

ORIGINAL ARTICLE

Punctate Administration of Ficin as a human and animal model of non-histaminergic itch

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Abstract

Background Ficin, a cysteine protease derived from fig-tree latex, has been reported to elicit itch and nociceptive sensations, though quantitative sensory studies are lacking. Cowhage containing the pruritic cysteine Mucunain, on the contrary, has been widely studied as activating polymodal nociceptors and eliciting a histamine-independent itch.

Objectives We tested whether ficin in heat-inactivated cowhage spicules would elicit itch and nociceptive sensations in humans, and analogous behaviours in mice, which are similar to those evoked by native cowhage, and whether these behaviours in mice were dose-dependent when ficin was injected intradermally.

Methods Human volunteers rated the magnitude of itch and nociceptive sensations evoked by either native cowhage spicules or heat-inactivated spicules soaked in 1, 10 or 100 mg/mL ficin (0.03, 0.3 and 3 ng of ficin in spicule tip), applied to forearm. In mice, itch-like scratching and nociceptive-like wiping were recorded in response to either native cowhage, to heat-inactivated spicules that were either inactive or contained 100 mg/mL ficin, or to intradermal injections of 1.25, 2.5 or 5 µg/5 µL, each treatment applied to the cheek.

Results The dose of 100 mg/mL ficin in spicules evoked comparable magnitudes of itch, nociceptive sensations and areas of cutaneous dysesthesia as native cowhage in humans and comparable itch-like scratching and pain-like wiping behaviours in mice. But to elicit similar behaviours when injected intradermally in mice a greater amount of ficin (1.25 µg) was required.

Conclusion Spicule delivery or intradermal injection of ficin elicits behaviours in mice that model itch and nociceptive sensations in humans, suggesting that ficin may be useful in translating mechanistic research on the neural mechanisms of pruritic and nociceptive effects of cysteine proteases between the two species.

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Conflicts of Interest

None to declare.

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Introduction

Cysteine proteases applied to the skin can activate pruriceptive nociceptors and elicit a sensation of itch in humans and itch-like

scratching in mice.^{1–3} Here, we explored the use of ficin, a cysteine protease from the latex of a fig tree, as a pruritogen in an experimental model of itch and nociceptive sensations in both species. Ficin belongs to the class of ‘papain-like’ cysteine proteases such as papain from papayas, bromelain from pineapples,

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mucunain from the pruritic hairs (spicules) of cowhage and Der p1 from house dust mites.^{4,5} The same class includes some endogenous cysteine proteases including Cathepsin S, expressed in the dermis,⁶ and associated with clinically significant itch.⁷

When the tips of cowhage spicules are superficially inserted into the skin in humans, the mucunain produces a histamine-independent itch without a weal and typically with no neurogenic flare.^{8–11} Itch is accompanied by nociceptive sensations of pricking/stinging and burning. Analogous itch- and pain-like sensory behaviours were observed in mice in response to a cowhage spicule applied to the cheek.¹² In humans, cowhage-evoked itch was also bound to elicit cutaneous dysesthesias in the surrounding skin. These dysesthesias are characterized by itch to touch (alloknesis) and enhanced pricking-evoked itch and pain (hyperknesis and hyperalgesia).⁹

Heat-inactivated cowhage spicules that were soaked in a solution of recombinant mucunain or Cathepsin S, dried and applied to the skin elicited the same itch and nociceptive sensations as cowhage in humans.^{1,13} Early anecdotal reports that showed other cysteine proteases, including ficin and papain, also elicit itch in humans.¹⁴ But the effects of different concentrations of soaking solution have not been compared in humans for their effectiveness in eliciting itch, nociceptive sensations and cutaneous dysesthesias. Nor have different doses of ficin been compared for their sensory effects with native cowhage in humans or in mice.

