Study of the Percutaneous Penetration of Flurbiprofen by Cutaneous and Subcutaneous Microdialysis after Iontophoretic Delivery in Rat

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ABSTRACT: The percutaneous penetration of flurbiprofen delivered by iontophoresis was investigated in the hairless rat. Unbound concentrations of flurbiprofen in dermis and subcutaneous tissue were continuously measured by on-line microdialysis. Simultaneously, a conventional blood sampling was performed. Linear microdialysis probes were implanted in dermis and in subcutaneous tissue at a depth of 398.3 ± 15.3 and 1878 ± 35.8 μm, respectively. Commercial patches were used to deliver flurbiprofen for 15 min at a current density of 0.4 mA/cm^2. *In vivo* recoveries of both probes, determined by using naproxen as retrodialysis calibrator, were 26.0 ± 0.3 and $72.9 \pm 0.7\%$ for dermal and subcutaneous probe, respectively. After iontophoretic delivery, a gradient in mean tissue unbound concentrations was observed, with a C_{max} in dermis of 8.7 ± 0.4 μg/mL as compared with subcutaneous C_{max} of 0.5 ± 0.1 μg/mL. The area under the unbound concentration curve in dermis was 13-fold higher than that in the subcutaneous tissue. Total plasma concentration curves showed a rapid absorption phase with a T_{max} of 30 min and C_{max} of 1.8 ± 0.1 μg/mL. In conclusion, iontophoresis delivery was demonstrated to be efficient to deliver a high amount of flurbiprofen in dermis and underlying tissue with a fast input rate whereas maintaining a low plasma exposure. © 2004 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 94:144–152, 2005
Keywords: iontophoresis; microdialysis; transdermal drug delivery; skin

INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely prescribed for patients with rheumatic disease.[1] They are among the most commonly prescribed drugs worldwide and are responsible for approximately one-fourth of all adverse drug reaction reports.

Flurbiprofen is a potent propionic acid-derived NSAID that has been widely available in tablet form since 1977. Although flurbiprofen has potent pharmacological activities after oral administration, it also has adverse effects secondary to prostaglandin inhibition: gastropathy, prolonged bleeding time, fluid retention, and acute renal insufficiency.[2] Considering the fact that flurbiprofen is often used for a long period, it is desirable to reduce these side effects. Transdermal administration of flurbiprofen could achieve therapeutic concentrations in the tissues subjacent to the site of application whereas maintaining low plasma concentrations. Because NSAID-mediated toxicity is often dose related, reduction in plasma concen-

trations should also decrease the risk of potentially serious systemic adverse effects. Furthermore, avoidance of the gastrointestinal tract should mitigate the common direct toxicities that occur secondary to high local concentrations of NSAID in the gastrointestinal tract.

For this purpose, flurbiprofen is formulated in the form of a topical patch (10×14 cm) containing 40 mg of flurbiprofen. TransAct LAT® flurbiprofen (Local Action Transcutaneous) is designed to be applied to skin overlying sites, such as the shoulder, elbow, knee, or ankle affected by painful and inflamed acute musculoskeletal conditions. After a single application of a LAT flurbiprofen patch for 12–14 h in volunteers, flurbiprofen penetrated slowly and in small quantities into the systemic circulation.[3,4] The relative bioavailability of flurbiprofen absorbed from the 40-mg patch is about 4% of that of a 50-mg tablet. A large gradient in mean tissue concentrations of flurbiprofen was reported by Frostick et al.[5] after LAT flurbiprofen application. Based from samples taken during an arthroscopy, the highest concentration was measured in the skin (77.8 µg/g), followed by subcutaneous fat (7.2 µg/g) and the synovium (1.4 µg/g).[5] The main limitations of the LAT flurbiprofen patch are the long application time and the high interindividual variability observed in the pharmacokinetics of flurbiprofen due to individual skin properties.[4]

Iontophoresis is a noninvasive and safe technique, which uses a mild electric current to facilitate transdermal delivery of a variety of drugs, including NSAIDs. Typically, current density in the range of $0.1–0.5$ mA/cm^2 is applied for minutes or hours. The principle of iontophoresis is based on electrorepulsion and electroosmosis.[6,7] Electrorepulsion refers to the mechanism whereby a charged molecule moves away from the electrode of the same polarity into the skin. Electroosmosis, however, is the result of the convective solvent flow produced by the application of an electrical potential gradient across the skin charged negatively under physiological conditions. In addition to the common advantages of transdermal drug delivery, iontophoresis offers the following benefits: i) the dosage regimen can be tailored at a programmed rate for a bolus or continuous delivery because the delivery of drug is controlled by current application; ii) a fast input rate of drug can be achieved because the drug is transported by an electrical force and not by a concentration gradient; and iii) a reduction of interindividual variability is obtained because the rate of drug delivery

is proportional to the current.[8,9] Based on these advantages, iontophoretic transdermal delivery of flurbiprofen could be an interesting alternative to passive permeation methods.

