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Topical Delivery of Fluconazole: In Vitro Skin Penetration and Permeation Using Emulsions as Dosage Forms

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ABSTRACT We investigated in vitro skin penetration and permeation of fluconazole from emulsions containing different penetration enhancers. Fluconazole permeation was high (15–65% of the applied dose) across hairless mouse skin and low (8–9%) across pig ear skin. Permeation across mice skin from a formulation containing propyleneglycol and isopropyl myristate was significantly higher than that observed with the paraffin oil and propyleneglycol or Transcutol® emulsions. With pig skin, the paraffin oil or isopropyl myristate and propyleneglycol emulsions showed similar skin permeation and penetration. However, these emulsions provided epidermal concentrations higher than the minimal inhibitory concentrations for most dermatophytes.

KEYWORDS Fluconazole, Skin permeation, Topical treatment and delivery, Formulations, Emulsions, Skin fungal infections

INTRODUCTION

Superficial skin infections caused by fungus are among the most common diseases. Dermatophytes, which usually cause such infections can be classified in three genera; *Microsporum*, *Trichophyton* and *Epidermophyton*. These fungi are inherently not pathogenic, but when the host's cellular defense or skin function is altered, colonization, infection and disease can occur (Odom, 1997). Generalized infections by dermatophytes have occurred with extensive cutaneous damage due to usage of immunosuppressive drugs. HIV-positive and immunosupressed patients are especially susceptible to fungal skin infections (Odom, 1994; Virgili et al., 2002).

Topical treatment is recommended in cases of dermatophytoses by noninflammatory tinea corporis, tinea cruris, tinea faciei, tinea manuum and tinea pedis (Drake et al., 1996). Clinical efficacy of topical antifungal therapy depends on the drug ability to penetrate into the stratum corneum (SC) and the duration of treatment (Piérard et al., 1996). The efficacy of topical imidazoles (clotrimazole, ketoconazole, econazole and miconazole), which are more fungistatic than fungicidal, is related to the duration of treatment and, as a consequence, to the maintenance of the minimal inhibitory concentrations (MIC) for a prolonged time (Brenman & Leyden, 1997). Long lasting

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treatments make compliance difficult and increase relapse rates. Systemic therapy is recommended in cases of hyperkeratotical and disseminated lesions, chronic infections or in those cases in which patients do not respond to topical treatment (Drake et al., 1996).

Fluconazole, a hydrophilic bis-triazole, is an antimycotic with a broad spectrum that has been used in the treatment of dermatophytoses by oral administration (Lesher, 1999). It is reliably absorbed after oral administration with systemic bioavailability over 90%. After oral dosing (50 mg daily for 12 days or 150 mg once a week for 2 weeks), fluconazole accumulates in eccrine sweat and diffuses rapidly and extensively in the SC. Fluconazole concentration in the skin (SC and epidermis + dermis without SC) is higher than in the serum and its elimination from SC is considerably slower than from serum or plasma (Faergemann & Laufen, 1993; Wildfeuer et al., 1994). The highest concentration is achieved in SC and it is still high 1 week after completion of the treatment. This concentration is much higher than MIC for most dermatophytes (Faergemann, 1999). The prolonged skin retention of fluconazole has been attributed to its high affinity to SC due to an interaction between fluconazole and keratin (Klimke & Schäfer-Korting, 1997). However, fluconazole skin distribution after topical administration has been little investigated.

Mathy et al. (2005) reported that fluconazole unbound concentration in dermis, using dermal microdialysis, was higher than the MIC against *Candida albicans*. However, this concentration was obtained after the application of an infinite dose (0.5 g of Difucan® Gel to a surface of 58 mm^2; applied dose = 833 mg/cm^2) using rodent skin, a limited model to simulate human skin. On the other hand, application of infinite dose does not mimic the use conditions. Recently, it has been shown that reduction of skin penetration enhancer from topical formulations, applied at clinically relevant doses (finite), would limit its penetration enhancement effect (Trottet et al., 2004).

