

Feasibility of Measuring Lipophilic or Protein-bound Drugs in the Dermis by *In Vivo* Microdialysis after Topical or Systemic Drug Administration

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Our aim was to assess the microdialysis technique for determining *in vivo* drug levels of a lipophilic and a protein-bound model drug in the dermis. Forearm skin of healthy volunteers received topical 2% fusidic acid or 0.1% betamethasone-17-valerate formulations twice daily as occluded treatment on irritative dermatitis. Microdialysis sampling in the dermis after 48 h was without measurable drug. Hairless rats received maximized treatment with occluded applications of 10% fusidic acid or 4% betamethasone-17-valerate in ethanol for 72 h followed by microdialysis. Mean levels of betamethasone-17-valerate were 11–45 ng/ml; fusidic acid was not measurable. Systemic administration in clinical doses to rats was without measurable drug levels; increasing doses to 312 mg/kg of fusidic acid and 158 mg/kg of betamethasone-17-valerate yielded betamethasone-17-valerate levels of 25–44 ng/ml and fusidic acid levels of 10–90 ng/ml. This study demonstrates the challenges arising when using microdialysis for measuring *in vivo* drug levels. For the drugs chosen it was necessary to administer very high systemic doses or apply a high topical drug concentration to obtain measurable drug levels in the dialysates. Drug levels were in the nanomolar range and demonstrated reproducible and dynamic monitoring of *in vivo* drug levels in the skin. Using microdialysis for sampling highly protein-bound or lipophilic drugs in the skin requires very sensitive analytical methods, and the sensitivity of the analysis is likely to be the limiting factor. *Key words:* betamethasone-17-valerate; fusidic acid; human; penetration.

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For evaluation of the efficacy and toxicity of drugs used in the treatment of skin diseases, basic knowledge of the pharmacokinetic profile of the drug *in vivo* within the skin is desirable. *In vivo* studies of drugs within the skin have so far been hampered by the lack of techniques permitting drug sampling without local tissue destruction (1). Most drugs used for the treatment of skin diseases have therefore been characterized by pharmacokinetic studies of plasma-vs-time profiles or by systemic distribution and subsequent excretion of radiolabelled drug after topical application, as described by Feldmann & Maibach (2). Drug levels in the skin can be studied by the suction blister fluid technique, by shave or punch biopsies or by indirect methods, e.g. correlation to a drug effect such as vasoconstriction in the case of topical steroids (3) or by correlation to drug content in stratum corneum strippings (4).

Microdialysis (Fig. 1) is a new technique which allows sam-

pling of intercellular fluid from tissues under minimally invasive *in vivo* conditions. Originally developed for neurotransmitter sampling in experimental rat brain pharmacobehavioural studies (5), the technique has the advantage of a high temporal resolution, providing that the compound studied is accessible in a high enough concentration (6). Microdialysis sampling is performed by placing an ultra-thin semipermeable “artificial vessel”, commercially made or constructed in the laboratory using a single fibre from a haemodialysis cylinder, in the tissue. This probe is then perfused with a pH-buffered perfusate (with added glucose, protein or other substances relevant to the diffusion of the compound studied) at a well-controlled flow. Unbound substances in the extracellular fluid will enter the slowly flowing perfusate in the lumen of the probe by passive diffusion over the membrane according to their concentration gradient, size and charge of the molecules. Large molecules (more than 20 kDa) such as proteins and enzymes cannot cross the membrane type used in this study and will therefore not be present in the perfusate, called the dialysate once it has perfused the membrane length. After continuous sampling at regular intervals the dialysate can be analysed, with little or no sample preparation, by HPLC or other very sensitive methods (7). The concentration of a given compound or drug in the dialysate will thus reflect the free, diffusible concentration in the extracellular fluid. The dialysate data should be interpreted in connection with the *in vitro* recovery characteristics of the drug or compound studied and with respect to the experimental conditions which influence recovery of the drug, such as composition of the perfusate, perfusate flow rate and membrane area/length.

