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Percutaneous absorption of drugs used in atopic eczema: pimecrolimus permeates less through skin than corticosteroids and tacrolimus

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Abstract

For treatment of skin diseases with topical drugs, penetration of the agents into the relevant layers of the skin is required. Permeation through the skin should, however, be kept to a minimum, in order to avoid the risk of systemic side effects. Here we compared the in vitro skin penetration and permeation of two novel drugs used in the therapy of atopic eczema (pimecrolimus and tacrolimus) and three representative corticosteroids (betamethasone-17-valerate, clobetasol-17-propionate, and diflucortolon-21-valerate). Drug concentrations of pimecrolimus and corticosteroids in human skin were found to be in the same order of magnitude. Permeation of pimecrolimus through human skin was, however, lower by factors of 70–110 as compared to the steroids. When pimecrolimus was compared with tacrolimus in human, pig, or rat skin, similar concentrations of the two compounds were measured in the skin, whereas permeation of pimecrolimus through skin was consistently lower by factors of 9–10. Lipophilicity was found to be highest for pimecrolimus, its octanol–water distribution coefficient being higher by factors of 8 and 25–450 than that of tacrolimus and the corticosteroids, respectively. The low permeation of pimecrolimus may be explained by its higher lipophilicity (compared to tacrolimus and the corticosteroids) and higher molecular weight (compared to steroids). In conclusion, pimecrolimus appears to have a favourable skin penetration/permeation profile, featuring a low degree of percutaneous absorption.

Keywords: Dermatologic agents; Eczema; Percutaneous absorption; Skin

1. Introduction

In dermatology, topical drugs are used to treat diseased areas of the skin with the intention to confine the distribution of the applied active agent to the affected cutaneous tissue. Permeation of topical drugs through the skin leading to uptake into the systemic circulation is generally not desired, and may, in some instances, lead to systemic side effects of topical treatments. As an example, topical corticosteroids, besides their well-known local side effects, may have systemic adverse effects such as hypothalamic–pituitary–adrenal axis suppression, Cushing's syndrome, femoral head osteonecrosis, and cataracts (Jackson, 1978; Takeda et al., 1988; Fisher, 1995; Abma et al., 2002; Brazzini and Pimpinelli, 2002). Thus, an ideal topical drug for treatment of inflammatory skin diseases should be able

Abbreviations: Elog D_{oct}, experimental octanol–water partition coefficient

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to pass the stratum corneum and to reach therapeutically relevant concentrations in the epidermis/dermis without leading to high serum levels and systemic exposure.

While topical corticosteroids have been the mainstay in the treatment of atopic eczema, two topical drugs have recently been introduced as novel therapeutic options, namely pimecrolimus (SDZ ASM 981, Elidel®) and tacrolimus (FK 506, Protopic®) (Nghiem et al., 2002). While both drugs act as inhibitors of calcineurin, their pharmacological profile is different: (i) tacrolimus, when used systemically, is a potent immunosuppressive drug enhancing graft survival after organ transplantation (Spencer et al., 1997); (ii) pimecrolimus is a selective pro-inflammatory cytokine inhibitor specifically developed for treatment of inflammatory skin diseases (Meingassner et al., 1997; Grassberger et al., 1999; Eichenfield et al., 2002) with low potential to affect systemic immune response as compared to tacrolimus (Stuetz et al., 2001).

In the present study, we performed a direct comparison of the skin pharmacokinetics of representative topical corticosteroids with those of the new drugs, pimecrolimus and tacrolimus. We determined the intrinsic ability of the test compounds to penetrate into and to permeate through human or animal skin in vitro. In addition, we measured the lipophilicity of the test compounds and propose that it may represent an important factor governing their skin penetration.

2. Materials and methods

2.1. Materials

Betamethasone-17-valerate, clobetasol-17-propionate, and diflucortolone-21-valerate were purchased from Sigma. Pimecrolimus and tacrolimus were produced at Novartis Pharma.

2.2. Skin donors and skin preparation

Full thickness rat skin was obtained from 8–12-week-old hairless female animals (strain ICO:OFA hr-hr); after suffocation with CO_2, the skin was dissected and subcutaneous tissue was removed. Skin of domestic pigs (weighing about 15 kg) was dissected after euthanasia with Ketavet/T-61; the skin was dermatomed to 0.4 mm with an Aesculap dermatome; thus so-called split-thickness skin was prepared, consisting of epidermis and the upper part of the dermis.

