Fluconazole Distribution in Rat Dermis following Intravenous and Topical Application: A Microdialysis Study

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ABSTRACT: The objective of this study was to investigate the skin distribution of fluconazole, a water-soluble antifungal agent, following intravenous (i.v.) and topical administration in the awake freely moving rat. Following i.v. bolus injection of fluconazole (10 mg/kg), a dual-site microdialysis sampling was performed in jugular vein and dermis in five rats. In addition, cutaneous absorption was studied by dermal microdialysis sampling following topical application of Diflucan® Gel 0.5% to 12 rats. Fluconazole microdialysate concentrations were measured by on-line HPLC. To calibrate in vivo the probes, a fluorinated analog (UK-54737) of fluconazole was used as retro-dialysis marker after demonstrating that recoveries were no different. Following i.v. bolus injection, fluconazole rapidly penetrates into the dermis. Cutaneous microdialysis sampling provided dermal concentrations of fluconazole, which were very similar to the unbound plasma concentrations determined by vascular microdialysis. The distribution equilibrium was rapidly achieved with a dermis-to-plasma partition coefficient of 1.02 ± 0.04 (n = 5). Following topical application of 0.5 g of Diflucan Gel® containing 0.5% of fluconazole, active unbound concentrations in dermis were measured by cutaneous microdialysis for 11 h after application. The area under the curve (AUC) of fluconazole in dermal dialysate was relatively constant to an implantation depth of approximately 350 µm. Below this depth, the AUC progressively decreased with increasing implantation depth of the probe. Finally, this study shows that cutaneous microdialysis is an effective and minimally invasive tool to evaluate the dermal pharmacokinetics of fluconazole following intravenous or topical administration.

Keywords: fluconazole; skin; topical; microdialysis; rat; distribution; cutaneous; pharmacokinetics

INTRODUCTION

Fluconazole, [2-(2,4,-difluorophenyl)-1,3-bis(1H-1, 2, 4-triazol-1-yl)propan-2-ol], an orally active antifungal agent, is used in the treatment of superficial and systemic candidiasis and in the treatment of cryptococcal infections in patients with the acquired immunodeficiency syndrome (AIDS). It acts by blocking the synthesis of ergosterol, an essential component of the fungal cell membrane.1 As for antibiotics, the determination of unbound active concentrations of antifungal agents at the site of infection, particularly in the dermis, is more relevant than the measurement of plasma concentrations. Hence, techniques that directly measure drug concentrations in the target tissue are more appropriate than
indirect estimations of tissue levels from plasma concentrations. A limited number of techniques is available for direct assessment of drug concentrations in dermis. These include the skin blister-fluid method and biopsy followed by tissue homogenization. However, ethical considerations, cost, and lack of reproducibility limit the applicability of these techniques in pharmacokinetics. Moreover, these techniques only allow to determine the total concentrations of drug, whereas the pharmacological activity relates to the unbound concentration.

Overcoming the inherent limitations of the above-mentioned techniques, microdialysis is an in vivo sampling technique that has become very popular in conventional pharmacokinetic studies. During the last decade, cutaneous microdialysis has been successfully applied to assess cutaneous drug delivery, indicating the large potential for skin penetration evaluation of topical formulations. Considering that the technique has been shown to be minimally invasive, to result in concentration–time profiles with a high temporal resolution directly from the target organ for cutaneous delivery and samples more effectively water-soluble molecules, it appears especially suited to study the skin penetration of fluconazole following intravenous and topical application. Indeed, the low lipophilicity ($\log P_{\text{octanol}} = 0.5$) and the low plasma protein binding (12%) make fluconazole an ideal candidate for microdialysis sampling.

Distribution of fluconazole has been studied in various human tissues including in the skin by punch biopsy or by the suction blister method following administration of 50 mg once daily for 12 days. Recently, microdialysis and the suction blister fluid were used to investigate the uptake of fluconazole into the interstitial fluid of human subcutaneous tissue after ingestion of a single dose of 200 mg.

In the treatment of superficial and localized infections, topical application of antifungal agents seems to be the first choice and the more convenient treatment. Like other azole derivatives, fluconazole was formulated for topical application as a gel containing 0.5% of the active substance (Diflucan® Gel). However, there are no data available regarding the distribution of fluconazole in dermis following topical application.