Here, we compared sensory behavioural responses of humans and mice to ficin in heat-inactivated spicules. We compared itch and nociceptive sensations evoked by different doses of ficin with sensations evoked by native cowhage. We then measured analogous itch and nociceptive site-directed behaviours of mice in response to cowhage spicules, ficin in spicules and to ficin injected intradermally. A major outcome of this study is that the delivery of ficin by heat-inactivated spicules or by intradermal injection provides an effective behavioural model for producing sensory behaviours in mice that are analogous to those obtained by spicule application in humans. Analogous behaviours in humans and mice are useful for translating research findings between the two species on the role of cysteine proteases in eliciting non-histaminergic itch and pain.

Methods

Psychophysical studies in humans

Participants Nine females and 10 males were tested using protocols by the Institutional Review Board of Yale University School of Medicine. The exclusion criteria were a history of allergies, dermatological or neurological conditions, or recent administration of antihistamines, corticosteroids or analgesic

drugs. Prior to any experiments, each subject was provided with description of the procedures and signed the informed consent.

Pruritogens Ficin was obtained as a lyophilized powder (1.2 U/mg, product F6008) from Sigma-Aldrich (St. Louis, MO). Spicules were obtained from cowhage (*Mucuna Pruriens*) from India.¹⁵ Chemically inactive spicules were produced by autoclaving native cowhage spicules to inactivate the pruritic protease, mucunain.¹ The inactive spicules were soaked in concentrations of 1, 10 or 100 mg/mL of ficin in saline. A specially designed spicule inserter⁹ was used to insert the tips of a row of 10–12 spicules about 0.2 mm into the skin.¹⁶ After testing, the number of spicules inserted into the skin was counted under a dissecting microscope. Based on the length and volume of a spicule tip,¹⁶ the amounts of ficin for the concentrations of 1, 10 and 100 mg/mL were estimated to be 0.03, 0.3 and 3 ng, respectively. Subjects were also tested using inserters containing native cowhage and tested with inactive cowhage spicules.⁹ The order of delivery of each type of spicule was randomized and blinded.

Quality and perceived intensity of pruritogen-evoked sensations At 30 s intervals, subjects used a computer mouse to rate the perceived intensity of itch, pricking/stinging, and burning by positioning a cursor along a labelled magnitude scale^{17,18} presented on a computer-screen via DAPSYS 8 software (<http://www.dapsys.net/>). The scale consisted of a vertical line with labels of 'no sensation', 'barely detectable', 'weak', 'moderate', 'strong', 'very strong' and 'strongest imaginable sensation' positioned in a quasi-logarithmic manner. Ratings were recorded for 20 min or until there were no sensations for three consecutive 30 s periods.⁹

From ratings of each sensory quality we obtained: 1) peak magnitude, 2) duration of sensation defined as the time from the onset of sensation to the first of the three consecutive ratings of zero or until the end of the 20 min and 3) area under the curve (AUC). For AUC and duration, only data for subjects with peak ratings of greater than zero in response to each treatment were used.

Cutaneous dysesthesias After each session, mechanical stimuli were delivered along 8 radial lines that intersected at the site of spicule application to determine the borders of dysesthesias.^{8,9,18} Dysesthesias include: 1) aloknesis, defined as itch elicited by a gentle stroke with a cotton swab; 2) hyperknesis, enhanced pricking-evoked itch elicited by an indentation of a Von-Frey filament with a bending force of 20 mN and 50- μ m tip-diameter and 3) hyperalgesia, greater than normal pricking pain elicited by an indentation of a Von-Frey filament with a 200- μ m tip and delivering 80 mN. The areas of dysesthesia were mapped on the skin and measured using ImageJ software (V1.51j8, NIH,

Bethesda, Maryland). A minimal area of 2 cm² was required for inclusion in analyses.

Behavioural studies in mice

Animals C57BL/6 mice 6–8 weeks old were obtained from Charles River Laboratories (Wilmington, MA). Males weighed 20–25 g, and females 15–20 g. During brief anaesthesia for a maximum of 10 min with isoflurane (2% in 100% oxygen), each cheek was shaved two days before an experiment. Each mouse was habituated once daily for five days to the experimental environment. Each mouse was placed in a separate, clear, plastic container, with four angled mirrors to enable a five-sided view. Each mouse's behaviour was recorded by a camcorder positioned above the container inside a soundproof room consistent with previous settings.^{19,20} Experimental procedures were approved by Yale Institutional Animal Care.

