INVESTIGATIVE REPORTS

Cutaneous Microdialysis in Man: Effects of Needle Insertion Trauma and Anaesthesia on Skin Perfusion, Erythema and Skin Thickness

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Cutaneous microdialysis is a method of measuring endogenous and exogenous compounds in the dermal interstitial fluid. The microdialysis probe is inserted in the dermis using a guide cannula.

The insertion trauma was studied in dorsal forearm skin in a total of 28 human healthy volunteers. Twenty-four volunteers received local anaesthesia (Xylocain® 10 mg/ml) in both forearms and a microdialysis probe was inserted in one of the arms. In 12 volunteers the insertion trauma and the effect of anaesthesia on skin blood flow and erythema were studied by laser Doppler perfusion imaging, Minolta Chromameter CR 200® and Dermaspectrometer®. In the other 12 subjects trauma-induced oedema and effects on skin thickness were studied by ultrasound imaging. In addition, a microdialysis probe was inserted without prior anaesthesia in 4 volunteers, and the effects on skin blood flow and erythema were investigated.

Significant increases in skin blood flow, erythema and skin thickness were found after insertion of the microdialysis probe. Local anaesthesia prior to the insertion reduced the effects of trauma. Probe depth in dermis did not influence the effects of trauma. At least 90–120 min is required after insertion in order to allow the vascular reaction to needle trauma to return to the baseline range. Key words: laser Doppler perfusion imaging; ultrasound imaging; Dermaspectrometer; Minolta Chromameter.

(Materials and Methods)

Subjects

Twenty-eight healthy volunteers, without a history of atopic dermatitis, allergy or asthma, were included in the study. Subjects were excluded if they had any skin disease or an allergic reaction towards lidocaine.

Significant increases in skin blood flow, erythema and skin thickness were found after insertion of the microdialysis probe. Local anaesthesia prior to the insertion reduced the effects of trauma. Probe depth in dermis did not influence the effects of trauma. At least 90–120 min is required after insertion in order to allow the vascular reaction to needle trauma to return to the baseline range. Key words: laser Doppler perfusion imaging; ultrasound imaging; Dermaspectrometer; Minolta Chromameter.

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Cutaneous microdialysis is a new field in dermatological research. The technique allows in vivo study of the skin as an alternative or supplement to the skin blister technique (1), the tape stripping technique (2), skin biopsies (2) and the traditional in vitro permeation studies (3).

Microdialysis is a sampling technique to sample substances in the extracellular space. The technique was developed in neurosciences to study neural transmitters in laboratory animals (4). Recently cutaneous microdialysis was used to study ethanol absorption across the skin (5), histamine release in the skin (6) and transdermal drug transport of nicotine (7). Our ultimate aim is to use cutaneous microdialysis to study drug penetration.

The single lumen microdialysis probe consists of a tubular dialysis membrane, glued to an effluent and efferent tubing. The probe is inserted within the dermis using a guide cannula. We
Parts 1 and 2 kept throughout the study. Three measurements were taken each time. Chromameter and then with the Dermaspectrometer. This order was immediately after blood flow measurements, first with the Minolta Chromameter and the Dermaspectrometer. Erythema was measured were taken each time. The mean values will be used.

The study was divided into three parts:

**Study of effect of local anaesthesia and of insertion of a microdialysis probe, Part 1**

Twelve healthy volunteers (7 males and 5 females, mean age 24 years, range 21–28 years) a microdialysis probe was inserted in the dorsal forearm of both arms. The lidocaine was dispersed in marked areas of 3 × 4 cm. In one of the arms a microdialysis probe was inserted in the middle of the test area to a length of 3 cm. The other arm served as a control arm to determine the effect of insertion of a microdialysis probe on the total skin erythema index is expected to have a roughly linear relationship with 50, 60, 80, 100 and 120 min after insertion. The arm that was only anaesthetized was measured at the same time. Prior to skin thickness measurements an ultrasonic coupling gel was applied to the test area. Three B-scans were taken at every time from different positions along the intradermal probe. The Dermascan-C was operated with a linearly increasing gain, adjusted for each subject in order to individualize and optimize the measurement. A typical gain curve was operated at 15 dB to 30 dB. The probe head of the Dermascan-C was oriented so that the scan line was 90 degrees to the dialysis membrane. A-mode scanning of the microdialysis probe was used to measure skin thickness, defined as the distance between the epidermis entrance echo and the exit of the microdialysis membrane. Three scans at every time were used to determine the mean of the probe depth.

