INVESTIGATIVE REPORTS

Microdialysis vs. Suction Blister Technique for *In vivo* Sampling of Pharmacokinetics in the Human Dermis

EVA BENFELDT[1], JØRGEN SERUP[2] and TORKIL MENNÉ[1]

[1]*Department of Dermatology, University of Copenhagen, Gentofte Hospital, Hellerup and* [2]*Department of Dermatological Research, Leo Pharmaceutical Products, Denmark*

Our aim was to simultaneously investigate 2 techniques for *in vivo* sampling of peripheral compartment pharmacokinetics after systemic administration of acetylsalicylic acid. Ten volunteers were given 2 g acetylsalicylic acid orally. Blood samples and dialysates from 4 microdialysis probes inserted in the dermis of the forearm were collected for 5 h and suction blisters were raised 1–3 h after dosing. In microdialysates, both acetylsalicylic acid and the metabolite salicylic acid were measurable in the absence of hydrolysing enzymes. The mean C_{max} (maximum concentration) of total, unbound salicylic acid was 9.5 µg/ml in microdialysates, 13.2 µg/ml in suction blister fluid and 56.5 µg/ml in plasma. Mean T_{max} (time to C_{max}) for salicylic acid was 188 and 161 min in plasma and microdialysates, respectively. The dermis-to-plasma C_{max} ratio was 0.16 ± 0.04 (mean $\pm$ SD) by microdialysis sampling and 0.25 ± 0.09 by the suction blister fluid method. Close correlations ($p < 0.01$) were found between C_{max} of salicylic acid in microdialysates and plasma, and between C_{max} of salicylic acid in suction blister fluid and plasma. The 2 techniques were in excellent accordance with even closer correlation between maximum concentrations obtained by microdialysis and suction blister fluid sampling ($p < 0.001$). However, comparing the tolerability of the sampling procedure, ease of analysis, and detail in chronology, microdialysis is superior for sampling *in vivo* pharmacokinetics in the dermis. *Key words: dermal drug levels; acetylsalicylic acid; salicylic acid; protein binding; systemic drug administration.*

(Accepted February 25, 1999.)

Acta Derm Venereol 1999; 79: 338–342.

E. Benfeldt, Department of Dermatology, University of Copenhagen, Gentofte Hospital, Niels Andersensvej 65, DK-2900 Hellerup, Denmark.

In this study, we wanted to evaluate and compare 2 different techniques for the determination of *in vivo* dermal drug levels by sampling at the level of the target organ.

Suction blisters have been an established method for more than 30 years and the method principle relies on the separation of the epidermis from the dermis along the lamina lucida due to the application of prolonged suction to the skin surface. The fluid drawn into this newly-formed compartment can be sampled and analysed for the content of either topically or systemically administered drug, or for inflammatory mediators, inflammatory cells or other compounds (1). The suction blister fluid (SBF) has been found to correspond to average interstitial tissue fluid of the dermis (2) and the protein content has been found to be $29 \pm 4\%$ of the content in plasma (3).

Microdialysis is a sampling method which has been used for the last decade, initially in pharmaco-behavioural rat brain studies (4), but the application of which has been expanded to the study of both endogenous and exogenous compounds in research, drug trials and the clinical setting. The technique has the advantage of being minimally invasive when applied to sampling in the skin, and it provides highly reproducible real-time chronological sampling with a short time resolution. The tolerability of microdialysis sampling is high, and pharmacokinetic studies of up to 3 days duration have been performed in patients (5). Microdialysis samples extracellular fluid in the tissue in which the probe is placed, but excludes larger molecules and proteins from the sample. For highly protein-bound drugs, the technique requires very sensitive methods of analysis as the drug concentration in the samples will be reduced accordingly (6).

As a model drug we chose acetylsalicylic acid (ASA, aspirin), which is the most widely used systemic drug today (7). After oral administration, ASA is rapidly hydrolysed to salicylic acid (SA) by ubiquitous esterases, also present in the blood. The aim of the present study was threefold:

1. To study the relationship between pharmacokinetics in plasma and the skin, as measured by dermal microdialysis.
2. To study the relationship between pharmacokinetics in plasma and the skin, as measured by the suction blister method.
3. To establish the correlation between results obtained with the 2 sampling methods for studying skin pharmacokinetics.

