JOURNAL OF

Pharmacy and Pharmacology

VOLUME 51

NUMBER 3

MARCH 1999

ISSN 0022-3573

Royal Pharmaceutical Society of Great Britain

Journal of Pharmacy and Pharmacology

Published by The Royal Pharmaceutical Society of Great Britain
1 Lambeth High Street, London SE1 7JN Telephone 0171-735 9141 FAX 0171-820 3917
E-Mail jpp@rpsgb.org.uk http://dspace.dial.pipex.com/jpp/
Editorial Office for Japan: Department of Pharmaceutics, Kanazawa University, Takara-machi 13-1, Kanazawa 920

CODE: 0022-3573/90.

Annual joint subscriptions (1999) to Journal of Pharmacy and Pharmacology and Pharmacy and Pharmacology Communications: UK £450; Europe £470; America and Japan $790; rest of world £490. Single issues: UK £30; Europe and rest of world £35; America and Japan $56.

All orders should be sent to: The Pharmaceutical Press, PO Box 151, Wallingford, Oxon OX10 8QU, UK. Telephone +44 (0)1491 829 272; FAX +44 (0)1491 829 292; E-Mail rpsgb@cabi.org

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J. Pharm. Pharmacol. 1999, 51: 271–278
Received August 25, 1998
Accepted November 2, 1998

Drug Permeation through the Three Layers of the Human Nail Plate

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Abstract

The in-vitro permeation characteristics of a water soluble model drug, 5-fluorouracil, and a poorly water soluble model drug, flurbiprofen, were investigated through three layers of the human nail plate (namely, the dorsal, intermediate and ventral nail plates), using a modified side-by-side diffusion cell. The dorsal-filed nail plate, the ventral-filed nail plate and the dorsal-and-ventral-filed nail plate were prepared to known thicknesses and then used with the full-thickness nail plate to investigate the permeation characteristics of each single layer.

Most of the lipids in the human nail plate were found in the dorsal and ventral layers. The rank orders of the permeation fluxes for 5-fluorouracil and flurbiprofen were both: dorsal-and-ventral-filed nail plate > dorsal-filed nail plate > ventral-filed nail plate > full-thickness nail plate. With respect to 5-fluorouracil permeation through each single layer, the permeability coefficient of the intermediate layer was higher than those of other single layers. However in the case of flurbiprofen, the permeability coefficient of the ventral layer was higher than other single layers. The diffusion coefficients of 5-fluorouracil and flurbiprofen in the dorsal layer were the lowest of any single layer. The drug concentration in each layer was estimated using each respective permeation parameter. The drug concentration in the nail plate was observed to be dependent on the solubility and the flux of the drug.

From these findings, we suggest that the human nail plate behaves like a hydrophilic gel membrane rather than a lipophilic partition membrane and that the upper layer functions as the main nail barrier to drug permeation through its low diffusivity against the drugs.

Although the human nail plate is generally considered to be composed of either one layer or three layers, the three-layer model is more widely accepted (Spearman 1978; Dawber 1980). The upper, the middle and the lower layers of the human nail plate are called the dorsal, intermediate and ventral nail plates, respectively (Figure 1). In addition, the tissue under the nail plate, called the nail bed, consists of viable epidermis. Information regarding the distribution of fungi in the human nail plate is required for the treatment of onychomycosis trichophytica. It has been reported that the dorsal nail plate, the ventral nail plate, the subungual keratin and the eponychium are infected by fungi such as *Trichophyton rubrum* and *Trichophyton mentagrophytes* (Sagher 1948; Jillson & Piper 1957; Akiba 1971). Of these nail components, it is the ventral nail plate and the subungual keratin that are found to be infected in most cases. Therefore, it is of great importance to understand the specific properties of each layer in the human nail plate.

