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Histamine H_4 Receptor Antagonists Ineffective against Itch and Skin Inflammation in Atopic Dermatitis Mouse Model

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TO THE EDITOR

Histamine is the best known pruritogen in humans and the most commonly used experimental itch-causing substance. It induces increased itch responses in the lesional skin of atopic dermatitis (AD) patients compared with normal skin (Ikoma *et al.*, 2006). However, histamine H_1 receptor (H_1R) antagonists frequently fail to relieve the itch in AD patients as well as it does in patients with systemic diseases such as kidney and liver diseases. The lack of amelioration by high-potency H_1R antagonists of different types in patients with itch suggests that other systems are involved (Ikoma *et al.*, 2006; Ständer and Weisshaar, 2012).

Involvement of histamine H_4 receptor (H_4R) in histamine-evoked itch in animal models has been reported (Dunford *et al.*, 2007; Thurmond *et al.*, 2008). However, the therapeutic efficacy of H_4R antagonists on the H_1R antagonist-resistant itch in AD is poorly understood. We therefore examined the therapeutic effects of H_4R antagonists on itch and skin inflammation in AD using NC/Nga mice, a mouse model of AD that has been previously described (Tanaka *et al.*, 2012).

Male NC/Nga mice (Charles River Japan, Yokohama, Japan), 10 weeks old, were maintained in clean condition. All animal procedures were approved by the institutional Animal Care and Use Committee of Juntendo University Graduate School of Medicine. It is generally accepted worldwide that AD patients are highly sensitized to house dust mite allergens (Sanda *et al.*, 1992); and that house dust mite *Dermatophagoides farinae* body (Dfb) and feces are well-known major environmental allergens (Matsuoka *et al.*, 1995). We used a Dfb ointment-induced AD-like mouse model (Dfb-NC/Nga) to evaluate the therapeutic efficacy of H_4R antagonists against itch-related behavior (scratching) and dermatitis in a mouse model of AD.

Dermatitis was induced by application of Dfb ointment (Biostir, Kobe, Japan) twice a week for 3 weeks as described (Yamamoto *et al.*, 2009). Severity of skin lesion was graded according to the criteria as described (Matsuda *et al.*, 1997). Animals that received repeated application of Dfb ointment to their skin (Figure 1a) showed higher dermatitis scores than controls after 3 weeks (data not shown). After the induction, transepidermal water loss was measured using a Tewameter TM210 (Courage and Khazawa, Cologne, Germany) and scratching behavior was observed for 2 hours using a MicroAct (Neuroscience, Tokyo, Japan) as described (Inagaki *et al.*, 2003). Dfb-NC/Nga mice showed significant loss of transepidermal water and more scratching bouts (data not shown).

We examined effects of H_4R antagonists, JNJ7777120 and JNJ28307474, on dermatitis and scratching behavior in Dfb-NC/Nga mice. In mice, antagonist JNJ28307474 shows a longer plasma half-life than JNJ7777120 (Thurmod *et al.*, unpublished observations). Mice that scored over 5 for dermatitis severity were treated by either intraperitoneal injection with a vehicle (20% dimethylsulphoxide and 80% 2-hydroxypropyl-β-cyclodextrin in saline) or H_4R antagonists (10 or 30 mg kg^{-1}) three times per week for 3 weeks (Figure 1a). Dermatitis score was assessed after each Dfb application, and the data were expressed as fold change values over score of dermatitis before H_4R antagonist treatment (baseline) in each group. No significant amelioration of dermatitis followed treatment by either of these H_4R antagonists (Figure 1b and c). In addition, scratching behavior was recorded before and after H_4R antagonist treatment (# shown in Figure 1a). The data were expressed as fold change values over number of scratching bouts before the treatment (baseline) in each group. Behavior analyses revealed neither treatment inhibited scratching behavior (Figure 1d and e). Moreover, the fold change value of scratching bouts significantly increased by treatment of 30 mg kg^{-1} JNJ28307474 (Figure 1e). Treatment with JNJ7777120 or JNJ28307474 had no effect on locomotion activity (Kamo *et al.*, unpublished observations).

