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

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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 Cmax (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 Tmax (time to Cmax) for salicylic acid was 188 and 161 min in plasma and microdialysates, respectively. The dermis-to-plasma Cmax ratio was 0.16 ± 0.04 (mean ± SD) by microdialysis sampling and 0.25 ± 0.09 by the suction blister fluid method. Close correlations (p < 0.01) were found between Cmax of salicylic acid in microdialysates and plasma, and between Cmax 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.

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MATERIAL AND METHODS

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.

\[
RR = \frac{C_{\text{dialysate}} - C_{\text{perfusate}}}{C_{\text{medium/tissue}} - C_{\text{perfusate}}}
\]  

(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 vitro microdialysis recovery study**

As *in vitro* 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 anesthetised 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/Hitachi 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

\[
[\text{Total SA}] = [\text{ASA}’0.767] + [\text{SA}]
\]

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 C18, 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...
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<sub>max</sub> (maximum concentration) of unbound SA was 8.3 ± 2.7 μg/ml in microdialysates, and C<sub>max</sub> of ASA was 5.5 ± 2.8 μg/ml (n = 9, total no of probes = 36). Mean T<sub>max</sub> (time to C<sub>max</sub>) for ASA and SA was 87 ± 48 and 205 ± 52 min in microdialysates. The total SA concentration in microdialysates showed a C<sub>max</sub> of 9.5 ± 3.1 μg/ml and a T<sub>max</sub> 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
Comparison of the 2 methods for sampling drug concentrations in the skin in vivo

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

A study (11) found that the recoveries can be assumed to be largely similar in this study (although not performed per se).

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 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.
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.

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