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Onychomycosis trichophytica has been treated mainly with oral antifungal medication (Piepponen et al 1992; Villars & Jones 1992). With systemic treatment, an antifungal drug may be delivered from the blood in the dermis under the nail bed and the nail matrix to the ventral nail plate and subungual keratin (Matthieu et al 1991). On the other hand, antifungal drugs are not delivered to the ventral nail plate and the subungual keratin following topical treatment because of low nail-plate permeability. Each layer has unique physical

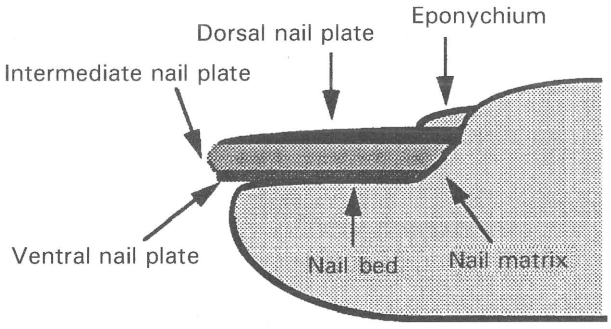

Figure 1. Schematic diagram of the human nail plate.

properties and, therefore, drug permeabilities through each layer may be different. Only a few in-vitro drug permeation studies have been performed on the human nail plate. Mertin & Lippold (1997a) reported that the human nail plate and the keratin membrane from bovine hooves (the model membrane of the human nail plate), behave like hydrophilic gel membranes. Walters et al (1983, 1985) also suggested that the human nail plate behaves like a hydrophilic gel membrane. Furthermore, they suggested that it has an additional lipophilic route for permeation.

In the present study, the distribution of lipids in the human nail plate was examined in detail. In order to investigate the nail permeability of a water soluble model drug, 5-fluorouracil, and a poorly soluble model drug, flurbiprofen, a modified side-by-side diffusion cell was used. The drug permeation characteristics of each single layer were investigated by filing upper or lower layers, or both, of the human nail plate. 5-Fluorouracil and flurbiprofen permeations, from the ventral to the dorsal nail plate, were compared with those from the dorsal to the ventral nail plate. The amounts of 5-fluorouracil and flurbiprofen in the nail plate were also compared with those calculated from the permeation parameters of each drug.

Materials and Methods

Materials

5-Fluorouracil was obtained from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). Flurbiprofen was supplied by Kaken Pharmaceutical Co., Ltd (Chiba, Japan). Triolein (practical grade), cholesterol and vaniline (for measuring total lipid) and Sudan Black B (for lipid staining) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Other reagents were obtained from commercial sources.

Preparation of nail plates

Tip nail pieces were obtained from the fingers of healthy volunteers using nail clippers. A lot of nail pieces, clipped from the same volunteer (male, aged 24 years) were used in the permeation study and for microscopic observation. Forty nail pieces obtained from other healthy volunteers (10 males, mean age 24 years) were used in the measurement of total lipid. The thicknesses of nail pieces were measured with a micrometer (Mitutoyo Corp., Japan) equipped with pointed metal attachments. The thickness ratio of each layer of the human nail plate (dorsal : intermediate : ventral) was assessed to be 3 : 5 : 2 after examining several reports (Jillson & Piper 1957; Spearman 1978; Stüttgen & Bauer 1982). The dorsal-filed nail plate, the ventral-filed nail plate and the dorsal- and ventral-filed nail plate were prepared to known thicknesses with sand paper.

Determination of solubilities and nail plate/vehicle partition coefficients of drugs

5-Fluorouracil and flurbiprofen aqueous suspensions were mixed with a magnetic stirrer at 37°C. After 24 h, each suspension was subjected to filtration (Ekicrodisc 3; German Sciences Japan, Ltd, Tokyo). The filtrate was immediately diluted with distilled water or methanol to obtain samples for analysis.

The finger nail pieces (full-thickness nail plate, ventral-filed nail plate, dorsal-filed nail plate and the dorsal-and-ventral-filed nail plate) were weighed with an electronic balance (JL-200, Chyo Balance Corp.). They were immersed in half-concentration solutions (1 mL) of 5-fluorouracil or flurbiprofen solubility at 37°C for 48 h. After removal of the nail piece from each solution, the concentration of the solution was measured. The drugs in the nail pieces were extracted with methanol (10 mL) three times. The extracted solvent was evaporated under nitrogen gas at 60°C. Subsequently, 1 mL of distilled water or methanol was added to obtain samples for analysis. We assumed that the weight (volume) ratio of each layer of the nail plate was 3 : 5 : 2. The apparent partition coefficient of the drugs was calculated as the concentration ratio in the nail plate/vehicle at 37°C.

