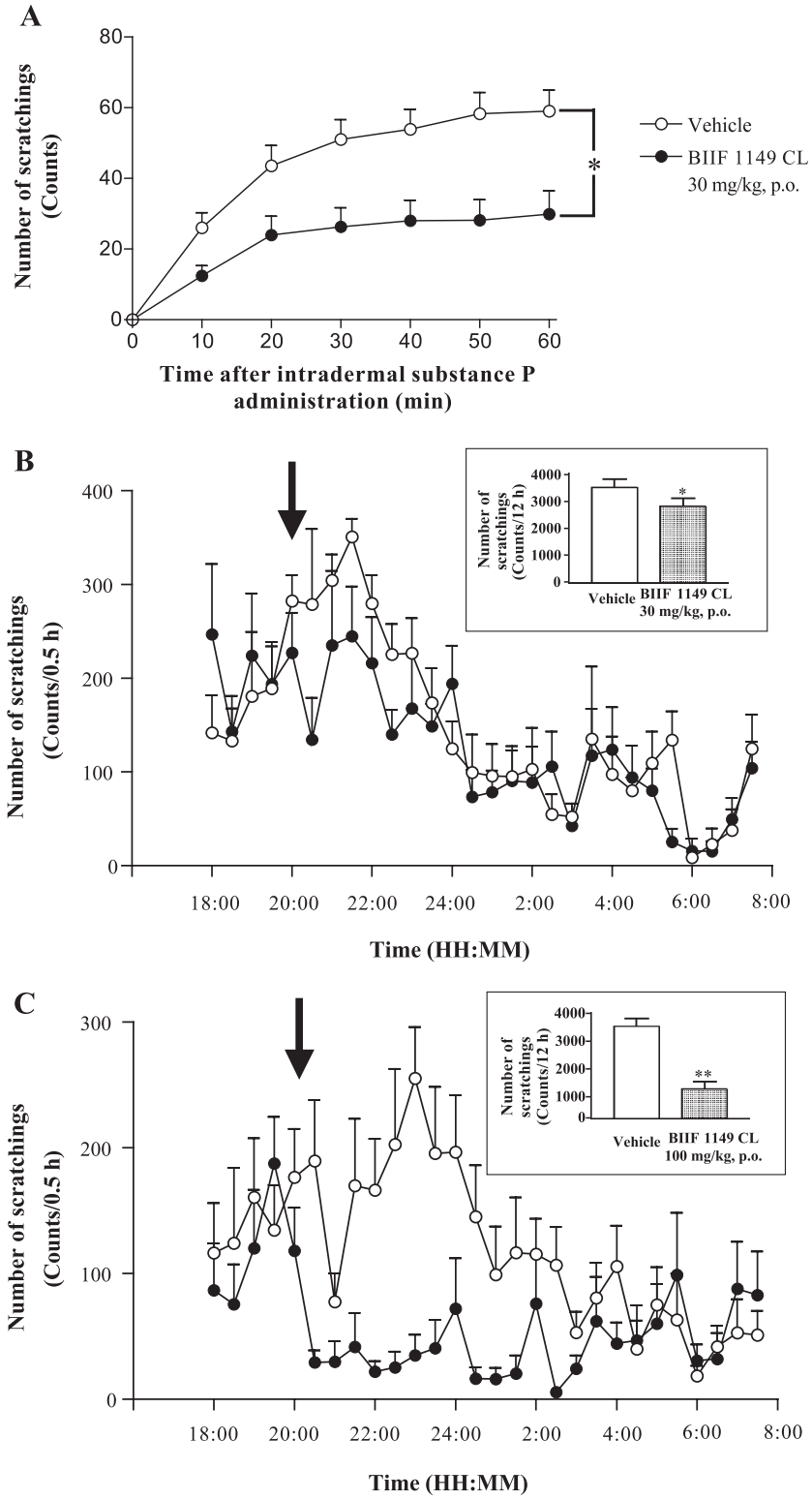

Fig. 2. (A) Effect of BIIF 1149 CL (30 mg/kg, p.o.) on intradermal substance P (150 μg/site)-induced scratching behaviour in ddY mice. The mice were orally administered distilled water (open symbol) or BIIF 1149 CL (closed symbol). Scratching behaviour represents the cumulative value at 0, 10, 20, 30, 40, 50 and 60 min after substance P treatment. Values are shown as means ± S.E.M. for 20 animals. The significance of differences compared with the vehicle group was determined with the unpaired *t*-test. $*P<0.05$. (B, C) Effect of oral administration of distilled water (open symbol), BIIF 1149 CL, 30 and 100 mg/kg (closed symbol), on scratching behaviour in picrylchloride-treated NC/Nga mice. The number of instances of scratching behaviour was determined every 30 min after treatment. Each value represents the mean ± S.E.M. for 11–12 mice. The insets of the graphs show the total number of scratchings (area under the curve, AUC). The significance of differences compared with the vehicle group in AUC was determined with the paired *t*-test; $*P<0.05$, $**P<0.01$. The arrow indicates the administration of drug or the vehicle.

histamine results in a lower itching rate (Heyer et al., 1989) and smaller axon reflex flares in atopic dermatitis (Giannetti and Girolomoni, 1989), suggesting that pruritogens other than histamine are also important. Substance P is one of the most potent endogenous pruritogenic peptides (Andoh et al., 2001; Gibbs et al., 2001). It induces the degranulation of mast cells (Hägermark et al., 1978; Farber et al., 1986), and the itching induced by substance P is thought to be mediated by not only histamine (Hägermark et al., 1978) but also by tumor necrosis factor-α, prostaglandin E_2, etc., released from human mast cells (Gibbs et al., 2001; Okabe et al., 2001). A significant correlation between plasma substance P level and disease activity was found using three different scoring systems for atopic dermatitis (Toyoda et al., 2002). These results and those of our study suggest a role of substance P not only in the