

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.

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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 afferent and efferent tubings. The probe is inserted within the dermis using a guide cannula. We

have earlier demonstrated in hairless rats that insertion of a microdialysis probe results in increased skin blood flow and histamine release (8). Increase in skin thickness due to oedema formation was also found in rat skin following probe insertion (9). It was concluded that a minimum equilibration period, i.e. the time required for the skin to return to normal state, of 30 min after probe insertion was necessary.

The aim of the present study was to determine the equilibration period in human skin by skin blood flow, erythema and skin thickness measurements. In addition, the effect of local anaesthesia prior to the insertion was studied.

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. The subject was not allowed to use any local or systemic medication, including oral contraception, or to use any skin care products 24 h before the investigation.

All subjects gave their informed consent. The study was approved by the regional Ethics committee (KA 95198g).

Insertion of the microdialysis probe

Microdialysis membranes obtained from an “artificial” kidney (Gambro GFE 12, Gambro Dialysaten AG, Hechingen, Germany, outer diameter 216 µ, wall thickness 8 µ) were inserted horizontally (intradermally) within the dermis, using a guide cannula, 21-Gauge (length 40.0 mm i.d. 0.80 mm). The tubular microdialysis membrane was inserted through the guide cannula. The guide was then withdrawn, leaving the membrane horizontally within the dermis. The length of the membrane was 3 cm. Inlet and outlet tubings were then glued to the microdialysis membrane, completing a microdialysis probe.

Laser Doppler perfusion imaging of cutaneous blood flow

The blood flow in the skin was imaged and measured by laser Doppler perfusion imaging, LDPI (PIM 1.0, Lisca Development AB, Linköping, Sweden). PIM 1.0 is a computer-controlled system, which performs two-dimensional scanning of the tissue blood perfusion using a low power He–Ne laser. Backscattered Doppler-shifted light, caused by moving red blood corpuscles, is detected and converted to an electrical signal, which is linearly proportional to tissue blood perfusion (10). The measurements of the test site provide a colour-coded image on the display. Results are expressed in arbitrary units. Data analysis can be performed using manufacturer’s software. The scan head was positioned 17.5 cm above the test area. The resolution was set at high and the background threshold at 6.1.

The spatial resolution was 0.74 mm². The orientation of the scanning was parallel to the arm. The LDI version 2.5 software was used.

Minolta chromameter

Skin colour and erythema were measured by the Minolta Chromameter CR 200® (Osaka, Japan). The light source is a high-power xenon arc lamp. Measurements are based on the CIE-system and the parameter a^* , which is a measure of erythema (11). This system was developed as a correlate to the colour perception of the human eye. The apparatus provides good accuracy and reproducibility. Before measurement on a subject the Minolta Chromameter was calibrated using a white calibration tile. During measurements the apparatus was kept in a perpendicular fashion to the skin surface, and the head of the instrument only touched the skin surface slightly.

Dermaspectrometer

Erythema was also measured by the Dermaspectrometer® (Cortex Technology, Hadsund, Denmark), which operates with a spectral measuring principle. Erythema and melanin indices are derived from the reflection of the object in a selected band of the spectrum. The erythema index is expected to have a roughly linear relationship with red blood cells in the upper dermis (12). Before measurement on a subject, calibration of the Dermaspectrometer was performed with a white standard surface. During measurements the measuring head was held perpendicularly to the skin and only touched the skin very softly.

Ultrasound examination of the skin

Skin thickness was measured by 20 MHz ultrasound scanning, using the Dermascan-C (Cortex Technology, Hadsund, Denmark). High-frequency ultrasound is a non-invasive method for *in vivo* imaging of the skin structure (13). The accuracy of this method for skin thickness measurements is about 0.1 mm (14).

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 26, range 20–37 years) were bilaterally anaesthetized with 3 ml subcutaneously injected lidocaine (Xylocain®, 10 mg/ml, Astra, Sweden) 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 possible effect of lidocaine. The arms were randomised as regards right and left arm. Six subjects had the probe inserted in the left arm and 6 in the right arm.

Non-invasive measurements were performed before and after anaesthesia and probe insertion on both arms in parallel. After injection of lidocaine 10 min elapsed before the probe was inserted, allowing the effect of anaesthesia to develop. After the experiment the depth of the probe was measured by ultrasound imaging, see below.