The determination of unbound active concentrations of flurbiprofen underlying the site of application, particularly the skin and subcutaneous tissue, is more relevant than the measurement of total plasma concentrations. This is particularly true for drugs such as flurbiprofen which present a high plasma protein binding and are mostly confined to the central compartment.[10,11] Techniques that directly measure tissue drug concentrations are more appropriate for pharmacokinetic studies than indirect estimations of tissue levels from plasma concentrations. As alternative to the subcutaneous implantation of a disk-shaped agar gel in which cutaneously delivered drug is measured at different time points,[12] (sub-) cutaneous microdialysis offers the advantage to assess the percutaneous penetration of drug continuously and under minimally invasive conditions.[13,14] Furthermore, microdialysis has already been demonstrated to be suitable for the evaluation of iontophoresis delivery.[15,16] Indeed, both iontophoresis and microdialysis work well with polar molecules and recovery of the probe is not modified by current application.[17]

The objective of the present study was to investigate the *in vivo* percutaneous penetration of flurbiprofen after iontophoresis delivery. To measure *in vivo* the unbound active concentrations of flurbiprofen in dermis and in subcutaneous tissue, a dual-site sampling by microdialysis was performed with simultaneous conventional blood sampling.

MATERIALS AND METHODS

Reagents

Flurbiprofen, naproxen, ketamine, and xylazine were purchased from Sigma Chemical Co. (St. Louis, MO). Solvents were high-performance liquid chromatography (HPLC) grade and all other chemicals used were analytical reagent grade.

Surgical Procedure

Hairless male rats (Iffa Credo, Saint-Germain, France), 10–12 weeks old, weighing 315 ± 30 g (mean $\pm$ SD, $n = 5$), were anesthetized with a ketamine/xylazine mixture (90:10 mg/kg), 1 day

before the pharmacokinetic study, to implant cannulae of polyurethane tubing (Access Technology, Skokie, IL) in the left jugular vein (0.6 mm i.d. × 0.9 mm o.d.) using standard surgical procedures. The animals were allowed to recover from the surgery overnight. The described experimental procedures in the rats were approved by the University Animal Experimentation Ethics Committee.

Microdialysis Probes

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 5000 Da. The fiber (20-mm length) was glued at both ends to a piece of silicone tubing (0.012 in. i.d., 0.025 in. o.d.; Specialty Manufacturing Inc., Saginaw, MI) using B-210 cyanoacrylate glue (3M, Brussels, Belgium).[11]

Dermal Microdialysis Probe

The linear dermis microdialysis probes were manufactured using the same Hemophane® dialysis fiber as the one used for 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.[14]

Implantation of the Microdialysis Probes in the Rat and Microdialysis System

Two hours before starting the experiment, the animals were anesthetized with a mixture of 4 mg/kg droperidol and 0.08 mg/kg fentanyl injected subcutaneously. The skin of the dorsal region in the rat was punctured horizontally by a 23-gauge intravenous (iv) needle (i.d. 0.6 mm, length 30 mm) for subcutaneous implantation and by a 26-gauge iv needle (i.d. 0.45 mm, length 12 mm) for dermal implantation.[14] The linear microdialysis probe was inserted through the guide needle. The needle was then withdrawn leaving the dialysis membrane in the subcutaneous tissue or in the dermis.

Rats were placed in a freely awake moving system of microdialysis (CMA/120, Stockholm, Sweden) for the total duration of the experiment

and had free access to food and water during the microdialysis sampling period.

