International Journal of Pharmaceutics, 27 (1985) 193–203
Elsevier

IJP 00914

Topical absorption of methotrexate: role of dermal transport

Ovais Siddiqui *, Michael S. Roberts and Alan E. Polack
School of Pharmacy, University of Tasmania, Hobart, Tasmania 7001, (Australia)

(Received December 14th, 1984)
(Modified version received July 15th, 1985)
(Accepted July 16th, 1985)

Key words: methotrexate – skin absorption – dermal blood flow – epidermal transport

Summary

The relative contribution of epidermal and dermal transport of methotrexate was examined in an attempt to define the concentration of methotrexate likely to be found in the viable epidermis. Dermal transport was examined using human dermis and an in vivo rat model. Epidermal transport was assessed using excised human stratum corneum. Methotrexate was rapidly absorbed into the dermis from aqueous solutions, its clearance being facilitated by both the dermal blood supply and diffusion into the dermis. The aqueous solution pH affected the rate of penetration of methotrexate through the stratum corneum. Maximal steady-state concentration in the viable epidermis estimated from the epidermal permeability coefficient and clearance were found to be lower than the concentrations reported as being required for the effective management of psoriasis.

Introduction

Research in the field of percutaneous absorption has gained considerable prominence in recent years through the development of topically applied dosage forms for systemic drug delivery and the related interest in the design of topical products for

* *Present address:* Controlled Drug-Delivery Research Center, College of Pharmacy, Rutgers University, Busch Campus, PO Box 789, Piscataway, N.J. 08854.
Correspondence: A.E. Polack, School of Pharmacy, University of Tasmania, GPO Box 252C, Hobart, Tasmania, Australia 7001.

0378-5173/85/$03.30 © 1985 Elsevier Science Publishers B.V. (Biomedical Division)

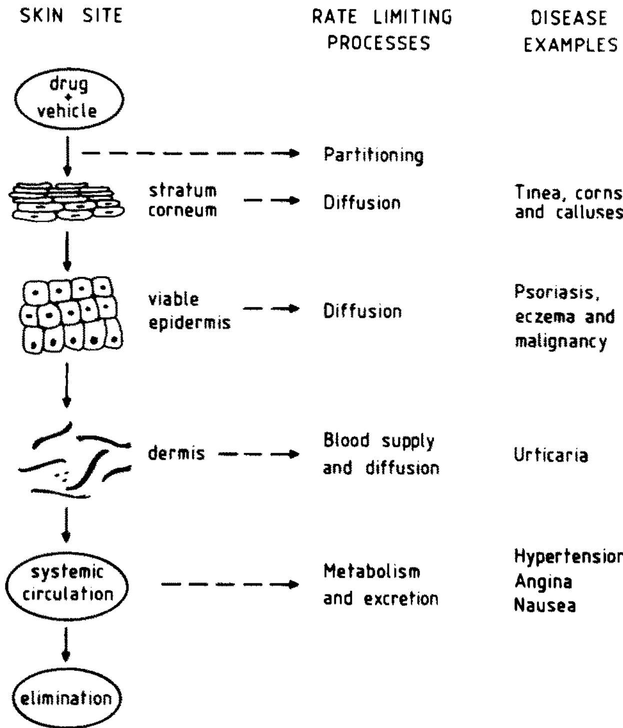

Fig. 1. Rate-limiting steps in the absorption of drugs across the skin and sites of action required for topical medication.

the treatment of local disorders. Most studies on topical absorption have quantified transport through the epidermis. Limited attention in work related to topical drug application has, however, been given to dermal transport.

The topical efficacy of any drug will depend upon the concentration that can be attained in the epidermis. For drugs used in psoriasis the site of action is in the Malphigian layer (stratum spinosum and stratum germinativum). The concentration of any drug at this site is, however, dependent on the rate of its delivery to and clearance from the site (Fig. 1). On the other hand, for the antifungal agents the site of action is in the stratum corneum (Fig. 1) and the rate of penetration into the stratum corneum alone is important (Scheuplein, 1978; Roberts et al., 1978).

