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Transcutaneous sampling of ciprofloxacin and 8-methoxypsoralen by electroporation (ETS technique)

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ABSTRACT

The novel technique of transcutaneous sampling of drugs by electroporation was developed to study the dermatokinetics of ciprofloxacin and 8-methoxypsoralen. The selected drugs differ in their aqueous solubility and also with respect to the extent of protein binding. Ciprofloxacin (15 mg/kg) was administered i.v. through tail vein, whereas 8-methoxypsoralen (5 mg/kg) was given by oral administration, in hairless rats and the time course of drug concentration in the plasma was determined. Drug concentration in the dermal extracellular fluid (ECF) was determined by ETS and microdialysis sampling techniques. The extent of penetration into dermal ECF for ciprofloxacin and 8-methoxypsoralen was found to be ~19–32% and ~13–23%, respectively. The drug concentration in the dermal ECF determined by ETS and microdialysis did not differ significantly from each other and so as were the pharmacokinetic parameters. The results show that ETS can be utilized as a potential technique for sampling of drugs from the dermal ECF.

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1. Introduction

Determination of drug levels in the dermal extracellular fluid (ECF) is extremely important for efficient treatment of skin infections and other disorders (Tegeder et al., 2002; Bielecka-Grzela and Klimowicz, 2005). Conventional methods of drug sampling in the skin include biopsy, skin blister fluid and cutaneous microdialysis sampling techniques (Wise et al., 1984; Ault et al., 1992; Muller et al., 1996; Alguire and Mathes, 1998; Brunner et al., 2002). Skin biopsy sampling and skin blister fluid sampling are both invasive in nature and also have limitation with the number of samples that could be obtained. Presently, microdialysis is the most widely used method for sampling of unbound drug from dermal ECF. However, it is also an invasive technique which makes it unsuitable for implementation in routine therapeutic drug monitoring.

Electroporation is one of the promising electrically mediated techniques to enhance the transdermal drug delivery. It is a technique in which reversible permeabilization of skin is brought about by application of short electrical pulses (Lombry et al., 2000; Denet and Preat, 2003; Murthy et al., 2004). ETS involves sampling of drugs from dermal ECF by facilitating reverse diffusion of drug in the direction of dermis to stratum corneum. The applicability of the ETS technique in case of sampling acyclovir, salicylates and diagnostic analyte glucose has already been demonstrated (Murthy et al., 2005; Murthy and Zhang, 2008; Srinivasa Murthy et al., 2008).

In the present study ciprofloxacin and 8-methoxypsoralen which are used in treatment of skin infections and pigmentation disorders, respectively are selected as model drugs. Ciprofloxacin, a broad spectrum antibacterial agent belonging to the group of fluoroquinolones is effective against microbial infections localized in skin. The time course of ciprofloxacin in the skin needs to be monitored for determination of the frequency and dose from safety and efficacy perspectives (Brunner et al., 1999; Tsai and Wu, 2001; Brunner et al., 2002; Bielecka-Grzela and Klimowicz, 2005).

8-methoxypsoralen, a furocoumarinic is used in conjuction with UV radiation (PUVA therapy) for the treatment of dermatoses like vitilgo and psoriasis (Mays et al., 1985; Susanto et al., 1986; Ketchum et al., 1990). The effectiveness of 8-methoxypsoralen depends on ultraviolet A irradiation, and optimum response will be elicited only when the drug concentration in skin is over the effective concentration. For this reason, it is imperative to determine drug concentration in skin to achieve better PUVA therapy (Gazith et al., 1978; Tegeder et al., 2002; Brautigam et al., 2003).

The two drugs ciprofloxacin and 8-methoxypsoralen possess different physicochemical properties. They differ in their extents of protein binding. For the purpose of assessing the validity of the technique, the two drugs were administered by different

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routes in this project (ciprofloxacin by intravenous bolus and 8-methoxypsoralen by oral route).

2. Materials and methods

2.1. Chemicals

Ciprofloxacin hydrochloride, 8-methoxypsoralen were purchased from Sigma–Aldrich Inc. (St. Louis, MO), phosphate-buffered saline (PBS, pH 7.4) premixed powder was obtained from EMD Chemicals (Gibbstown, NJ), and all other chemicals were obtained from Fischer Scientific (Fairway, NJ).

