Acta Derm Venereol (Stockh) 1998; 78: 10–14

Cutaneous Microdialysis in the Rat: Insertion Trauma Studied by Ultrasound Imaging

LOTTE GROTH[1,2], AKSEL JØRGENSEN[1] and JØRGEN SERUP[1]

[1]*Department of Dermatological Research, Leo Pharmaceutical Products, Ballerup, and* [2]*Department of Pharmaceutics, The Royal Danish School of Pharmacy, Copenhagen, Denmark*

Microdialysis is a method by which compounds can be studied in the extracellular space in skin, *in vivo*. The microdialysis probe is inserted in the dermis using a guide cannula. It is expected that intradermal oedema associated with insertion trauma as well as the probe depth can influence the results of microdialysis studies. The aim of the present study was to assess the effects of insertion trauma and, additionally, the probe depth by ultrasound. High-frequency (20 MHz) ultrasound is a non-invasive method for measuring the thickness and echostructure of skin. Hairless rats were anaesthetized with either halothane ($n=7$) or pentobarbital sodium ($n=6$). The insertion of the microdialysis probe resulted in a 38% relative increase in skin thickness. At 120 min the skin thickness had not reached the pre-insertion value. Thus, significant skin thickening, representing traumatic oedema, developed due to insertion of the microdialysis probe in the skin. The microdialysis probe could be inserted reproducibly in the lower dermis. *Key words: skin; ultrasound; halothane; pentobarbital sodium.*

(Accepted June 25, 1997.)

Acta Derm Venereol (Stockh) 1998; 78: 10–14.

L. Groth, Department of Dermatological Research, Leo Pharmaceutical Products, DK-2750 Ballerup, Denmark.

Microdialysis is a technique to monitor levels of compounds in the extracellular space over time (1). The method has been used in experimental neuropharmacology for two decades, but is has only recently been adopted in dermatological research (2, 3). Cutaneous microdialysis can be used to study *in vivo* skin penetration of drugs from topical formulations.

A microdialysis probe is a tubular semipermeable dialysis membrane, connected to afferent and efferent tubings. The microdialysis probe is inserted in the dermis using a guide cannula. This causes trauma in the skin (4). Effects of trauma, especially oedema and vasodilatation, may influence the skin penetration and the microdialysis process with consequences for the results of the microdialysis experiment. Furthermore, the trauma may release various mediators, e.g. histamine, which cause the oedema. It is thus important to determine the period the skin needs in order to recover after probe insertion.

High-frequency ultrasound is an advanced method for two-dimensional *in vivo* imaging of the skin (5). By using B-mode scanning *in vivo* distances can be measured on a purely non-invasive basis, including skin thickness and the distance from the probe to the skin surface, i.e. the probe depth. Ultrasound examination of the skin has been used as a standard method to investigate irritant reactions, allergic reactions and various types of oedema in human and animal skin (6–9).

In this study the effect of insertion of a microdialysis probe on skin thickness was measured in rats by ultrasound. In addition, the probe depth was measured to evaluate the reproducibility of the insertion procedure. Probe depth in the dermis is also expected to influence the results of a drug penetration experiment.

MATERIALS AND METHODS

Experimental conditions

Two groups of rats were studied, since anaesthesia might influence reactions of trauma, as the two anaesthetics may influence the vascularisation and the release of mediators differently.

Method of anaesthesia

Halothane anaesthesia. Seven hairless rats (200 g, female, OFA-hr/hr, IFFA CREDO) were used. The rats were anaesthetized with halothane (Halothan "halocarbon", Halocarbon Laboratories, North Augusta, SC, USA), evaporated by a vaporizer (PPV sigma, Penlon LTD., Abingdon, England) and connected to an open face mask. Oxygen and nitrous oxide were delivered with halothane at a total flow rate of 700 ml/min (1 : 1). At the induction of the anaesthesia the halothane concentration was 4–5%. Subsequently the concentration was adjusted depending on depth of anaesthesia, starting from approximately 2%. After induction the rats were placed on a temperature controller (CMA/150, CMA/Microdialysis, Stockholm, Sweden).

Pentobarbital sodium (Mebumal, DAK) anaesthesia. Six hairless rats (200 g, female, OFA-hr/hr, IFFA CREDO) were anaesthetized with 50 mg/kg pentobarbital sodium, injected in the peritoneal cavity, and placed on a temperature controller as mentioned above.