Pruritogens Three groups of 11–12 male mice received spicules applied to the skin of one cheek in the manner described for humans. Each group received either 1) inactive, autoclaved spicules, serving as a control, 2) spicules soaked in 100 mg/mL ficin (maximal dose applied in our human testing or 3) native cowhage spicules. Placement and number of spicules inserted were counted immediately.

Another 26 mice, 12 males and 14 females were each intradermally injected with ficin in ascending doses of 0 (saline vehicle/control), 1.25, 2.5 and 5 µg in 5 µL saline every 24 h, alternating cheeks. After several days, the male mice received a dose of ficin 10 µg/5 µL. These male mice were also tested with a dose of 2.5 µg/5 µL of ficin combined with 50 µM of E-64, a highly selective cysteine protease inhibitor.^{21,22}

Behavioural measurements After spicule application or injection, we measured two site-directed behaviours: (i) the number of itch-like scratching bouts with the hind paw, and (ii) the number of pain-like wipes with the forepaw as described.²⁰ No mice were excluded from the study after testing. The experimenter was blinded to the experimental conditions.

Statistical analyses

The data for males and females were combined due to considerable overlap and small sample size. For logarithmic transformations of data, a value of 1.0 was first added to each score (magnitude ratings of humans and behavioural responses of mice) to avoid the log of a score of zero. D'Agostino-Pearson omnibus was used for distribution and normality. Because the assumptions required for statistical comparisons using parametric tests were not met, nonparametric analyses were used in this study. For repeated measures, Friedman tests were used with post hoc Dunn's multiple comparisons to test the differences in effects of each dose. Wilcoxon signed-rank tests were used to

compare the effects of two different treatments. For unpaired analyses, data were tested with the Mann–Whitney U test for two samples and, for more than two, with the Kruskal–Wallis test followed by Dunn's multiple comparisons.

GraphPad Prism 8 software (San Diego, CA) was used. $P < 0.05$ was considered significant.

Results

Psychophysical tests in humans

The time course of magnitude ratings of sensations evoked by spicules of ficin and cowhage The temporal profiles (Fig. 1) of the perceived intensity of itch and the nociceptive sensations of pricking/stinging and burning were similar for different doses of ficin and for cowhage. As in prior studies using spicules of cowhage or another pruritogen, average ratings of the magnitude of itch and nociceptive sensations reached an early peak within a few minutes followed by a slow decline.^{9–11,16}

From the ratings of each subject in response to each of the four spicule treatments we obtained logarithmic transformations of the maximal (peak) rating plus one of perceived intensity, the area under the rating curve (AUC) and the duration of ratings (Fig. 2).

There was a significant effect of ficin dose on a) the peak magnitude ratings of itch, pricking/stinging and burning, b) the AUCs for itch and for pricking/stinging and c) the duration only for itch (Table S1). Each significant main effect occurred because the median response for each parameter was always significantly greater in response to 100 than to 1 mg/mL despite not differing from the response to 10 mg/mL. However, when comparisons between the two higher doses in the whole sample of subjects including those that did not report sensations for 1 mg/mL, the 100 mg/mL dose evoked significantly greater AUCs than 10 mg/mL for itch and pricking/stinging though not for burning (Table S2).

There were no significant differences between native cowhage and either of the higher doses of ficin in the peak rating, AUC or duration of itch, pricking/stinging or burning (Fig. 2, Table S2).

Skin reactions and dysesthesias surrounding spicule applications There was no indication of a weal or flare produced by spicules with any dose of ficin or by native cowhage, suggesting an absence of histamine release. The only significant effect of ficin dose was the larger area of hyperalgesia after 100 vs. 10 mg/mL. There were no significant differences between the highest dose of ficin and native cowhage in the areas of allodynia, hyperknesis or hyperalgesia (Table S3).

