Drug Development and Industrial Pharmacy, 23(1), 99-106 (1997)

COMMUNICATION

Effect of Various Physical/Chemical Properties on the Transdermal Delivery of Cyclosporin Through Topical Application

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ABSTRACT

The purpose of this study was to evaluate the effect of (A) skin stripping (B) transdermal enhancer and (C) iontophoresis, on the in vitro transdermal delivery of cyclosporin. An in vitro transdermal study through hairless mouse skin using a selected cyclosporin topical formulation was also conducted. Results show that the permeation coefficient of cyclosporin was increased as the skins were stripped more times. Among the transdermal enhancers, azone, salicyclic acid, dimethyl sulfoxide, sodium lauryl sulfate and Tween 20; azone, and dimethyl sulfoxide were found to significantly increase the cyclosporin delivery; while salicylic acid, sodium lauryl sulfate and Tween 20 had no apparent effects. In further studies to define the optimum concentration of the above enhancers, the greatest effect was determined to be 1% for azone and 5% for dimethyl sulfoxide. Constant voltage iontophoresis was proven to be effective in enhancing the cyclosporin transdermal delivery. Data show that an increase in the permeability was observed when the voltage was increased from 1 to 7 V. The results of in vivo topical application of a selected cyclosporin formulation to hairless mouse skin indicate that both blood and skin concentration reached maximum at about 36 hr after application, and that the cyclosporin concentration in the skin was constantly higher (10 times at the peak maximum) than its corresponding blood concentration at the same time intervals.

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INTRODUCTION

Psoriasis is a common dermatological disease characterized by areas of thickened, erythematous skin lesions. The lesions are most often seen on the back, elbows, knees, and scalp. They may present in various forms clinically, but they are classically raised, nonitching, silvery white, and scaly. When removed, a needle-type bleeding spot appears and is called the Auspitz sign (1). The cause for psoriasis is not clear at this moment; however, about one third of the patients have a family history of psoriasis and it is believed to be associated with genetics.

Cyclosporin (cyclosporin A) is a cyclic undecapeptide of fungal origin. It is one of a group of cyclosporins produced by the fungus *Tolypocladium inflatum* Gams or *Cylindrocarpon lucidum* Booth. Cyclosporin is poorly soluble in water (<0.04 mg/ml at 25°C), but is generally soluble in lipids and alcohol (2). It is pharmacologically active as an immunosuppressive agent and is normally used in the prevention of organ rejection following transplant surgery. The successful use of cyclosporin in the treatment of psoriasis was first described by Muller and Herrmann (3). Further cases of successful induction of remission in severe palmoplantar psoriasis (4) and pustular psoriasis (5) by the administration of cyclosporin were also reported. The clinical efficacy of cyclosporin in severe psorisis is now well recognized (6). An oral solution of 100 mg/ml; soft gelatin capsules of 25 mg, 50 mg, and 100 mg; and an intravenous injection of 50 mg/ml are currently available in the market for the treatment of psoriasis. Although oral cyclosporin has shown significant effects on the treatment of psoriasis, unsteady absorption and poor bioavailability often create unpredictable results (7). Furthermore, severe side effects caused by the prolonged use of oral cyclosporin have made this therapeutical route questionable. Topical application of cyclosporin has regenerated the researcher's interest since it not only targets the local lesions directly with little bioavailability problem but reduces the systemic side effects from oral administration.

The purpose of this study was to evaluate the feasibility of topical application of cyclosporin and also to study the effects of various transdermal enhancers and iontophoresis on the transdermal delivery of cyclosporin across hairless mouse skin. An in vivo topical application of cyclosporin in a selected formula was also studied.

