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DEVELOPMENT AND OPTIMIZATION OF A METHOTREXATE TOPICAL FORMULATION

George C. Hwang, Alice Y. Lin, Wen Chen, and Richard J. Sharpe
Arcturus Pharmaceutical Corporation, 260 West Cummings Park
Woburn, MA 01801

ABSTRACT

A topical methotrexate (MTX) formulation that would achieve optimal drug buildup in the epidermis and diminish potential systemic toxicity could be of great utility in the treatment of a variety of hyperproliferative skin disorders. In an attempt to develop an optimized MTX topical formulation containing pharmaceutically acceptable excipients, a 2^3 factorial design was used to investigate the effects of a fatty alcohol, propylene glycol, and ethanol on the *in vitro* skin permeation and uptake of MTX. *In vitro* skin permeation studies were performed using excised human epidermis mounted in flow-through diffusion cells. The results of steady-state flux and skin uptake of MTX from these formulations ranged from 0.035 to 0.315 $\mu g/cm^2/hr$ and 1.146 to 7.929 $\mu g/cm^2$, respectively. Response surface analysis was used to determine the optimum formulation in terms of skin permeation and uptake of MTX.

An optimized cream formulation was developed and compared to a gel formulation containing 3% Azone in hairless mice to evaluate the uptake of MTX in the treated and untreated skin sites as well as the distribution of MTX in the blood and liver following topical application. The results of the *in vivo* study demonstrated the localization of MTX at the treated site for both formulations without significant uptake of MTX in the distant untreated epidermis and dermis. The levels of MTX in the blood and liver following topical application of the optimized cream were significantly less than those of the gel formulation with 3% Azone.

INTRODUCTION

Methotrexate (MTX) is an effective systemic chemotherapeutic agent for the treatment of psoriasis and possibly cutaneous T-cell lymphoma (CTCL). However,

the risk of hepatic fibrosis and other systemic toxicity, such as bone marrow suppression, has precluded its use in all but the most severe cases of these diseases (1). In considering the potential severe toxicity associated with systemic administration of MTX, a topical formulation might be of greater utility for the treatment of psoriasis and other hyperproliferative skin disorders, including malignant, pre-malignant and benign conditions. Examples of possible disease targets for a topical formulation include basal cell and squamous cell carcinoma, Kaposi's sarcoma, certain metastatic tumors to the skin, actinic keratosis, and warts in addition to psoriasis and CTCL. Such a topical formulation must be optimized for skin flux to reduce systemic toxicity. To be therapeutically effective in treating hyperproliferative skin disorders, the topical formulation must achieve sufficient skin uptake to elicit a clinical response.

The results of clinical trials of various MTX topical preparations for the treatment of psoriasis have been variable (2-6) despite data suggesting that MTX acts directly on the psoriatic plaque rather than systemically at a distant site (7-8). The variable clinical effectiveness of topical MTX therapy in psoriasis might be attributed to the following two main factors: (i) the high resistance of the stratum corneum to MTX which prevents sufficient percutaneous absorption of MTX necessary to inhibit epidermal hyperproliferation, and (ii) the rapid clearance of MTX from viable epidermis to the dermis followed by rapid uptake into the dermal vasculature (9,10).

Various vehicles and skin permeation enhancers have been utilized to improve the percutaneous absorption of MTX (11-15). A recent study has shown that a topical formulation of MTX with Azone (1-dodecylazacycloheptan-2-one), a known skin permeation enhancer, has somewhat beneficial effects in the treatment of psoriasis (16). However, little effort has been made to maximize the skin uptake of MTX in the development of topical formulations.

The objective of this project was to use GRAS (Generally Recognized As Safe) components for the development of an optimal topical MTX formulation which could achieve therapeutically effective skin uptake of MTX with minimum skin flux. A factorial study was performed to investigate the effects of key components in the formulation on the *in vitro* skin permeation and uptake of MTX. In addition, *in vivo* percutaneous absorption studies were performed in hairless mice to evaluate the uptake of MTX in the treated and untreated skin sites as well as the distribution of MTX in the blood and liver following topical application of the optimized formulation.

MATERIALS

MTX was obtained from Orion Corporation, Fermion (Finland). ^{3}H-MTX was purchased from New England Nuclear (MA) at a specific activity of 38.3 Ci/mmol and purity of 99.7%. Azone was provided by Whitby Research Inc. (VA). All excipients used in the topical formulations were supplied by Spectrum

Chemical Manufacturing Corp. (CA) and all conformed to USP and/or NF requirements. Skin samples for *in vitro* skin permeation/uptake studies were obtained from healthy females undergoing reduction mammoplasty operations. Hairless mice (male and female, strain SKH-1, 7-9 weeks old, Charles River Laboratories, MA) were used in the *in vivo* percutaneous absorption study.

