International Journal of Pharmaceutics, 25 (1985) 85–93
Elsevier

IJP 00838

Effect of pH and solubility on in vitro skin penetration of methotrexate from a 50% v/v propylene glycol–water vehicle

R. Vaidyanathan, M.G. Chaubal and R.C. Vasavada

School of Pharmacy, University of the Pacific, Stockton, CA 95211 (U.S.A.)

(Received October 19th, 1984)
(Modified version received and accepted January 16th, 1985)

Key words: skin penetration – methotrexate – effect of pH and solubility – propylene glycol – HPLC assay

Summary

In vitro percutaneous absorption of methotrexate from a saturated solution in a 50% v/v propylene glycol–water vehicle was examined across suitably characterized human cadaver skin. A detailed study of solubility and pH parameters in the range of 2–6 pH units was conducted. A clear trend towards increasing steady-state penetration rate with increasing pH was evident. Relatively high lag times were observed even at optimal pH values emphasizing the importance of prolonged application of drug to achieve therapeutic concentrations. From a vehicle at pH 2, the steady-state rate of penetration was very low and the data suggested drug binding with stratum corneum. Although the drug solubility in the vehicle was considerably lower at pH 4 than at pH 2–3, the steady-state rate and extent of penetration were higher. Both drug solubility and steady-state penetration rate were significantly higher at pH 5.29 than at pH 4. Increasing the vehicle pH to 6.34 increased the drug solubility as well as steady-state rate of penetration but the percent amount penetrated declined. The lag times were also large at this pH. Relatively high drug solubility and gradually increasing contribution of shunt pathways probably account for this result. In view of the observed pH–solubility profile, for a 50% v/v propylene glycol–water vehicle, a pH between 4 and 5 would appear to provide the most favorable environment for passive diffusion since the concentration of unionized methotrexate would be optimal.

Correspondence: R.C. Vasavada, School of Pharmacy, University of the Pacific, Stockton, CA 95211, U.S.A.

Introduction

Methotrexate, an antineoplastic agent, is also effective in the control of recalcitrant psoriasis. It has been shown to selectively inhibit DNA synthesis in psoriatic epidermal cells, thus decreasing the mitotic activity (Weinstein and Velasco, 1972; Flaxman et al., 1977). Several investigators have conducted clinical studies with topical methotrexate (Condit, 1961; Nurse, 1963; Fry and McMinn, 1967; Comaish and Juhlin, 1969). A review of these studies revealed significant differences with respect to clinical efficacy and mechanism of action. Newbold and Stoughton (1972) studied percutaneous penetration of methotrexate through hairless mouse skin and human skin under controlled pH conditions. Stewart et al. (1972) reported rapid in vivo absorption of topically applied methotrexate through hairless mouse skin, but could not achieve comparable results through human skin. McCullough and coworkers (1975, 1976) found that ester derivatives of methotrexate were more active than the parent compound, but percutaneous penetration was no more than 2% of the applied dose in vehicles containing 80% dimethylsulfoxide, 25% dimethylacetamide, 0.1% retinoic acid, or 2.5% C-10 methyl sulfoxide. These and other studies have served to emphasize the importance of pH and vehicle effects on percutaneous absorption of methotrexate.

This study was undertaken to study the effects of physicochemical factors such as pH, solubility, and vehicle composition on percutaneous penetration of methotrexate.

Materials and Methods

Solubility studies

The solubility of methotrexate USP [1] in a series of propylene glycol–water mixtures varying from 20% to 80% v/v propylene glycol [2] was determined in triplicate at $22 \pm 0.5°C$ (constant room temperature) by equilibrating a suspension of an excess of drug in 25 ml of appropriate solvent blend for 6 days in 50 ml amber flasks. The aliquot was filtered through 0.22 μm membrane filter prior to analysis by HPLC.

