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Antipruritic Effect of Cold-induced and Transient Receptor Potential-agonist-induced Counter-irritation on Histaminergic Itch in Humans*

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A frequent empirical observation is that cold-induced counter-irritation may attenuate itch. The aim of this randomized, single-blinded, exploratory study was to evaluate the counter-irritation effects of cold-stimulation and topical application of transient receptor potential TRPA1/M8-agonists (trans-cinnamaldehyde/L-menthol, respectively), on histamine-induced itch, wheals and neurogenic inflammation in 13 healthy volunteers. Histamine 1% was applied to the volar forearms using skin prick-test lancets. Recorded outcome-parameters were itch intensity, wheal reactions, and neurogenic inflammation (measured by laser-speckle perfusion-imaging). Homotopic thermal counter-irritation was performed with 6 temperatures, ranging from 4°C to 37°C, using a 3 × 3-cm thermal stimulator. Chemical "cold-like" counter-irritation was conducted with 40% L-menthol and 10% trans-cinnamaldehyde, while 5% doxepin was used as a positive antipruritic control/comparator. Cold counter-irritation stimuli from 4°C to 22°C inhibited itch in a stimulus-intensity-dependent manner ($p < 0.05$) and, to a lesser extent, also wheal reactions and neurogenic inflammation. Chemical "cold-like" counter-irritation with both L-menthol and trans-cinnamaldehyde had antipruritic efficacy similar to doxepin ($p < 0.05$). Cold-induced counter-irritation had an inhibitory effect on histaminergic itch, suggesting that agonists of cold transduction receptors could be of potential antipruritic value.

Key words: itch; histamine; cold stimulation; TRPs; counter-irritation; TRPM8; TRPA1.

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Itch is an unpleasant skin sensation that evokes scratching and frequently manifests as a chronic distressing symptom in association with diseases such as urticaria, atopic dermatitis, psoriasis, and uraemia (1, 2). Chronic itch profoundly impacts on quality of life in affected patients and, due to a point prevalence of 8–13.5%, in combination with largely suboptimal treatments; itch represents a substantial socioeconomic burden (3–5). Itch is transmitted in 2 separate pruritic pathways with distinct primary afferent populations (6–9). A subdivision of pruriceptive mechano-insensitive C-fibres (CMi) convey histaminergic itch (induced in the present study), whereas a subdivision of pruriceptive polymodal C-fibres (PmC) transmit so-called non-histaminergic itch (10). The peripheral fibres synapse with central projections in the superficial layer of the spinal dorsal horn, from where the pruriceptive signals are transmitted to the thalamus and parabrachial nuclei (11–13).

While scratching usually provides transient itch relief, it is thought to prolong exacerbations and be involved in chronification of itch by secondary mechanisms such as xerosis, inflammation, and infection, a phenomenon dubbed the vicious itch-scratch-itch cycle (14–16). However, the transient relief of itch achievable by introducing a mild painful or non-painful counter-irritation, such as scratching, a mechanism mediated by central segmental inhibition (17) via bhlhb5-interneurons in the spinal dorsal horn (18), signifies the existence of an effective endogenous mechanism capable of greatly attenuating itch. It has been shown experimentally that controlled thermal and mechanical painful stimuli inhibit itch and development of dysesthesias, such as alloknesis (10, 15, 19–21). In addition, patients with itch frequently report that it is reduced by cold-stimulation (11, 22–25). This has led to the idea that counter-irritation with non-painful/painful cooling or equivalent chemical agonists of cold thermosensory receptors, e.g. L-menthol, which is already in limited use, might be a feasible approach to alleviate chronic itch (12, 24). This approach is known from research into similar mechanisms related to pain-processing, where counter-irritation has been widely studied (13, 26, 27). A frequently used counter-irritation modality is homotopically-applied cold, which is often effective in relieving inflammatory pain, a phenomenon dubbed cold analgesia. However, evidence from psychophysical studies in patients with itch or using surrogate models of itch in healthy volunteers on the effect of cold or chemical "cold-like" counter-irritation is limited and contradictory (21, 24, 28, 29). For instance,

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Bromm et al. (29) showed a strong antipruritic effect of mild non-noxious cooling and topical L-menthol, but aspects of these results were not reproduced in a later study with similar methodology (24, 30). The receptors conveying thermal sensations are known as transient receptor potential (TRP) channels and can be activated by a range of thermal and chemical stimuli (31–33). For cold thermosensation, TRPM8 is activated at ≤23–27°C and TRPA1 is activated at ≤15–17°C. Moreover, activation of TRPM8 and TRPA1 can be achieved by the use of their natural agonists L-menthol and trans-cinnamaldehyde (CA), respectively (27, 34–38), which provides an opportunity to study the potential gating of itch-signalling induced by homotopic cold counter-irritation using thermal and chemical stimuli comparatively.