The present study aimed to evaluate the topical delivery of fluconazole after application of a clinically relevant dose of emulsions. These delivery systems were selected for their wide use as dosage forms in topical therapy of fungal skin infections. The influence of a lipophilic (isopropyl myristate) and two hydrophilic propyleneglycol (PG) or diethyleneglycol monoethyl ether (TCL, Transcutol®) skin penetration enhancers, incorporated into emulsions, was investigated (Moser et al., 2001a). In vitro skin penetration (topical delivery) and permeation studies were carried out in Franz diffusion cells using hairless mice skin and pig ear skin. Hairless mice skin has been used in our studies only as a preliminary step to the protocols that will include experiments in pig ear skin.

MATERIAL AND METHODS

Material

Fluconazole (Libbs Farmacêutica, São Paulo, Brazil), propyleneglycol (PG, Basf, Ludwigshafen, Germany), diethyleneglycol monoethyl ether (TCL, Transcutol®, Gattefossé, France), paraffin oil and isopropyl myristate (Sigma, St. Louis, MO), polyoxyethylene 3 lauryl ether (Laureth–3, Oxiteno, São Paulo, Brazil), Carbomer 940 (B.F. Goodrich Company, Cleveland, Ohio, USA) and sodium hydroxide (Merck, Germany) were used in the preparation of the emulsions. Other materials were polyoxyethylene 20 oleyl ether (Sigma, St. Louis, MO), PEG-8 caprylic/capric glycerides (Labrasol®, Gattefossé, France) and HPLC grade methanol (J.T. Baker, Phillipsburg, New Jersey, USA). All other chemicals used were analytical reagent grade.

Solubility of Fluconazole

The solubility of fluconazole was determined in water, ethanol, PG, PEG-8 caprylic/capric glycerides and TCL. An excess amount of fluconazole was added to 10 mL of each solvent and submitted to magnetic stirring for 24 hr at room temperature (23 ± 1°C). The mixtures were then centrifuged at 3000 rpm for 5 min and aliquots of the supernatant were filtered through a 0.45 μm Millipore filter. Supernatant samples were diluted with the mobile phase and high-performance liquid chromatography (HPLC) was used for evaluation of the fluconazole concentration (see below).

Preparation of Emulsions

The emulsions were prepared according to the formula shown in Table 1. Aqueous and oily phases were prepared separately. Aqueous phase, composed by Carbomer 940 and sodium hydroxide, and oily phase, composed by Laureth-3 and isopropyl myristate or

TABLE 1 Composition (% weight/weight) of the o/w Emulsions Containing Fluconazole

Formulations/ ingredients	A	B	C	D
Paraffin oil (PO)	10	10	–	–
Isopropyl myristate (IPM)	–	–	10	10
Laureth–3	2	2	2	2
Carbomer 940	0.25	0.25	0.25	0.25
NaOH	0.08	0.08	0.08	0.08
Fluconazole	1	1	1	1
Propyleneglycol (PG)	–	10	–	10
Transcutol® (TCL)	10	–	10	–
Water qs	100	100	100	100

paraffin oil, were separately heated at 75°C. At this temperature, the phases were mixed using an Eurostar® agitator (Ika, Staufen, Germany) at 900 rpm for 15 min. The emulsions were then cooled to 40°C. Subsequently, fluconazole, previously dissolved in PG or TCL, was added. No fluconazole crystal was observed, suggesting that it was completely dissolved in the emulsions. Homogeneity of fluconazole concentration in the emulsion was controlled by HPLC analysis. The results confirmed that the fluconazole concentration in the emulsions was 1.0% by weight.

In Vitro Skin Permeation

In vitro skin penetration (uptake) and permeation experiments were performed on Franz diffusion cells (membrane surface area of 1.77 cm^2 and volume of the receptor fluid of 6.7 mL). Skin was excised from 60 to 70-day-old male hairless mice (strain HRS/J Jackson Laboratories, Bar Harbor, ME) or from ears of 4-month-old pigs, obtained in a local slaughterhouse. Mice were euthanized by cervical rupture, the dorsal skin was excised with a scalpel and the subcutaneous fat was removed. The skin was visually examined in order to detect damages and mounted on Franz diffusion cells.

