CONFLICT OF INTEREST

The authors state no conflict of interest.

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Fabien Pelletier[1,2,3,4], Francine Garnache-Ottou[1,3,4], Fanny Angelot[1,3,4], Sabeha Biichlé[1,3,4], Chrystelle Vidal[5], Philippe Humbert[1,2,4], Philippe Saas[1,3,4,5], Estelle Seillès[1,3,4,7] and François Aubin[2,6,7]

[1]Université de Franche-Comté, UMR645, IFR133, Besançon Cedex, France; [2]Service de Dermatologie, CHU de Besançon, Besançon, France; [3]Établissement Français du Sang Bourgogne Franche-Comté, UMR645, Plateforme de Biomonitoring, Besançon, France; [4]INSERM, UMR645, Besançon Cedex, France; [5]Centre d'Investigation Clinique CBT506, CHU de Besançon, Besançon, France and [6]Université de Franche Comté, EA3181, IFR133, Besançon, France
E-mail: fabien.pelletier@univ-fcomte.fr
[7]These authors contributed equally to this work.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Antipruritic Effects of TRPV1 Antagonist in Murine Atopic Dermatitis and Itching Models

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TO THE EDITOR

Transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel widely expressed in skin tissues, including keratinocytes and peripheral sensory nerve fibers (C and Aδ). Activated by noxious heat, capsaicin, or endogenous inflammatory mediators, TRPV1 can provoke neuropeptide releases and propagate neurogenic inflammation, which ultimately contributes to the development of diverse dermatoses and pruritus (Hutter *et al.*, 2005; Shim *et al.*, 2007; Imamachi *et al.*, 2009). Recently, we demonstrated that a novel and potent TRPV1 antagonist, PAC-14028 ((E)-N-((R)-1-(3,5-difluoro-4-methanesulfonylamino-phenyl)-ethyl)-3-(2-propyl-6-trifluoromethyl-pyridine-3-yl)-acrylamide) can alleviate atopic dermatitis (AD)-like symptoms through the acceleration of skin barrier recovery (Yun *et al.*, 2011). Of note, we discovered that PAC-14028 could also suppress scratching behavior significantly. Severe itch symptom is a hallmark of AD and at the same time, the representative unmet medical need in diverse skin diseases (Steinhoff *et al.*, 2006). Here, we investigated the antipruritic effects of PAC-14028 and explored the mechanism underlying them to examine the utility of a TRPV1 antagonist as a novel antipruritic therapy.

AD-like symptoms were induced in male NC/Nga mice (8-week old, twice a week for 3 weeks) with the repeated topical application of allergen, *Dermatophagoides farina* (Df) extract, the major species of house dust mites, on the shaved dorsum as previously described (Bae *et al.*, 2010). All animal experiment procedures were approved by the AmorePacific Institutional Animal Care and Use Committee. PAC-14028, which is a potent and selective TRPV1 antagonist as determined by resiniferatoxin-induced Ca^{2+} influx assay in rat TRPV1-expressed CHO cells (Figure 1a) and capsaicin-induced Ca^{2+} influx in dorsal root ganglia