The objective of the present study was to investigate the skin penetration of fluconazole in a freely moving rat model. For this purpose, the distribution of fluconazole in dermis and plasma of rat following i.v. bolus injection at a dose of 10 mg/kg was first studied by using the on-line microdialysis double-site sampling technique. Subsequently, cutaneous absorption of fluconazole following topical application of the commercial Diflucan® Gel was studied by dermal microdialysis.

**MATERIALS AND METHODS**

**Materials**

Fluconazole and UK-54373, a fluorinated analog of fluconazole used as retrodialysis calibrator and internal standard, were generously provided by Pfizer Central Research (Sandwich, Kent, UK). Diflucan Gel® containing 0.5% of fluconazole was generously provided by Pfizer Italy. Acetonitrile HPLC ultragradient grade, methanol HPLC grade, and trifluoroacetic acid (TFA) were purchased from J.T. Baker (Deventer, The Netherlands). Sodium chloride, disodium hydrogen phosphate dihydrate, and di-ammonium hydrogen phosphate of analytical grade were purchased from Merck (Darmstadt, Germany).

Hairless male rats (Ifna Credo, Saint-Germain, France), 10–12 weeks old, weighing 315 ± 30 g ($n = 17$) were used. Food (type A04; U.A.R., Epinay-sur-Orge, France) and tap water were provided ad libitum.

**Microdialysis Probes**

**Jugular Microdialysis Probe**

A concentric vascular probe CMA-20 (10-mm membrane length, 20-kDa molecular weight cutoff) (CMA, Stockholm, Sweden) was implanted in the right jugular vein of the rat with the use of a guide cannula.

**Dermis Microdialysis Probe**

The linear microdialysis probes were manufactured using a Hemophane® dialysis fiber (210 μm i.d., Gambro AB, Lund, Sweden) with a molecular weight cutoff of 5000 Daltons. The fiber (10 mm length) was glued at one end to a piece of silicone tubing (0.012 inch i.d., 0.025 inch o.d., Specialty Manufacturing Inc., Saginaw, MI) using B-210 cyanoacrylate glue (3M, Brussels, Belgium).

The skin of the dorsal region of the rat was punctured with a 26-gauge intravenous needle (i.d. 0.45 mm, length 12 mm). The linear microdialysis
probe was inserted through the guide cannula. The guide was then withdrawn leaving the dialysis membrane in the dermis. After implantation in the rat, a second piece of silicone tubing was glued at the other extremity of the dialysis fiber.

**In Vitro Evaluation of the Recovery of the Probes**

**Recovery by Gain In Vitro**

To characterize the transfer rate of the probes, in vitro recoveries of fluconazole and UK-54373 (retrodialysis marker) were assessed. A CMA-20 10 mm microdialysis probe \( n = 3 \) was placed in a 2-mL vial containing well-stirred phosphate buffer 0.01 M at pH 7.4 isotonised with NaCl 8.288 g/L at three different concentrations of fluconazole or UK-54373 (5, 10, and 20 \( \mu \)g/mL). The vials were immersed in a thermostatic water bath at 37°C. The dermal linear probe with a 10 mm membrane \( n = 3 \) was placed in a plastic barrel of a 1-mL syringe of which one end was sealed with silicone glue and the other with a rubber cap. A micromagnetic stir bar encapsulated in Teflon\( ^{R} \) (2 mm in diameter, 7 mm length, part number 58948-584) (Nalge Nunc International, VWR, Belgium) was used to obtain well-stirred conditions inside the plastic barrel. This setup allowed to keep the membrane of the linear probe in a straight line during the calibration process. The complete system was immersed in a thermostatic water bath at 37°C. The 1-mL syringe equipped with the probe was filled with isotonic phosphate buffer pH 7.4 at three different concentrations of fluconazole or UK-54373 (5, 10, and 20 \( \mu \)g/mL). The probes were perfused at 0.5 \( \mu \)L/min with isotonic phosphate buffer pH 7.4 containing fluconazole or UK-54373 at two different concentrations (5 and 10 \( \mu \)g/mL). The microdialysate was collected every 12 min for a total of 8 h and directly injected into the HPLC system to determine the concentration of fluconazole and UK-54373 in the dialysate \( C_{out} \). The recovery was determined from the ratio of the concentration lost to the initial concentration in the perfusate \( C_{in} \) according to eq. 2.