Insertion of the microdialysis probe

A microdialysis membrane obtained from an "artificial" kidney (Gambro GFE 12, Gambro Dialysaten AG, Hechingen, Germany, outer diameter 216 μm, wall thickness 8 μm) was inserted horizontally (intradermally) within the dermis using a guide cannula, 21-Gauge, i.d. 0.80 mm, length 40.0 mm. The microdialysis membrane was inserted through the guide cannula. The guide was then withdrawn, leaving the 4-cm membrane horizontally within the dermis. Two microdialysis probes were inserted into the skin of each of the 7 rats anaesthetized with halothane, i.e. one probe on each side of the back. In each of the 6 rats anaesthetized with pentobarbital sodium one probe was inserted on the right side of the back.

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. The instrument consists of three main parts: the C-probe, the elaboration and visualization system and the memorizing and data-storing system. The probe is surrounded by water and sealed at the point of contact with an ultra-thin plastic diaphragm. The C-probe only touches the skin surface very gently and no rubbing is involved. The intensity of the reflection echoes is evaluated by the microprocessor and is visualized as a colour-coded two-dimensional B-mode image (Fig. 1). In A-mode interfaces are shown as well-defined peaks. The accuracy of this method for skin thickness measurements is about 0.1 mm (10).

Skin thickness measurements. Skin thickness was measured prior to and immediately after probe insertion, followed by measurements at 15, 30, 45, 60, 90 and 120 min. Three B-scans were taken at every time point from different positions along the intradermal probe.

 ISSN 0001-5555

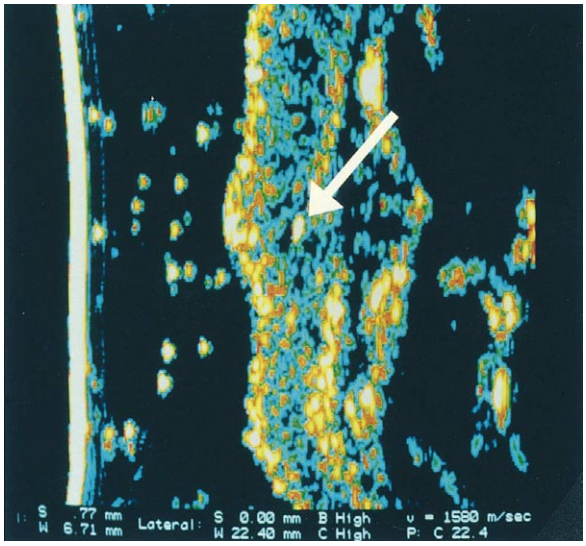

Fig. 1. A B-mode scan of rat skin. The marked *white dot* represents the microdialysis membrane within the dermis.

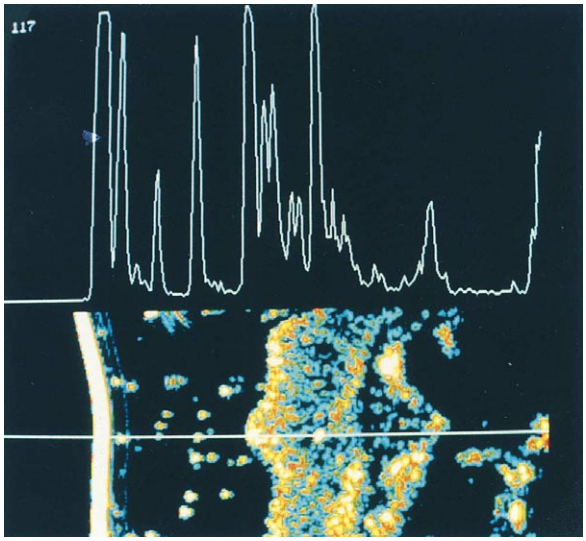

Fig. 2. A-mode scan (*upper half*) and B-mode scan (*lower half*) of rat skin. An entrance echo (*left*) corresponding to the epidermal surface and the interphase between the dermis and the subcutaneous tissue (*right*) are seen. Approximately in the middle an echo of the microdialysis membrane is seen.

