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In vivo Tolerance Assessment of Skin after Insertion of Subcutaneous and Cutaneous Microdialysis Probes in the Rat

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Key Words
Skin · Microdialysis · Subcutaneous · Dermis · Transepidermal water loss · Laser Doppler velocimetry · Tolerance · Rat

Abstract
The purpose of the study was to evaluate the trauma induced by insertion of the linear microdialysis probe in the subcutaneous and dermal tissue in the rat and to check if the microdialysis probe insertion affects transdermal drug delivery. Non-invasive bioengineering methods (TEWL, Laser Doppler Velocimeter, Chromameter) as well as histology were combined to characterize these effects. The results showed that the dermal and subcutaneous insertion of microdialysis probes did not change skin permeability, blood flow and color, confirming the safety of this technique. The probe depth did not influence the trauma. No significant physical damage after probe insertion was noticed. Thus, the present work validates the use of microdialysis in dermatopharmacokinetics studies after topical or systemic drug delivery.

Introduction

Microdialysis is an in vivo sampling technique to monitor the extracellular concentrations of exogenous or endogenous compounds. The principle of the microdialysis technique is based on the passive diffusion of compounds down a concentration gradient across the semi-permeable membrane of a dialysis fiber. Originally developed and applied in brain research [1], microdialysis has become during the last decade a common sampling method in conventional pharmaco-

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kinetic studies. The most important advantages of microdialysis are: (1) increased sampling frequency to characterize the concentration-time profile with a discriminating resolution, (2) reduction of the number of experimental animals, (3) because the dialysis fiber prevents diffusion of proteins, samples are sufficiently purified for further analysis, (4) possibility to study pharmacokinetics in awake freely-moving animals in almost real time by on-line analysis of microdialysates by using various techniques. Microdialysis sampling is a powerful tool to continuously monitor the extracellular unbound drug concentrations in different 'compartments' of the body. Indeed, microdialysis probes can be implanted in virtually any body organ or tissue [2–4].

Schaefer et al. [5] reviewed the different methods used to study dermopharmacokinetics after topical or systemic drug delivery. Cutaneous and subcutaneous microdialysis can be used to compare the penetration of different drugs into the skin, to study the effects of different vehicles on the penetration of drug, to determine how much drug enters into diseased skin compared to normal skin, and to measure drug concentration in the skin after systemic administration. In addition, the microdialysis technique can be used to deliver drug to the skin and to study skin metabolism.

Cutaneous microdialysis in which a probe is inserted within the dermis allows in vivo study of drug penetration as an alternative or supplement to the skin blister technique [6], the tape stripping technique and skin biopsies [7].

Cutaneous microdialysis was used to study ethanol absorption across the skin [8], histamine release in the skin [9], transdermal drug transport of nicotine [10] and the bioequivalence of topically applied drug in a microemulsion vehicle [11].

Subcutaneous tissue is an attractive sampling site for several reasons: (1) this tissue is relatively uniform, (2) the extracellular fluid is in constant flux with the systemic circulation, and (3) the implantation of the probe in subcutaneous tissue is easy [12]. Subcutaneous microdialysis sampling was used to study the pharmacokinetics of drugs as caffeine and theophylline [13], acetaminophen [12] and flurbiprofen [14] in rats. The technique was also used in humans to study the subcutaneous interstitial concentrations of antibiotics [15] and valproic acid [16].

In both cases, insertion of the linear microdialysis probe and its subsequent presence in the skin or in the subcutaneous tissue may affect the reactivity of the skin. Moreover, tissue changes in the surrounding of the probe membrane may affect the recovery characteristics and the measurement of the analyte. So before starting the sampling by the microdialysis technique, an equilibration period after probe insertion is usually required [17].

The effects of the probe insertion can be classified as (1) direct trauma to cells and tissue by guide and probe insertion, (2) modifications of the blood perfusion by *axon reflex*, and (3) inflammatory or 'foreign body' reactions to the probe [18].