Permeation studies

To overcome large variations in nail permeabilities due to individual differences in barrier properties, nail pieces (20–35 mg) from the third finger and the fifth finger of the same volunteer were used in

the permeation studies of 5-fluorouracil and flurbiprofen, respectively. The full-thickness nail plate, the dorsal-filed nail plate, the ventral-filed nail plate and the dorsal-and-ventral-filed nail plate were sandwiched between 2 adapters made of polypropylene with an O-shaped ring (effective diffusion area, 0·049 cm^2) and mounted in a side-by-side diffusion cell (2·5 mL or 1·5 mL) with a water jacket connected to a water bath at 37°C. 5-Fluorouracil- or flurbiprofen-suspension was applied to the dorsal nail plate side of the diffusion cell, and the ventral nail side was filled with distilled water or 40% polyethylene glycol 400 (to maintain the sink condition). In the case of vehicle application to the ventral nail plate side, 5-fluorouracil- or flurbiprofen-suspension was placed in the ventral nail plate side compartment, and the dorsal nail plate side was filled with distilled water or 40% polyethylene glycol 400. No preservative was added because the receiver solution was clear even at the end of the experiment. The permeation was measured by sampling the receiver side solution at predetermined times. The experimental period was 7 days (5-fluorouracil) or 19 days (flurbiprofen) because of the low nail permeability of drugs.

Measurement of total lipid

Total lipid was measured according to the method of Knight et al (1972). After nail pieces from the fingers of healthy volunteers were prepared into full-thickness, ventral-filed, dorsal-filed and dorsal-and-ventral-filed layers, they were immersed in a chloroform–methanol (3 : 1) mixture for 24 h to extract lipids. The extracted solvent was evaporated under nitrogen gas at 50°C. Sulphuric acid (300 μL) was added to the sample and heated in boiling water for 5 min. After cooling to room temperature, 2 mL of phosphovaniline test solution (0·6% w/v aqueous vaniline solution/phosphoric acid; 1 : 4) was added and the mixture was incubated for 15 min at 37°C. Then, the absorbance was measured at 535 nm using a spectrophotometer (UV-160A, Shimadzu Seisakusho, Kyoto, Japan). A mixture of cholesterol and triolein (3 : 1) was used as a reference standard.

Observation of lipid distribution

A nail piece from the first finger of a healthy volunteer was used to observe nail plate lipid distribution. After the nail plate was frozen in an embedding medium (Tissue-Tek; Sakura Finetechnical Co., Ltd, Tokyo), it was sliced with a microtome (IEC Model Minotome Microtome Cryostat; International Equipment Company). A section of this nail plate was stained with Sudan Black B in isopropyl alcohol (5 mg mL^{-1}) for 30 min. After being washed with 50% methanol for about 30 s, it was observed under a microscope (New VANOX, Olympus, Tokyo).

Analytical method

5-Fluorouracil and flurbiprofen levels were determined by high-performance liquid chromatography (HPLC). Sample solutions were injected into the HPLC instrument, which was composed of a pump system (LC-10A, Shimadzu Seisakusho, Kyoto, Japan), a UV detector (SPD-10A, Shimadzu), a fluorescence detector (RF-10AXL, Shimadzu), a Chromatopack (C-R5A, Shimadzu), a system controller (SCL-10A, Shimadzu), an auto injector (SIL-10A, Shimadzu) and a reverse phase column (Inertsil ODS 250 mm×4·6 mm i.d., GL Sciences Inc., Tokyo). The mobile phase for flurbiprofen was 0·1% phosphoric acid/acetonitrile (40 : 60), the flow rate was 1 mL min^{-1} and fluorescence detection was conducted at an excitation wavelength of 260 nm and an emission wavelength of 313 nm. The mobile phase for 5-fluorouracil was 0·1% phosphoric acid/acetonitrile (98 : 2), the flow rate was 1 mL min^{-1}, and UV detection was conducted at a wavelength of 270 nm.

Results and Discussion

Lipid distribution in the nail plate

The lipid content in the human nail plate was found to be much lower than that in the stratum corneum of skin (Walters & Flynn 1983). However, various lipids such as long-chain fatty acids, free fats, cholesterol, squalene and phospholipids are present in the human nail plate (Spearman 1978; Hirose et al 1990). It is thought that the lipid distribution and concentration in the human nail plate may affect the drug permeation, particularly the partition of drugs into the membrane.

