Distribution to the Skin of Penciclovir After Oral Famciclovir Administration in Healthy Volunteers: Comparison of the Suction Blister Technique and Cutaneous Microdialysis

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Penciclovir is a drug active against herpes simplex viruses located in the epidermis basal layer. The aim of this study was to compare the suction blister technique and microdialysis as methods to measure the penciclovir concentration in the skin after a single dose (250 mg) of its prodrug, famciclovir. Suction blister fluid, microdialysates and plasma were sampled from 11 healthy volunteers for 5 h after famciclovir administration. Both the suction blister technique and microdialysis showed that penciclovir reaches the skin in concentrations sufficient to inhibit herpes virus replication. The maximum concentration in both suction blister fluid and in microdialysate was observed later than in plasma. The microdialysis concentration was decreased by cooling of the skin surface and by adrenaline-mediated vasoconstriction. The microdialysis recovery of penciclovir was studied with respect to the flow-rate of perfusion medium through the microdialysis probe. Microdialysis and the suction blister technique can be used to study the time-concentration profile of penciclovir in the skin and microdialysis allows a continuous sampling of the drug for a prolonged time after administration. *Key words: famciclovir; vasoconstriction; dermal drug concentration; temperature; microdialysis.*

Famciclovir is the well-absorbed oral prodrug of penciclovir, a potent and selective antiviral agent, with activity against varicella-zoster virus (VZV) and herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) (1). The pharmacological profile of penciclovir is well documented and its pharmacokinetics has been studied both in healthy volunteers and in patients with herpes zoster (2, 3). The high selectivity of penciclovir is due to the participation of viral thymidine kinase in its conversion to penciclovir triphosphate, which is the active metabolite. Famciclovir is used for systemic treatment of herpes virus infections, while penciclovir as such is used for topical treatment. The dose regimens are very different for the 2 routes of administration: famciclovir is given twice daily while penciclovir cream is applied every second hour (except during the night). The rational behind the topical treatment regimen is not clear.

Knowledge about the ability of antiviral agents to penetrate into the tissue containing the infected cells and factors affecting the transport is of considerable interest in allowing for a rational drug development. Commonly, only *in vitro* antiviral activity and plasma concentrations are the bases for choosing doses and dose regimens in phase II and III trials. However, the relationships between the drug concentration in the target tissue and the clinical, pharmacodynamic or pharmacological effect are usually overlooked. In the case of penciclovir it is of considerable interest to know the concentrations in the basal cell layer of epidermis, where HSV is located. One method, which allows measurement of the tissue concentration, is the suction blister technique (4). The blisters can be produced by applying a vacuum to the skin, e.g. the Dermovac®, and the suction blister fluid and the stratum corneum-epidermis sheets can be used for analysis of the drug concentration. The suction blister fluid corresponds roughly to the interstitial fluid and is used and validated in several pharmacokinetic studies (4). Another method to measure unbound extracellular drug concentrations in the dermis is microdialysis (5–7). Both methods have been successfully used to study the concentration profile of different pharmacologically active compounds.

The aim of this study was to compare the concentration of penciclovir by simultaneous microdialysis sampling in the dermis and in suction blister fluid following a single dose of famciclovir in healthy volunteers. We also studied the influence of varying blood circulation and skin temperature on the distribution of penciclovir to the dermis and investigated the relationship between the dialysate concentration and the flow rate of the perfusion medium through the dialysis probe.

MATERIALS AND METHODS

Drugs

Famciclovir (Famvir 250 mg, SmithKline Beecham, UK) was obtained from the hospital pharmacy. Penciclovir was a generous gift from Medivir AB, Huddinge, Sweden. Ringer solution (Pharmacia & Upjohn AB, Sweden) and adrenaline (Adrenalin NM Pharma, NM Pharma AB, Sweden) were obtained from the hospital pharmacy.

Volunteers

Eleven healthy volunteers were selected from the volunteer panel at the Department of Clinical Pharmacology, Huddinge Hospital, Huddinge, Sweden. The study was approved by the local ethics committee composed of clinicians and lay advisers. The mean (±SEM) age of volunteers was 32±2 years (6 males and 5 females). All subjects were free from significant skin disease and they were not allowed to use any skincare products for 24 h prior to the investigation. All subjects gave their informed consent.