The pig ears were cleaned in running water immediately after excision. The hair from the outer region of the ears were removed with an electric hair clipper and then the skin was carefully separated from cartilage using a scalpel (Diembeck et al., 1999; Dick & Scott, 1992). Subsequently, adipose subcutaneous tissue was removed and the skin was visually examined to detect any damage. After drying it with a tissue, the skin was immediately mounted on the diffusion cells or frozen at –20°C for a maximum period of 4 weeks (Moser et al., 2001b).

The skins were placed horizontally on Franz diffusion cells, separating the cell in two compartments; donor and receptor. Sink conditions were obtained in the receptor compartment with phosphate-buffered saline (PBS; pH 7.4) containing 0.01% of $HgCl_2$ as preservative. During the experiment, receptor compartment was continuously homogenized using a stirring magnetic bar. The temperature of the skin was kept at 32°C using a water circulation system.

In order to simulate usage conditions, experiments were performed in finite dose. The skin, mounted in the cell, was allowed to rest for an hour before the application of the emulsions. Fresh receptor liquid was added to receptor compartment after replacement of the buffer. The application of the emulsions was performed as described previously (Ferreira et al., 1995). The emulsions were applied on skin (62 ± 2 mg; $n = 12$), using a Eppendorf automatic pipette, and it was evenly distributed on the skin surface with the aid of a small glass rod. The exact dose applied (54 ± 2 mg; $n = 12$) was determined by weighing the small glass rod before and after application. Serial sampling was performed after 2, 4, 6 and 8 hr through total removal of the receptor fluid and refilled with fresh solution. Receptor fluid (PBS) was directly injected into HPLC for determination of the fluconazole concentration (see below).

Evaluation of Skin Penetration/ Uptake (Topical Delivery)

After the experiment (8 hr), the excess of formulation was removed by washing the treated surface twice with 500 µL of a nonionic surfactant (polyoxyethylene-20 oleyl ether at 1% in water) and twice with 500 µL of water. It has been postulated that this nonionic surfactant does not interfere with the barrier function of the skin (Bronaugh & Stewart, 1984). Residues of the emulsions were removed with a cotton swab. Visual inspection after washing showed complete removal of the residual film formed, mainly, by the oily phase of the emulsions. The washing

solution, pipette tips and cotton swab were added to a bottle containing 50 mL of mobile phase. Fluconazole concentration in this solution was determined by HPLC. Previous experiments, carried out immediately after application of the preparations, were used to validate extraction procedure. Fluconazole recovery was 97 ± 3% ($n = 3$).

To separate the epidermis from dermis, the skin fragments were attached to a cork support with pins. The epidermis was separated from dermis with a scalpel (Surber et al., 1991; Ferreira et al., 1995). The separation of epidermis from dermis with the scalpel was relatively easy after 8 hr. The difference in the visual aspects of the two layers makes the procedure possible (epidermis is dark due to melanin while dermis is white). Validity of the procedure was confirmed by the uniformity in weights. The average weights obtained for epidermis and dermis fragments were 23 ± 2 mg and 55 ± 5 mg, respectively.