METHODS

Formulation Development and Optimization

Preliminary studies had demonstrated that the major excipients in the topical formulation affecting the *in vitro* skin permeation and uptake of MTX were a fatty alcohol (X_1), propylene glycol (X_2), and ethanol (X_3). A 2^3 factorial design was used to examine the effects of low (-1) and high (+1) levels of these independent variables on the *in vitro* skin permeation and uptake of MTX. As presented in Table 1, eight different formulations containing 0.44% (w/w) MTX and ^{3}H-MTX with various levels of the above three components in an aqueous gel vehicle were prepared for *in vitro* skin permeation and uptake studies.

The formulation was further optimized based on the following considerations: (i) *in vitro* skin permeation and uptake of MTX, (ii) physical stability of the formulation, (iii) cosmetic/aesthetic quality, and (iv) *in vivo* percutaneous absorption of MTX.

In Vitro Skin Permeation Study

In vitro skin permeation studies were performed using excised human epidermis mounted in flow-through diffusion cells (Crown Bio Scientific, NJ). The epidermal layer was separated from full-thickness skin after heating the skin in 60°C water for one minute. A circle piece of the resultant epidermal membrane was placed on the ledge of the receiver cell and the top was held tightly in place by the donor cell. Approximately 0.1 g of test formulations were applied to the surface of the stratum corneum. A 0.05M isotonic phosphate buffer (pH 7.4) was continuously pumped through the receiver cell at the flow rate of 2 ml/hr and maintained at 32 ± 0.5°C. The amount of MTX permeated through the epidermis was measured at various time points, up to 20 hours, by liquid scintillation counting of the receiver fluid.

In Vitro Skin Uptake Study

At the end of the skin permeation studies, the residuals of the test formulations on the epidermal surface were removed by rinsing with 50% ethanol solution. The epidermal membranes were then removed from the diffusion cells. The epidermal membranes were then wiped and rinsed with 50% ethanol solution and distilled water to remove any traces of MTX that had not permeated into the

TABLE 1
A 2^3 Factorial Design for Three Variables in MTX Topical Formulations

Formulation No.	Variable Level		
	X_1[a]	X_2[b]	X_3[c]
1	-1	-1	-1
2	-1	-1	-1
3	-1	+1	-1
4	-1	+1	+1
5	+1	-1	-1
6	+1	-1	+1
7	+1	+1	-1
8	+1	+1	+1

[a] % (w/w) of the fatty alcohol: 1 and 9.
[b] % (w/w) of propylene glycol: 10 and 40.
[c] % (w/w) of ethanol: 0 and 30.

skin. The epidermal membrane was solubilized in 1 ml of Solvable solution (DuPont, DE) and the amount of MTX retained in the epidermis was quantified by liquid scintillation counting.

In Vivo Percutaneous Absorption Study

Approximately 50 mg of the test formulation containing 0.44% (w/w) of MTX and ^{3}H-MTX (7-10 x 10^{-4} μCi/μg) was applied to the backs of hairless mice in a Hill Top Chamber (Hill Top Biolabs, Inc., OH) which was glued to the skin by a cyanoacrylate adhesive. The chamber was further secured in place with a non-irritating adhesive tape (Tegaderm, 3M, MN). After 20 hours of application, mice were anesthetized and blood samples were collected by cardiac puncture. Mice were sacrificed by cervical dislocation and then exsanguination. The treated skin surface was wiped with wet gauze and stripped twice with Scotch tape (3M, MN) to remove any traces of MTX that had not permeated into the skin.

Skin biopsies (0.92 cm^2) were taken from the treated and distant untreated sites, and the epidermis was separated from the dermis by heating the skin on a 60°C hot plate for one minute. A small portion of liver was dissected and weighed (approximately 200-300 mg) from each animal. The blood and tissue samples were solubilized in Solvable solution and decolorized by 30% H_2O_2 as needed. MTX levels in the blood, epidermis, dermis, and liver were quantified by liquid scintillation counting.