The Dermascan-C was operated with a linearly increasing gain, adjusted for each rat in order to individualize and optimize the operation of the instrument. A typical gain curve was 25dB to 55dB. The velocity of ultrasound in the skin was set at 1,580 m/s. Ultrasonic coupling gel was applied to the test area. The ultrasound probe was oriented so that the scan line was 90 degrees to the tubular dialysis membrane. A-mode scanning of the area over the microdialysis probe was used to calculate 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 inbuild software of the Dermascan C. The individual scans were stored on floppy disks. All assessments were based on the mean of three recordings of any inserted probe.

Probe depth measurements. The probe depth, i.e. the distance from the skin surface to the dialysis membrane, was measured on the B-scans used for total skin thickness measurements. The microdialysis membrane is echo-dense in structure and can easily be visualized by ultrasound (Fig. 1). 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, which can be calculated by image analysis (Fig. 2). Triple measurements along every probe at any time served to assess the variation in probe depth, i.e. intra-probe variation in depth.

Animal experiments were performed at a constant room temperature of 25°C. Temperature and humidity in the animal room were recorded by a thermohygrograph throughout the study. Three ml saline were delivered to the rats every hour during the experiment.

Statistical analysis

Increase in skin thickness after probe insertion was evaluated as the difference between skin thickness before and after cannula insertion. Additionally, the difference between the skin thickness before and 15 min after insertion was calculated. A one-sample *t*-test was used to analyze the increase in skin thickness. Probability of <0.05 was considered significant. Finally, to elucidate if skin thickness reached the pre-insertion value over time, the difference between skin thickness before and at 120 min after insertion was calculated.

RESULTS

Effect of insertion of the microdialysis probe on total skin thickness as a measure of traumatic oedema

Halothane group. The mean basal skin thickness (before insertion) on the back of the rats was $1.12\,\text{mm} \pm 0.08$, range 1.00–1.26 mm. Fig. 3 shows mean skin thickness as a function of time following 14 probe insertions. Increase in skin thickness after probe insertion is obvious. The skin thickness peaks around 15–30 min. The mean increase in skin thickness was 0.32 ± 0.14, range 0.08–0.61 ($p < 0.05$, difference between thickness before and immediately after probe insertion). The

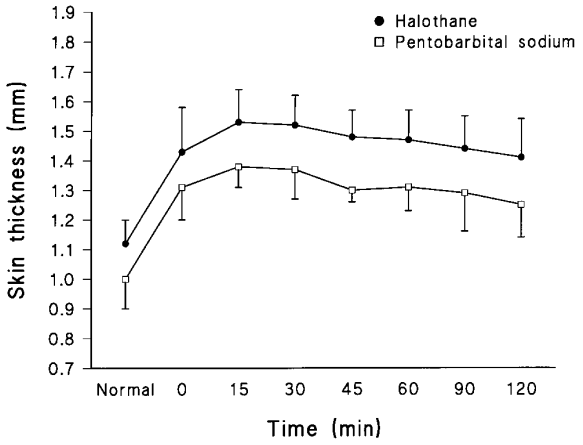

Fig. 3. Mean skin thickness in rats anaesthetized with halothane ($n = 14$ insertions) and pentobarbital sodium ($n = 6$ insertions) as a function of time before and after insertion of a microdialysis probe (mean $\pm$ SD). *Normal*: before insertion. *0*: immediately after insertion. *Solid circles*: halothane. *Open squares*: pentobarbital sodium.

increase 15 min after probe insertion was 0.42 ± 0.09, range 0.30–0.63 ($p < 0.05$). This corresponds to an increase of 37.5% above basal skin thickness. The mean difference between the pre-insertion thickness and the thickness 120 min after probe insertion was 0.28 ± 0.10, range 0.12–0.45. Thus, at 120 min the skin thickness had not reached pre-insertion value.

The microdialysis membrane has an outer diameter of 0.216 mm. If the probe was subtracted from the skin measurements, the skin thickening at 15 min was still significant, i.e. 0.20 mm ± 0.09, range 0.08–0.40.

Pentobarbital sodium group. The skin thickness before insertion was 1.00 mm ± 0.10, range 0.85–1.13 mm. The mean skin thickness as a function of time following six probe insertions is shown in Fig. 3. After insertion the skin thickness increased, mean skin thickening 0.31 mm ± 0.07, range 0.21–0.42 ($p < 0.05$) in 6 rats. Fifteen minutes after the insertion the mean increase was 0.38 mm ± 0.09, range 0.27–0.50 ($p < 0.05$), which is a relative increase of 38.0%. The increase was still significant even if the outer diameter of the probe was subtracted, i.e. skin thickening 0.17 mm ± 0.09, range 0.05–0.26.