Methotrexate has been shown to be effective in the management of psoriasis after oral or systemic administration (Schaefer et al., 1982). In order to avoid systemic toxicity there has been considerable interest in the possibility of administering methotrexate topically (Comaish and Juhlin, 1969; Wallace et al., 1972; Weinstein, 1977; Wantzin and Thomsen, 1983). Most investigators agree however that topically applied methotrexate is ineffective (Weinstein, 1977; Wallace et al., 1978; Weinstein, 1981; Ball et al., 1982). On the other hand Fry and Mcminn (1967) claimed that topical methotrexate is effective against psoriasis. Most studies quantifying the absorption rates of methotrexate through human skin have been limited to reporting

epidermal permeability data obtained with intact human skin in vitro (Wallace et al., 1978; Ball et al., 1982).

We have in the present work attempted to determine the relative importance of the epidermal and dermal barriers in the topical effectiveness of methotrexate.

Materials and Methods

Methotrexate (Amethopterin), Methotrexate [3′,5′,7-^{3}H]sodium salt (250 mCi/mmol), tritiated water (2.5×10^6 dpm/g) were purchased from Sigma Chemicals, U.S.A.; Amersham International, U.K. and Packard Instruments U.S.A., respectively. Biofluor (New England Nuclear) was used as liquid scintillation cocktail. Stable plasma protein solution (SPPS; 5 g plasma proteins in 100 ml buffered solution at pH 7.0) was a gift from Commonwealth Serum Laboratories, Trypsin (salt free, crystalline, lypholized) was purchased from Boehringer Mannheim, F.R.G. All other reagents were of analytical grade. Constant ionic strength buffers (0.1) were used. All pH's were measured on a Metrohm Herisau E520, pH meter. Hooded Wistar rats (male, approximately 300 g) were used in the in vivo dermal studies.

Purification of methotrexate

The radioactive [^{3}H]methotrexate was purified prior to use. Samples were injected into a high-pressure liquid chromatographic system (Waters Associates), consisting of a Bondapak phenyl column (300 mm $\times$ 3.9 mm), a Model 441 detector ($\lambda = 313$ nm) with acetonitrile: water buffered to pH 4.6 (11 : 89) being used as the mobile phase and a flow rate of 2 $\text{ml} \cdot \text{min}^{-1}$. This method is similar to that reported by Canfell and Sadee (1980). A retention time of 6 min was obtained for methotrexate. The purified methotrexate fraction was collected and the mobile phase subsequently evaporated under vacuum.

Thin-layer chromatography was used to validate the purity of the radioactive methotrexate and also to verify the identity of the labelled substance penetrating through the human stratum corneum. Corrections were made for quenching, and counts per minute (cpm) were converted into disintegration per minute (dpm).

In vitro apparatus

The type of glass cell used is shown in Fig. 2. The clamped cells were immersed in a water bath at 25 ± 0.5°C and a constant stirring rate of 40 rpm was maintained in both the compartments using a synchronized motor and external magnets. The maximum capacity of each of the receptor and donor compartments (Fig. 2) is 3.5 ml. The surface area of stratum corneum exposed to the cell is 4.5 cm^2.

Skin samples

Samples of human skin, including the subcutaneous fat, approximately 25 cm $\times$ 6 cm, were removed from the mid abdominal region of caucasian cadavers aged 50–78 years, within 48 h of death and stored at -20°C. The subcutaneous fat was carefully trimmed and the method of Kligman and Christophers (1963) was adapted to