Table 1 shows the total-lipid concentration (C_L) in the ventral-filed, dorsal-filed, dorsal-and-ventral-filed and full-thickness layers. The ventral-filed and dorsal-filed layers had a high total-lipid concentration; about 1·5 and 3·0 times those observed in the dorsal-and-ventral-filed layer. The total-lipid concentrations of each single layer were calculated from the experimental data of the ventral-filed, dorsal-filed and dorsal-and-ventral-filed layers (Table 1). The total-lipid concentration in the ventral layer was the highest in the human nail plate. In contrast, the intermediate layer had a low

Table 1. Experimental (a) and calculated lipid (b) concentration in the nail plate.

Experimental Layer (thickness ratio)	Ventral-filed (3 : 5)	Dorsal-filed (5 : 2)	Dorsal-and-ventral-filed (5)	Full-thickness (3 : 5 : 2)
C_L ($\mu g\,mg^{-1}$)	4·33 ± 0·45	8·76 ± 1·15	2·83 ± 0·28	7·58 ± 1·20
Calculated Layer (thickness ratio)	Dorsal (3)	Intermediate (5)	Ventral (2)	Full-thickness (3 : 5 : 2)
C_L ($\mu g\,mg^{-1}$)	6·82	2·83	23·6	8·50

C_L = total lipid concentration. Each value in Table 1 represents the mean ± s.e.m. (n = 10).

total-lipid concentration. The rank order of the total lipid concentration in each single layer was ventral layer > dorsal layer > intermediate layer. The total lipid concentration in the full-thickness nail plate (Table 1) was calculated using those of each single layer. This lipid concentration was very similar to the value for the full-thickness nail plate obtained from the experiment.

Figure 2 shows a micrograph of a human nail plate cross section. The lipids in the nail plate were stained with Sudan Black B. Sudan Black B can stain most lipids, such as neutral fats and phospholipids. Most lipids were observed in the upper and lower parts of the human nail plate. Moreover, uneven parts of the ventral side contained a lot of lipids, whereas no lipids were observed in the intermediate part of the nail plate.

From these results, we suggest that the dorsal and ventral layers in the human nail plate contain some lipids, whereas the intermediate layer, which is the main nail body of the human nail plate, contains few lipids.

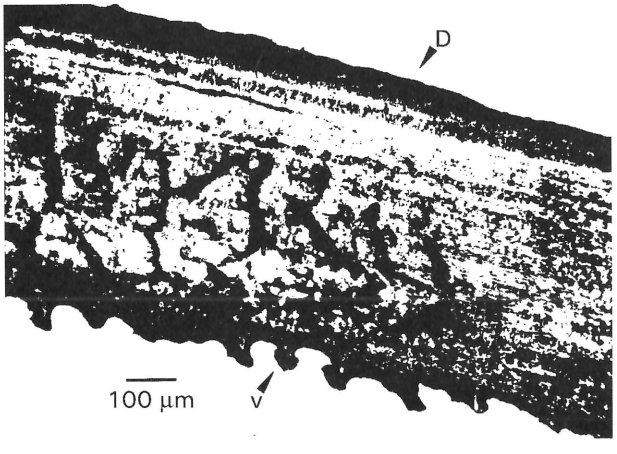

Figure 2. Micrograph of a human nail plate. D: dorsal nail plate side. V: ventral nail plate side. This nail plate section was stained with Sudan Black B.

Permeation parameters of each layer

The data analysis is based on Fick's first law:

$$dQ/dt = D_m \Delta C/h \quad (1)$$

where dQ/dt is the steady state permeation rate, D_m is the diffusion coefficient of the drug in the membrane, ΔC is the concentration differential of the drug in the membrane, and h is the membrane thickness. In the case of a sink condition, ΔC in Equation 1 can be replaced with the product of the solubility of the drug in the donor solvent (C_V) and the membrane/donor vehicle partition coefficient of the drug (K_m):

$$dQ/dt = D_m K_m C_V/h \quad (2)$$

The permeability coefficient of the drug (P) is given by:

$$P = D_m K_m/h \quad (3)$$

Figure 3 shows the permeation profiles of 5-fluorouracil and flurbiprofen through the ventral-filed, dorsal-filed, dorsal-and-ventral-filed and full-thickness nail layers. The rank orders for the 5-fluorouracil and flurbiprofen fluxes were both: dorsal-and-ventral-filed layer > dorsal-filed layer > ventral-filed layer > full-thickness layer. The lag times for 5-fluorouracil and flurbiprofen permeations through full-thickness nail plates were about 2·5 and 11 days, respectively. Mertin & Lippold (1997a) also suggested that steady-state permeations of nicotinic acid esters through human nail plates were obtained after 10–80 h. In addition, they reported that steady-state permeation of chloramphenicol, from an aqueous suspension or a nail lacquer, through the human nail plate occurred after a lag time of 200 h and 400 h, respectively (Mertin & Lippold 1997b).

Table 2 shows the 5-fluorouracil and flurbiprofen permeation parameters of the ventral-filed, dorsal-filed, dorsal-and-ventral-filed and full-thickness nail layers. The apparent permeability coefficients of each drug through these nail plates were calcu-

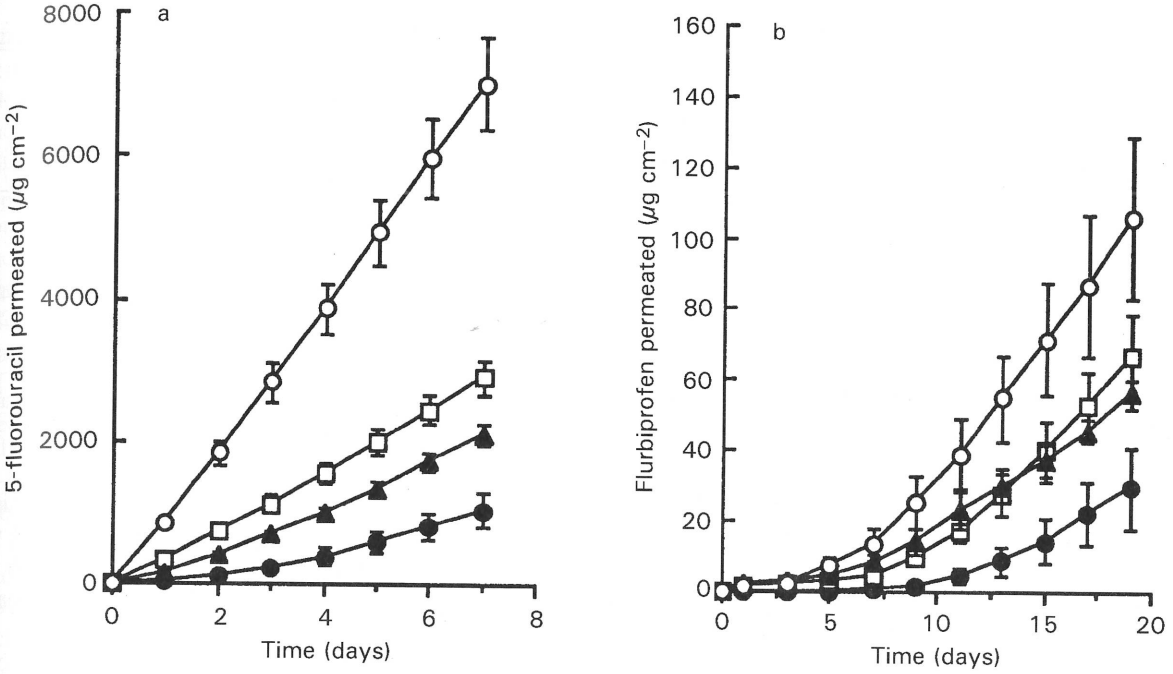

Figure 3. Permeation profiles of 5-fluorouracil (a) and flurbiprofen (b) through ventral-filed (▲), dorsal-filed (□), dorsal-and-ventral-filed (○) and full-thickness (●) nail layers. Each value represents the mean ± s.e.m. (n = 3–5).

