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Evaluation of In-vivo Transdermal Absorption of Cyclosporin with Absorption Enhancer Using Intradermal Microdialysis in Rats

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Abstract

The purpose of this study was to evaluate the effect of absorption enhancer on in-vivo transdermal absorption of cyclosporin using intradermal microdialysis in rats. Cyclosporin oily solutions (0·5, 2, 8% w/v) were prepared from Sandimmun (10% w/v oily oral preparation of cyclosporin) by diluting with olive oil. 1-[2-(Decylthio)ethyl]azacyclopentan-2-one (HPE-101) and glycerin were added to the cyclosporin formulation as an absorption enhancer at various concentrations between 1 and 20%. These formulations were applied to the shaved abdomen of rats treated with intradermal microdialysis at a flow rate of 2·5 μL min^{-1} for 6 h.

Cyclosporin was immediately detected and attained a plateau in the dermal dialysate after topical application of cyclosporin oily solution alone. Cyclosporin levels in the dialysate increased with increasing cyclosporin concentrations in the formulation from 0·5 to 8% (w/v). HPE-101 did not influence cyclosporin absorption at concentrations less than 6% (w/v). Addition of 10% (w/v) HPE-101 significantly enhanced an apparent absorption rate of cyclosporin by 4·9 times. However, 20% (w/v) HPE-101 did not show the enhancing activity. On the other hand, addition of glycerin at concentrations of 6, 10, and 20% (v/v) significantly enhanced an apparent absorption rate of cyclosporin by 3·0, 6·4, and 6·9 times, respectively. The time lag for cyclosporin absorption was less than 0·21 h in all tested cases.

This microdialysis study shows that glycerin is a suitable enhancer for improving the in-vivo cyclosporin absorption from the skin.

Cyclosporin is an efficient and clinically well established immunosuppressive agent. The systemic administration of cyclosporin has been shown to be extremely beneficial in treating several dermatological diseases, especially psoriasis; however, the systemic use of cyclosporin is limited by its toxic effects mainly on the kidney and liver (Heule et al 1992), and there has been considerable interest in the efficacy of topical use of cyclosporin for psoriasis. However, topical cyclosporin application has not shown the expected response in any clinical cases because of its low dermal penetrability.

Microdialysis is an in-vivo sampling technique permitting the determination of test substances with minimal tissue damage from the extracellular space of most body tissues. This technique allows direct monitoring of an unbound drug in the dermis in-vivo. In previous studies, we have successfully demonstrated the pharmacokinetics and transdermal absorption of drugs topically applied to rats by the use of an intradermal microdialysis technique (Matsuyama et al 1994a, b).

The aim of this study was to evaluate the effect of an absorption enhancer, 1-[2-(decylthio)ethyl]azacyclopentan-2-one (HPE-101) or glycerin, on the transdermal absorption of cyclosporin using an intradermal microdialysis technique in rats.

Materials and Methods

Materials

Sandimmun, 10% (w/v) oily oral preparation of cyclosporin, was purchased from Sandoz Pharma Ltd. (Basel, Switzerland). HPE-101 was supplied by Hisamitsu Pharmaceutical Co., Inc. (Tsukuba, Japan). Glycerin and olive oil were obtained from Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan). All other chemicals were of special reagent grade.

Various concentrations of cyclosporin oily solutions (0·5, 2, 8% w/v) were prepared from Sandimmun by diluting with olive oil. HPE-101 (1, 3, 6, 10, 20% w/v) or glycerin (1, 3, 6, 10, 20% v/v) was added to 2% (w/v) cyclosporin oily solution.

Microdialysis system

The microdialysis system consisted of a CMA/100 microinjection pump (Carnegie Medicin, Stockholm, Sweden) and CMA/10 microdialysis probes with a dialysis membrane (a length of 10 mm and an outer diameter of 0·5 mm)(Carnegie Medicin). The probe was connected to the microinjection pump and was perfused continuously with Ringer's solution (pH 6·5) at a rate of 2·5 μL min^{-1} during the experimental period.