\[
\text{RL}(\%) = \left( \frac{C_{out}}{C_{in}} \right) \times 100 \quad (2)
\]

**Recovery by Loss In Vitro**

The CMA-20 and the linear probes were placed in a 2-mL vial and a 1-mL syringe, respectively, containing well-stirred drug-free isotonic phosphate buffer pH 7.4 at 37°C. The probes \( n = 3 \) were perfused at three different concentrations (5, 10, and 20 \( \mu \)g/mL) of fluconazole or UK-54373 at a flow rate of 0.5 \( \mu \)L/min. Perfusate was analyzed by on-line HPLC to determine fluconazole or UK-54373 concentration \( C_{out} \). The recovery by loss (RL) was calculated as:

\[
\text{RG}(\%) = \left( \frac{C_{in} - C_{out}}{C_{in}} \right) \times 100 \quad (1)
\]

**In Vivo Evaluation of the Recovery of the Probes**

**In vivo** recovery was determined by the retrodialysis method or “internal reference technique,” which consists of adding a retrodialysis calibrator to the perfusate and measuring the rate of delivery of this compound. This in vivo calibration method relies on the assumption that the recovery of the analyte is quantitatively the same as the delivery of the retrodialysis calibrator.

One dermal probe was implanted in the dorsal region of hairless rats and a CMA-20 probe placed in the jugular vein \( n = 3 \). Each probe was perfused at 0.5 \( \mu \)L/min with isotonic phosphate buffer pH 7.4 containing fluconazole or UK-54373 at two different concentrations (5 and 10 \( \mu \)g/mL). The microdialysate was collected every 12 min for a total of 8 h and directly injected into the HPLC system to determine the concentration of fluconazole and UK-54373 in the dialysate \( C_{out} \). The recovery was determined from the ratio of the concentration lost to the initial concentration in the perfusate \( C_{in} \) according to eq. 2.

**Probe Depth Measurement**

To measure the probe depth after implantation and to determine the accurate position of the microdialysis probe in the dermis, histological slices were performed. 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.

**In Vivo Microdialysis Experiments following Intravenous Bolus Injection**

To study the distribution of fluconazole in cutaneous tissue of the rat, an i.v. bolus injection (10 mg/kg) with simultaneous vascular and dermal microdialysis sampling was performed. Hairless male rats \( n = 5 \) were anesthetized with a ketamine/xylazine mixture (90/10 mg/kg) 1 day before the pharmacokinetic study to implant
a CMA-20 probe in the right jugular vein. Cannulae of polyurethane tubing (Access Technology, Skokie, IL) were implanted in the left jugular vein (0.6 mm i.d. × 0.9 mm o.d.) (for i.v. bolus injection) and in the left femoral vein (0.3 mm i.d. × 0.6 mm o.d.) (for blood sampling) 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.

Two hours before starting the experiment, a linear microdialysis probe was implanted in the dermis of the dorsal region of the rat. Both probes were connected to a microinjection pump (CMA/100, Stockholm, Sweden) and perfused with isotonic phosphate buffer pH 7.4 at a flow rate of 0.5 \( \text{mL/min} \). The retrodialysis marker, UK-54373, was introduced in the perfusate at a concentration of 0.75 \( \mu \text{g/mL} \).

During the pharmacokinetic study, the rat was placed in a containment system (CMA/120, Stockholm, Sweden) allowing the free movement and access to water and food. Dialysates from both probes were directly collected into the injection loops and alternately injected into the microbore HPLC system every 12 min during a period of 480 min after intravenous bolus of fluconazole at a dose of 10 mg/kg. Blood samples (0.25 mL) were obtained from the femoral vein before dosing and at 5, 10, 15, 30, 45, 60, 90, 120, 240, 360, and 480 min after dosing, and the plasma was harvested and stored frozen at \(-20^\circ \text{C}\) until analysis.

