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The effect of repetitive topical applications of local anesthetics (EMLA) on experimental pain and itch (histaminergic and nonhistaminergic)

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Background: The effects of repeated topical applications of local anesthetics are poorly investigated as they may, in addition to analgesia, impact peripheral nerve endings in a cumulative manner. In the present study, the effects of 6 repetitive applications of eutectic mixture of lidocaine (EMLA 2.5% and prilocaine 2.5%) were investigated on experimentally induced pain, histaminergic and nonhistaminergic itch, and neurogenic inflammation.

Methods: Four skin areas on the forearms of 24 subjects were randomized to receive 3 hours of application of EMLA or placebo twice a day for 3 consecutive days. After each application, superficial blood perfusion (SBP), mechanical (mechanically evoked itch, mechanical pain threshold, and mechanical pain sensitivity), and thermal sensitivity (warm detection threshold, heat pain threshold, and suprathreshold heat sensitivity) were assessed. After the last application of EMLA/placebo, histamine and cowhage was applied (2 areas each) and itch and pain intensity and SBP were assessed.

Results: After 3 hours of EMLA application, significant mechanical and thermal hypoalgesia were found with no cumulative efficacy over the 3 days. EMLA alone had no effect on SBP. Significantly increased SBP, reduced cowhage-induced itch, but the unaffected histamine-induced itch was found when applying EMLA ahead of histamine and cowhage.

Conclusions: EMLA induced a reduction of mechanical and thermal sensitivity without a cumulative-dose effect. EMLA reduced nonhistaminergic itch and pain but not the experimentally provoked histaminergic itch. Selective action of EMLA on polymodal C-fibers could explain these effects.

Keywords: histamine, cowhage, itch, EMLA, topical anesthetics

Local anesthetic eutectic mixture of lidocaine (EMLA) is a eutectic mixture of lidocaine 2.5% and prilocaine 2.5% used to anesthetize the skin, for example before minor surgical procedures, vaccination of children[1], as pretreatment of topical capsaicin[2] or postherpetic neuralgia[3]. Pharmacodynamic studies showed that single applications of EMLA-induced graded analgesia to experimental pain stimuli depending on the application time[4–6].

Side effects of local anesthetics include an increase of intracellular calcium concentration via external influx or release from intracellular stores[7], and when applied repeatedly, rearrangement of microtubules or mitochondrial dysfunction[8]. Thereby repeated topical applications may cause a longer-lasting impact on the function of the peripheral nerve terminals including nociceptors[9]. It has been demonstrated that 42 days of repeated treatment with a lidocaine patch of 5% causes a reduction of nerve fiber density in the superficial layers of the epidermis in healthy subjects[10] even though the observed decrease in mechanical sensitivity is probably due to acute desensitization since it was restored 2 days after the end of lidocaine application[10].

Moreover, EMLA action is characterized by a biphasic response of superficial blood perfusion (SBP), with a reduction followed by an increase of SBP, but the exact timing of these 2 phases is still unknown[11]. A previous study[12], showed increased erythema and redness 30–60 minutes after EMLA application in atopic dermatitis (AD) patients compared with healthy subjects. For this reason, we wanted to assess the SBP after EMLA alone and in combination with pruritogens.

The clinical use of EMLA also extends to the treatment of both chronic pruritus and neuropathic pain conditions[3,13], but the effect of local anesthetics on pruritus has only been poorly investigated with limited evidence[13]. Itch transmission follows 2 pathways: histamine-dependent or histamine-independent. Histaminergic itch is transmitted by a subgroup of mechano-insensitive C-fibers, mostly localized in the vascularized

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dermis[14], that, according to previous human studies in which the response of the fibers after the stimulation with histamine[15] and capsaicin[16] was tested, probably express histamine receptors (H1-R and H4-R) and transient receptor potential vanilloid 1 (TRPV1)[17–20]. Nonhistaminergic itch is mainly mediated by the activation of 2 families of receptors: the protease-activated receptors (PAR2 and PAR4) and the Mas-related G protein-coupled receptors (rodent MrgprA3, MrgprC11, and human MrgprX1)[17,20,21]. The downstream target of both families is TRPA1 (transient receptor potential ankyrin 1), and its activation induces the transmission through the mechano-heat sensitive C-fibers also known as polymodal C-fibers (PmC-fibers), due to their ability to perceive different kind of stimuli[17,22–24]. These fibers are mostly localized in the epidermis as demonstrated by studies reporting the absence of cowhage-induced itch after epidermal removal[25,26].

Antihistamine treatment generally abolishes histamine-dependent itch, but not nonhistaminergic itch[13,27–31] which causes major challenges in the management of itch related to, for example, AD.

The aims of this placebo-controlled, experimental, mechanistic study were to investigate the effect of repetitive application of EMLA cream on experimental evoked pain intensity and experimentally induced itch by histamine and cowhage as models for the histaminergic and nonhistaminergic itch.