2.2. In vitro transcutaneous sampling studies

The *in vitro* diffusion studies were carried out in Franz diffusion cells (FDC) (Logan Instruments Ltd., Somerset, NJ) using hairless rat skin excised from the abdomen region. Hairless rat skin is considered to be a good model for topical and transdermal drug delivery studies due to the similarity between the rat and human skin with respect to lipid content and water uptake properties (Morimoto et al., 1992). Moreover, a good correlation of permeation data between the hairless rat model and human skin models have been reported by several research groups in the past (Wester and Maibach, 1993). The skin was mounted on the diffusion cell in such a way that the epidermis side of the skin was in contact with upper sampling compartment and dermal side with the lower reservoir compartment. The active diffusion area of FDC was 0.64 cm^2. Ag/AgCl electrode wires of 2 mm diameter (In Vivo Metric, CA) made in the form of circular rings were placed 2 mm away from skin in both sampling and reservoir compartments. The sampling compartment and the reservoir compartment were filled with 0.4 and 5 ml PBS, respectively and the skin was allowed to equilibrate for an hour. The AC electrical resistance of the epidermis was measured by placing a load resistor R_L (100 kΩ) in series with the epidermis. The voltage drop across the whole circuit (V_O) and across the skin (V_S) was measured using an electrical set up consisting of a waveform generator and a digital multimeter (Agilent Technologies, Santa Clara, CA). For measuring resistance, voltage of 100 mv was applied at 10 Hz and the skin resistance in kΩ was approximated from the formula

$$R_s = \frac{V_S R_L}{V_O - V_S} \quad (1)$$

where R_S is the skin resistance and R_L is the load resistor in kΩ. The piece of skin, which had a resistance greater than 20 kΩ cm^2 was used for the experiment.

Later, the sampling compartment was replaced with fresh 0.4 ml of PBS and reservoir compartment was filled with 5 ml of ciprofloxacin solution prepared in PBS (2.5–40 μg/ml). In case of 8-methoxypsoralen, the sampling compartment was replaced with PBS:ethanol (50:50) and the reservoir compartment was filled with 5 ml of 8-methoxypsoralen solution (5–40 μg/ml) prepared in PBS:ethanol (50:50). Thirty square electrical pulses each of 10 ms duration at 120 V/cm^2, 1 Hz was applied using ECM 830 Electro Square Porator (BTX Harvard apparatus, Holliston, USA). The electrical resistance of the skin was measured immediately after application of the electrical pulses to ensure skin permeabilization. The solution from the sampling compartment was withdrawn 15 min after application of electrical pulses and the amount of drug was analyzed by HPLC.

2.3. Ex vivo plasma protein binding

The blood was collected by cardiac puncture in rats and the plasma was separated by centrifugation at 2000 × *g* at 4 °C. Rat plasma was spiked with drug to prepare samples of concentration ranging from 1 to 40 μg/ml. The spiked plasma samples were thoroughly mixed by vortexing and allowed to equilibrate for 12 h at 4 °C. After equilibration, protein free plasma was obtained by carrying out ultra filtration (Millipore Centrifree® filtration units) by centrifugation of 0.5 ml of plasma at 2000 × *g* for 20 min (Schaefer et al., 1996; Murthy et al., 2005). The amount of unbound drug present in the filtrate was measured by HPLC after suitable dilution with PBS.

2.4. In vivo studies

The *in vivo* experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Mississippi (Protocol # 07-004). The *in vivo* studies were carried out in hairless rats (Taconic, Hudson, New York) (250–300 g) under ketamine (80 mg/kg) and xylazine (10 mg/kg) anesthesia administered intraperitoneally.

Blood sampling, ETS and microdialysis sampling was carried out in the same group of rats ($n = 6$). The samples by all three procedures were obtained at the same time points in each rat simultaneously. Ciprofloxacin solution (150–180 μl) of 15 mg/kg prepared in sterile isotonic saline was administered by i.v. into tail vein as a bolus injection, whereas in case of 8-methoxypsoralen, solution (415–500 μl) of 5 mg/kg prepared in 50:50 of PBS:ethanol was given by oral gavage using a ball ended feeding needle.

2.4.1. Cutaneous microdialysis

For cutaneous microdialysis, a 20G needle was inserted intradermally through a distance of 1 cm and penetration depth of 2 mm. A linear microdialysis probe of 5 mm membrane length and 30 kDa cutoff molecular weight (BASi, West Lafayette IN) was inserted through this needle and the needle was withdrawn leaving the probe implanted in the dermal tissue. The proper placement of probe in the dermis was confirmed by making an incision at the site of probe implantation after completion of the study. Any experiment, in which the probe was not placed horizontally at the intended depth was not considered.