The aim of the present exploratory study was to quantify the dose-response relationship between homotopic cold stimulations or chemical "cold-like" stimulations and their counter-irritation effect on experimentally induced histaminergic itch, wheals, and neurogenic inflammation in healthy volunteers. The antihistamine doxepin (5%, topical pretreatment) was used as a comparator.

METHODS (for complete details see Appendix S1[1])

Test subjects and study design

Thirteen healthy subjects (mean age 22.8 ± 3 years; 8 males, 5 females) participated in and completed the study after providing informed consent. The regional ethics committee of Northern Jutland approved the study protocol (N-20140078). All subjects participated in 2 90-min sessions with a minimum 24 h interval between sessions. The study was conducted in a randomized and single-blinded manner with the order of experimental interventions and arm dominancy randomized. Thus, histamine challenges and homotopic counter-irritation stimuli always alternated between the right and left arm. In total, 6 histaminergic itch provocations and 6 randomized counter-irritation stimuli (thermal/chemical) or control conditions were performed in each of the 2 experimental sessions (**Fig. 1**).

[1]https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-2447

Thermal sensory thresholds, itch induction, itch assessment, and counter-stimulation

Prior to induction of itch in the first of the 2 experimental sessions, individual cold sensitivity was established using quantitative sensory testing (QST). The cold detection threshold (CDT) and cold pain threshold (CPT) were assessed with a baseline temperature of 32°C and ramp stimuli decreasing at a rate of 1°C/s. When the subjects pressed a button denoting CDT or CPT, the temperature returned to the baseline temperature at a rate of 5°C/s.

Itch was induced using a solution of 1.0% histamine dihydrochloride (Allergopharma, Reinbek, Germany) and a standard shouldered 1-mm tip skin prick test (SPT) lancet. A drop of histamine solution was applied to the centre of a 3 × 3 cm square on the skin of the forearm and a custom-made SPT lancet-mount (Aalborg University, Denmark) delivered ≈ 200 g of pressure for 2–3 s (41).

The subjects continuously expressed itch intensity on a 10-cm visual analogue scale ($VAS_{0–10}$) after each itch provocation, with 0 being labelled as "no itch" and 10 being labelled as "worst imaginable itch". The VAS was sampled 4 times/min for the first 5 min of each application. Area under the curve (AUC) was calculated for the 0–2 min (before thermode-induced cold counter-irritation) and at 2–5 min (during cold-counter stimulation). At 3 or 5 min after each interventional condition, the subjects were asked to rate the mean pain experienced as a consequence of the counter-irritation on a $VAS_{0–10}$, with 0 being "no pain" and 10 "worst imaginable pain".

Thermal counter-irritation stimuli and thermal threshold assessments of cold sensitivity (CDT and CPT) were conducted using the same 3 × 3-cm Thermal Stimulator probe attached to a Medoc Main Station (MMS). Thermal counter-irritation was applied at 6 temperatures: 37, 32, 28, 22, 12, and 4°C. The counter-irritation was commenced 1 min and 55 s post-introduction of histamine and lasted for 3 min and 5 s.