EXPERIMENTAL

Materials

The following materials were used: cyclosporin oral solution (Sandoz Pharm., Ltd., USA, lot 657MFD0491); 0.9% sodium chloride solution (Nan Kwang Chem., Taiwan, lot D2100C); acetonitrile (Mallinckrodt Chem., USA, lot UN1648); methanol (Mallinckrodt Chem., USA, lot UN1230); azone (Whiby Research Inc., USA, lot 2810K); sodium lauryl sulfate (Santoku Chem., Japan, lot 800712); Lutrol® FC127 (Hui Ming Pharm., Inc., Taiwan, lot 17B222Q); olive oil (Wako Chem., Japan, lot PTN 5382); polyoxyethylene 20 oleyl ether (Brij® 99, Sigma Chem., USA, lot 62H3529); Scotch Magic Tape™ 810 (3M, USA, lot 7378-6); salicylic acid (Yakuri Chem, Japan, lot 365853); propylene glycol (Dow, USA, lot 44910); isopropyl myristate (Merck, Germany, lot 1294441); and polyoxyethylene 20 sorbitan monooleate (Tween® 20, Yakuri Chem., Japan, lot 35624404).

Methods

Preparation of Skin Samples

Male hairless mice aged from 6 to 8 weeks and weighing 24.1 ± 3.4 g (n = 24) were euthanatized by ether. Their abdominal skin was then cleaned and removed from the mouse body. The removed skin was further treated by removing extra-endodermal tissue and blood vessels. The skins were stored immediately after preparation at -20°C until use. The frozen skin was taken from the freezer and equilibrated to room temperature before being placed into the diffusion cells for transdermal studies.

In Vitro Transdermal Studies

A vertical diffusion apparatus (Tong Hong Instruments, Taiwan) consisting of the donor and jacketed receptor cells with an aperture of 0.785 cm^2 was used in the in vitro transdermal studies. For iontophoresis studies, two platinum electrodes with one end connected to a constant-voltage electric source (Model 7651, YoKogama Electric Corp., Japan) and the other end looped into a cross area of 0.1 cm^2 circle were inserted into the donor and receptor cells, respectively. The receptor cell was first filled with 10 ml 20/80 methanol/water solution followed by mounting the mouse skin between two cells. The donor cell was placed on top of the skin and filled with drug dosage to start the experi-

ment. The study temperature was maintained by circulating thermostated water through the jacketed receptor cell at 32 ± 0.5°C throughout the experiment. A sample of 0.2 ml at each designated time interval was withdrawn for analysis. The removed sample was immediately replaced with the same amount of 20/80 methanol/water solution and corrected for its concentration.

Selection of Cyclosporin Topical Formulation

Various topical formulations containing cyclosporin as an active ingredient at different concentrations were prepared according to the formulas listed in Table 1.

The topical solution from these formulations was placed into the donor cell of the diffusion device and the 20/80 methanol/water solution was used in the receptor cell. Mouse skin that had been stripped 20 times by Scotch tape was mounted between two half cells and used in this study. Samples were withdrawn from the receptor cell for a period of 24 hr and analyzed by a high-performance liquid chromatographic (HPLC) method. Formula 24, which consisted of 10% (v/v) cyclosporin oral solution, 30% water, 35% propylene glycol, 20% Lutrol FC 127, and 5% isopropyl myristate, was determined to deliver the most cyclosporin through the mouse skin and was selected to be the model formula for most of the studies.

Transdermal Enhancer Effect Study

Various types of enhancers including azone, salicylic acid, dimethyl sulfoxide, sodium lauryl sulfate, and Tween 20 were incorporated into the model formula at concentrations of 1%, respectively. Transdermal studies were conducted for each formula to determine the effect of different enhancers on the delivery of cyclosporin. Varying concentrations of enhancer in the range 0–10% for azone, 0–10% for dimethyl sulfoxide, and 0–10% salicylic acid, were used in the model formula, respectively, to determine the optimum concentration of each enhancer to achieve the highest transdermal rate of cyclosporin. A sample of 0.2 ml was removed from the receptor cell at each designated time interval for HPLC analysis. The removed sample was immediately replaced with the same amount of 20/80 methanol/water solution and corrected for concentration.