Animal experiments

An intradermal microdialysis experiment was carried out as described previously (Matsuyama et al 1994a, b). Briefly, male SPF Wistar rats, 280–320 g, were anaesthetized with an intraperitoneal injection of urethane (1·5 g kg^{-1} as 300 mg mL^{-1}). The abdominal fur of the rats was shaved using a Thrive Model 900 animal clipper (Daitoh Electric Co., Tokyo, Japan), with care taken not to damage the skin. The skin was incised over the dermis in the shaved abdominal region, followed by the intradermal insertion of an introducer

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assembled by inserting the L-shaped needle into the tubing. After setting the introducer under the skin, the L-shaped needle was withdrawn, followed by insertion of a microdialysis probe. The tubing was torn off by pulling upward and outward. After completion of probe implantation, a hemispherical glass reservoir with an inner diameter of 20 mm was placed over the shaved abdominal region. At 1 h after probe implantation, 2 mL of drug solution was applied to the reservoir. The dialysate samples were collected into a small sample tube every hour for 6 h.

Analysis of cyclosporin in dialysate

The level of cyclosporin was analysed by the Abbott TDx automated analyser (Abbott Laboratories, Irving, USA) based on fluorescence polarization immunoassay. The TDx assay reagent set for cyclosporin was purchased from Abbott Laboratories (Abbott Park, USA).

Pharmacokinetic analysis

The apparent absorption profile across the skin was constructed by plotting the cumulative amount of cyclosporin in the dermal dialysate against time. The apparent rate of transdermal absorption was calculated from the slope of linear portion of the absorption profile. The lag time was defined as the intercept on the time axis extrapolated from the absorption profile as described previously (Matsuyama et al 1994b). Statistical analysis was carried out using Student's *t*-test.

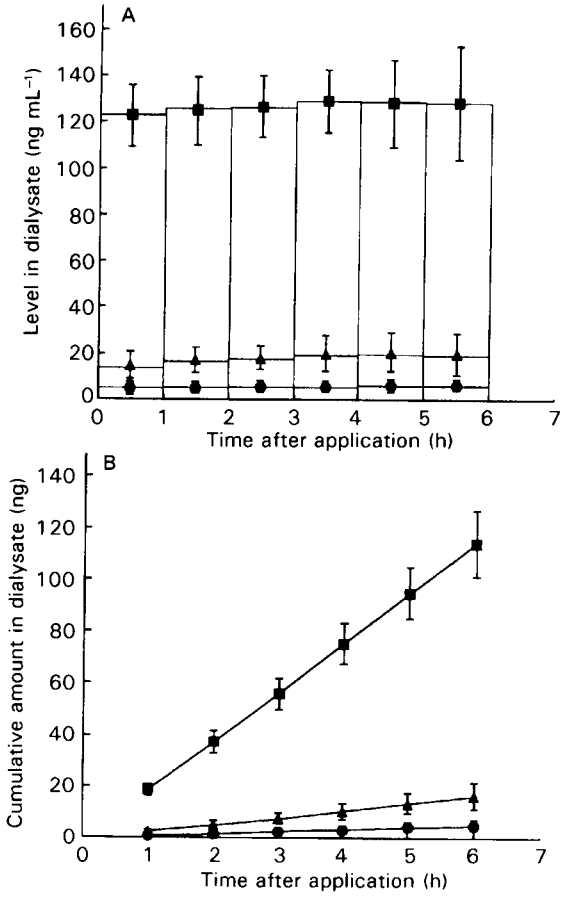

FIG. 1. Level (A) and cumulative amount (B) profiles of cyclosporin in the dermal dialysate after topical application of cyclosporin oily solutions without an enhancer in rats. ● 0·5%; ▲ 2%; ■ 8% (w/v) cyclosporin. Data represent the mean ± s.e.m. of 5 rats.

Results

In-vivo transdermal absorption of cyclosporin

Level and cumulative amount of cyclosporin in the dermal dialysate after topical application of its oily solutions (0·5, 2, 8% w/v) without an enhancer are shown in Figs 1A and 1B, respectively. Cyclosporin was immediately detected and attained a plateau after application. The level and cumulative amount of cyclosporin in the dermal dialysate increased with increasing cyclosporin concentrations in the formulation from 0·5 to 8% (w/v).