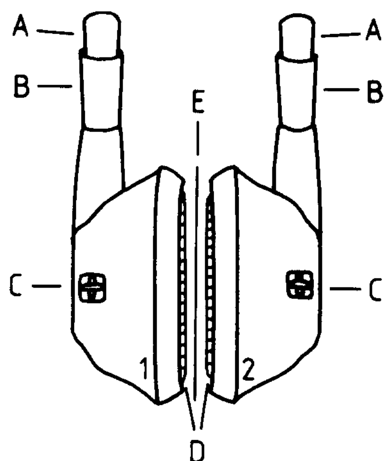

Fig. 2. Diagram of the apparatus used in permeation experiments. A = glass stoppers; B = sampling ports; C = magnetic fleas; D = wire mesh; E = stratum corneum. A spring-loaded clamp is used to hold the donor (1) and receptor (2) compartments together.

remove the epidermis (60°C) and the stratum corneum (0.1% trypsin in 0.05M trizma buffer at pH 7.90 for 50 min). The transparent sheet of stratum corneum obtained was washed several times with water, dried overnight at room temperature and stored at −20°C prior to use. The stratum corneum was allowed to thaw overnight at room temperature and rehydrated by immersion in water for 1 h (Swarbrick et al., 1982) prior to being placed in the permeation cell with the dermal side towards the receptor compartment. The stratum corneum was supported in this position by wire mesh (Fig. 2). A thin film of silicone lubricant (Apiezon-AP100) was spread on the lapped glass surfaces of the cells to provide a water-tight glass to membrane seal.

In vitro permeation through the stratum corneum

The donor compartment contained a mixture of a freshly prepared cold methotrexate and [^{3}H]methotrexate to give final concentrations of 0.03% or 0.003% of the solute containing more than 1 μCi of [^{3}H]methotrexate. Isotonic sodium chloride was used as the receptor fluid for all the experiments. Samples were taken at regular intervals from the receptor side and occasionally from the donor side. The permeation experiments were run over a period of 25–30 h.

Receptor (0.5 ml) and donor (0.1 ml) aliquots were added to 10 ml biofluor for liquid scintillation counting (Rackbeta II, LKB, Wallac, Finland). Receptor volume was replaced by the addition of an equal volume of fluid immediately after the sample was removed.

All the experiments were performed at least in duplicate and all the permeation runs were carried out in the dark to ensure minimum methotrexate decomposition.

The apparent permeability coefficient (k_p) was calculated using Eqn. 1 (Scheuplein, 1978; Roberts et al., 1978; Wallace et al., 1978):

$$k_p = J_{ss}/C_v \quad (1)$$

where J_{ss}, is the steady-state flux, is equal to the steady-state slope of the plot of cumulative amount permeated against time and C_v is the total donor (unionized and ionized) concentration. Lag time was determined by extrapolation of the steady-state portion of the above plot to the time axis. The units of k_p are $cm \cdot h^{-1}$.

The integrity of the stratum corneum was examined at the end of each run by quantifying the permeation of Congo red and/or tritiated water from aqueous solutions through the stratum corneum.

Concentration in the stratum corneum at steady-state

At the end of each run the permeation cell was dismantled and the superficial substance on the exposed area was removed by washing. The stratum corneum was then immersed in fresh receptor fluid in an amber coloured bottle (without any agitation). The desorption fluid was replaced a number of times over a period of about 72 h until it gave a count of zero. The total amount of methotrexate in the stratum corneum was calculated by counting of the pooled desorption solutions.

In vitro and in vivo dermal kinetics studies

The apparatus shown in Fig. 2 was also used for all in vitro dermal studies. Dermal specimen were prepared by trimming the subcutaneous fat of full thickness human skin and the method of Kligman and Christophers (1963) was adapted to remove the epidermis by heat (60°C). The dermis was placed in the permeation cell and the penetration of methotrexate was followed in the manner described above for stratum corneum.

The dermal absorption of methotrexate in vivo was carried out using an approach similar to that described by Levy and Rowland (1974) for subcutaneous absorption. Dermal absorption was assessed in 4 anaesthetized and 4 dead animals. The dead animals were sacrificed with an overdose of ether while 4 others were anaesthetized with an intraperitoneal injection of urethane (as 25% aqueous solution, 1.5 mg/g). Hair in the abdominal region of all rats was shaved with small animal clippers and a depilatory agent (Nair; Carter and Wallace Australia) was applied to the region for 10 min to remove the residual hair. The method of Frosch and Kligman (1977) was used to remove the epidermis. The minimal blistering time with 1:1 aqueous solution of ammonium hydroxide was found to vary between 50 and 90 min for the abdominal skin of the rats.