Methods

Subjects and study design

Twenty-four healthy subjects were recruited (16 males and 8 females, aged 18–32). Exclusion criteria included pregnancy or lactation, skin disease, use of medications that may affect the trial (such as analgesic drugs, sodium channel modulators, or antihistamines), acute or chronic itch or pain, allergy to lidocaine, prilocaine, or other local anesthetics. In accordance with the Helsinki Declaration, all subjects signed an informed consent form, and the regional Ethics Committee approved the protocol. The study was designed as a randomized, single-blinded, controlled trial including 3 sessions over a period of 3 days. The forearms of each subject were divided into 2 squared areas (4 × 4 cm, 4 cm apart). During the first session, 2 areas were treated with EMLA cream, and 2 areas were treated with a placebo cream for 3 hours. After 3 hours, measurement of neurogenic flare and quantitative sensory tests were performed followed by a second EMLA/placebo application period of 3 hours. Measurement of neurogenic flare response and quantitative sensory tests were repeated at the end of the session. The second session was performed after 24 hours and was identical to the first session. The third session took place 24 hours after the second session and was identical to sessions 1 and 2. At the end of this last session, histamine or cowhage was randomly applied, one at a time, in order to have 2 areas (EMLA/placebo) treated with histamine and 2 areas treated with cowhage (**Fig. 1**). The application of EMLA and placebo creams was randomized between right and left forearm. In addition, the histamine and cowhage applications were randomized so that each arm received only histamine or cowhage (**Fig. 1**).

The induction parameters, assessment of microvascular reactivity, and QSTs are described in Supplementary Material (Supplemental Digital Content 1, http://links.lww.com/ITX/A15).

Statistical analysis

The SPSS (v26, IBM Corporation) software was used to perform statistical analysis. Data were tested for normality using the Shapiro-Wilk normality test. To account for the lack of normality, the data were log-transformed. Data were analyzed using repeated measure analysis of variance (RM-ANOVAs) followed by the Sidak post hoc test. P-value ≤ 0.05 was considered statistically significant. RM-ANOVAs were constructed using these factors: treatment (placebo/EMLA), day (first, second, and third day), application (first and second application), pruritogen (histamine/cowhage), and time (pre-pruritogens and postpruritogens application for SBP analysis or every 30 seconds of 9 min of VAS only for itch/pain temporal profile analysis). Graph plotting was realized in GraphPad Prism 6 (GraphPad Software Inc.).

Results

All the 24 participants concluded the experimental procedure without reporting any safety issues during and/or after the study. For all the tests, in Supplementary Table 1 (Supplemental Digital Content 1, http://links.lww.com/ITX/A15) are reported as mean and SD.

Mechanical sensitivity

EMLA caused mechanical hypoesthesia/hypoalgesia (**Figs. 2A–C**). EMLA statistically reduced the mechanically evoked itch as compared with placebo after each application (main effect of treatment: $F_{1,\ 22} = 23.38$; Sidak, EMLA vs. placebo $P < 0.001$; **Fig. 2A**). The values of mechanically evoked itch did not change within the 3 days of experiment nor between applications for neither EMLA nor placebo ($P = 0.99$).

EMLA increased the mechanical pain threshold (MPT, **Fig. 2B**) and mechanical pain sensitivity (MPS, **Fig. 2C**) compared with placebo on all days (MPT: day × treatment: $F_{2,\ 46} = 3.70$; Sidak, EMLA vs. placebo at day 1, 2, and 3, $P < 0.001$; MPS: day × treatment $F_{2,\ 46} = 5.21$; Sidak, EMLA vs. placebo at day 1, 2, and 3, $P < 0.001$), without difference between days (MPT: $P = 0.47$, MPS: $P = 0.33$).

Thermal sensitivity

EMLA significantly decreased the thermal sensitivity (**Figs. 2D–F**). EMLA increased the warm detection threshold (WDT, **Fig. 2D**) and the heat pain threshold (HPT, **Fig. 2E**) compared with placebo (WDT: main effect of treatment; $F_{1,\ 23} = 47.86$; Sidak, EMLA vs. placebo $P < 0.001$; HPT: main effect of treatment; $F_{1,\ 23} = 21.11$; Sidak, EMLA vs. placebo $P < 0.001$). In both applications of the 3 days, the WDT and HPT did not change over time (WDT: $P = 0.75$; HPT: $P = 0.16$).

Suprathreshold heat sensitivity was significantly lower in the EMLA areas with respect to placebo areas (main effect of treatment: $F_{1,\ 23} = 59.71$; Sidak, EMLA vs. placebo $P < 0.001$; **Fig. 2F**).