The extraction procedure of fluconazole from skin fragments was carried out according to the technique described by Faergemann (1999), with some modifications. The epidermis and dermis fragments were placed in a glass vial that contained, respectively, 2 and 4 mL of mobile phase. The mixture was rested for 12 hr and there after epidermis was grounded with a Ultra-Turrax T 25 (IKA, Staufen, Germany) homogenizer at 24,000 rpm for 2 min, whereas dermis was vortexed for 1 min. Subsequently, the samples were centrifuged at 1000 g for 5 min. After centrifugation, an aliquot of 1 mL of supernatant was transferred to a conical glass tube containing 2 mL of 1*M* HCl. The tube was vortexed and 4 mL of ethyl acetate was added. The mixture was homogenized and centrifuged at 1000 g for 3 min. The aqueous phase was transferred to a clean conical glass tube. Next, 1 mL of 1*M* NaOH and 4 mL of ethyl acetate were added and the mixture was vortexed for 2 min and then centrifuged at 1000 g for 3 min. The organic phase was transferred to a glass vial and evaporated to dryness under nitrogen flow at 45°C. The residue was dissolved in 2 mL of the mobile phase and the solutions were filtered through 0.45 μm Millipore filter. A 50 μL aliquot of this solution was injected into the chromatograph. Two methods were used to validate fluconazole extraction procedure from skin fragments. The first was carried out as described previously (Diembeck, 1999). A known amount of fluconazole in ethanol (50 μL of a solution at 10 mg/mL) was applied to the surface of skins ($n = 5$), mounted in Franz cells, for 120 min under experimental conditions. Next, the samples (epidermis, dermis, receptor fluid and surface) were extracted as described in experimental protocols (see above). Recovery results showed accuracy of 105 ± 1.6% and 92 ± 3.6% for hairless mouse skin and porcine ear skin, respectively.

High-Performance Liquid Chromatography (HPLC)

Fluconazole concentration was determined by reverse phase HPLC. A Waters chromatograph coupled with a 515 model Waters pump, 717 plus automatic injector and a 2487 model UV detector at 260 nm was used. A Lichrospher RP-18 (5 μm), 250 × 4 mm column was used with a solution of methanol/0.025*M* phosphate buffer pH 7.0 (45 : 55) as mobile phase, 1.0 mL/min flow rate and 50 μL injection volume. The retention time for fluconazole was 5.1 ± 0.2 min. The method was calibrated in the range of 2 to 32 μg/mL. Regression equation and linearity (r^2) were $y = -802.2 + 5{,}394x$ and 0.9999, respectively. Sample chromatograms of blank receptor fluid and skin matrix reveal that there are no UV absorbing endogenous substances in the same retention time as for the fluconazole. Hence, there are no interferences in the receptor fluid or skin matrix for fluconazole determination. The assay procedure has been validated to establish selectivity, accuracy (100.5 ± 2.2%, $n = 5$) for receptor fluid, precision, limits of detection and quantification. The precision was evaluated through the repeatability test compared to standard solutions. Statistical analysis showed low values of relative standard deviation (RSD < 2.0%) and, therefore, a satisfactory precision. Detection and quantification limits were 0.5 μg/mL and 2.0 μg/mL, respectively.

Statistical Analysis

Data of skin penetration (topical delivery) and permeation represent mean ± standard deviation (SD). Comparison among mean values was carried out using ANOVA. The differences were considered as statistically significant at $p < 0.05$.

RESULTS

Fluconazole solubility in various solvents/vehicles is presented in Table 2. Solubility was low in water (5.5 mg/mL) and high in PG (147 ± 1.0 mg/mL) and TCL (146 ± 5.9 mg/mL). Fluconazole solubility increased as water < PEG-8 caprylic/capric glycerides < ethanol < PG ~ TCL. Therefore, solubility in PG and TCL was similar and these two substances were selected for dissolution and incorporation of fluconazole into emulsions. In the absence of PG or TCL, fluconazole was insoluble in the emulsions (visible solid excess and presence of crystals), while, in presence of these solvents, fluconazole was completely dissolved (absence of crystals).

Fluconazole permeation across mouse skin is shown in Fig. 1. Fluconazole permeation was high and ranged from 15–65% of the applied dose. Permeation after 8 hr from an emulsion containing isopropyl myristate and propyleneglycol (356 ± 40 μg) was significantly higher than that observed for the other formulations. Fluconazole permeation from formulation C (206 ± 41 μg) was slightly higher than that from formulation B (145 ± 23 μg), nevertheless, the difference between them was not statistically significant. Formulation A presented the lowest permeation after 8 hr (82 ± 27 μg). Therefore, fluconazole permeation from formulations C and D, both containing isopropyl myristate as oily phase, was greater than that observed for formulations A and B, in which paraffin oil was used. In addition, PG, but not TCL, increased mouse skin permeation of fluconazole. Permeation from formulation B (PG) was greater than that observed with the formulation A (TCL), both containing paraffin oil as oily phase. A similar phenomenon was observed when formulation D was compared with formulation C (both containing isopropyl myristate as oily phase). When the data of skin permeation were analyzed as percentage of the applied dose (data not presented), similar conclusions were obtained.