Skin Stripping Effect Study

The stripped skins were prepared by obtaining full mouse skins and stripping the surfaces with a tape for 10, 20, and 30 times, respectively. Topical cyclosporin solution was prepared by diluting 10 ml of cyclosporin oral solution (100 mg/ml) into 100 ml with olive oil to make 10 mg/ml. The mouse skin was mounted between donor and receptor cells. One milliliter of the diluted cyclosporin solution was pipetted into the donor cell of the diffusion apparatus with the receptor cell filled with 10 ml 20/80 methanol/water solution. A sample of 0.2 ml was removed from the receptor cell at each designated time interval for HPLC analysis. The removed sample was immediately replaced with the same amount of 20/80 methanol/water solution and corrected for concentration.

Iontophoresis Effect Study

The model cyclosporin topical solution was used in this study. The iontophoresis study was carried out by supplying a constant voltage of 1, 3, 5, and 7 V with a 1 hr on–1 hr off cycle for a total of 6 hr. A sample of 0.2 ml was removed from the receptor cell at each designated time interval for HPLC analysis. The removed sample was immediately replaced with the same amount of 20/80 methanol/water solution and corrected for concentration.

In Vivo Animal Study

Hairless mice age between 6 and 8 weeks, and of average weight of 22.3 ± 3.5 g (n = 6) were used. An area of 2.25 cm^2 in the abdominal site was cleaned for study. The model formula of cyclosporin was applied to the cleaned region of each hairless mouse for the duration of the designated time period. Blood samples of 0.3 ml to 0.5 ml were collected from the heart of the mouse immediately after euthanatization by cervical dislocation. Samples at initial time and 2, 24, 36, 48, 72, and 120 hr after dose administration were obtained for cyclosporin analysis. The skin area subjected to dose administration was also removed and cleaned for residual drug on the skin surface before analyzing for cyclosporin concentration in the skin tissue.

Cyclosporin Analysis

In vitro transdermal samples of cyclosporin were analyzed by a HPLC system. This system was equipped with an isocratic pump (Model LC-6A, Shimadzu, Japan), a C_{18} column (Lichrospher 100 RP-18.5μ, Merck, West Germany) maintained at 70 ± 1°C, an UV detector (Model SPD-6AV, Shimadzu, Japan) at 215 nm, an

Table 1

Composition of Various Cyclosporin Topical Solutions Used in the Transdermal Studies

Formula No.	Ingredients, % v/v					
	CyA[a]	Water	PG[b]	FC127[c]	IM[d]	Brij 99[e]
1	35	35	10	20	—	—
2	27	35	20	18	—	—
3	20	35	30	15	—	—
4	35	42	—	23	—	—
5	38	32	—	19	11	—
6	32	43	—	20	5	—
7	25	30	30	15	—	—
8	10	27	35	17	11	—
9	30	40	—	20	10	—
10	24	45	—	15	16	—
11	21	40	—	14	25	—
12	10	70	—	20	—	—
13	10	35	35	20	—	—
14	10	25	35	20	10	—
15	10	60	—	20	10	—
16	14	19	40	22	5	—
17	12	19	37	20	12	—
18	12	23	30	20	5	10
19	10	29	29	16	10	6
20	11	22	32	18	11	6
21	10	33	29	18	10	—
22	10	32	28	25	5	—
23	10	25	40	20	5	—
24	10	30	35	20	5	—

[a]Cyclosporin oral solution.
[b]Propylene glycol.
[c]Poloxamer 407 (Lutrol® FC127).
[d]Isopropyl myristate.
[e]Polyoxyethylene 20 oleyl ether.

autosampler (Model SIL-6A, Shimadzu, Japan), and a data station (Model C-R6A, Shimadzu, Japan). The mobile phase was composed of 30% (v/v) acetonitrile and 70% water, and delivered at a flow rate of 1.0 ml/min. The relative retention time was found to be 9.5 min. The correlation coefficient of the linearity in the concentration range of 0.06 μg/ml to 6 μg/ml was greater than 0.999. The within-day variation at the same concentration range was determined to be 1.55%.