Behavioural tests in mice

The numbers of site-directed behaviours evoked by spicules of ficin and cowhage Different groups of mice received inactive

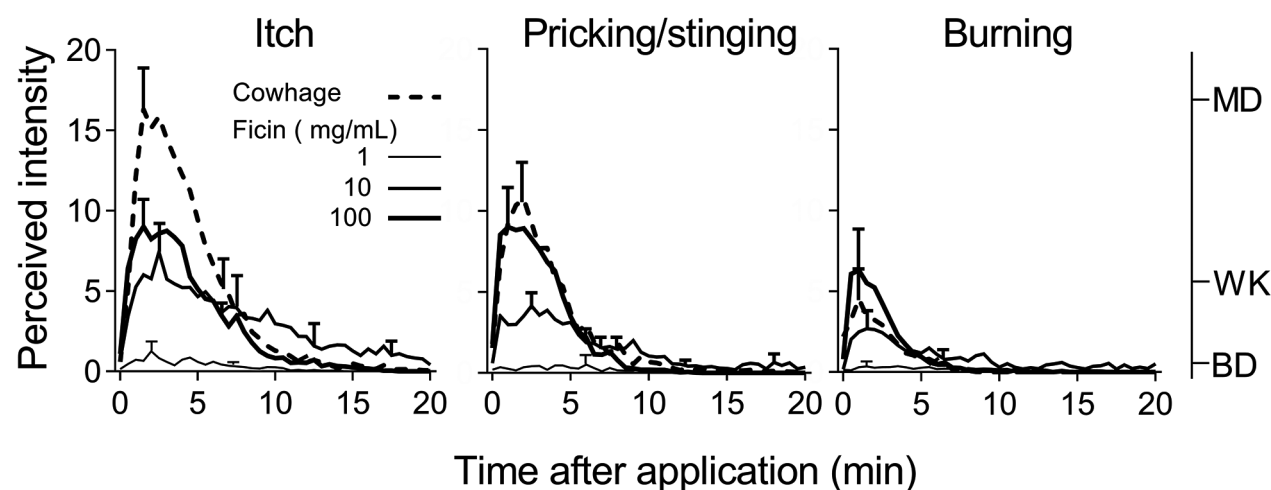


Figure 1 Similarities in the time course of itch and nociceptive sensations evoked by spicules of cowhage and spicules delivering different amounts of ficin applied to the forearm in humans. The ratings of each sensory quality reached a peak magnitude within the first three minutes and then gradually decreased in similar manner over time. Ficin spicules were previously soaked in solutions of 1, 10 and 100 mg/mL and respectively labelled as 1 Ficin, 10 Ficin and 100 Ficin (solid lines) (41.9 μ M, 419 μ M and 4.19 mM). For each ficin dose and for native cowhage (dashed line), the mean rating of the perceived intensities of itch (a) pricking/stinging (b) and burning (c) were obtained at successive intervals of 30 s. SEMs are presented every 5 min starting at the highest mean rating for each type of spicule application. The right vertical axis marks the position of three verbal descriptors ('MD' for moderate, 'WK' for weak and 'BD' for barely detectable) shown in correspondence with the ratings of mean perceived intensity indicated on the left vertical axis. Inactive cowhage-evoked barely detectable itch and pricking/stinging in 3 subjects but no sensations in 16.

spicules, native cowhage or spicules soaked in 100 mg/mL of ficin. The ficin dose was chosen based on the human testing where, similar to cowhage, it had evoked itch in every human subject and a nociceptive sensation in all but one. The median number of spicules successfully inserted was 4.

There were significant differences between groups in the number of bouts of scratching and the number of wipes (Fig. 3, Table S4). Spicules of ficin and cowhage each elicited greater numbers of scratching bouts and wipes than inactive spicules. In contrast, there were no significant differences between cowhage and ficin in scratching or wiping.

The number of site-directed behaviours evoked by intradermal injection of ficin There was a significant effect of dose on the number of scratching bouts and the number of wipes (Fig. 4, Table S5). Each non-zero dose of ficin evoked significantly more scratching bouts and wipes than the saline vehicle. There were no differences between ficin doses of 1.25 and 2.5 μ g in the numbers of scratching bouts and wipes. Ficin 5 μ g elicited more scratching bouts but not more wipes than either ficin 2.5 or 1.25 μ g.