**In Vivo Microdialysis Experiments following Topical Application of Diflucan Gel**

The distribution of fluconazole in the cutaneous tissue of the rat following topical application was also studied. To prevent the topically applied gel from seeping through the probe entrance and outlet in the skin, an HPLC screw cap (9 mm, part no. WAT072711, Waters Corp., Milford, MA), used as a container, was filled with the weighed amount of Diflucan Gel (500 mg) and glued, with tissue glue Veltbond (BAS, West Lafayette, IN). An infinite occluded dose of 0.5 g of Diflucan Gel (0.5% fluconazole) was applied to a surface of 58 mm\(^2\) in the dorsal region of the rat just above the implanted dermal probe. The gel was applied during the total duration of the experiment.

The dermal dialysates were collected every 20 min and analyzed by on-line microbore HPLC for a total of 11 h. The retrodialysis marker, UK-54373, was introduced in the perfusate at a concentration of 0.75 \( \mu \text{g/mL} \).

**On-Line Analysis of Microdialysate Samples by Microbore HPLC**

The on-line analysis of the microdialysate samples was previously described. Briefly, the HPLC system consisted of a Kontron Instruments Model 422 pump (Kontron, Milan, Italy), an Ultimate TM UV-vis detector (LC Packings, Amsterdam, The Netherlands) equipped with an UZ-View TM Capillary flow cell (LC Packings, Amsterdam, The Netherlands) with a path length of 30 mm, and an illuminated volume of 540 nL, a data acquisition system Kromasystem 2000 Version 1.83 (Bio-Tek Kontron Instruments S.r.l, Milan, Italy), and a microbore 10-port valve Cheminert TM C2-1000D with 0.2-mm diameter channels (Valco Instruments Co.Inc., Houston, TX) with a pneumatic actuator model A36 (Valco Instruments Co.Inc, Houston, TX). The 10-port valve was fitted with 2 PEEK loops of 1 \( \mu \text{L}\) (i.v. bolus experiments) or 5 \( \mu \text{L}\) (topical application experiments). The HPLC pump was converted to deliver a microflow by interfacing the pump with a microflow processor (Accurate, LC Packings, Amsterdam, The Netherlands).

The separation was performed on a microbore Nucleosil \(^{3\times}\) C\(_{18}\) HD column, 150 × 1 mm i.d., with a 3-\( \mu \text{m}\) particle size and a 100-Å pore size (Macherey-Nagel, Düren, Germany) protected by an Nucleosil \(^{3\times}\) C\(_{18}\) HD guard column at ambient temperature. The mobile phase was composed of 20 mM di-ammonium phosphate buffer \((\text{NH}_4)_2\text{HPO}_4\) (adjusted to pH 7.0 with phosphoric acid 85% v:v) and acetonitrile (75:25, v:v). The flow rate through the microbore column was maintained at 40 \( \mu \text{L/min}\) and the absorbance was measured at 210 nm with AUFS = 0.2 (after i.v. bolus) and 0.05 (after topical application).

The assay was precise, with inter- and intra-assay relative standard deviation values of 0.64 and 0.71% in the range of 0.15–20 \( \mu \text{g/mL}\), respectively. The LOQ for fluconazole was 0.15 \( \mu \text{g/mL}\) with a bias less than 10%.

**HPLC Assay of Plasma Samples**

Fluconazole plasma concentrations after i.v. bolus injection were determined by HPLC with UV detection and UK-54373 as internal standard. Spiked plasma calibrators were prepared at six
different concentrations of fluconazole (0.5, 1, 5, 10, 20, and 25 µg/mL) by adding appropriate amounts of a stock solution of fluconazole (1 mg/mL) in methanol evaporated to dryness under a gentle nitrogen stream at 40°C to blank plasma.

Plasma samples and plasma calibrators were extracted by using Oasis-HLB 30 mg cartridge 1 ml (Waters Corp., Milford, MA). The sorbent was first conditioned with 1 mL of methanol and equilibrated with 1 mL of ultrapure water. Plasma samples (0.1 mL) were diluted with 0.1 mL of phosphate buffer 0.01 M pH 7.0 containing UK-54373, the internal standard, at 10 µg/mL. The diluted plasma alkalinized by adding 20 µL of a 2% ammonia solution 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 15% (v/v) methanol in ammonia solution 2% (pH 11), followed by a wash with 1 mL of ultrapure water and subsequently by 1 mL of 15% (v/v) methanol in phosphoric acid solution 0.001 M (pH 3). The final elution of fluconazole and internal standard was performed with 1 mL of methanol containing 2% of TFA (apparent pH 2.15). Before evaporation at 40°C under a gentle stream of nitrogen, the eluate was filtered on a 0.22-µm PVDF filter. The residue was reconstituted in 0.1 mL of chromatographic mobile phase, and 1 µL was injected onto the microbore HPLC system.

