SHORT COMMUNICATION

Papain as a Potential New Experimental Model of Non-histaminergic Itch

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Chronic itch is a major symptom in several disorders, such as neuropathic conditions, systemic diseases and inflammatory dermatological disorders (1). Antihistamines are often unsuccessful to treat chronic itch (2), which confirms a prevalence of non-histaminergic itch in pathological itch conditions. In the research field, histamine and cowhage are the 2 most used models to induce itch. They differ in the stimulation of specific thin primary afferents. Histamine activates mechano-insensitive C-fibres (CMi-fibres) expressing histamine-receptor 1 (H1R) and transient receptor potential vanilloid 1 (TRPV1) on their surface (3), while cowhage activates protease-activated receptors 2-4 (PAR2-4) expressed on polymodal c-fibres (PmC fibres) (4). Moreover, they also differ in the cutaneous reactions induced after application (5). In fact, the induction of flare and wheal reactions is characteristic only of mechano-insensitive C-fibres activation (6). Even though cowhage has become the "gold standard" pruritogen in studies involving histamine-independent itch, it is associated with several limitations: inaccessibility on the commercial market, inter- and intra-batch variations in potency, and challenges to standardizing the amount of spicules delivered to the skin (2). These considerations emphasize the need for a more standardized non-histaminergic experimental itch model. Papain, a cysteine protease from the raw fruit of the papaya plant, is known to produce rapid itch in the absence of wheal and flare, by binding human proteinase-activated receptors 2 and 4 (PAR-2/4), similar to the action of mucunain, the active component of cowhage (7). Studies strongly imply that PARs may be involved in pathological itch disorders (8).

The aim of the current study was to develop a potential, non-histaminergic itch model based on papain. For this purpose, different concentrations of papain solution (10, 50, and 100 µg of papain in 20 µl distilled water) introduced by skin prick test (SPT) lancets, repeated pricks through SPT (1, 5 and 25 SPT pricks), and inactivated cowhage spicules soaked in papain solution (5 mg/ml) were used. Itch and pain induced by papain were evaluated, followed by the measurement of superficial blood perfusion and mechanical and thermal sensitivities.

METHODS (see Appendix S1)

RESULTS

Nineteen participants finished the study successfully without reporting any adverse reactions during or after the termination of the study. Subject number 20 was removed and considered an outlier, since her values for cold and warm detection thresholds were outside the reference values (9).

Papain-soaked inactivated cowhage spicules, compared with vehicle and all the other conditions, caused significantly increased peak itch intensity ($\chi^2(7)=59.028$; $p<0.001$) (**Fig. 1**A) and area under the curve (AUC) itch (χ^2 $(7)=59.096$; $p<0.001$) (Fig. 1B). A visual inspection of the temporal profile of itch (Fig. 1C) found that papain-soaked spicules evoked robust itch. In all the other conditions (different concentrations and number of SPT) no differences in peak itch intensities and AUC itch were present compared with vehicle (Fig. 1A–B).

A main effect of conditions of peak pain intensities ($\chi^2(7)=21.774$; $p<0.01$) and AUC of pain ($\chi^2(7)=22.794$; $p<0.01$) was found (Fig. S1A–B). However, *post hoc* analysis could not reveal the exact differences. From a visual inspection of the temporal profile of pain, 25 pricks induced a rapidly occurring pain, peaking at 2 min (Fig. S1C).

Concerning the superficial blood perfusion (SBP), an overall main effect of condition for the mean SBP was found ($F_{3,55}=3.118$; $p<0.05$) although the exact difference was not detected after a *post hoc* comparison (Fig. 1D). The peak of SBP was statistically higher in the area of 25 pricks compared with vehicle ($F_{7,126}=9.236$, $p<0.01$), 10 µg, 50 µg, 100 µg papain, and 1 prick ($p<0.001$) and in the area of 5 pricks compared with 50 µg papain ($p<0.05$; Fig. 1E).

No differences were present in any of the conditions for touch pleasantness ($p=0.676$, Fig S2A). In the mechanically evoked itch, a difference between the spicules area and the area with 5 pricks was found ($F_{7,126}=2.127$; $p<0.05$; Fig. S2B). Moreover, no differences were present for the mechanical pain threshold ($p=0.053$; Fig. S2C), while the mechanical pain sensitivity in the area with 50 µg papain was significantly higher than in the area with 1 prick ($F_{4,65}=7.144$, $p<0.05$), and in the area with 100 µg papain compared with the 1, 5, and 25 pricks areas, and cowhage area ($p<0.001$; Fig. S2D).