The purpose of the present study was (1) to investigate the effect induced by insertion of the linear microdialysis probe in the subcutaneous and dermal tissue in the rat, (2) to evaluate the safety of the implantation procedure, (3) to compare the trauma after probe insertion in the subcutaneous tissue and in the dermal tissue, and (4) to check if the microdialysis probe insertion affects transdermal drug delivery. Non-invasive bioengineering methods to assess skin barrier function transepidermal water loss (TEWL), cutaneous blood flow (laser Doppler velocimeter, LDV), and erythema (Chromameter) as well as histology were combined to characterize these effects.

Materials and Methods

Animals

Hairless male rats 8 weeks old were housed in standard cages at room temperature (IOPS mutant from Iffa Credo, France). Standard laboratory food (A04, UAR-France) and water were given ad libitum.

Rat Treatment

Experiments were carried out in an assigned laboratory room with controlled temperature (20 °C) and relative humidity (50%). Animals were anesthetized before experiments and if necessary before a measurement with Thalamonal® (Fentanyl 50 µg/ml, Droperidol 2.5 mg/ml; Janssen Pharmaceutica, Belgium).

Microdialysis Probe

Subcutaneous Microdialysis Probe. The linear subcutaneous microdialysis probes were manufactured using a Hemophane® dialysis fiber (210 µm i.d., Gambro AB, Lund, Sweden) with a molecular weight cut-off of 5,000 D. The fiber (20 mm length) was glued at both ends to a piece of silicone tubing (0.012 inch i.d., 0.025 inch o.d.; Specialty Manufacturing Inc., Saginaw, Mich., USA) using B-210 cyanoacrylate glue (3M, Brussels, Belgium).

Dermis Microdialysis Probe. The linear dermis microdialysis probes were manufactured using the same Hemophane® dialysis fiber as the subcutaneous microdialysis probes. The fiber (10 mm length) was glued at one end to a piece of silicone tubing using the B-210 cyanoacrylate glue. After implantation in the rat, a second piece of silicone tubing was glued at the other extremity of the dialysis fiber.

Implantation of the Microdialysis Probe in the Rat

The skin of the dorsal region in the rat was punctured horizontally with a 16-gauge intravenous cannula (i.d. 1.7 mm, length 45 mm) for subcutaneous implantation or with a 26-gauge intravenous cannula (i.d. 0.45 mm, length 12 mm) for dermal implantation. The linear microdialysis probe was inserted through the guide cannula. The guide was then withdrawn, leaving the dialysis membrane in the subcutaneous tissue or in the dermis.

Non-Invasive Bioengineering Methods

Transepidermal Water Loss. The measurement of transepidermal water loss (TEWL) is an noninvasive method for assessing the skin barrier [19–20]. TEWL was measured by using a Tewameter TM 210 (CK electronic GmbH, Köln, Germany). The probe of Tewameter was maintained in contact with the skin for 1 min in order to get stable TEWL value, measurements being taken during the last 30 s. Each measurement is the result of the average of the registered values during the last 30 s. The values of TEWL are expressed in $g/m^2 \cdot h$.

Laser Doppler Velocimeter. Cutaneous blood flow was measured by a Laser Doppler Velocimeter (Periflux, PF3, Perimed, Sweden) [21]. The probe was placed directly on the skin and maintained in contact for at least 1 min. The values were recorded when a constant signal was observed. Results are expressed in arbitrary units.

Chromameter. Skin color and erythema were measured by the Minolta Chromameter CR-200 (Minolta, Osaka, Japan). The skin color is expressed by three-dimensional coordinates in which a* represents the red-green axis, b* the yellow-blue axis and L* the black-white axis (brightness) [22]. Only a* and L* were studied in order to determine change in brightness or erythema. Before measurement the Minolta Chromameter was calibrated by using a white calibration tile. During measurements the apparatus was perpendicularly kept to the skin surface, and the head of the instrument only touched the skin surface slightly.