RESULTS AND DISCUSSION

Development of MTX Topical Formulations

Three pharmaceutically acceptable excipients, a fatty alcohol, propylene glycol, and ethanol in an aqueous gel vehicle, were evaluated for their effects on the *in vitro* skin permeation and uptake of MTX by a 2^3 factorial design. As shown in Table 2, the results of steady-state flux and skin uptake of MTX from eight formulations ranged from 0.035 to 0.315 $\mu g/cm^2/hr$ and 1.146 to 7.929 $\mu g/cm^2$, respectively. The steady-state flux and skin uptake of MTX were changed significantly by altering the composition of the formulations.

The optimization procedure was facilitated by fitting an empirical equation to the experimental results. The equation that can be constructed from a 2^3 factorial experiment is of the following form:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad \text{[Eq. 1]}$$

where Y_i is the measured response (steady-state flux or skin uptake of MTX), β_i is the regression coefficient, and X_i is the coded level of the independent variable.

Table 3 summarizes the results of the regression analysis after fitting Eq. 1 to each measured response. Response surfaces (Figures 1 and 2) were generated from the best regression models. Regression analysis and response surface plotting were performed with the Statgraphics software package (version 7 for DOS, Manugistics, MD). Figures 1 and 2 illustrate the effects of excipients on the steady-state flux and skin uptake of MTX, respectively, while one of the independent variables is fixed at the high level.

As indicated by the magnitude of the regression coefficients in Table 3 and visual inspection of the response surfaces, ethanol (X_3) had the greatest effect on the *in vitro* skin permeation and uptake of MTX. As the level of ethanol was increased in the formulation, the steady-state flux and skin uptake of MTX were increased. However, increasing the levels of the fatty alcohol (X_1) and propylene glycol (X_2) generally resulted in a decrease of skin uptake of MTX. Within the inference space of the design, the fatty alcohol and propylene glycol had little effect on the steady-state flux of MTX. Significant interactions between excipients were evident for the steady-state flux and skin uptake of MTX. The two-level design can make predictions only in a linear fashion and these are usually an approximation. If curvature in the response with changes in independent variables is present, the response may be misrepresented outside the confines of the design.

Optimization of MTX Topical Formulations

The optimum topical MTX formulation was defined as one having high skin uptake and minimum flux of MTX as well as acceptable physical stability and cosmetic/aesthetic quality. From the above results, a gel formulation (designated

TABLE 2
Results of *In Vitro* Skin Permeation and Uptake Studies for MTX Topical Formulations

Formulation No.	Steady-State Flux[a] ($\mu g/cm^2$)	Amount of MTX in Epidermis[a] ($\mu g/cm^2$)
1	0.051 ± 0.014	1.146 ± 0.293
2	0.315 ± 0.208	7.929 ± 2.235
3	0.053 ± 0.026	2.209 ± 0.284
4	0.196 ± 0.083	4.284 ± 0.535
5	0.035 ± 0.008	2.087 ± 0.828
6	0.219 ± 0.054	5.059 ± 0.529
7	0.120 ± 0.045	1.428 ± 0.188
8	0.244 ± 0.053	4.167 ± 1.692

[a] Average ± SD of 3 determinations

TABLE 3
Summary of Regression Results for the Measured Response

	Regression coefficient value for response Yi	
Coefficient	Steady-State Flux	Skin Uptake
$ß_0$	0.1541	3.5529
$ß_1$	N[a]	-0.3677
$ß_2$	N	-0.5025
$ß_3$	0.0892	1.8211
$ß_{11}$	N	N
$ß_{12}$	0.0281	N
$ß_{23}$	-0.0232	-0.6175
$ß_{13}$	N	-0.3933
$ß_{123}$	N	0.5593
r[b]	0.8048	0.9229
F[c]	11.0308	14.3650
p[d]	0.0002	0.0001

[a] N indicates that the regression coefficient was not significant at $\alpha < 0.2$
[b] Multiple correlation coefficient
[c] Mean square regression/mean square residual
[d] The attained significance level for the model