**Study of effect of insertion of a microdialysis probe without prior anaesthesia, Part 3**

In 4 subjects (3 males and one female, mean age 27 years, range 22–32 years) a microdialysis probe was inserted in the skin of the dorsal forearm. The probe was placed in the centre of the 3 × 4 cm area. Left and right arm were randomized. Two had the probe inserted in the right arm and 2 in the left arm, without prior anaesthesia. Non-invasive measurements were performed before and after probe insertion. After the experiment the depth of the probe was measured by ultrasound imaging. Three scans at every time were used to determine the mean of the probe depth.

**Statistical analysis**

**Parts 1 and 2.** The values on the left and right forearm were compared at each time by a paired t-test, and sites with and without needle insertion were compared.
inserted were analyzed. Local anaesthesia without probe insertion was used as a baseline.

**Part 3.** The difference before and after insertion of the probe was compared at each time by paired *t*-tests.

In addition, in each study a *t*-test was performed in order to evaluate if probe depth had any influence on the measured responses (skin blood flow, skin colour, erythema and skin thickness).

**RESULTS**

*Study of effect of local anaesthesia and of insertion of a microdialysis probe, Part 1*

Significant increases in skin blood flow (*p* < 0.01), skin colour (*a*<sup>*</sup>, erythema, Minolta) (*p* < 0.01) and erythema (Dermaspectrometer) (*p* < 0.01) were found after the insertion of the microdialysis probe (Figs. 1–3). The skin blood flow did not normalize until 90–120 min after insertion. At least 60–80 min were needed for the erythema to normalize, as measured with the Minolta. The time-to-normalization for erythema measured by the Dermaspectrometer was longer, i.e. 100 min.

*Study of effect of insertion of a microdialysis probe on the total skin thickness as a measure of traumatic oedema, Part 2*

Increase in skin thickness was observed following insertion of the microdialysis probe (Fig. 4). The difference between the arm with and without insertion of a probe was highly significant throughout the experiment (*p* < 0.01). The outer diameter of the membrane, 0.216 mm, contributed of course to skin thickening. However, even if this constant was subtracted, the difference remained significant at any time. The reaction peaked 10 min after probe insertion and dropped to a constant level, which corresponded to a relative skin thickening of 38%.

*Study of effect of insertion of a microdialysis probe without prior anaesthesia, Part 3*

Increase in skin blood flow (*p* < 0.01), skin colour (*p* < 0.01) and erythema (*p* < 0.01) was found after probe insertion (Figs. 1–3). Baseline blood flow was reobtained after 90–120 min. Difference before and 40 min after needle insertion was non-significant for skin colour, indicating that pre-insertion level was reached. However, care must be taken to base the normalisation time entirely on the obtained *p*-values, since this study was more variable than the study with local anaesthesia (Part 1). The same considerations are relevant for erythema measurements. The difference in erythema was no longer significant 30–40 min after insertion of the probe. Figs. 2–3 suggest that skin colour and erythema did not return to baseline until after 100 min.

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*Fig. 1.* Mean skin blood flow (Volt) in human skin as a function of time before and after insertion of a microdialysis probe (mean ± SD). *n* = 12 insertions for Part 1 (anaesthesia), *n* = 4 for Part 3 (no anaesthesia). Solid square: no probe, Part 1 (control arm), solid circle: probe inserted, Part 1, open circle: probe inserted, Part 3. B: baseline before insertion, B/A: baseline after anaesthesia, 0: immediately after probe insertion.

*Fig. 2.* Mean skin colour in human skin as a function of time before and after insertion of a microdialysis probe (mean ± SD). Symbols, see Fig. 1.

*Fig. 3.* Mean erythema in human skin as a function of time before and after insertion of a microdialysis probe (mean ± SD). Symbols, see Fig. 1.