Half of a permeation cell (Fig. 3) was placed on the exposed dermis (4.5 cm^2) using a medical adhesive (Hollister, Chicago, U.S.A.) and supported by a retort stand. The solution (3.5–4 ml) in the half-cell was maintained at 37 ± 1°C by direct heating of the outer glass surface of the cell with a wrapped electric wire (Fig. 3). The solution was stirred at a constant rate of 40 rpm using an external synchronized motor and a glass rod with paddles. Methotrexate, 0.003% solution pH 7.2 (pH of the dermis is between 7.1 and 7.3; from Katz and Poulsen 1971), was used in both in vitro and in vivo dermal studies. The reduction in concentration in the donor compartment was monitored with time using high-pressure liquid chromatography (Canfell and Sadee, 1980).

The steady-state unbound concentration (C_{ss}) likely to be achieved in the viable

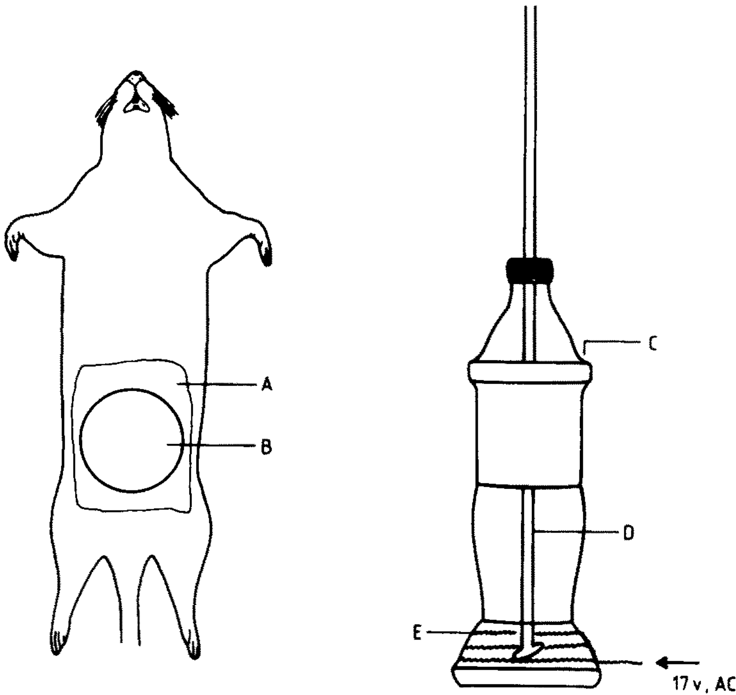

Fig. 3. Schematic representation of the animal and abdominal dosing area where the glass cell was placed. A = shaved area; B = dosing area (4.5 cm^2); C = sampling port; D = glass stirrer; E = wrapped electric wire to maintain the temperature of the solution at 37°C. Glass half-cell was placed over the dosing area and held by a retort stand.

epidermis was estimated using the clearance into the dermis and the rate of permeation through the stratum corneum (Roberts et al., 1982):

$$C_{ss} = \frac{J_{ss}A}{Cl} = \frac{k_p C_v A}{Cl} \tag{2}$$

where k_p is the permeability of the stratum corneum of area A, C_v is the concentration of the solute in the solution applied to the stratum corneum, J_{ss} is the steady-state flux and Cl is the clearance of the unbound solute from the viable epidermis. In this study, Cl was estimated from the disappearance of the aqueous solution applied to the dermis:

$$Cl = kV \tag{3}$$

where k is the rate constant for disappearance from volume V. The minimum rate of input required (R_0) to achieve a given steady-state concentration C_{ss} in the viable epidermis was obtained by rearranging Eqn. 2:

$$R_0 = C_{ss}Cl \tag{4}$$

Results and Discussion

Fig. 4 shows the percentage of methotrexate remaining in solution following application to human and rat dermis. A monoexponential decline was observed in studies. The rate of disappearance of methotrexate was found to depend on the volume of solution applied, mean rate constants of 0.04 and 0.022 h^{-1} being found for the disappearance of methotrexate into human dermis for 4 and 6 ml, respectively. The corresponding values of clearance (Eqn. 3) obtained for these volumes are 0.15 and 0.13 $ml \cdot h^{-1}$, respectively, and are not significantly different ($P > 0.05$, t-test). In the pharmacokinetic approach proposed by Rowland and Tozer (1980), clearance and volume are also independent variables. According to this approach, the *unbound* concentration of drug in the viable epidermis is only dependent on the rate of methotrexate delivery to this site and the clearance from this site. The total concentration of methotrexate is determined not only by these parameters but also by the binding of methotrexate to other components in this viable epidermis.

Table 1 shows the clearances obtained for the dermal studies. The clearance of methotrexate from the aqueous solution into human dermis is relatively unaffected by temperature (Table 1). In contrast, the transport of a number of compounds through stratum corneum has been shown to be temperature dependent (Schaefer, 1982).

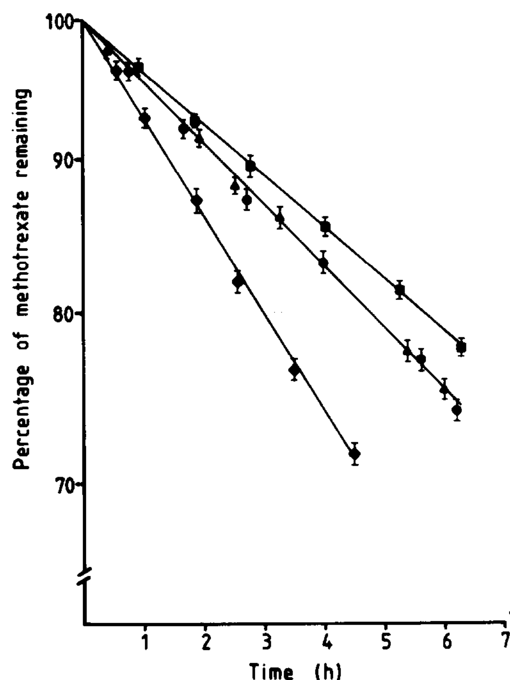

Fig. 4. Disappearance of methotrexate into the dermis (log scale). Mean ±S.D., n = 4. ■, human dermis, 25°C; ▲, human dermis, 37°C; ●, dermis of dead rat, 37°C; ◆, dermis of anaesthetised rat, 37°C.

TABLE 1

CLEARANCE OF METHOTREXATE INTO THE DERMIS FROM AQUEOUS SOLUTIONS CONTAINING 0.003% METHOTREXATE

Dermal preparation	Clearance [a] (ml/h)
Human (25°C) postmortem	0.12 ± 0.011
Human (37°C) postmortem	0.15 ± 0.012
Rat (37°C) sacrificed	0.20 ± 0.011
Rat (37°C) anaesthetized	0.30 ± 0.013

[a] Mean ± S.D. (n = 4).