lated using Equations 2 and 3. In all permeation studies, C_V values are given in saturated concentrations of 5-fluorouracil (17·1 mg mL^{-1}) and flurbiprofen (27·7 μg mL^{-1}) at 37°C, because donor vehicles are aqueous suspensions. Although the ventral-filed nail layer had approximately the same 5-fluorouracil permeability coefficient as the dorsal-filed layer, the dorsal-and-ventral-filed layer had a higher permeability coefficient than those layers. On the other hand, the flurbiprofen permeability coefficient of the dorsal-and-ventral-filed layer was also higher than those of the ventral-filed and dorsal-filed layers. The nail plate/vehicle partition coefficients of each drug were calculated as concentration ratios in the ventral-filed, dorsal-filed, dorsal-and-ventral-filed and full-thickness layers/water at 37°C, respectively. The nail plate/vehicle partition coefficients of 5-fluorouracil and flurbiprofen were not very different between the ventral-filed, dorsal-filed and dorsal-and-ventral-filed layers. The diffusion coefficients of each drug in the ventral-filed, dorsal-filed, dorsal-and-ventral-filed and full-thickness nail layers were calculated using Equation 3. The diffusion coefficients of 5-fluorouracil and flurbiprofen in the ventral-filed layer were lower than those in the dorsal-filed and dorsal-and-ventral-filed layers.

The permeation parameters of each single layer (dorsal, intermediate or ventral) were computed using those of the ventral-filed, dorsal-filed and dorsal-and-ventral-filed layers. Flynn et al (1974) proposed that the diffusional resistance, R_i, in the ith layer can be defined by:

$$R = 1/P_i = h_i/(D_iK_i) \quad (4)$$

In multiple layers, the total diffusional resistance (R_T) may be computed by:

$$R_T = 1/P_T = h_1/(D_1K_1) + h_2/(D_2K_2) + \ldots + h_n/(D_nK_n) \quad (5)$$

Table 3 shows the permeation parameters of 5-fluorouracil and flurbiprofen for each single nail layer. The permeability coefficient of each single layer was calculated using Equation 5. For 5-fluorouracil, the intermediate layer had a high permeability coefficient compared with the other single layers. In contrast, the ventral layer had a high permeability coefficient for flurbiprofen. The permeability coefficients for 5-fluorouracil and flurbiprofen through the dorsal layer were low. The nail plate/vehicle partition coefficients of the drugs for each single layer were calculated using those of the ventral-filed, dorsal-filed and dorsal-and-

Table 2. Permeation parameters of 5-fluorouracil and flurbiprofen through the human nail plate.

Drug Parameter	Layer (thickness ratio)			
	Ventral-filed (3 : 5)	Dorsal-filed (5 : 2)	Dorsal-and-ventral-filed (5)	Full-thickness (3 : 5 : 2)
5-Fluorouracil				
h (μm)	396.0	346·5	247·5	495·0
P ($\times 10^7$ cm s^{-1})	2·51 ± 0·18	3·07 ± 0·22	7·12 ± 0·65	1·49 ± 0·27
Km	0·53 ± 0·04	0·49 ± 0·01	0·56 ± 0·02	0·54 ± 0·06
Dm ($\times 10^8$ cm^2 s^{-1})	1·87	2·18	3·13	1·37
Flurbiprofen				
h (μm)	347·2	303·8	217·0	434·0
P ($\times 10^6$ cm s^{-1})	1·83 ± 0·11	2·76 ± 0·43	3·59 ± 1·28	1·45 ± 0·54
Km	1·07 ± 0·15	1·13 ± 0·24	0·98 ± 0·22	1·47 ± 0·32
Dm ($\times 10^8$ cm^2 s^{-1})	5·92	7·42	7·96	4·29

Each value represents the mean ± s.e.m. (n = 3–5). Apparent permeability coefficients were calculated using Equations 2 and 3. h is the nail membrane thickness; P is the permeability coefficient; Km is the membrane/donor vehicle partition coefficient of the drug; Dm is the diffusion coefficient of the drug in the nail membrane.