TABLE 2 **Solubility of Fluconazole in the Tested Solvents Determined by HPLC[a]**

Solvents	Solubility (mg/mL) ± SD[b]
Water	5.5 ± 0.5
Ethanol	120 ± 3.9
Propyleneglycol (PG)	147 ± 1.0
Transcutol® (TCL)	146 ± 5.9
Labrasol®	71 ± 1.0

[a]Lichrospher RP-18 (5 μm), 250 × 4 mm column, methanol/0.025 *M* phosphate buffer pH 7.0 (45:55) mobile phase, 260 nm, 1.0 mL/min flow rate and 50 μL injection volume;
[b]sd, standard deviation.

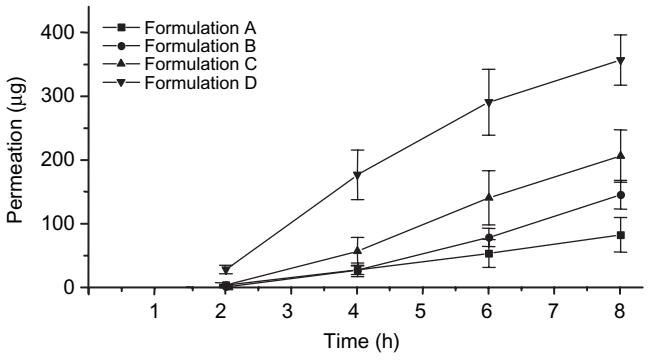

FIGURE 1 **In Vitro Skin Permeation of Fluconazole From Emulsions Across Mouse Skin. The Formulations, Containing 1% w/w of Fluconazole, Were Applied Over Biopsies of Mouse Skin Mounted in Franz Cells. Each Point Represents Average (*n* = 3) ± sd.**

Data on skin distribution of fluconazole (permeation and penetration into epidermis and dermis after 8 hr, surface and total recovery) are shown in Table 3. Total recovery of fluconazole ranged from 90–91% of the applied dose. Epidermal penetration of fluconazole from formulation B (67 ± 16 μg) was approximately double than that observed for the other formulations, which presented epidermal delivery ranging from 28 ± 6 to 37 ± 6 μg. Dermal penetration of fluconazole from the formulations B (19 ± 2 μg) and C (24 ± 11 μg) was significantly higher than that observed from the formulations A (11 ± 3 μg) and D (13 ± 3 μg). Thus, fluconazole skin uptake (epidermis and dermis) was higher from formulation B (paraffin oil + PG), whereas skin permeation was higher from formulation D (isopropyl myristate + PG) in comparison to those of the other formulations. Therefore, the emulsions B and D were selected for the experiments using pig skin, a relevant model of the human skin.

Fluconazole permeation from formulations B and D across pig skin is shown in Fig. 2. Fluconazole permeation across pig skin was lower than that observed in mice skin and ranged from 8–9% of the applied dose. Fluconazole distribution in pig skin (permeation after 8 hr, penetration into epidermis and dermis) is presented in Table 4. Fluconazole permeation after 8 hr from formulation D (48 ± 15 μg) was similar to that observed for formulation B (43 ± 6 μg). Fluconazole

TABLE 3 **Skin Penetration and Permeation of Fluconazole From Emulsions[a] Across Hairless Mice Skin**

Form.	Permeation (μg)	Penetration Epidermis	(μg) Dermis	Surface (μg)	Recovery (μg)	(%)
A	82 ± 27	29 ± 5	11 ± 3	356 ± 42	478 ± 43	90 ± 6
B	145 ± 22	67 ± 16	19 ± 2	237 ± 36	485 ± 26	90 ± 5
C	206 ± 41	37 ± 6	24 ± 11	255 ± 43	522 ± 31	90 ± 5
D	356 ± 40	28 ± 6	13 ± 3	101 ± 23	498 ± 24	91 ± 2

[a]62 ± 2 mg of the emulsions were applied on skin and it was evenly distributed with the aid of a small glass rod; exact dose applied (54 ± 2 mg) was determined by weighing the small glass rod before and after application.