Human abdominal cadaver skin was obtained from the National Disease Research Interchange (Philadelphia, PA), kept frozen at −80 °C and used within 2 months after autopsy. After thawing, the skin was dermatomed, as for porcine skin. In two experiments, skin from either the back of a 64-year-old female Caucasian (comparison of pimecrolimus versus steroids) or from the upper thigh of a 22-year-old male Caucasian (comparison of pimecrolimus versus tacrolimus) was used.

2.3. Penetration assay

Percutaneous penetration was studied in vitro using static Franz-type diffusion cells. The exposed skin area was 2.54 cm^2, and the volume of the receptor chamber was 5.8 ml. Phosphate buffered saline:ethanol (3:1) was used as receptor phase, maintaining sink conditions throughout the experiment (maximum receptor concentrations of the drugs were at least 30-fold above their solubility in the receptor). All experiments were performed at 32 °C in triplicates for 48 h. An infinite dosing regimen was used: the test compounds were applied to the epicutaneous side of the skin in propylene glycol or in propylene glycol:oleylalcohol (9:1) at a concentration of 1% (w/v) in a volume of 300 μl. The solubility of the five test compounds in propylene glycol is between 1.8 and 2.5% and in propylene glycol:oleylalcohol (9:1) between 3.6 and 4.4% as determined by HPLC analysis; thus, by selecting 1% as concentration in the application solution for all compounds, they were applied at approximately equal thermodynamic activity.

Samples of 100 μl were withdrawn from the receptor phase at four–eight time points during the 48-h experiment and replaced by fresh receptor fluid. After addition of an internal standard, these samples were analysed directly (see below). At the end of the experiment at 48 h, the skin was taken from the diffusion cells and the stratum corneum was removed by 5 strippings (rat skin) or 20 strippings (porcine and human skin) with transparent adhesive tape (Kores, Spain). Specimens from the stripped skin were taken with a biopsy punch, weighed and then analysed as described below.

2.4. Sample analysis

Skin samples containing pimecrolimus or tacrolimus were homogenized in buffer solution pH 10 (Merck) using a Potter S homogenizer (B. Braun Bio-tech, Germany); the homogenates were spiked with an internal standard and then extracted with *tert*-butylmethyl ether. In the case of the corticosteroids, 0.1 M sodium phosphate, pH 7.0, was used as buffer, and the homogenates were extracted with ethyl acetate. Extracts were evaporated and the residues were subjected to analysis. Skin extracts and receptor samples containing pimecrolimus or tacrolimus were analysed using LC-MS/MS with a Hewlett-Packard 1090 M HPLC coupled to a Finnigan LCQ mass spectrometer with an ESI ion-source. The limits of quantification for pimecrolimus and tacrolimus were 10 ng/ml in receptor fluids and were 150 ng/g in skin samples. Samples containing steroids were analysed using a Hewlett-Packard 1090 M HPLC with UV detection at 236 nm. Concentrations of both the corticosteroid esters and the free active corticosteroids (being formed to a minor extent by hydrolysis during the experiment) were calculated. Reported skin concentrations and permeation rates represent the sum of the values for ester and free steroid. The limits of quantification for the steroids were 250 ng/ml in receptor fluids and were 1.25 μg/g in skin samples. Skin concentrations were calculated by comparing the area of drug versus the area of internal standard. Calibration curves were prepared for the test compound in the respective skin homogenates, and analysed by linear regression. Calculation of flux was done as described by Schmook et al. (1993).

2.5. Determination of Elog D_{oct}

Distribution coefficients between buffer, pH 7.4, and octanol were determined experimentally using the method of Lombardo et al. (2001). Briefly, a set of 20 reference compounds with known octanol–water partition coefficients ($\log D_{\mathrm{oct}}$) were chromatographed on a Supelcosil LC-ABZ column (4.6 mm × 50 mm). The mobile phase consisted of 20 mM MOPS buffer, pH 7.4, and methanol in varying proportions from 30 to 60%; a 0.25 % (v/v) amount of octanol was added to methanol and octanol-saturated water was used to prepare the buffer. The capacity factors extrapolated to a 0% concentration of acetonitrile ($\log k'_{\mathrm{w}}$) were plotted against $\log D_{\mathrm{oct}}$; a linear correlation was obtained. Using this correlation and experimental $\log k'_{\mathrm{w}}$ values for the test compounds, the experimental $\log D_{\mathrm{oct}}$ values were determined. Determinations were done in triplicate and standard deviations were calculated.