The probe inlet and outlet were connected to a dual-channel swivel (Instech Laboratories Inc., Plymouth Meeting, PA) using PEEK® tubing (0.65-mm o.d. × 0.12-mm i.d.; CMA). The inlet of the swivel was then connected to a syringe pump (CMA/100) whereas the outlet was linked to a 10-port pneumatic valve. The probe was perfused with isotonic phosphate buffer pH 7.4 containing naproxen (200 ng/mL), as retrodialysis calibrator, at a flow of 1.5 μL/min.[11]

In Vivo Evaluation of the Recovery of the Probes

In vivo recovery of flurbiprofen was determined by the retrodialysis method or "internal reference technique" which consists in adding a retrodialysis calibrator to the perfusate and measuring the rate of delivery of this compound. The principle of this *in vivo* calibration method relies on diffusion being quantitatively equal in both directions through the membrane.[18,19] The choice of naproxen as a retrodialysis calibrator for *in vivo* calibration of the probe to sample flurbiprofen was fully validated in previous studies[10,11] in which the recovery of flurbiprofen was demonstrated to be quantitatively the same as the delivery of naproxen.

After its implantation in the subcutaneous tissue or in the dermis, the probes were perfused at a flow rate of 1.5 μL/min with isotonic phosphate buffer pH 7.4 containing naproxen (200 ng/mL), as a retrodialysis calibrator. Analyte concentrations were measured in the microdialysate samples (C_{out}) every 11 min for 8 h. The recovery was determined from the ratio of the concentration lost to the initial concentration in the perfusate (C_{in}):

$$\text{Recovery}_{in\ vivo} = [(C_{in} - C_{out})/C_{in}] \times 100$$

Iontophoresis

To study the percutaneous penetration of flurbiprofen delivered by iontophoresis, a dual-site sampling by subcutaneous and cutaneous microdialysis with simultaneous blood sampling was performed in six hairless male rats.

The GelSponge® pad of an iontophoretic patch with a contact area of 7.2 cm^2 (Iogel™ Small; IOMED Inc., Salt Lake City, UT) was saturated with 1.4 mL of flurbiprofen solution at 2 mg/mL in 0.04 M HEPES buffer pH 7.4. Therefore, the applied dose of flurbiprofen was 2.8 mg corresponding to 8.9 mg/kg (for a mean weight of 315 g).

The patch was applied on the dorsal region of the rat above the implanted probes and maintained in place with the help of a flexible wrist support during the current application. The patch was connected to the cathode of the custom-made constant current generator. A direct current of 3.1 mA (0.4 mA/cm^2) was applied during 15 min. After this period, the iontophoretic patch was withdrawn and the skin carefully cleaned with cotton wool to remove any excess of gel. The application site was not covered during the sampling period.

In all pharmacokinetic studies, after implantation of the probe and before the administration of flurbiprofen, a period of 90–120 min was required to stabilize the skin blood flow which may have been perturbed by the insertion of the steel mandrel during the implantation procedure.[14] Blood samples (250 μL) obtained from jugular cannula were collected into K$_2$-ehtylenediamine-tetraacetic acid tubes at the following times: 0 (blank), 2.5, 5, 10, 15, 30, 45, 60, 120, 240, 360, and 480 min. Blood samples were immediately centrifuged and the plasma stored at −20°C until analysis.

Probe Depth Measurement

To measure the probe depth after implantation and to determine the accurate position of the microdialysis probe in the dermis and subcutaneous tissue, histological slices were prepared. After biopsy, the tissue was fixed in a 4% formalin solution and embedded in paraffin wax. Sections were cut perpendicularly to the surface of the skin. Tissue processing and staining with hematoxylin/eosin stain were performed following standard procedures.

The probe depth was measured by using an optical microscope equipped with a graduated lens.

On-line Analysis of Microdialysate Samples by HPLC

The chromatographic system consisted of an HPLC pump (model 420; Kontron Instruments, Milan, Italy), a programmable fluorescence detector (Spectrasystem FL2000; Spectraphysics, San Jose, CA), a Varian model 4290 integrator (Varian Instruments, Walnut Creek, CA), and a 10-port pneumatic valve (Valco Instruments Co. Inc., Houston, Texas) fitted with two PEEK$^®$ injection loops 10.5 μL (Bioanalytical Systems Inc, Con-