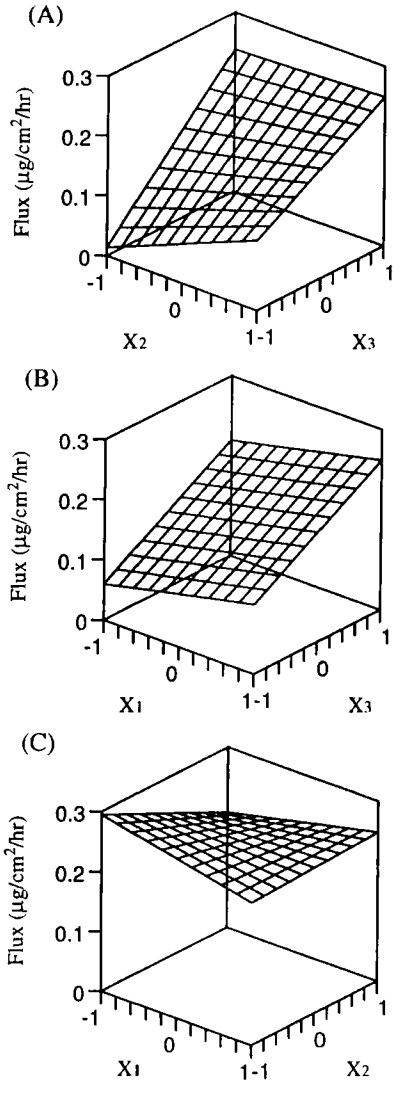

FIGURE 1

Response surfaces showing the effects of various levels of two excipients on the steady-state flux of MTX while the third excipient, X_1-a fatty alcohol (A); X_2-propylene glycol (B); X_3-ethanol (C), is fixed at the high level.

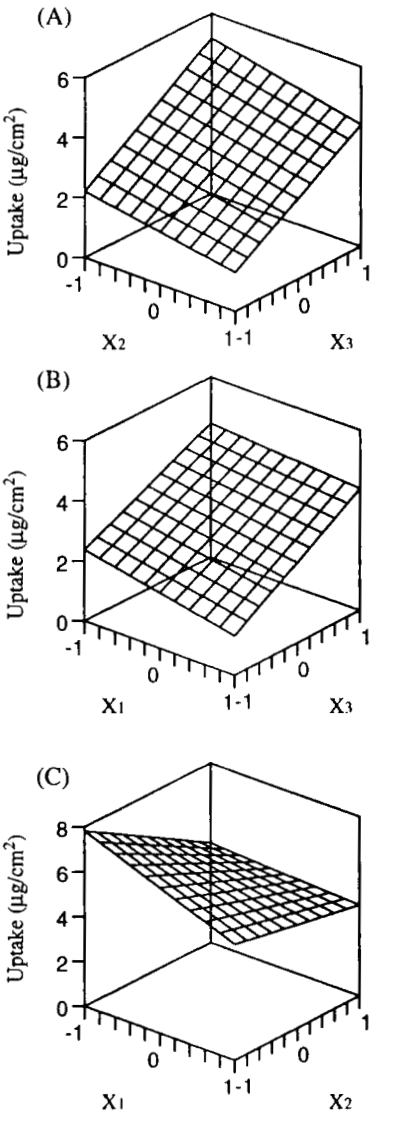

FIGURE 2

Response surfaces showing the effects of various levels of two excipients on the skin uptake of MTX while the third excipient, X_1-a fatty alcohol (A); X_2-propylene glycol (B); X_3-ethanol (C), is fixed at the high level.

as A) containing 0.44%(w/w) of MTX and the optimum levels of the fatty alcohol, propylene glycol, and ethanol was prepared. In addition, two other types of topical formulations containing the same concentration of MTX were prepared to evaluate the effects of different types of semisolid vehicles on the *in vitro* skin permeation and uptake of MTX. The compositions of these three prototype formulations are listed as follows.

1. Formulation A - an aqueous gel containing the fatty alcohol, propylene glycol, ethanol, antioxidant, and preservatives.
2. Formulation B - a cream containing the fatty alcohol, propylene glycol, nonionic primary emulsifiers, secondary emulsifier, antioxidant, and preservatives.
3. Formulation C - a modified PEG ointment containing a fatty acid, propylene glycol, and isopropyl myristate.

The values of *in vitro* steady-state flux and skin uptake of MTX obtained from the above three formulations are presented in Table 4. The results indicated that formulation C had the highest flux but the lowest skin uptake among the three formulations. The cream formulation (B) could achieve comparable skin uptake of MTX to the gel formulation (A) with less flux through the skin.

Based on the consideration that the high alcohol content of the gel formulation might cause skin irritation in long-term therapy and potential difficulty in its large-scale manufacture, formulation B was selected for further development and optimization.

Three other formulations were developed by modifying the key components in formulation B in an attempt to attain an elegant topical product with optimal skin flux and uptake of MTX. The variation of components in these formulations is listed in Table 5.

A phase separation was observed in the preparation of formulation B2, hence this formulation was not examined in the *in vitro* skin permeation study. The results of steady-state flux and skin uptake of MTX for the other formulations are summarized in Table 6. The results indicated that steady-state flux and skin uptake of MTX were not significantly different among these formulations. However, based on the evaluation of cosmetic/aesthetic qualities, formulation B1 was selected as the potential optimal formulation for further evaluation.