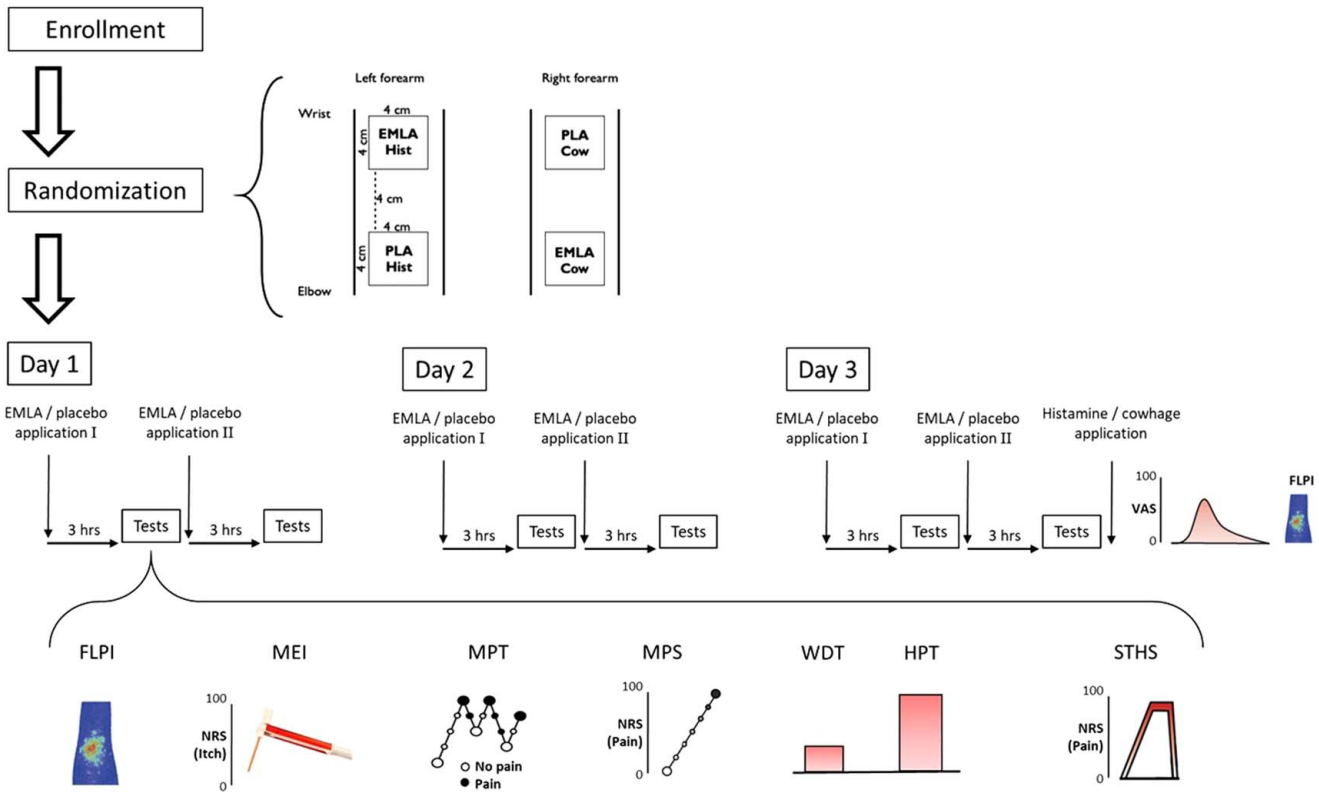

Figure 1. Overview of the experimental protocol. Following enrollment, 4 volar forearm areas on 24 subjects were randomized to EMLA or placebo pretreatment. After 3 hours, the cream was removed, and tests were performed. EMLA or placebo cream were applied again for 3 hours followed by tests. The day after, all the interventions were repeated. The third day all the interventions were repeated followed by the application of histamine or cowhage, 9 minutes of VAS scale measurement and FLPI measurement. EMLA indicates eutectic mixture of lidocaine; FLPI, full-field laser perfusion imaging; HPT, heat pain threshold; MEI, mechanically evoked itch; MPT, mechanical pain threshold; MPS, mechanical pain sensitivity; NRS, numerical rating scale; STHS, suprathreshold heat sensitivity; VAS, visual analog scale; WDT, warmth detection threshold.

Effects of EMLA on neurogenic inflammation

Mean and peak SBP and flare areas are shown in **Figure 3**. There was no difference between placebo and EMLA in mean, peak, and flare (mean: $P = 0.208$, peak: $P = 0.157$, flare: $P = 0.322$; **Figs. 3A–C**).

After histamine and cowhage application, the SBP increased in both placebo and EMLA areas (mean: main effect of time, $F_{1,\ 23} = 97.69$; Sidak, prepruritogens vs. postpruritogens application $P < 0.001$; peak: main effect of time, $F_{1,\ 23} = 142.4$; Sidak, prepruritogens vs. postpruritogens application $P < 0.001$; flare: main effect of time, $F_{1,20} = 162.42$; Sidak, prepruritogens vs. postpruritogens application $P < 0.001$; **Figs. 3D–F**). The SPB induced by histamine is always higher than the one induced by cowhage [mean: time × pruritogen $F_{1,\ 23} = 60.9$, Sidak, histamine (postpruritogens application) vs. cowhage (postpruritogens application), $P < 0.001$. Peak: time × pruritogen $F_{1,\ 23} = 8.51$; Sidak, histamine (postpruritogens application) vs. cowhage (postpruritogens application), $P < 0.01$. Flare: time × pruritogen $F_{1,\ 20} = 66.82$; Sidak, histamine (postpruritogens application) vs. cowhage (postpruritogens application), $P < 0.001$]. In the areas pretreated with EMLA the SBP after pruritogens was increased compared with the placebo areas (mean: time × treatment $F_{1,\ 23} = 14.05$; Sidak, EMLA + histamine and EMLA + cowhage vs. histamine and cowhage $P < 0.01$. Peak: time × treatment $F_{1,\ 23} = 44.99$; Sidak, EMLA + histamine and EMLA + cowhage vs. histamine and cowhage $P < 0.001$. Flare: time × treatment $F_{1,\ 20} = 46.93$; Sidak, EMLA + histamine and EMLA + cowhage vs. histamine and cowhage $P < 0.001$).