These profiles of the drug cumulative amounts in the dialysate were pharmacokinetically analysed. The results are shown in Table 1. The in-vivo apparent absorption rate of cyclosporin across the skin increased in proportion to the topically applied concentration as shown in Fig. 2. The coefficient of correlation among them was 0·991. The time lag for cyclosporin absorption was always less than 0·21 h.

Effects of enhancers on cyclosporin absorption

The effect of various concentrations of HPE-101 on cumulative amount profile and total recovery for 6 h of cyclosporin in the dermal dialysate are shown in Figs 3A and 3B, respectively. In-vivo apparent absorption parameters for cyclosporin are summarized in Table 2. HPE-101 did not influence the apparent absorption rate of cyclosporin across the skin at

Table 1. In-vivo apparent absorption parameters for cyclosporin after topical application of cyclosporin oily solutions in rats.

Cyclosporin concentration (w/v %)	Apparent absorption rate in dialysate (ng h^{-1})	Lag time (h)
0·5	0·85 ± 0.39	0·21 ± 0·14
2·0	2·78 ± 0·89	0·18 ± 0·22
8·0	19·02 ± 2·08	0·06 ± 0·05

Values represent the mean ± s.e.m. of 5 rats.

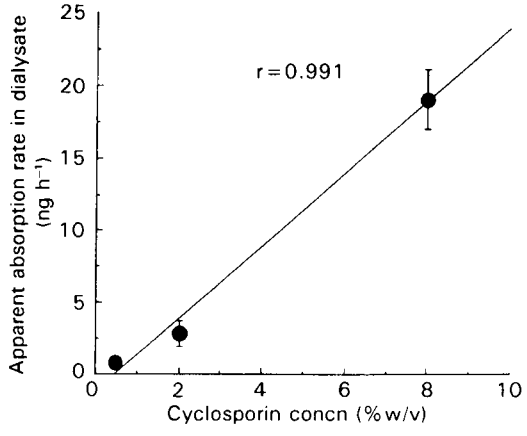

FIG. 2. Correlation between topically applied concentration and in-vivo apparent absorption rate of cyclosporin. Data represent the mean ± s.e.m. of 5 rats.

concentrations less than 6% (w/v). HPE-101 significantly enhanced the apparent absorption rate of cyclosporin by 4·9 times at 10% (w/v). However, 20% (w/v) HPE-101 showed no enhancing activity.

The effects of glycerin concentration on cumulative amount profile and total recovery of cyclosporin in the dialysate are shown in Figs 4A and 4B, respectively. Table 3 summarizes pharmacokinetic parameters for cyclosporin profiles in the dermal dialysate. Glycerin at concentrations of 6, 10, and 20% (v/v) significantly enhanced the apparent absorption rate of cyclosporin by 3·0, 6·4, and 6·9 times, respectively. The enhancing activity of glycerin increased gradually with increasing concentrations up to 10% (v/v) and then attained a plateau.

The time lag for cyclosporin absorption was also less than 0·21 h in all tested cases with an enhancer.

Discussion

Hermann et al (1988) have reported that cyclosporin is unable to penetrate excised human skin and that its poor skin penetrability is not influenced by addition of any penetration enhancer, such as Azone, polyvinyl pyrrolidine, propylene glycol, or ethanol. On the other hand, Duncan et al (1990) and Cole et al (1988) showed that cyclosporin could penetrate excised human skin when a test solution of cyclosporin was prepared from its commercial product by diluting with olive