Dose administration

After an overnight fast, each subject received a single oral dose of famciclovir (1 tablet of Famvir 250 mg). Tap water (100 ml) was taken with the dose.

Collection of samples

Blood. Venous blood (10 ml) was collected before dosing and 30, 90, 150, 210, 270 min after administration. Blood was taken in

heparin tubes and was centrifuged (3, 000 g) after 20 min at room temperature in order to obtain plasma.

Suction blister fluid. Prior to drug administration 5 suction blisters were produced by application of a special dome-shaped Dermovac® cap (Ventipress Oy, Lappenranta, Finland) to the inside of the left upper arm of each volunteer. The Dermovac® cap was switched to a pressure about 300 mmHg below the atmospheric pressure for 2 h continuously. After 2 h 5 blisters had developed, containing 50‑‑150 µl fluid. The suction blister fluid was sampled from one blister at each time-point (30, 90, 150, 210, 270 min) after famciclovir administration.

Microdialysis. Microdialysis probes were made in the laboratory using a membrane (cut off 6,000 Da) from an "artificial" kidney (GFS Plus 16, Gambro AB, Sweden) as previously described (5). A microdialysis probe was inserted horizontally intradermally using a 22-gauge guide cannula, i.d. 0.7 mm and length 50 mm. Prior to sampling of dialysate the guide was withdrawn leaving the probe horizontally within the dermis. The length of the membrane lying in the dermis was around 30 mm. Three to four probes were inserted in each subject without using of any pharmacological anaesthetics. In some volunteers hypnosis was used to reduce pain.

Study A: Influence of blood circulation on the concentration of penciclovir in the microdialysate

In each volunteer ($n = 7$) 3 probes were inserted on the volar aspect of the left forearm with a distance of around 30 mm between the probes. Probe 1 was then perfused with a Ringer solution and served as a control probe to the other two. Probe 2 was perfused with a Ringer solution containing adrenaline at a concentration of 0.2 mg/ml and probe 3 was perfused with a Ringer solution and the skin over probe 3 was chilled by application of a soft rubber container with cold water during the whole experiment. The temperature of the skin over the probe 3 was maintained around 20°C and was controlled by a temperature probe (Physitemp MT-23/3, Physitemp Instruments Inc., USA), inserted into the dermis close to probe 3 at the same time as microdialysis probes and it was connected to temperature reader (Physitemp BAT-12, Physitemp Instruments Inc., USA). All dialysis probes were perfused continuously at 1 µl/min by a syringe pump (CMA 100, CMA Microdialysis AB, Sweden) and dialysate was collected at 20 min intervals for 5 h. Famciclovir was administered 1 h after inserting the probes, when the vascular reaction to needle trauma had decreased (5, 7).

Study B: Influence of perfusion flow rate on the concentration of penciclovir in the microdialysate

In volunteers 8–11 4 microdialysis probes were inserted on the volar aspect of the left forearm. All probes were perfused by a Ringer solution, 2 of the probes with a flow rate of 1 µl/min and the other 2 with a flow rate of 0.5 µl/min. Dialysates were collected at 30 min intervals for 5 h.

Sample analysis

Microdialysates were analysed directly, without purification steps by isocratic reversed-phase high performance liquid chromatography (HPLC) with UV-detection (254 nm). A 150×2.1 mm 5 micron particle size C_{18} column (Chromtech AB, Sweden) was used in the study. The mobile phase consisted of a 0.05 M ammonium phosphate buffer at pH 7.0, containing 10% (v/v) methanol (8). Penciclovir was easily separated from endogenous compounds in the dialysates.

A 20 µl volume of each fraction of suction blister fluid was used for analytical measurement. Proteins were precipitated by adding of 8 µl 6% $HClO_4$ and after centrifuging at 3,500 g for 5 min, 15 µl of the fluid fraction was injected on the HPLC column. Penciclovir was easily separated from endogenous compounds in the suction blister fluid.

Blood samples were purified by reversed phase solid-phase extraction and then analysed by HPLC with fluorescence detection as previously described (9).

Statistical analysis

The microdialysis samples were collected in 20 (or 30) min fractions which can be represented in the graphs as the midpoint of the sampling period, i.e. 10, 30,...290 min (or 15, 45,...285 min) (10). Data are presented as a range or as median $\pm$ standard errors of means. For some comparisons microdialysis data were pooled to give the same time resolution as blood and blister samples.