Effect of EMLA-pretreatment on histaminergic and nonhistaminergic provoked itch

The itch profile is shown in **Figures 4A–C**. EMLA did not induce any changes in histamine-provoked itch for either peak intensity or the area under the curve (AUC) (peak: $P = 0.168$, AUC: $P = 0.867$; **Figs. 4A, B**). In contrast, EMLA had a significant effect on itch induced by cowhage by reducing both peak and AUC (peak: treatment × pruritogen $F_{1,\ 23} = 12.18$; Sidak, $P < 0.01$; AUC: treatment × pruritogen $F_{1,\ 23} = 5.38$; Sidak, $P < 0.001$). Moreover, the cowhage-induced peak itch intensity was significantly higher than the histamine-induced peak (Sidak, $P < 0.01$) and EMLA reduced the peak intensity of cowhage to the level of histamine. The results were confirmed by the graph of the temporal profile of itch (**Fig. 4C**). Histamine and histamine + EMLA showed the same profile. Cowhage compared with cowhage + EMLA has a higher itch intensity from 30 to 240 seconds [time (every 30 s) × treatment × pruritogen: $F_{4,\ 95} = 3.73$; Sidak, 30 s $P < 0.05$, from 60 to 180 s $P < 0.001$, 210 and 240 seconds $P < 0.01$]. Moreover, cowhage induced higher itch intensity than histamine from 30 to 210 seconds (Sidak, 30 s $P < 0.01$, 60 s $P < 0.05$, from 90 to 150 s $P < 0.01$, 180 and 210 s $P < 0.05$), but cowhage + EMLA