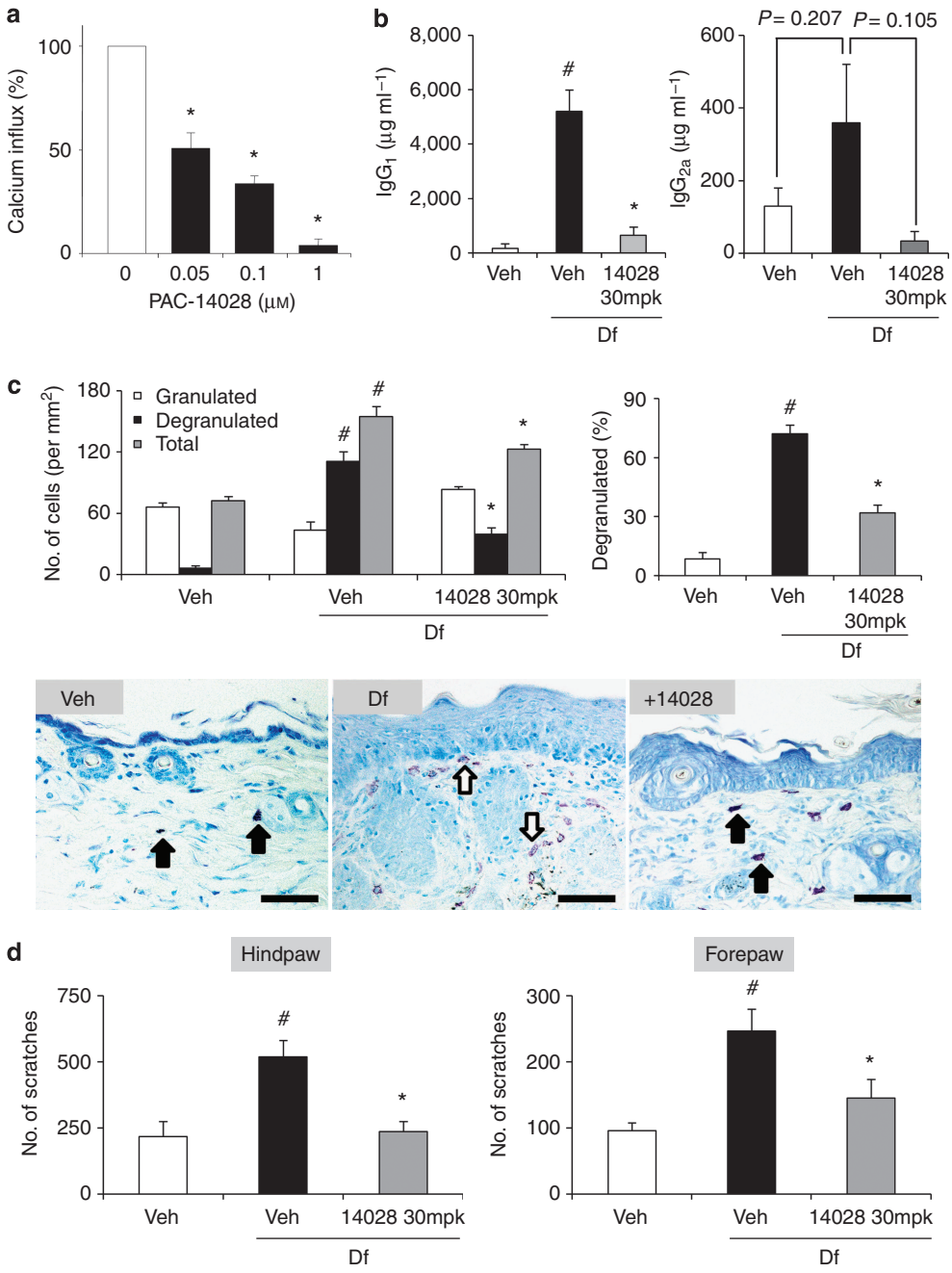

Figure 1. Effects of TRPV1 antagonist, PAC-14028, on Df-induced AD-like symptoms in NC/Nga mice. (**a**) Resiniferatoxin-induced Ca^{2+} influx in rTRPV-expressed CHO cells. (**b**) Serum IgG_1 and IgG_{2a} levels measured with ELISA kits. IgG_{2a} (BD Bioscience, Mississauga, ON, Canada), IgG_1 (Bethyl Laboratories, Montgomery, TX). (**c**) The granulated (black arrows) and degranulated (white arrows) mast cells in the AD-like skin lesion stained with toluidine blue. Scale bar is 50 μm. (**d**) The scratching behaviors in NC/Nga mice. The number of scratches with hindpaws (for 5 hours) and forepaws (for 2 hours) were counted using MicroAct (Neuroscience, Tokyo, Japan) and digital video camcorder, respectively. The data are presented as means ± SE ($N = 5$–6). #Significantly different from vehicle (Veh) control group ($P < 0.05$). *Significantly different from Df group ($P < 0.05$). AD, atopic dermatitis; Df, *Dermatophagoides farina*; PAC-14028, (E)-N-((R)-1-(3,5-difluoro-4-methanesulfonylamino-phenyl)-ethyl)-3-(2-propyl-6-trifluoromethyl-pyridine-3-yl)-acrylamide; TRPV1, transient receptor potential vanilloid type 1; Veh, vehicle control; 14028, PAC-14028.

neurons as reported in our previous study (Yun *et al.*, 2011), was administered daily (30 mg kg^{-1} orally dispersed in 0.5% Tween 80 and 1% methylcellulose). Serum IgG_1, a T_H2 cell-associated immunoglobulin, was significantly increased in the Df group, whereas serum IgG_{2a}, a marker of T_H1 immune response (Chan *et al.*, 2001), was marginally insignificant (Figure 1b), reflecting that T_H2 type dermatose was developed by multiple challenges of Df. Consistently with the development of AD-like symptoms, bouts of scratching increased along with larger number of mast cells in the dermis of Df-applied animals (Figure 1c and d). Importantly, both the proportion and total number