The inlet tube was connected to an injection pump (BASi, West Lafayette, IN) and perfusion was set to a flow rate of 2 μl/min. The probe was equilibrated for 30 min (Groth and Serup, 1998a,b; Schnetz and Fartasch, 2001). The perfusate was PBS for studies involving ciprofloxacin and PBS:ethanol (50:50) for that of 8-methoxypsoralen. Two samples were collected before drug administration. The drug was administered after equilibration of the probe. Subsequently the microdialysis samples were collected continuously at every 15 min interval including at time points corresponding to ETS and plasma sampling at 30, 60, 120, 180, 240, 300 and 360 min.

The microdialysis probe recovery was determined *in vitro* by placing microdialysis probe in PBS containing known drug concentration and perfusing the probe with PBS (for ciprofloxacin) and 50:50 PBS:ethanol (for 8-methoxypsoralen) at flow rate of 2 μl/min. The dialysate coming out of the probe was collected every 15 min and analyzed for drug content (Dempsey et al., 1997; Leveque et al., 2001). The in vitro recovery rate was calculated using the formula:

$$\%\,\text{Relative Recovery} = \left(\frac{\text{Concentration of dialysate}}{\text{Concentration of sample}}\right) \times 100 \quad (2)$$

2.4.2. Transcutaneous sampling by electroporation

A custom made sampling cell was fixed using an adhesive (Krazy glue, Elmers products Inc., Ohio) on the back of the rats. The sampling cell was fitted with a Ag/AgCl electrode and the counter electrode was secured just adjacent to the cell on the surface of

the skin using a micropore surgical tape (3 M Healthcare, MN). The skin was hydrated with 100 μl of saline for 5 min before each sampling and was replaced with 100 μl of sampling buffer (PBS for ciprofloxacin, PBS:ethanol (50:50) for 8-methoxypsoralen). One blank sample was collected before drug administration, and subsequent samples were collected at 30, 60, 120, 180, 240, 300 and 360 min. For ETS procedure, thirty electrical pulses each of 10 ms duration at 120 V/cm^2, 1 Hz was applied and the sampling fluid remained in the chamber for 15 min after pulsing.

2.4.3. Plasma pharmacokinetics of drugs

For plasma pharmacokinetic studies, 100 μl of blood was collected by retro orbital bleeding before injection of drug and before each episode of transcutaneous sampling and cutaneous microdialysis. The blood samples were diluted with 200 μl of PBS and plasma was separated by centrifugation at 2000 × *g*. In case of ciprofloxacin, plasma samples were subjected to protein precipitation with equal volume of 0.5 M perchloric acid, followed by centrifugation at 10,000 × *g* for 10 min and drug content was analyzed by HPLC (Bielecka-Grzela and Klimowicz, 2005). 8-methoxypsoralen in the plasma was extracted with methylene chloride for 30 min followed by centrifugation at 5000 × *g* at 4 °C for 10 min, the organic phase was separated and evaporated to dryness at room temperature under nitrogen and the residue was dissolved in PBS:ethanol (50:50) and analyzed by HPLC (Monbaliu et al., 1981).

2.5. Analytical method

The amounts of ciprofloxacin and 8-methoxypsoralen present in plasma, ETS and microdialysis samples were analyzed by high-performance liquid chromatography. The HPLC system (Waters, MA) consisted of a chromatographic pump (Waters 1525) and an autosampler (Waters 717 plus).

Analysis of ciprofloxacin samples were carried out using a fluorescence detector (Waters 2475) at an excitation wavelength of 278 nm and emission wavelength of 445 nm. Symmetry® C18 column (4.6 mm × 150 mm) was used and the mobile phase consisted of a mixture of acetonitrile and 0.1 M aqueous monopotassium phosphate solution adjusted to pH 2.5 with orthophosphoric acid (15:85, v/v) with flow rate of 2 ml/min (Bielecka-Grzela and Klimowicz, 2005). The linearity range was between 1 and 1000 ng/ml (R^2 = 0.99).

Analysis of 8-methoxypsoralen samples were carried out using a UV detector (Waters 2487) at 248 nm using Symmetry® C18 column (4.6 mm × 150 mm). The mobile phase consisted of a mixture of methanol, acetonitrile and water (2:30:68) and the flow rate was 1 ml/min (Monbaliu et al., 1981; Kappes et al., 2003). The linearity range was between 10 and 1000 ng/ml (R^2 = 0.99).