Assay method

Methotrexate content of various samples was analyzed by high-performance liquid chromatography (Tong et al., 1975). The chromatograph was equipped with a 600-psi pump [3], a variable wavelength detector [4] and a loop injector [5]. A 30 cm long, 3.9 mm inner diameter stainless steel column with μ-Bondapak as the sta-

[1] Lot 1260-A0922, Lederle Laboratories, Pearl River, NY.
[2] J.T. Baker Chemicals, Phillipsburg, NJ.
[3] Waters 6000-A pump, Waters Associates, Milford, MA.
[4] Model 450, Waters Associates, Milford, MA.
[5] UGK injector, Waters Associates, Milford, MA.

tionary phase [6] was used. An automated integrator system [7] was used to determine the areas under the curve. Injection volume was maintained constant with the aid of a gas tight microsyringe [8].

The eluent was 85% 0.005 M ammonium acetate [9] in water (pH 5.0) and 15% v/v in acetonitrile [9].

Preparation of skin

Skin penetration studies were conducted using human abdominal skin obtained at autopsy. Skin samples were readied for penetration experiments according to the method reported previously (Iyer and Vasavada, 1979).

Diffusion through skin

The details of the skin diffusion assembly were described previously (Iyer and Vasavada, 1979). The diffusion area was 2.01 cm^2. The epidermal side was covered with Saran Wrap following the application of 1 ml of saturates solution of methotrexate in 50% PG–water. Normal saline [10] was pipetted into the skin cell bathing the dermal side and was maintained at $37 \pm 0.5°C$. During the experiment, each cell was kept covered with aluminum foil to minimize photodecomposition of methotrexate.

All experiments were conducted for a period of 14 days. The receptor fluid was completely removed at selected intervals and the chamber was refilled with fresh normal saline. At the end of the penetration study, the remaining portion of the applied dose was removed and the epidermal surface was then washed with three 1-ml portions of 50% propylene glycol in water. The skin was removed from the cell and the circular portion of the skin in contact with bathing fluid was cut out with surgical scissors. The epidermis and dermis were easily separated by means of forceps and collected separately in two amber-colored flasks. The epidermal portion was placed in 7.5 ml of 50% propylene glycol in water. The dermal portion was cut into very small pieces and suspended in 15 ml of 50% aqueous propylene glycol. Following a 48-h stirring period, solutions were assayed for methotrexate by HPLC. All samples were filtered through a disposable 0.22 μm filter prior to dilution (if necessary) and injected on the column. In order to overcome interference from peaks due to impurities from skin samples, a more polar eluant solvent, consisting of 90% v/v 0.005 M ammonium acetate in water (pH 5.0) and 10% v/v acetonitrile was used in conjunction with a flow rate of 0.9 ml/min.

Results and Discussion

Chromatograms of methotrexate USP used in this study showed six peaks as shown in Fig. 1. Peak IV was identified as that of methotrexate by spiking with an

[6] Waters Associates, Milford, MA.
[7] Data Module, Waters Associates, Milford, MA.
[8] 800 series, Hamilton, Reno, NV.
[9] HPLC grade, J.T. Baker Chemicals, Phillipsburg, NJ
[10] Abbott Laboratories, North Chicago, IL.

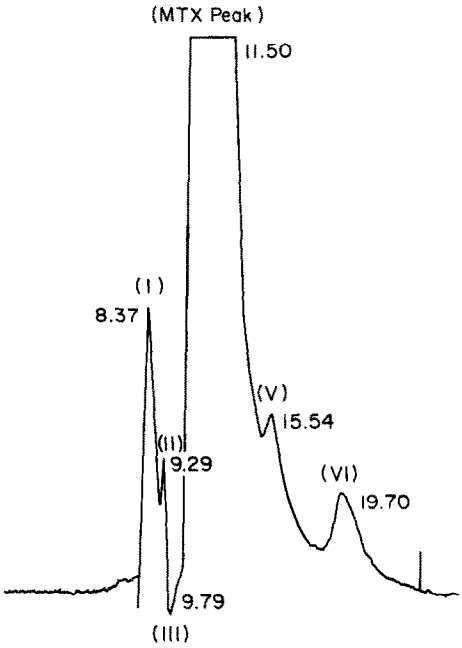


Fig. 1. Chromatogram of methotrexate in 85% v/v 0.005 mol/l ammonium acetate in water (pH 5.0) and 15% v/v acetonitrile.

authentic sample. The purity of methotrexate USP was calculated to be 95.5% anhydrous.