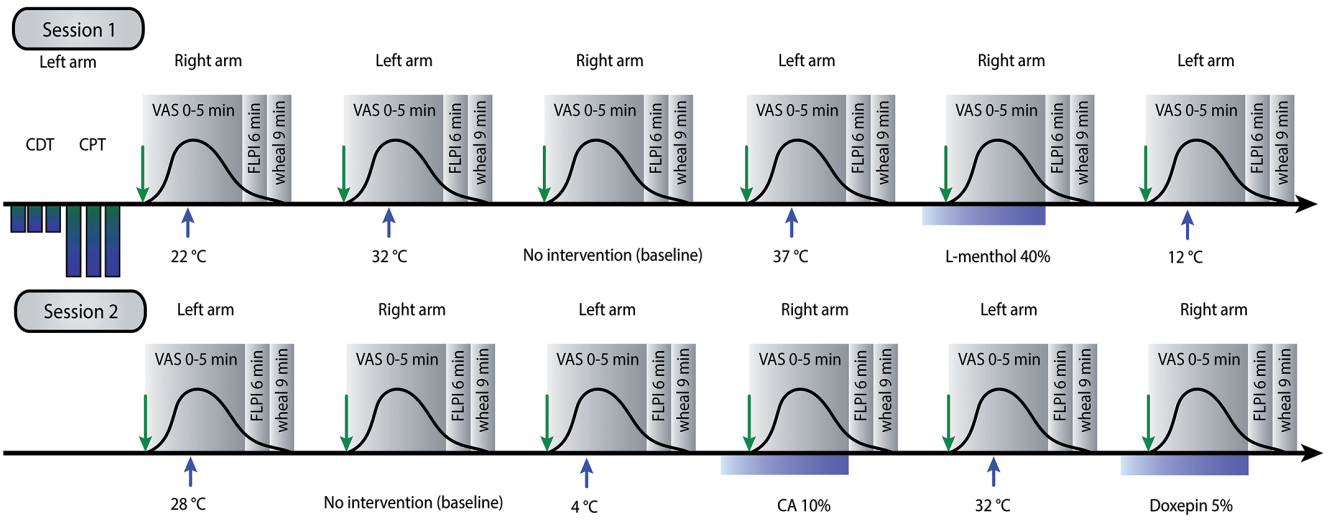

Fig. 1. Overview of the experimental set-up of sessions 1 and 2. The illustration exemplifies the order of experimental procedures conducted in a single subject. Note that the actual order of thermal/chemical interventions was randomized under the condition that applications always switched from arm to arm. *Green arrows*: skin prick testing lancet application of histamine. *Blue arrows*: onset of the experimental thermal interventions. *Wave shapes*: temporal profiles of the itch intensity. L-menthol, CA, and doxepin were applied by pretreatment (see Methods). CA: trans-cinnamaldehyde, CDT: cold detection threshold; CPT: cold pain threshold; FLPI: full-field laser perfusion imaging; VAS: visual analogue scale.

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Chemical counter-irritation was induced with 40% L-menthol (>99.9%, TRPM8-agonist) and 10% CA (>99%, TRPA1-agonist), (both Sigma Aldrich, Broendby, Denmark). These were dissolved in 96% ethanol and topically applied to the skin using a 3 × 3 cm cotton pad on a plastic sheet attached to the arm by medical tape. The substances were applied to the skin 7 min before itch induction to achieve a high level of counter-irritation during the application period. Lastly, 5% doxepin (Region Hovedstadens Apotek, Copenhagen, Denmark) was applied to the skin under occlusion at least 60 min before the histamine application, as a comparator.

Neurogenic inflammation (superficial blood flow) and wheal reactions

To assess neurogenic inflammation, speckle contrast imaging (Moor FLPI, Moor Instruments, Axminister, UK) was recorded 6 min after each histamine application. The imager was placed with a 50-cm distance to the application area, and the pictures were analysed with the appertaining proprietary software. Infrared thermography (A40, FLIR systems, Wilsonville, OR, USA) was conducted to ensure that the control condition of 32°C was within an appropriate temperature range, i.e. close to the normal physiological temperature of the subject. Wheals were measured horizontally and vertically 9 min after every application.

Statistical analysis

All statistical analyses were performed using SPSS version 22 (IBM, New York, USA). The primary outcome was considered to be the intensity of itch 2–5 or 0–5 min after 1% histamine injections with/without counter-stimuli or control interventions and analysed with a repeated measures analysis of variance (RM-ANOVA) test using the Sidak *post hoc* correction for multiple paired comparisons. Comparisons were made between experimental interventions and their respective control; in particular, the thermal counter-stimuli were statistically compared with the 32°C condition. Secondary outcome parameters were wheal and neurogenic inflammation, which were analysed similarly to the primary outcome. Tests were considered significant at $p < 0.05$, and highly significant at $p < 0.01$. All results are presented as the arithmetic means ± standard error of the mean (SEM).

RESULTS

The 13 healthy test subjects recruited for the experiment all completed both sessions of the study without experiencing any adverse reactions during or after the study.

Quantitative sensory thermal tests

The mean CDT was measured to 27.89 ± 1.05°C, i.e. very close to the least intense cold counter-irritation condition at 28°C, while the mean CPT was measured to 6.36 ± 1.87°C, and generally exhibited high inter-individual variation.

