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Study of the Effective Dose of a Topical Antifungal Agent, Omoconazole Nitrate, on the Basis of Percutaneous Pharmacokinetics in Guinea-pigs and Mice

TERUSHI HASHIGUCHI, AKIE RYU, TOSHIO ITOYAMA, KATSUHISA UCHIDA* AND HIDEYO YAMAGUCHI*

*Tosu Research Laboratories, Hisamitsu Pharmaceutical Co., Inc., 408, Tashiro, Tosu, Saga 841, and *Institute of Medical Mycology, Teikyo University, 359, Otsuka, Hachioji, Tokyo 192-03, Japan*

Abstract

The clinically useful optimum dose of omoconazole nitrate, a topical antifungal agent, has been examined by analysing the percutaneous pharmacokinetics of the drug to assess its pharmacological activity in an in-vivo study. Creams containing omoconazole nitrate were prepared on a pilot basis.

The therapeutic effect of the omoconazole nitrate creams was examined in an in-vivo pharmacological dermatophytosis infection model in guinea-pigs. Creams containing 0·25% or higher concentrations of omoconazole nitrate resulted in significant inhibition compared with no treatment and with vehicle-treated controls. In the mycological examination no growth of dermatophytes was observed for creams containing 1% or higher concentrations. In an in-vitro hairless mouse skin-permeability test a non-linear least squares program based on a fast inverse Laplace transform algorithm was used to calculate the partition and diffusion parameters of omoconazole nitrate in the stratum corneum and viable epidermis. The time-course of drug concentrations in the skin of the guinea-pig, estimated on the basis of these parameters, led to predictions that percutaneous drug concentrations on the guinea-pig would require 10 or more days to reach equilibrium in the skin; that drug concentrations in the corneum-viable epidermis border, where dermatophytes are considered to grow, would exceed the minimum effective concentration when 0·1% or higher concentration creams were used; and that for binding to keratin drug concentrations would reach the practical minimum effective concentration when creams containing 0·5% or more omoconazole nitrate were used.

These results show that partition and diffusion parameters obtained from in-vitro skin permeation studies can be used to predict in-vivo percutaneous pharmacokinetics and to estimate therapeutically effective concentrations.

Topical antifungal agents are usually highly lipophilic, and accumulate in the skin (Lüker et al 1984; Uchida & Yamaguchi 1993; Tomura & Takahashi 1995), which blocks their transfer into the body almost completely (Schaefer & Stüttgen 1976; Patzschke et al 1983). Therefore, topical application is considered as an excellent method of application of these drugs from the viewpoint of safety. However, such administration easily leads to overdose, provoking skin irritation and other adverse reactions.

The therapeutic efficacy of an antifungal agent depends on its local concentration at the infected site. Therapeutic doses of the drug should be established on the basis of the effective local drug concentration which can be achieved after administration.

Omoconazole nitrate is used as a topical antifungal agent in Europe (Zirngibl et al 1988). To estimate the required concentration of omoconazole nitrate in the skin, we have studied the pharmacokinetics of the topically applied drug, and its therapeutic efficacy.

Materials and Methods

Materials

Omoconazole nitrate, an imidazole-derived topical antifungal agent (Fig. 1) developed by Siegfried Pharma (Zofingen, Switzerland) was provided by that company. Liquid paraffin, isopropyl myristate, polyglycerine fatty acid ester and polyoxyethylene sorbitan monooleate (tween 80) were obtained from Nikko Chemicals (Tokyo, Japan). Sabouraud dextrose medium was purchased from Nissui Pharm (Tokyo, Japan). Cycloheximide, mezlocillin and sisomicin were purchased from Wako Pure Chemical (Osaka, Japan). Acetonitrile used was of HPLC grade and other reagents were of analytical grade.

Female hairless mice (Hr-/Kud) and female Hartley guinea-pigs were obtained from Kyudo (Tosu, Japan).

Trichophyton mentagrophytes TIMM1189, originally isolated from a clinical specimen and preserved in Teikyo University Institute of Medical Mycology (Hachioji, Japan), was used to infect guinea-pigs.