Recovery calculation

The *in vivo* recovery (R) of each dialysis probe was calculated in the following way. The plasma concentration was taken as a measure of the extracellular concentration in the skin at C_{max}, estimated by curve fitting, in the peripheral compartment (skin). The rational behind this is the assumption that the concentration in the dermis extracellular fluid increases when the plasma concentration is higher and decreases when the plasma concentration is lower. The C_{max} in the dermis should therefore equal the plasma concentration at that time-point. A second-degree polynomial curve was fitted to microdialysis data to estimate C_{max} and T_{max}. The plasma concentration was obtained from the graph by means of a straight line. Recovery was been calculated as

$$R = C_d/C_{pl},$$

where C_d is the C_{max} of penciclovir in microdialysate obtained from the graph and C_{pl} is the penciclovir concentration in plasma at the same time point.

It has previously been shown that recovery is a function of the perfusion medium flow-rate (Φ) and depends on the probe length (L) and on the recovery constant (k). Thus,

$$R = 1 - e^{-k^*L/\Phi},$$

where k depends on the probe geometry, diffusion over the microdialysis membrane, diffusion in the tissue and active processes in the tissue (11). This constant, which is substance- and tissue-specific but flow-rate independent, can be found from a linear fitting $Y = k^*X$, where $Y = \ln(1 - R)$ and $X = L/\Phi$.

RESULTS

Concentration comparison between plasma, suction blister fluid and microdialysate

The median concentration vs. time curves ($n = 10$, 1 volunteer was excluded due to missing suction blister samples) are shown in Fig. 1. The maximum concentration (C_{max}) varied between 0.85 and 4.38 µM and between 2.76 and 5.16 µM for the dialysate at flow rate 1 µl/min ($n = 10$) and 0.5 µl/min ($n = 4$), respectively. The C_{max} varied between 1.67 and 5.15 µM for the suction blister fluid and between 3.59 and 8.06 µM for plasma. The time to maximum concentration (T_{max}) was 150 min in the dialysate, from 150 to 210 min in suction blister fluid and 90 min in plasma. Penciclovir was eliminated with a half-life of 1.8 ± 0.1 h from plasma. Clearance calculated for plasma data was 0.59 ± 0.05 l/h/kg. The distribution volume was 1.46 ± 0.10 l/kg. The mean residence time of penciclovir was 3.5 ± 0.2 h in plasma. The half-life obtained from dialysate data was 2.6 ± 0.6 h.
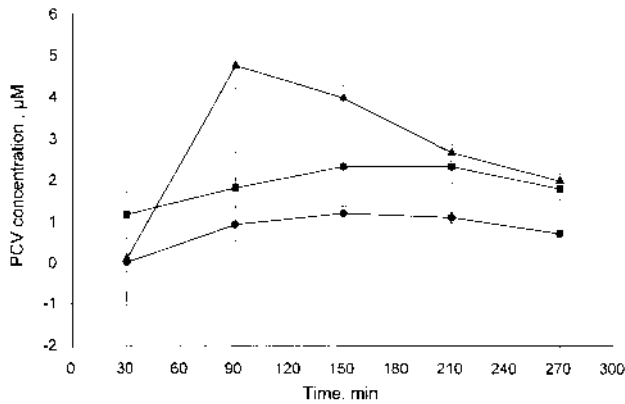
Fig. 1. Time-median (±SEM) concentration curves for plasma (tri-angles), suction blisters fluid (squares) and microdialysate at flow-rate 1 μl/min (circles) from healthy volunteers after a single dose of famciclovir (n = 10).
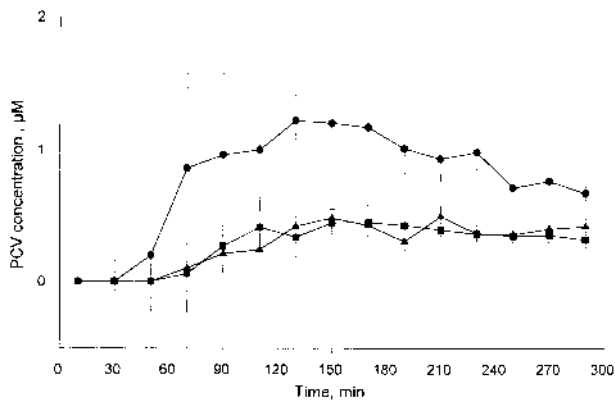

Fig. 2. Time-median (±SEM) concentration curves for microdialy-sis probes: control (circles), perfused with adrenaline (squares) and cold (triangles) (n = 4).