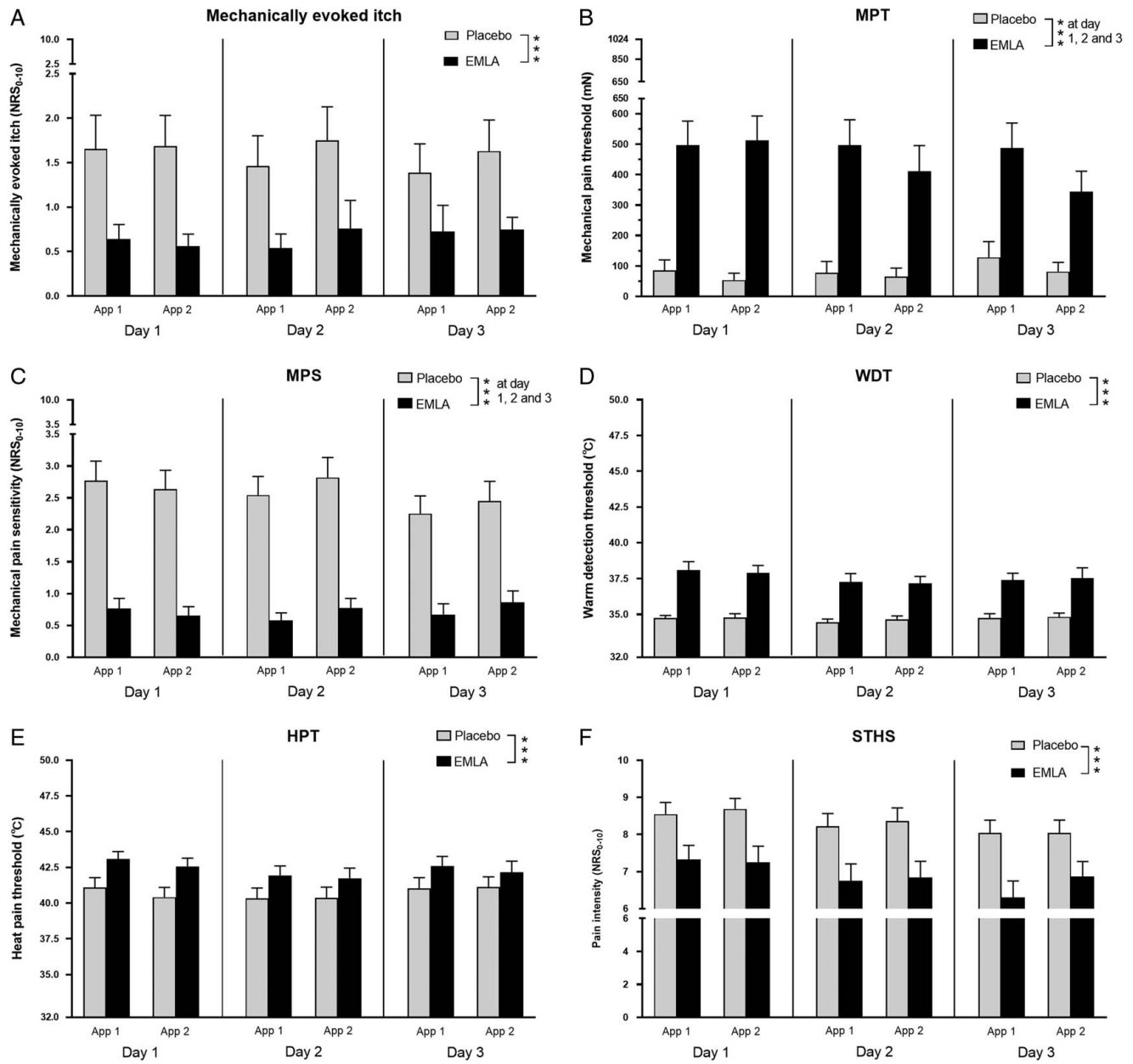

Figure 2. Mechanical and thermal sensitivity. Changes in mechanical and thermal sensitivity after the application of EMLA and placebo cream (3 h of application, twice a day for 3 consecutive days). A, Mechanically evoked itch: in the graph is indicated the itch intensity on a NRS (0–10) perceived by the subjects after a mechanical stimulation. B, MPT in the graph indicates the force in mN in which subjects started to feel pain after a mechanical stimulation. C, MPS in the graph indicates the pain intensity on a NRS (0–10) perceived by the subjects after a mechanical stimulation. D, WDT in the graph indicates the temperature in °C in which subjects started to feel warm. E, HPT in the graph indicates the temperature in °C in which subjects started to feel pain to an ascending heat stimulus. F, STHS in the graph indicates the pain intensity on a NRS (0–10) perceived by the subjects after a heat stimulation. Significance indicators: for MEI, WDT, HPT, and STHS is shown as the main effect of treatment (significant differences are pooled across the 3-d of treatment), EMLA versus placebo $^{***}P < 0.001$. For MPT and MPS, the interaction treatment × day is shown and the significance indicator ($^{***}P < 0.001$) represents the difference between EMLA and Placebo present at day 1, 2, and 3. Placebo = gray and EMLA = black. Values are presented as mean + SEM. EMLA indicates eutectic mixture of lidocaine; HPT, heat pain threshold; MEI, mechanically evoked itch; MPT, mechanical pain threshold; MPS, mechanical pain sensitivity; NRS, numerical rating scale; STHS, supra-threshold heat sensitivity; WDT, warmth detection threshold.

showed no differences at any time point compared with histamine and histamine + EMLA.

Effect of EMLA-pretreatment on histaminergic and nonhistaminergic provoked pain

The pain profile is shown in **Figures 4D–F**. Cowhage induced a significantly higher pain intensity than histamine for both the peak intensity and AUC curve (peak: main effect of pruritogen $F_{1,\ 23} = 9.61$, Sidak, histamine vs. cowhage $P < 0.01$; AUC: treatment × pruritogen $F_{1,\ 23} = 5.59$; Sidak cowhage vs. histamine in placebo areas $P < 0.05$; **Fig. 4D, E**). EMLA reduced the AUC of cowhage-induced pain (Sidak $P < 0.05$). In addition, there was no difference between cowhage + EMLA and histamine + EMLA on the pain peak and AUC. Regarding the

