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A Quantitative Comparison of the Effect of Local Analgesics on Argon Laser Induced Cutaneous Pain and on Histamine Induced Wheal, Flare and Itch

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A quantitative comparison was made of the effect of infiltration of local analgesics and topical analgesic cream (EMLA) on laser-induced pain and histamine-induced wheal, flare and itch. Wheal and flare were quantified by planimetry and analgesia was quantified by the pricking pain threshold to argon laser stimulation. The intensity of histamine-induced itch was scored on a 4-point scale. Local analgesics had no effect on the wheal area. The flare reaction was abolished by infiltrating lignocaine, and gradually inhibited by increased application times of EMLA. Itch was abolished after local lignocaine infiltration, but not significantly reduced after EMLA cream applied for less than 120 min, although the skin was anaesthetized to laser-induced pain. The reduction of flare area correlated to the level of analgesia, which may therefore reflect the cutaneous responsiveness to neurogenic inflammation. It is suggested that itch and pricking pain are mediated by different populations of nerve fibres, as itch can be evoked even when the sensation of pricking pain is abolished. Surgery, skin prick tests and other traumatic procedures should therefore be performed under local anaesthesia to reduce neurogenic inflammation. ***Key words: Pain measurements; Skin.***

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Initiation of acute inflammatory skin reactions may be mediated by histamine release from mast cells by a direct action on cutaneous vessel walls and by chemoattraction. The reaction is characterized by a central wheal and a peripheral vascular dilatation (1), the flare, which already early in this century was thought to be caused by a neurogenic component (2). This reaction may involve an antidromic flow of chemical substances through cutaneous afferent sensory nerves. These substances may be small peptides, among which substance P (SP), calcitonin gene related peptide, vasointestinal peptide and somatostatin have been identified. SP may induce peripheral wheal and flare either by releasing histamine (3), or directly by a histamine-like effect. The neuropeptides act as peripheral chemical neurotransmitters, and may cause both initiation and propagation of inflammatory processes, whereas histamine has been shown to be unable to sustain inflammation *per se* (4).

The actual role of C-fibre innervated free nerve endings (polymodal nociceptors) in neurogenic inflammatory responses of the skin has been shown both in animals (5) and in humans (6, 7). When substance P is depleted from free nerve endings by capsaicin treatment, the inflammatory responses are reduced or abolished, and therefore the neurogenic component of inflammation is dependent on an intact function of the neuropeptide-containing nerve endings (8).

The purpose of the present study was to investigate the physiological connection between cutaneous itch, and reactions to experimental cutaneous pain and inflammation following gradual analgesia of the skin (9). The results indicate that potentially traumatic procedures that release inflammatory mediators could be performed under local analgesia to eliminate the neurogenic part of inflammation.

MATERIALS AND METHODS

Volunteers

The investigation included 11 healthy, non-atopic volunteers, 5 females (age range 29–50 years, mean 34 years) and 6 males (age 24–38 years, mean 32 years). The volunteers were tested for urticarial dermographism and were all found negative. The experiments were performed in two sessions separated by a one week interval (experiments 1 and 2). All volunteers gave their informed consent according to the Declaration of Helsinki.

Analgesia

Intradermal infiltrations of 2 ml 1% lignocaine (Lidocaine, DAK, Denmark) were performed using a 28G cannula. Analgesia was also induced by topical application of EMLA cream (Astra AB, Sweden), a *e*utectic *m*ixture of *l*ocal *a*nalgesics (lignocaine 2.5% and prilocaine 2.5%), in which the two analgesics form the oily-phase in an oil-in-water emulsion. 1.5 g/cm^2 of the cream was applied to the skin under a semipermeable plastic film occlusion (Tegaderm, 3M, UK).

Histamine skin prick

Standardized histamine skin prick test devices were used (Phazet, Pharmacia AB, Sweden), corresponding to 10 μg of histamine (10). The tests were performed in the centre of both anaesthetized and untreated control areas 7 min after local infiltration of lignocaine, and 5 min after removal of EMLA cream and plastic film occlusion.

