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Research paper

Topical delivery of cyclosporin A: an in vitro study using monoolein as a penetration enhancer

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Abstract

Topical delivery of cyclosporin A (CysA) is of great interest for the treatment of autoimmune skin disorders, but it is frequently ineffective due to poor drug penetration in the skin. The present study was aimed at investigating whether the presence of monoolein (a lipidic penetration enhancer) in a preparation of propylene glycol can improve CysA delivery to the skin. CysA was incorporated in a propylene glycol preparation containing 5–70% (w/w) of monoolein. The topical (to the skin) and transdermal (across the skin) delivery of CysA were evaluated in vitro using porcine ear skin mounted in a Franz diffusion cell. CysA was quantified by UV-HPLC. At 5%, monoolein increased only the transdermal delivery of CysA. At 10%, it increased both topical and transdermal delivery. When the concentration of monoolein was further increased (20–70% w/w), an interesting phenomenon was observed: the topical delivery of CysA was still elevated but its transdermal delivery was substantially reduced. It was concluded that monoolein (in propylene glycol formulations) can promote the topical delivery of CysA, with reduced transdermal delivery.

Keywords: Monoolein; Cyclosporin A; Topical delivery; Penetration enhancer; In vitro permeation

1. Introduction

Cyclosporin A (CysA) is an immunosuppressive cyclic undecapeptide [1] that has been used clinically for the treatment of inflammatory and autoimmune diseases, including skin disorders like psoriasis [2,3]. Despite its therapeutic action, systemic administration of CysA has several shortcomings, often associated with the drug's variable pharmacokinetics, narrow therapeutic index, and large number of side effects [4,5]. Topical delivery of CysA is a promising strategy to treat skin disorders, since it avoids several side effects associated with systemic delivery [4,6]. However, such a strategy has been described as ineffective in most cases [7,8], and its ineffectiveness has been attributed to an inadequate drug penetration in the skin [4].

The penetration of CysA in the skin is not easily achieved due to its lipophilicity ($\log P_{\text{octanol/water}} = 2.92$), large molecular weight (1202 Da), and cyclic molecular structure [9,10]. To optimize the topical delivery of CysA, it is necessary to use techniques that reduce the diffusional resistance of the stratum corneum. Many studies have used physical and chemical techniques to disrupt the stratum corneum barrier [4,6,9,11–15]. By using a physical method (electroporation), Wang et al. [13] were able to obtain a high penetration of CysA in the skin associated with low transdermal delivery. Although chemical methods are likely to be more viable clinically, the vast majority of formulations tested to date were aimed at increasing the transdermal (not topical) delivery of CysA [6,9]. This would result in drug diffusion to the blood stream and manifestation of its undesirable, systemic effects. To develop a formulation that promotes the topical delivery of CysA associated with poor transdermal delivery is still a challenge.

In the present study, we tested whether formulations containing monoolein promote the topical delivery of CysA

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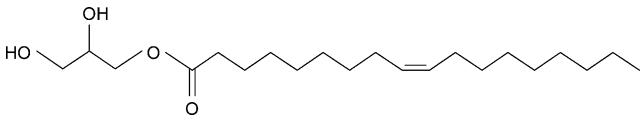

Fig. 1. Monoolein chemical structure.

(assessed in vitro using porcine ear skin). Monoolein is a monoacylglyceride (Fig. 1), structurally similar to oleic acid, a well-known skin penetration enhancer for peptides [16,17]. Due to its ability to remove skin ceramides, increase lipid fluidity in the stratum corneum, and solubilize lipophilic compounds, monoolein has been reported to enhance the skin penetration of several compounds such as urea, indomethacin, and steroids [18,19].

2. Materials and methods

2.1. Materials

Monoolein (Myverol 18–99) was supplied by Quest International (Norwich, NY, USA). Acetonitrile and methanol were purchased from Ominsolve (Merck, Darmstadt, Germany), propylene glycol from Synth (Diadema, SP, Brazil), and CysA from Boechel Co. (Hamburg, Germany).