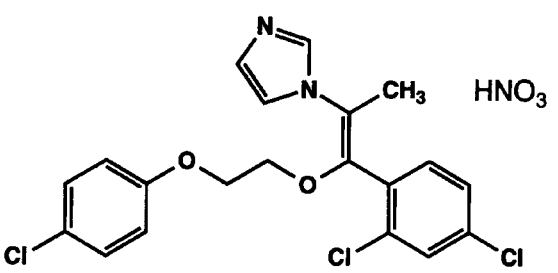

FIG. 1. The structure of omoconazole nitrate.

Correspondence: T. Hashiguchi, Tosu Research Laboratories, Hisamitsu Pharmaceutical Co., Inc., 408, Tashiro, Tosu, Saga 841, Japan.

Preparation of cream

Liquid paraffin and isopropyl myristate were mixed with omoconazole nitrate. Glycerine and water were then added to the mixture and polyglycerine fatty acid ester was further used as an emulsifier to formulate the preparations as water-in-oil creams; creams were prepared containing 0·25, 0·5, 1·0 and 2·0% omoconazole nitrate.

Evaluation of therapeutic efficacy

Therapeutic efficacy was performed by the method of Fujita (1992). The backs of female Hartley guinea-pigs, 300–350 g, were shaved with electric clippers. Adhesive tape was pressed on to two 5 cm^2 shaved areas on each animal and then pulled off vigorously to depilate the skin completely and to abrade its upper horny layer. Each of the regions was inoculated with a conidial suspension of *T. mentagrophytes* (25 μL containing 1×10^6 cells). Once-a-day topical application of omoconazole nitrate cream was commenced on day 5 post-infection and continued for 14 consecutive days. Cream preparations containing a daily dose of 0·3 g were applied uniformly using sterilized spatulas to the entire infected regions of the skin. Their therapeutic effect was evaluated by scoring at the site of infection. A four-score evaluation criterion was used, the score being 0 when no symptoms, e.g. redness, were noted at the infection site, and scores of +1 to +4 depending on the severity of the symptoms. Two days after the last treatment all animals were killed under ether anaesthesia and the skin of the infected sites was excised and cut into 10 small blocks. Subsequently, all blocks were implanted on to a Sabouraud dextrose agar plate containing 500 μg mL^{-1} cycloheximide, 50 μg mL^{-1} mezlocillin and 50 μg mL^{-1} sisomicin, and incubated at 27°C for 10 days. Skin blocks yielding fungal growth were regarded as culture-positive, and infected sites with more than one culture-positive skin block were considered fungus-positive. In addition, the intensity of infection was given a score of 0 to 10 according to the corresponding number of culture-positive skin blocks among the ten skin blocks studied.

In-vitro skin-permeability test

The back skin of 7-week-old female hairless mice was excised immediately before the permeation study and was mounted between donor and receptor cells in a diffusion cell (Loftsson & Bodor 1981) with a receptor volume of 5 cm^3 and effective diffusion area of 0·785 cm^2. The stripped-skin pieces were prepared by stripping the excised skin 20 times with Scotch tape (3M, Tokyo, Japan). Phosphate buffer (0·05 M, pH 7·4) containing 3% tween 80 and 0·025% sodium alginate, was used as the receptor phase, and 1·0% omoconazole nitrate cream was applied on the corneum side of the murine skin in the donor phase. The receptor phase was perfused at a rate of 0·75 mL h^{-1}, at 32°C. The perfusate was collected at intervals and the drug content assayed by HPLC.

Analytical method

HPLC analysis of omoconazole nitrate was performed with a model LC-10A chromatograph (Shimadzu, Kyoto, Japan); the detection wavelength was 254 nm. A 150 mm × 4·6 mm i.d. ODS-120T column (Toso, Tokyo, Japan) was used for the analysis and a 1:1 (v/v) mixture of acetonitrile and 2% aqueous acetic acid containing 5 mM sodium heptanoate was used as the mobile phase. The flow rate was 1·0 mL min^{-1} and the temperature 40°C. The retention time of omoconazole nitrate was approximately 14·6 min and the standard calibration curve was linear over the concentration range 1-100 μg mL^{-1} of the solution.