Monitoring of wheal and flare reactions

The areas of the wheal and flare reactions were measured 7 min after the skin pricks. The circumferences of both reactions were traced with a pen and transferred to a transparent plastic film. The areas were measured with a digital planimeter (Videoplan, Kontron, FRG). During the experiments the skin temperature on the forearms was monitored and kept at 33 ± 2°C using an infrared heater.

Laser stimulation

The output from an argon laser (Spectra Physics 168, USA) was transmitted to the skin via a quartz fibre. Output power could be adjusted from 50 mW to 3.5 W. The highest laser intensity used in the present experiment was 3.0 W, because intensities above this level could cause superficial burns on the volar part of the forearm. This limit was defined as the skin destruction level (SDL). The wavelengths of argon laser light were 0.488 μm (blue) and 0.515 μm (green). An external laser power meter (Ophir, Israel) was used to measure the dissipated output power at skin level. A continuous low-energy beam (50 mW) from the argon laser visualized the stimulation site. The stimulus pulse duration was 200 ms and the beam diameter 3 mm. Parameters which may influence the measured pain threshold, i.e. skin thickness, reflectance of laser light from the skin surface, and skin temperature were measured throughout the experiment. If these parameters changed during the experiments the pain threshold was compensated for this variability according to our earlier suggestions (11).

Pain threshold

The pain threshold to an argon laser stimulation was defined as a distinct, sharp pinprick without any burning after-sensation. The threshold was calculated as a mean of five ascending and five descending series of stimulations (11). The pain threshold was measured 3 min after removing the EMLA cream and the plastic film occlusion, and 5 min after local infiltration of lignocaine.

Itch rating

The intensity of histamine-induced itch was quantified on a 4-point scale: 0, no sensation; 1, mild itch; 2, moderate itch; 3, intense itch, wish to scratch. The itch intensity was scored 7 min after histamine skin prick.

Statistics

For statistical analysis, Wilcoxon's test was used. A probability of less than 5% was considered significant.

Experimental protocols

Experiment 1. Small anaesthetized areas (15 cm^2). Six 15 cm^2 circular target areas were marked on the volar side of the forearms. The areas were randomly allocated for control, infiltration, and EMLA application. Initially the pain threshold was measured in each area, and a histamine prick was performed in the control area. EMLA cream was applied for 30, 60, 90, and 120 min. After removal of EMLA cream the pain threshold was determined and a histamine skin prick was performed.

Experiment 2. Large anaesthetized areas (350 cm^2). Data obtained in experiment 1 showed that EMLA cream applied even for 120 min did not suppress the itch sensation, whereas the laser-induced pain was completely inhibited. An anaesthetized area of 15 cm^2 was generally sufficient to avoid reappearance of the flare outside the anesthetized area. In 2 volunteers, however, a peripheral reappearance of the flare and itch was observed. To evaluate the effect of the size of anaesthetized skin areas, a 15 cm broad lane of EMLA cream was applied around one forearm (area approximately 350 cm^2). The cream was applied under occlusion for 60 and 120 min, and the intensity of itch was scored 7 min after histamine skin pricks. Also, histamine skin pricks were applied simultaneously to the contralateral arm as controls. The itch intensity was rated and compared after each histamine prick on both arms.

RESULTS

Wheal reaction

The central wheal reaction was unaffected by local infiltration of lignocaine or by EMLA cream application.

Flare reaction

Compared with the control area, lignocaine infiltration reduced the flare area significantly by 91% ($p<0.01$, Fig. 1). Application of EMLA cream on intact human skin reduced the histamine flare area (Fig. 1). The inhibition of the flare area was dependent on the duration of EMLA application. Application times of 30, 60, and 90 min showed a linear reduction of the flare area. When EMLA cream was applied for 60 min or longer, the flare areas were significantly reduced ($p<0.01$) compared with the control flare areas. The reduction in flare area after 90 and 120 min did not differ significantly from the 60 min application. Also, no significant difference in flare area was seen after intradermally injected lignocaine or after the 90 and 120 min of EMLA applications. The degree of erythema of the flare did not