Efficacy of counter-irritation

The temporal development of itch in response to 1% histamine was highly reduced by the thermode-induced cold counter-irritation. Immediately after histamine injection, all test subjects rated rapidly increasing itch intensity, reaching a mean of 3.3 ± 1.2 ($VAS_{0–10}$), immediately before application of cold-stimuli. Statistical analysis of the AUC after cold stimulation revealed significant reductions in itch intensity for all temperatures ($p < 0.05$ or < 0.01), except for 32°C and 37°C, which both caused insignificant reductions in itch. VAS scores after the histamine application without interventions remained between 3.3 ± 1.1 and 3.8 ± 1.3, at 2 and 4 min, respectively (**Fig. 2a**). There were no significant differences in the comparison between itch intensity AUC from 0–2 min post-histamine application ($p > 0.6$), indicating lack of bias between conditions. Inter-individual variation in the intensity of the histamine-provoked itch was high, i.e. the highest peak itch intensity score of the baseline condition was VAS = 8.2 and the lowest was VAS = 1.0. Similarly, all chemical counter-irritations, L-menthol ($p \leq 0.05$), CA ($p \leq 0.01$) and doxepin ($p < 0.01$) applied by pre-treatment, caused a significant and pronounced anti-pruritic effect in comparison with the baseline application of histamine (Fig. 2b). The anti-pruritic effect size of the chemical interventions varied between –48.5 ± 12.1% (for L-menthol) and –73.6 ± 10.4% (for CA), but no significant differences were found between effect sizes for any of the substances.

When comparing thermode-induced cold counter-irritation interventions with 32°C, adjusting for the mechanical pressure stimulation introduced by the weight of the probe, only cold stimulation at 22, 12, and 4°C caused significant decreases in itch intensity, signifying that both cold-stimuli in the innocuous and noxious range were sufficient to decrease histaminergic itch (see Fig. 2c). Several of the applied counter-irritations gave rise to mild pain, with a mean VAS = 0.3 ± 0.2 for all interventions. CA resulted in a pain score of VAS = 1.7 ± 0.5, 4°C resulted in 1.2 ± 0.3, and 12°C stimulation resulted in VAS = 0.5 ± 0.3. All other interventions were rated VAS ≤ 0.5. This is well aligned with the fact that CA, 4°C, and 12°C also gave rise to the most profound anti-pruritic effects.

Neurogenic inflammation (superficial blood perfusion) and wheals after histamine application

The mean skin temperature assessed by infrared thermography prior to any tests was 32.67 ± 0.33°C, i.e. very close to the chosen control temperature of 32°C and with low inter-individual variability. The neurogenic inflammation, also known as axon-reflex-flare was, for all thermal conditions, most intense in the immediate injection area; 1–2 cm surrounding the injection site where a 3.2-fold increase in superficial skin perfusion was observed, while the flare progressively normalized approximately 4.0–4.5 cm away from the injection site. All thermal applications ≤28°C resulted in a significant decrease in skin perfusion compared with baseline, ($p < 0.05$), **Fig. 3a**, but only 22°C and 12°C stimuli reduced the