Skin thickening was fully developed after only 15–30 min after probe insertion. After 120 min the skin thickness had not reached the basal level. The difference between the pre-insertion skin thickness and thickness 120 min after probe insertion was 0.25 ± 0.13, range 0.10–0.45.

Probe depth

Halothane group. The dialysis membrane was detected very easily with the ultrasound. Fig. 4 shows the mean probe depth (14 microdialysis membranes, probe depth measured from epidermal surface to probe echostructure within the dermis) as a function of time in the same plot as the mean total skin thickness. The probe depth was dependent of skin thickness. As the skin thickness increases, the probe depth becomes larger. The mean probe depth of the 14 insertions was 0.92 mm ± 0.12, showing little variation in insertion.

No significant linear relationship between probe depth and the increase in skin thickness 15 min after probe insertion was found.

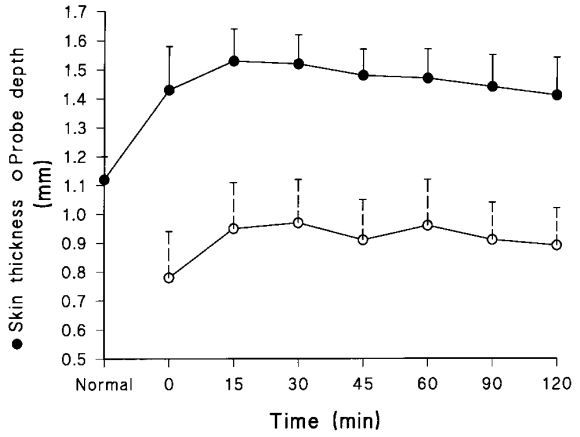

Fig. 4. Mean probe depth, i.e. the distance from the microdialysis probe to skin surface, as a function of time before and after insertion of a microdialysis probe ($n = 14$ microdialysis membranes) (mean ± SD). Mean skin thickness from the same experiments is also plotted. Rats anaesthetized with halothane. *Normal*: before insertion. *0*: immediately after insertion. *Open circles*: probe depth. *Solid circles*: skin thickness.

Pentobarbital sodium group. The mean probe depth of six insertions was 0.78 mm ± 0.07, range 0.70–0.85. Probe depth depended on skin thickness, as the probe depth followed skin thickness.

Intra-probe variation in depth

In both groups the variation of probe depth of any single probe, measured at three different sites along the probe, was occasionally high (e.g. 0.43 mm, 0.68 mm, 1.06 mm; mean 0.72 mm ± 0.32 mm), illustrating that the probe cannot be inserted in exactly the same depth along the full four centimetres of length. The microdialysis probe was generally inserted in the lower level of the dermis.

The mean probe depths in the halothane- and pentobarbital sodium-anaesthetized groups were different, 0.92 mm and 0.78 mm, respectively. However, the basal skin thickness also differed between the two groups of rats, as the rats in pentobarbital sodium anaesthesia had a thinner basal skin thickness.

Laboratory experiments, probe echostructure and reflection

The microdialysis membrane was placed on a plane material and the distance between the outermost peak of the membrane and the background material was 0.22 mm by ultrasound scanning. The exact outer diameter of the probe is 0.216 mm, i.e. in close agreement with ultrasound. Thus, probe depth measurements with A-mode scanning of closest probe reflection were accurate, with no special acoustic artifacts.

DISCUSSION

We found a significant increase in skin thickness after probe insertion in rats anaesthetized with halothane or pentobarbital sodium. The oedema and resultant thickening appeared rapidly and were fully developed 15–30 min after insertion. Oedema was still prominent 120 min after probe insertion. No significant difference in skin thickening between rats anaesthetized with halothane and pentobarbital sodium was found.

The microdialysis probe was inserted reproducibly in the lower dermis. None of the probes were placed in the subcutaneous tissues. The reproducibility of the insertion procedure is most important, since the microdialysis process of a skin-penetrating drug is dependent of probe depth. Andersson et al. (11) studied skin penetration of ethanol by cutaneous microdialysis. The concentration of ethanol in the dialysate decreased with increasing probe depth. Based on our practical experience it is possible to comprehend a special manual feeling of probe depth during the insertion, but it is still difficult to control the exact position of the probe along the full four centimetres of insertion length.