MATERIAL AND METHODS

Chemicals

ASA tablets (Albyl®) 500 mg were obtained from Leo Pharmaceutical Products Ltd. SA (>99% purity) was obtained from Merck, Germany.

Microdialysis equipment

We used microinjection pumps (CMA/100, CMA/Microdialysis AB, Stockholm, Sweden) and probes manufactured from single fibres of hollow dialysis membrane (Gambro GFE 18, Gambro Dialysaten AG, Hechingen, Germany; outer diameter 216 µm, wall thickness 8 µm, molecular cut-off 2 kDa). The membrane was glued to nylon connecting tubing (Portex, Berck-sur-Mer, France) using cyanoacrylate (Super Attak, Lock-tite, Denmark). Membrane length accessible to microdialysis was 3 cm. Probes were sterilized by immersion in 70% alcohol for 20 min. Perfusate consisted of a sterile phosphate buffer with added glucose 2.5 mM (Gentofte University Hospital pharmacy). Perfusate flow rate was 5 µl/min and samples were collected in fixed 20 min fractions.

In vitro microdialysis recovery

The *in vitro* relative recovery (RR) is independent of the concentration of the drug and is expected to be constant during experiments.

$$RR = \frac{C_{dialysate} - C_{perfusate}}{C_{medium/tissue} - C_{perfusate}} \quad (1)$$

The dialysis process can be reversed by adding the drug to the perfusate instead of to the medium. The principle of this method relies on the assumption that the diffusion process is quantitatively equal in both directions through the semipermeable membrane (8). By perfusing the dialysis probe with a known concentration of SA, the relative loss of SA from the perfusate to the medium can be calculated using equation 1.

In vitro microdialysis experiments were conducted with probes placed in SA in known concentrations of 1.0, 4.0, 10.0, 20.0 and 100.0 μg/ml at 35°C, stirred at 350 rpm, using a perfusate flow rate of 5 μl/min (*n* = 19).

In vitro loss was studied by perfusing probes with SA in known concentrations of 1.0, 10.0 and 100.0 μg/ml, using the same experimental set-up (*n* = 7).

In vivo microdialysis recovery study

As *in vivo* recovery may differ substantially from *in vitro* recovery (9), *in vivo* recovery of SA was established. By perfusing the dialysis probe with a known concentration of SA, the relative *in vivo* loss of SA from the perfusate to the dermis can be calculated using equation 1 and used as an estimate of *in vivo* recovery. Two subjects had microdialysis probes (2 each, total *n* = 4) inserted in the dermis of the left volar forearm. The probes were perfused for 4 h with the usual perfusate with 10 μg/ml SA added.

Subjects

We investigated 10 healthy volunteers: 5 women and 5 men, age 25–50 years. None of the subjects were allergic to aspirin or local anaesthesia. The volunteers had used no topical creams for 2 days prior to the experiment and took no medication. All subjects gave written informed consent and the study was approved by the Copenhagen County ethics committee (Ref. KA 97021s) and by the Danish Medicines Agency (J.nr. 5312-133-1997).

Experimental protocol

Local anaesthesia (1% Xylocaine®, Astra, Sweden) was injected in the dermis/s.c. tissue around (but not into) a demarcated area on the volar aspect of the left forearm (volume < 10 ml). Four probes were inserted in parallel in the anaesthetised skin by means of a 21 G guide cannula, and each outlet placed directly in a collecting glass vial, fixed to the skin by tape. Perfusion was started at low flow for a period of 1 h after probe insertion to allow the insertion trauma to subside. An intravenous cannula (18 G Venflon®, Ohmeda AB, Sweden) was inserted in a hand vein and a baseline blood sample was taken. The subject was given 2 g acetylsalicylic acid with a glass of water (t = 0) and microdialysis and blood sampling was started. Blood samples were drawn at the mid-point of the microdialysis sampling interval. After 1 h, a disposable suction blister chamber (Dermovac® Blistering Device, Ventipress Oy, Finland) was taped to the inner aspect of the upper arm (also left arm) and connected to controlled suction of 250 mmHg. After 2 h of suction most subjects had developed 5 fluid-filled blisters, which were emptied using a Mantoux syringe. In some subjects, additional suction time was necessary to obtain fluid in the blisters. Blood and microdialysis sampling was continued for 5 h.