The numbers of scratching bouts and wipes evoked by the dose of 1.25 μ g were comparable to, i.e., not significantly different from, those elicited by the spicules with 100 mg/mL ficin (Mann–Whitney U tests on log transformed data). Thus, the larger amounts of ficin injected elicited behaviours comparable to

those evoked by the smaller amounts, which were estimated as available in the tips of spicules.

To explore whether proteolysis contributed to the behavioural responses to intradermal injection of ficin, 12 of the mice (males) were injected with 2.5 μ g of ficin plus the cysteine protease inhibitor E-64. When combined with the inhibitor vs. given alone, the combination evoked significantly fewer bouts of scratching without affecting the number of wipes (Table S6). It would be useful, in future, to test whether E-64 blocks all sensations evoked by ficin when delivered by spicules to humans, as it did when combined with mucunain.¹

In another test, these male mice received, in the other cheek, an injection of 10 μ g/5 μ L of ficin. This dose evoked significantly more wipes than the previously delivered dose of 5 μ g of ficin but with no significant difference in the number of scratches (Table S6). This highest dose also produced a lesion with slight bleeding in half of the mice.

Discussion

We found that spicules of ficin and cowhage-evoked similar itch- and nociceptive-like sensory responses in humans and in mice. Previous studies found that native cowhage and heat-inactivated spicules containing recombinant mucunain, Cathepsin S, histamine or capsaicin each evoked both itch and nociceptive sensations in humans^{1,13,16} and, for histamine and capsaicin, itch- and pain-like site-directed behaviours in mice.¹²

