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Effect of vehicles and enhancers on the topical delivery of cyclosporin A

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Abstract

Topical delivery of cyclosporin a (CysA) is of great interest for the treatment of autoimmune skin disorders. The purpose of this study was to investigate the effect of various vehicles and enhancers on the topical delivery across rat skin. The topical (to the skin) delivery of CysA was evaluated in vitro using rat skin mounted in a Franz diffusion cell. CysA was analyzed by UV-HPLC. As vehicles, CysA vehicle containing 40% ethanol showed significantly enhanced deposition of CysA into the stratum corneum (SC) and deeper skin, as compared to other vehicles. The efficiency of the vehicles to improve the topical delivery of CysA was sequenced in the order of: 40% ethanol > ethyl oleate > Transcutol > isopropyl myristate > ethanol > Labrasol > propylene glycol > Lauroglycol FCC. Next, we tested effect of pre-treatment with chemical enhancers on the penetration of CysA. The permeation-enhancer effect of enhancers was in the following order: 10% menthol ≈ 0.05% SLS > 5% Azone > 5% NMP > 5% DEMO. Moreover, chemical enhancers shortened the lag time of the penetration of CysA into deeper skin. The present study suggests that the suspension of 40% ethanol containing 0.5% drug can more effectively enhance the topical delivery of CysA after skin pre-treatment with 10% menthol or 0.05% SLS.

Keywords: Cyclosporin A; Vehicle; Enhancer; Pre-treatment; Topical

1. Introduction

In topical and transdermal formulations, selection of a suitable vehicle is very important as it can affect both drug release and percutaneous absorption. Factors that contribute to the selection of a suitable vehicle are: (a) solubility of the drug in the vehicle; (b) release of the drug from the vehicle into the skin; (c) enhancement of drug penetration through the SC (Kikwai et al., 2002). The use of penetration enhancers is a long-standing and widely used approach to increase transdermal and topical delivery (Moster et al., 2001). Diffuse through skin is controlled by the outermost layer, the stratum corneum. Some of its properties may be manipulated by application of a penetration enhancer to the skin. Thus, an effective penetration enhancer may increase the diffusion coefficient of the drug into the SC, or could improve partitioning between the formulation and the SC. On the other hand, a good enhancer may act by increasing the effective concentration of the drug in the vehicles (Barry, 1991).

Cyclosporin A (CysA) is a nonpolar cyclic oligopeptide consisting of 11 amino acids. Over the past years, CysA has been evaluated for numerous potential applications in dermatology. It is effective in the treatment of alopecia areata and psoriasis, when administered systemically by i.v. injection or orally (Hultsch et al., 2005; Vc, 2004). However, long-term systemic administration of CysA has been noted to produce harmful effect such as hypochromic, granulomatous, hepatitis and proximal renal tubular cell damage (Guzzo, 1997; Zachariae et al., 1997). This drawback has led to the exploration of investigations using CysA to achieve local immune suppression. Topical delivery of CysA is hindered by its physicochemical properties and the barrier property of stratum corneum (SC) (Duncan et al., 1990; Choi and Flynn, 1995). Many studies have used physical and chemical techniques to disrupt the stratum corneum barrier (Verma and Fahr, 2004; Lopes et al., 2005; Tran et al., 1999; Boinpally et al., 2004; Wang et al., 1998). CysA in a topically applied oily solution containing isopropyl alcohol and propylene glycol formulations was reported effective in alopecia areata (Parodi et al., 1995). Verma et al. (2004) also suggested that 0.5% CysA in a liposomal formulation had promising potential as a topical treatment for alopecia areata in human. There were no systemic effects or detectable blood concentrations of CysA in any of

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these studies. In psoriasis, although the desquamative process increases the thickness of the horny layer, the associated inflammation of the dermis permits faster drug removal with than healthy skin (Roenigkjk and Maibach, 1998; Anigbogu et al., 1996). Consequently, delivery systems that preferentially retain the drug into the skin will be the best candidate for topical applications. However, much attention has been paid for increasing the transdermal delivery of CysA (Guo et al., 2000a; Boinpally et al., 2004).

The objective of the present study was to design an effective topical delivery system of CysA. We first investigated the effects of various hydrophilic and lipophilic vehicles on the dermal delivery of CysA across rat skin. The vehicles used in the study were: propylene glycol (PG), ethyl oleate, isopropyl myristate (IPM), Labrasol™, Transcutol™, Lauroglycol FCC (LFCC) and 40% ethanol (EtOH). Next, the effect of penetration enhancers on the topical delivery of CysA was tested. The enhancers we tested were: 10% menthol, 5% Azone, 5% NMP, 5% DMS0 and 0.05% SLS.