Histology

To investigate tissue response to probe implantation, histology studies were performed at various time points. Probes were implanted during 2, 4, 8, 12, 24, 36, 72 h and 8 days, after which the animal was sacrificed and the skin with subcutaneous tissue excised. The tissue was fixed in a 4% formalin solution during approximately 7 days and embedded in paraffin wax. Sections 3 µm thick were cut perpendicularly to the surface of the skin. Tissue processing and staining with hematoxylin/eosin stain were performed following standard procedures.

Experiments

The dorsal region of each rat was divided in three sites: (1) a control site with no treatment, (2) a site where just a guide cannula was implanted and withdrawn, and (3) a site where the microdialysis probe was implanted for 8 h in subcutaneous tissue (first group of 8 rats) or in dermis (second group of 8 rats). Probes were perfused with an isotonic phosphate buffer pH 7.4 (PBS = Na_2HPO_4 3.191 g/l; NaH_2PO_4 0.775 g/l; NaCl 5.58 g/l) at 2 µl/min. Measurements of biophysical parameters were performed at three sites of the dorsal skin of each rat in the order: skin color, TEWL, LDV before and at time intervals until 24 h after implantation.

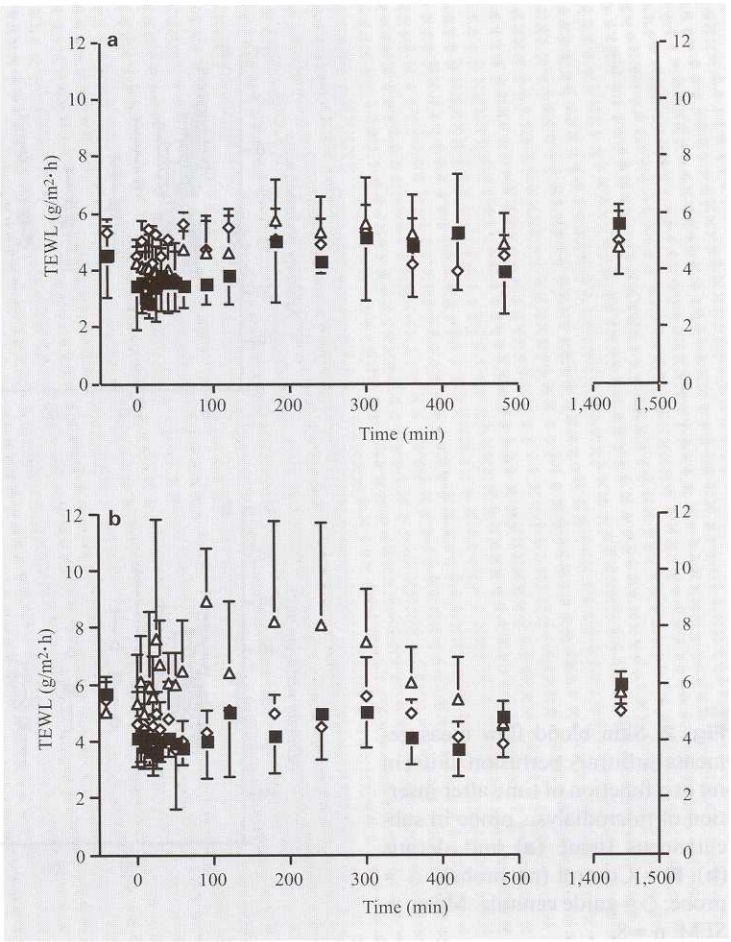

Fig. 1. Transepidermal water loss (TEWL) values in rat as a function of time after insertion of microdialysis probe in subcutaneous tissue (**a**) and dermis (**b**). ■ = Control (no probe); △ = probe; ◇ = guide cannula. Mean ± SEM, n = 8.

Statistics

Data were validated by using the Dixon test. Values in the text, figures and tables are expressed as mean ± SEM of the measurements of the 8 rats. A one-way ANOVA and Dunnet's test were used to compare the different treatments by using the Statistical Software SPSS 10.0.5. (SPSS Inc., Chicago, Ill., USA). A p value ≤ 0.05 was considered significant.