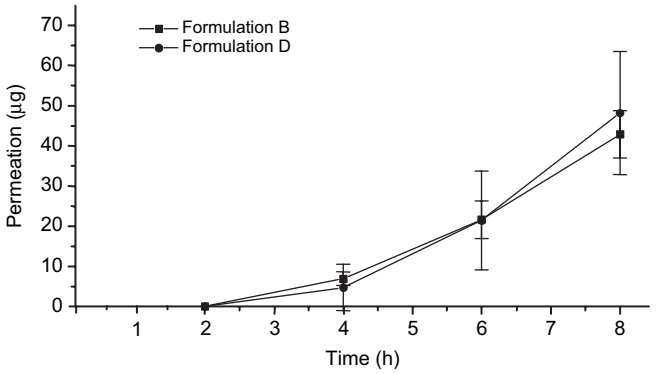

FIGURE 2 **In Vitro Skin Permeation of Fluconazole From Emulsions Across Pig Skin. The Formulations, Containing 1% w/w of Fluconazole, Were Applied Over Biopsies of Pig Skin Mounted in Franz Cells. Each Point Represents Mean (*n* = 3) ± sd.**

topical delivery (epidermal and dermal penetration) was also similar to emulsions B and D. Total recovery of fluconazole ranged from 97–98% of the applied dose (Table 4).

Epidermal (59 ± 6 μg) and dermal (58 ± 29 μg) penetration of fluconazole from formulation D in pig skin was higher than that observed in mouse skin (28 ± 6 μg and 13 ± 3 μg, respectively). This can be explained by the fact that fluconazole permeation from formulation D across mouse skin was about 60% of the applied dose, whereas permeation across pig skin was only 9%. Thus, a significant decrease of the drug concentration in the donor compartment was observed in the experiments with mouse skin. Table 5 shows the fluconazole concentrations (μg of drug/g of tissue) for formulations B and D after 8 hr in pig skin. Epidermal (2930 ± 12 μg/g) and dermal (135 ± 50 μg/g) concentrations of fluconazole from formulation D were higher than those observed from formulation B.

DISCUSSION

The treatment of superficial fungal skin infections can be topical and/or systemic. Although, in certain circumstances systemic therapy may be necessary, fungal skin infections frequently respond to topical antifungal therapy and this modality occupies a prominent position in acute lesions and in those of limited extension (Degreef & De-Doncker, 1994). Antifungal therapy with oral fluconazole is well known (Lesher, 1999), however, its potential for topical treatment of skin infections has been little investigated.

The topical formulations for the treatment of skin infections must provide proper concentrations of the drug in target site for therapeutic activity. In the case of superficial fungal skin infections, in which the main location of the pathogen is in epidermis, the drug must penetrate into the SC in proper concentrations to inhibit the fungus growth (Alberti et al., 2001).

TABLE 4 **Skin Penetration and Permeation of Fluconazole From Emulsions[a] Across Pig Ear Skin**

Form	Permeation (μg)	Penetration Epidermis	(μg) Dermis	Surface (μg)	Recovery (μg)	(%)
B	43 ± 6	56 ± 0.4	27 ± 4	401 ± 10	526 ± 11	97 ± 2
D	48 ± 15	59 ± 6	58 ± 29	375 ± 30	540 ± 14	98 ± 2

[a]62 ± 2 mg of the emulsions were applied on skin and it was evenly distributed with the aid of a small glass rod; exact dose applied (54 ± 2 mg) was determined by weighing the small glass rod before and after application.

TABLE 5 Epidermal and Dermal Concentration of Fluconazole in Pig Ear Skin

Formulations	Concentration (μg drug/g tecidue) epidermis	dermis
B	2,53 ± 18	62 ± 4
D	2,93 ± 12	135 ± 50

Thus, the aim of this study was to evaluate the skin permeation and penetration (topical delivery) of fluconazole after topical administration.