2. Material and methods

2.1. Materials

CysA was procured from Sigma Chemical. Labrasol™, Transcutol™ and Lauroglycol FCC were gifts from Gattefosse (Cedex, France). IPM, laurocapram (Azone), *N*-methyl pyrrolidine (NMP), dimethyl sulfoxide (DMSO) and menthol were obtained from Shanghai Chemical Reagent Corporation (Shanghai, China). All other chemicals were of either regent or high-performance liquid chromatography (HPLC) grade.

2.2. Analytical methodology for CysA

CysA was analyzed by reversed phase HPLC using JASCO 1500 series. The column was a Diamond C_{18} column (5 μm, 4.6 mm × 200 mm). The mobile phase was an acetonitrile–water–phosphoric acid (750:250:1) mixture with a flow rate of 1.0 ml/min. The detection wavelength was set at 210 nm and oven at 70 °C. The retention time of CysA was 13.5 min. No interference of the other formulation components was observed. All samples were filtered through a 0.45 μm pore size membrane filter before injection.

2.3. In vitro permeation studies

The abdominal skins were obtained from male rats weighing, 200 ± 20 g. After hair was removed with a depilatory, the skins were excised. The subcutaneous fat was removed, and then the skins were washed. To ensure intact skin barrier function, the electrical resistivity of the skin was measured. The samples showing abnormally low electrical resistance were discarded. The skins were placed in a refrigerator at 4 °C overnight, and then used for the experiments. The permeation experiments were performed using modified Franz diffusion cells with diffusion area of 0.785 cm^2 and a receiver volume of 8.0 ml. Normal saline containing 20% ethanol was used as the receiver medium. The receptor chambers were thermostated at 32 °C and the solution in the receptor chambers was stirred continuously at 300 rpm. A solution of 0.5% (w/w) CysA was made with each vehicle by agitating on an environment shaker at 32 ± 1 °C for 24 h. The formulations (500 μl) containing 0.5% (w/w) CysA were gently placed in donor chamber. All experiments were carried out with non-occluded donor compartments.

Menthol (10%, w/w), SLS (0.05%, w/w), Azone (5%, w/w), NMP (5%, w/w) and DEMO (5%, w/w) were used as chemical enhancers. These enhancers were dissolved in ethanol except SLS (in water). Five hundred microliters of the enhancer solution was placed in the donor compartment of the diffusion cell for the skin pre-treatment. The enhancer solution was removed after 2 h of incubation, the remaining enhancer on the surface of skin was removed and the skin was washed with receiver medium for 10 times, then the percutaneous penetration experiment was carried out.

Finally, the amount of drug accumulated into the skin was recovered using the following procedure. To separate the SC from the remaining epidermis (E) and dermis (D), skin sections were subjected to tape stripping. The skin was striped with 20 pieces of adhesive tape, and the tapes containing the SC were immersed in 3.0 ml of methanol and vortex-stirred for 2 min. The remaining tissue, |E + D|, was cut in small pieces, vortex-mixed for 2 min in 1.0 ml of methanol, and subject to three sonication cycles of 30 min each in an ultrasound bath. The resulting mixture was then filtered using 0.45 μm membrane, and CysA was then quantified by HPLC. It was observed that the sonication step did not affect the stability of CysA. The concentration of drug in deeper skin was an index of topical delivery, whereas the concentration in receptor phase was an index of transdermal delivery.

2.4. Statistical analysis

All skin permeation experiments were repeated four times and data were expressed as the mean value ± S.D. Statistical data were analyzed by one-way analysis of variance (ANOVA). A multiple comparison test was used to compare different formulations, and a *P*-value of 0.05 was considered to be significant.

3. Results and discussion

The penetration of a drug through skin layers can be predicted by its diffusivity and solubility properties (Barry, 2001, 2004). The deposition of CysA into the stratum corneum and deeper skin at 12 h post-application are presented in Fig. 1. Forty percent ethanol was considered as the best vehicle, both in terms of the deposition of CysA of the SC and deeper skin. Compared to ethanol, 40% ethanol showed an eight-fold higher accumulation of CysA in the SC ($P < 0.05$). Ethanol is one of the most widely used solvents in transdermal drug delivery systems and is used in some of the commercial transdermal patches to improve solubility and enhance the permeation of drugs (Kobayashi et al., 1994). There are several mechanisms that have been proposed for ethanol action on skin depending on its concentration and the physicochemical properties of the permeant (Kadir et al.,