In human drug studies, microdialysis has been used to determine pharmacokinetics of propranolol (8), caffeine (9), gentamycin and paracetamol (10) and theophylline (11), sampled in muscle and subcutaneous tissues. The technique has also been assessed for monitoring free valproic acid in ambulatory epileptic patients (12). Microdialysis in the dermis of humans (for method and editorial, see Groth (13) and Church et al. (14)) has successfully been used to describe the rising levels of nicotine under a nicotine patch for transdermal administration (15, 16). Cutaneous penetration of topical lidocaine and prilocaine in EMLA[®] cream (17) and of ethanol (18) has also been shown.

In this study, we assess the feasibility of using the microdialysis technique for the determination of drug levels in the skin after topical and systemic drug administration of 2 model drugs (Table I). Fusidic acid is an antibiotic widely used for both topical and systemic treatment of staphylococcal skin infections. Betamethasone-17-valerate is a corticosteroid with well-documented topical penetration (19), also given systemically as betamethasone. The drugs are examples of a highly protein-bound substance (fusidic acid) and a lipophilic steroid hormone.

Table I. Characteristics of model drugs

Drug	MW Da	Protein binding	pKa	Charge at pH 7.4	Solubility mg/ml water	Log P ^a
Betamethasone-17-valerate	476.6	Low ^b	—	Neutral	0.0093	3.5
Fusidic acid	516.7	97%	5.35 ^c	Anion	0.30	2.68

^a Octanol/water coefficient at pH 7.4.

^b Siddiqui et al. J Pharmacokinet Biopharm 1989; 17: 405–424.

^c Fusidic acid ionizes and becomes the fucidate salt at pH > 5.3.

In the study of topically administered drugs, SLS pretreatment, inducing irritant dermatitis, has been used to create barrier damage mimicking skin disease and thus enhanced penetration (20, 21).

MATERIALS AND METHODS

Chemicals

Sodium lauryl sulphate (>99% purity) was obtained from the Sigma Chemical, St. Louis, MO, USA. Fucidin[®] cream (fusidic acid 2%, Leo Pharmaceutical Products Ltd.) and Betnovat[®] cream and Betnovat[®] ointment (betamethasone-17-valerate 0.1%, Glaxo-Wellcome) were used as is. Betamethasone-17-valerate and fusidin (as fusidic acid or sodium fucidate) were obtained from Leo Pharmaceutical Products Ltd.

Animals

Thirty-two female Sprague-Dawley hairless rats (hr/hr, weighing 200 ± 20 g) were studied. They were housed at constant ambient conditions ($23 \pm 0.5^\circ\text{C}$) with free access to food and water. After pretreatment or application of topical treatment, animals were housed in individual cages.

Human volunteers

Three healthy volunteers (2 men age 49 and 56, 1 woman age 27 years) were studied. The volunteers had used neither topical nor systemic medication for 2 weeks prior to the experiment.

Pretreatment

In humans, patch treatment on both forearms with the standard irritant sodium lauryl sulphate (SLS, 2% w/v in water) was applied in

extra large Finn Chambers 19 mm in diameter (Epitest Ltd. Oy, Finland) for 24 h. After removal of the patch and gentle washing, topical treatment with the test drug was started.

In rats, irritant dermatitis on 1 side of the trunk was induced by application of 600 μl 10% SLS onto 3 layers of filter paper measuring 2.5×3.0 cm, kept in place on the skin by a frame of Comfeel[®] (Coloplast A/S, Kokkedal, Denmark). The area was sealed by impermeable tape (Blenderm[®], Coloplast A/S, Kokkedal, Denmark). After 2 h the SLS was washed off and the area left unoccluded until the start of topical treatment 16 h later.

Topical treatment

In humans, treatment with Fucidin[®] cream or Betnovat[®] cream was randomized between forearms. Each forearm was treated on 2 areas: the area pretreated with SLS was treated with daily application of approximately 200 mg cream under continuous occlusion using a 19 mm Finn Chamber and Micropore tape (3M Health Care, St. Paul, MN, USA). A demarcated area of uncompromised skin, 19 mm in diameter, was treated with open application of approximately 200 mg cream twice daily. Topical treatment was given for 48 h.