gleton, Cheschire, UK). The injection valve was configured to collect directly the dialysate samples of flurbiprofen from the subcutaneous and dermal probes into the injection loops and to inject alternately the samples every 11 min onto a Nucleodur C$_{18}$ Gravity^{TM} column (125 × 2.0 mm i.d., 3 μ; Macherey-Nagel, Düren, Germany). Therefore, in pharmacokinetic sense, the temporal resolution for flurbiprofen in a given tissue (subcutaneous tissue or dermis) was 22 min with this double-site sampling set-up. The mobile phase consisted of monopotassium phosphate buffer 30 mM (pH 2.5) and acetonitrile (52:48, v/v) and was delivered at 0.2 mL/min. The eluate was monitored using the following excitation (ex) and emission (em) wavelengths: 262 nm (ex) and 356 nm (em) from 0 to 4 min (naproxen), and 258 nm (ex) and 310 (em) from 4 to 10 min (flurbiprofen).

The inter- and intra-day RSDs of the slopes of six calibrations curves were 5.2 and 3.1%, respectively. No significant difference was observed between intra- and inter-day values of the slopes (analysis of variance, $p > 0.05$). The limit of detection and quantification were 4.9 and 8.2 ng/mL, respectively, with an average bias <10%.

HPLC Assay of Plasma Samples

Flurbiprofen plasma concentrations were determined by HPLC with fluorescence detection and naproxen as internal standard. Spiked plasma calibrators were prepared at eight different concentrations of fluconazole (50, 100, 250, 500, 750, 1000, 1500, and 2000 ng/mL) by adding appropriate amounts of a stock solution of flurbiprofen (200 ng/mL) in methanol evaporated to dryness under gentle nitrogen stream at 40°C, to blank rat plasma.

Plasma samples and plasma calibrators were extracted by using 1-mL cartridges containing 30 mg of Oasis-HLB (Waters, Milford, MA). The sorbent was first conditioned with 1 mL of methanol and equilibrated with 1 mL of ultrapure water (Sation 9000; Vel, Leuven, Belgium). Plasma samples (0.1 mL) were diluted with 0.1 mL of phosphate buffer 0.01 M pH 7.0 containing naproxen, the internal standard at 625 ng/mL. The diluted plasma acidified by adding 2 μL of a phosphoric acid solution 85% v/v was applied to the column under light vacuum (3 to 4 in. Hg on the gauge). The column was then washed with 1 mL of 5% (v/v) methanol in acetic acid solution 2% (apparent pH 3), followed by a wash with 1 mL of

70% (v/v) methanol in acetic acid solution 2%, and subsequently by 1 mL of ultrapure water. Final elution of flurbiprofen and internal standard was performed with 1 mL of 90% (v/v) methanol in ammonia solution 2% (apparent pH 11). Before evaporation at 40°C under a gentle stream of nitrogen, the eluate was filtered on a 0.22-μm polyvinylidene fluoride filter. The residue was reconstituted in 0.25 mL of chromatographic mobile phase, and 10.5 μL was injected onto the narrow-bore HPLC system by using an autosampler (Gilson model 231, Villers-le-Bel, France) equipped with a 500-μL syringe.

Recovery of flurbiprofen from plasma was calculated by comparing the slopes of the calibration curves of the calibrators in plasma with the slope of the same standards prepared in the mobile phase and injected directly. Oasis-HLB sorbent allowed obtainment of a recovery of flurbiprofen and naproxen of 92.1 ± 3.9 and $89.7 \pm 3.6\%$, respectively, over the studied concentration range (50–2000 ng/mL). These values of recoveries satisfactorily meet the acceptance criteria.[20]

The inter- and intra-day RSDs of the slopes of six calibration curves were 6.3 and 3.8%, respectively. No significant difference was observed between intra- and inter-day values of the slopes (analysis of variance, $p > 0.05$). The limit of detection and quantification were 34 and 57 ng/mL, respectively, with an average bias <10%.

Data Analysis

Pharmacokinetic parameters were determined by the noncompartmental analysis (WinNonlin 3.1; Pharsight Corp., Mountain View, CA). Area under the curve (AUC) values were calculated using the linear trapezoidal rule from 0 to t (last microdialysate or blood sampling time) with extrapolation to infinity (plasma or dialysate concentration at time t divided by slope λ_z). Terminal dialysate or plasma half-time ($t_{1/2z}$) was calculated as $0.693/\lambda_z$ where λ_z is estimated by linear regression of the terminal log-linear phase of the dialysate or plasma concentration-time curve. Systemic bioavailability after iontophoretic delivery (F) was determined from the ratio of the AUC value in plasma after iontophoretic delivery to the AUC value after iv bolus. This last AUC value presently used for the F topical calculation was determined in a previous study after iv bolus administration of flurbiprofen at a dose of 20 mg/kg in hairless rat.[11] For microdialysis data, the midpoint of the sampling interval was used as time-point for calculations. The analyzed dialysate concentrations were corrected for estimated *in vivo* recovery (based on the loss of retrodialysis marker) at each sampling interval. Values in the text are expressed as the mean $\pm$ SEM.