The clearance of methotrexate into the dermis was higher for the rat dermis than the human dermis (Table 1). An important factor controlling dermal clearance was the dermal blood supply, the clearance of methotrexate being 50% higher in anaesthetised than in dead rats (Fig. 4, Table 1). The use of anaesthetized and dead rats to demonstrate the role of blood supply in drug absorption has been previously used by Levy and Rowland (1974). These workers reported a biphasic disappearance profile for local anaesthetics applied to subcutaneous tissue and proposed a partitioning of these agents into the subcutaneous tissue with a subsequent loss to the blood stream. A biphasic profile is most likely when significant accumulation in the dermis/subcutaneous tissue occurs. As the dermis is essentially aqueous (Katz and Poulsen, 1971), significant accumulation of methotrexate after application as an aqueous solution is not likely and hence a nonexponential profile. Abolition of the dermal blood supply only partly reduces dermal clearance, suggesting that this clearance consists of both diffusion in the dermis and a parallel removal by the dermal blood supply. Methotrexate was quantified in the receptor compartment in human dermal studies, the rate constant for appearance ($0.0282\ h^{-1}$) at early times being less than the rate constant for disappearance ($0.04\ h^{-1}$). The slower rate of appearance is consistent with the transport being diffusion controlled.

Table 2 shows the steady-state flux and lag times obtained for methotrexate applied to the stratum corneum. The flux and the lag times are pH dependent; with increase in pH the flux was found to decrease and the lag time to increase. Larger concentrations of residual methotrexate were found at lower pH values. In this work the effect of receptor composition on the permeation of methotrexate through human stratum corneum was also evaluated. The rate of penetration was independent of the type of receptor fluid used. The fluxes for 0.003% methotrexate (pH 5.8) was $8.75\ ng \cdot cm^{-2} \cdot h^{-1}$ for isotonic sodium chloride and $8.90\ ng \cdot cm^{-2} \cdot h^{-1}$ for an aqueous protein solution (SPPS). In all studies, the cumulative percentage recovered in the receptor solution was less than 5%. A one way analysis of variance of in vitro stratum corneum flux data (Table 2) revealed that the steady-state flux and lag times were dependent upon the degree of ionization of methotrexate in aqueous solution ($F = 1032$, df = 4/10, $P < 0.05$).

Table 3 shows the apparent unbound steady-state concentrations estimated for the viable epidermis. These values are derived from dermal absorption (Table 1) and

TABLE 2

EFFECT OF pH ON THE PERMEATION OF METHOTREXATE THROUGH HUMAN STRARUM CORNEUM AT 25±1°C FOR AQUEOUS SOLUTIONS CONTAINING 0.003% METHOTREXATE

pH (25°C)	Number of runs	Lag time (h)	Steady-state flux, J_{ss} $(ng/cm^2/h)^d$	Concentration in stratum corneum at steady-state, $C_{st.c}$ (ng/mg)
3.4 [a]	2	0.8	13.0 (12.6, 13.5)	190±10
3.6 [b]	2	1.6	11.0 (10.0, 11.7)	410±16
5.0 [a]	2	4.3	10.0 (10.1, 9.8)	500±10
5.8 [c]	3	10.0	8.8 (8.6, 8.8, 9.0)	60±10
8.0 [a]	4	11.3	2.2 (1.9, 2.3, 2.4, 2.0)	50± 5

[a, b and c] Sorensen's, Walpole's acetate and Sorensen's phosphate buffers, respectively.
Weight of stratum corneum (4.5 cm^2) = 72±0.016 mg (n = 4).
[d] The numbers in parentheses represent individual J_{ss} values.

the stratum corneum permeability (Eqn. 1) calculated from values of flux (Table 2). The resulting unbound steady-state concentrations range between 0.5 and 0.03 $\mu g \cdot ml^{-1}$, the highest concentrations being observed for acidic solutions applied to skin in which no blood supply exits (Table 3). A minimal theoretical concentration of less than 1 $\mu g \cdot ml^{-1}$ for methotrexate in the viable epidermis has been suggested as being required for effective management of psoriasis (Schaefer et al., 1982). As the estimated concentrations given in Table 3 are lower than this value, the present results are consistent with the suggestion that methotrexate passes very rapidly through the skin thereby preventing the attainment of a concentration sufficient to inhibit the epidermal DNA synthesis necessary to maintain psoriasis (Schaefer et al., 1982). More recently it has been reported that inhibition of polymorphonuclear leucocyte activity (Walsdorfer et al., 1983) is also important. According to Eqn. 4 a