Ultrasound measurement of probe depth and skin thickness

At the end of the experiment, skin thickness and probe depth in the dermis was measured in triplicate by 20 MHz ultrasound scanning using the Dermascan-C (Cortex, Hadsund, Denmark).

Microdialysate analysis

The concentration of SA and ASA in dialysates was measured by HPLC. An 80 μl sample was acidified by adding 125 μl 0.08 M phosphoric acid. An aliquot of 150 μl was injected into a Merck/Hitashi LaChrom HPLC system. A LiChrosphere 100 RP-8, 5 μm, 125 × 4 mm i.d. column was used for separation and ASA and SA were quantified at 234 nm using peak area measurements. Isocratic elution with methanol/0.005M phosphate buffer, pH 2.2: 35/65 was used. The retention times were 4.5 and 10.5 min for ASA and SA, respectively. The limit of quantification was 10 ng/ml for both compounds. Concentrations below this limit were arbitrarily set to 5 ng/ml. The total SA concentration in the samples was calculated by the equation

$$[Total\ SA] = [ASA^* 0.767] + [SA]$$

Analysis of samples containing both SA and lignocaine showed no compounds interfering with the detection of SA.

Suction blister fluid analysis

A volume of 20 μl of the SBF samples was for used for analysis of total protein concentration by a manual biuret method (10). The remaining blister fluid was protein precipitated by adding acetonitrile and centrifuged. The supernatant was analysed in triplicate by HPLC as above.

Blood sample analysis

After centrifugation, serum was pipetted into vials and frozen. After protein precipitation and centrifugation, the SA concentration in the supernatant was measured by HPLC. A 20 μl sample was injected into a TSP HPLC system with a Waters Bondapak C_{18}, 10 μm, 300 × 4.6 mm i.d. column for separation. SA was determined at 238 nm and quantified by peak height measurements. O-toluic acid was used as internal standard and the eluent was 42% v/v methanol in 0.005% v/v acetic acid. The limit of quantification was 3 μg/ml.

RESULTS

In vitro microdialysis recovery

The relative recovery of SA was 79% ± 2% (mean ± SD, *n* = 19), and the relative loss of SA was 75% ± 3% (mean ± SD, *n* = 7). Although there is a 5% difference between these recoveries, they can be regarded as similar for practical purposes.

In vivo microdialysis recovery study

In vivo microdialysis of SA gave a relative loss (delivery to the tissue from the perfusate) of 24% ± 4% (mean ± SD, *n* = 4) using equation 1 for calculation. The *in vivo* loss, which is an indirect measure of the *in vivo* microdialysis recovery of SA when using the same probe type in the same tissue, was constant during the 4 h of the experiment. The relative recovery value was subsequently used for calculating the absolute concentration of SA in the dermal interstitial fluid from the SA concentration in the microdialysis samples.

Pharmacokinetic study: microdialysates

In microdialysis samples, the concentration of both ASA and its metabolite SA were measurable due to the absence of hydrolysing enzymes in the sample. Once the microdialysis sample has been collected, no further hydrolysis occurs as shown by Steele et al. (11), and the sample is amenable to