RESULTS AND DISCUSSION

Probe Depth Measurement

Histological examination of hairless rat skin showed that probe insertion did not result in significant physical damage to the subcutaneous tissue. The linear probe was implanted in the subcutaneous tissue at 1878.0 ± 35.8 μm ($n = 5$), below the smooth skeletal muscle layer which separates the hypoderm from the subcutaneous tissue. As previously reported,[11] the implantation procedure of the subcutaneous probe showed a good reproducibility of implantation depth characterized by an interindividual RSD of 5% ($n = 5$).

In dermis, the linear probe was implanted in the upper dermis at an average depth of 398.3 ± 15.3 μm ($n = 5$). The use of a thinner guide cannula (26G) allowed insertion of the probe more superficially with minimal tissue perturbation and good reproducibility in the implantation depth of the dermal probe [interindividual RSD of 10.2% ($n = 5$)].[14]

In Vivo Evaluation of the Recovery of the Probes

The *in vivo* recoveries of the subcutaneous and dermal probe were continuously monitored during the experiment by using the *in vivo* loss of naproxen. *In vivo* loss of naproxen was $26.0 \pm 0.3\%$ ($n = 95$) and $72.9 \pm 0.7\%$ ($n = 90$) for the dermal and subcutaneous probe, respectively. A very similar mean value ($71.5 \pm 0.9\%$) for the *in vivo* retrodialysis of naproxen was obtained for subcutaneously implanted microdialysis probes in a previous study in which flurbiprofen (20 mg/kg) was administered intraperitoneally or iv.[11] This shows that the iontophoretic current does not affect the diffusion characteristics of the microdialysis probes. This has also been reported by Stagni et al.[17] who used fluorescein as a retrodialysis calibrator.

The significant difference observed between the *in vivo* recovery of dermal and subcutaneous probes is explained by the difference of the membrane length (10 mm for dermal probe versus

20 mm for subcutaneous probe) and by the tortuosity factor dependent on the structure and biological nature of the target tissue.[21−23]

The use of a retrodialysis calibrator to monitor continuously the *in vivo* recovery allows control in real time of any small fluctuations of *in vivo* recovery during the experiment. In the present work, *in vivo* recovery fluctuations within rats were $4.9 \pm 0.5\%$ for dermal probes and $4.4 \pm 0.6\%$ for subcutaneous probes. This relatively constant dialysis efficiency of both probes after implantation and during all the experiments is illustrated in Figure 1. The mean interindividual RSDs for dermal and subcutaneous probes were 10.5 ± 0.7 and $12.7 \pm 0.8\%$, respectively.