Skin blood flow measurements. Skin blood flow was measured by the LDPI in normal skin (before any treatment), after lidocaine was injected and immediately after the probe insertion, followed by measurements at 10, 20, 30, 40, 50, 60, 80, 100 and 120 min after insertion. The arm that was only anaesthetized was measured at the same time as the one with the membrane inserted. The test area was 22×44 measuring points (a total of 968 measuring points in the 3×4 cm area), which equals the analysis area. The site of insertion and exit of the probe were marked with blue ink. They were seen as grey points in the colour-coded image and they were not included in the calculation.

Erythema measurements. Erythema was measured using the Minolta Chromameter and the Dermaspectrometer. Erythema was measured immediately after blood flow measurements, first with the Minolta Chromameter and then with the Dermaspectrometer. This order was kept throughout the study. Three measurements were taken each time. The mean values will be used.

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

Twelve healthy volunteers (7 males and 5 females, mean age 24 years, range 21–28 years) were anaesthetized with 3 ml subcutaneously injected lidocaine (Xylocain®, 10 mg/ml, Astra, Sweden) in both forearms. The lidocaine was dispersed in marked areas of 3×4 cm using 3 injections. 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 plain lidocaine. The arms were randomised as regards right and left arm. Six subjects had the probe inserted in the left arm and 6 in the right arm.

Skin thickness was measured before anaesthesia, after anaesthesia and after probe insertion on both arms in parallel. After injection of lidocaine 10 min elapsed before the probe was inserted, allowing the anaesthesia to develop. The probe depth was also determined.

Skin thickness measurement. Skin thickness was measured before anaesthesia, after lidocaine was injected and immediately after the insertion of the probe, followed by measurements at 10, 20, 30, 40, 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 interface between dermis and subcutis. Ultrasound image analysis of scans was performed using the ROI function and the inbuilt software of the Dermascan-C.

Probe depth. The probe depth, i.e. the distance from the skin surface to the dialysis membrane, was measured on the scans used for total skin thickness measurements. The microdialysis membrane is echodense in structure and can easily be visualized by ultrasound. The microdialysis membrane appears as a white hyper-reflecting dot. In A-mode scan probe depth corresponds to the vertical distance between the epidermis entrance echo and the echo 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.

Blood flow measurements. LDPI scans (22×44 measuring points) were performed prior to and immediately after probe insertion, followed by measurements at 10, 20, 30, 40, 50, 60, 80, 100 and 120 min. The site of insertion and exit of the probe were marked with blue ink. They were seen as grey points in the colour-code image and they were not included in the calculation. The analysis area was 22×44 measuring points.

Erythema measurements. Erythema was measured before and after probe insertion using the Minolta Chromameter and the Dermaspectrometer and following LDPI scans as described above, i.e. first Minolta and then the Dermaspectrometer. Three measurements were taken each time. The mean values will be used.

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

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^* , 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%.

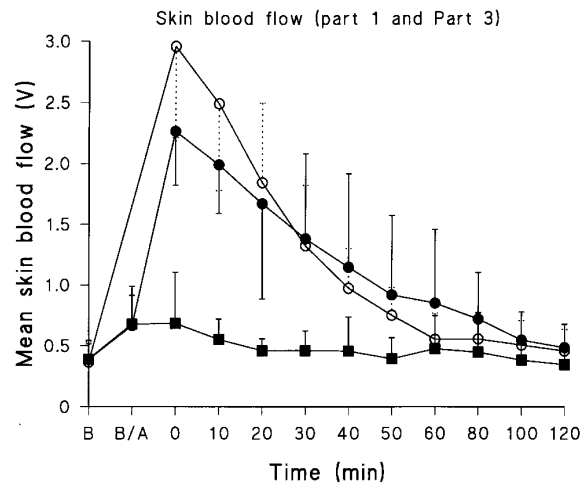


Fig. 1. Mean skin blood flow (Volt) in human skin as a function of time before and after insertion of a microdialysis probe (mean \pm SD). $n = 12$ insertions for Part 1 (anaesthesia), $n = 4$ for Part 3 (no anaesthesia). Solid square: no probe, Part 1 (control arm), solid circle: probe inserted, Part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