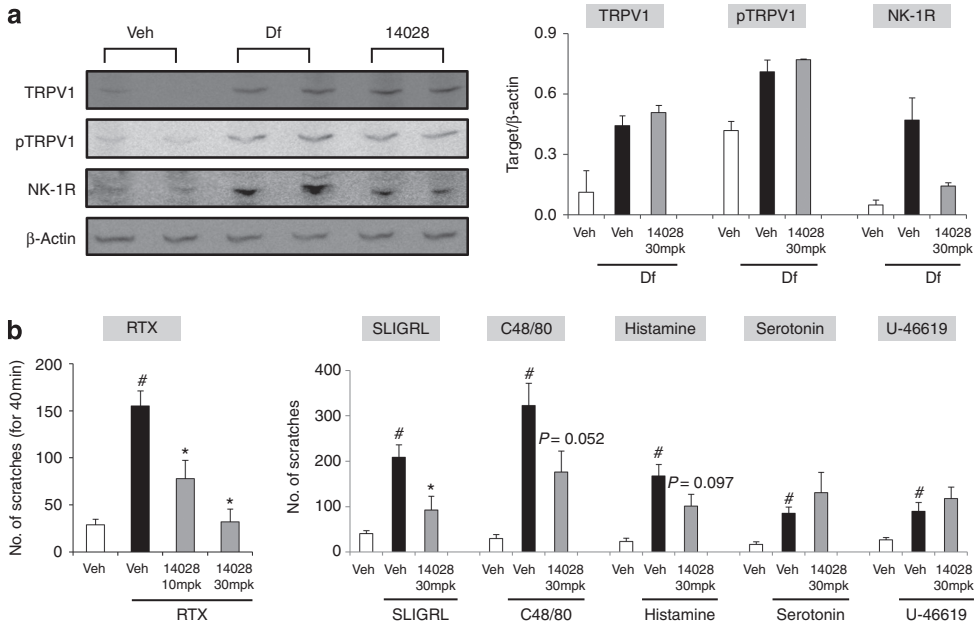

Figure 2. Effects of TRPV1 antagonist, PAC-14028, on the expression of TRPV1, pTRPV1, and NK-1R, and scratching behaviors induced by diverse pruritogens. (**a**) The levels of TRPV1, pTRPV1, and NK-1R in the atopic dermatitis-like skin lesion. (**b**) Scratching behaviors induced by intradermal injection of diverse pruritogens in male ICR mice. PAC-14028 was administered orally at 1 hour before the pruritogen injection. For c48/80, PAC-14028 was pretreated once daily for 3 days. Immediately after the injection of pruritogens, scratching behaviors with hindpaws were quantified for 40 minutes. The data are presented as means ± SE ($N = 4$–10). #Significantly different from vehicle control group ($P < 0.05$). *Significantly different from respective pruritogen group ($P < 0.05$). Histamine, 300 nmol 50 μl^{-1}; serotonin, 300 nmol 50 μl^{-1}; and U-46619, 10 nmol 50 μl^{-1}. c48/80, compound 48/80 (100 μg 50 μl^{-1}); Df, *Dermatophagoides farina*; NK-1R, neurokinin-1 receptor; PAC-14028, (E)-N-((R)-1-(3,5-difluoro-4-methanesulfonylamino-phenyl)-ethyl)-3-(2-propyl-6-trifluoromethyl-pyridine-3-yl)-acrylamide; pTRPV1, phosphorylated transient receptor potential vanilloid type 1; RTX, resiniferatoxin (10 ng 50 μl^{-1}); SLIGRL, SLIGRL-NH_2 (50 μg 50 μl^{-1}); Veh, vehicle control; 14028, PAC-14028.

of degranulated mast cells increased significantly in the Df-treated group. Mast cell degranulation leads to the release of granule-associated pruritogenic mediators, including histamine, serotonin, and protease-activated receptor-2 (PAR-2) activating tryptase into skin tissue, initiating the itch symptoms (Caughey, 2007; Shim *et al.*, 2007; Costa *et al.*, 2008; Imamachi *et al.*, 2009). Notably, the treatment of PAC-14028 significantly attenuated the degranulation of mast cells and scratching behaviors along with reduced IgG_{2a}, indicating that PAC-14028 can suppress AD-associated pruritus and prevent the development of T_H2 type dermatoses.