2.2. Formulations tested

Different quantities of melted monoolein (40 °C) were added to propylene glycol, so that the final concentration of monoolein was 0, 5, 10, 20, or 70% (w/w). Immediately thereafter, CysA was incorporated in the formulations to achieve a concentration of 4% (w/w). CysA was used at similar concentrations by other authors for topical administration [4,8,11].

The monoolein-containing propylene glycol formulations were analyzed by polarized light microscopy at different temperatures, since the phase behavior of monoolein-based systems can be influenced by temperature [20]. The formulations were examined using an Axioplan 2 Imaging Pol polarized light microscope (Carl Zeiss, Germany) fitted with a Mettler hot stage. Each formulation was heated at 1 °C/min over the temperature range of 25–40 °C. In this temperature range, all formulations were characterized as isotropic liquids and no phase transformation was observed (data not shown).

2.3. Analytical methodology for CysA

CysA was assayed by HPLC using a Shimadzu equipment, which consisted of a Model LC10 AD solvent pump, a Rheodyne injector, a 20 μL loop, a Model SPD-10A variable wavelength UV detector, a Model CTO-10A column oven, and a Model SCL-10A controller system. The separation was performed by a Lichrospher 100 RP-18 column (5 μm, Merck, Darmstadt, Germany), which was equipped with a RP-8 precolumn (Merck, Darmstadt, Germany) and equilibrated at 60 °C. A mobile phase of 67% acetonitrile and 33% water (flow rate of 1 mL/min) was used, and CysA was detected at 210 nm. Under these conditions, the retention time of CysA was 9.1 min. The area under the peak was used to calculate the concentration range of CysA. Linearity was achieved for concentrations of between 0.15 and 500.00 μg/mL, with correlation coefficient (r) of 0.9998. The quantification limit of this HPLC assay was 0.15 μg/mL, with less than 5.15% intra-day variation, and less than 2.77% inter-day variation. The error was less than 5.50%. These values are considered adequate for analytical assay [21]. Using this HLPC procedure, unidentified peaks were not detected.

2.4. In vitro permeation studies

The topical and transdermal delivery of CysA were assessed in an in vitro model of porcine ear skin, as previously described [22]. Briefly, the skin from the outer surface of a freshly excised porcine ear was carefully dissected (making sure that the subcutaneous fat was maximally removed), stored at −20 °C, and used within a month. On the day of the experiment, the skin was mounted in a Franz diffusion cell (diffusion area of 1.77 cm^2; Hanson Research, Chatsworth, CA, USA), with the stratum corneum (SC) facing the donor compartment (where the formulation was applied) and the dermis facing the receptor compartment, which was filled with 7.0 ml of receptor medium (100 mM phosphate buffer, pH 7.2, containing 10% ethanol). Ethanol was used to increase the solubility of CysA, which was 30.33 ± 1.84 μg/mL in this medium. Previous studies have used receptor phases containing up to 33% of ethanol [6,9,15]. The receptor phase was under constant stirring; it was maintained at 37 ± 0.5 °C by a water jacket. This temperature was selected based on the facts that the receptor phase is in contact with the deepest skin layers and that the deep body temperature of humans is maintained between 36.2 and 37.2 °C during the circadian cycle [23]. It is noteworthy that monoolein formulations show no phase transition when the temperature is risen from 25 °C (ambient) to 37 °C (receptor phase; see above). To achieve higher reproducibility, the skin samples were pre-hydrated for 2 h before applying the formulation. Each formulation (100 mg) was applied to the surface of the SC.

At the end of the experiment, skin surfaces were thoroughly washed with distilled water to remove excess formulation and carefully wiped with tissue paper. To separate the SC from the remaining epidermis (E) and dermis (D), skin sections were subjected to tape stripping. The skin was stripped with 15 pieces of adhesive tape (Scotch Book Tape, n. 845, 3M, St Paul, MN), and the tapes containing the SC were immersed in 5 mL of methanol

and vortex-stirred for 2 min. The methanol phase was filtered using a 0.45 μm membrane, and the resulting filtrate assayed for CysA. The remaining tissue, |E+D|, was cut in small pieces, vortex-mixed for 2 min in 2 mL of methanol, and bath-sonicated for 30 min. The resulting mixture was then filtrated using 0.45 μm membranes, and CysA was determined in the filtrate. The amount of CysA that permeated across the skin was extracted from samples (500 μL) of receptor phase withdrawn at 3, 6, 7.5, 9, 10.5, or 12 h post-application, using 3 mL of chloroform. After the chloroform phase was evaporated, the residue was suspended in 50 μL of an acetonitrile–water solution (67:33 v/v; mobile phase), and CysA was quantified by HPLC assay. The extraction procedure presented a recovery of 96.8%.