In this study, we found that treatment with H_4R antagonist (JNJ7777120 or

Abbreviations: AD, atopic dermatitis;; Dfb, Dermatophagoides farinae body; H_1R, histamine H_1 receptor; H_4R, histamine H_4 receptor

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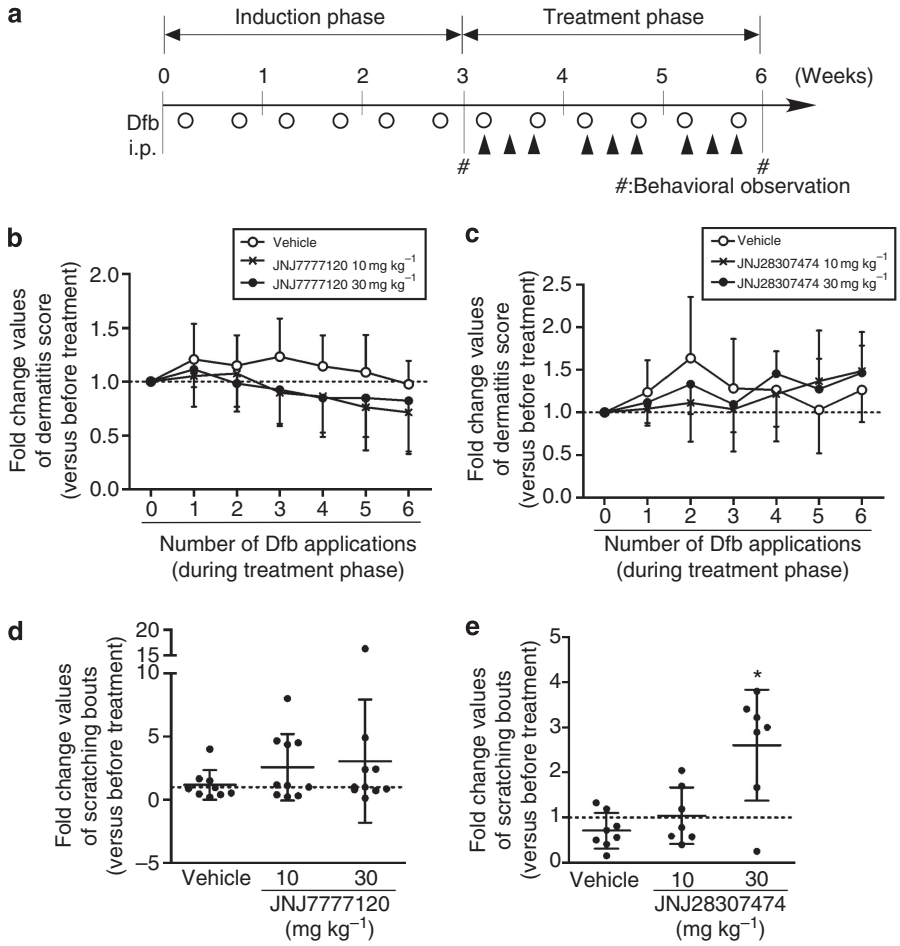

Figure 1. Effects of histamine H_4 receptor (H_4R) antagonists on dermatitis and scratching behavior in *Dermatophagoides farinae* body (Dfb)-NC/Nga mice. (**a**) Following repeated application of Dfb ointment for 3 weeks, vehicle or H_4R antagonist was administered intraperitoneally (i.p.) three times per week for 3 weeks. Scratching behavior was recorded before and after treatment (#). (**b**, **c**) Dfb-NC/Nga mice were intraperitoneally administered 10 and 30 mg kg^{-1} JNJ7777120 or 10 and 30 mg kg^{-1} JNJ28307474. No significant improvement of dermatitis was observed in either JNJ7777120 (**b**) or JNJ28307474 (c). (**d**, **e**) Scratching bouts were not inhibited by either JNJ7777120 (**d**) or JNJ28307474 (**e**). The fold change value of scratching bouts significantly increased by treatment at 30 mg kg^{-1} JNJ28307474 (**e**, $*P<0.001$). Data (means ± standard deviation (SD), $n=7$–10) were compared by one-way analysis of variance and Bonferroni's multiple comparison test.