The increase in skin thickness is believed to be due to oedema formation of the skin, as a result of the insertion trauma created by the cannula. During the experiments low echogenic picture elements were obvious, especially 15 to 30 min after probe insertion. The black and blue areas, which correspond to low echogenicity on the ultrasound image, appeared around the probe (Fig. 5). It has previously been demonstrated that the number of low echogenic pixels is proportional to the degree of skin oedema, since the low

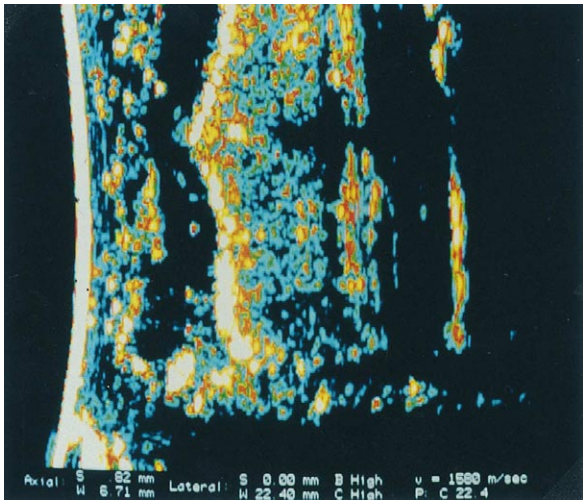

Fig. 5. Ultrasound image of rat skin 15 min after probe insertion. Note the low echogenic area around the probe. Colour scale of echogenicity: white > yellow > red > green > blue > black.

echogenic part of the image is proportional to the water content in the tissue (6). However, we did not systematically quantify the number of low echogenic pixels in the image, as Gniadecka et al. (12) did.

Serup (13) studied skin thickness among other parameters of skin-prick histamine weals. Healthy persons were prick-tested with three concentrations of histamine chloride (0.1 mg/ml; 1.0 mg/ml; 10 mg/ml). Relative increases in weal parameters, as measured between low and high concentrations of histamine, were 139% in weal diameter and 46% in skin thickness. This result of skin prick test with histamine on skin thickness corresponds well with the relative mean skin increase of 37.5% and 38% after probe insertion using halothane and pentobarbital sodium, respectively. Histamine release is known to be involved in the traumatic reaction to probe insertion (14).

In the present study the total skin thickness included the inserted microdialysis membrane. However, due to the elastic pretension of the skin the hypothetical increase in total skin thickness following probe insertion (the presence of the probe in the dermis) is believed to be less than the outer diameter of the microdialysis membrane, i.e. 0.216 mm.

It is possible that dermal oedema affects recovery and drug concentration in the dermis. It is believed that dermal oedema may impair oxygen and nutrient exchange between capillaries and cells, since tissue swelling results in increased diffusion distance (15). Dykstra et al. (16) observed oedema after probe implantation in a rat brain. Oedema increases the volume fraction of the extracellular space surrounding the dialysis membrane, thereby increasing the area available for diffusion of drugs towards the membrane and the recovery. The same phenomenon was seen in human skin. Thus, recovery of glucose was increased during the implantation trauma phase and after challenging the skin with histamine, which induced a weal (17).

The trauma in rat skin after insertion of a microdialysis probe has also been studied by laser Doppler image scanning of cutaneous blood flow (14). It was concluded that the skin vasculature was stabilized 30 min after probe insertion, as the skin blood flow and histamine release have then reached the baseline level. In this study the thickening of the skin remained constant in the entire period of the experiment, i.e. 2 h. It is not known for how long the oedema will be present and how the oedema progresses following removal of the probe. The oedema seems to immediately reach a steady-state after probe insertion with a supposed constant recovery. However, it is uncertain to which degree the changed volume fraction influences recovery. Most likely the oedema formation is not an important variable as compared with other variables interfering *in vivo* studies of rats. If a couple of hours have to elapse for the oedema to vanish and the microdialysis to start, the duration of the whole experiment would be longer than acceptable in relation to keeping the animals anaesthetized. Thus, for practical reasons our group decided to start the microdialysis experiment 30 min after insertion, based on the skin blood flow and histamine studies combined with the present observations of trauma-related oedema.