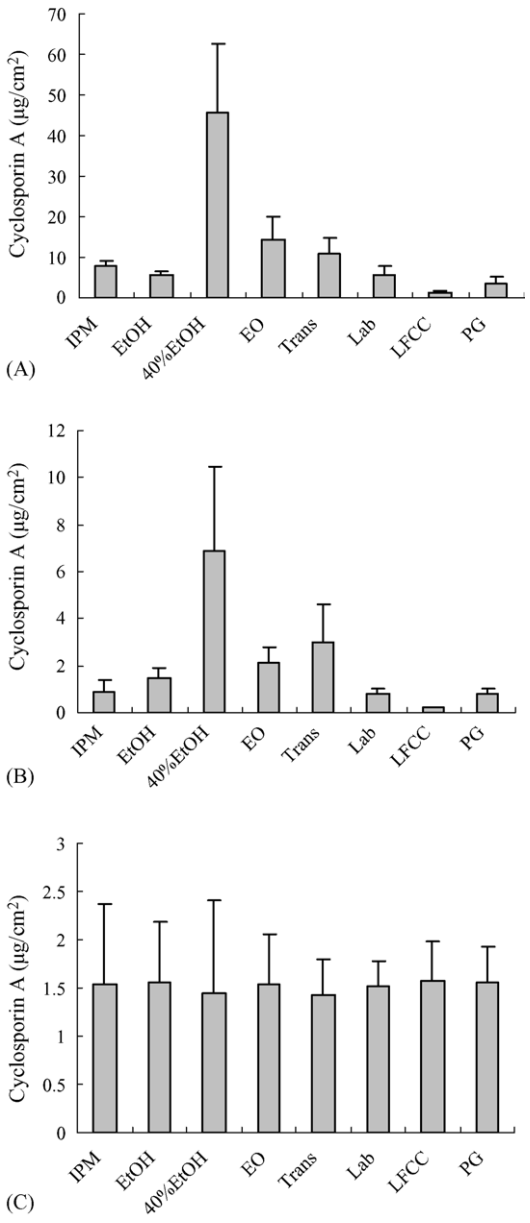

Fig. 1. Effect of various vehicles on the skin penetration of cyclosporin A. The drug concentration in the SC is shown in (A); the concentration in the |E + D| is depicted in (B); The concentrations in receptor phase are shown in (C); $n = 4$.

1987; Panchagnula et al., 2001). It has been generally observed from literature that at lower concentration of ethanol (20–70%, v/v), permeation of lipophilic drugs was enhanced (Williams and Barry, 1992). In addition, experiment conducted by Pershing et al. (1990) suggested a relationship between the ethanol concentration and the absorption of drug into epidermis at lower ethanol concentration. Verma et al. (2004) reported that ethanol alone might not deliver necessarily amount of CysA to elicit a meaningful pharmacological response. At higher concentrations of ethanol, lipids were extracted from the SC, which led to a decrease of deposition of CysA observed with 100% ethanol.

Ethyl oleate provided the second highest SC deposition among the vehicles that we tested. This result might be explained by the different lipid packing properties of unsaturated *cis* fatty acid and ester, forming a sharp kink at the double bond (Small, 1984). They were reported to function by partitioning into the lipid region of the SC, disrupting the structure and lipid fluidity of the SC (Kim et al., 1993). The result revealed that ethyl oleate might increase the diffusion of CysA into the SC and the deeper skins.

Several studies have suggested that Transcutol creates a depot in the stratum corneum and upper epidermal layers (Ritschel et al., 1991). Tran et al. (1999) were successful in achieving site-specific immunosuppression using a topical formulation of CysA containing Transcutol, Labrasol, PG and ethanol. Compared to Transcutol, Labrasol appeared to be a weak penetration enhancer as shown by low concentrations of CysA in SC and |E + D|. It probably has a high affinity for CysA, resulting in a poor release of CysA to the skin.

IPM showed a comparable concentration of CysA to ethanol in the skin. This is probably because it only caused increased fluidity of lipid bilayers of the SC (Pillai et al., 2004). PG was a solubilizer for CysA and decreased topical delivery compared to ethanol. Among all of the vehicles, LFCC showed the minimal concentrations of CysA to the skin.

We also studied the effect of 40% ethanol, ethyl oleate and Transcutol on the topical delivery of CysA as a function of time. The penetration of 40% ethanol in SC and |E + D| was almost linearly improved as a function of time. On the other hand, ethyl oleate and Transcutol did not enhance the penetration of CysA to the skin significantly after 3 h (Fig. 2).