JNJ28307474) provided no significant inhibition of scratching behavior or amelioration of dermatitis in Dfb-NC/Nga mice. This result is consistent with a recent study in a canine model of AD induced by Df house dust mites (Bäumer *et al.*, 2011). Meanwhile, another study using a model of allergic dermatitis reported that H_4R antagonist JNJ7777120, but not H_1R antagonists, showed both anti-inflammatory and anti-pruritic effects in fluorescein-5-isothiocyanate-induced dermatitis model BALB/c mouse (Cowden *et al.*, 2010). The contact dermatitis model had several features common with AD (Takeshita *et al.*, 2004). Moreover, a more recent study showed that H_1R antagonist olopatadine and H_4R antagonist JNJ7777120 improved scratching behavior and skin inflammation in a model of allergic dermatitis induced by repeated challenges with picryl chloride on the dorsal back of NC/Nga mice (Ohsawa and Hirasawa, 2012). Thus, although treatment with JNJ28307474 at high doses might induce itch because of off-target effects via histamine H_3 receptors or 5-hydroxytryptamine receptors (Cowden *et al.*, 2010), these findings suggest that H_4R antagonists are effective against itch of dermatitis in the hapten-induced model, but not in the Dfb ointment-induced model. In addition, a recent study using microdialysis reported elevated levels of histamine in the induction phase after topical administration of Df allergen, but not high levels in the late reactions (Bäumer *et al.*, 2011). Taken together, these findings imply that histamine may have only a minor role with other mediators or mechanisms responsible for itch and inflammatory reaction in the pathologic process of AD induced by environmental allergens such as a dust mite (Ikoma *et al.*, 2006; Ständer and Weisshaar, 2012; Kabashima, 2013).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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The *BRAF* V600K Mutation Is More Frequent than the *BRAF* V600E Mutation in Melanoma *In Situ* of Lentigo Maligna Type

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TO THE EDITOR

Lentigo maligna melanoma (LM, i.e., *in situ* melanoma) together with its invasive form lentigo maligna melanoma (LMM) is a subtype of malignant melanoma with specific and distinct characteristics compared with the other subtypes of malignant melanoma (superficial spreading melanoma, nodular melanoma, and acral lentiginous melanoma). In contrast to other subtypes, LM predominantly occurs on chronically sun-damaged skin of elderly people and is the most frequent malignant melanoma found in the face (Cohen, 1995). Another characteristic feature of LM is the location of atypical melanocytes along the dermal–epidermal junction, where they are arranged in solitary units or small nests. Because of these features, the proportion of tumor cells within the lesion is usually rather small, rendering molecular characterization challenging.

Mutations in the *BRAF* gene have been shown to be a common, early event in melanoma development (Ko and Fisherm, 2011) and because of recent advances in the treatment of metastatic melanoma, the mutational status of *BRAF* has become therapy decisive (Sosman *et al.*, 2012). So far, only little information is available on the *BRAF* V600E and *BRAF* V600K mutations in LM. Therefore, the objective of this study was to determine the occurrence of mutations in codon 600 of the *BRAF* gene in a large cohort of LM patient samples using a highly sensitive PCR method (Stadelmeyer, 2012).

We analyzed tissue samples from 61 patients (36 women and 25 men; median age, 73.9 years; age ranging from 43.5 to 94.5 years). A total of 59 patients had LM and two patients had a lesion with invasive tumor cells (i.e., LMM). LM-adjacent (normal) skin was available from 39 samples, and DNA was extracted and analyzed for the presence of *BRAF* mutations as described in the Supplementary Information.

Abbreviation: BRAF, v-raf murine sarcoma viral oncogene homolog B1

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