Results

Using several non-invasive bioengineering techniques, skin biophysical parameters were measured for at least 8 h after insertion of a guide cannula and a microdialysis probe in subcutaneous tissue or in dermis, and were compared to the control site with no treatment.

Transepidermal Water Loss

Skin barrier function was evaluated by the TEWL measurement. No significant increase in TEWL was observed following subcutaneous insertion of guide cannula and microdialysis probe versus control site (fig. 1a). The dermal guide cannula insertion did not induce TEWL changes during 24 h ($p > 0.05$) (fig. 1b). TEWL values after dermal probe

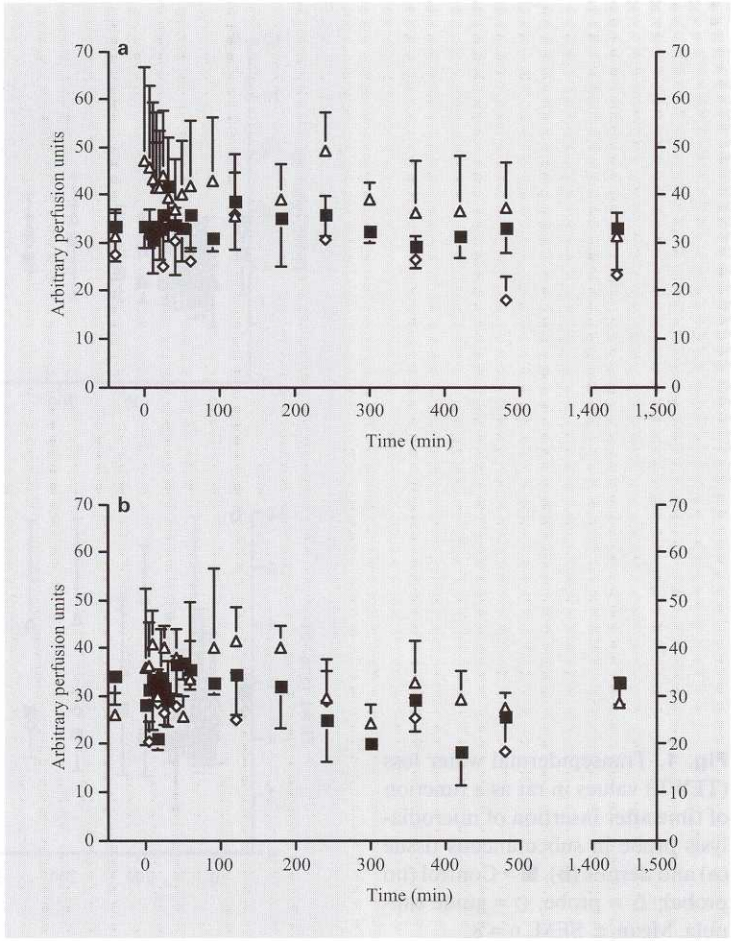

Fig. 2. Skin blood flow measurements (arbitrary perfusion units) in rat as a function of time after insertion of microdialysis probe in subcutaneous tissue (**a**) and dermis (**b**). ■ = Control (no probe); △ = probe; ◇ = guide cannula. Mean ± SEM, n = 8.

insertion were generally slightly higher during the first 6 h of the experiment than those recorded at the control site. However, there was no significant difference ($p > 0.05$).

Laser Doppler Velocimeter

The vascular effects of trauma induced by the implantation of a microdialysis fiber in the skin were monitored by the measurement of laser Doppler velocimetry. The insertion of guide cannula and microdialysis probes in subcutaneous ($p > 0.05$) and cutaneous ($p > 0.05$) tissues did not modify the skin blood flow as compared to control values (fig. 2a, b).

Minolta Chromameter

Erythema after probe insertion was studied by the skin color parameter. No significant increase in redness a* was observed after insertion of guide cannula ($p > 0.05$), subcutaneous ($p > 0.05$) and dermal ($p > 0.05$) microdialysis probes versus control sites (fig. 3a, b).