Fig. 1. Time-concentration curves, showing the dialysate concentrations of salicylic acid (SA) (□), acetylsalicylic acid (ASA) (■), and the total salicylate content (× - - ×), sampled simultaneously by the 4 microdialysis probes in one individual.
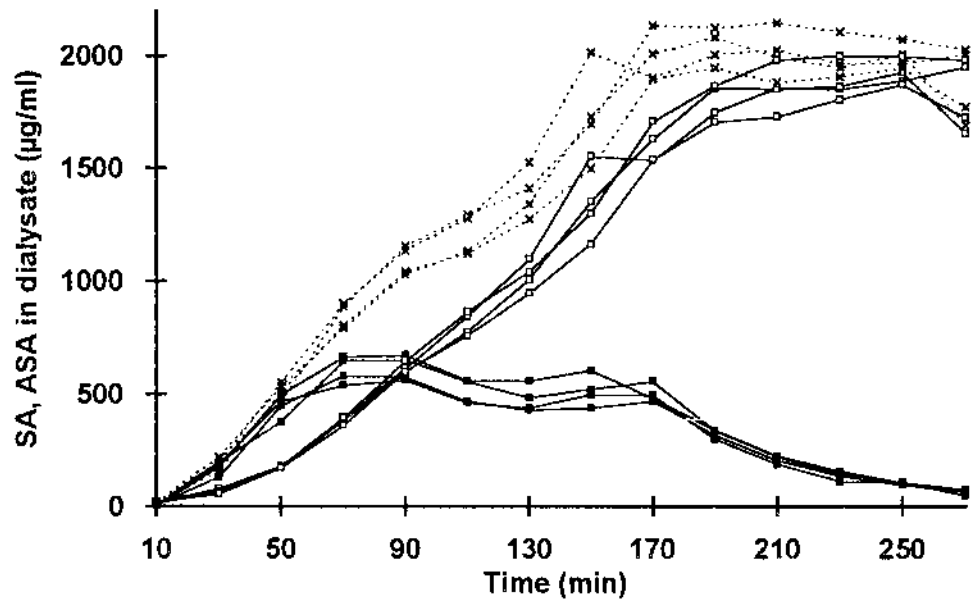

Fig. 2. Time-log concentration curves for mean total salicylic acid (SA) concentration in plasma (●), suction blister fluid (−) and microdialysis samples (◆), following oral administration of 2 g acetylsalicylic acid (ASA) to healthy volunteers at $t = 0$ ($n = 10$). Error bars are SD of original measurements. Microdialysate concentrations are also shown corrected for *in vivo* recovery of SA (- -), as is the unbound drug concentration in plasma (. . .). Suction blister fluid concentrations are uncorrected, total SA concentrations.
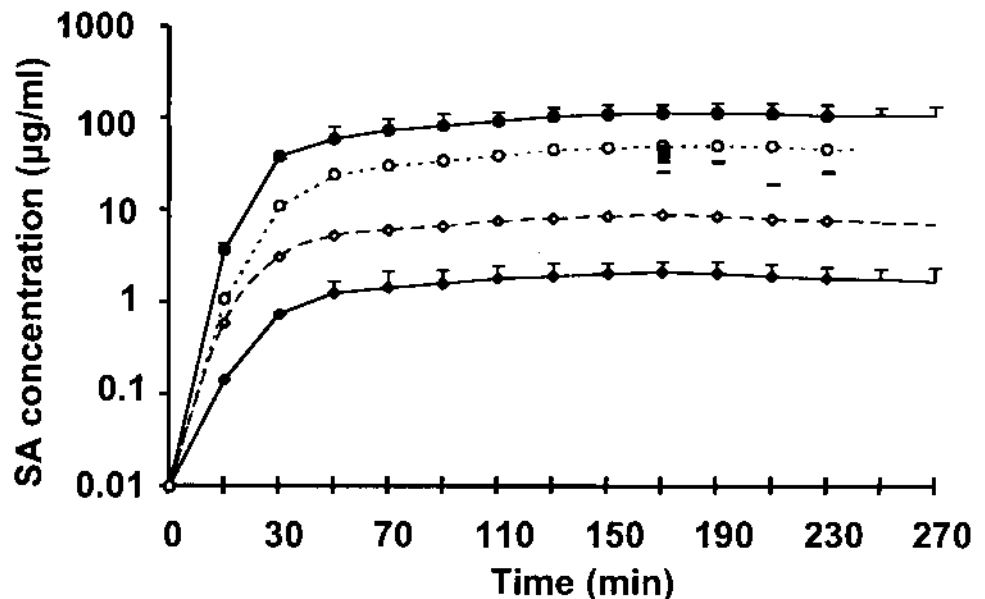
direct injection onto the chromatographic column. A representative time-concentration curve, showing the concentration of SA and ASA sampled by the 4 probes in a single individual, is shown in Figure 1. In 1 subject, the HPLC analysis of microdialysates displayed high concentrations of a non-identifiable compound which interfered with SA/ASA quantification. The mean C_{max} (maximum concentration) of unbound SA was 8.3 ± 2.7 µg/ml in microdialysates, and C_{max} of ASA was 5.5 ± 2.8 µg/ml ($n = 9$, total no of probes = 36). Mean T_{max} (time to C_{max}) for ASA and SA was 87 ± 48 and 205 ± 52 min in microdialysates. The total SA concentration in microdialysates showed a C_{max} of 9.5 ± 3.1 µg/ml and a T_{max} of 161 ± 50 min. The intrasubject variability in total SA concentration measured by microdialysis sampling was 10%, which was a third of the intersubject microdialysis variability of 30%.