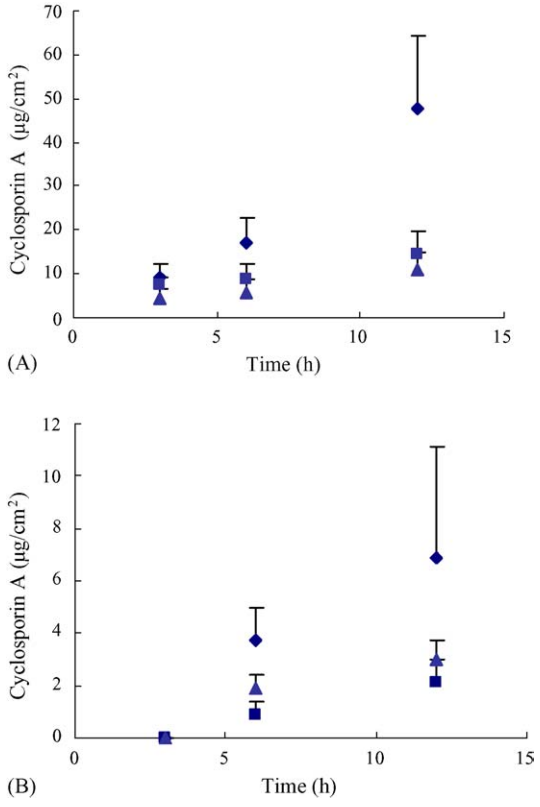

Fig. 2. Time-course of the effect of vehicles on the skin penetration of cyclosporin A. The drug concentration in the SC is shown in (A); the concentration in the |E + D| is depicted in (B); $n = 4$. (♦) Forty percent ethanol; (■) ethyl oleate; (▲) Transcutal P.

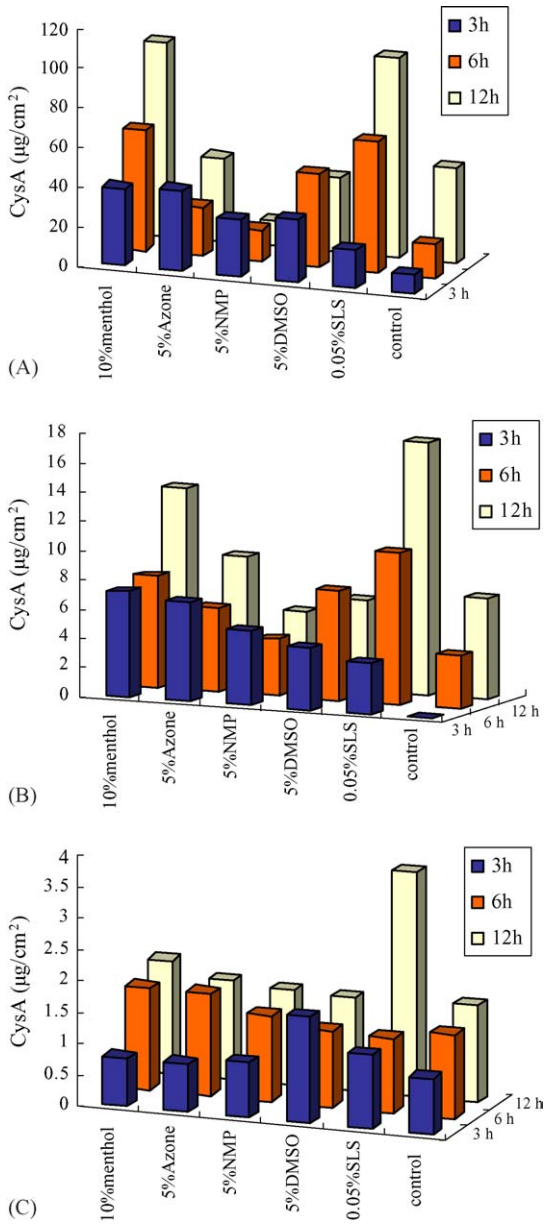

Fig. 3. Effect of various enhancers on the skin penetration of cyclosporin A. The drug concentration in the SC is shown in (A); the concentration in the |E + D| is depicted in (B); the concentration in receptor phase is shown in (C); $n = 4$.