TABLE 3

ESTIMATED STEADY-STATE CONCENTRATION (C_{ss}) IN THE VIABLE EPIDERMIS CALCULATED FROM EQN. 2 USING THE DATA GIVEN IN TABLES 1 AND 2.

pH of aqueous solution applied to stratum corneum	Dermal preparations, C_{ss} (Mg/ml)			
	Human 25°C postmortem	Human 37°C postmortem	Rat 37°C sacrificed	Rat 37°C anaesthetized
3.4 [a]	0.49	0.39	0.30	0.20
3.6 [b]	0.45	0.36	0.27	0.18
5.0 [a]	0.38	0.30	0.23	0.15
5.8 [c]	0.35	0.28	0.21	0.14
8.0 [a]	0.10	0.06	0.05	0.03

[a, b and c] As in Table 2.

flux of about 60 $\mu g \cdot cm^{-2} \cdot h^{-1}$ is required from the stratum corneum to achieve the minimum effective concentration of methotrexate in the viable epidermis. This flux is about 5 times that observed in this study where aqueous solutions were applied to isolated stratum corneum at 25°C. In psoriasis, although the structural alterations in the stratum corneum facilitate faster permeation, the dermis also undergoes morphological changes (Handley et al., 1983) with a likely increase in clearance.

High concentrations of methotrexate (50–510 $ng \cdot ml^{-1}$) were found in the stratum corneum after topical application (Table 2). As these concentrations are much higher than the minimal theoretical concentration required in the viable epidermis, it appears that removal of methotrexate by the dermis rather than penetration into the stratum corneum is more important in determining the efficacy of topical methotrexate. The data obtained for the pH dependence of epidermal penetration (Table 2) is consistent with results reported previously for full thickness hairless mouse skin (Wallace et al., 1978).

Our unpublished work on the mechanism of methotrexate permeation through excised human stratum corneum indicates that both the unionized and ionized species of methotrexate can permeate excised human skin. The dependence of lag time on the degree of ionisation of methotrexate in aqueous solution conforms to the above hypothesis. It has also been found that ionized methotrexate permeates excised stratum corneum as ion pairs with the likely route of skin penetration being through the cells of the stratum corneum (intracellular route).

Considerably enhanced penetration may be achieved with an occlusive dressing which: (a) raises the temperature of the stratum corneum; and (b) promotes hydration of the stratum corneum (Idson, 1978). The concentration in the viable epidermis may be increased by a reduction in dermal clearance by the use of vasoconstrictors and adjustment of the pH of the formulation applied to the skin (Table 3).

The results obtained in the present study suggest that the rate of the loss into the dermis may be an important factor in determining the overall rate of disappearance of methotrexate following topical application to the epidermis. The results reported here support the hypothesis (Schaefer et al., 1982) that it is not the inability of methotrexate to permeate the stratum corneum which is the reason for its topical ineffectiveness even at high pH. The present data suggests that methotrexate is removed rapidly after passing through the stratum corneum, thereby preventing the attainment of a sufficient concentration in the Malphigian layer to inhibit the epidermal DNA synthesis necessary to maintain psoriasis. It is also possible that significant dermal clearance may also be a determining factor in the efficacy of other topical medications.

References

Ball, M.A., McCullough, J.L. and Weinstein, G.D., Percutaneous absorption of methotrexate: effect on epidermal DNA synthesis in hairless mice. J. Invest. Dermatol., 79 (1982) 7–10.

Canfell, C. and Sadee, W., Methotrexate and 7-hydroxymethotrexate: serum level monitoring by high performance liquid chromatography. Cancer Treat. Rep., 64 (1980) 165–169.

Comaish, S. and Juhlin, L., Site of action of methotrexate in psoriasis. Arch. Dermatol., 100 (1969) 99–105.

Frosch, P.J. and Kligman, A.M., Rapid blister formation in human skin with ammonium hydroxide. Br. J. Dermatol., 96 (1977) 461–473.