Analysis of data from in-vitro pharmacokinetic studies

The pharmacokinetics of omoconazole nitrate in the hairless mouse skin was analysed by the MULTI (FILT)-based two-membrane (corneum and viable epidermis) model at infinite dose on the basis of the data on cumulative amount permeating the skin in an in-vitro excised hairless mouse skin study (Yamashita et al 1993). By Fick's second law of diffusion, the solution in the dimension of Laplace is given by:

$$Q = \frac{P_3P_4C_0}{s(P_4D_1\sinh D_1\cosh D_2 + P_3D_2\cosh D_1\sinh D_2)} \quad (1)$$

where $D_1=(sP_1^{-1})^{1/2}$, $D_2=(sP_2^{-1})^{1/2}$, Q is the cumulative amount of drug, C_0 is the initial drug concentration in the preparation, and the diffusion parameters P_1 and P_2 and the partition parameters P_3 and P_4 are:

$$P_1 = D_S/L_S^2,\ P_2 = D_d/L_d^2,\ P_3 = K_SL_S \text{ and } P_4 = K_dL_d \quad (2)$$

where D_S and D_d are diffusion coefficients in the stratum corneum and viable epidermis, L_S and L_d are the thickness of the stratum corneum and viable epidermis, and K_S and K_d are, respectively, the partition coefficients between the preparation and the corneum layer and between the preparation and the viable epidermis. The partition and diffusion parameters obtained were used to correct for the thickness of the guinea-pig skin, and the time-course of drug concentrations in the guinea-pig skin were thus simulated.

Results

Therapeutic effects on in-vivo tinea model in guinea-pigs

The dose-dependent therapeutic effects observed are shown in Fig. 2. Lesional symptoms started to be reduced significantly on days 8 to 10 post-infection when treated with 0·25–2% omoconazole nitrate creams. Almost complete symptomatological cure was achieved by treatment with 1 or 2% creams by the end of the 14-day experiment. The results of mycological

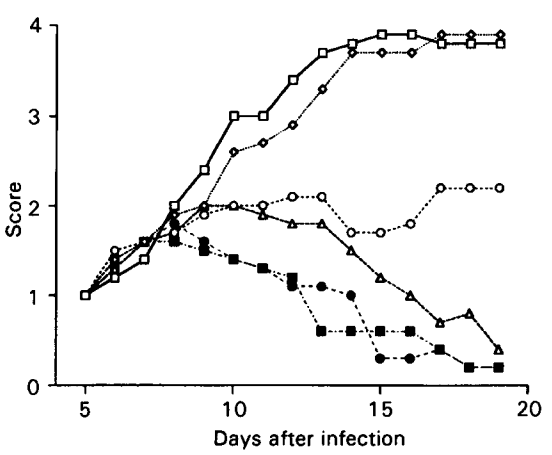

FIG. 2. Therapeutic activity of cream containing different concentrations of omoconazole nitrate. Each value is the mean of ten examinations: □, no treatment; ◇, 0%; ○, 0·25%; △, 0·5%; ●, 1·0%; ■, 2·0%.

Table 1. Therapeutic activity, measured in culture studies, of cream containing different concentrations of omoconazole nitrate.

Treatment	Number of positive blocks
No treatment	9·9 ± 0·1
0% omoconazole nitrate	9·0 ± 0·8
0·25% omoconazole nitrate	8·3 ± 0·8
0·5% omoconazole nitrate	1·5 ± 0·9*
1·0% omoconazole nitrate	0*
2·0% omoconazole nitrate	0*

Each value is the mean ± s.d. *$P < 0·01$, significantly different (U-test) from result for 0%.

examination of the infected sites performed on day 14 post-infection are shown in Table 1. The animals treated with cream containing 0·5% omoconazole nitrate yielded positive fungal cultures, although the number of fungi recovered was significantly lower than that of vehicle-treated animals. On the other hand, all animals treated with creams containing 1 or 2% omoconazole nitrate were culture-negative, suggesting that when these active creams were applied, the drug concentrations at the infected sites in the stratum corneum reach a level sufficient to inhibit fungal growth completely.