Recovery of fluconazole 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 to obtain a recovery of fluconazole and UK-54373 of 88.5 ± 4.2% and 85.5 ± 5.2%, respectively, over the studied concentration range (0.5 to 25 µg/mL). These recovery values satisfactorily met the acceptance criteria.17

The inter- and intraday RSDs of the slopes of six calibration curves were 2.4 and 1.3%, respectively. No significant difference was observed between intra- and interday values of the slopes and the intercepts (ANOVA, p > 0.05). The LOD and LOQ were 0.63 and 1.06 µg/mL, respectively. The bias at the LOQ was less than 10%.

Data Analysis

Pharmacokinetic parameters were determined by the noncompartmental approach18 by using WinNonlinPro 4.1 (Pharsight Corp. Mountain View, CA). AUC values were calculated using the linear trapezoidal rule from 0 to t (last microdialysate or blood sampling time) with extrapolation to infinity (plasma concentration at time t divided by slope λz). Terminal dialysate or plasma half time (t1/2z) was calculated as 0.693/λz where λz is estimated by linear regression of the terminal log-linear phase of the dialysate or plasma concentration–time curve. Clearance (CL) and the volume of distribution (Vd) were calculated with standard pharmacokinetic equations. The extent of fluconazole distribution into the dermis was quantified using the dermis to plasma partition coefficient (Kp), which was estimated as follows:19

\[ K_p = \frac{AUC_{dermis}}{AUC_{plasma}} \]

Following topical application of an infinite occluded dose of fluconazole, area under the unbound dermis concentrations of fluconazole from 0 to the last sampling time was calculated because it was not possible to determine λz.

Data were validated by using the Dixon test. Mean values of pharmacokinetic parameters were compared by the ANOVA with HSD-Tukey post hoc comparisons. A p-value of 0.05 or less was considered significant.

RESULTS AND DISCUSSION

In Vitro Evaluation of the Recovery of the Probes

Although UK-54373 is a fluorinated analog of fluconazole and was previously demonstrated to be useful as retrodialysis calibrator,20 we compared the in vitro recovery by gain (RG) and by loss (RL) for fluconazole and UK-54373 for CMA-20 and linear probes (n = 3) (Table 1). The RG between fluconazole and UK-54373 was not significantly different for either probe (paired t-test, p = 0.83 for the jugular probe and p = 0.81 for the dermal probe). Likewise, RL between both compounds was not significantly different for either probe (paired t-test, p = 0.50 for the jugular probe and p = 0.85 for the dermal probe). Moreover, the ratio (%) of UK-54373 recovery to fluconazole recovery was around 100%, indicating that the in vitro dialysis characteristics of UK-54373 are similar to those of fluconazole.

Furthermore, there was no statistical difference between RG and RL for either compound (paired t-test, p = 0.12 for the CMA-20 probe and p = 0.23 for the linear probe). Finally, the RG of fluconazole was not statistically different from the RL of
UK-54373 (paired \( t \)-test, \( p = 0.11 \) for the CMA-20 probe and \( p = 0.65 \) for the linear probe).

The linear relationship (\( R^2 = 0.999 \)) between the concentration in the surrounding solution and in the dialysate over the studied concentration range for both analytes shows that the diffusion through the membrane is independent of the fluconazole or UK-54373 concentration.

In conclusion, UK-54373 can be used as the retrodialysis calibrator for estimating the recovery of fluconazole.

**In Vivo Evaluation of the Recovery of the Probes**

The in vivo relative recovery between fluconazole and UK-54373 for the two probes was determined at two different concentrations of fluconazole or UK-54373 (5 and 10 \( \mu \)g/mL) (\( n = 3 \)).

The recoveries by loss for fluconazole were 40.5 ± 5.5% (mean ± SD) for the CMA-20 probe and 33.5 ± 3.9% for the linear probe. The RL of fluconazole was not significantly different from that of UK-54373 for each type of probe (paired \( t \)-test; Table 2).