*Fig. 4.* Mean skin thickness in human skin as a function of time before and after insertion of a microdialysis probe (mean ± SD). Symbols, see Fig. 1.
Skin thickness was relatively constant. A mean skin thickness of 0.98 ± 0.13 mm for Parts 1, 2 and 3, respectively. Probe depth was relatively constant at 1.30 ± 0.13 mm (n = 24) was found, which indicates that the probe was placed in the lower part of the dermis. However, B-mode scanning showed that the probe was located within the reticular dermis in every case. The reason for the large probe depth in one study, i.e. the study without prior anaesthesia, might be explained by reactions to pain influencing the procedure.

Probe depth had no influence on the effects of trauma in any parts of the study.

**DISCUSSION**

Human skin was affected by insertion of a microdialysis probe with respect to skin blood flow, skin colour, erythema and skin thickness. Except for skin thickness, the reactions to the insertion appeared momentarily.

The local anaesthesia reduced the vascular effects of microdialysis probe insertion. Pipkorn & Andersson (15) stated that topical dermal anaesthesia inhibited the flare but not the weak response to allergen and histamine prick-test. Injection of a local anaesthetic subcutaneously exerted a minor effect on the vasculature in the present study; however, this effect was overshadowed by the much stronger effects of needle trauma.

The precise time until the skin had normalized concerning skin blood flow, skin colour and erythema is not well defined. It would be erroneous to estimate the period of equilibration simply by the p-values, as large variations were found. However, the study indicates that skin blood flow required 90–120 min to stabilize after probe injection, with no difference related to anaesthesia. Erythema reached baseline level approximately 90 min after insertion of the probe in the anaesthetized skin. Skin thickness was constantly increased and may not be normal until the microdialysis probe is removed. The study period should have been longer to make it possible to estimate when the skin thickness normalized. For practical reasons the skin thickness was not taken in consideration when the equilibration period was determined.

Skin thickening is mainly due to oedema formation in the skin. Low echogenic picture elements were observed in the ultrasound image around the probe. These picture elements are known to be proportional to the degree of dermal oedema (16). The oedema formation around the microdialysis probe may increase the diffusion area and thereby result in increased recovery (17, 18). However, we suppose that the degree of oedema is constant during the presence of the probe in the dermis, without any further influence on recovery.

After insertion of a commercial microdialysis probe in the skin Anderson et al. (19) also found increased skin perfusion measured by LDPI. After 60 min the skin perfusion was close to resting level. The increase in skin perfusion depended on probe depth, i.e. the increase was more pronounced with a more superficial probe level, though their observation was only based on two individuals. We could not demonstrate any relationship between probe depth and trauma.

In the study of Anderson et al. the vascular effects due to the insertion disappeared when the skin was anaesthetized before the insertion. We only found a reduction of the vascular effects when local anaesthesia was used. This difference can be explained by difference in study design. We used the dorsal forearm, which ensures a more comfortable position during the experiment, and the local anaesthesia was given as a subcutaneous injection, in contrast to the ventral forearm and intracutaneous injection of anaesthetic used by Anderson et al. The intracutaneous injection might block the axon reflexes within the dermis more efficiently.

Petersen et al. (17) suggested an equilibration period of 90–135 min after insertion of a probe, similar to the one we
use, according to laser Doppler flowmetry. Kroqstad et al. (20) did not find complete normalization of the skin perfusion after insertion of a probe like ours. They speculated that axon reflexes were evoked by mechanical manipulation of the probe, since local anaesthesia was not used prior to insertion.

In the hairless rat increased skin blood flow and skin thickness were also apparent after insertion of a microdialysis probe. After approximately 30 min the skin perfusion was stable and close to the baseline level, i.e. a faster normalization than in human skin. Normalization in skin thickness did not occur, comparable to human skin.

CONCLUSION

Increase in skin blood flow, skin colour (redness) and erythema and skin thickness was demonstrated after insertion of a microdialysis probe. A 90–120-min period of equilibration is required in human skin, allowing the vascular reactions of the skin to stabilize. Local anaesthesia prior to insertion reduced the vascular effects of needle insertion trauma. The local anaesthesia injection itself induced a minor trauma which, however, declined rapidly. Probe depth did not have any influence on the needle insertion trauma.

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