The recovery for ciprofloxacin was 96.90 ± 1.98% and that of 8-methoxypsoralen was 95.81 ± 1.57%. HPLC method of ciprofloxacin had an intra- and inter-day precision of <6% with an accuracy >95% and the limit of detection was 1 ng/ml. 8-methoxypsoralen HPLC method had an intra- and inter-day precision of <8% and accuracy of >94% with limit of detection being 10 ng/ml.

2.6. Data analysis

The pharmacokinetic parameters were calculated using non-compartmental pharmacokinetic model. The terminal elimination rate constant (λ_z) was determined from the slope of terminal exponential phase of the logarithmic plasma concentration–time curve. The elimination half life ($t_{1/2}$) was calculated using $0.693/\lambda_z$. The area under the curve (AUC_{0-t}) was caclulated using the trapezoidal rule and $AUC_{0-\infty}$ was obtained by adding C_{last}/λ_z to AUC_{0-t}.

In case of 8-methoxypsoralen, the maximum plasma concentration (C_{max}) and the time to reach maximum plasma concentration (T_{max}) were determined from the concentration–time curves.

The statistical analysis was carried out using GraphPad Instat 3 software and the t-test was selected for comparing the parameters obtained from ETS and microdialysis techniques. $p < 0.05$ was considered as level of significance. The data points shown in graphs are an average of six trials with error bars representing standard deviation.

3. Results and discussion

Solubility of drug in the sampling fluid is one of the key determinants of recovery of drug in case of both ETS and microdialysis. PBS was found to be an appropriate solvent for sampling ciprofloxacin. However, in the case of 8-methoxypsoralen, the the recovery was poor with PBS which was likely due to the low water solubility of 8-methoxypsoralen (water solubility of 8 methoxy psoralen is ~55.8 μg/ml). The recovery of 8-methoxypsoralen was improved with 50:50 PBS:ethanol system in both ETS and microdialysis techniques.

Electroporation leading to permeabilization of the skin is indicated by the drastic drop in electrical resistance of the skin. The drop in electrical resistance has been reported to be due to formation of transient aqueous pathways in the lipid domain of the stratum corneum. The aqueous pathways reseal and the skin will regain its barrier property (thus, its original electrical resistance). The extent of drop in electrical resistance and the duration required for the skin to recover is determined by the applied electrical protocol. With the protocol of 120 V, 10 ms, 30 pulses applied in this project, the drop in skin resistance was ~70 ± 5%. There was no significant recovery in skin resistance during the sampling time of 15 min. However, the skin resistance recovered within few hours reflecting the fact that the electrical protocol was not too aggressive to bring about irreversible impairment of the skin barrier. This was in agreement with the observations in our previous studies carried out across the rat skin and porcine epidermis (Murthy et al., 2005; Murthy and Zhang, 2008; Srinivasa Murthy et al., 2008).

Transcutaneous sampling was carried out across the untreated skin (did not apply electrical pulses) which served as control set of experiment. In case of untreated skin (control samples), detectable level of drugs could not be sampled within the sampling duration of 15 min. In case of ETS, detectable amounts of drug was sampled even at low reservoir drug concentrations. The amount of drug sampled in 15 min following application of electrical pulses was plotted against the corresponding reservoir drug concentrations (Figs. 1 and 2). A linear relationship (R^2 = 0.96 and 0.98 for ciprofloxacin and 8-methoxypsoralen, respectively)was observed between the amount of drug sampled and the reservoir drug concentration, indicating that the subdermal drug concentration can be represented by ETS samples. The percentage recovery by ETS (slope X 100) was 4.8 ± 0.8% and 7.2 ± 1.3% for ciprofloxacin and 8-methoxypsoralen, respectively. The recovery of 8-methoxypsoralen was less (~0.5%) when ETS was carried out with PBS alone.