Cutaneous Penetration of Flurbiprofen after Iontophoresis Delivery

To date, this is the first *in vivo* study in which an on-line dual-site sampling by microdialysis was used to investigate the percutaneous penetration of a drug after iontophoretic delivery. The dual-site sampling set-up allowed us to characterize simultaneously the unbound flurbiprofen concentrations in dermis and subcutaneous tissue below the application site. After iontophoresis delivery, a gradient in tissue concentrations of flurbiprofen was observed. Figure 2 shows the mean semilogarithmic concentration-time profiles for total flurbiprofen in plasma and unbound flurbiprofen in the extracellular spaces of subcutaneous tissue and dermis in five rats after iontophoresis delivery with simultaneous microdialysis and blood sampling. Unbound concentrations of flurbiprofen
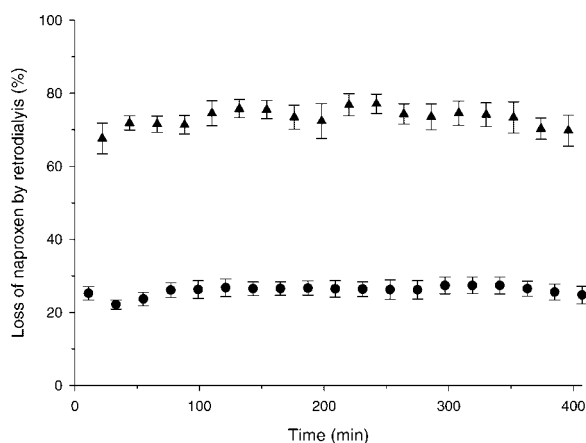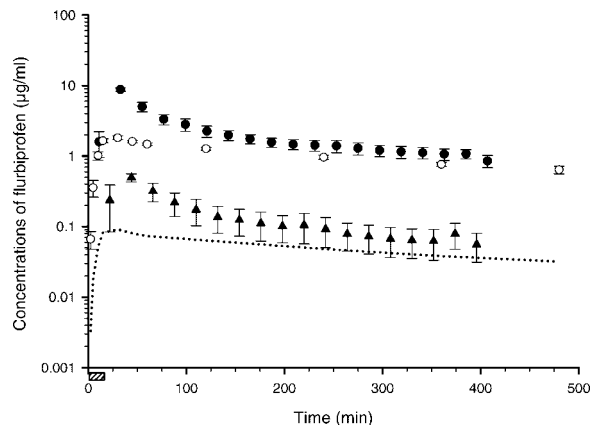
Figure 2. Semilogarithmic presentation of the concentration-time profiles of total flurbiprofen in plasma ($\bigcirc$) and unbound flurbiprofen in subcutaneous tissue ($\blacktriangle$) and in dermis ($\bullet$) after iontophoresis delivery. The dotted line represents calculated unbound plasma concentrations based on an unbound fraction (fu) of 0.5%. The hatched box represents the period of time during which the current was applied. For microdialysis data, the midpoint of the sampling interval was used as time-point for calculations and plotting. Values are the mean $\pm$ SEM ($n = 5$).

in dermis reached relatively high levels with an average C_{max} of 8.7 ± 0.4 µg/mL in comparison with a subcutaneous C_{max} of 0.5 ± 0.1 µg/mL (Table 1). The area under the unbound concentration curve in dermis was 13-fold higher than that in the subcutaneous tissue. Unexpectedly, unbound concentrations of flurbiprofen in dermis stayed 10-fold higher than unbound concentrations in subcutaneous tissue. A similar observation was reported after topical administration of ibuprofen in humans.[24] Indeed, by using microdialysis sampling in subcutaneous tissue (probe depth of 4−5 mm) and skeletal muscle, Tegeder et al.[24] showed that subcutaneous unbound concentrations of ibuprofen were approximately 4000 times higher than the estimated unbound concentrations in plasma. Based on the measurement of total concentrations in plasma and tissue, Singh and Roberts[25] showed that iontophoretic delivery of lidocaine and salicylic acid resulted in higher concentrations of drugs in skin, dermis, and subcutaneous tissue below the application site than those observed in plasma to a depth of 3−4 mm. A similar observation was reported by Hui et al.[26] for diclofenac sodium after iontophoretic delivery in rabbit. In this study, the comparison between diclofenac concentrations measured beneath the application site and the

Figure 1. Loss (%) of naproxen by retrodialysis for subcutaneous ($\blacktriangle$) and dermal ($\bullet$) probes. Values are the mean $\pm$ SEM ($n = 5$).

Table 1. Flurbiprofen Pharmacokinetic Parameters Determined Using Conventional Blood Sampling, Subcutaneous and Dermal Microdialysis Sampling after Iontophoresis Delivery

	Plasma	Subcutaneous Microdialysis[a]	Dermal Microdialysis[a]
AUC_{0-t} ($\mu g \times h/mL$)	8.2 ± 0.3^{b}	1.1 ± 0.2	14.0 ± 1.2
AUC_{∞} ($\mu g \times h/mL$)	14.4 ± 1.5	1.3 ± 0.5	17.4 ± 1.7
Percent of extrapolated area[c]	43	23	24
$T_{1/2z}$ (h)	6.4 ± 0.9	4.4 ± 0.3^{d}	4.5 ± 0.2^{d}
C_{max} ($\mu g/mL$)	1.8 ± 0.1	0.5 ± 0.1	8.7 ± 0.4
T_{max}^{f} (min)	30	44	33
F^{e}	0.11	n.d.	n.d.