Pharmacokinetic study: plasma samples and suction blister fluid

In the blood, the hydrolysis of ASA to SA is a rapid, dose-dependent process with a half-life of less than 10 min. Thus, in the plasma samples only SA could be detected, and in suction blisters only a non-quantifiable concentration of ASA could be detected (limit of quantification 10 ng/ml).

In order to obtain comparable levels of unbound SA, measurements of SA concentrations in plasma and SBF in the present study were corrected to unbound SA levels using the degree of binding applicable to the SA concentration in each sample. Data regarding the plasma protein binding of ASA and SA were obtained from 2 *in vitro* studies of SA/ASA plasma protein binding (11, 12), in which the binding has been determined both by ultrafiltration and microdialysis. In the

present study, plasma and SBF concentrations of SA were between 2 and 166 µg/ml, and in this concentration range SA is between 50% and 70% protein bound (the higher the SA concentration, the lower the protein binding). The unbound SA concentration in SBF has been calculated using the same protein binding data as for plasma samples.

The C_{max} of SA was 13.2 ± 5.7 µg/ml in SBF and 56.5 ± 18.9 µg/ml in plasma. Mean T_{max} for SA was 188 ± 52 min in plasma. Figure 2 shows the mean time-log concentration curves for SA in blood and microdialysis samples over the 5 h. SBF concentrations have been plotted at the time of actual sampling, which was delayed in some subjects due to slow blister formation ($n = 9$; in 1 subject suction blisters did not form). The protein content in SBF was 25.4 ± 3.9 mg/ml, and there was no correlation between protein content and SA concentration in SBF, nor was there any difference in SA concentration in SBF or in SBF/plasma ratio between normal and slow blistering subjects ($p > 0.10$).

Comparison of the 2 methods for sampling drug concentrations in the skin in vivo

The dermis-to-plasma C_{max} ratio was 0.16 ± 0.04 by micro-dialysis sampling and 0.25 ± 0.09 by the suction blister fluid method. The positive correlation between C_{max} of unbound SA in plasma and unbound SA measured by microdialysis and SBF, respectively, is shown in Figure 3. The highly significant correlation between C_{max} of unbound SA in microdialysates and in SBF is shown in Figure 4.

No interferences were found in the blank samples taken at $t = 0$. The ultrasound scans of the probes inserted confirmed that all probes were situated in the dermis.