The free drug present in the blood plasma equilibrates rapidly with the skin ECF. In other words, the extent of distribution of drug into peripheral tissues is known to be determined predominantly by the plasma protein binding. Generally, the drugs that show high protein binding penetrate less into the peripheral compartment. *Ex vivo* plasma protein binding studies were carried out for both ciprofloxacin and 8-methoxypsoralen to assess the relationship between the protein binding and tissue penetration. The plasma protein binding of ciprofloxacin was found to be 20.65 ± 3.93% at concentrations between 1 and 40 μg/ml which was in aggreement with 16–40% reported by other research groups previously

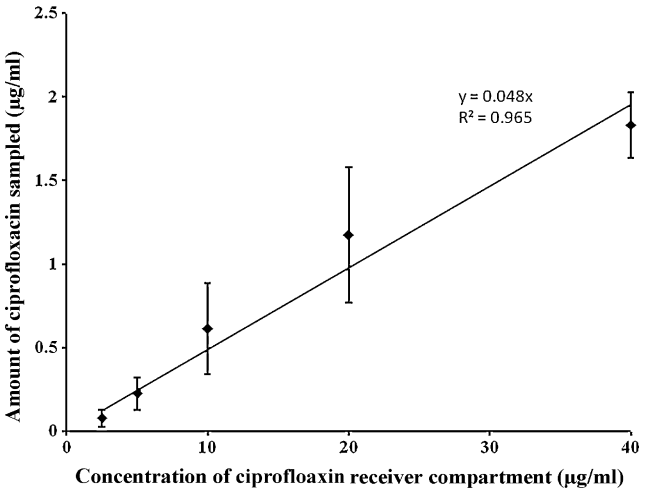

Fig. 1. Correlation between ciprofloxacin concentration (2.5–40 µg/ml) in the reservoir compartment and amount sampled by ETS across hairless rat skin in vitro. The data points represent an averages of $n = 6 \pm$ S.D.

(Nouaille-Degorce et al., 1998; Zhu et al., 1999; Singh and Mehta, 2006). The fraction of 8-methoxypsoralen bound to plasma was $80.97 \pm 6.85\%$. This is comparable to 91.4% reported by Pibouin et al. (1987). The two drugs chosen for this project differ significantly with respect to their protien binding property. One of the major objectives of this project was to assess the workability of the ETS technique in case of drugs which exhibit different extents of protein binding.

The *in vitro* recovery of ciprofloxacin and 8-methoxypsoralen by microdialysis probe were found to be $3.47 \pm 0.35\%$ and $16.41 \pm 3.32\%$, respectively. Generally microdialysis is regarded as less suitable for sampling lipophilic drugs because, microdialysis method of drug sampling involves perfusion of aqueous sampling fluid through the dialysis probe during which the exchange of ingredients between the perfusion fluid and tissue fluid takes place. The perfusate liquids are preferably aqueous and therefore when the drug to be sampled is highly lipophilic, the recovery of the drug with the aqueous perfusion liquids would be relatively very less (Malonne, 1999; Schnetz and Fartasch, 2001; Kreilgaard, 2002). It is not recommended to use organic solvents as the perfusate could

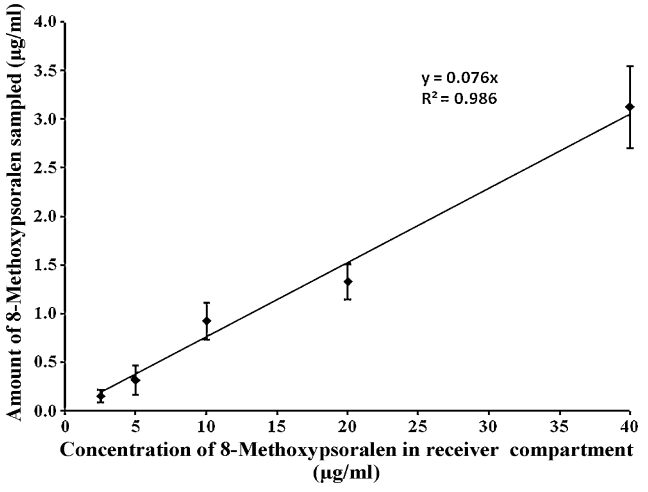

Fig. 2. Correlation between 8-methoxypsoralen concentration (2.5–40 µg/ml) in the reservoir compartment and amount sampled by ETS across hairless rat skin in vitro. The data points represent an averages of $n = 6 \pm$ S.D.