ventral-filed nail layers and the weight ratio of each layer (3 : 5 : 2). The partition coefficient for 5-fluorouracil in the ventral layer was lower than those in the dorsal and intermediate layers. The rank order of the partition coefficients for 5-fluorouracil was intermediate layer > dorsal layer > ventral layer, whereas the rank order of the flurbiprofen partition coefficients was the reverse of this. In addition, rank orders of these partition coefficients for each drug are correlated with those of the total lipid concentrations in the three layers of the human nail plate. The diffusion coefficients of the drugs in each single layer was calculated using Equation 3. The dorsal layer had low diffusion coefficients for 5-fluorouracil and flurbiprofen compared with the other single layers. The diffusion coefficients for 5-fluorouracil and flurbiprofen in the intermediate layer were the highest of those for any single layer. The permeation parameters of the full-thickness nail plate (as shown in Table 3) were calculated by substituting those of each single layer, according to Equation 5. They agreed with the experimental permeation parameters of the full-thickness nail plates obtained from the permeation studies for both drugs (as shown in Table 2).

From these results, the drug permeation characteristics of each single layer can be summarized as follows: the dorsal layer is characterized by a low diffusivity of drugs; the intermediate layer is characterized by low lipophilicity; and the ventral layer is characterized by high lipophilicity. We suggest that the main nail barrier to drug permeation may be the low diffusivity of drugs in the dorsal layer. The difference between the nail plate/vehicle partition coefficients for 5-fluorouracil and flurbiprofen was small because of the low lipid content in the human nail plate. Therefore, this suggests that the human nail plate behaves like a hydrophilic gel membrane rather than a lipophilic partition membrane. This suggestion agrees with

Table 3. Calculated permeation parameters of 5-fluorouracil and flurbiprofen through the human nail plate.

Drug Parameter	Layer (thickness ratio)			
	Dorsal (3)	Intermediate (5)	Ventral (2)	Full-thickness (3 : 5 : 2)
5-Fluorouracil				
h (μm)	148·5	247·5	99·0	495·0
P ($\times 10^7$ cm s^{-1})	3·87	7·12	5·39	1·71
Km	0·48	0·56	0·30	0·48
Dm ($\times 10^8$ cm^2 s^{-1})	1·21	3·13	1·80	1·75
Flurbiprofen				
h (μm)	130·2	217·0	86·8	434·0
P ($\times 10^6$ cm s^{-1})	3·71	3·59	11·90	1·58
Km	1·22	0·98	1·51	1·16
Dm ($\times 10^8$ cm^2 s^{-1})	2·63	7·96	3·42	5·91

Each value represents the mean ± s.e.m. (n = 3–5).Permeability coefficients of each single layer were calculated using Equation 5. h is the nail membrane thickness; P is the permeability coefficient; Km is the membrane/donor vehicle partition coefficient of the drug; Dm is the diffusion coefficient of the drug in the nail membrane.

the findings of Walters et al (1983, 1985) and Mertin & Lippold (1997a).

Estimation of drug concentration in each layer

For the treatment of fungal infections in the nail plate, information concerning antifungal drug concentrations in the nail plate, particularly the ventral nail plate, is of great importance. However, it is very difficult to obtain detailed information about these concentrations. Consequently, the drug concentration in each layer was estimated from the permeation parameters of each drug, obtained in earlier studies. In order to obtain information regarding the treatment of fungal infection in the nail plate by oral antifungal medication, 5-fluorouracil and flurbiprofen permeations from the back of the nail plate were also investigated.

5-Fluorouracil ($9{\cdot}54 \pm 0{\cdot}72\ \mu g\ cm^{-2}\ h^{-1}$) and flurbiprofen ($0{\cdot}13 \pm 0{\cdot}03\ \mu g\ cm^{-2}\ h^{-1}$) fluxes from the ventral to the dorsal nail plate were approximately the same values as those (5-fluorouracil: $9{\cdot}19 \pm 1{\cdot}63\ \mu g\ cm^{-2}\ h^{-1}$; flurbiprofen: $0{\cdot}14 \pm 0{\cdot}05\ \mu g\ cm^{-2}\ h^{-1}$) from the dorsal to the ventral nail plate. The amount of 5-fluorouracil ($13{\cdot}98 \pm 1{\cdot}25\ \mu g$) in the full-thickness nail plate from the ventral to the dorsal nail plate was also nearly the same as that ($14{\cdot}33 \pm 0{\cdot}69\ \mu g$) from the dorsal to the ventral nail plate. However, the amounts of flurbiprofen in the full-thickness nail plates were not detectable in either case.