[a]Calculated based on unbound concentration-time profiles.
[b]Values are listed as the mean $\pm$ SEM of five rats.
[c]Percentage of extrapolated area is calculated as follows: $[1- (AUC_{0-t}/AUC_{\infty})] \times 100$.
[d]Not significantly different (paired t test, $p > 0.05$).
[e]Systemic bioavailability from iontophoretic delivery (F) (see section Data Analysis) was calculated as follows: $F = \left(\dfrac{AUC_{ionto} \times Dose_{iv}}{AUC_{iv} \times Dose_{ionto}}\right)$
[f]T_{max} parameter for microdialysate data depends on the sampling time of the dual-site sampling set-up.
n.d., not determined.

contralateral site (nondosed site) have demonstrated that the increased tissue concentrations at the iontophoretic delivery site could not be attributed to redistribution from the blood. The dual-site sampling by microdialysis could be a powerful tool to sort out the respective contributions of iontophoresis delivery and redistribution from plasma to subcutaneous tissue.

Despite a different applied dose of flurbiprofen (8.9 mg/kg), iontophoretic delivery leads to relatively similar unbound concentrations in the subcutaneous tissue to those observed after iv administration of a 20 mg/kg dose.[11] We previously reported unbound subcutaneous C_{max} and $AUC_{unbound}$, after iv bolus injection at a dose of 20 mg/kg, of 0.7 ± 0.1 $\mu g/mL$ and 1.6 ± 0.2 $\mu g \cdot h/mL$, respectively, versus 0.5 ± 0.1 $\mu g/mL$ and 1.1 ± 0.2 $\mu g \cdot h/mL$ after iontophoresis delivery (present study).

Simultaneous to the microdialysis sampling, a conventional blood sampling was performed with the first blood sample obtained after 2.5 min of current application in order to characterize the plasma absorption phase. Indeed, the rapid absorption phase observed in the total plasma concentration curve corresponds to the 15-min period during which the current was applied (Fig. 1). Once the current is switched off, plasma concentrations continue to increase, reaching a peak plasma concentration of 1.8 ± 0.1 $\mu g/mL$ at 30 min. The systemic bioavailability of flurbiprofen after iontophoretic delivery could be calculated based on

the previously published AUC after administration of an iv bolus dose of this NSAID. The calculated systemic bioavailability of flurbiprofen after iontophoretic delivery was 0.11 (see Table 1). Based on the unbound fraction of flurbiprofen (fu) in rat plasma determined *in vivo* by vascular microdialysis, unbound plasma concentrations of flurbiprofen were calculated by using a mean fu value of 0.5%. Although the plasma binding of flurbiprofen is concentration dependent, it was demonstrated that at low total plasma concentration (<26 $\mu g/mL$) of flurbiprofen, *in vivo* plasma binding was 99.5% and constant.[10] The unbound concentrations in subcutaneous tissue are relatively similar to the calculated unbound concentrations in plasma, especially in the terminal phase. This suggests that the unbound concentrations of flurbiprofen in the subcutaneous compartment are in equilibrium with those in the plasma compartment.

A well-known advantage of iontophoresis over the patch system or gel is to reduce the variability attributed to individual skin differences.[27] With iontophoresis delivery, the administration of drug is mainly controlled by the application of iontophoretic current which mitigates the interindividual variability. In the present work, mean interindividual RSDs for pharmacokinetic parameters such as AUC_{0-t} and C_{max} varied in the range of 9.2–10.5 and 11.4–15.5%, respectively. After a single application of LAT flurbiprofen in humans, mean RSDs of 23.5 and 37.2% for AUC_{0-t}

and C_{max}, respectively, were reported by Taburet et al.[4]

CONCLUSIONS

In conclusion, this is the first *in vivo* study in which a dual-site sampling by microdialysis was successfully performed to investigate the percutaneous penetration of flurbiprofen after iontophoresis delivery. Unbound flurbiprofen concentrations in dermis and subcutaneous tissue were simultaneously characterized with a good temporal resolution and with minimum trauma. The dual-site sampling allowed us to characterize in real time the gradient in flurbiprofen concentrations between dermis and subcutaneous tissue. Iontophoresis was demonstrated to be efficient to deliver flurbiprofen in the skin with a fast input rate and a low absolute bioavailability of 0.11.

ACKNOWLEDGMENTS

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