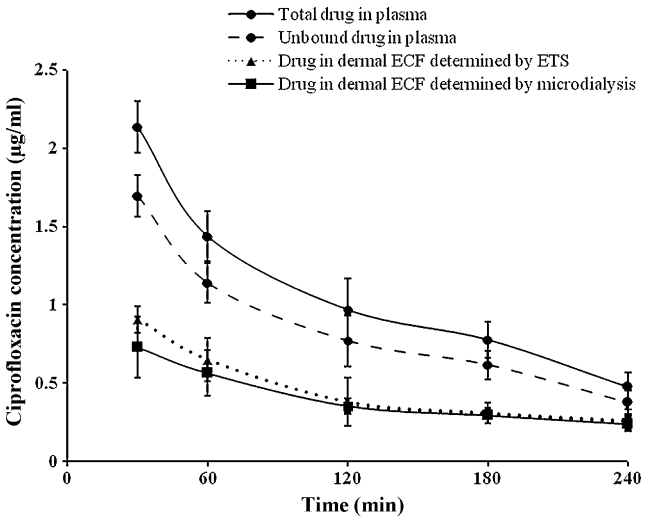

Fig. 3. Time course of ciprofloxacin in rat plasma determined by blood sampling and dermal ECF determined by microdialysis and ETS technique following administration of 15 mg/kg ciprofloxacin i.v. bolus. The data points represent an average of $n = 6 \pm$ S.D. Blood sampling, ETS and microdialysis were carried out simultaneously on each rat at the same time points.

alter the dialysis membrane properties and may also cause potential toxicity to the tissue. However, in this study, we found that the permeability of the dialysis membrane did not change significantly even after immersing the membrane for over 24 h in the 50:50 alcohol:water system.

In case of ETS, as the technique is noninvasive and the sampling fluid is in contact with the tissue for a short period of time during drug sampling, use of hydroalcoholic systems pose relatively less severe risk as compared to microdialysis. This shows that ETS technique could be used to sample even lipophilic drugs, by changing the the sampling fluid constitution appropriately. This is considered as one of the the advantages of ETS over microdialysis technique.

In the current experiments, as the animals were anesthetized, there were no notable irritation symptoms due to electroporation or microdialysis. There was no inflammation due to application of electrical pulses or insertion of probe. However, there was slight bleeding during the implantation of the probe which stopped within 1 or 2 min completely.

From both microdialysis and ETS techniques, the amount of ciprofloxacin and 8-methoxypsoralen present in the dermal ECF in rats was calculated using the amount sampled and the corresponding recovery values as follows:

$$\text{Dermal ECF concentration} = \left[\frac{\text{sample concentration}}{\text{percentage recovery}}\right] \quad (3)$$

Fig. 3 represents the concentration time profile of ciprofloxacin (15 mg/kg) in plasma and dermal ECF (determined by ETS and cutaneous microdialysis) following i.v. bolus administration. The pharmacokinetic parametes calculated from non-compartmental analysis are given in Table 1.

Table 1
Mean pharmacokinetic parameters of ciprofloxacin (unbound) in plasma and dermal ECF (determined by ETS and microdialysis) following administration of 15 mg/kg i.v bolus in hairless rats ($n = 6 \pm$ S.D.).

Parameter	ETS	Microdialysis	Plasma
T_{max} (min)	30	30	30
C_{max} (µg/ml)	0.906 ± 0.046	0.729 ± 0.196	1.695 ± 0.131
$AUC_{0-\infty}$ (min µg/ml)	88.29 ± 20.41	89.71 ± 25.18	240.33 ± 29.59
$t_{1/2}$ (min)	55.84 ± 13.44	82.36 ± 22.54	124.12 ± 25.99

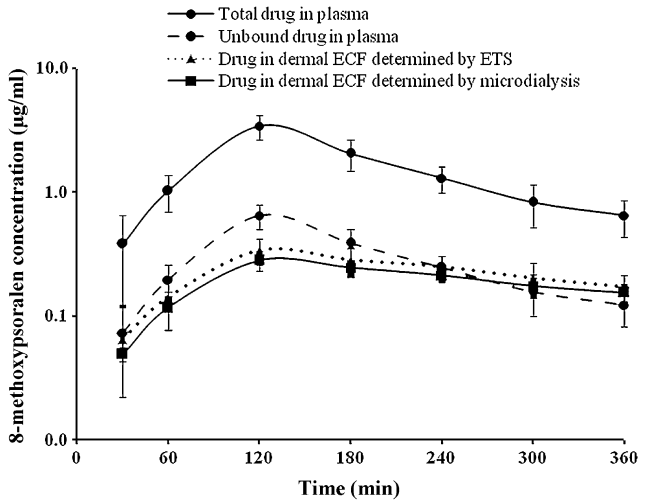

Fig. 4. Time course of 8-methoxypsoralen in rat plasma determined by blood sampling and dermal ECF determined by microdialysis and ETS technique following oral administration of 5 mg/kg 8-methoxypsoralen. The data points represent an average of $n = 6 \pm$ S.D. Blood sampling, ETS and microdialysis were carried out simultaneously on each rat at the same time points.