CONCLUSION

(1) It was possible to measure rat skin thickness by 20 MHz ultrasound scanning and identify a 0.216 μm microdialysis membrane inserted in the skin.
(2) Ultrasound examination of rat skin showed a mean skin thickness on the back of $1.08\,\text{mm} \pm 0.10$ (mean $\pm$ SD, $n = 20$).
(3) Traumatic oedema and skin thickening develop immediately after microdialysis probe insertion. Thickening remains fairly constant at a level around 30%. At 120 min after insertion the skin thickness had not normalised to the pre-insertion level.
(4) The two anaesthetics halothane and pentobarbital sodium apparently had no influence on oedema formation due to cannula insertion.
(5) Insertion of the microdialysis probe was performed reproducibly in the lower dermis. However, intra-probe variation in depth of a single probe along the whole 4-cm insertion length occurs.
(6) It was preferred not to start the microdialysis procedure until 30 min after cannula insertion as standard procedure, as this provides a more steady experimental situation.

REFERENCES

1. Ungerstedt U. Measurement of neurotransmitter release by intercranial dialysis. In: Marden CA, editor. Measurement of neurotransmitter release in vivo. New York: John Wiley & Sons, Inc.; 1984. p. 81–105.
2. Anderson C, Andersson T, Molander M. Ethanol absorption across human skin measured by in vivo microdialysis technique. Acta Derm Venereol (Stockh) 1991; 71: 389–393.
3. Müller M, Schmid R, Wagner O, Osten v.B, Shayganfar, Eichler HG. In vivo characterization of transdermal drug transport by microdialysis. J Controlled Release 1995; 37: 49–57.
4. Anderson C, Andersson T, Wårdell K. Changes in skin circulation after insertion of a microdialysis probe visualized by laser Doppler perfusion imaging. J Invest Dermatol 1994; 102: 807–81.
5. Serup J, Caddying J, Fullerton A, Gniadecka M, Gniadecki R. High-frequency ultrasound examination of the skin: introduction and guide. In: Serup J, Jemec GBE, editors. Handbook of non-invasive methods and the skin. Boca Raton: CRC-Press; 1995. p. 241–258.

6. Seidenari S, Di Nardo A. B scanning evaluation of irritant reaction with binary transformation and image analysis. Acta Derm Venereol (Stockh) 1992; Suppl 175: 9–13.
7. Seidenari S, Di Nardo A, Pepe P, Giannetti A. Ultrasound B scanning with image analysis for assessment of allergic patch test reactions. Contact Dermatitis 1991; 24: 216–222.
8. Seidenari S, Zanella C, Pepe P. Echographic evaluation of sodium lauryl sulphate (SLS)-induced irritation in mice. Contact Dermatitis 1994; 30: 41–42.
9. Beck JS, Spence VA, Lowe JG, Gibbs JH. Measurement of skin swelling in the tuberlin test by ultrasonography. J Immunol Methods 1986; 86: 125–130.
10. Serup J. Quantification of acrosclerosis measurement of skin thickness and skin-phalanx distance in females with 15 MHz pulsed ultrasound. Acta Derm Venereol (Stockh) 1984; 64: 35–40.
11. Andersson T, Anderson C, Boman A, Molander M. Cutaneous microdialysis technique for the demonstration of the in vivo absorption kinetics of hydrophilic solvents applied to human skin. Linköping University Medical Dissertations. Thesis No. 456. Linköping, Sweden, 1995.
12. Gniadecka M, Serup J, Søndergaard J. Age-related diurnal changes of dermal oedema: evaluation by high-frequency ultrasound. Br J Dermatol 1994; 131: 849–855.
13. Serup J. Diameter, thickness, area, and volume of skin-prick histamine weals. Measurement of skin thickness by 15 MHz A-mode ultrasound. Allergy 1984; 39: 359–364.
14. Groth L. Cutaneous microdialysis. Methodology and validation. Acta Derm Venereol (Stockh) 1996; Suppl 107: 1–54.
15. Fagrell B. Microcirculation disturbances – the final cause for venous leg ulcers. Vasa 1982; 11: 101–103.
16. Dykstra KH, Hsiao JK, Morrison PE, Bungay PM, Mefford IN, Sculley MM, et al. Quantitative examination of tissue concentration profiles associated with microdialysis. J Neurochem 1992; 58: 931.
17. Petersen LJ, Kristensen JK, Bülow J. Microdialysis of the interstitial-water space in human skin in vivo: quantitative measurement of cutaneous glucose concentrations. J Invest Dermatol 1992; 99: 357–360.