skin lesions, but also in the itching of atopic dermatitis. Andoh et al. (1998) reported that substance P caused scratching behaviour in ddY mice, which are insensitive to histamine for causing scratching behaviour, and L-668,169 (cyclo[Gln-D-Trp-(NMe)-Phe(*R*))-Gly(ANC-2)-2-Leu-Met]$_2$: a tachykinin NK_1 antagonist) inhibited this scratching behaviour. It suggests that substance P might be an important mediator in itching. BIIF 1149 CL is a novel tachykinin NK_1 antagonist whose receptor affinity (K_i values) for human tachykinin NK_1 receptors and rat native receptors is 0.12 and 2.1 nM, respectively (personal information). We showed here that BIIF 1149 CL significantly reduced scratching behaviour in mice, presumably by antagonizing the action of substance P (tachykinin NK_1 antagonistic effect). However, there is no report showing that tachykinin NK_1 antagonists inhibit scratching behaviour in atopic dermatitis disease models. In general, picrylchloride-induced dermatitis is used as a contact dermatitis model. However, repeated picrylchloride treatment induces atopic-like dermatitis in BALB/c mice (Nagai et al., 1999) and NC/Nga mice (Taniguchi et al., 2003). In this study, therefore, we used picrylchloride-induced NC/Nga mice as a model to examine whether substance P might be a crucial mediator in scratching behaviour in atopic dermatitis. BIIF 1149 CL at a dose of 100 mg/kg, p.o., inhibited scratching behaviour completely in picrylchloride-treated mice. This is the first study to show that a tachykinin NK_1 antagonist can inhibit scratching behaviour in an atopic dermatitis model, and the results indicate that substance P might be an important pruritogen in atopic dermatitis. Further, tachykinin NK_1 antagonists may have potential for controlling the itching of patients with atopic dermatitis.

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References

Andoh, T., Nagasawa, T., Satoh, M., Kuraishi, Y., 1998. Substance P induction of itch-associated response mediated by cutaneous NK1 tachykinin receptors in mice. J. Pharmacol. Exp. Ther. 286, 1140–1145.