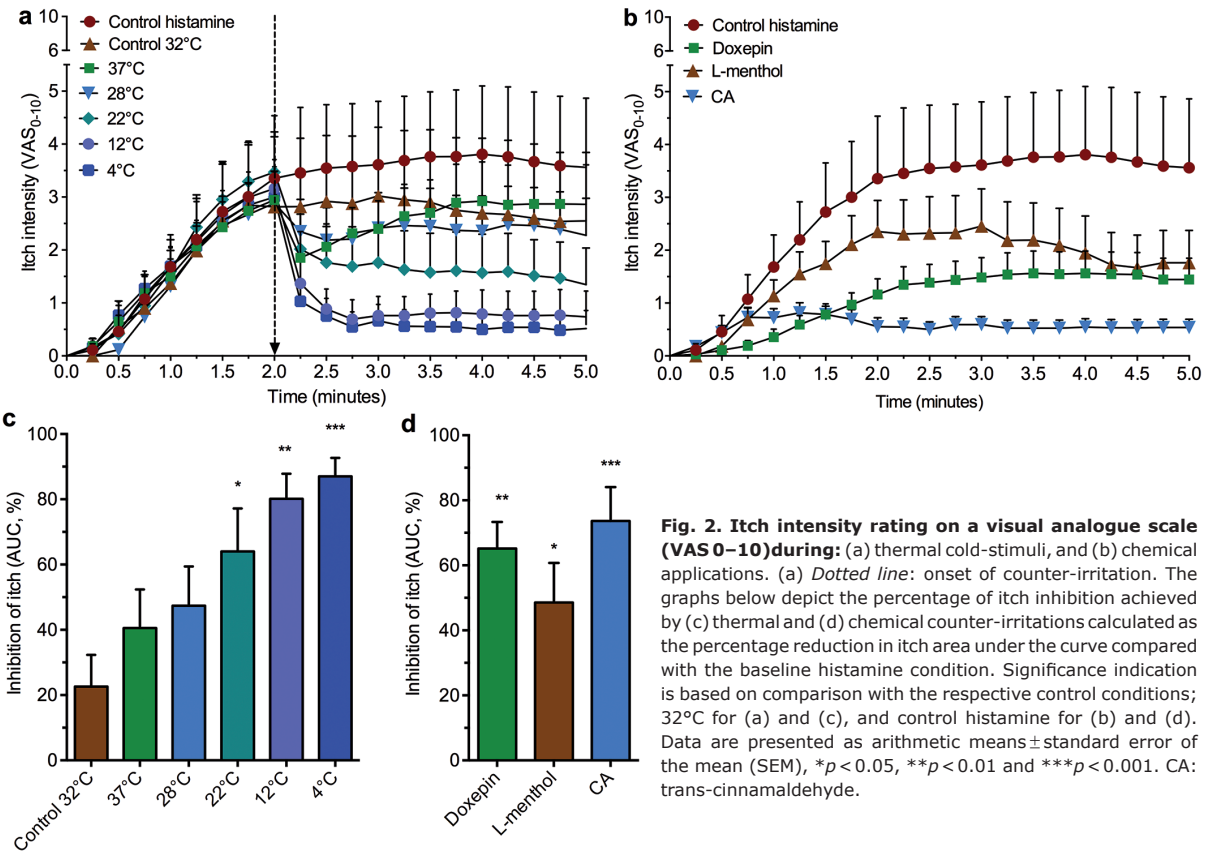

Fig. 2. Itch intensity rating on a visual analogue scale (VAS 0–10)during: (a) thermal cold-stimuli, and (b) chemical applications. (a) *Dotted line*: onset of counter-irritation. The graphs below depict the percentage of itch inhibition achieved by (c) thermal and (d) chemical counter-irritations calculated as the percentage reduction in itch area under the curve compared with the baseline histamine condition. Significance indication is based on comparison with the respective control conditions; 32°C for (a) and (c), and control histamine for (b) and (d). Data are presented as arithmetic means ± standard error of the mean (SEM), $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. CA: trans-cinnamaldehyde.