Simulation of pharmacokinetics in the guinea-pig skin

The cream preparation containing 1% omoconazole nitrate was used in the in-vitro hairless mouse skin permeability test. For stripped skin the amount permeating was almost twice that for intact skin (Fig. 3). Percutaneous drug concentrations were analysed on the basis of this permeated amount using the two-membrane model at infinite dose. It was demonstrated that omoconazole nitrate easily permeated into the corneum layer of the skin, with a very low diffusion coefficient in this layer and low distribution from the corneum layer into the viable lower layers, i.e. omoconazole nitrate was transferred very slowly into the skin tissue from the surface (Table 2). The time-course of drug concentrations in the guinea-pig skin were simulated by correcting the partition and diffusion coefficients obtained in the corneum and viable lower layers for the thickness of the guinea-pig skin. The partition and diffusion coefficients obtained in the excised hairless mouse skin were presumed not to be significantly different from those for the guinea-pig skin, because the mouse and the guinea-pig are taxonomically related animals and the structure and chemical composition of the skins of these two species of rodent are similar. On the basis of this assumption, simulation was conducted by considering that the thicknesses of the corneum layer and viable epidermis layers in guinea-pigs are 18·6 and 20·8 μm, respectively (Hayashi et al 1991). The results of the simulation study using the corrected absorption parameter are shown in Fig. 4. In guinea-pigs omoconazole nitrate was, in the early stages, retained in the corneum layer at a high concentration relative to that in the viable epidermis. Omoconazole nitrate was then gradually transferred into the viable epidermis layer, the concentration gradient requiring more than 10 days to reach equilibrium. Because drug concentrations at the corneum-viable epidermis border were considered to be critical for therapeutic efficacy, the time-course of omoconazole nitrate concentrations in this area was simulated for several creams as shown in Fig. 5. The minimum effective concentration of omoconazole nitrate required for action against *T. mentagrophytes* is approximately 10–40 μg mL^{-1} (Itoyama et al 1993). Therefore, it was estimated that the 0·1% cream would require consecutive application for ten or more days for drug concentrations in the corneum-viable epidermis border to reach the minimum effective concentration.

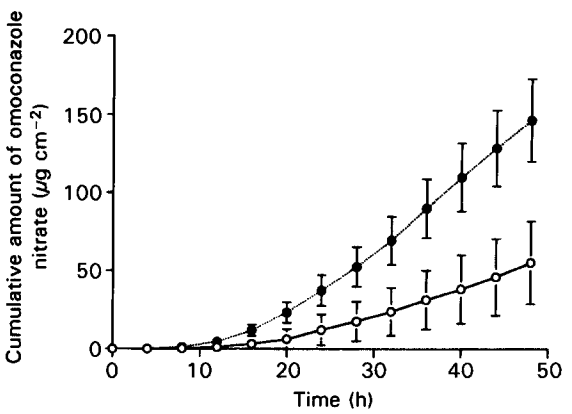

FIG. 3. Percutaneous absorption profiles, through intact and stripped hairless mouse skin, for cream containing 1% omoconazole nitrate. Each value is the mean from three determinations. ○, Intact skin; ●, stripped skin.

Discussion

It is thought that binding to keratin is concerned in the accumulation of antifungal agents in the skin. The antifungal activity decreases because of the presence of keratin protein (Freedman et al 1962; Arika et al 1990; Aljagre et al 1991; Niwano et al 1996). When we performed the binding experiment between omoconazole nitrate and keratin, the binding ratio of omoconazole nitrate to keratin was found to be ~80%.

Table 2. Partition and diffusion parameters for omoconazole nitrate in excised hairless mouse skin.

Parameter	Cream containing 1% omoconazole nitrate
Partition coefficient between the preparation and the corneum layer	26·9
Diffusion coefficient in the stratum corneum ($cm^2\ h^{-1}$)	$9·52 \times 10^{-9}$
Partition coefficient between the preparation and the viable epidermis divided by the partition coefficient between the preparation and the corneum layer	0·089
Diffusion coefficient in the viable epidermis ($cm^2\ h^{-1}$)	$1·13 \times 10^{-5}$