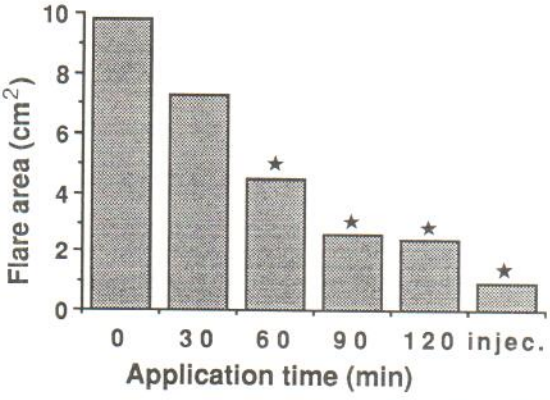

Fig. 1. Gradual inhibition of the histamine-induced flare areas following topical application (0, 30, 60, 90, and 120 min) of the analgesic cream (EMLA) and intradermal injection (*injec*) of lignocaine. Significant ($*p<0.01$) reduction of flare areas vis-à-vis normal, non-anaesthetized skin.

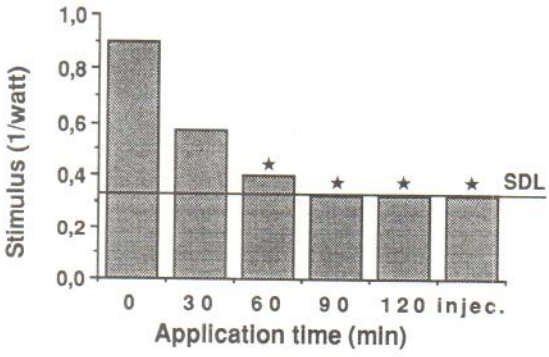

Fig. 2. Gradual decrease in excitability of pain-sensitive nerve fibres in topical EMLA- and lignocaine infiltration analgesia. *Ordinate*: 1/Watt of laser irradiation, eliciting a minimal pricking pain sensation. *SDL*: Skin Destruction Level of laser energy (Watts), at which the laser energy is sufficient to cause a skin burn. Significant ($*p<0.05$) reduction of excitability of pain-sensitive nerve fibres.

differ from that of the control flares. Also, the limits of the flare reactions were equally sharp and demarcated in the anaesthetized areas as in the control flares.

In 2 subjects the flare reappeared outside the circular 15 cm^2 anaesthetized area (EMLA 120 min), resulting in a 'halo flare reaction'. A histamine prick, placed on normal skin close to the border of an anaesthetized area (EMLA 120 min), resulted in a flare reaction confined to the normal, non-anaesthetized area.

Pain threshold

Total analgesia was defined as inability to feel warmth or pain when the argon laser stimulus intensity was increased to skin destruction level (3.0 W). Local infiltration of lignocaine caused total analgesia in all volunteers.

The pain thresholds increased linearly in response to increased EMLA application time and were significantly elevated after 60 min of EMLA cream application ($p<0.05$). In 5/11 volunteers, complete analgesia was obtained after 60 min of application, and after 120 min of application total analgesia was obtained in all cases (Fig. 2).

The intensity of itch perception

Intradermal lignocaine infiltration significantly reduced the mean itch intensity, from 2.1 to 0.2 on the subjective itch score scale.

In experiment 1 in which anaesthetized areas of 15 cm^2 were used, the itch rating following EMLA application did not change significantly. The mean itch ratings were 2.2, 2.2, 2.1, 2.1, and 1.9 after applications of 0, 30, 60, 90 and 120 min, respectively (Fig. 3).

In experiment 2 the anaesthetized area was increased more than 20-fold (to 350 cm^2). The mean itch rating was 2.3 before EMLA application. After 60 and 120 min EMLA application the mean itch ratings were 1.9 and 1.7, respectively. None of the volunteers experienced total inhibition of itch. Itch provoked by histamine skin pricks on the control areas (at 0 min, 60 min and 120 min) were rated 2.2, 2.1, and 2.3. The volunteers reported that itching generally persisted longer on the EMLA-treated areas, sometimes lasting up to 30 min (Fig. 4).

