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Iontophoresis of lecithin vesicles of cyclosporin A

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Abstract

Site-specific immunosuppression with topical cyclosporin A (CsA) has broad clinical implications in the treatment of skin disorders like psoriasis, pyoderma gangrenosum, lichen planus, cutaneous graft-versus-host disease and contact hypersensitivity and the temporary treatment of skin allografts on burn wounds. However, like any other peptide drug, its skin delivery is hindered by the barrier property of stratum corneum and the physicochemical properties of CsA. We have attempted to deliver CsA across human cadaver epidermis in vitro using colloidal systems like microemulsion and lecithin vesicles and iontophoresis. Although, passive diffusion did not result in permeation of quantifiable amounts of CsA, anodal iontophoresis of the negatively charged colloidal systems facilitated the permeation. Electroosmosis and compromised epidermis might have contributed to the higher skin flux. Lecithin vesicles were better than microemulsion for the iontophoretic delivery of CsA and appear to have potential in site-specific immunosuppression.

Keywords: Cyclosporin A; Site-specific immunosuppression; Topical delivery; Lecithin vesicles; Microemulsion; Iontophoresis; Electroosmosis

1. Introduction

Cyclosporin A (CsA) is an established immunosuppressant. Long-term systemic administration of CsA may result in harmful but reversible side effects such as hypochromic, microcytic anemia, marrow hypoplasia, lymphopenia, granulomatous hepatitis and proximal renal tubular cell damage (Whiting et al., 1983; Thomson et al., 1984). Because of this drawback, attempts are being made to achieve localized site-specific immunosuppression using topical CsA (Hewitt and Black, 1996). Topical CsA has been shown to eliminate contact hypersensitivity in guinea pigs, to decrease histopathological evidence of rejection in corneal allografts in rabbits and rats, and to be effective in treating alopecia areata and contact hypersensitivity in humans (de Prost et al., 1986; Parodi and Rebora, 1987; Aldridge et al., 1986; Foets et al., 1985; Behrens-Baumann et al., 1986; Williams et al., 1987; Tran et al., 1999). Dual skin allograft studies in rats have shown significant prolongation of survival in topical CsA treated allografts as compared with vehicle treated ones (Black et al., 1988, 1990, 1991; Tatem et al., 1995; Llull et al., 1995).

Several formulations of topical CsA are being tested in recent years for superior efficacy and reduced side

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effects (Guo et al., 2000; Tran et al., 1999; Black et al., 1988, 1990). However, like any other peptide, its skin penetration/ permeation is hindered by the barrier property of stratum corneum and the physicochemical properties of CsA. Lecithin vesicles are being reported to be capable of delivering macro molecular protein/peptide drugs including CsA in recent years (Guo et al., 2000; Cevc et al., 1998; Boinpally et al., 2001). We have investigated the ability of colloidal systems (lecithin vesicles and microemulsion) to deliver CsA across human cadaver epidermis through passive diffusion and/or iontophoresis.

2. Materials and methods

2.1. Materials

CsA sample was a gift from Novartis Pharma AG, Basel, Switzerland. Soya phosphatidylcholine, sodium cholate, ethyl alcohol and chloroform were purchased from Sigma Scientific and methanol from Fischer Scientific. NEORAL® capsules were a gift from Dr. Patricia M. LoRusso, Karmanos Cancer Institute, Detroit, MI, USA. Human cadaver skin was obtained from the skin bank of the department of dermatology, Detroit Medical Center, Detroit, MI, USA and stored at −80 °C until used.

2.2. Methods

2.2.1. Formulations tested

(1) Lecithin vesicles of CsA: 440 mg soya phosphatidylcholine, 35 mg CsA and 150 mg sodium cholate were dissolved in a 1:1 mixture of methanol and chloroform. The solution was dried under vacuum using a rotary evaporator and the dried film was hydrated with 10 ml of 0.9% sodium chloride solution in water. The preparation was then sonicated for 10 min in a bath sonicator to reduce the vesicle size. (2) Microemulsion of CsA: prepared by dissolving the contents of 100 mg cyclosporin soft gelatin capsule (NEORAL®) in 25 ml water. (3) 3.5 mg/ml suspension of CsA in normal saline containing 20% ethanol.