The concentrations of drug in SC and |E+D| were indexes of topical delivery, whereas the concentration in the receptor phase was an index of transdermal delivery.

2.5. Statistical analyses

The results are reported as means ± SD. Data were statistically analyzed using nonparametric tests. The Mann–Whitney test was used to compare two experimental groups. The Kruskal–Wallis test (followed by Dunn post-hoc test) was used to compare more than two experimental groups. The level of significance was set at $P < 0.05$.

3. Results and discussion

The penetration of a drug through skin layers can be predicted by its diffusivity and solubility properties [24,25]. Lipophilic drugs such as CysA generally present high partition in the stratum corneum. However, their penetration in viable epidermis is low, presumably due to their accumulation in the SC. Different strategies can be used to increase the topical or transdermal delivery of drugs [25].

In the present study, we verified whether the penetration enhancer monoolein (5–70% w/w), promotes topical and/or transdermal delivery of CysA. We first studied the effect of monoolein at 10% (w/w) on the delivery of CysA as a function of time. Compared to the control formulation, the preparation containing monoolein enhanced the topical delivery of CysA within several hours: the penetration of CysA in |E+D| was significantly ($P < 0.05$) augmented at 6 h post-application, whereas the penetration in SC was significantly ($P < 0.05$) enhanced at 12 h post-application (Fig. 2). The same preparation also enhanced the transdermal delivery of CysA: the presence of CysA in the receptor phase was barely detected before 6 h and significantly ($P < 0.05$) enhanced 9 h post-application (Fig. 2). The relatively long lag time for the permeation of CysA across the skin is probably due to its high molecular weight and lipophilicity. This finding agrees with previous studies on the in vitro transdermal delivery of CysA [6,9].

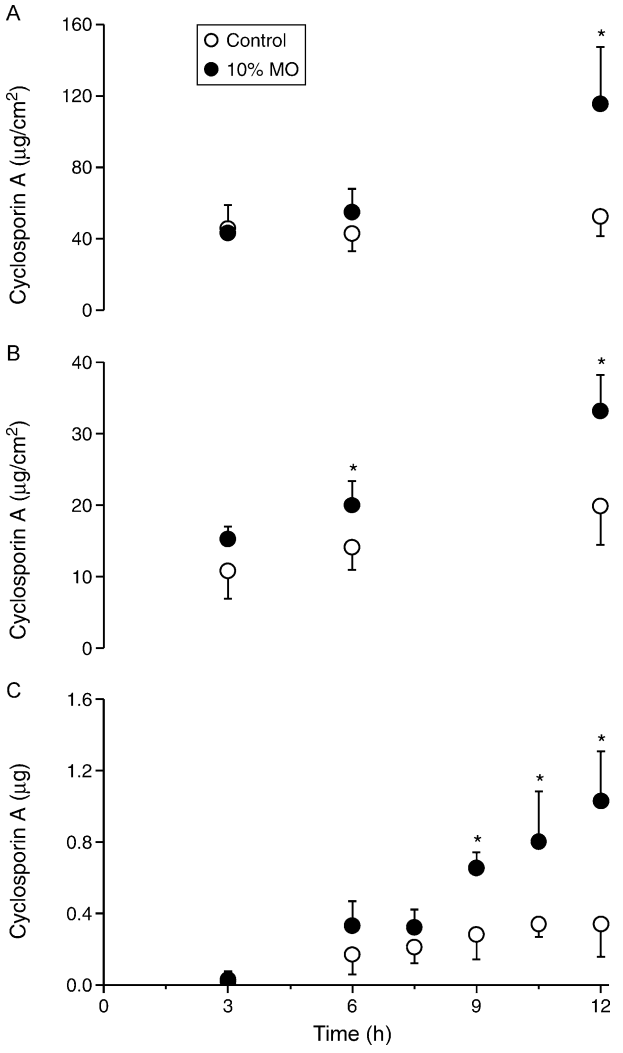

Fig. 2. Time-course of the effect of monoolein (MO) or control formulation on the skin penetration and transdermal delivery of cyclosporin A. The drug concentration in the SC is shown in **A**; the concentration in the [E+D] is depicted in **B;** the concentration in the receptor phase (an index of transdermal delivery) is shown in **C**. $n = 6$.