In rats, topical treatment was applied to a 2.5×3.0 cm demarcated area on the side of the trunk; 60 mg of cream/ointment or 400 μl of solution applied onto 3 layers of filter paper in a Comfeel framework was administered. Occlusion was achieved using impermeable tape (Blenderm[®], Coloplast A/S, Denmark) to cover the treated area. A bandage of Band-a-Rete[®] (no 2. Artsana, Como, Italy) covered the dressing and prevented the animal from tearing it off. The animals were treated on both sides of the trunk. Treatment was given twice daily for 48 or 72 h and consisted of Fucidin[®] cream (fusidic acid 2%), 10% fusidic acid in ethanol, Betnovat[®] ointment (betamethasone-17-valerate 0.1%), or 4% betamethasone-17-valerate in ethanol, with or without pretreatment and occlusion (see Results).

Systemic treatment

Rats were given oral administration of suspensions using a feeding tube. Treatment was given 90 min prior to microdialysis sampling and was: fusidic acid 60 mg/kg, fusidic acid 80 mg/kg, fusidic acid 312.5 mg/kg, betamethasone-17-valerate 15 mg/kg, betamethasone-17-valerate 60 mg/kg, or betamethasone-17-valerate 158 mg/kg.

Control experiments

Two rats received neither topical nor systemic treatment prior to microdialysis.

Microdialysis equipment

We used microinjection pumps (CMA/100, CMA/Microdialysis AB, Stockholm, Sweden) and probes manufactured by us, using single fibres of hollow dialysis membrane (Gambro GFE 18, Gambro Dialysaten AG, Hechingen, Germany; outer diameter 216 μm , wall thickness 8 μm , molecular cut-off 2 kDa). The membrane was glued to nylon connecting tubing (Portex, Berck-sur-Mer, France) using cyanoacrylate (Super Attak, Lock-tite, Denmark).

For *in vitro* experiments and rat experiments a connection (PEEK tubing, MF5366, CMA/Microdialysis) was used from probe to vial to prevent drug adherence to tubing. Membrane length accessible to

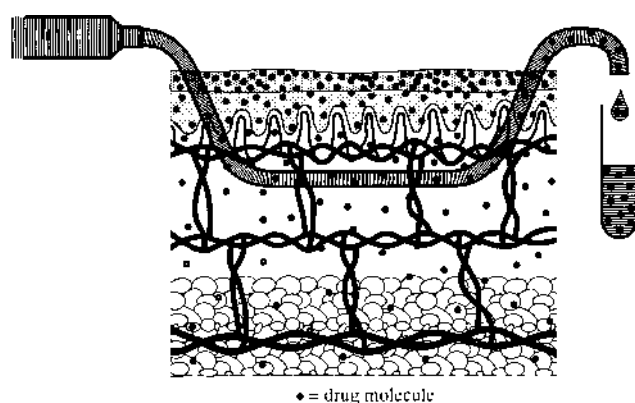


Fig. 1. Principle of microdialysis in the dermis. The semipermeable, hollow dialysis membrane is inserted in the middle of the dermis. The perfusate flow from the syringe is controlled by the microdialysis pump. Drug molecules penetrate the skin and passively diffuse into the lumen of the membrane. The dialysate is collected at fixed intervals.

microdialysis was 3 cm in all experiments. Probes for *in vitro* and human use had been sterilized by immersion in 70% alcohol for 20 min.

Perfusate consisted of sterile Ringer's solution (DAK, Denmark) or a sterile phosphate buffer with added glucose 2.5 mM (Gentofte University Hospital pharmacy). Perfusate flow rate was either 2 or 3 µl/min (see Results). Samples were collected at fixed intervals, either 30 or 40 min, giving sample sizes of 80–90 µl for analysis.

In vitro microdialysis

Relative recovery establishes probe efficiency with regard to the substance analysed. Relative recovery is defined as the dialysate concentration relative to the drug concentration in the medium surrounding the probe. *In vitro* microdialysis experiments with sodium fucidate in concentrations from 2 to 50 µg/ml in perfusate at 37°C, stirred at 350 rpm, showed a relative recovery of 44% (3 cm probe, 3 µl/min flow). For betamethasone-17-valerate the relative recovery under the same conditions was 38%.