Histology

Histological examination of hairless rat skin showed that probe insertion did not result in significant physical damage to the skin and the probes were implanted in the dermis

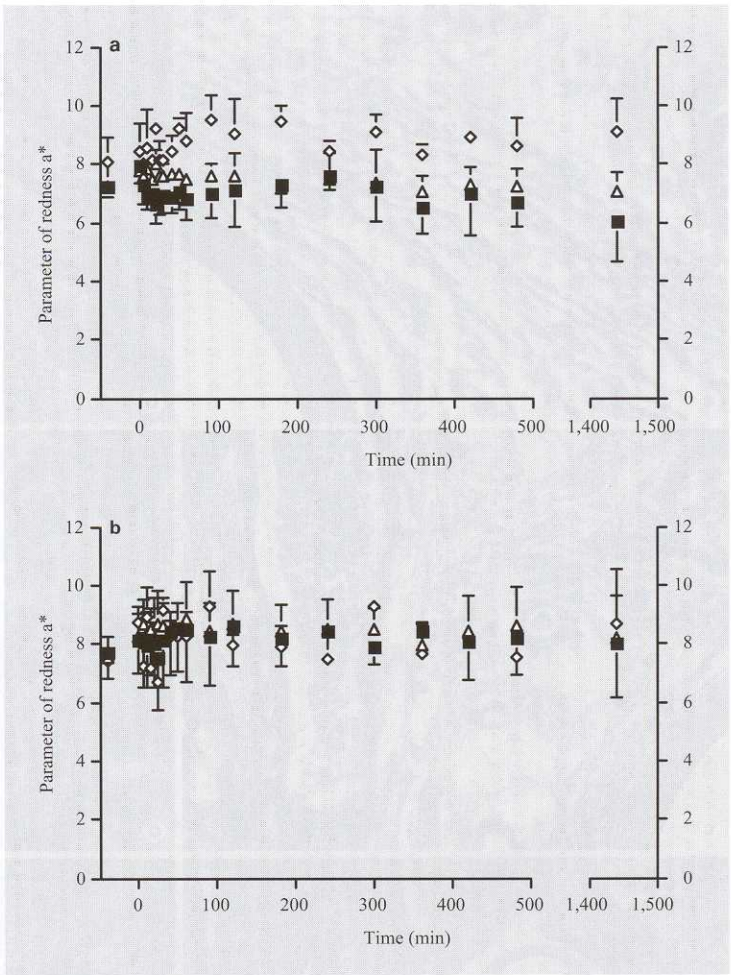

Fig. 3. Evaluation of erythema (redness a* parameter) in rat as a function of time after insertion of microdialysis probe in subcutaneous tissue (**a**) and dermis (**b**). ■ = Control (no probe); △ = probe; ◇ = guide cannula. Mean ± SEM, n = 8.

at about 0.3 mm or in the subcutaneous tissue at about 1.8 mm from the surface of the skin. The reproducibility of the insertion procedure is important, since the microdialysis recovery of skin penetrating drug may be dependent on probe depth. Even though it is possible to place the probe reproducibly in the subcutaneous tissue, in the case of dermal insertion, however, it is still difficult to control the exact position of the probe along the full insertion length. Practical experience can reduce variation in probe depth.

Two hours after probe implantation, there was no evidence of either edema or substantial tissue disruption (fig. 4a, b). 8 h after probe implantation, infiltration of lymphocytes at the site of the probe was observed and increased with time (fig. 4c, d). Changes in the cells surrounding the probe appeared approximately 24 h after probe insertion and were seen as an elongation of cells and an attachment of cells to the membrane (fig. 4e, f). After 8 days, a noticeable fibrosis had developed around the membrane of the probe to form a scar tissue (data not shown).