We used both mice and pig skin to evaluate fluconazole penetration and permeation. For mice skin, PG but not TCL increased permeation as well as penetration (formulations B versus A; Table 3). PG and TCL markedly increased fluconazole solubility. Considering that fluconazole solubilities in PG and TCL were similar, its solubilities in the formulations could also be expected to be similar. Thus, differences in skin penetration and permeation between these formulations across mice skin could mainly be ascribed to a skin penetration enhancer effect instead of thermodynamic activity of the drug in the vehicle.

In addition, isopropyl myristate promoted skin permeation but not penetration more than that observed from paraffin oil emulsion. The association of two skin permeation enhancers, one hydrophilic (PG) and other lipophilic (isopropyl myristate), increased fluconazole permeation across mice skin. It is well known that the association of two skin penetration enhancers promote marked increase in drug permeation (Moser et al., 2001a). However, skin penetration was higher for paraffin oil emulsion than that for isopropyl myristate emulsion. This was attributed to a significant decrease of the fluconazole concentration in donor compartment, since permeation from formulation D represented about 60% of the applied dose.

Unlike mouse skin, no statistical significant difference was found between the emulsions containing isopropyl myristate or paraffin oil in pig skin (Table 4). The effect of skin penetration enhancers depends on the vehicle and frequently these substances are applied at relatively large doses (Trottet et al., 2004). Our experiments were performed with low doses of formulations containing a low concentration of isopropyl myristate (10% w/w) Thus, the amount of penetration enhancer applied could not be sufficient to improve drug permeation in pig skin. On the other hand, it has been shown that skin penetration enhancer effect can be more easily observed in mouse skin than in pig skin (William & Barry, 1998).

The fluconazole permeation was high (15–65% of the applied dose) across mice skin and low in pig skin (8–9%). In fact, it has been demonstrated that mice skin is more permeable than human or pig skin and that differences between these species were more pronounced for hydrophilic than for lipophilic compounds (Roy et al., 1994). It has been shown that results obtained from mice skin cannot be easily extrapolated to human skin (Simon & Maibach, 2000). Thus, experiments in pig skin, a relevant model for human skin, were included.

There are several methods to determine the drug quantity in skin after permeation studies (Touitou et al., 1998). These techniques include the drug extraction from skin (epidermis and dermis), stripping or horizontal sectioning. Extraction methods represent an easy, fast and cheap option. One of the limitations of these techniques is the difficulty in validating the removal of the excess of non-absorbed formulation/drug. A removal validation carried out immediately after the application of formulations provides limited information in case of preparations containing volatile constituents. In our experiments, the complete removal of the formulation excess was evinced by the disappearance of residual oily film after the cleaning procedure. Therefore, fluconazole skin concentration could be attributed to topical delivery.

Skin distribution of fluconazole has been reported after oral administration, however, data on topical administration are poor. Fluconazole concentration (bound and unbound) in SC (73 μg/g) and epidermis-dermis biopsy (2.93 μg/g) was high after oral administration of a tablet (Faergemann & Laufen, 1993; Wildfeuer et al., 1994; Faergemann, 1999). After topical application of an infinite dose in rat skin, unbound dermal concentration (637.1 ng/mL) was higher than MIC of fluconazole against *C. albicans* (Mathy et al., 2005). In our investigations, fluconazole concentration (μg drug/g tissue) in pig epidermis, the target site for dermatomycoses, after application of a finite dose, was also high. Although our studies have evaluated different layers, epidermal (with SC) and dermal delivery, rather than SC and epidermis + dermis (without SC) as in those previous studies (Faergemann, 1999), the concentrations reached were much higher than those obtained after oral administration. Thus, our data

showed, for the first time, that skin concentrations, higher than fluconazole MIC for most dermatophytes, might be obtained after topical administration of drug from dosage forms applied at clinically relevant doses using a skin model that can be related to human skin.

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