The solubility of methotrexate USP in propylene glycol–water mixtures at $22 \pm 0.5°C$ was found to vary from 0.1752 ± 0.0048 mg/ml in 20% v/v aqueous propylene glycol to 1.4567 ± 0.0363 mg/ml in 80% v/v aqueous propylene glycol. A semi-log plot of solubility (S) against percent propylene glycol showed a linear relationship described by Eqn. 1.

$$\ln S(\text{mg/ml}) = 0.0362(\%\ \text{propylene glycol}) - 2.482\ldots \quad (1)$$

The pH of aqueous propylene glycol systems (20–80% v/v) containing methotrexate showed minimal variation (4.13–4.47). The solubility and pH of methotrexate USP in 50% v/v aqueous propylene glycol are listed in Table 1. A semi-log plot of solubility against pH showed a V-shaped curve with minimum solubility occurring around pH 4.

The equations 2 and 3, respectively, describe the variation of molar solubility of methotrexate as a function of molar concentration of hydrochloric acid and potassium hydroxide over the concentration range studied.

$$\ln(\text{M of HCl}) = 1626 \times (\text{molar solubility of MTX}) - 8.5571\ldots \quad (2)$$

$$\text{Molar solubility of MTX} = 0.1930\ (\text{M of KOH}) + 0.0013\ldots \quad (3)$$

TABLE 1

SOLUBILITY AND pH OF METHOTREXATE U.S.P. IN 50% v/v PROPYLENE GLYCOL IN AQUEOUS MEDIUM

Aqueous medium	pH			Solubility 22 ± 0.5°C (mg/mg)
	Aqueous medium	50% v/v PG in aqueous medium	Satd. soln. of MTX in 50% v/v PG–aqueous med.	
0.001 M potassium hydroxide	11.02	10.62	4.33	0.6536
0.01 M potassium hydroxide	12.12	11.78	5.29	1.4776
0.02 M potassium hydroxide	12.18	12.06	5.84	2.4188
0.05 M potassium hydroxide	12.56	12.48	6.34	4.9636
0.001 M hydrochloric acid	3.02	3.22	3.87	0.4633
0.005 M hydrochloric acid	2.36	2.53	2.98	0.8976
0.01 M hydrochloric acid	2.14	2.31	2.56	1.1211
0.03 M hydrochloric acid	1.72	1.88	1.98	1.4059

The penetration data for saturated solution of methotrexate in 50% propylene glycol in water (Table 2) were analyzed by plotting Q (amount penetrated per unit area) against time. The distribution of methotrexate at the end of 14 days is shown in Table 3. From the results, it is quite evident that methotrexate is absorbed to a widely variable extent by excised human skin specimens from abdominal region of different donors. The rate of drug penetration through skin samples with damaged stratum corneum was 5–10 times that reported for intact skin samples.

These results demonstrate that the intact stratum corneum is probably the principle effective barrier for the passage of methotrexate through excised human skin. The water-bearing tissues of dermis offer the least resistance. Furthermore, the diffusional process through skin could be regarded as the rate-limiting step and not

TABLE 2

IN VITRO SKIN PENETRATION DATA OF METHOTREXATE

Set [a]	Run [b]	Applied concentration [d] (μg/ml)	Steady-state penetration rate [c] ($\mu g \cdot cm^{-2} h^{-1}$)	Lag time (h)
I	1	627.1	0.1992	190
II	2	566.5	0.1272	158
	3	566.5	0.1122	176
III	4	566.6	0.2190	187
	5	566.5	0.1015	223
IV	6	627.1	0.3692	–
	7	627.1	0.3663	80

[a] Set numbers refer to skin specimens from different donors.

[b] Runs 2 and 3, 4 and 5, and 6 and 7 are duplicate runs.

[c] All steady-state values were computed from the regression line drawn from the data for each run by using a TI-55 calculator; $r > 0.990$

[d] Saturated solution of methotrexate in 50% propylene glycol–water vehicle.