The plasma elimination half life of ciprofloxacin in the present study was found to be 124.12 ± 25.99 min. This was comparable to 102.3 ± 26.2 min and 91 ± 8.6 min reported by Nouaille-Degorce et al. (1998) and Tsai and Wu (2001), respectively.

Fig. 4 represents the concentration time profile of 8-methoxypsoralen in plasma and dermal ECF (determined by ETS and cutaneous microdialysis) following oral administration of 5 mg/kg of 8-methoxypsoralen. The pharmacokinetic parametes calculated from non-compartmental analysis are given in Table 2.

The C_{max} and T_{max} following oral administration of 5 mg/kg of 8-methoxypsoralen to hairless rats was found to be 0.64 ± 0.14 μg/ml and 120 min, respectively which was comparable to that reported by Roelandts et al. Roelandts et al have reported that following oral adminstration of 10 mg/kg of 8-methoxypsoralen to rats, C_{max} and T_{max} were ~1.2 μg/ml and 150 min, respectively (Roelandts et al., 1983).

The tissue penetration was determined from the ratio of $AUC_{0-\infty}$ of unbound drug in dermal ECF to the $AUC_{0-\infty}$ of total drug (bound + unbound) in plasma. The extent of tissue penetration of 8-methoxypsoralen from ETS and cutaneous microdialysis was found be 18.41 ± 3.76 and 18.47 ± 3.30% respectively,which very well agrees with the percentage protein binding (80.97 ± 6.85%). In contrast, ciprofloxacin, penetration into skin tissue was found to be 26.31 ± 4.54 and 26.99 ± 7.60% with ETS and cutaneous microdialysis, respectively which did not correlate with the protein binding of 20.65 ± 3.93%. It is likely that in case of ciprofloxacin, there are additional factors other than protein binding that determine the extent of penetration of drug into peripheral tissues. In agreement with our data, despite a less extent of protein binding (~30%), ciprofloxacin has been reported to penetrate only ~30–60% into the interstitial fluid in humans (Brunner et al., 2002; Singh and Mehta, 2006).

The point to point comparison of the drug concentration in the dermal ECF and the dermatokinetic parameters, i.e. C_{max}, T_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ determined by ETS and microdialysis technique for ciprofloxacin and 8-methoxypsoralen did not differ significantly (t-test, $p < 0.05$) (Tables 1 and 2). This provides validity to the ETS technique for sampling drugs like ciprofloxacin and 8-methoxypsoralen. The study also demonstrates that ETS could be implemented in dermatokinetic investigation regardless of route of administration of drugs.

Successful development of a sampling device based on the ETS concept would be very useful in carrying out dermatokinetic investigations of drugs. The noninvasiveness of the technique permits frequent sampling of drugs which helps in relatively more precise calculation of the kinetic parameters. In addition the technique could be used to sample drugs in routine therapeutic drug monitoring. ETS is a potential technique for cutaneous sampling of drugs and analytes and could replace the invasive blood sampling procedure in future.

Table 2
Mean pharmacokinetic parameters of 8-methoxypsoralen (unbound) in plasma and dermal ECF (determined by ETS and microdialysis) following oral administration of 5 mg/kg in hairless rats ($n = 6 \pm$ S.D.).

Parameter	ETS	Microdialysis	Plasma
T_{max} (min)	120	120	120
C_{max} (μg/ml)	0.338 ± 0.080	0.280 ± 0.051	0.644 ± 0.142
$AUC_{0-\infty}$ (min μg/ml)	116.08 ± 29.22	116.10 ± 22.85	120.72 ± 36.25
$t_{1/2}$ (min)	243.83 ± 18.43	237.76 ± 15.83	97.15 ± 6.72

4. Conclusion

ETS is a potential noninvasive technique for sampling of drugs from the dermal ECF. The results of this project have shown the workability of ETS in case of drugs with different solubility and different degree of protein binding. In addition the present work also demonstrates the workability of the ETS technique in case of intravenous and oral routes of drug administration.

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