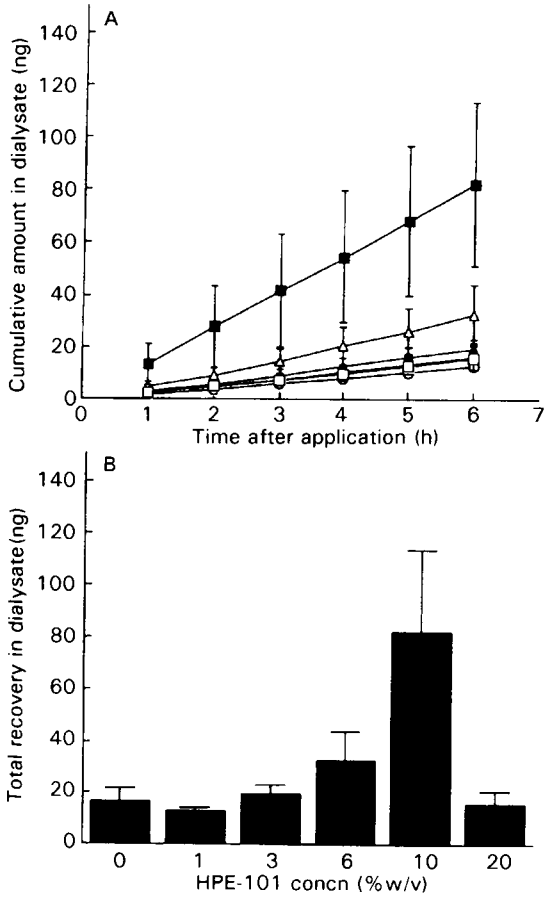

FIG. 3. Effect of HPE-101 concentration on cumulative amount profile (A) and total recovery for 6 h (B) of cyclosporin in the dermal dialysate after topical application of 2% (w/v) cyclosporin oily solutions with various concentrations of HPE-101 in rats. ● 0%; ○ 1%; ▲ 3%; △ 6%; ■ 10%; □ 20% (w/v) HPE-101. Data represent the mean ± s.e.m. of 3–5 rats.

Table 2. In-vivo apparent absorption parameters for cyclosporin after topical application of 2% cyclosporin oily solutions with various concentrations of HPE-101 in rats.

HPE-101 concentration (w/v %)	Apparent absorption rate in dialysate (ng h^{-1})	Lag time (h)
0	2·78 ± 0·89	0·18 ± 0·22
1	2·09 ± 0·21	0·10 ± 0·10
3	3·27 ± 0·65	0·18 ± 0·16
6	5·40 ± 1·93	0·15 ± 0·04
10	13·67 ± 5·17*	0·14 ± 0·19
20	2·64 ± 0·71	0·18 ± 0·13

Values represent the mean ± s.e.m. of 3–5 rats. *$P < 0.05$ compared with value obtained without HPE-101.

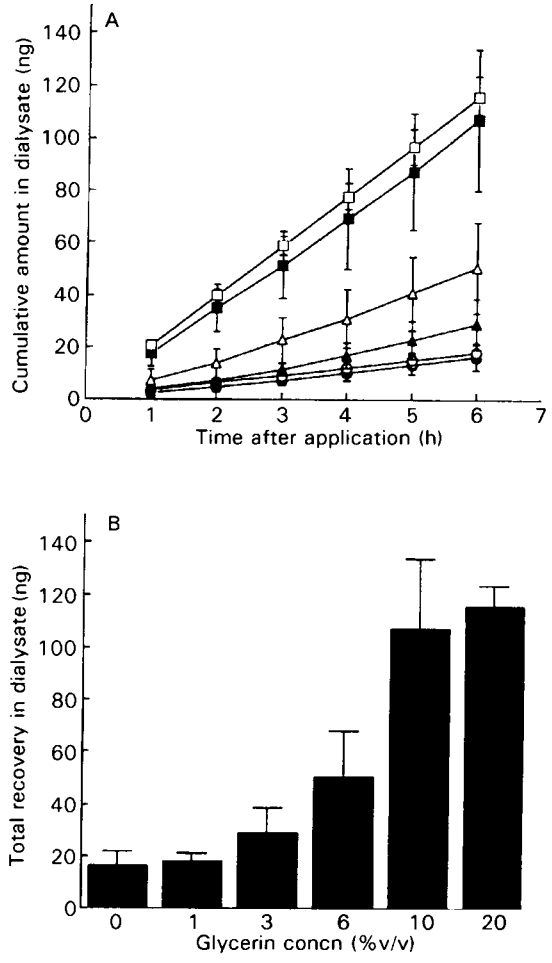

FIG. 4. Effect of glycerin concentration on cumulative amount profile (A) and total recovery for 6 h (B) of cyclosporin in the dermal dialysate after topical application of 2% (w/v) cyclosporin oily solutions with various concentrations of glycerin in rats. ● 0%; ○ 1%; ▲ 3%; △ 6%; ■ 10%; □ 20% (v/v) glycerin. Data represent the mean ± s.e.m. of 3–5 rats.