Andoh, T., Katsube, N., Maruyama, M., Kuraishi, Y., 2001. Involvement of leukotriene B(4) in substance P-induced itch-associated response in mice. J. Invest. Dermatol. 117, 1621–1626.

Aramori, I., Morikawa, N., Zenkoh, J., O'Donnell, N., Iwami, M., Kojo, H., Notsu, Y., Okuhara, M., Ono, S., Nakanishi, S., 1994. Subtype- and species-selectivity of a tachykinin receptor antagonist, FK888, for cloned rat and human tachykinin receptors. Eur. J. Pharmacol. 14 (269), 277–281.

Farber, E.M., Nickoloff, B.J., Recht, B., Fraki, J.E., 1986. Stress, symmetry, and psoriasis: possible role of neuropeptides. J. Am. Acad. Dermatol. 14, 305–311.

Giannetti, A., Girolomoni, G., 1989. Skin reactivity to neuropeptides in atopic dermatitis. Br. J. Dermatol. 121, 681–688.

Gibbs, B.F., Wierecky, J., Welker, P., Henz, B.M., Wolff, H.H., Grabbe, J., 2001. Human skin mast cells rapidly release preformed and newly generated TNF-alpha and IL-8 following stimulation with anti-IgE and other secretagogues. Exp. Dermatol. 10, 312–320.

Greaves, M.W., Wall, P.D., 1996. Pathophysiology of itching. Lancet 348, 938–940.

Hägermark, Ö., 1992. Peripheral and central mediators of itch. Skin Pharmacol. 5, 1–8.

Hägermark, Ö., Hökfelt, T., Pernow, B., 1978. Flare and itch induced by substance P in human skin. J. Invest. Dermatol. 71, 233–235.

Heyer, G., Hornstein, O.P., Handwerker, H.O., 1989. Skin reactions and itch sensation induced by epicutaneous histamine application in atopic dermatitis and controls. J. Invest. Dermatol. 93, 492–496.

Nagai, H., Inagaki, N., Tanaka, H., 1999. Role of IgE for the onset of allergic cutaneous response caused by simple chemical hapten in mice. Int. Arch. Allergy Immunol. 118, 285–286.

Okabe, T., Hide, M., Koro, O., Nimi, N., Yamamoto, S., 2001. The release of leukotriene B4 from human skin in response to substance P: evidence for the functional heterogeneity of human skin mast cells among individuals. Clin. Exp. Immunol. 124, 150–156.

Pradier, L., Habert-Ortoli, E., Emile, L., Le Guern, J., Loquet, I., Bock, M.D., Clot, J., Mercken, L., Fardin, V., Garret, C., 1995. Molecular determinants of the species selectivity of neurokinin type 1 receptor antagonists. Mol. Pharmacol. 47, 314–321.

Stander, S., Steinhoff, M., 2002. Pathosphysiology of pruritus in atopic dermatitis: an overview. Exp. Dermatol. 11, 12–24.

Taniguchi, Y., Kohno, K., Inoue, S., Koya-Miyata, S., Okamoto, I., Arai, N., Iwaki, K., Ikeda, M., Kurimoto, M., 2003. Oral administration of royal jelly inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. Int. Immunopharmacol. 3, 1313–1324.

Toyoda, M., Nakamura, M., Makino, T., Hino, T., Kagoura, M., Morohashi, M., 2002. Nerve growth factor and substance P are useful plasma markers of disease activity in atopic dermatitis. Br. J. Dermatol. 147, 71–79.

Wahlgren, C.F., 1991. Itch and atopic dermatitis: clinical and experimental studies. Acta. Derm.-Venereol., Suppl (Stockh) 165, 1–53.

Wahlgren, C.F., Hägermark, Ö., Bergström, R., 1990. The antipruritic effect of a sedative and a non-sedative antihistamine in atopic dermatitis. Br. J. Dermatol. 122, 545–551.

Yamaguchi, T., Nagasawa, T., Satoh, M., Kuraishi, Y., 1999. Itch-associated response induced by intradermal serotonin through 5-HT2 receptors in mice. Neurosci. Res. 35, 77–83.