In vivo transdermal samples of whole blood were examined by a fluorescence polarization immunoassay (FPIA) due to its high sensitivity of detection. A TDX analytical instrument (Abbott Laboratory) was used in this type of study. The performance of this analysis was checked by repeated injections of a control cyclosporin standard of 150 mg/ml. Results show that three determinations of 146.43 mg/ml, 140.84 mg/ml, and 136.60 mg/ml were all within the acceptable range of 135–165 mg/ml with reproducibility of less than 4%. The residual cyclosporin in the tissue after topical administration was analyzed also by a HPLC method. This method was the same as the method used for in vitro study except that a mobile phase of 65% acetonitrile and 35% water was employed. The relative retention time was found to be 12.5 min. The correlation coefficient of the linearity in the concentration range of 0.6 μg/ml to 24 μg/ml was greater than 0.999. The within-day variation at the same concentration was determined to be 1.36%.

The recovery of the tissue analysis was carried out by comparing the amount of cyclosporin determined from the skin after extraction to the actual amount of cyclosporin spiked into the skin. At concentrations of 0.6, 1.2, and 24.0 μg/ml, the average recovery rate was found to be 74%.

RESULTS AND DISCUSSION

The permeation coefficient of a drug through a skin barrier is obtained from the plot of the accumulated transported drug concentration against time. Dividing the slope of the above plot by skin contact area and the drug concentration in the donor site yields the permeation coefficient of the drug in the skin. The intercept of the plot in the time axis is the lag time for drug penetration. The apparent permeation coefficient (P) is calculated from the following equation:

$$P = \frac{(\Delta C)}{(S)(C_d)(\Delta t)}$$

where P is apparent permeation coefficient, ΔC is concentration gradient, S is the exposed area of diffusion, and C_d is the drug concentration in the donor cell.

Selection of Cyclosporin Topical Formulation

Preliminary screening of various formulas consisting of different ratios of cyclosporin oral solution, purified water, Lutrol FC127, propylene glycol, and isopropyl myristate was carried out. The formula from the above study which achieved the highest transdermal rate was selected as the candidate for further studies. Based on these results, formula 24—consisting of 10% (v/v) cyclosporin oral solution, 30% purified water, 20% Lutrol FC 127, 35% propylene glycol, and 5% isopropyl myristate—was selected as the model formulation for the other experiments conducted in this study.

Effect of Transdermal Enhancers

Transdermal delivery of cyclosporin in the model formula containing different 1% (w/v) transdermal enhancers is depicted in Table 2. The results show that the inclusion of azone or dimethyl sulfoxide in the model formula increased the transdermal delivery rate of cyclosporin, but salicylic acid, sodium lauryl sulfate and Tween 20 did not.

Table 2

Transdermal Rate of Cyclosporin in the Model Formula Containing Different Enhancers at 1% (w/v) Concentration

Skin Enhancer	Permeation Coefficient[a] (10^9 cm sec^{-1})
Control	0.38 ± 0.01
1% azone	2.65 ± 0.03
1% salicylic acid	0.31 ± 0.03
1% dimethyl sulfoxide	0.54 ± 0.01
1% sodium lauryl sulfate	0.40 ± 0.01
1% Tween 20	0.36 ± 0.03

[a] $n = 3$.

Further investigation to determine the most effective concentration of azone (0.5–10%), dimethyl sulfoxide (1–10%), and salicylic acid (0.5–5%) was carried out, and the results are shown in Tables 3–5. The optimum concentration of the enhancers with greatest enhancing effect of cyclosporin transportion in the studies was determined to be 1% for azone and 5% for dimethyl sulfoxide. Salicyclic acid was found to have no effect in the studied concentration range.

