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Comparison of Franz cells and microdialysis for assessing salicylic acid penetration through human skin

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Abstract

The purpose of the present study was to compare Franz cells (FC) and microdialysis (MD) for monitoring the skin absorption of salicylic acid (SA). The influence of pH on SA flux was also assessed by these two techniques.

Excised abdominal human skin was used in the experiments. SA was dissolved in phosphate buffer solutions of pH 2, 5 and 7 (2 mg/ml). SA concentrations in the receptor FC solutions and in MD samples were assessed by high performance liquid chromatography (HPLC).

The results demonstrate that the flux of SA decreased with increased pH. The profiles permeation determined by Franz cells and microdialysis were similar. However, whatever the pH, the SA flux was higher with microdialysis than with Franz cells.

The results showed that SA percutaneous permeation conformed to the pH partition hypothesis. The flux of SA was different when it was determined by the two techniques. The collect of SA, by these two techniques is different. The results of the two techniques are compared and discussed.

Keywords: Skin permeation; Franz cells; Microdialysis; Salicylic acid; Human skin; pH partition

1. Introduction

Skin penetration studies play an essential role in the optimisation of drug and formulation design in dermal and transdermal delivery. Therefore, the experimental use of in vitro permeation techniques such as Franz type diffusion cells (FC) and microdialysis (MD), is highly important. Furthermore, there have been very few comparisons between FC and MD studies. A comparison could help in the validation of MD in vivo. These systems permit the evaluation of the dermal kinetics of drug uptake and diffusion. In addition, MD allows the estimation of drug concentrations in the deeper skin layers (Wagner et al., 2000). Exact quantification of drug concentrations in the skin is complex, difficult and involves invasive techniques such as biopsies. The use of excised human skin mounted on suitable in vitro test systems may be a good alternative.

The FC system is widely used because of its low cost. It is less time consuming and it is reproducible (Franz, 1975; Sartorelli et al., 2000). However, the MD technique can be employed ex vivo as well as in vivo. The MD principle can be compared to an artificial blood vessel. MD sampling is performed by placing a tubular MD membrane in the selected region (dermis,

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hypodermis), parallel to the skin surface. The probe, which is permeable to water and small molecules, is continuously perfused with a physiological buffer solution at a low flow rate. Unbound substances present in the skin can cross the membrane and enter the lumen probe in proportion to a concentration gradient (Le Quellec et al., 1995).

The aim of this work was to compare FC and MD techniques in monitoring the transport of SA through the skin. The second objective was to determine if the effect of ionisation upon the transport of SA across human skin is the same by these two techniques.

2. Materials and methods

2.1. Chemicals

Salicylic acid (SA), of 99% purity, was purchased from Sigma (Paris, France). KH_2PO_4 and NaH_2PO_4 were obtained from Merck (Strasbourg, France). Phenobarbital, used as internal standard (IS), was purchased from Cooperation Pharmaceutique Française (Melun, France).

2.2. Donor solution

SA was dissolved in phosphate buffer of pH 2, 5 and 7 (0.05 M). Any pH adjustment required was performed using 2 M sodium hydroxide or 2 M hydrochloric acid. The concentration of SA was 2 mg/ml (Smith and Irwin, 2000).

2.3. Skin sample preparation

Excised human skin from female patients, who had undergone abdominal plastic surgery, was used (44±5 years old, mean ± S.D.). Immediately after excision the skin was wrapped in aluminium foil and stored in polyethylene bags at −18 °C until use. Under these conditions the skin is stable with regards to the penetration of drugs, as well the thickness of the stratum corneum, over a time period of 3 and 6 months, respectively (Harison et al., 1984; Bronaugh et al., 1986; Schaefer and Loth, 1996). One night before the experiments, they were placed in a refrigerator at +4 °C. To compare FC and MD experiments, under the same conditions, samples from the same donors of the same region were used for the two techniques. The skin samples were cut in two. Half of the samples were used for FC tests, in these the subcutaneous tissue was removed 1h before the experiments and the skin was cut into (3 cm × 3 cm) pieces. The other half was used for the MD experiments. For the penetration experiments, skin was thawed cleaned with cotton which was soaked with Ringer solution.