Due to the tortuosity of the diffusional path of the drug in the tissue,\(^{21} \) the in vivo RL of fluconazole and UK-54373 was significantly less than the RL of the same compound determined in vitro. The ratio (%) of UK-54373 to fluconazole recoveries close to 100% indicates that the retrodialysis of UK-54373 can be used to estimate in vivo the probe recovery in blood and dermis for fluconazole.

**Probe Depth Measurement**

Histological examination of hairless rat skin showed that probe insertion did not result in significant inflammation or extravasations of red blood cells to the skin. The linear probes were implanted at an average depth of 383.5 ± 118.5 \( \mu \)m (\( n = 17 \)). Based on the histological observations, the probe was implanted in the upper half of the dermis.

**Fluconazole Distribution Studies following i.v. Bolus Injection**

To study the distribution of fluconazole in cutaneous tissues of the rat, fluconazole was administered to rats by i.v. bolus injection at a dose of 10 mg/kg. A double sampling by microdialysis was alternately performed in the dermis and in...
blood to measure the unbound concentrations of fluconazole. Simultaneously, a conventional blood sampling was also carried out to determine total plasma concentrations of fluconazole.

The *in vivo* loss of the retrodialysis calibrator (UK-54373) was used to monitor the recovery of the probe during the experiment. *In vivo* recovery for the jugular probes and dermal probe were 39.2 ± 5.2% (mean ± SD, n = 85) and 31.6 ± 6.7% (n = 85), respectively. Mean intraindividual RSDs of *in vivo* recovery were 6.8 ± 0.7% and 10.7 ± 1.2% (mean ± SD, five rats) for the dermal and jugular probe, respectively.

Following i.v. bolus injection, fluconazole rapidly penetrated into the dermis. Indeed, the fluconazole unbound concentration–time profiles determined by using intravenous and dermal microdialysis were similar (Fig. 1) and characterized by a monoexponential decline within the range 4.6 and 10.4 μg/mL, which is substantially higher than the range of reported MICs of 0.2–0.8 μg/mL for *Candida albicans*. Furthermore, the unbound fluconazole concentrations in blood and dermis microdialysates were not different from the total plasma concentrations of fluconazole because the plasma protein binding of fluconazole is negligible (about 12%). The distribution equilibrium was rapidly achieved with a dermis-to-plasma partition coefficient of 1.02 ± 0.04. This value is in agreement with those previously reported by Haneke in humans by using the blister–fluid technique (range from 1.04 to 1.14).

Recently, a value of 1.16 ± 0.22 for the human subcutaneous interstitial fluid-to-plasma partition coefficient was reported by Sasongko et al. after a single oral dose of 200 mg. The rapid distribution of fluconazole in dermis suggests that low lipophilicity combined with a low plasma protein binding may be a key requirement for achievement of therapeutic levels at this site.

Pharmacokinetic parameters are summarized in Table 3. No statistical difference (ANOVA, *p* > 0.05) was observed between various parameters calculated from vascular and dermal microdialysis sampling.

**Fluconazole Distribution Studies following Topical Application of Diflucan® Gel**

For the treatment of superficial and localized fungial infections, fluconazole, like other azole antifungal drugs, was formulated for topical application as a gel containing 0.5% of the active substance. The present report is the first study in which dermal microdialysis is used to investigate the cutaneous penetration of fluconazole following topical application (Fig. 2). The *in vivo* recovery of the dermal probe was continuously monitored during the experiment by using the *in vivo* loss of the retrodialysis calibrator. *In vivo* loss of UK-54373 was 37.4 ± 6.9% (mean ± SD, n = 204). Unlike reported by Kreilgaard for cutaneous delivery of prilocaine and lidocaine, recovery fluctuations were relatively small. Indeed, the mean intraindividual R.S.D. was 7.1 ± 2.9% (mean ± SD, n = 12). Although this within-experiment recovery fluctuation is lower than 10%, the monitoring of the *in vivo* recovery of the probe at each time during the experiment is relevant to improve the reproducibility of the data obtained by microdialysis sampling. In the previous (sub-)cutaneous microdialysis studies, *in vivo* recovery was estimated by the retrodialysis method with the substance of interest in separate experiments prior to or at the end of the sampling period, which does not allow a continuous control of the probe’s performance during the experiment. In other words, continuous monitoring of the *in vivo* retrodialysis of a calibrator is relevant as a quality control during the experiment.