No significant main effect was present for either cold detection threshold ($p=0.386$; Fig. S2E) or warm detec-

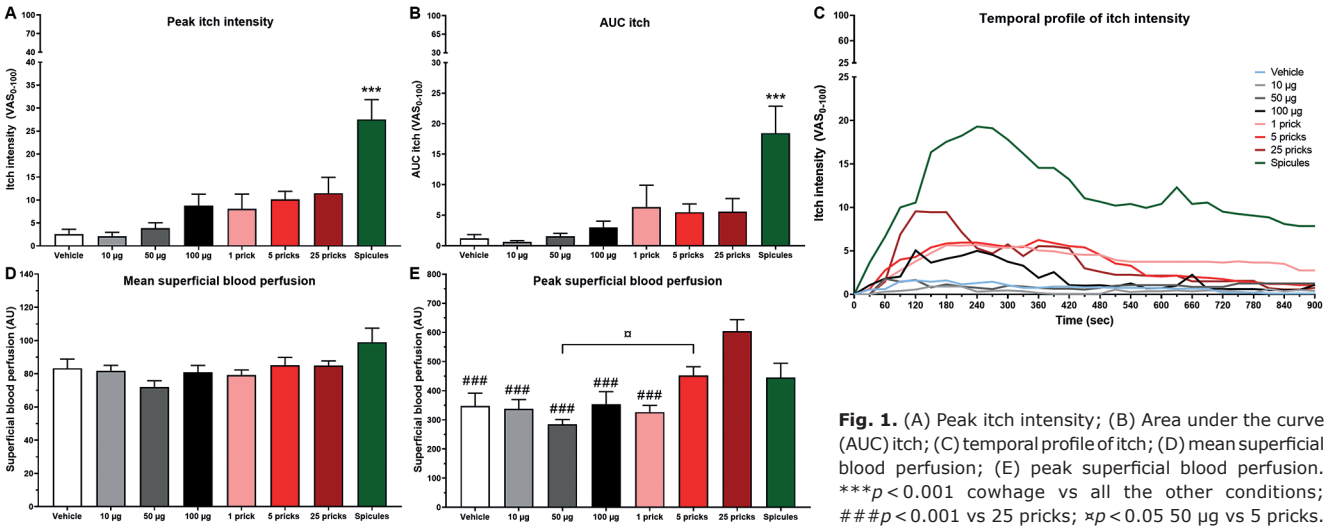

Fig. 1. (A) Peak itch intensity; (B) Area under the curve (AUC) itch; (C) temporal profile of itch; (D) mean superficial blood perfusion; (E) peak superficial blood perfusion. ***$p < 0.001$ cowhage vs all the other conditions; ###$p < 0.001$ vs 25 pricks; ¤$p < 0.05$ 50 µg vs 5 pricks.

tion threshold ($p = 0.787$; Fig. S2F). Cold pain threshold in the area of 5 pricks was significantly lower compared with 100 µg papain ($\chi^2(7) = 32.438$; $p < 0.001$; Fig. S2G). An overall significant main effect of condition was present for heat pain threshold ($F4,64 = 4.902$; $p < 0.01$; Fig. S2H) and supra-threshold heat sensitivity ($\chi^2(7) = 21.577$; $p < 0.001$; Fig. S2I). *Post hoc* analysis was unsuccessful to detect the exact differences.

DISCUSSION

These results demonstrate that papain is an effective pruritogen when applied through inactivated spicules. Papain-soaked spicules may have been more effective than SPT with papain, regardless of the number of pricks, to activate PmC-fibres due to their skin localization. In fact, spicules only breach the keratinous layer of the skin since PmC-fibres transmitting non-histaminergic itch reside more superficially than CMi-fibres transmitting histaminergic itch, most likely activated through SPT (5, 10). Moreover, papain did not induce pain at any concentration or number of performed SPT, except for 25 pricks. However, this is more likely due to the penetrational damage to the skin rather than activation of PAR receptors.

The absence or weak presence of a flare reaction excludes the activation of histamine-sensitive CMi-fibres that branch closely with dermal capillaries to cause neurogenic inflammation around the application area (11). The superficial microtrauma caused by the repetitive SPT, and not the direct activation of CMi-fibres, could be the direct cause of the increased superficial perfusion present in the 25 pricks area. Thus, it represents indirect evidence of the stimulation of PmC-fibres. That papain activates a non-histaminergic pathway remains to be reproduced during antihistamine pretreatment.