2.4. SA experiments using Franz type cells (FC)

Skin pieces were placed in all glass Franz type diffusion cells (Type FDC 200, Sommerville, NJ, USA). The absorption surface area was 3.14 cm^2. 200 μl of the SA solution (2 mg/ml) were deposited using a Hamilton micro-syringe on the donor chamber. The 8 ml volume of the receptor chamber of FC was filled with a receptor solution containing human albumin, diluted with a pH 7.4 phosphate buffer (1.4%). This concentration was chosen since it is similar to the interstitial fluid of the skin (Vermeer et al., 1975). The receptor chambers of the diffusion cells were surrounded with a water bath maintained throughout the experiment at 37 °C, corresponding to the normal skin temperature. The receptor solution was continuously agitated with a magnetic stirrer. During the experiments, the donor compartments and sampling arms were occluded to prevent evaporation.

The fluid in the receptor FC chamber was removed at different periods of time (15, 30, 45, 60, 120, 180, 240 and 300 min) and replaced by a new phosphate buffer solution. Samples were stored at −20 °C after sampling, prior to analysis.

2.5. Microdialysis experiments

2.5.1. Microdialysis system

The MD system consisted of a CMA/100® syringe pump (Phymep, Paris, France), and a CMA/140® microfraction collector. The MD probes (CMA/100®) have a 20 kDa cut off with a polycarbonate membrane (length 10 mm).

2.5.2. Determination of the relative recovery of salicylic acid

For ex vivo recovery, a No Net Flux method was used (Lonnroth et al., 1987; Petersen, 1995). Three microdialysis probes were inserted into abdominoplasty

skin fragments ($n = 2$, 42 ± 2 years old) and were perfused with different concentrations of SA (from 10 to 100 μg/ml). The difference between the concentration in the dialysate and the perfusate was plotted in relation to the concentration in the perfusate. In this way, a linear relationship was established and the slope represented the SA recovery and the SA concentration, in the medium, was characterized by the intercept with the *x*-axis.

2.5.3. Determination of SA penetration into human dermis by microdialysis

Six probes were inserted into human dermis of each skin sample 0.9 ± 0.1 mm below the skin surface. They were perfused with phosphate buffer solution (pH = 7.4, 0.05 M) at a flow rate of 3 μl/min. This value was optimum to collect SA. MD probes were perfused with buffer for an initial 1 h period to reach equilibrium. Then, SA solution (pH = 2, 5 or 7) was applied onto the skin surface (200 μl of the SA solution (2 mg/ml)). MD samples and FC were collected at the same time intervals. During the experiments, the donor compartments were occluded to prevent evaporation. The skin was maintained at 37 °C during the experiments.

2.6. Determination of salicylic acid concentration in receptor solutions by high performance liquid chromatography (HPLC)

2.6.1. Franz cell samples

Three milliliters of FC receptor solution were mixed with 4 ml of acetonitrile and 3 ml of phosphate buffer pH 2.83 and 40 μl of phenobartbital (50 μg/ml). This mixture was vortexed and the organic phase was evaporated with nitrogen vapour. The residue was dissolved in 40 μl of phosphate buffer pH 2.83 and 20 μl of this mixture was injected into HPLC system. To compare the FC results with those of MD, we needed to take in consideration the diluton factor. Therefore, the concentration obtained after the dilution was multiply by 0.04 ml and divided by 3 ml.

2.6.2. Microdialysis samples

40 μl of MD perfusate solutions were mixed with 40 μl of phenobarbital (50 μg/ml) and 20 μl of this new solution were injected in the HPLC system.

A Merck/ Hitachi A55 HPLC system was used to assess SA. A RP18 column (5 μm, 125 mm × 4 mm) was used for molecular separation. A 25/40/35 (v/v/v) mixture of acetonitrile/ phosphate buffer (pH 2.83; 0.05 M)/water was the composition of the mobile phase (Pirola et al., 1998). At a flow rate of 1 ml/min, the retention time of SA and phenobarbital (internal standard, IS) were 3.4 and 6.0 min, respectively. The UV detector was set at 230 nm. Eight standard solutions between 0.3 and 25 μg/ml were analysed in triplicate to determine the linearity of the assay. Peak height ratios of SA and phenobarbital (IS) were used for calculations. The concentrations of SA in the receptor solutions were found directly from the standard calibration graph ($r \geq 0.99$) and the method provided good linearity over a concentration range of 0.3–25 μg/ml.