Fry, L. and Mcminn, R.M.H., Topical methotrexate in psoriasis. Arch. Dermatol., 96 (1967) 483–488.

Handley, A., Black, D. and Marks, R., Mechanical properties of dermis in psoriasis. Br. J. Dermatol., 108 (1983) 240–241.

Idson, B., Hydration and percutaneous absorption. Curr. Probl. Dermatol., 7 (1978) 132–141

Katz, M. and Poulsen, B.J., Absorption of drugs through skin. In Brodie, B.B. and Gillette, J.R. (Eds.) Handbook of Experimental Pharmacology, Vol. 28, Concepts in Biochemical Pharmacology, Part I, Springer-Verlag, New York, 1971, Ch. 7.

Kligman, A.M. and Christophers, E., Preparation of isolated sheets of human stratum corneum. Arch. Dermatol., 88 (1963) 702–705.

Levy, R.H. and Rowland, M., Absorption kinetics of a series of local anaesthetics from rat subcutaneous tissue. J. Pharmacokin. Biopharm., 2 (1974) 313–355.

McCullough, J.L., Snyder, D.S., Weinstein, G.D., Friedland, A. and Stein, B., Factors affecting human percutaneous penetration of methotrexate and its analogues in vitro. J. Invest. Dermatol., 66 (1976) 103–107.

Roberts, M.S., Anderson, R.A., Swarbrick, J. and Moore, D.E., The percutaneous absorption of phenolic compounds: the mechanism of diffusion across stratum corneum. J. Pharm. Pharmacol., 30 (1978) 486–490.

Roberts, M.S., Favretto, W.A., Meyer, A., Reckmann, M. and Wongseelashote, T., Topical bioavailability of methyl salicylate. Aust. N.Z. J. Med., 12 (1982) 303–305.

Rowland, M. and Tozer, T.N., Clinical Pharmacokinetics, Concepts and Applications, Lea and Febiger, Philadelphia, 1980, Ch. 5 and 6.

Schaefer, H., Zesch, A. and Stuttgen, G., Skin permeability. Springer-Verlag, Berlin-Heidelberg, 1982, pp. 827–829.

Scheuplein, R., Skin permeation. J. Physiol. Pathophysiol. Skin, 5 (1978) 1693–1730.

Stewart, W.D., Wallace, S.M. and Runikis, J.O., Absorption and local action of methotrexate on human mouse skin. Arch. Dermatol., 106 (1972) 357–361.

Swarbrick, J., Lee, G. and Brom, J., Drug permeation through human skin: I. Effect of storage conditions of skin. J. Invest. Dermatol., 78 (1982) 63–66.

Wallace, S.M., Runikus, J.O. and Steart, W.D., The effect of pH on in vitro percutaneous penetration of methotrexate: correlation with solubility and partition coefficient. Can. J. Pharm. Sci., 13 (1978) 66–68.

Wallace, S.M., Stewart, W.D. and Runikus, J.O., Percutaneous penetration and clinical efficiency of local methotrexate (amethopterin) in psoriatic patients. Clin. Res., 20 (1972) 214.

Walsdorfer, U., Christophers, E. and Schroder, J.M., Methotrexate inhibits polymorphonuclear leucocytes chemotaxis psoriasis. Br. J. Dermatol., 108 (1983) 451–456.

Wantzin, G.L. and Thomsen, K., Acute paronychia after high dose methotrexate therapy. Arch. Dermatol., 119 (1983) 623–624.

Weinstein, G.D., Methotrexate. Ann. Int. Med., 86 (1977) 199–204.

Weinstein, G.D., McCullough, J.L., Eaglstein, W.H., Golub, A., Cornell, R.C. Stoughton, R.B., Clendenning, W., Zackheim, H., Maibach, H., Kulp, K.R., King, L., Baden, H.P., Taylor, J.S. and Deneau, D.D., A clinical screening programme for topical chematherapeutic drugs in psoriasis. Arch. Dermatol., 117 (1981) 388–393.