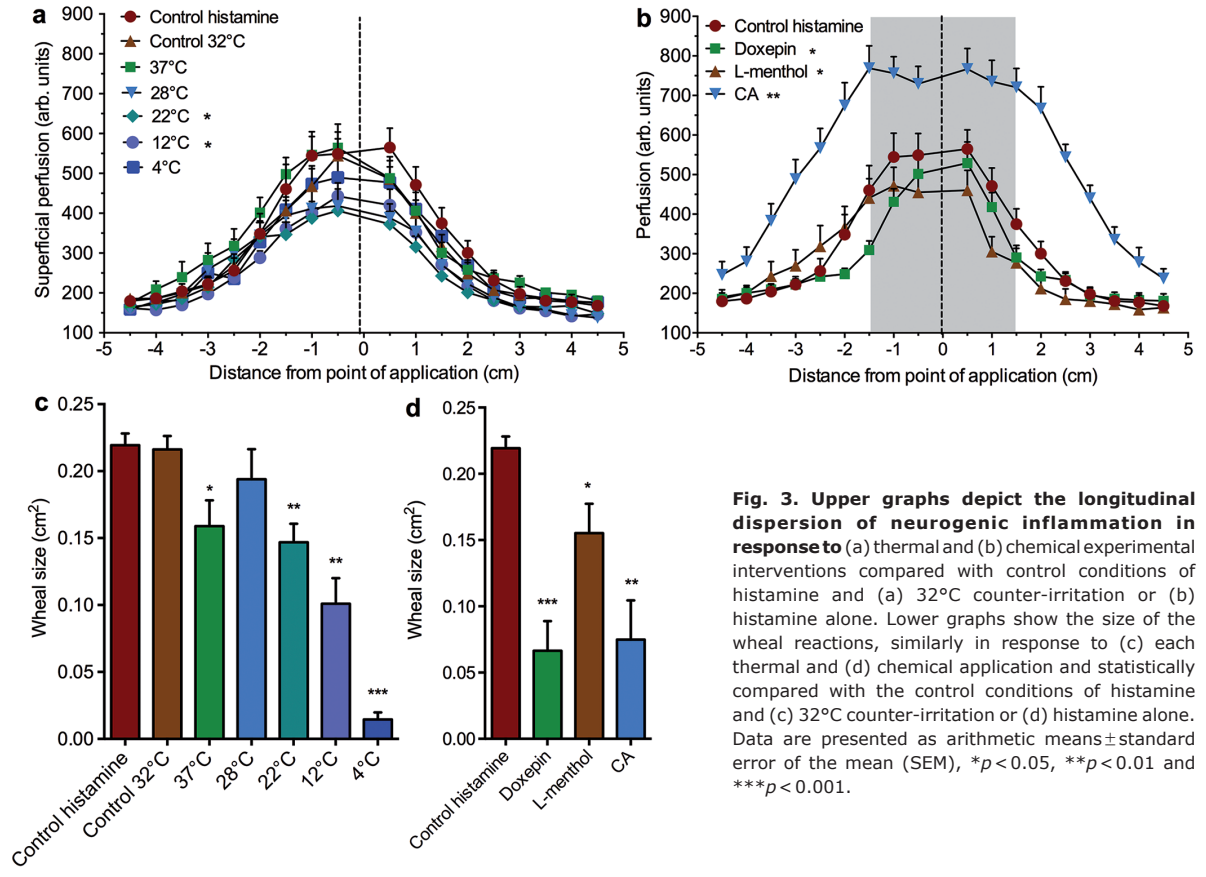

Fig. 3. Upper graphs depict the longitudinal dispersion of neurogenic inflammation in response to (a) thermal and (b) chemical experimental interventions compared with control conditions of histamine and (a) 32°C counter-irritation or (b) histamine alone. Lower graphs show the size of the wheal reactions, similarly in response to (c) each thermal and (d) chemical application and statistically compared with the control conditions of histamine and (c) 32°C counter-irritation or (d) histamine alone. Data are presented as arithmetic means ± standard error of the mean (SEM), $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

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neurogenic flare significantly compared with the 32°C control condition ($p<0.05$). For the chemical counter-irritation, the TRPA1-agonist CA expectedly resulted in a very large neurogenic inflammatory reaction (Fig. 3b), $p<0.001$, as described previously (27, 36, 44). Doxepin and L-menthol both reduced the neurogenic inflammation by a moderate, but significant, extent ($p<0.05$). The intensity and dispersion of the neurogenic inflammation assessed by speckle contrast imaging is shown in Figs 3a and b. Wheal reactions occurred under all experimental conditions, but were significantly decreased by thermal counter-irritation compared with the 32°C control condition ($p<0.01$), with the exception of the 28°C stimulation (Fig. 3c). Both at 32°C and without any counter-irritation, the wheals were measured to 0.22 ± 0.01 cm^2 on average. The decreases during thermal counter-irritation varied; from the lowest -0.06 ± 0.01 cm^2 at 37°C, to the highest -0.20 ± 0.01 cm^2 at 4°C, representing the almost total abolition of the reaction. For the chemical counter-irritations, both 40% L-menthol ($p<0.05$) and, to a much greater extent, 10% CA, and 5% doxepin ($p<0.001$), reduced the wheal reactions (Fig. 3d).

Correlational analyses

Correlational relationships were assessed between the magnitude of experienced anti-pruritic effect for any of the thermal or chemical counter-irritations and the QST parameters assessing individual cold hypersensitivity, i.e. CDT and CPT, as well as between the neurogenic flare and the itch intensity. There were no significant correlations between these groups of parameters; however, a nearly significant positive association was found between CPT and itch inhibition at 12°C ($p=0.061$, $n=12$), indicating that individuals exhibiting high cold pain sensitivity, i.e. high absolute CPT values, experienced more itch relief from cold counter-irritation near their pain threshold.

DISCUSSION

The present study investigated the inhibitory effect of homotopic, monophasic cold-stimuli, and TRP-agonist-induced activity on histaminergic itch and vascular responses. For thermode-induced cold counter-irritation, a stimulus-response relationship was established for itch inhibition, starting at 22°C and increasing stepwise towards 4°C, while stimulation at 28°C and 37°C did not significantly inhibit histamine-induced itch perception compared with the control condition at 32°C. The chemical counter-irritations resulted in significant itch inhibition, the most effective being 10% CA followed by 40% L-menthol. The 5% topical doxepin applied as antipruritic control and comparator gave rise to inhibition of itch at a magnitude similar to 10% CA and 40% L-menthol. Listed in order of highest antipruritic effect the counter-irritations: 4°C, 12°C, 10% CA, 22°C, 40% L-menthol, as well as the positive control, 5% doxepin, all gave rise to a total reduction in itch of more than 40% compared with respective control conditions. In addition, stimuli with 22°C, 12°C, but not 4°C, in addition to doxepin and L-menthol reduced the local neurogenic inflammatory histamine-evoked response.