Table 3. In-vivo apparent absorption parameters for cyclosporin after topical application of 2% cyclosporin oily solutions with various concentrations of glycerin in rats.

Glycerin concentration (v/v %)	Apparent absorption rate in dialysate (ng h^{-1})	Lag time (h)
0	2·78 ± 0·89	0·18 ± 0.22
1	2·93 ± 0·50	0·04 ± 0.05
3	4·78 ± 1·67	0·19 ± 0.24
6	8·39 ± 2·78*	0·21 ± 0·11
10	17·67 ± 4·46**	0·05 ± 0·05
20	19·21 ± 1·37**	0·01 ± 0·01

Values represent the mean ± s.e.m. of 3–5 rats. *$P < 0.05$, **$P < 0.01$ compared with value obtained without glycerin.

oil. Duncan et al (1990) also showed that the addition of 2% (v/v) Azone and 18% (v/v) propylene glycol to 5% (w/v) cyclosporin oily solution significantly enhanced the penetration of cyclosporin across the excised skin. However, Cole et al (1988) showed that a penetration of cyclosporin through the excised skin was not improved by the use of 20% (v/v) propylene glycol as an enhancer. This discrepancy might be caused by a difference of experimental conditions. It has been reported that in-vitro permeation methods using excised skin are influenced by hydration (Hinz et al 1989) and microbial growth (Sloan et al 1991). An in-vivo method using microdialysis is independent of these problems.

Our in-vivo study showed that the apparent absorption of cyclosporin across living rat skin attained steady state immediately after topical application of its oily solution alone. This finding agrees with a previous report of cyclosporin penetrating excised skin without the time lag and achieving steady state immediately when using an oily solution prepared from Sandimmun (Duncan et al 1990).

The appropriate selection of an absorption enhancer is an important factor for enhancing transdermal drug delivery. The present study clearly indicated that HPE-101 or glycerin significantly enhanced the absorption of topically applied cyclosporin into living rat skin. We have previously demonstrated that HPE-101 is a potent absorption enhancer for a hydrophilic drug, methotrexate (Matsuyama et al 1994a), and a lipophilic drug, valproate (Matsuyama et al 1994b).

Glycerin exerts a penetration-enhancing effect which is attributed to its humectant properties. However, glycerin has not been used as an absorption enhancer for topical formulations although it is a safe and clinically well established additive. Generally the activity of an enhancer increases gradually and then attains a plateau with increasing enhancer concentration and this was observed for glycerin. Yano et al (1992) have also reported that HPE-101 produces a continuous increase in enhancing activity for dermal penetration of indomethacin at concentrations up to 3% (w/v), above which the enhancing activity reaches a plateau. However, the present study showed that there was an optimum HPE-101 concentration for enhancing the transdermal absorption of cyclosporin. A similar observation was reported by Niazy et al (1989) when they examined the effect of Azone concentration on percutaneous penetration of dihydroergotamine. Solubilization of drug by an enhancer decreases thermodynamic activity of drug in the formulation and results in a decrease of drug penetration.

Physicochemical properties of vehicles also influence not only drug penetration but also enhancer penetration. The apparent enhancing activity of enhancing vehicle on drug penetration must be defined as a balance of these combining effects. The presence of the optimum HPE-101 concentration for enhancing the cyclosporin absorption is probably related to the combining effect of HPE-101 on the animal skin and on the physicochemical properties of the cyclosporin oily solution.

Recently, topical application of cyclosporin with or without absorption enhancers has been tested for psoriasis, although most researchers have failed to gain the expected response because of the low penetrability of cyclosporin (Gilhar et al 1988; Hermann et al 1988; Bousema et al 1990). The present study showed high enhancing activity of glycerin on transdermal absorption of cyclosporin. Since glycerin is a safe and clinically well established additive, it is a suitable enhancer for improving the in-vivo cyclosporin absorption from the skin.

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