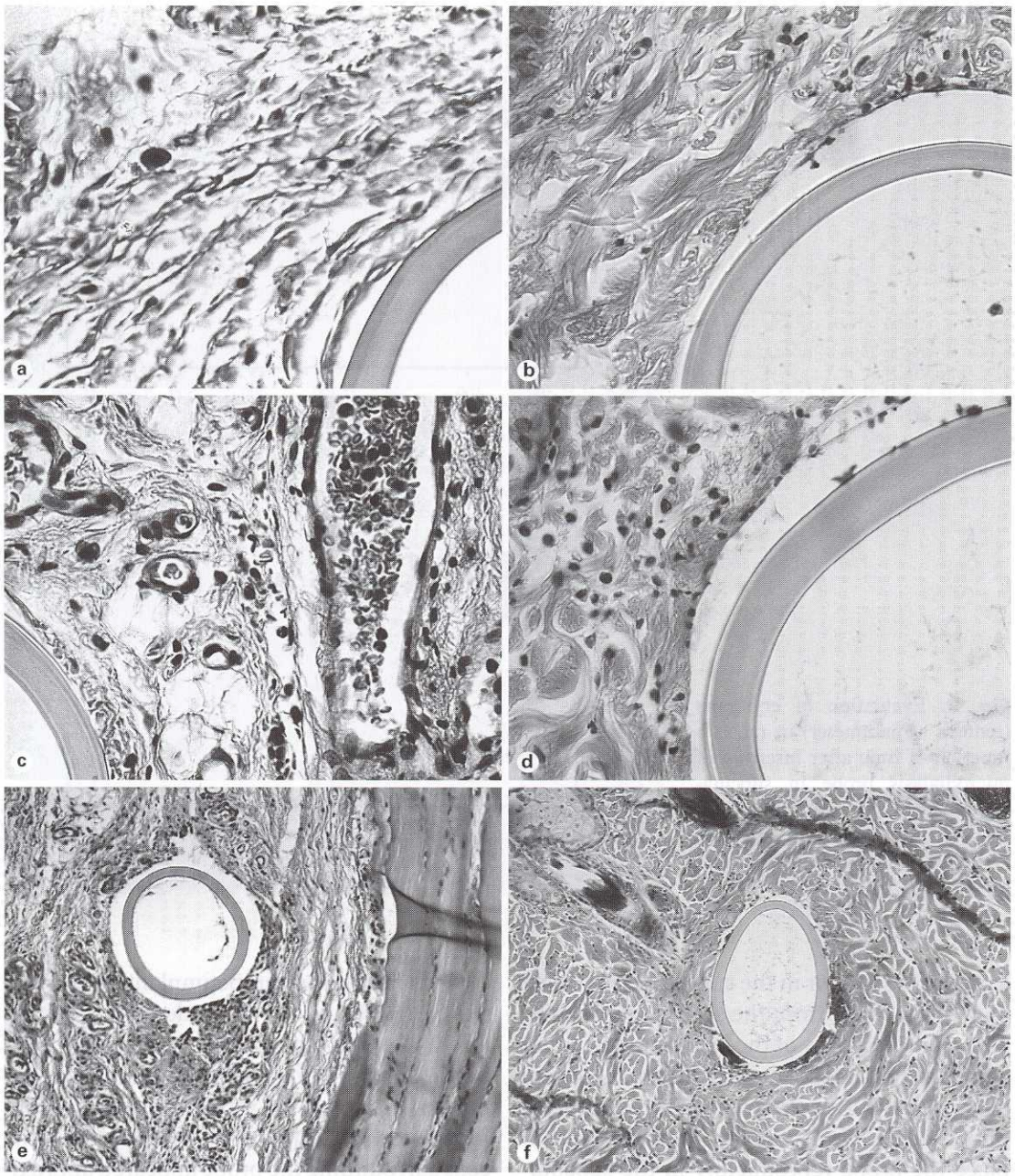

Fig. 4. Histological photos of the excised rat tissue showing the effects of probe implantation in subcutaneous tissue after 2 h (**a**), 8 h (**c**) and 24 h (**e**); and in the dermis after 2 h (**b**), 8 h (**d**) and 24 h (**f**). **a–d** ×40. **e**, **f** ×10.