Antipruritic effect of thermode-evoked cold and cold-like chemical stimulations

Few studies have attempted to assess or quantify the antipruritic effect of cold stimulation at different temperatures or chemical "cold-like" stimulation, despite the fact that cold-induced alleviation of itch is commonly reported in patients (4, 45). While it has frequently been observed that painful cold (or heat) stimulation results in inhibition of itch (46–48), the effect of non-painful thermode-induced cold counter-irritation or TRP-modulation by, for example, L-menthol, is less clear. Bromm et al. (29) found a significant decrease mediated by 1% L-menthol on histamine-induced itch; however, this finding was not reproduced in a subsequent study despite a 10-fold increase in L-menthol concentration. Similarly, studies have reported that both homotopic and heterotopic (in the same dermatome) innocuous cold-stimuli significantly attenuate histaminergic itch (21, 29), while 2 other studies, applying similar methodology, have reported that itch is aggravated by innocuous cold stimulations at 25°C (28, 30). The present data supports the prevailing notion that homotopic innocuous cold is sufficient to significantly reduce experimentally evoked itch. While cold analgesia of cutaneous and deep tissue pain is thought to rely on a combination of anti-nociceptive gating, decreased nerve conductivity, and diminished oedema, the antipruritic effect of cold-stimulation is probably less multifactorial. Since L-menthol does not change the actual temperature of the skin (required to affect neuronal conductivity), but decreases itch by a magnitude similar to thermal stimuli at 22°C and 12°C, it is likely that lateral inhibition from cold-encoding Aδ-fibres is the primary mechanism behind the observed antipruritic effect. This proposition is also well supported by the fact that antipruritic efficacy slightly weaker than presented here has been observed when applying the thermal counter-stimulation 3-cm distally to the site of itch induction, hereby assuring limited interference from potential local inhibitory mechanisms (21).

In the present study, counter-irritation with 4°C and 10% CA were both found to evoke mild pain during the application period; an effect likely to be mediated by counter-irritancy through TRPA1-positive cold C-nociceptors (36, 49, 50). This is well in line with previous studies on the effect of painful counter-irritation of experimentally induced itch, wherein particularly painful mechanical stimuli (i.e. controlled scratching) and noxious

heat stimuli have been shown to significantly reduce itch (15, 20, 21). The mean of the observed CDT was on par with previously reported data for healthy controls (27, 40), while the mean CPT at 6.4°C was slightly below the usual reports of 10–13°C (27, 40). This is probably a consequence of CPT being a QST measure characterized by very high inter-individual variability (40) in combination with a relatively small sample size. A tendency towards a positive correlation was found between CPT and itch inhibition at 12°C ($p=0.061$), indicating that individuals with high cold pain sensitivity, might be more susceptible to effective cold-induced itch relief.

Vasomotor manifestations of thermode-evoked cold and cold-like stimulations

For the thermal stimulations, a significant inhibitory effect on the neurogenic inflammatory response to histamine was found for stimulation at 22°C and 12°C, but not 4°C. Cutaneous cold-stimuli are well known to cause local vasoconstriction and, if sufficiently strong and persistent, subsequent transient paradoxical vasodilation after 3–4 min of stimulation. This, and the short delay between removal of the thermal probe and laser speckle contrast image recordings, could explain why cold-stimuli below the painful threshold (22°C and 12°C) appear to inhibit neurogenic inflammation, while stimulation above the pain threshold at 4°C, did not cause a significant decrease in neurogenic inflammation. In addition, painful cold stimulation is thought to entail TRPA1 activation, although this remains disputed (51), and TRPA1 exhibits co-expression with TRPV1 on peptidergic nociceptive C-fibres capable of generating axon-reflex flare (52, 53). This means that the paradoxical lack of inhibition of neurogenic inflammation at 4°C could be a consequence of responses being masked by additional recruitment of nociceptive C-units, beyond those CMi-fibres activated by histamine-introduction. Thermal stimulations ≤22°C stimulus-intensity-dependently decreased wheal reactions compared with the 32°C control condition. Wheal reactivity has not previously been assessed for a range of cold stimulations. Mechanistically, the observed vasoconstriction is probably mediated by a direct cold-induced smooth muscle contraction in the most superficial dermal capillaries (54).