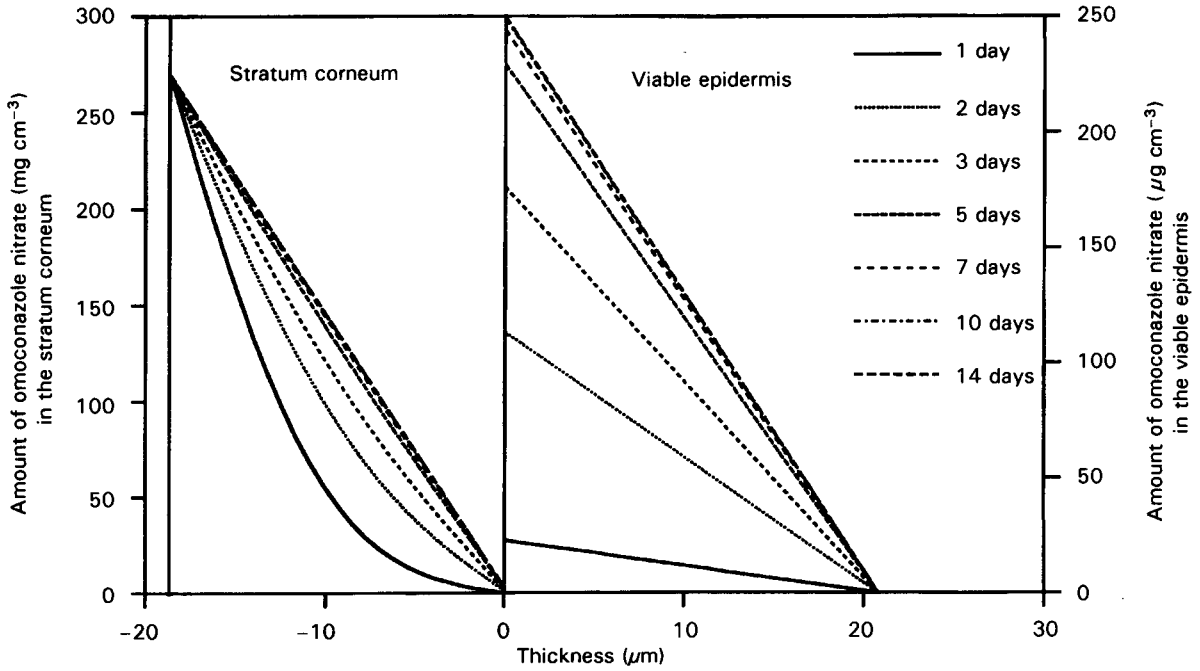

FIG. 4. Simulation of omoconazole nitrate concentration in the guinea-pig skin when applied as 1·0% cream. Each line was calculated by use of partition and diffusion parameters obtained from in-vitro permeation studies of excised hairless mouse skin.

Taking this into account, the practical minimum effective concentration of omoconazole nitrate required in the border would be 50–200 g cm^{-3}. Consequently, from the results, it was supposed that the 0·5% or higher concentration preparations were required to ensure therapeutic effect.

The symptomatological therapeutic effects and mycological examination of experimental dermatophytosis in guinea-pigs confirmed that the therapeutic effect of the 1 and 2% creams was satisfactory, revealing that these results are almost consistent with those from simulation of percutaneous drug concentrations.

The target site and the effective pharmacological concentration as manifested by the practical minimum effective concentration, which must take into account binding of drug to the skin, are clearly obvious for the topical antifungal agents used in dermatological therapy. Therefore, prediction of in-vivo percutaneous pharmacokinetics on the basis of partition and diffusion parameters obtained in the in-vitro skin permeation study was considered useful in estimating the therapeutically effective concentration of the preparation.

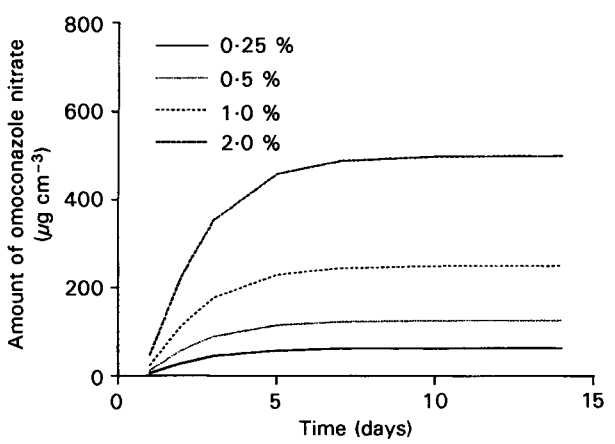

FIG. 5. Simulation of omoconazole nitrate concentration in the stratum corneum-viable epidermis border. Each line was calculated by use of partition and diffusion parameters obtained from in-vitro permeation studies of excised hairless mouse skin.

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