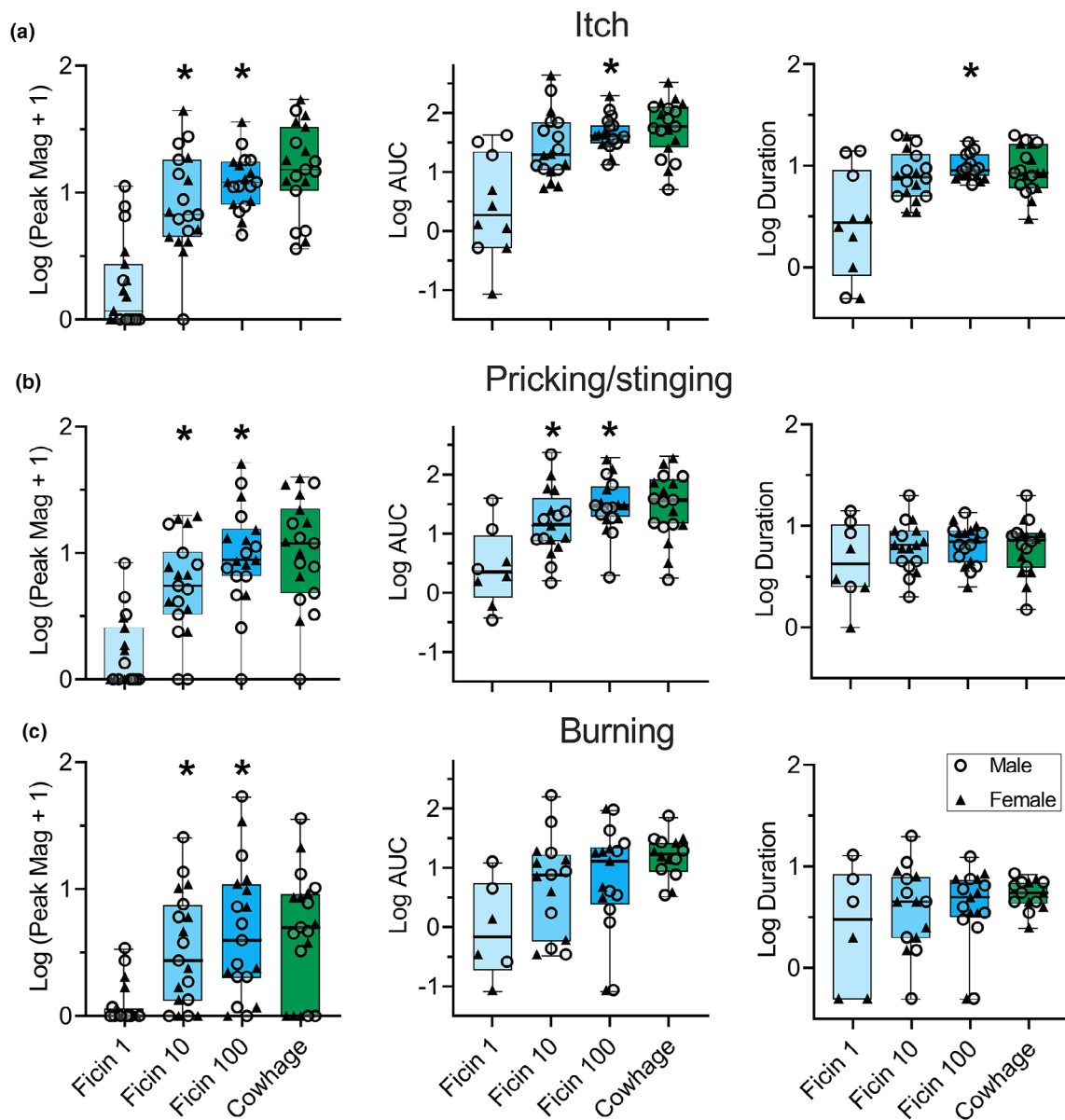


Figure 2 Ficin in spicules evoked dose-dependent magnitude ratings of itch and nociceptive sensations that, at the highest dose, were indistinguishable from ratings of native cowhage. Each symbol is a value obtained from the ratings of itch (a) pricking–stinging (b) and burning (c) obtained from a single subject (open circles and closed triangles for females and males, respectively) in response to spicules previously soaked in 1, 10 and 100 mg/mL of ficin (labelled ficin 1, 10 and 100 (blue box) and to spicules of native cowhage (green box). For better graphical representation of values that differed over a large range, the peak magnitude rating of the perceived intensity of each quality was logarithmically transformed after first adding a value of 1 and labelled as ‘log (peak mag +1)’. For subjects with peak ratings greater than zero for a given sensory quality, the log area under the rating curve (AUC), and log duration (in minutes) were also obtained. Each box spans the interquartile range (25% to 75%); the horizontal line inside the box is the median for data of both genders combined. Ten subjects reported non-zero ratings of itch at each dose of ficin. For these subjects, there was a significant effect of dose on the median peak rating and AUC for itch and pricking/stinging but not burning and not for the duration of any sensory quality (Friedman’s tests, Table S1). Asterisks indicate significant differences between the lowest dose of 1 and doses of 10 or 100 (post hoc Dunn’s multiple comparisons). All 19 subjects experienced itch and one or both nociceptive ratings to cowhage and to ficin doses of 10 and 100. Median values for each of these were neither significantly different from each other (Table S1) nor from those elicited by cowhage (Table S2) in peak magnitude, AUC or duration (Wilcoxon signed-rank tests). Not shown here are the peak ratings for inactive cowhage spicules which elicited significantly less peak itch and peak pricking/stinging than ficin 1 (Wilcoxon signed-rank tests, $P < 0.05$).

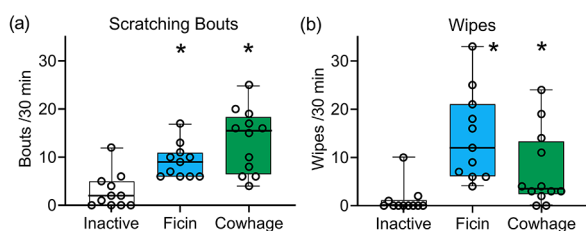


Figure 3 Ficin and cowhage elicited comparable total numbers of scratching bouts and wipes but significantly more of each than inactive spicules. Each symbol represents the total number of bouts of scratching (a) and wipes (b) of each male mouse occurring during the 30 min observation period after application of either chemically inactive spicules ($n = 11$ mice, no colour), inactivated spicules with 100 mg/mL of ficin ($n = 11$, shaded blue) or native cowhage spicules ($n = 12$, shaded green). Each box spans the interquartile range (25% to 75%) and the horizontal line inside the box is the median. The group medians were statistically compared for scratching and for wipes using Kruskal–Wallis analyses of variance followed by Dunn's multiple comparisons. The asterisk indicates a significant difference between responses to inactive spicules vs. spicules of ficin or cowhage. There were no significant differences in either scratching bouts or wipes between cowhage and ficin (Table S4).