Aδ-fibres are considered to be the most suitable mediators of hyperknesis, defined as an increased itch response provoked by a normally itching stimulus, while mechanical sensitivity is mostly mediated by the PmC-fibres (12). However, the lack of significant hyperknesis and difference in mechanical sensitivity induced by papain is probably related to its modest itch-inducing ability. Data regarding thermal sensory tests showed that papain did not alter cold or heat sensitivities. This is probably related to a limited expression of PAR-2/-4 receptors and the lack of affinity of papain to TRP receptors, in particular TRPV1- and TRPM8-expressing fibres, responsible for heat and cold detection, respectively (13).

Regardless of the results, this study presents few limitations: (*i*) the risk of pricking the same spot more than once during the repeated pricks. However, the SPT instrument was weight-controlled making it a highly standardized method. (*ii*) The unstandardized amount of cowhage spicules inserted into the skin. Nonetheless, this method presents the advantage, with respect to cowhage, of standardizing the amount of substance delivered by each spicule. (*iii*) This study was conducted only in a small sample including 19 subjects over a short period of time. Nevertheless, regarding itch perception, all the subjects in this study reported itch after the application of papain through inactivated spicules, and only 3 of them reported mild pain. After the 25 SPT, the majority of the subjects reported pain, often accompanied by mild pruritus. Based on these considerations, we expect that, even with a bigger sample size, our conclusions about spicules soaked in papain induced itch and 25 SPT induced pain will not change. It is possible to speculate that by increasing the papain concentration applied by 25 SPT, a higher itch intensity could be perceived by the subjects.

In conclusion, this study showed that papain could be a potential non-histaminergic itch model that induces modest pruritus. Further studies are needed to explore the full potential of papain, in particular by increasing the concentration used.

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The authors have no conflicts of interest to declare.

REFERENCES

1. Ständer S, Weisshaar E, Mettang T, Szepietowski JC, Carstens E, Ikoma A, et al. Clinical classification of itch: a position paper of the International Forum for the Study of Itch. Acta Derm Venereol 2007; 87: 291–294.
2. Andersen HH, Elberling J, Arendt-Nielsen L. Human surrogate models of histaminergic and non-histaminergic itch. Acta Derm Venereol 2015; 95: 771–779.
3. Imamachi N, Park GH, Lee H, Anderson DJ, Simon MI, Basbaum AI, et al. TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. Proc Natl Acad Sci U S A 2009; 106: 11330–11335.
4. Lee SE, Jeong SK, Lee SH. Protease and protease-activated receptor-2 signaling in the pathogenesis of atopic dermatitis. Yonsei Med J 2010; 51: 808–822.
5. Johanek LM, Meyer RA, Hartke T, Hobelmann JG, Maine DN, LaMotte RH, et al. Psychophysical and physiological evidence for parallel afferent pathways mediating the sensation of itch. J Neurosci 2007; 27: 7490–7497.
6. Weisshaar E, Kucenic MJ, Fleischer AB. Pruritus: a review. Acta Derm Venereol 2003; Suppl 213: 5–33.
7. Reddy VB, Lerner EA. Plant cysteine proteases that evoke itch activate protease-activated receptors. Br J Dermatol 2010; 163: 532–535.
8. Steinhoff M, Neisius U, Ikoma A, Fartasch M, Heyer G, Skov PS, et al. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. J Neurosci 2003; 23: 6176–6180.
9. Harju EL. Cold and warmth perception mapped for age, gender, and body area. Somatosens Mot Res 2002; 19: 61–75.
10. Wooten M, Weng HJ, Hartke T v, Borzan J, Klein AH, Turnquist B, et al. Three functionally distinct classes of C-fibre nociceptors in primates. Nat Commun 2014; 5: 4122.
11. Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjörk HE. Specific C-receptors for itch in human skin. J Neurosci 1997; 17: 8003–8008.
12. Andersen HH, Akiyama T, Nattkemper LA, van Laarhoven A, Elberling J, Yosipovitch G, et al. Alloknesis and hyperknesis – mechanisms, assessment methodology, and clinical implications of itch sensitization. Pain 2018; 159: 1185–1197.
13. Dai Y. TRPs and pain. Semin Immunopathol 2015; 38: 277–291.
14. Simone DA, Alreja M, Lamotte RH. Psychophysical studies of the itch sensation and itchy skin ("alloknesis") produced by intracutaneous injection of histamine. Somatosens Mot Res 1991; 8: 271–279.