DISCUSSION

Wheal reaction

The central wheal reaction, which is a direct histamine effect on vessel walls, was not reduced by infiltrating local analgesics. Similar results were reported by Jancso et al. (12) and by Pipkorn & Andersson (13), who applied EMLA cream for one hour. We extended the EMLA application time to 2 h without any effect on the wheal. In vitro studies have shown that local analgesics inhibit rat mast cell release of inflammatory mediators (14), but this effect could not be demonstrated in humans.

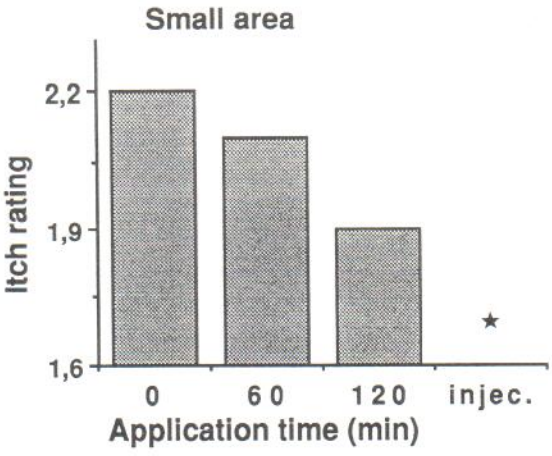

Fig. 3. Subjective rating of itch intensity on *small* anaesthetized skin areas (15 cm^2) after various EMLA cream application times, and after local infiltration of lignocaine. Only lignocaine infiltration suppressed the itch sensation. Significant (*$p<0.05$) reduction of itch sensation.

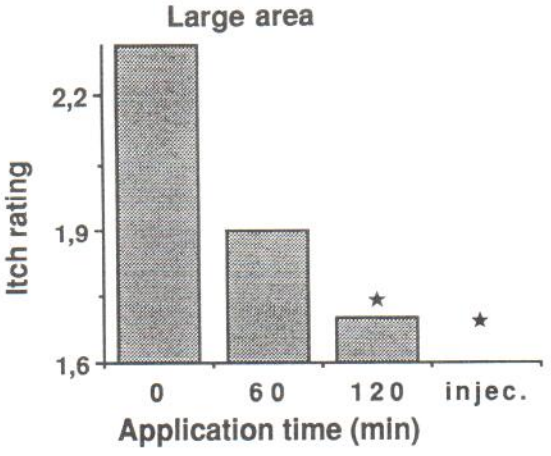

Fig. 4. Subjective rating of itch intensity on *large* anaesthetized skin areas (350 cm^2) after different EMLA cream application times, and after local infiltration of lignocaine. EMLA cream applied for 120 min significantly reduced the itch intensity, while only lignocaine infiltration suppressed the itch sensation. Significant (*$p<0.05$) reduction of itch sensation.

Flare reaction

Two models may describe cutaneous flare reactions. The first model assumes that the erythema is mediated through a neuron short arc axon reflex by ortho- and antidromic propagation of the axon potential to other branches of the same nerve fibre (3, 15). In another model (16) the spread of flare is due to a cascade of mediator release between neurons arranged in parallel. Support for the model of neurally mediated spread rather than the cascade model comes from an experiment (17) in which capsaicin-induced flare on the forehead in humans did not spread across the midline to the area innervated by the contralateral trigeminal nerve. Also, according to the model of neurally mediated flare reaction, the flare is suggested to be confined to the most distal arborizations of the neurons located near the inflamed area, and therefore the flare should be sensitive to local analgesics. The extent of flare areas can then be limited by two anatomic elements: 1) the size of the skin area supplied by the collateral axon network of fibres which terminate near the histamine skin prick, and 2) the spatial distribution of blood vessels innervated by collaterals of fibres terminating near a histamine skin prick.