The effect of the probe depth on the amount of drug collected by the probe is still a subject of debate in the literature. Whereas most other studies have employed *in vivo* ultrasound scanning measurements of probe depth, the current...
study employs measurements on histological sections. The preparation process is likely to introduce some shrinkage of the tissue, but because this can be expected to be symmetrical and reproducible, the analysis of probe depth correlations will be similar to studies employing other methods for probe depth measurement. Although most authors have not found a correlation between penetrated drug levels in the skin and probe depth,24,26–29 Benfeldt et al.25 reported a weak significant correlation ($r = 0.5$, $p = 0.04$) between salicylic acid penetration and probe depth (0.5–1.1 mm) in an investigation on the cutaneous delivery of salicylic acid in the hairless rat. Nevertheless, this correlation was not confirmed in a similar human study.24 In a recent Simonsen’s study,30 no influence of probe depth (ranging from 0.7 to 1.1 mm) on the cutaneous penetration of salicylic acid derivatives in hairless rat was observed. As previously reported by Stagni et al.,29 and recently reported by Simonsen et al.,30 the more variations in probe depth there is, the more significant correlations may be found. In the present study, the influence of the probe implantation depth on the amount of fluconazole sampled by the probe was investigated by plotting the AUC$_{0–674}$min in function of the probe depth (Fig. 3). The probe depth varied between 0.23 to 0.46 mm. The AUC$_{0–674}$min was relatively constant and independent of implantation depth to a depth of approximately 350 μm, which corresponds to the upper half of the dermis. Below this depth, AUC$_{0–674}$min decreased progressively and a significant correlation was found between AUC$_{0–674}$min and the probe depth (Pearson coefficient $= -0.962$, $p < 0.01$) (Fig. 3). This particular profile may be explained by the fact that the cutaneous microcirculation is organized as two horizontal plexuses. The upper plexus is situated just below the dermoepidermal junction, and the second is localized just above the junction

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<th>Table 3. Fluconazole Pharmacokinetic Parameters Determined Using Conventional Blood Sampling, Intravenous and Dermal Microdialysis Sampling following i.v. Bolus 10 mg/kg</th>
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<tr>
<td>Blood Sampling$^c$</td>
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<tr>
<td>AUC (μg × min/mL)</td>
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<td>$T_{1/2}$ (min)</td>
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<td>CL (mL/min/kg)</td>
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<td>$V_d$ (L/kg)</td>
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$^a$Calculated based on unbound concentration–time profiles.
$^b$No significant difference was observed between the various parameters calculated from the different sampling techniques ($p > 0.05$, ANOVA, Tukey post hoc comparisons).
$^c$Not determined.

Figure 2. Fluconazole unbound concentration–time profiles in dermis following topical application of a dose of 0.5 g of Diflucan® Gel in rat (●). Mean ± SEM (n = 12).

Figure 3. Area under the curve of dermal microdialysates from 0 to 674 min in the function of the probe depth. Each point (●) represents the calculated AUC$_{0–674}$ in one experiment after topical application.
between the dermis and subcutaneous fat, the latter being constituted by larger vessels to which the upper plexus drain. Therefore, when the implantation probe depth increases, the blood flow increases because the diameter of blood vessels increases and as well as the dermal clearance of the drug. However, besides the increase of blood vessel diameter, others important factors such as the presence or the lack of fenestrations in the capillary wall and the density of blood vessels in the various dermis layers should also be considered.

Following topical application of a dose of 0.5 g of Diflucan Gel containing 0.5% of fluconazole, active unbound concentrations in dermis were measured by cutaneous microdialysis. The AUC of fluconazole in dermal dialysate was relatively constant to an implantation depth of approximately 350 μm. Below this depth, the AUC progressively decreased with increasing implantation depth of the probe.

Finally, this study shows that cutaneous microdialysis is an effective and minimally invasive tool to evaluate the dermatopharmacokinetics of drugs following systemic or topical administration.

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