Because both the topical and transdermal delivery of CysA were enhanced 12 h post-application, this time point was chosen to evaluate the influence of monoolein concentration on these parameters.

Fig. 3 shows the influence of monoolein concentration on the delivery of CysA. At 5% (w/w), monoolein significantly ($P < 0.05$) increased the transdermal delivery of CysA compared to control formulation, but had no significant effect on the topical delivery of this drug. At 10% (w/w), monoolein significantly increased the topical delivery of CysA, to both SC and |E+D| ($P < 0.05$ and 0.01, respectively). It also increased the transdermal delivery of the drug ($P < 0.01$). When the concentration of monoolein was further increased to 20 and 70% (w/w), an interesting phenomenon was observed: significant increases in the concentrations of CysA in SC ($P < 0.05$ for 20% and $P < 0.01$ for 70%) and |E+D| ($P < 0.05$ for 20 and 70%)

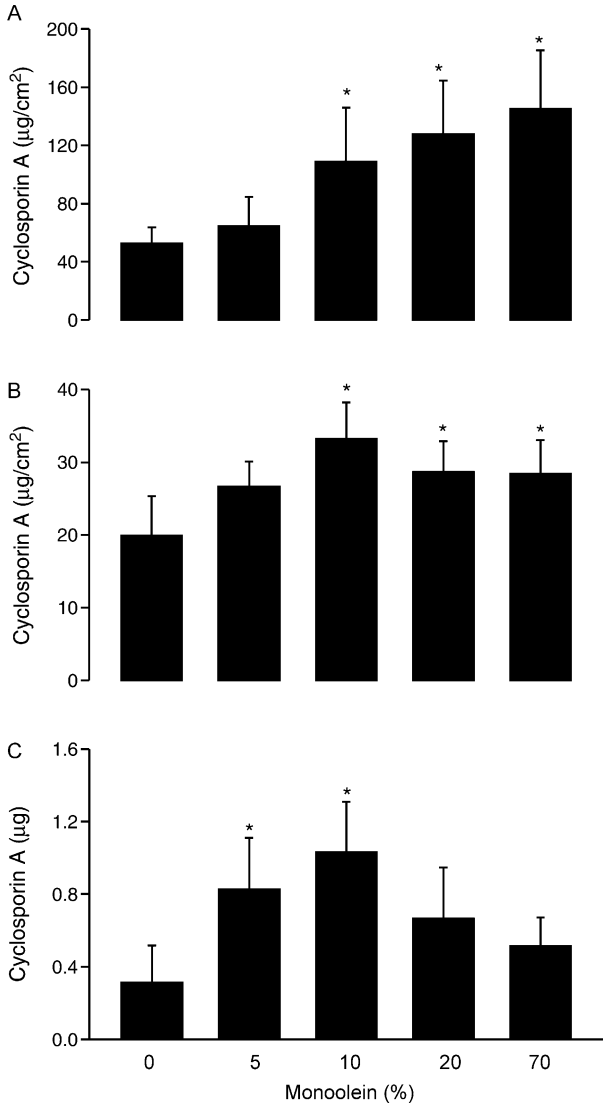

Fig. 3. Concentration-dependent effects of monoolein on the skin penetration and transdermal delivery of cyclosporin A. The drug concentration in the SC is shown in **A**; the concentration in the [E+D] is depicted in **B;** the concentration in the receptor phase (an index of transdermal delivery) is shown in **C**. $n=6$.