2.6. Molecular modelling

The pimecrolimus and tacrolimus structures (determined by X-ray) were extracted from the Novartis corporate database and were aligned using the Sybyl molecular modelling package (Version 6.8, Tripos Associates). Electron density surfaces were generated for both molecules using the MOLCAD program (Heiden et al., 1993a), and various physicochemical properties such as lipophilicity (Ghose and Crippen, 1986) and the electrostatic potential distribution were mapped onto them (Heiden et al., 1993b). The surface-mapped properties were displayed using Sybyl and compared visually.

3. Results

We used an in vitro system to compare the skin penetration properties of pimecrolimus, tacrolimus, and selected topical steroids. In this model system, skin is mounted in Franz-type diffusion cells and solutions of the test compounds are applied to the epicutaneous side of the skin (Schmook et al., 2001). Two parameters are obtained: (i) the rate of permeation (flux) of the test compound through the skin into the receptor fluid; (ii) the concentration of the compound in the skin (epidermis and dermis, after removal of stratum corneum). Permeation rates are calculated by taking samples from the receptor phase at various time points, while the skin concentration reported here refers to the 48-h time point at the end of the experiment.

3.1. Comparison pimecrolimus versus topical steroids

When pimecrolimus was applied to the skin as 1% solution in propylene glycol, skin concentrations and skin permeation were undetectable with our an-

alytical methods (limits of detection: 150 ng/g and 0.4 ng/cm^2/h, respectively). In contrast, skin concentrations of betamethasone-17-valerate, clobetasol-17-propionate, and diflucortolon-21-valerate were in the range of 12–18 μg/g and flux was 80–140 ng/cm^2/h. When applying the compounds in propylene glycol containing 10% oleyl alcohol, concentration of pimecrolimus in human skin was of the same order of magnitude as for the steroids (about 40–60 μg/g), with levels of pimecrolimus (about 20 μg/g) being lower by factors of about 2–3 (Fig. 1A). Permeation through human skin was low for pimecrolimus (5.2 ng/cm^2/h), the rate being lower by factors of about 70–110 as compared to the steroids (340–570 ng/cm^2/h; Fig. 1B).

3.2. Comparison pimecrolimus versus tacrolimus

Concentrations of the two compounds in the skin after application in propylene glycol:oleyl alcohol (9:1) were found to be similar for rat, porcine, and human skin (Fig. 2A). In contrast, the permeation rates of pimecrolimus through the skin were lower by factors of 9–10 as compared with the respective rates of tacrolimus (Fig. 2B). As an example, Fig. 3 shows the time-dependent increase of tacrolimus and pimecrolimus in the receptor fluid after permeation through human skin.

3.3. Lipophilicity of test compounds

In order to rationalize the observed differences in skin permeation, we measured the Elog D_{oct} value as a measure of lipophilicity. Pimecrolimus showed the highest value in the series of test compounds (Table 1), being 450-fold more lipophilic than clobetasol-17-propionate and eight-fold more lipophilic than tacrolimus.

Table 1
Elog D_{oct} values of test compounds

Test compound	Elog D_{oct}[a]
Pimecrolimus	6.99 ± 0.05
Tacrolimus	6.09 ± 0.04
Betamethasone-17-valerate	4.74 ± 0.02
Clobetasol-17-propionate	4.34 ± 0.02
Diflucortolon-21-valerate	5.60 ± 0.03

[a] Means of triplicate determinations ± standard deviation.