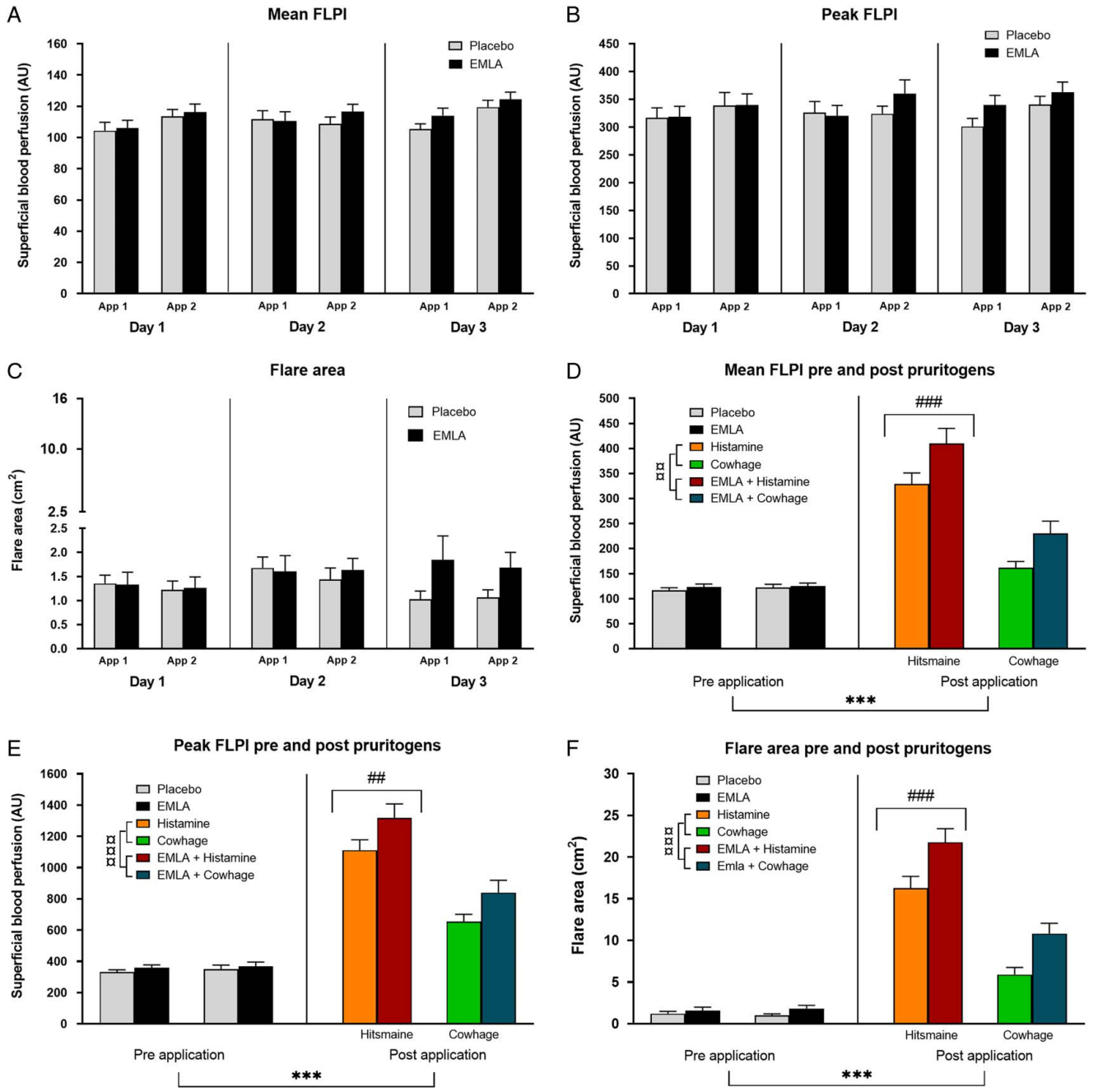

Figure 3. *Superficial blood perfusion.* Changes in superficial blood perfusion assessed by full-field laser perfusion imaging (FLPI). A, Mean of SBP for each ROI (region of interest, equivalent to the predefined cream application area). B, Peak of SBP for each ROI. C, Flare area size. D, Mean of SBP for each ROI prepruritogens and postpruritogens application. E, Peak of SBP for each ROI prepruritogens and postpruritogens application. F, Flare area size prepruritogens and postpruritogens application. Significance indicators: histamine (pool: histamine and EMLA + histamine) versus cowhage (pool: cowhage and EMLA + cowhage) $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$; pruritogens (pool: histamine and cowhage) versus pruritogens + EMLA (pool: EMLA + histamine and EMLA + cowhage) $^{\alpha\alpha}P < 0.01$; preapplication of pruritogens (pool of all the areas preapplication of pruritogens) versus postapplication of pruritogens (pool of all the areas postapplication of pruritogens) $^{***}P < 0.001$. Placebo = gray and EMLA = black; histamine = orange and histamine + EMLA = red; cowhage = light green and cowhage + EMLA = dark green. Values are presented as mean + SEM. EMLA indicates eutectic mixture of lidocaine; FLPI, full-field laser perfusion imaging.

temporal profile (**Fig. 4F**), EMLA delayed the development of histamine-induced pain, and at 30 seconds time points, pain in the histamine + EMLA area was lower than in the histamine area [time (every 30 s) × treatment × pruritogen $F_{3,\ 72} = 3.80$, Sidak $P < 0.05$]. From 60 seconds the 2 pain profiles were similar. The pain profile of cowhage was significantly higher ($F_{1,\ 23} = 5.72$, $P < 0.05$) than the pain reported in the other 3 areas. The difference between cowhage and histamine was significant from 60 to 180 seconds (Sidak, $P < 0.01$). The differences between cowhage and cowhage + EMLA were significant from 120 to