TABLE 3

RELATIVE DISTRIBUTION OF METHOTREXATE 14 DAYS AFTER APPLICATION

Set [a]	Run [b]	Amount penetrated (%)	Washings of epidermis (%)	Applied dose recovered		Accountability (%)
				% Epidermis	% Dermis	
I	1	23.79	49.22	13.88	11.56	98.45
II	2	41.75	48.47	2.77	1.87	94.86
	3	33.56	54.27	3.45	1.76	93.04
III	4	14.34	62.85	1.65	2.16	81.00
	5	5.43	84.88	2.18	0.88	93.37
IV	6	45.72	44.58	1.03	2.26	93.59
	7	34.80	52.10	1.02	2.53	90.45

[a] Set numbers refer to skin specimens from different donors.
[b] Runs 2 and 3, 4 and 5, and 6 and 7 are duplicate runs.

the release of drug from the vehicle. It is possible that the values for distribution of methotrexate at the end of the 14-day period could reflect relative solubilities rather than permeability differences. Some of the studies on percutaneous penetration of methotrexate (Newbold and Stoughton, 1972; McCullough et al., 1976) were carried out for comparatively shorter periods of time not exceeding 20 h. The results of the present work, especially the observed high lag times (100–200 h) demonstrate the inability of methotrexate to reach significant steady-state levels over short periods of time. The penetration rates calculated from data based upon short durations of study could lead to erroneous conclusions. The high values of lag times indicate the importance of prolonged exposure to methotrexate in order to achieve steady-state and possibly therapeutically effective concentrations in clinical situations.

Effect of pH on in vitro percutaneous penetration

If passive diffusion is the predominant mechanism for percutaneous penetration of methotrexate, then changes in vehicle pH would be expected to influence the in vivo percutaneous absorption of methotrexate through human cadaver skin, since transport by passive diffusion is generally maximized when the drug is present in the unionized form. A set of 5 saturated solutions of methotrexate in 50% v/v propylene glycol–water media having pH values of 1.98, 2.98, 3.87, 5.29 and 6.34 were chosen for this study. A saturated solution of methotrexate in 50% propylene glycol–water (pH 4.12) was used as control. Skin diffusion studies were conducted in an identical manner to that described before in this text. In all, three sets of penetration experiments were carried out. In each set, six skin diffusion studies were conducted —one for each pH. The limitation was dictated by the size of the skin samples available from the same site. While steady-state penetration rates showed considerable variation between different skin specimens, a clear trend towards increasing rates of penetration with increasing pH was evident.

In the pH range 1–3, both the rate and extent of drug penetration were low while the amount of methotrexate retained by stratum corneum was quite high (Tables 4

TABLE 4

EFFECT OF pH ON RELATIVE DISTRIBUTION OF METHOTREXATE 14 DAYS AFTER APPLICATION

pH	Applied concentration (μg/ml)	Amount penetrated [a] (%)	Washings of epidermis [a] (%)	Applied dose recovered [a]		Percent accountability [a]
				% Epidermis	% Dermis	
1.98	1405.9	11.27	49.48	21.58	3.22	85.5
2.98	897.6	14.84	67.22	4.45	2.14	88.65
3.87	463.3	46.94	53.80	1.36	2.88	104.98
4.12	566.5	31.62	53.17	1.23	2.31	88.34
5.29	1477.6	51.56	38.37	1.82	4.50	96.25
6.34	4963.6	24.69	73.26	1.05	4.36	103.36

[a] The values represent mean of three separate experiments.

and 5) suggesting drug binding with components of stratum corneum. However, caution should be used in linking the extent of ionization of the molecule with drug binding in the stratum corneum since it could not be established that the pH level in the tissues was similar to that of the applied solution. The nitrogens at position 1, 5 and 10 of the methotrexate molecule (Fig. 2) with respective pK_a values of 5.71, −1.5 and 0.5 (Poe, 1977) could be conceivably involved in this interaction since they would be positively charged in this pH range. It should be noted that for the system investigated, the pK_a values may be somewhat shifted from those reported for the aqueous system. Drug penetration by transepidermal as well as transfollicular routes would be adversely affected. Although the drug solubility in the vehicle was considerably lower at pH 4 than at pH 2–3, the steady-state rate and extent of penetration were higher: between pH 4.12 and pH 5.29 a sharp rise in drug solubility and penetration rate was observed. This would be consistent with the predicted