Effects of adrenaline and temperature on the skin dialysate concentrations

The median concentration vs. time curves for dialysate from the control probe, the probe perfused with adrenaline solution and the probe in cold skin during the whole experiment (n = 4, 3 volunteers have been excluded from the total of 7 due to missing data) are shown in Fig. 2. The temperature in the cold skin region was around 20°C. The observed total vasoconstriction around the probe perfused with adrenaline solution was 570 ± 150 mm^2. The areas under the curve of penciclovir were 3.61 ± 0.57 μmol*h/l, 1.22 ± 0.32 μmol*h/l and 1.03 ± 0.22 μmol*h/l for control, adrenaline and cold skin, respectively. By Student t-test for dependent samples the adrenaline-treated probes and the probes in the cooled skin are significantly different from the control probes with respect to AUC ($p = 0.022$ and $p = 0.009$, respectively).

Influence of flow-rate on the penciclovir microdialysis recovery

The median concentration vs. time curves for dialysates from probes perfused with 0.5 and 1 μl/min are shown in Fig. 3. The recoveries of penciclovir over the microdialysis mem-brane were found to be (mean ± SEM) 0.732 ± 0.061 at flow rate 0.5 μl/min and 0.391 ± 0.056 at flow rate 1 μl/min. The diffusion constant **k** was found to be 0.0221 ± 0.0039 mm^2/
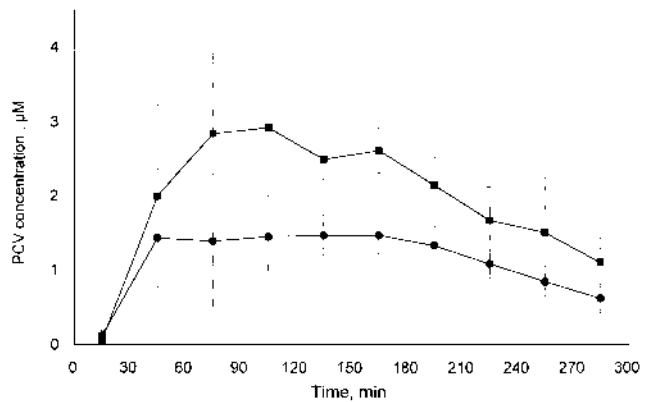
Fig. 3. Time-median (±SEM) concentration curves for microdialy-sis probes, perfused at flow rate 1 μl/min (circles) and 0.5 μl/min (squares) (n = 4).

min, when the probe length is expressed in mm and the flow-rate of the perfusion medium in μl/min.

DISCUSSION

Famciclovir is a well-absorbed oral form of penciclovir, which is rapidly converted to penciclovir by removal of 2 ester groups and enzymatic oxidation in the 6-position of the purine ring. The clinical pharmacokinetics of famciclovir and penciclovir have been studied extensively in healthy volunteers, patients with herpes zoster and elderly individuals. However, the concentra-tion of penciclovir in the skin has not previously been studied. The *in vitro* concentration (IC50) of penciclovir needed to inhibit virus replication has been found to be around 1 μM for HSV-1, 6 μM for HSV-2 and 12 μM for VZV (1), suggesting that these penciclovir concentrations should be attained in the basal layer of epidermis, where the virus is located, in infected patients. Among known methods, microdialysis and the suction blister technique are most suitable to investigate the concentra-tion of antiviral agents in the skin *in vivo*.

In this study it was found that the concentration profiles of penciclovir in plasma, dialysate and in suction blister fluid are different. C$_{max}$ was higher in plasma compared with dialysate and blister fluid. T$_{max}$ was shorter in plasma (90 min) than in dialysate and in suction blister fluid. T$_{max}$ was similar in these 2 fluids (150 min in microdialysate and 150–210 min in suction blister fluid). The half-life of penciclovir in the dermis calculated from microdialysis data (2.6 h) was slightly longer than in plasma (1.8 h), possibly due to a depot function of the skin.