As shown previously, the applied control, doxepin, drastically reduced neurogenic inflammation and wheal reactions, conceivably by direct inhibition of H1R on CMi-fibres (6, 43, 55, 56). L-menthol was also observed to reduce neurogenic inflammation and wheal formation, probably either via TRPM8-induced capillary constriction (57–59) or via potential TRPV1-cross-desensitization (60–63), given that TRPV1 is highly involved in downstream processing of histaminergic signalling (64). Interestingly, topical L-menthol, occasionally in much lower concentrations (above or equal to 1%), has previously been found to reduce histaminergic and CA-induced neurogenic flare (27, 29); however, contradictory evidence does exist (24). The concentration of 10% CA used in the current study induced a stronger neurogenic inflammatory response than histamine, through direct activation of TRPA1-positive nociceptors (36, 65). On the other hand, CA profoundly reduced wheal formation and, in fact, abolished wheal formation completely in 5 subjects. The wheal reaction is based on localized dermal protein extravasation and is likely to wane when the applied histamine and extravasation products are sufficiently diluted by cutaneous blood perfusion (8). This process was conceivably augmented by the 5-fold increase in superficial blood perfusion induced by CA pre-treatment and a similar mechanism could be relevant for the slight, but significant, decrease in wheal size observed at 37°C.

Study limitations

High concentrations of topically applied ethanol are suspected to entail minor somatosensory, but not neurogenic, inflammatory changes, although previously published results are conflicting (27, 36, 66) and the supposed mechanism is unclear (67). The present study did not include a vehicle condition for application of the chemical substances, for the following reasons: (*i*) even in the few studies that do find somatosensory changes following ethanol application, the effect is very subtle (36, 68); (*ii*) there is no spontaneous sensation associated with ethanol when applied as in the present study (12, 68); and (*iii*) a previous study failed to find any effect of 80% ethanol on histaminergic itch (24). Nonetheless, it cannot be excluded that topical ethanol and the vehicle cream for application of doxepin have minor antipruritic effects, especially since ethanol is a TRPV1 agonist (69). Similarly, although the 32°C stimulation was chosen because it is known to be an approximation of the physiologically normal skin temperature, a significant decrease in itch was observed compared with histamine application without thermal counter-irritation, presumably due to the ongoing mechanical counter-stimulus from the thermal stimulator probe. Ideally, cold counter-irritation should be applied without concomitant activation of mechanoreceptive Aβ-fibres, but this remains a technological challenge. CA produced spontaneous burning/pricking pain and extensive neurogenic inflammation in a number of subjects, which is known to drastically reduce homotopic itch. Future studies should include a titration of CA to a concentration at which it provides somatosensory counter-irritation without provoking spontaneous pain and neurogenic inflammation. In this context, it is a concern that lower concentrations of topical CA alone have previously been shown to have pruritogenic effects in humans (12). It should be kept in mind that itch and pain sensations were not quantified similarly in the present study; while itch was scored continuously as it occurred, subjects had to

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recall the pain intensity of the previously applied counter-irritation stimuli, immediately after the stimuli had ended. As a result, comparisons can be made within the resulting itch and pain scores, but not across the sensory modalities. Lastly, the present study applied a histaminergic human surrogate model of itch conveyed by a subset of pruriceptive CMi-fibres. Future studies should ascertain whether itch induced by a non-histaminergic model, such as the mucunain-model (cowhage) (8, 12), and thus activating PmC-fibres, also known to transmit pain under certain circumstances (70), would be susceptible to effective cold-induced itch suppression.

Conclusion

The thermal cold-stimuli applied in this study inhibited histaminergic itch in a stimulus-intensity-dependent manner of which the painful thermode-provoked stimulations had the highest antipruritic effect. A similar inhibitory tendency was observed on wheal reactions and neurogenic inflammation. In addition, the applied chemical proxies of cold-stimuli, L-menthol and trans-cinnamaldehyde, had significant inhibitory effects on histaminergic itch with an antipruritic effect-size comparable to that of 5% topical doxepin. Thermal counter-stimuli or selective and potent TRPA1-/TRPM8-agonists, titrated to concentrations evoking counter-irritation at sub-pain threshold levels could be potential antipruritic treatment modalities, particularly for those chronic itch patients reporting itch alleviation by cooling.

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