2.2.2. Characterization of the colloidal systems

The size and Zeta potential of both the microemulsion as well as the lecithin vesicles of CsA were characterized using a Zetasizer 3000 HS (Malvern Instruments, UK). Encapsulation efficiency of CsA in lecithin vesicles was determined by a modified method reported by Fang et al. (2001). Briefly, the preparation was filtered using 0.025-μm PTFE (Millipore, USA) filter and washed with deionized water twice. The cake was suspended in methanol to extract CSA and quantified by HPLC method. To account the mass balance, the drug in the aqueous filtrate was also quantified. The percentage of drug encapsulated was calculated from the ratio of drug in the lipid vesicles to the total amount of drug in the formulation.

2.2.3. Separation of human epidermis

The epidermis was separated from full thickness human skin that was obtained from skin bank and frozen until use. Immersing the full thickness skin in water at 60 °C for 2 min and gently peeling the epidermis, which was then separated as an intact sheet, achieved separation of epidermis at the epidermal/dermal junction. The physical integrity of the epidermal sheets was assessed by visual examination and receiver fluid permeation across epidermis from receiver to donor chamber of diffusion cell under mild pressure created due to the continuous flow of receiver fluid into and out of the cell. Integrity of barrier function in the heat-separated epidermis was tested through trans-epidermal resistance measurements.

2.2.4. Permeation/flux studies

Modified Franz cells, with diffusion area of 1.768 cm^2 and a receiver volume of 8 ml were used in transport studies and all experiments were done in triplicate. Normal saline containing 20% ethanol was used as the receiver medium. A suitable size of heat separated human epidermis was cut and mounted in the Franz cell, with the stratum corneum side facing the donor. After filling the donor and receiver compartments with the CsA formulation and the receiver fluid respectively, the receiver compartment was connected to a reservoir and sample collector through a peristaltic pump. Samples were collected continuously, separated by predetermined time intervals and the CsA content in them was estimated by HPLC method using external standard.

2.2.5. Flux calculations and statistics

Amount of CsA released in successive 1.5-h intervals was obtained from the cumulative amount re-

leased at different time intervals and the flux was calculated per unit area and time. Cumulative amount of CsA released at the end of 22.5 h and the maximum flux values for different formulations were compared using analysis of variance and multiple comparison procedures (the Tukey test) employing SigmaStat, Version 2.03 at $P < 0.01$.

2.2.6. Preparation of electrodes

Silver wire of 0.5 mm diameter was folded at one end three times to create a loop of 1 cm length. Then the electrode was rinsed in concentrated HCl, followed by water to remove surface contamination. The plating solution 1 M KCl in 1N HCl was placed in a beaker with a Teflon stir bar along with a silver wire folded as a plate as the counter electrode. The wire electrode was then placed in the beaker with the plating solution parallel to the silver plate. The negative lead of the power supply unit was attached to the silver plate and the positive to the silver wire. The current input was set at 7.5 mA for 1 h. After plating, the electrode was rinsed in water and stored in 1 M NaCl until use.

2.2.7. Iontophoresis

The iontophoresis experiments were conducted using the same set-up as for the passive diffusion studies. Prior to the experiment the Ag/AgCl electrodes were placed in the diffusion cells such that the electrodes were 1.8 cm away from the skin. The power supply leads from the Phoresor® iontophoretic device were connected to the electrodes, the positive lead to the electrode in the donor cell for anodal iontophoresis and negative lead to the electrode in the donor cell for cathodal iontophoresis. Current of different intensities 0.28 and 0.57 mA/cm^2 was passed from 0 to 5 h and passive diffusion was continued after that for about 20 h.