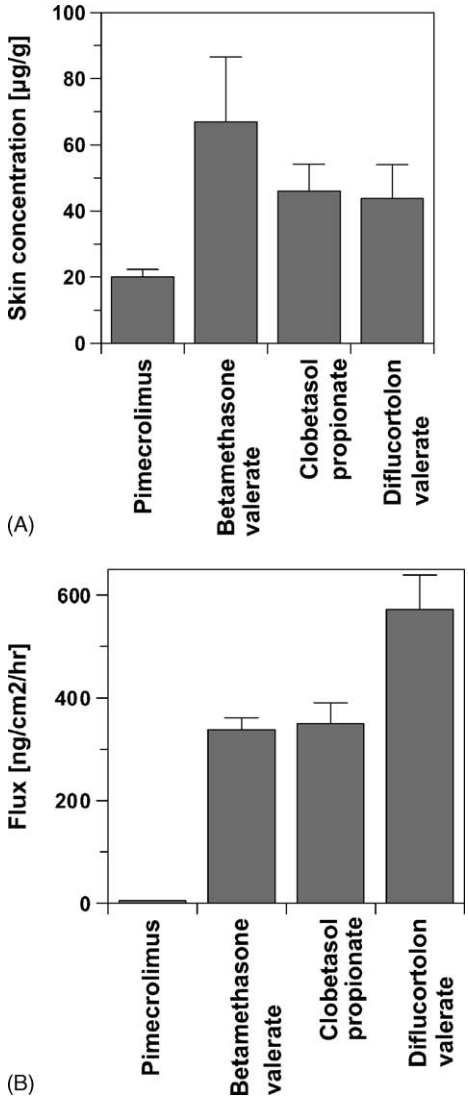

Fig. 1. Penetration of pimecrolimus and of selected topical steroids into human skin in vitro after application as 1% solutions in propylene glycol:oleyl alcohol (9:1). (A) Skin concentrations (epidermis and dermis, after removal of stratum corneum). (B) Permeation rates.

Fig. 4 shows the structural differences between tacrolimus and pimecrolimus. While the surface charge distribution of the two molecules were very similar (data not shown), the lipophilicity distributions revealed two areas of significantly higher lipophilicity for pimecrolimus as compared with tacrolimus (see Fig. 5).

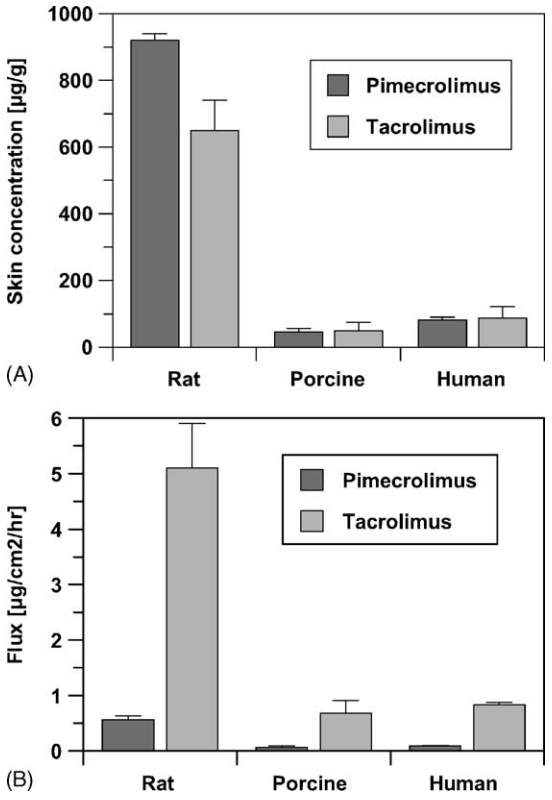

Fig. 2. Penetration of pimecrolimus and tacrolimus into rat, pig, and human skin in vitro after application as 1% solutions in propylene glycol:oleyl alcohol (9:1). (A) Skin concentrations (epidermis and dermis, after removal of stratum corneum). (B) Permeation rates.

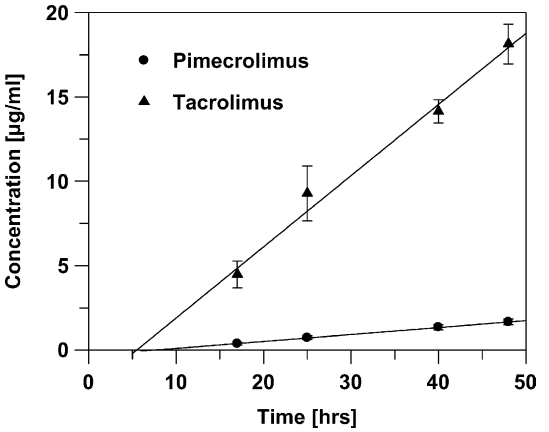

Fig. 3. Increase of pimecrolimus and tacrolimus concentrations in the receptor fluid following permeation through human skin in vitro.