2.7. Statistics

All data were calculated and presented as mean ± S.D. The statistical significance between SA concentrations obtained by FC and MD techniques was determined by two tailed, unpaired test. A value of $P < 0.05$ was considered significant.

3. Results and discussion

3.1. Salicylic acid relative recovery determined by microdialysis

It is well established that the quantity of a compound recovered by MD is only a fraction of the quantity present in the tissue (Le Quellec et al., 1995). The results are usually expressed in terms of the relative recovery (RR). Benfeldt et al. (1999) have determined in vitro and in vivo SA recovery. In vitro work consisted in placing a microdialysis probe in a vial containing SA. The probe was perfused with a buffer solution and the collected SA allowed the determination of the in vitro relative recovery. According to these authors, in vitro SA recovery was equal to $80 \pm 3\%$ ($n = 13$) while in vivo SA recovery was $24 \pm 4\%$ ($n = 4$). As in vitro and in vivo RR differed, according to Benfeld et al. (1999), ex vivo RR of SA was conducted in the current work by using human skin samples (Fig. 1). It was $28.1 \pm 3\%$ ($n = 6$). The ex vivo recovery value was very similar to the in vivo RR value ($P = 0.07$, unpaired test).

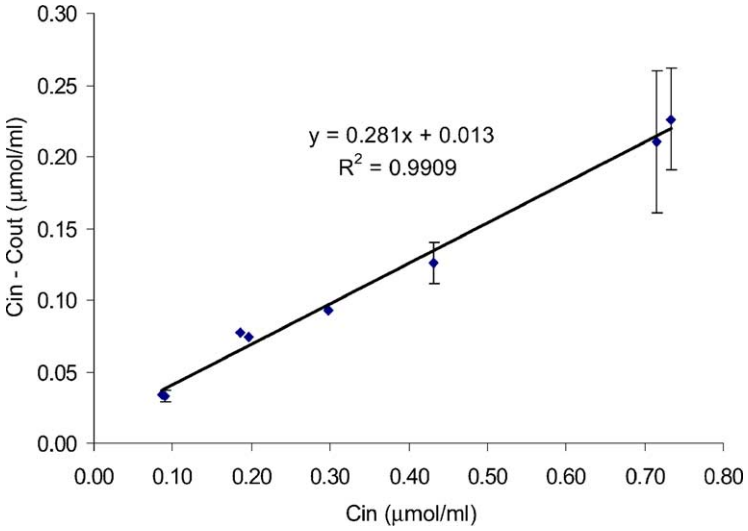

Fig. 1. SA recovery determined by NNF method. C_{in} represents the SA concentration in the perfusate and C_{out} the SA concentration in the microdialysis samples. Each value represents mean ± S.D. ($n = 6$). The slope of the curve represents the relative recovery of SA.

3.2. Salicylic acid permeation determined by Franz cells and microdialysis

Cumulated permeated amounts of SA determined by MD and FC are showed in Figs. 2 and 3. The results demonstrate that, up to 2 h, SA penetration was significantly higher with MD than with FC technique, irrespective of pH ($P < 0.05$). Different hypotheses may be suggested: firstly, according to Ault et al. (1992) the insertion of the cannula guide, which permitted the implantation of the probe, may affect the SA permeated amount. Secondly the efficiency of salicylic removal from the dermis is thought to be better for the MD technique (Schnetz and Fartasch, 2001). In the FC technique the SA diffuses through both epidermis and dermis membranes. In microdialysis, SA

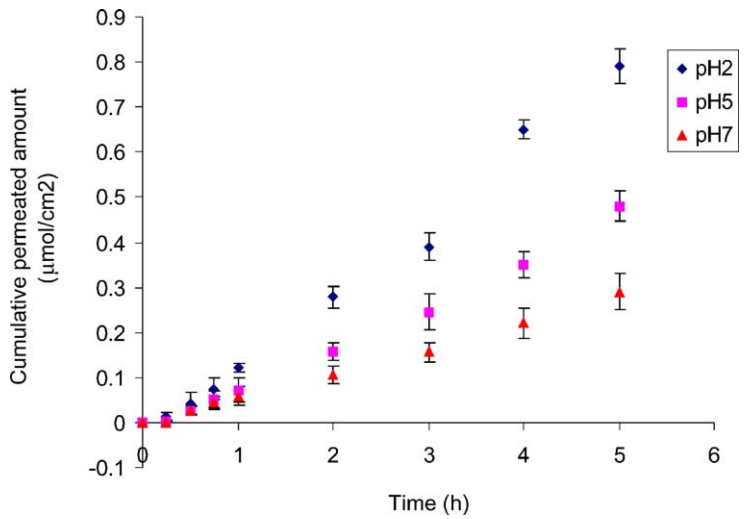

Fig. 2. Cumulative permeated amount of salicylic acid determined with microdialysis technique. Each value represents the mean ± S.D. ($n = 24$).