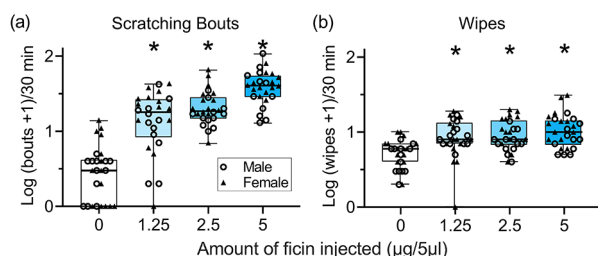


Figure 4 Total numbers of scratching bouts and wipes evoked by intradermal injection of ficin depended on the dose. Each of 26 mice was injected into alternating cheeks every 24 h with ficin of 0 (saline as the control vehicle, no colour), 1.25, 2.5, and 5 µg/5 µL, delivered in ascending order of dose (shaded blue). For better graphical representation of values that differed over a large range the total number of bouts of scratching (a) and number of wipes (b) for each mouse was logarithmically transformed after first adding a value of 1 and labelled as 'log (bouts + 1)' or 'log (wipes + 1)'. Each data point is a value for a single mouse (open circles and closed triangles for males and females, respectively). Each box spans the interquartile range and the horizontal line within is the median response for both sexes combined. The asterisk indicates a significant difference between the median number evoked by a dose of zero in comparison with doses greater than zero as determined by Friedman's tests followed by *post hoc* Dunn's multiple comparisons (Table S5).

We found that spicules of ficin and cowhage produced comparable areas of alloeknesis, hyperknesis and hyperalgesia in humans without producing skin reactions such as weals. In

mice, although behavioural hyperalgesia has been observed after an injection of Cathepsin S,²³ pruritic and nociceptive dysesthesias have yet to be tested after application of pruritogen-containing spicules.

We found that 100 mg/mL of ficin was required to elicit itch and nociceptive sensations in humans and analogous scratching and wiping site-directed behaviours in mice that, for each species, were similar to the sensations and behaviours elicited by native cowhage. Intradermal injection of ficin dose-dependently evoked similar behaviours but required larger amounts in relation to the amount estimated as available in the tips of spicules. For example, injection of 1.25 µg elicited scratching and wiping behaviours comparable to those elicited by spicules soaked in 100 mg/mL ficin but is 425 times the amount of 3 ng estimated as contained in the tip of a single spicule or 104 times the combined amounts in the median of 4 inserted spicules. A pruritogen may be more effective and less of it required if delivered more superficially by spicule or, alternatively, by the skin prick test^{11,24} than by intradermal injection.

Behaviours evoked by intradermal injection of ficin are similar to those previously reported for the cysteine protease, Cathepsin S. When Cathepsin S was injected into the nape of the neck in mice²⁵ the lowest amount of 1 µg elicited scratching that was comparable to that evoked by our lowest injected amount of ficin of 1.25 µg. A higher dose of Cathepsin S, either 80 µM²⁵ or 20 µg³ each elicited scratching and wiping similar in magnitude to those evoked by our injection of 5 µg of ficin. But whether these higher amounts of Cathepsin S evoked injury like the lesion generated by 10 µg of ficin in our study was not reported. A higher concentration of protease might result in a lesion due to increased enzymatic activity and/or the site-directed behaviours it elicits.