2.2.8. HPLC method of analysis

The CsA assay was based on a method reported in literature and slightly modified to avoid any interference by solvents (Abisch et al., 1982). Briefly, the method employed a reverse-phase HPLC (Hewlett-Packard) with a 4.6 mm × 250 mm All-Tech C-18 column maintained at 70 °C. A mobile phase consisting of 55% acetonitrile, 25% methanol and 20% water was used. A flow rate of 1 ml/min was used and CsA was monitored by UV detection at 210 nm. The retention time of CsA was 5.4 min. The area under the peak was used to calculate the concentration of CsA and linearity was achieved over the concentration range of 0.325–10 μg/ml with a limit of detection of 0.2 μg/ml.

3. Results

The mean (±S.D.) size of lecithin vesicles and microemulsion globules of CsA were 88.1 ± 5 nm and 27.5 ± 0.2 nm, respectively. The mean (±S.D.) Zeta potential of lecithin vesicles and microemulsion globules of CsA was −55.9 ± 5 mV and −13.1 ± 1.3 mV, respectively. Both lecithin vesicles as well as the microemulsion globules of CsA were negatively charged. However, the microemulsion globule size was smaller and the ZP was lower than that of lecithin vesicles. Of the CsA used, 96.3% was encapsulated in the lecithin vesicles and the remaining 3.7% was present in the external aqueous medium. Thus, in presence of lecithin and cholate, the aqueous solubility of CsA was increased to 120 μg/ml.

Influence of electric current on the mean cumulative amount of CsA permeated across cadaver epidermis in vitro from the different formulations tested is shown in Fig. 1. Passive diffusion did not result in the permeation of quantifiable amounts of CsA into

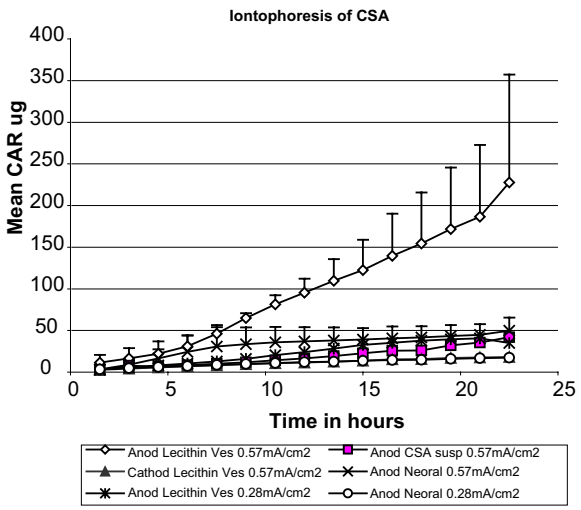

Fig. 1. Plots of mean cumulative amounts of cyclosporin A released vs. time across cadaver epidermis in vitro.

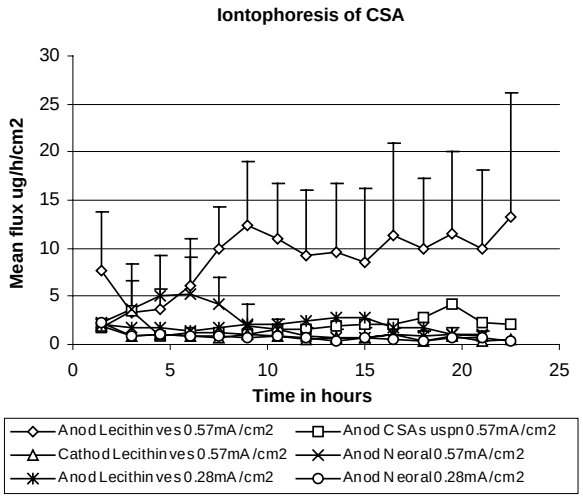

Fig. 2. Plots of mean flux of cyclosporin A across cadaver epidermis vs. time in vitro.