DISCUSSION

Whereas both *in vitro* and *in vivo* recovery of SA has been determined in the present study, the recovery of ASA has not been established. However, since the *in vitro* relative recoveries of both SA and ASA have been determined in
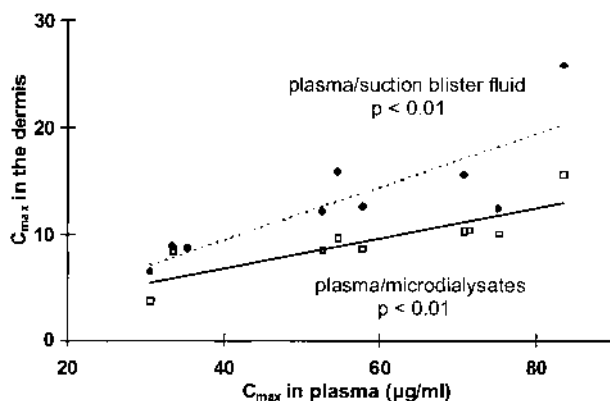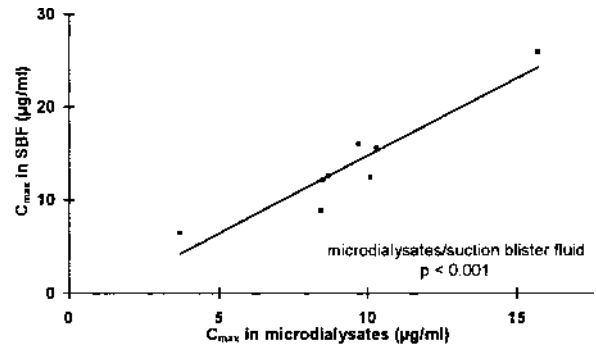

Fig. 4. Correlation between maximum unbound SA concentrations measured by microdialysis and by suction blister fluid method. Each point represents data from 1 individual.

another study (11) and found to be 11.3 (SA) and 12.5 (ASA), the recoveries can be assumed to be largely similar in this study (although not performed *per se*).

We have used protein binding data from the literature for estimating free drug concentrations in plasma and SBF (taking the different protein content into account). It has been demonstrated in a pharmacokinetic study of bendroflumethiazide, which is also essentially solely bound to albumin, that the protein binding is not very different (84 vs. 76%) in plasma and SBF (13).

In the present study, the production of suction blisters caused moderate discomfort with slight pain and itching, usually perceived at the peak time of blister formation. The insertion of microdialysis probes was not perceived due to the use of local anaesthesia. Microdialysis probes can be inserted in the dermis without anaesthesia, and the discomfort is similar to what is perceived during ordinary blood sampling (5, 14).

In drug distribution studies, suction blisters may be raised before (13, 15, 16) or after (17) the drug administration, and single or multiple dosing may be used. In a recent study by Müller et al., peripheral tissue kinetics of theophylline was followed in cantharidine-induced skin blisters, saliva and microdialysis samples from subcutaneous and muscle tissue following a single i.v. dose to healthy volunteers (15). Subcutaneous and muscle tissue drug levels, sampled by microdialysis, closely correlated with unbound plasma levels, whereas the data from saliva and blister fluid did not correlate with free plasma values of theophylline. It was concluded that microdialysis is a reliable technique for sampling of unbound peripheral compartment concentrations, superior to saliva and skin blister concentration measurements in this regard, and was suggested that skin blisters should not be employed for this purpose. Using the same design, the peripheral pharmacokinetics of paracetamol following an oral dose has also been studied, showing close correlations between pharmacokinetics in serum and both blister fluid and microdialysates, whereas saliva sampling was less useful (16). Paracetamol is a drug with a low protein binding, whereas theophylline displays a protein binding of around 60%. The usefulness of any method for sampling pharmacokinetics in the peripheral compartment will depend on the pharmacokinetic properties of the drug, including the protein binding in the blood and tissues. For SA, we have been able to demonstrate very close correlations between concentrations

Fig. 3. Correlation between maximum unbound salicylic acid (SA) concentration in plasma versus maximum unbound SA concentration measured by microdialysis (□) and suction blister fluid method (◆). Each point represents data from 1 individual. Regression lines (best fit) are shown for microdialysates (full drawn line) and suction blister fluid (dotted line).

in the dermis and plasma by both methods, although the calculated absolute free drug concentrations were very different in plasma and dermis (as can be seen in Figure 2).