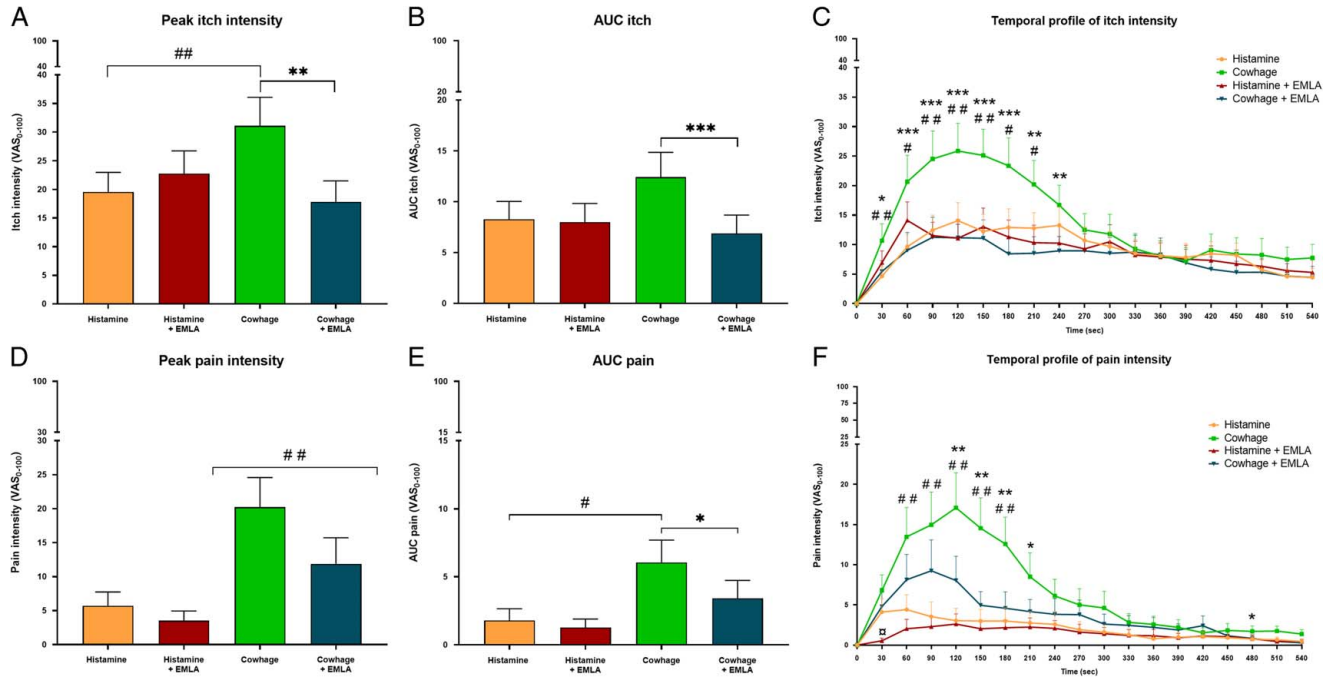

Figure 4. Itch and pain intensities on a VAS (0–100) perceived by the subjects after the application of histamine and cowhage with EMLA or placebo pretreatment. A, Peak itch intensity. B, AUC itch. C, Temporal profile of itch intensity. D, Peak pain intensity. E, AUC pain. F, Temporal profile of pain intensity. Significance indicators: cowhage versus cowhage + EMLA $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; histamine versus histamine + EMLA $^{\circ}P < 0.05$; in peak pain intensity graph: histamine (pool: histamine and EMLA + histamine) versus cowhage (pool: cowhage and EMLA + cowhage) $^{\#\#}P < 0.01$; in peak itch intensity and AUC pain graphs: cowhage versus histamine $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. Histamine = orange and histamine + EMLA = red; cowhage = light green and cowhage + EMLA = dark green. Values are presented as mean + SEM. EMLA indicates eutectic mixture of lidocaine; VAS, visual analog scale.

210 seconds (Sidak, from 120 to 180 s $P < 0.01$, 210 s $P < 0.05$). There were no differences at any time point between histamine + EMLA and cowhage + EMLA.

Discussion

Repeated EMLA applications over 3 days caused mechanical and thermal hypoalgesia but did not result in a cumulative analgesic effect. EMLA did not per se change superficial blood flow and flare area but caused increased blood perfusion and flare after both histamine and cowhage stimulations. Moreover, repetitive applications of EMLA decreased consistently itch and pain to nonhistaminergic stimulation but not to histamine.

Effect of EMLA on mechanical and thermal sensitivity

In the present study, the application of EMLA for 3 hours induced a reduction of mechanical sensitivity assessed by using von Frey filament and a pinprick set (for itch and pain perception, respectively). In both cases, the mechanical sensitivity was profoundly decreased in EMLA-treated areas compared with the areas treated with a placebo. The repetitive applications did not completely abolish the mechanical sensitivity which remains the same during the whole experiment, consequently, a cumulative dose effect was not present. Similar results were obtained for thermal sensitivity. A single EMLA application increased warm and pain detection thresholds and decreased pain intensity of the suprathreshold heat sensitivity with no cumulative effect over the 3 days.

Local anesthetics block the discharge in Aδ-fibers and C-fibers[32], by the block of voltage-gated sodium channels[33,34]. The consequence of the binding of a local anesthetic is that the sodium channel passes to an "inactivated state," from which direct activation is impossible[32]. In this way, the frequency of the opening of the Na^+ channels decreases. A 3-day application, 6 hours per day, of EMLA, probably did not induce a decrease in fiber density[10]. This could explain why a dose-cumulative response was not found in the present study. The alteration of thermal sensitivity is probably due to the effect of local anesthetics on the TRP channels. In particular, TRPA1 and TRPV1 are directly activated by lidocaine (probably through a different domain with respect to the vanilloid-binding domain)[10,35] and this activation enhances the influx of calcium ions in the cells. It was also proposed that, as TRPV1 activators, local anesthetics cause desensitization when applied for a prolonged time[35]. It was suggested that the TRPV1 activation by lidocaine needs PI $(4,5)P_2$, and its depletion consequent to the increase of PLC activity (always induced by local anesthetics) leads to the desensitization of TRPV1 induced by lidocaine[35].