For comparison, the microdialysis experimental equipment used gives *in vitro* recoveries of 80% for salicylic acid and 75% for glucose.

In vivo microdialysis

In humans, local anaesthesia was achieved using s.c. injection of approximately 3.5 ml of 1% lidocaine (Xylocaine®, Astra, Sweden) around (but not into) each of the treated areas. In rats, microdialysis experiments were performed under general anaesthesia with pentobarbital sodium 50 mg/kg given as intraperitoneal injection with supplementary injections of 10 mg/kg every 90 min. During the experiments animals were placed on a temperature controller (CMA/150, CMA/Microdialysis AB, Stockholm, Sweden).

In all experiments, 2 probes were inserted in each treated area. They were inserted in the dermis by means of a 21 G guide cannula. Perfusion was started following an equilibration period of 1 h to allow insertion trauma to subside, as previously shown by us (13). Perfusion of the probes was continued for 4 h.

Dialysate analysis

Sample size was 80 µl from dialysate samples of 90 µl. Concentrations of fusidic acid and betamethasone-17-valerate were determined by HPLC using a Merck/Hitachi LaChrom HPLC system. A Supersphere 100 RP-18, 4 µm, 125 × 2 mm narrow-bore column was used for separation. The mobile phase was acetonitril/methanol/0.05 M phosphoric acid 50/10/40, run at 0.3 ml/min for 3 min, then 0.6 ml/min for 5 min. Both compounds were detected at 245 nm and the peak heights used for quantitation. Retention time was 3.8 min for betamethasone-17-valerate and 6.3 min for fusidic acid. With dialysate samples of 80 µl, the limit of quantitation was 5 ng/ml for betamethasone-17-valerate and approximately 10 ng/ml for fusidic acid.

RESULTS

In human microdialysis experiments, none of the dialysates obtained after 48 h of treatment contained measurable levels of fusidic acid or betamethasone-17-valerate. This also applied to the skin treated with twice daily drug application under occlusion on irritative dermatitis.

In rat experiments using topical application of the commercially available formulations, the dialysates were without measurable levels of the drugs applied. In experiments using maximized topical treatment (10% solution of fusidic acid in ethanol or 4% solution of betamethasone-17-valerate in ethanol) we found measurable levels of betamethasone-17-valerate (Table II).

In some of the experiments an artefact was observed, as drug concentrations in the first and second dialysate samples were unexpectedly high. This might be due to contamination with residues of the topically applied betamethasone-17-valerate during insertion of the guide cannula. It was sought to minimize this by washing the skin with ethanol and water at the point of insertion and by using a stiletto in the cannula during insertion. Samples from more than 100 min of microdialysis show drug concentrations indicating that a steady-state concentration had been established (Fig. 2). Although indicated by mean drug concentrations, SLS provocation prior to topical treatment did not significantly increase drug concentrations in the dialysates (at a level of $p < 0.05$). The variation between samples from different probes in one rat was small compared to the variation between rats.

Using systemic administration of the drugs to rats in doses similar to the relevant clinical doses in man gave no measurable drug levels in the dialysates. Increasing doses stepwise was necessary to obtain measurable drug levels in the dialysates (Table III). Systemically administered betamethasone-17-valerate was measurable when a dose of 158 mg/kg was administered 90 min prior to starting the experiment.

For systemically administered fusidic acid 312.5 mg/kg, drug levels for the 2 probes shown in Fig. 3 demonstrated pharmacokinetics suggestive of a monoexponential decline with initial values of approximately 75 ng/ml and half-lives of 4.22 h and 1.93 h, respectively.

Ultrasound scanning (Derma scan-C, Cortex Technology, Hadsund, Denmark) of the probes inserted confirmed that all probes were situated in the dermis. Microdialysis in the dermis of untreated rats showed no compounds interfering with HPLC analysis of the drugs.