Comparison between Dermis and Subcutaneous Tissue

For all biophysical parameters, we compared the trauma induced by the insertion of microdialysis probe in dermis and subcutaneous tissue. There was no significant difference in LDV values ($p > 0.05$), in TEWL values ($p > 0.05$) and in skin redness values ($p > 0.05$). Small changes observed by histological examination were found to be similar in both cases.

Discussion

This report provides an in vivo safety investigation of microdialysis probe insertion in skin and the comparison of trauma induced in dermis and subcutaneous tissue.

When compared to a control site, the dermal and subcutaneous insertion of microdialysis probes did not alter skin functions: TEWL, LDV and skin redness parameters did not change significantly.

The barrier function of the skin, assessed by TEWL measurements, was not affected by the probe insertion in the dermal or subcutaneous tissue, indicating that for at least 24 h after probe implantation, there was no important change in skin permeability.

Skin blood flow is an important parameter since blood flow changes in the skin may have effects on dermal pharmacokinetics [23]. In contrast with our results of cutaneous experiments, Groth et al. [17] showed that the dermal probe insertion increased skin blood flow in rats for about 30 min. The difference in the results could be explained by the use in the present study of a thinner guide cannula (26G) in order to insert the microdialysis probe as superficially as possible. In the case of subcutaneous experiments, in spite of the use of a 21G guide cannula, no change in skin blood flow was observed: as the scanning laser Doppler beam penetrated the skin superficially, these results indicated that there was no perturbation of skin blood flow in the surroundings above the subcutaneous probe, placed at a depth of about 1.8 mm [14].

Skin redness is a measure of erythema. Groth and Serup [24] showed that at least 60–80 min after dermal probe insertion were needed for the normalization of the erythema in human skin. The reasons presented above, i.e. the use of a thinner guide cannula in cutaneous experiments and the depth of probe implantation in subcutaneous experiments, could explain the absence of skin color changes in rats measured by the Chromameter.

For experiments requiring long-term implantation, an inflammatory response by the tissue may be expected. The inflammatory response is similar for most tissue [25]. Histological studies were performed at various time points to investigate skin inflammatory response to probe implantation. Examination of rat skin biopsies confirmed that the probes were placed intradermally or subcutaneously. There was no sign of inflammatory reaction or physical disruption of the skin after 8 h of dialysis, confirming the fact that the microdialysis probe implantation induced only minor tissue alterations. This is in agreement with the previous findings that inflammation does not occur before prolonged dialysis and the minor alterations observed do not seriously affect the microdialysis measurements [26, 27].

No significant difference in LDV, TEWL and skin color values were found between dermal and subcutaneous probe insertion, indicating that probe depth did not have any influence on the trauma. The implantation of microdialysis probes in dermis and subcutaneous tissue are equivalent in terms of trauma. Thus, the tissue to insert the probe will be chosen according to the accessibility and the interest of the tissue studied.

The present study validates microdialysis for transdermal drug delivery use: microdialysis may allow measurements of molecules in the skin in vivo, following a topical or a systemic administration, with minor trauma.

Conclusion

Tolerance issues of cutaneous and subcutaneous microdialysis were addressed by noninvasive evaluation directly in vivo in rats and by histological investigations. The measurements of the biophysical parameters, i.e. LDV, TEWL, skin color and the histological examination indicate a good tolerance to probe insertion and confirm the safety of this technique. Moreover, the results point out the fact that both dermis and subcutaneous tissue are convenient and accessible for microdialysis probe insertion. Due to minimal tissue damage, dermal and subcutaneous microdialysis can provide continuous in vivo dermal or subcutaneous levels of endogenous substances as well as exogenous compounds, without affecting skin permeability and skin barrier properties. Thus, it can be a powerful in vivo skin penetration method to study dermopharmacokinetics after topical or systemic drug delivery.

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