TABLE 5

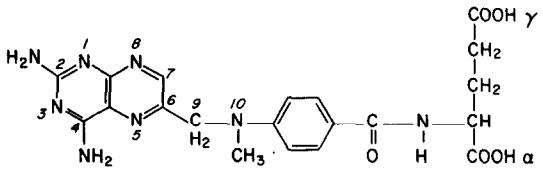
EFFECT OF pH ON IN VITRO PERCUTANEOUS PENETRATION OF METHOTREXATE

pH	Concentration [a] (μg/ml)	Steady-state penetration rate [b,c] ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	Lag time [b] (h)
1.98	1405.9	0.1588	59
2.98	897.6	0.1995	120.3
3.87	463.3	0.4064	101
4.12	566.5	0.3182	89
5.29	1476.6	1.6687	140
6.34	4963.6	2.5819	142

[a] Saturation solubility.

[b] The values represent average of three separate experiments.

[c] All steady-state values were computed from the regression line drawn from the data for each run at each pH by using a TI-55 calculator; $r = 0.990$.



4-Amino-N^{10}-methyl pteroylglutamic acid

Fig. 2. Chemical structure of methotrexate.

optimal concentrations of unionized methotrexate. At pH 6.34 the concentration of unionized drug would be very low (pK_a α-carboxyl = 4.3, pK_a γ-carboxyl = 5.5) (Wallace et al., 1978) yet the steady-state rate of penetration was highest observed. The lag times were also large. Relatively high solubility and gradually increasing contribution of shunt pathways probably account for this result. The earlier work of Wallace and Barnett (1978) has provided evidence for the existence of parallel penetration pathways for methotrexate. The pH values between 4 and 5 would appear to provide the most favorable environment for passive diffusion since the concentration of unionized methotrexate would be optimal in this pH range.

Acknowledgements

Presented at the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences 33rd National Meeting (San Diego, 1982). Abstracted in part from the dissertation of R.V. submitted in partial fulfillment of the Doctor of Philosophy degree requirements at the School of Pharmacy, University of the Pacific, Stockton, CA. Grateful acknowledgement is extended to Dr. Richard E. Jones, Genentech, South San Francisco and to Dr. Stanley Penzotti, Lederle Laboratories, NY.

References

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

Condit, P.T., The onset of action of amethopterin. Science, 134 (1961) 1421.

Flaxman, A.B., Harper, R.A. Chiarello, S. and Feldman, A.M., Effects of methotrexate on proliferation of human keratinocytes in vitro. J. Invest. Dermatol., 68 (1977) 66–69.

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

Iyer, B.V. and Vasavada, R.C., In vitro skin penetration of triamcinolone acetonide from lanolin alcohols–ethyl cellulose films. Int. J. Pharm., 3 (1979) 247–260.

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

Newbold, P.C.H. and Stoughton, R.B., Percutaneous absorption of methotrexate. J. Invest. Dermatol., 58 (1972) 319–322.

Nurse, D.S., Effects of antimetabolites in epidermal structures. Arch. Dermatol., 87 (1963) 258–265.
Poe, M., Acidic dissociation constants of folic acid, dihydrofolic acid and methotrexate. J. Biol. Chem., 252 (1977) 3724–3728.
Poulsen, B.J., Young, E., Coquilla, V. and Katz, M., Effect of topical vehicle composition on the in vitro release of fluocinolone acetonide and its acetate ester. J. Pharm. Sci., 57 (1968) 928–933.
Stewart, W.D., Wallace, S.M. and Runitis, J.O., Absorption and local action of methotrexate in human and mouse skin. Arch. Dermatol., 106 (1972) 357–361.
Tong, W.P., Rosenberg, J. and Ludlum, D.B., Purity of methotrexate. Lancet., Oct. 11 (1975) 719.
Wallace, S.M., Runikins, J.O. and Stewart, 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. and Barnett, G., Pharmacokinetic analysis of percutaneous absorption: evidence of parallel penetration pathways for methotrexate. J. Pharmacokin. Biopharm., 6 (1978) 315–325.
Weinstein, G.D. and Velasco, J., Selective action of methotrexate in psoriatic epidermal cells. J. Invest. Dermatol., 59 (1972) 121–127.
Weinstein, G.D. and McCullough, J.L., Effect of methotrexate esters on normal and psoriatic skin. Arch. Dermatol., 111 (1975) 471–475.