Table II. Microdialysate concentrations after topical treatment in rats

Drug and formulation	Pretreatment with SLS 10%	Duration of treatment	Occlusive treatment	ng/ml
Betamethasone-17-V 4% in ethanol	–	72 h	+	17 ± 1 ($n=1$, $dp=3$) ^a 26 ± 5 ($n=1$, $dp=4$) ^a 26 ± 15 ($n=1$, $dp=3$) ^a 19 ± 8 ($n=1$, $dp=3$) ^a
Betamethasone-17-V 4% in ethanol	+	72 h	–	30 ± 19 ($n=1$, $dp=3$) ^a 45 ± 23 ($n=1$, $dp=3$) ^a 11 ± 4 ($n=1$, $dp=4$) ^a

± = procedure done/not done.

n = number of rats, dp = total number of dialysis probes. Perfusate flow rate 2 µl/min, sampling interval 45 min.

^a Mean ± SD in samples from $t \geq 100$ min. See Fig. 2.

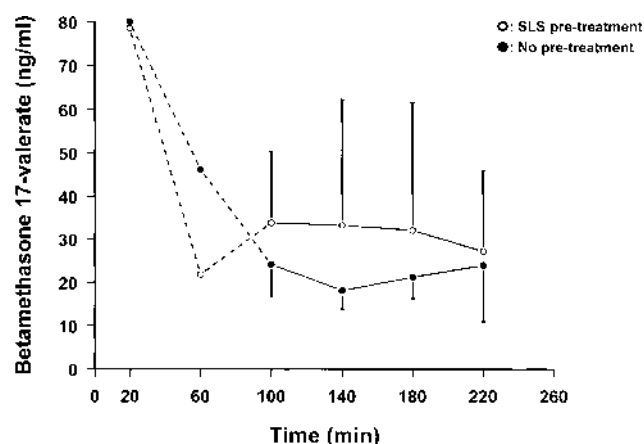


Fig. 2. Microdialysis of betamethasone-17-valerate after topical administration. Mean dialysate concentrations of betamethasone-17-valerate after topical treatment with 4% betamethasone-17-valerate in ethanol for 72 h in rats. SD are shown after $t \geq 100$ min, when the insertion contamination had washed away. Pretreated group: data from 3 rats and a total of 10 probes. No pretreatment group: data from 4 rats and a total of 13 probes.

DISCUSSION

In vitro studies of penetration and permeation of fusidic acid in human skin have shown that 2% of the fusidic acid applied in an ethanol solution permeates the intact skin (22) and that an intact horny layer offers marked resistance to permeation of sodium fucilate/fusidic acid. When the horny layer is damaged, permeation through as well as drug levels in the skin increases (23). *In vitro* studies generally show higher drug concentrations in the skin than *in vivo* studies due to the absence of drug metabolism and the wash-out effect of local blood perfusion. We were unable to measure free fusidic acid in the skin following even very aggressive topical treatment of skin with severe irritant dermatitis in rats.

Fusidic acid has been measured in human skin blister fluid after oral administration of 250 or 500 mg fusidic acid twice daily for 6 days, resulting in blister fluid concentrations of 21 ± 5 $\mu\text{g/ml}$ and 79 ± 11 $\mu\text{g/ml}$, respectively (24). Blister fluid includes both protein-bound and unbound drug fractions in contrast to samples obtained by microdialysis. As fusidic acid is 97% protein bound, the free drug concentration in blister

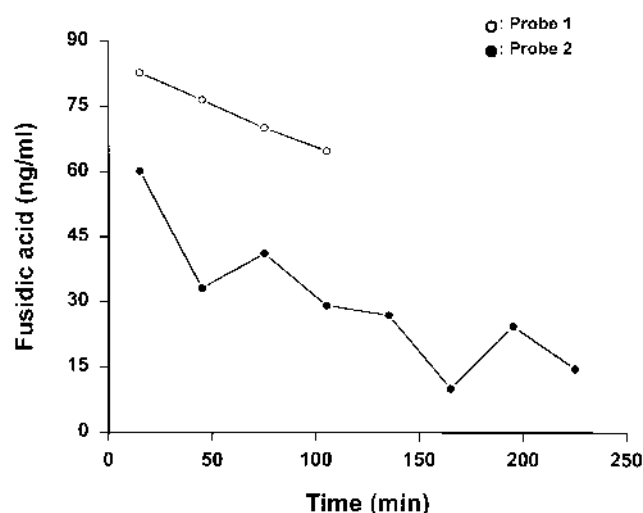


Fig. 3. Microdialysis of fusidic acid after systemic administration. Concentration of fusidic acid in dialysates from 2 probes following oral administration of 312.5 mg/kg fusidic acid to a rat. Probe 1 failed to function after 100 min.