The correlation found between drug concentrations in SBF and plasma in the present study has most likely been improved by the timing of the suction blister formation, planned to coincide with the peak salicylate concentration in plasma. The highly significant correlation between the 2 peripheral compartment sampling methods employed in this study demonstrates the potential usefulness of both techniques. However, we find microdialysis sampling superior to suction blisters for obtaining dermal pharmacokinetics, when factors such as the tolerability of the sampling procedure, the ease of analysis, and the detail of chronology and reproducibility are considered.

ACKNOWLEDGEMENTS

REFERENCES

1. Kiistala U. Suction blister device for separation of viable epidermis from dermis. J Invest Dermatol 1968; 50: 129–137.
2. Rossing N, Worm AM. Interstitial fluid: Exchange of macromolecules between plasma and skin interstitium. Clin Physiol 1981; 1: 275–284.
3. Vermeer BJ, Reman FC, Van Gent CM. The determination of lipids and proteins in suction blister fluid. J Invest Dermatol 1979; 73: 303–305.
4. Ungerstedt U. Measurement of neurotransmitter release by intracranial dialysis. In: Marsden CA, editor. Measurement of neurotransmitter release in vivo. Chichester; John Wiley & Son Ltd, 1984: 81–105.
5. Lindberger M, Tomson T, Ståhle L. Validation of microdialysis sampling for subcutaneous extracellular valproic acid in humans. Ther Drug Monit 1998; 20: 358–362.
6. Benfeldt E, Groth L. Feasibility of measuring lipophilic or protein-bound drugs in the dermis by in vivo microdialysis after topical or systemic drug administration. Acta Derm Venereol 1998; 78: 274–279.
7. Weissmann G. Aspirin. Sci Amer 1991; 1: 59–64.
8. Ståhle L, Segersvärd S, Ungerstedt U. A comparison between three methods for estimating the extracellular concentration of endogenous and exogenous compounds by microdialysis. J Pharmacol Methods 1991; 25: 41–52.
9. Ståhle L. Drug distribution studies with microdialysis I: Tissue dependent difference in recovery between caffeine and theophylline. Life Sci 1991; 49: 1835–1842.
10. Witt I, Trendelenburg C. Gemeinsame studie zur erstellung von richtwerten für klinisch-chemische kenngröβen im kindesalter. J Clin Chem Clin Biochem 1982; 20: 235–242.
11. Steele KL, Scott DO, Lunte CE. Pharmacokinetic studies of aspirin in rats using in vivo microdialysis sampling. Anal Chim Acta 1991; 246: 181–186.
12. Herrera AM, Scott DO, Lunte CE. Microdialysis sampling for determination of plasma protein binding of drugs. Pharm Res 1990; 7: 1077–1081.
13. Schäefer-Korting M. Skin blister fluid – an access to the peripheral compartment. Arzneim.-Forsch./Drug Res 1985; 35: 1828–1831.
14. Groth L, Serup J. Cutaneous microdialysis in man: effects of needle insertion trauma and anaesthesia on skin perfusion, erythema and skin thickness. Acta Derm Venereol 1998; 78: 5–9.
15. Müller M, Brunner M, Schmid R, Putz EM, Schmiedberger A, Wallner I, Eichler HG. Comparison of three different experimental methods for the assessment of peripheral compartment pharmacokinetics in humans. Life Sci 1998; 62: 227–234.
16. Brunner M, Schmiedberger A, Schmid R, Jäger D, Piegler E, Eichler HG, Müller M. Direct assessment of peripheral pharmacokinetics in humans: comparison between cantharides blister fluid sampling, in vivo microdialysis and saliva sampling. Br J Clin Pharmacol 1998; 46: 425–431.
17. Korting HC, Schäefer-Korting M, Roser-Maass E, Mutchler E. Determination of 8-methoxypsoralen levels in plasma and skin suction blister fluid by a new sensitive fluorodensitometric method. Arch Dermatol Res 1982; 272: 9–20.