We can calculate the drug amount in each layer from the permeation parameters of the drug. The flux of a drug through each single layer can be defined as follows:

$$dQ/dt = D_A(C_A - C'_A)/h_A = D_B(C_B - C'_B)/h_B = D_C(C_C - C'_C)/h_C \quad (6)$$

where $(C_A - C'_A)$, $(C_B - C'_B)$ and $(C_C - C'_C)$ are the concentration differentials of that drug in each layer (A = dorsal; B = intermediate; C = ventral). In a sink condition, C'_C can be assumed to be equal to zero. Therefore, the total amount of a drug (Q_T) in the full-thickness nail plate can be defined as follows:

$$Q_T/S = (C_A - C'_A)h_A/2 + (C_B - C'_B)h_B/2 + C_C h_C/2 \quad (7)$$

where S is the surface area of the nail plate. We calculated the mean drug concentration in each single layer using Equations 6 and 7.

Table 4 shows the amounts and mean concentrations of 5-fluorouracil and flurbiprofen in each layer, as estimated from the permeation parameters. The estimated amount of 5-fluorouracil in the full-thickness nail plate was similar to the experimental data. The 5-fluorouracil and flurbiprofen concentrations in the ventral nail layer that resulted from drug permeation data from the ventral nail plate were 4·2 and 45 times higher than those from the dorsal plate. Compared with the estimated 5-fluorouracil concentration in the full-thickness nail plate, the estimated flurbiprofen concentration in the nail plate was about 300 times lower. Since the nail plate behaves like a hydrophilic-gel membrane, flurbiprofen, a poorly water soluble drug cannot distribute into the nail plate to a greater extent than 5-fluorouracil, a water soluble drug. Drug permeation from the ventral nail plate displayed a high drug delivery rate compared with that from the dorsal nail plate, which is not affected by drug solubility.

Most antifungal drugs have the same or high lipophilicity (in addition to a higher molecular weight) than flurbiprofen. Therefore, the antifungal drug concentration in the ventral nail plate may be

Table 4. Estimation of 5-fluorouracil and flurbiprofen concentration in the human nail plate.

	5-Fluorouracil		Flurbiprofen	
	Amount (μg)	Concn ($\mu g\ mL^{-1}$)	Amount (μg)	Concn ($\mu g\ mL^{-1}$)
Dorsal to ventral				
Full-thickness	11·32	4657	22·60	10·61
Dorsal	4·84	6642	15·45	24·17
Intermediate	5·97	4911	6·80	6·38
Ventral	0·51	1045	0·35	0·83
Ventral to dorsal				
Full thickness	10·68	4396	37·73	18·00
Dorsal	1·49	2040	4·52	7·07
Intermediate	7·05	5808	17·34	16·28
Ventral	2·14	4401	15·87	37·24

Values were estimated from the permeation parameters.

lower than the estimated flurbiprofen concentration. However, if we have an adequate understanding of the nail barrier function, it is possible to achieve a suitable drug concentration for the treatment of fungal infection in the ventral nail plate, because recently developed antifungal drugs have low minimum inhibitory concentrations. Antifungal drugs such as ciclopirox and amorolfine achieve active concentrations in the nail plate after about one week (Ceschin-Roques et al 1991; Pittrof et al 1992). We reported earlier that *N*-acetyl-L-cysteine and 2-mercaptoethanol were able to enhance 5-fluorouracil and tolnaftate permeations through the human nail plate (Kobayashi et al 1998). Thus, a topical drug delivery system containing these enhancers may be suitable for the treatment of fungal infection in the nail plate.

Based on our results with 5-fluorouracil and flurbiprofen permeation studies, we suggest that the human nail plate behaves like a hydrophilic-gel membrane. In addition, we also show that the human nail plate is composed of three layers having differential barrier properties; drug diffusion in the upper layer is the main barrier in the nail plate. Lipid content in the nail plate is much lower than that in the stratum corneum of the skin, thus, the nail plate is unable to hold a high concentration of a poorly water soluble drug. In the treatment of nail plate fungus, an antifungal concentration in the ventral nail plate would be of great importance. Treatment success would also depend on the permeation and the solubility of the antifungal drugs used.

Acknowledgements

The authors wish to thank the volunteers at Josai University for supplying nail pieces.

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