fluid would be approximately 630 ng/ml after 250 mg b.d. dosage, which is 10 times the drug level found in dialysates after very high systemical doses to rats. Fusidic acid has an *in vitro* microdialysis recovery of 44%, but it is expected that the *in vivo* recovery will be somewhat lower (25). The remaining, larger difference might be explained by a more extensive metabolism in the rat as compared to man. This could be suggested by the apparently lower fusidic acid half-life of 2–4 h in our rat experiments, compared with the half-life of 5 h in a human study (26).

Using topical or systemical treatment with betamethasone-17-valerate in clinically relevant dosage, no drug could be measured in the dialysates. Betamethasone-17-valerate has a relative recovery of 38% under *in vitro* conditions (using an aqueous phosphate buffer both for perfusion and as drug suspension medium), but it is highly lipophilic and has a very low affinity for the aqueous perfusate compared to the tissues. *In vivo* sampling is therefore expected to be difficult due to decreased recovery (15, 27).

Following systemic administration of high doses of betamethasone-17-valerate, drug levels in the dermis of rats were

Table III. Microdialysate concentrations after systemic treatment in rats

Drug in suspension		Treatment	Drug level in dialysate ng/ml
Sodium fucilate	60 mg/kg	Twice daily for 72 h	Not detectable (n=2)
Sodium fucilate	80 mg/kg	Twice daily for 72 h	Not detectable (n=2)
Fusidic acid	312.5 mg/kg	Single dose	10–90 (dp=4, n=2) ^a
Betamethasone-17-V	15 mg/kg	Single dose	Not detectable (n=2)
Betamethasone-17-V	60 mg/kg	Single dose	Not detectable (n=2)
Betamethasone-17-V	158 mg/kg	Single dose	44 ± 10^b
			39 ± 20^b (dp=2, n=1)
			26 ± 7^b
			25 ± 4^b (dp=2, n=1)

n=number of rats, dp=total number of dialysis probes. Perfusate flow rate 3 $\mu\text{l/min}$, sampling interval 30 min.

^a See Fig. 3.

^b Mean $\pm$ SD.

comparable to the levels found after maximized topical treatment with betamethasone-17-valerate 4% in ethanol. In a human study comparing plasma concentrations of betamethasone-17-valerate after systemic or topical administration, oral treatment resulted in higher plasma concentrations. Plasma half-lives of betamethasone-17-valerate were 16.6 h and 8.1 h after topical and oral administration, respectively (19). We found steady-state dermal drug levels in the rats following topical administration, but sampling time was limited to a maximum of 6 h. SLS pretreatment has been shown to increase the percutaneous penetration of hydrocortisone by a factor of 2.6 in guinea pigs (21), but an increase in dialysate levels in our material was not significant at a level of $p < 0.05$.

In conclusion, we have assessed the microdialysis technique for measuring *in vivo* drug levels in the dermis following both topical and systemic treatment. Using fusidic acid as a model of a very protein-bound drug and betamethasone-17-valerate as a lipophilic model drug, we have shown that treatment with fusidic acid or betamethasone-17-valerate in clinically relevant dosage was without measurable free drug levels in the dermis. Maximizing treatment by administering very high systemic doses or creating extreme conditions of topical application was necessary to obtain measurable drug levels in the dialysates. The experimental conditions could have been optimized further by alterations in the composition of the perfusate, e.g. adding a protein or lipid compound to the perfusate, or by substantially reducing perfusion rate, thereby increasing *in vivo* recovery. However, both these solutions would create difficulties with regards to the analysis, introducing a protein content in the samples or reducing sample size below the required volume for injection and thus necessitating the development of an even more sensitive method of analysis than the very sensitive HPLC method we had access to (e.g. radiolabelling of the drugs, immunological assays or mass spectrometry).

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