The accumulation of CysA in the skin layers from 40% ethanol after pre-treatment with enhancers are showed in Fig. 3. The obtained values were compared to the drug accumulation analyzed in the SC and in the |E + D| after the same time of 0.5% suspension of the drug in 40% ethanol. As shown in Fig. 3, all of the enhancers we used can shorten the lag of the deposition of CysA into the |E + D|, indicating some increase in diffusivity of the drug through the SC. Compared to the control, skin pre-treatment with menthol, Azone, NMP, DMSO and SLS showed 4.24-, 4.43-, 3.12-, 3.44-, 2.05-fold higher accumulation of CysA in the SC at 3 h post-application ($P < 0.05$). The concentration of CysA in rat skin was increased as a function of time after menthol and SLS treatment. However, after pre-treatmemt with NMP, DMSO and Azone, the amount of CysA retained in the skin did not increase after 3 h. Pre-treatment of skin with menthol and SLS produced a statistically significant increment of the amount of CysA deposited in the skin in relation to the control ($P < 0.05$) between 1.88- and 2.51-fold and Azone pre-treatment increased the amount of CysA retained up to 1.24 times at 12 h post-application. In contrast, DMSO and NMP did not increase the amount of CysA retained in the skin.

Menthol is a monocyclic terpene with a pleasant taste and odor. It is widely used as a flavoring agent in oral dosage forms and as a fragrance with a mild antipruritic effect in topical formulations. Due to the pleasant taste associated with menthol, its use in transdermal drug delivery system may increase patient acceptability. The effect of menthol on transdermal absorption of several drugs has been reported (Williams and Barry, 2004). Menthol showed the highest enhancing activity in this study. It might involve their capacity to disrupt the bilayer of SC and form pools in the SC (Cornwell et al., 1996). Borrás-Blasco et al. (1997) reported that the effect of SLS as an enhancer depended on the lipophilicity value of the permeant. It was observed that the greater the enhancer effect, the lower the lipophilicity value of the penetrant. However, after pre-treatment with SLS, the amount of CysA deposited in skin was sharply increased compared to the control ($P < 0.05$). These results can be explained because, hydrophobic interaction of SLS alkyl chain with the skin structure left the end sulfate group of the surfactant exposed, creating additional sites in the membrane which could permit an increase in skin hydration (Ribaud et al., 1994).

Since there is no reported value for therapeutically required concentration of CysA to treatment of autoimmune diseases, we can only make a speculation. When applied with flexible lecithin vesicular containing 3.75% CysA onto the excised abdominal skin of mice non-occlusively in vivo, the deposition of the drug into the skin was about 1.35 ± 0.51 μg/cm^2 after 2 h and could retain to 24 h (Guo et al., 2000b). In comparison to that we obtained a 13-fold higher value in the deeper skin combined with the use of 40% ethanol vehicle and skin pre-treatment with menthol or SLS. Topical delivery (in vitro) of a 0.4% CysA in a lipid mixture containing ethanol in human skin was reported to have resulted in 4.079 μg/cm^2 in the SC and 0.042 μg/cm^2 in the deeper skin after 6 h non-occlusive application (Verma et al., 2004). The amount of the drug delivered into the skin was lower than we obtained, which could partly be due to the limited solubility of CysA in PBS, used as the receiver medium. Lopes et al. (2005) suggested that using monoolein as a penetration enhancer 4% CysA in propylene glycol formulations could promote the topical delivery up to about 140 μg/cm^2 in the SC and 30 μg/cm^2 in the deeper skin in vitro using porcine ear skin at 12 h post-application. Concerning the dose of the drug used by Lopes et al. was much higher than we used, 40% ethanol may be an appropriate vehicle for the topical delivery of CysA. Wang et al. (1998) have been able to obtain a high penetration of CysA in 40% ethanol vehicle into mice skin by electroporation in vitro. In our case, skin pre-treatment with menthol and SLS not only shortened the lag time of CysA into rat deeper skin but also enhanced the amount of CysA retained in the skin. Combination of physical method and these chemical enhancers might offer a synergistic effect. This approach can be effective for topi-

cal treatment of inflammatory skin diseases like psoriasis, atopic dermatitis and some hair follicle disorders.

In addition, the amount of CysA permeated through rat skin by various vehicles was only about 1.5 $\mu g/cm^2$ at 12 h post-application. The accumulation of CysA into deeper skin by 40% ethanol was 4.6 times higher than the amount of drug delivered across the skin ($P < 0.05$). After skin pre-treatment with enhancers, the transdermal delivery of CysA was not promoted except SLS. These results suggested that enhancers pre-treatment increase the topical delivery of the drug, but have no effect on the transdermal delivery (except SLS).

4. Conclusion

This study demonstrated that various vehicles could significantly influence the skin deposition of CysA. Forty percent ethanol, which showed best topical delivery of CysA into rat skin, may be used as potential vehicle for topical delivery of CysA. Pre-treatment with enhancers can shorten the lag time of the penetration of CysA into deeper skin. Ten percent menthol and 0.05% SLS can serve as efficient promoters of CysA localization into the skin. The enhanced skin accumulation of CysA could help significantly to optimize the targeting of the drug without of a concomitant increase of the systemic side effects.

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