It has been suggested by Siddiqui et al. (9) that a flux of about 60 ng/cm^2/hr is required from the stratum corneum to achieve the minimum effective concentration of MTX for the management of psoriasis. The study results above indicated that the optimized MTX cream formulation achieved this desirable skin flux. This minimal flux is of importance because it may be associated with limited systemic absorption of MTX and therefore reduce the potential for systemic toxicity.

In Vivo Percutaneous Absorption Study

The variable clinical efficacy of topical MTX therapy in psoriasis might be attributed to the rapid dermal clearance of the drug, thereby producing an inadequate concentration in the epidermis to inhibit the epidermal hyperproliferation (9). Since

TABLE 4
In Vitro Steady-State Flux and Skin Uptake of MTX for Three Prototype Formulations Containing 0.44%(w/w) MTX

Formulation	Steady-State Flux ($\mu g/cm^2/hr$)	Amount in Epidermis ($\mu g/cm^2$)
A	0.093 ± 0.087 (n=3)	10.321 ± 2.367 (n=4)
B	0.073 ± 0.028 (n=7)	5.898 ± 0.660 (n=6)
C	0.192 ± 0.051 (n=3)	2.467 ± 1.130 (n=3)

TABLE 5
Variation of Excipients in 0.44% MTX Cream Formulations

	% (w/w) Concentration		
Formulation	Fatty Alcohol	Secondary Emulsifier	White Wax
B	5	8	0
B1	10	0	3
B2	10	3	0
B3	10	3	3

TABLE 6
Effects of Excipients on *In Vitro* Steady-State Flux and Skin Uptake of MTX

Formulation	Steady-state Flux ($\mu g/cm^2/hr$)	Amount in Epidermis ($\mu g/cm^2$)
B	0.073 ± 0.028 (n=7)	5.898 ± 0.660 (n=6)
B1	0.061 ± 0.008 (n=6)	3.576 ± 1.453 (n=6)
B3	0.087± 0.014 (n=4)	4.058 ± 0.610 (n=4)

TABLE 7
Comparison of MTX Levels in Blood, Liver and Skin Following Application of 0.44%(w/w) MTX Topical Formulations in Hairless Mice

Tissue (MTX level)[a]	Optimized Cream	Azone Containing Gel
Treated Epidermis ($\mu g/cm^2$)	3.10 ± 0.84	4.96 ± 1.32
Treated Dermis ($\mu g/cm^2$)	1.14 ± 0.39	1.94 ± 0.70
Untreated Epidermis ($\mu g/cm^2$)	0.04 ± 0.02	0.01 ± 0.01
Untreated Dermis ($\mu g/cm^2$)	0.10 ± 0.08	0.02 ± 0.01
Blood ($\mu g/ml$)	0.07 ± 0.02	0.24 ± 0.15
Liver ($\mu g/g$)	0.49 ± 0.17	1.07 ± 0.49

[a] Average ± SD of 5 determinations

the dermal clearance cannot be assessed by *in vitro* skin permeation experiments, the need to perform an *in vivo* percutaneous absorption study in the development of an effective MTX topical formulation was indicated.

Therefore, the optimized cream formulation (B1) was further evaluated by comparing to a gel formulation containing 3%(w/w) Azone in an *in vivo* percutaneous absorption study. Table 7 summarizes the comparison of MTX levels in blood, liver and skin between the two formulations. The amount of MTX found in the epidermis is approximately two times that found in the dermis for both formulations. The amounts of MTX found in the epidermis and dermis treated with the optimized cream are approximately 60% of those treated with the Azone containing gel. The MTX levels in the blood and liver following topical application of the optimized cream are significantly less than those of the formulation containing Azone. The results indicated that the optimized cream formulation could achieve a comparable MTX concentration in the skin with less systemic absorption compared to the formulation containing Azone.

CONCLUSIONS

The use of the factorial experimental design is valuable in the development and optimization of complex topical formulations, particularly when components are believed to interact significantly. The optimized cream formulation demonstrated the localization of MTX in the treated skin without significant uptake of MTX in the untreated epidermis and dermis in the hairless mouse. Also, the optimized cream formulation showed less systemic absorption of MTX than the Azone containing formulation. A further evaluation of the optimized MTX topical formulation in human clinical trials of malignant, pre-malignant, and benign hyperproliferative skin diseases is warranted.

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