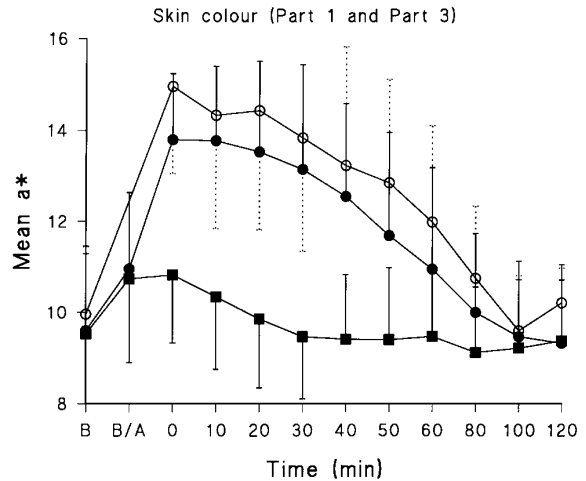


Fig. 2. Mean skin colour in human skin as a function of time before and after insertion of a microdialysis probe (mean \pm SD). Symbols, see Fig. 1.

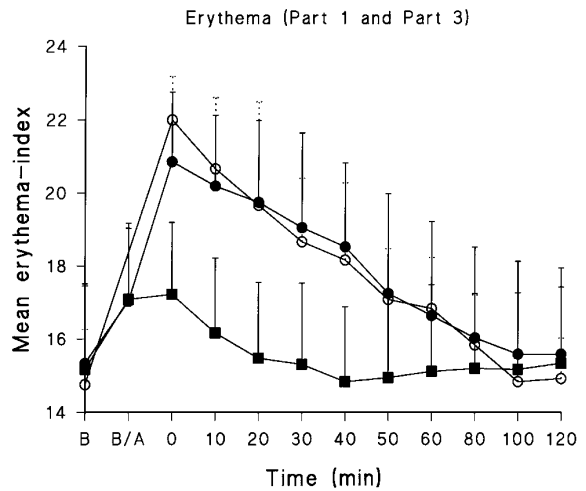


Fig. 3. Mean erythema in human skin as a function of time before and after insertion of a microdialysis probe (mean \pm SD). Symbols, see Fig. 1.

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.

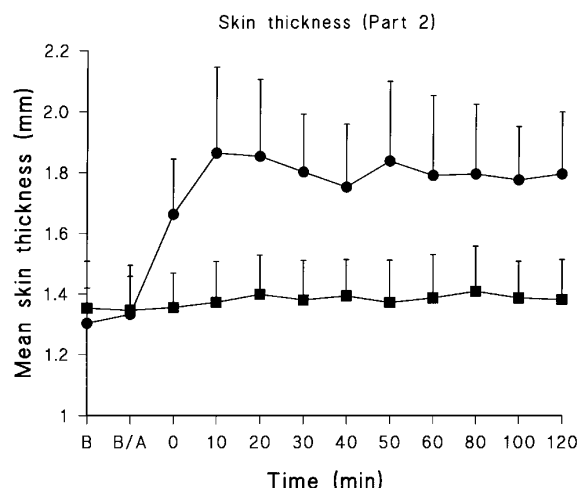


Fig. 4. Mean skin thickness in human skin as a function of time before and after insertion of a microdialysis probe (mean \pm SD), $n=12$ insertions for Part 2 (anaesthesia). Solid square: no probe, Part 2 (control arm), solid circle: probe inserted, Part 2. B: baseline before insertion, B/A: baseline after anaesthesia, 0: immediately after probe insertion.

Comparison of effects of insertion with or without prior anaesthesia.

Differences from baseline (before anaesthesia and insertion) for skin blood flow, skin colour and erythema as a function of time showed that, for all three parameters the mean differences from baseline were always greater in the study without prior anaesthesia as compared with the study with anaesthesia. For erythema six of ten differences at various measurements were significant, i.e. higher responses without prior anaesthesia (independent t -test at any time between Part 1 and Part 3, $p < 0.05$), whereas skin colour and blood flow showed a significant difference three times each. This shows that trauma after insertion of a microdialysis probe was reduced by the injection of local anaesthesia prior to insertion, as compared with no local anaesthesia.

Effect of local anaesthesia

Skin blood flow, skin colour and erythema after injection of lidocaine increased significantly. This effect was diminished 20 min after lidocaine was administered. No effect on skin thickness was found.

Probe depth

Probe depth was measured in every trauma study. The mean probe depth was 0.98 ± 0.22 mm, 0.79 ± 0.15 mm and 1.31 ± 0.13 mm for Parts 1, 2 and 3, respectively. Probe depth was relatively constant. A mean skin thickness of 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 larger probe depth in one study, i.e. the study without prior anaesthe-

sia, 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 weal 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. Krogstad 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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