The pharmacokinetic properties, such as C$_{max}$, areas under the curve, half-life, mean residence time, of penciclovir obtained from plasma data in this study are similar to previously published results (2). The difference in T$_{max}$, 90 min in our study and 45 min in a previously published study (2), can be explained by differences in study design. This study was not planned as a pharmacokinetic investigation and plasma was not sampled as often as in the previously published study.

The concentration of PCV obtained in the dermis of healthy volunteers after a single dose of famciclovir varied between volunteers from 3.8 to 7.1 μM (C$_{max}$ after adjusting for recovery calculated). It must be pointed out that the measured concentrations are the free (unbound) extracellular

concentrations and not the total concentration that is obtained in plasma and blister fluid analysis. In the present study this is not of great importance because compounds with low affinity to plasma proteins, such as penciclovir (protein binding reported less than 20%) will reach the same concentration in all three fluids. For other compounds this should be kept in mind in the interpreting of microdialysis data. Penciclovir as all other antiviral nucleoside analogues is active as its triphosphate ester and the phosphorylation takes place inside of the cells. Thus, the intracellular concentration of penciclovir triphosphate is more interesting from the virological point of view. However, penciclovir extracellular levels in the dermis may be regarded as corresponding to the culture medium concentration used when studying antiviral activity in *in vitro* experiments. In doing such comparisons, the variability among cell- and virus-strains in *in vitro* studies should be taken into account.

It has been shown previously that vasoconstriction influences the pharmacokinetics and dermal tissue distribution of compounds (12). In this study the influence of 2 types of vasoconstriction, pharmacological (adrenaline) and physiological (application of cold), on the concentration of penciclovir in the dermis was investigated. Both types of vasoconstriction resulted in a significantly decreased dialysate concentration, compared with the dialysate from the control probe. Probably, the decreased blood flow in a region of the skin lowers the transport of penciclovir molecules from blood vessels into this region and causes a decrease in the concentration of the drug in the dialysate. The other possible explanation to the decreased dialysate concentration is a reduced microdialysis recovery. The microdialysis recovery is dependent of temperature due to the temperature influence on mass transport in the tissue. Simultaneously measuring local blood flow and the influence of increased temperature on the dialysate concentration are points of interest in future studies of penciclovir in the skin.

Also the flow-rate of the perfusion medium through the dialysis probe influences the concentration of penciclovir in the dialysate. This phenomenon is a result of the changed microdialysis recovery of penciclovir. The decrease of the flow-rate from 1 µl/min to 0.5 µl/min resulted in almost a doubling of the concentration levels of penciclovir and in an increase of the areas under the curve of penciclovir. The data obtained on the recovery of penciclovir over the dialysis membrane and the diffusion coefficient (**k**) in this study allows calculation of the effect of changing the flow-rate and the length of the dialysis probe to get a higher recovery over the microdialysis membrane in future studies of penciclovir distribution to the skin. Thus, changing the flow-rate of the perfusion medium from 0.5 µl/min to 0.3 µl/min and increasing the length of the microdialysis membrane from 30 mm to 40 mm should increase the recovery from 73% to 95%.

In this study pharmacological anaesthesia was not used to avoid the influence of local anesthetics through the change of local blood flow on the distribution of penciclovir into the skin. The insertion of microdialysis probes was described by all volunteers, who did not receive hypnosis, as painful, but temporary (only during the time a guide cannula is inserted into the dermis). Hypnosis was effective in some subjects and partially effective in others. In the present study we did not investigate the effects of hypnosis systematically. The slightly increased local blood circulation was observed after probe insertion and faded within 30–60 min. Most of the volunteers were willing to participate in future microdialysis studies.

In summary, in this study it was demonstrated that penciclovir is distributed into the dermis in healthy volunteers in concentrations inhibiting herpes virus replication, following oral administration of a single dose of famciclovir. The concentration profiles of penciclovir in the microdialysate from the dermis and in suction blister fluid were similar and lagged somewhat compared with the time-concentration curve of the drug in plasma. Local vasoconstriction in the skin reduces the rate of drug distribution to the dermis. It was established that decreasing of the flow-rate of the perfusion medium through the microdialysis probe and increasing the probe length would increase the recovery of penciclovir in the skin, relevant to future studies of dermal drug concentrations.

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