There is little evidence in humans that the receptive fields of C-fibre innervated nociceptors in the skin are comparable to those found for the flare response areas. Torebjörk (18) examined the size of the receptor area for individual fibres in the skin by microneurographic recording, and found that the innervation field was in the order of 1 cm^2. The flare area to histamine is much larger, and is therefore not assumed to be caused by activity in a single nerve fibre alone. In the present study we found that the flare might reappear outside the EMLA anaesthetized area. This may be caused by collaterals from active fibres terminating in the anaesthetized area, and it was observed only in the small (15 cm^2) anaesthetized areas but not in the large (350 cm^2) areas.

Effect of local analgesics on the flare reaction

Application of local analgesics may selectively inhibit the flare component of the acute inflammatory reaction elicited by histamine skin pricks.

Injection of local analgesics and application of topical EMLA cream have previously been found to inhibit the flare reaction to histamine (12, 17). EMLA cream was only tested in a one hour application, which did not abolish the flare, and it was concluded that the flare was only partly a reflectory neural inflammatory response (12). In the present study, the EMLA application time was extended, and it is evident that the area of the flare can be inhibited quantitatively, depending on application time. Several mechanisms may explain this: 1) a gradual inhibition of vasomotor activity, 2) a quantitative membrane stabilization of mast cells (13, 19), and 3) a quantita-

tive inhibition of neuropeptide release from the distal terminations of the sensory nerve fibres (20). This inhibitory effect of local anaesthetics may have clinical applications in skin surgery and laser treatment of haemangiomas and teleangiectasias, where the neurogenic inflammatory induced vasodilation may be inconvenient. For this reason, it may be advantageous to use local anaesthetics even in general anaesthesia.

The actual mechanism of the inhibitory effect of local analgesics on the flare reaction is still not known. Lidocaine and prilocaine prevent transmembrane Na^+ and K^+ flux and thus depolarization of nervous membranes (21, 22), and in vitro studies show that mast cells are stabilized by inhibition of transmembrane Ca^{++} flux (23). Also, inhibition of antidromic secretion of neurotransmitters from small diameter sensory afferent nerves in the periphery of an inflamed area my be a consequence of a diminished orthodromic sensory response.

Effect of local analgesics on pain threshold

The analgesic effect of EMLA cream, evaluated by the pain threshold to argon laser light increased linearly as the flare reaction abated. The pain-sensitive cutaneous receptors play a decisive role in the inflammatory reaction. By using lasers for cutaneous pain stimulation, it was possible to elicit a sharp Aδ-fibre mediated pricking pain (11), and to correlate the excitability (thresholds) to the acute inflammatory responses. Our findings suggest that the excitability of the nociceptors may be a sensitive indicator for the cutaneous responsiveness to neurogenic inflammation.

Effect of local analgesics on itch perception

Itch is mediated by fine, unmyelinated C-fibres. Burning pain and itch have long been regarded as sharing a common neural mechanism (24, 25, 26), but it is still open to discussion whether these sensory modalities are mediated by the same sensory neurons or by separate populations of C-fibres (27). LaMotte et al. (28) recently showed that itch mediating fibres are a subpopulation of the C-fibres.

The neural basis of itch is therefore still controversial. Pipkorn & Andersson (13) found that 2 g of EMLA cream applied for one hour suppressed the itch sensation. We found a both application time- and area-dependent reduction of the itch intensity by EMLA, while infiltration of lignocaine strongly reduced the mean itch score. We suggest that itch and pricking pain are mediated in different fibres, because itch can be evoked from EMLA anaesthetized areas in which the sensation of pricking pain is abolished.

Clinical aspects

The present results indicate that procedures which release inflammatory mediators (plastic surgery, dermabrasio, laser treatment of haemangiomas and teleangiectasias, etc.), should be performed with local analgesia. This may inhibit the neurogenic component of local inflammation both during the tissue trauma and in the postoperative period. Since the reduction of flare area following histamine skin prick is correlated to the level of analgesia determined by laser stimulation, the excitability of the nociceptors (measured by pain thresholds) may indicate the actual cutaneous responsiveness to neurogenic inflammation. The wheal is not affected by local analgesics, and therefore in children, skin prick tests may be performed with EMLA analgesia without affecting the result of the test.

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