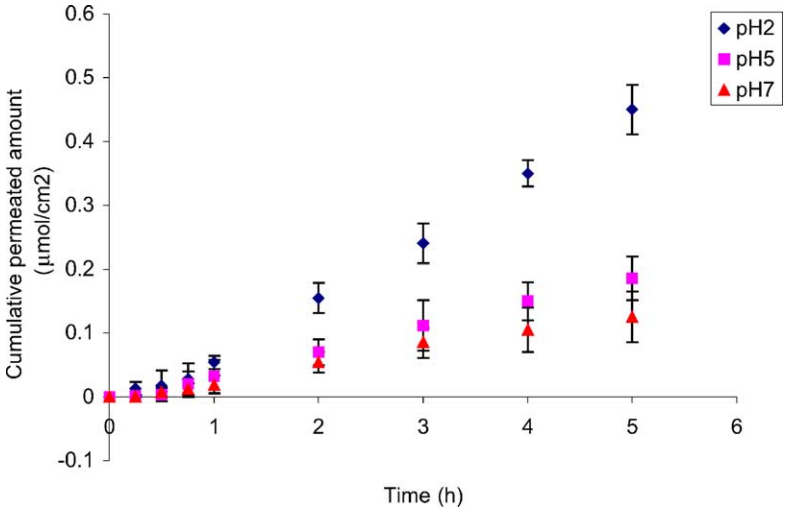

Fig. 3. Cumulative permeated amount of salicylic acid determined with Franz cells technique. Each value represent mean ± S.D. ($n = 24$).

penetrated the epidermis and only a part of the dermis layer, since the microdialysis probe was inserted in the dermis. Therefore, SA collected by Franz cells and microdialysis did not necessarily diffuse to the same skin depth.

3.3. Influence of pH on salicylic acid flux

The ionised part f_{ion}, can be calculated according to the following equation (Martin et al., 1975):

$$f_{ion} = \frac{1}{1 + 10^{(pK_a - pH)}}$$

The highest steady state flux was determined at pH 2 (Table 1). The pK_a for SA is 3.1. The results indicated that the SA flux significantly decreased with the amount of ionised form. This demonstrates that the topical delivery of SA obeys the pH–partition theory and transdermal permeation is due to the unionised species. Smith and Irwin (2000) demonstrated that SA percutaneous absorption was due to unionised species over a pH range 1.8−4.7. Thus, our results, over a pH range 2−7, were in good accordance with those of Smith and Irwin (2000). However, they contrast with those of Loftsson (1985) who proposed that increased ionisation can promote topical delivery of SA. This contrasting conclusion could be explained by the fact that Loftsson determined SA permeation profile during a long time (3–12 h). During this long period, the absorption profiles could have been influenced by the SA's keratolytic effect upon stratum corneum.

Table 1
Steady state flux of salicylic acid determined by microdialysis and Franz cells through a human skin membrane at different pH values ($n = 24$)

pH	Fraction ionised	Microdialysis flux (µmol/cm^2/h)	Franz cells flux (µmol/cm^2/h)
2	0.09	0.162 ± 0.006*	0.095 ± 0.010
5	0.99	0.095 ± 0.009*	0.04 ± 0.011
7	0.99	0.051 ± 0.008*	0.033 ± 0.006

* Values significantly different to those obtained with Franz cells ($P < 0.05$).

4. Conclusion

The permeation profiles determined by Franz cells and microdialysis were similar. However, the SA flux was higher with microdialysis than with Franz cells. The sampling depths of SA are different in these two techniques. The flux of SA decreased with increased pH. Apparently, for more efficient permeation SA should be unionised for delivery across the stratum corneum barrier.

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