Changes in SBP induced by EMLA and pruritogens

In this study, the application of EMLA did not induce any changes in SBP when it is applied alone. In a previous study, it was proposed that EMLA induces a biphasic response on skin blood flow[11]. EMLA induced a concentration-dependent vasoconstriction of peripheral microcirculation and, after a short time application of EMLA, blanching was observed[11]. After 1½ hours from application, maximal reduction of the SBP has been

found, but 4 hours of application is found to induce erythema with an increased SBP[11]. Moreover, 6 consecutive hours of EMLA application induced an increase of the SBP up to 148%[11]. The absence of changes in SBP found in the present study could be explained by assuming that the first SBP measurement was after 3 hours of application and in the middle of the biphasic response. The second measurement was after 3 more hours (so 6 in total), but between the 2 daily of applications, there was a break of ~45 minutes (time to run all the mechanical and thermal tests), and possibly the normal SBP was restored during this time avoiding the cumulative effect of the 2 applications. Moreover, it should be considered that the full-field laser perfusion imaging technique used in this study is more precise and sensitive than the specially constructed fiberoptic scanning reflectance spectrophotometer used in the previous experiment of 1989[11].

The pretreatment with EMLA induced an increase of SBP and flare in combination with the pruritogens. In a previous study, 30–60 minutes of EMLA application increased erythema and redness in patients with AD compared with healthy subjects[12]. It was speculated that AD skin allows a higher absorption of EMLA and hence could enhance the vascular reaction[12]. The PmC-fibers (activated by cowhage) weakly contribute to neurogenic inflammation, meanwhile, the CMi-fibers have a profound impact on both neurogenic inflammation in the area surrounding the application site and axon-reflex flare that occurs in an otherwise unprovoked surrounding area[36,37]. To induce vasodilatation, there is a release of neuropeptides, such as CGRP and substance P[38,39]. It could be hypothesized that the pretreatment with EMLA increases the engagement of C-fibers in neurogenic inflammation and the release of these neuropeptides.

Antipruritic and anesthetic effect of EMLA on histamine and cowhage stimulations

Histamine is the golden model for experimentally induced histaminergic itch[40], while mucunain (the active enzyme present in cowhage spicules[41]) induces nonhistaminergic itch[42]. Cowhage induced a more intense itch than histamine[43]. Pretreatment with EMLA cream in the present study decreased only the itch induced by cowhage. EMLA did not abolish completely the pruritus induced by cowhage but caused a reduction of the intensity. It has been proposed that PmC-fibers are located more superficially than CMi-fibers[44–46]. Moreover, the histamine-induced axon-reflex flare could indicate that histamine acts more deeply into the vascularized dermis[14,47]. Due to the different delivery methods and molecular weight of the pruritogens (cowhage ~36 kDa vs. histamine ~0.11 kDa[47]), histamine could diffuse directly to a deeper layer of the epidermis compared with mucunain[48]. Hence, it could be possible that local application of EMLA only affects the PmC-fibers and consequently, it reduces only non-histaminergic itch. EMLA application seems to have the same effect as an 8% capsaicin patch applied for 1 hour. One hour of capsaicin application reduces only the itch induced by cowhage and not the one induced by histamine. Nevertheless, after 24 hours of application, capsaicin induced a degeneration of the TRPV1-positive fibers and reduced both histaminergic and non-histaminergic itch[49]. Forty-two days of lidocaine patch application reduced the density of epidermal nerve fibers[10]. It could be possible that a longer application of EMLA may also reduce the histaminergic itch.

Study limitations

One limitation of this study is the lack of proper blinding for participants and investigators. After the first 3 hours of pretreatment, it was obvious to participants and investigator in which areas EMLA was applied due to the anesthetic action as the mechanical and thermal sensitivities were profoundly decreased. Furthermore, it was difficult to blind histamine and cowhage applications due to the different delivery methods.

Conclusion

EMLA application for 3 days, twice a day, induced a reduction of mechanical and thermal sensitivity without a cumulative-dose effect. In addition, EMLA alone did not induce changes per se in neurogenic inflammation. However, the combination of EMLA and pruritogens affected the microvascular reactivity and enhanced neurogenic inflammation. Moreover, EMLA reduced nonhistaminergic itch and pain but not the experimentally provoked histaminergic itch possible via a selective action of EMLA on PmC-fibers. Considering that nonhistaminergic itch is more difficult to treat, the present findings are of particularly clinical relevance for treating localize itch conditions resistant to antihistamines.

Ethical approval

The regional (Region Nordjylland) Ethics Committee approved the protocol (N-20190043).

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Author contribution

L.A.N. and S.L.V.: designed the study. G.E.A.: collected, analyzed the data, and wrote the manuscript draft. All authors discussed the results and commented on and approved the manuscript.

Conflict of interest disclosures

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Data availability statement

Data will be made available on reasonable request.

Statement of exclusivity

The data included in the present paper has not been published elsewhere.

Patient consent statement

In accordance with the Helsinki Declaration, all subjects signed an informed consent form.

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