Effect of Skin Stripping

The effect of skin stripping on the transdermal delivery of cyclosporin from the model formula is shown in Fig. 1. The permeation coefficient was determined to be 0.24×10^{-9} cm sec^{-1}, 1.73×10^{-9} cm sec^{-1}, 5.70×10^{-9} cm sec^{-1}, and 9.04×10^{-9} cm sec^{-1} for the skins stripped 0, 10, 20 and 30 times, respectively. The results indicate that the permeation coefficient was increased more significantly as the skin was stripped more times.

Effect of Iontophoresis

The results of the iontophoresis effect under constant voltage on the cyclosporin transdermal rate through hairless mouse skin are depiected in Fig. 2. An increase in the total transported amount of cyclosporin was observed as the voltage of the electric source was increased from 1 to 7 V.

In Vivo Animal Study

The concentrations of cyclosporin in the blood and skin areas after topical application of the model formula to the hairless mouse skin are shown in Table 6.

Table 3

Effects of Azone Concentration on the Transdermal Rate of Cyclosporin

Azone Concentration (%, w/v)	Permeation Coefficient[a] (10^9 cm sec^{-1})
0.0	0.35 ± 0.05
0.5	1.91 ± 0.06
1.0	2.68 ± 0.01
2.0	0.82 ± 0.02
3.0	0.90 ± 0.04
4.0	0.79 ± 0.06
5.0	0.90 ± 0.04
6.0	0.60 ± 0.04
7.0	0.49 ± 0.03
8.0	0.49 ± 0.01
9.0	0.48 ± 0.02
10.0	0.49 ± 0.02

[a]$n = 3$.

Table 4

Effects of Dimethyl Sulfoxide Concentration on the Transdermal Rate of Cyclosporin

Dimethyl Sulfoxide Concentration (%, w/v)	Permeation Coefficient[a] (10^9 cm sec^{-1})
0.0	0.36 ± 0.02
1.0	0.50 ± 0.03
2.0	0.57 ± 0.04
5.0	0.59 ± 0.05
7.0	0.43 ± 0.02
10.0	0.34 ± 0.01

[a]$n = 3$.

Table 5

Effect of Salicylic Acid Concentration on the Transdermal Rate of Cyclosporin

Salicylic Acid Concentration (%, w/v)	Permeation Coefficient[a] (10^9 cm sec^{-1})
0.0	0.41 ± 0.02
0.5	0.26 ± 0.04
1.0	0.32 ± 0.00
2.0	0.36 ± 0.02
5.0	0.29 ± 0.04

[a]$n = 3$.

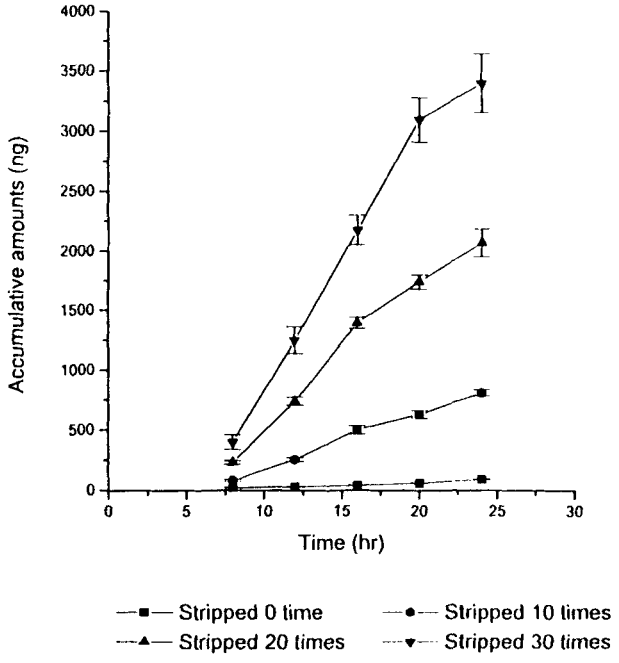

Figure 1. Effect of skin stripping on the transdermal delivery of cyclosporin from the model formula: ■, stripped 0 times; ●, stripped 10 times; ▲, stripped 20 times; ▼, stripped 30 times.