Prolonged contact with exogenous cysteine proteases can cause epithelial cells to release alarmins and proinflammatory cytokines that activate dendritic cells to promote a Th2-type allergic immunity.²⁶ Chronic exposure to ornamental fig plants, for example, has caused sensitization to fig aeroallergens and cross sensitization to certain fruits containing cysteine proteases.²⁷ With subsequent exposure in sensitized individuals, the same or similar allergens could, among other effects, cross-link immunoglobulin IgE/IgG, specific for the antigen on Fc receptors in mast cells causing the release of histamine and other inflammatory mediators. Histamine not only acts on H1 receptors on postcapillary venules (producing a weal) but also on the terminals of nociceptors including the mechanically insensitive nociceptors thought to peripherally release calcitonin gene-related peptide *via* a local axon reflex and to signal centrally to elicit itch.²⁸ There was no indication of a weal or flare produced by spicules with any dose of ficin nor native cowhage spicules. This is taken as evidence that the sensory effects occurred in the absence of histamine release.¹¹

Application of cysteine protease by spicules provides an alternate strategy of delivering cysteine proteases that likely avoids triggering strong dermal reactions. In the absence of prior sensitization, a cysteine protease, like ficin, can elicit itch by activating specific G protein-coupled receptors on pruriceptive nociceptors and on certain non-neuronal cutaneous cells. One such receptor that can activate mechanosensitive nociceptors²⁹ and certain non-neuronal cells³⁰ is the protease-activated receptor 2 (PAR2). In cultured dorsal root ganglion (DRG) neurons or transfected Hela cells, ficin, bromelain, papain and Cathepsin S each activated PAR2 receptors to increase intracellular calcium.^{4,23,25} Nociceptors might also be activated indirectly by activation of PAR2 receptors on keratinocytes which causes release of thymic stromal lymphopoietin that, in turn activates a subset of nociceptive-like DRG neurons.³¹

In addition to PAR2, certain Mas-related G protein-coupled receptors (Mrgprs) can be activated by one or more cysteine proteases.^{3,32} For example, in heterologous cells, mucunain, Cathepsin S and papain each activated the mouse receptor, MrgprC11. Mucunain and papain but not Cathepsin S activated a similar receptor from human. MrgprX1³, Mucunain and Cathepsin S but not papain activated MrgprX2 expressed in mast cells in human and possibly neurons.³² MrgprC11 is expressed in a subset of mechanosensitive nociceptors in the mouse.³³ Cowhage, containing mucunain, activates mechanosensitive cutaneous nociceptors in the monkey³⁴, mouse³⁵ and humans² but not mechano-insensitive nociceptors responsive to histamine in humans and monkey.²

We conclude, that delivery of the cysteine protease ficin via spicules or intradermal injection provide an effective model of itch and nociceptive sensations in both mice and humans. Ficin may be useful in translating mechanistic research of pruritic and nociceptive effects of cysteine proteases between the two species. A future direction would be to test whether ficin and mucunain are equally effective in activating MrgprC11 in mice and X1 in monkeys and humans and equally effective in eliciting action potentials from mechanosensitive pruriceptive nociceptors in each species.

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Author contributions

RHL, SS and NM made the ethical proposals and designs of studies. SS and NM contributed to psychophysical experiment. NM, NK, ZZ contributed to behavioural experiments. NK, SS, SO, NM contributed to preparation of chemicals. HFI and NM contributed to data management and statistics. RHL, NM and

HFI contributed to data analysis and manuscript drafting. NM, HFI, SO, NK, ZZ, SS and RHL contributed to manuscript revision.

Data availability statement

Data available upon reasonable request due to ethical restrictions.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Effects of the dose of ficin in spicules on the peak magnitude rating, the area under the rating curve (AUC), and the duration of each sensory quality.

Table S2. Comparisons between spicules of ficin, native cowhage and inactive spicules in the peak magnitude, area under the rating curve (AUC) and duration of each sensory quality.

Table S3. Comparison of the areas of allodynia, hyperalgesia and hyperalgesia produced by spicules of each ficin dose and native cowhage.

Table S4. Comparisons in mice of the numbers of scratching bouts and wipes directed toward an application of spicules that contained ficin (100 mg/mL), native cowhage spicules or were inactive.

Table S5. Comparisons in mice of the numbers of scratching bouts and wipes evoked by intradermal injections of different doses of ficin.

Table S6. Comparisons in mice of the numbers of scratching bouts and wipes between intradermal injections of 2.5 µg of ficin with and without E-64.