TRPV1 is upregulated in the AD-skin lesion, and the activation of TRPV1 causes the release of proinflammatory and pruritic mediators (Steinhoff *et al.*, 2004; Hutter *et al.*, 2005; Imamachi *et al.*, 2009). Therefore, we sought to determine the expression and/or phosphorylation of TRPV1 in AD-like skin lesions *in vivo*. Both TRPV1 and phosphorylated TRPV1 increased in the AD-like skin lesions of the Df-treated groups (Figure 2a), reflecting that the activity and functionality of TRPV1 may substantially increase in AD. The activation of TRPV1 leads to the release of substance P and subsequently upregulates neurokinin-1 receptor (Velazquez *et al.*, 2002; Hutter *et al.*, 2005). In addition to TRPV1 and phosphorylated TRPV1, neurokinin-1 receptor expression increased substantially in the AD-like lesion (Figure 2a), indicating that TRPV1 was activated and substance P was released in AD-like lesion. In contrast, although PAC-14028 treatment did not affect TRPV1 expression and phosphorylation, it significantly reduced the expression of neurokinin-1 receptor, reflecting that PAC-14028 suppressed the release of substance P through the blockade of TRPV1 activation. Substance P can contribute to the development of neurogenic inflammation and pruritus, and neurokinin-1 receptor antagonists could suppress scratching behaviors (Ohmura *et al.*, 2004; Yamaoka and Kawana, 2007), suggesting that antipruritic effect of TRPV1 antagonist in AD may be explained, at least in part, by the suppression of substance P release.

To further explore the mechanism underlying the antipruritic effect of PAC-14028, the effects on the scratching responses evoked by diverse pruritogens were examined. The scratching behaviors induced by resiniferatoxin, a TRPV1 agonist, were significantly inhibited by PAC-14028 treatment in a dose-dependent manner, confirming the efficacy of PAC-14028 against TRPV1-mediated pruritus. Interestingly, the scratching bouts evoked by PAR-2-activating peptide, SLIGRL-NH_2 (Shimada *et al.*, 2006), were also significantly attenuated by PAC-14028 (Figure 2b). This antipruritic effect of PAC-14028 on PAR-2-mediated pruritus might support the close relationship between PAR-2 activation and TRPV1 sensitization, which has been addressed by several studies in diverse inflammation, pain states, and especially pruritus (Costa *et al.*, 2008). Meanwhile, the scratching behaviors induced by mast cell degranulator, compound 48/80, and the representative

granule-associated pruritogenic mediator from mast cells, histamine, were markedly inhibited by PAC-14028, whereas those by serotonin or thromboxane analog, U-46619, were not affected. Partial attenuation of histamine-mediated itch response might reflect the partial agonistic effect of histamine on TRPV1 activation (Shim *et al.*, 2007), and the lack of antipruritic efficacy on the scratching behaviors evoked by serotonin or U-46619 was anticipated as these pruritogens work through G-protein-coupled receptors, including 5-HT receptors and TP receptor (Andoh *et al.*, 2007; Imamachi *et al.*, 2009), that have little cross talk with TRPV1-mediated pathways. Partial inhibition of PAC-14028 on compound 48/80-evoked itch may be explained by the presence of TRPV1-independent itch signals from serotonin or other pruritogenic mediators in mast cell granules, despite histamine- and tryptase-mediated (PAR-2 mediated) itch responses could be suppressed by TRPV1 blockade.

In conclusion, we found that TRPV1 antagonist, PAC-14028, can suppress scratching behaviors associated with AD-like symptoms through the inhibition of TRPV1 activation, of which expression and phosphorylation increase in AD-like skin lesion. Particularly, experiments with diverse pruritogens revealed that PAC-14028 could manifest antipruritic effects on TRPV1-, PAR-2-, and histamine-mediated scratching behaviors. Considering the frequent failure of conventional antipruritic therapy (i.e., antihistamines) in the management of severe itch symptoms, we believe that TRPV1 antagonists can be a novel antipruritic therapy that might satisfy the unmet medical need.

CONFLICT OF INTEREST

PAC-14028 is the patented compound of Amore Pacific Corporation (Patent PCT/KR07/003592: novel compounds, isomer thereof, or pharmaceutically acceptable salts thereof as vanilloid receptor antagonist, and pharmaceutical compositions containing the same).

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Jun-Won Yun[1], Jung A Seo[1], Won-Hee Jang[1], Hyun Ju Koh[1], Il-Hong Bae[1], Young-Ho Park[1] and Kyung-Min Lim[1]

[1]AmorePacific Corporation R&D Center, Medical Beauty Research Institute, Gyeonggi-do, Republic of Korea
E-mail: kimlim@amorepacific.com

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Global Analysis of BRAF^{V600E} Target Genes in Human Melanocytes Identifies Matrix Metalloproteinase-1 as a Critical Mediator of Melanoma Growth

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TO THE EDITOR

BRAF kinase has been found to be mutationally activated in up to 70% of benign nevi and melanomas (Davies *et al.*, 2002). It has been implicated as a critical mediator of melanoma development, with the V600E-activating mutation representing the most commonly mutated form of BRAF in nevi and melanomas (Pollock *et al.*, 2003). Despite strong evidence implicating BRAF kinase as a bona-fide oncogene

Abbreviations: EGFR, epidermal growth factor receptor; HPM, human primary melanocyte; MMP-1, matrix metalloproteinase-1; siRNA, small interfering RNA