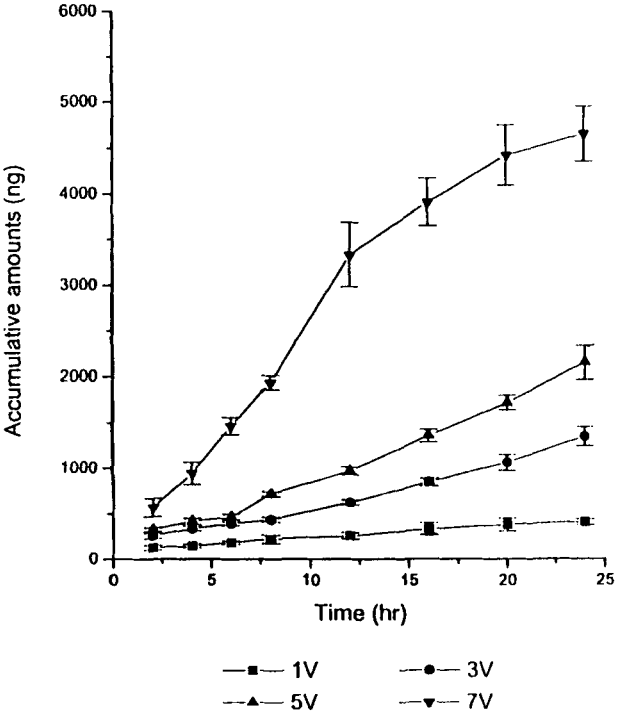

Figure 2. Effect of iontophoresis under constant voltage on the transdermal delivery of cyclosporin from the model formula: ■, 1 V; ●, 3 V; ▲, 5 V; ▼, 7 V.

The cyclosporin concentration–time curve in blood and tissue followed the same type of pattern. Maximum concentration was reached at 36 hr after administration and decreased dramatically afterward. Cyclosporin concentration in the skin was always 10 times higher than that in blood at the corresponding time intervals. This appears to indicate that topical application of cyclosporin for the treatment of psoriasis has a great potential; it not only localizes in the skin but reduces the risk of severe side effects which oral administration may cause.

Table 6

Concentrations of Cyclosporin in Whole Blood and Skin Tissue After In Vivo Administration of Cyclosporin Topical Solution

	Concentration[a] (ng/ml)	
Time (hr)	Whole Blood	Skin Tissue
12	17.99 ± 4.89	1046.4 ± 189.6
24	35.23 ± 11.59	2404.1 ± 1524.9
36	110.50 ± 49.83	12591.1 ± 6993.9
48	17.52 ± 4.26	2362.6 ± 1263.1
72	22.57 ± 5.73	2930.4 ± 1335.9
120	10.54 ± 3.18	2470.6 ± 386.6

[a] $n = 6$.

ACKNOWLEDGMENTS

The authors would like to gratefully thank the National Science Council, Republic of China, for the financial support of the following projects: NSC 81-0420-B016-08 and NSC 82-0412-B016-022.

REFERENCES

1. J. N. W. N. Baker, Lancet, 38, 227 (1991).
2. G. K. McEvoy (ed.), AHFS Drug Information 91, Am. Soc. Hosp. Pharm., Bethesda, 1991.

3. W. Muller and B. Herrmann, N. Engl. J. Med., 301, 555 (1979).
4. J. D. Bos et al., Lancet ii, 1500 (1989).
5. T. H. Van Joost et al., Br. Med. J., 114, 615 (1986).
6. H. M. Lewis et al., Br. J. Dermatol., 127(Suppl. 40), 18 (1992).
7. M. Lemaire, G. Maurer, and A. J. Wood, Pharmacokinet. Pharmacother., 11(5), 1303 (1991).