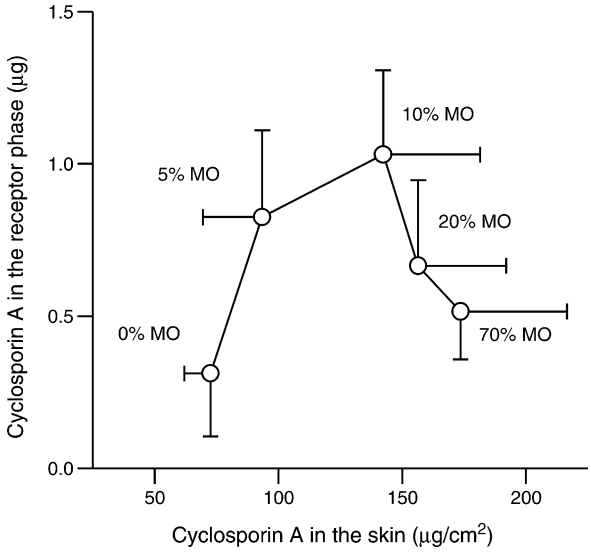

Fig. 4. Relationship between the concentrations of cyclosporin A in the receptor phase (an index of transdermal delivery) and total skin (an index of skin penetration) when preparations containing different concentrations of monoolein (MO) were used. $n=6$.

were seen, whereas the concentration of CysA in the receptor phase did not differ from that obtained with the control formulation. The dual effect of monoolein on the transdermal delivery of CysA can be clearly seen in Fig. 4.

These findings agree with previous studies reporting that monoolein acts as a penetration enhancer. Formulations containing monoolein have been shown to increase the topical and transdermal delivery of urea and indomethacin [18] as well as of progesterone [19]. Additionally, cubic phases made of monoolein increase the permeation of several drugs, including peptides, across the skin and buccal mucosa [26–28]. However, the present observation that monoolein at a concentration of 20% (w/w) or higher markedly increases topical delivery without promoting transdermal delivery of CysA is novel. Formulations containing high concentrations of monoolein may thus be a simple, relatively inexpensive method to obtain an optimal local pharmacological action of CysA on the skin, associated with minimal systemic side effects. Presumably, 20% monoolein would be better tolerated than 70%. However, higher concentrations (60–70%, w/w) of monoolein are used for the preparation of cubic phases, which have been increasingly investigated for topical [29,30], transdermal [26], vaginal [20], and buccal delivery of drugs [27,28,31]. To our knowledge, three previous studies have obtained high topical delivery of CysA along with low transdermal delivery. These studies, however, have used more complex approaches. Wang et al. [13] achieved such an effect using a physical method, electroporation. Verma and Fahr [32] achieved the effect using phospholipid vesicles containing 10% ethanol. Finally, Dowton et al. [33] observed the same effect employing ceramide-based liposomes.

The question then arises as to how relatively high concentrations of monoolein do not increase the transdermal delivery of CysA whereas low concentrations are very effective to do so. The answer to this question may lie on the fact that the activity of monoolein as a penetration enhancer depends not only on its actions on the skin, such as extraction of ceramides and enhancement of skin fluidity [18], but also on its physicochemical interaction with the drug of interest [34]. Due to their lipophilic nature, monoolein and CysA are likely to have a high affinity to each other. Hence, an interaction between monoolein (at high concentrations) and CysA may result in drug retention in the skin (where monoolein is better partitioned). Another possibility is that the solubility and, consequently, thermodynamic activity of the drug might have been changed when the concentration of monoolein

was increased. This is, nevertheless, improbable because the concentration of CysA in the preparations was at least 4 times lower than the solubility of the drug (data not shown), irrespectively of the monoolein concentration used.

In conclusion, we found that monoolein in propylene glycol formulations affects both the topical and transdermal delivery of CysA in a time- and concentration-dependent manner. Lower concentrations (up to 10%, w/w) enhance both the topical and transdermal delivery. However, higher concentrations (20% w/w or more) increase the topical delivery, but have no effect on the transdermal delivery. Formulations containing high concentration of monoolein may thus be a simple, relatively inexpensive method to achieve an optimal local pharmacological action of CysA on the skin, associated with minimal systemic side effects. This approach can be effective for topical treatment of inflammatory skin diseases like psoriasis, atopic dermatitis, and some hair follicle disorders.

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