4. Discussion

We compared the in vitro skin penetration properties of pimecrolimus to those of (i) representative corticosteroids and (ii) tacrolimus. Skin concentrations reached by both the steroids and the calcineurin inhibitors are of the same order of magnitude under the in vitro test conditions. In contrast, the propensity of pimecrolimus to pass through the skin into the receptor fluid is much lower than for the steroids: a factor of 70–110 was observed when directly comparing pimecrolimus with the steroids. By inference, it can be predicted that percutaneous absorption of pimecrolimus

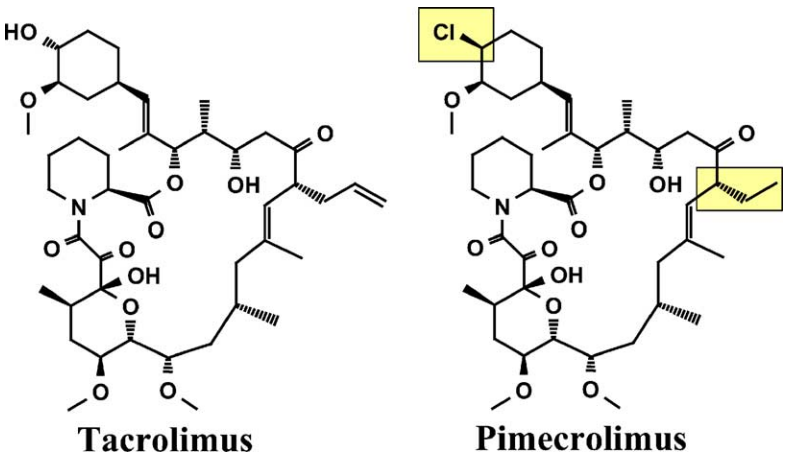

Fig. 4. Chemical structure of pimecrolimus and tacrolimus. Differences are highlighted in the pimecrolimus structure.

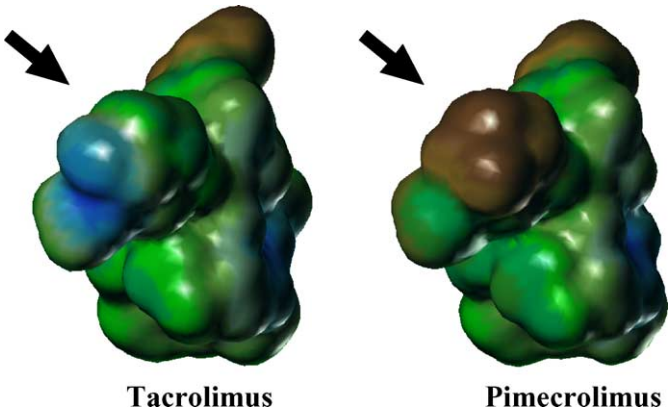

Fig. 5. Surface lipophilicity distribution of tacrolimus and pimecrolimus. Brown, green, and blue areas indicate lipophilic, intermediate, and hydrophilic regions, respectively. The location of markedly different lipophilicity of the two molecules are indicated by the arrows.

into the systemic circulation after topical application to patients will be significantly less than for the steroids.

When directly comparing pimecrolimus and tacrolimus, equal concentrations within the skin, but 9–10-fold lower concentrations of pimecrolimus in the receptor fluid were observed. Since sink conditions were maintained throughout the experiments and since the compounds were applied at approximately equal thermodynamic activity (see Section 2), these data indicate that the intrinsic capability of pimecrolimus and tacrolimus to cross the stratum corneum is similar, while further permeation into the receptor fluid is impaired in the case of pimecrolimus. This difference between the structurally related compounds may be explained by the different lipophilicity/hydrophilicity distribution within the molecules and the higher overall lipophilicity of pimecrolimus as compared to tacrolimus. Introducing increased lipophilicity to reduce systemic absorption of topical drugs is a well-known principle, which has also been exploited in the case of the corticosteroids, e.g. by esterification (Brazzini and Pimpinelli, 2002). In comparison with the corticosteroids investigated, the lipophilicity of pimecrolimus is higher by more than two log steps. These differences, together with the higher molecular weight (pimecrolimus: 810 Da, corticosteroids: ~470 Da), may explain the much lower skin permeation of pimecrolimus as compared to corticosteroids.

The data are in line with the low systemic exposure after topical application of pimecrolimus (Van Leent et al., 2002) observed in atopic dermatitis patients irrespective of severity of disease, extent of body surface treated, and duration of treatment (Kapp et al., 2002; Wahn et al., 2002). Thus, the results indicate that pimecrolimus, which due to its different pharmacological profile has per se a lower risk of systemic immunosuppression (Stuetz et al., 2001) and, as demonstrated here, shows an intrinsically low skin permeation, may offer a larger safety margin than other topical therapeutic options.

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