the receiver chamber from any of the formulations tested. However, anodal iontophoresis of all the formulations tested has resulted in the permeation of quantifiable amounts of CsA into the receiver compartment within 1.5 h, the first sampling point. Among all the formulations tested, the amount of CsA permeated in 22.5 h was highest from lecithin vesicles under anodal iontophoresis (0–5 h) at a current density of 0.57 mA/cm^2, followed by anodal iontophoresis of microemulsion. Comparison of the mean CsA released in 22.5 h from different formulations using ANOVA has revealed that the drug release from lecithin vesicle formulation under anodal iontophoresis was highest and significantly higher than all other formulations tested ($P < 0.001$). Iontophoretic trans-epidermal flux of CsA for different formulations tested is shown in Fig. 2. Interestingly, CsA flux from lecithin vesicles decreased during anodal iontophoresis, followed by an increase after stopping the passage of current. In contrast, the trans-epidermal flux of CsA from NEORAL® microemulsion increased with time during anodal iontophoresis followed by a gradual decline to attain a steady state. ANOVA of the mean maximum flux of CsA for different formulations has revealed that the flux for lecithin vesicle formulation due to anodal iontophoresis was highest and significantly different from that of other formulations tested ($P < 0.01$). Cathodal iontophoresis of lecithin vesicles of CsA has resulted in permeation of lowest amount of CsA across the epidermis (Fig. 1).

4. Discussion

Clinical potential of site-specific immunosuppression with topical CsA has been well recognized (Hewitt and Black, 1996). Tran et al. (1999) were successful in achieving site-specific immunosuppression using a topical formulation of CsA containing polyethylene glycol-8 glyceryl caprilate. However, several attempts to treat psoriasis using topical CsA with or without absorption enhancers have failed to give expected response mainly because of the low penetrability of CsA. Although CsA is not a suitable candidate for transdermal delivery according to the 500 Da rule (Bos and Meinardi, 2000), efforts are being made to deliver the drug across stratum corneum, the main barrier for permeation across skin. Hermann et al. (1988) failed to deliver CsA across excised human skin using penetration enhancers like Azone, polyvinyl pyrrolidine, propylene glycol or ethanol. In contrast to this, Cole et al. (1988) and Duncan et al. (1990) have demonstrated that CsA could penetrate excised human skin from a formulation containing olive oil. Further, significant enhancement in the penetration of CsA across excised skin was observed upon the addition of 2% (v/v) Azone and 18% (v/v) propylene glycol to 5% (w/v) CsA oily solution (Cole et al., 1988). Using intradermal microdialysis in rats, Nakashima et al. (1996) could demonstrate the ability of HPE-101 and glycerin to facilitate the permeation of CsA across the skin barrier. Absorption rate of CsA was significantly enhanced (by 4.9 times) at 10% (w/v) HPE-101, and 6.4 times by 10% glycerin. Although lecithin vesicles in the present study could deliver detectable amounts of CsA by passive diffusion, the amount released was below the limit of quantification due to the lower sensitivity of the HPLC method employed by us when compared to the immuno assay methods used by earlier workers (Wang et al., 1998).

According to 500-Da rule, only lipophilic molecules with MW less than 500 Da can permeate through the stratum corneum and thus large MW compounds like peptides are not suitable candidates for transdermal delivery (Bos and Meinardi, 2000). However, a few recent studies have revealed that peptide drugs can be

administered into systemic circulation transdermally using flexible lecithin vesicles (Guo et al., 2000; Paul et al., 1998; Cevc et al., 1998; Paul et al., 1995). Previous attempts by other investigators to deliver CsA topically using iontophoresis, electroporation have met with only limited success. Wang et al. (1998), although could succeed to deliver some CsA into the skin by electroporation, partitioning of CsA into the receiver compartment was not appreciable under the influence of electric current, which could be due to the limited solubility of CsA in PBS, used as the receiver medium. The equilibrium solubility of CsA in 0.9% saline and 20% ethanol was reported to be 0.18 ± 0.02 μg/ml, 60.61 ± 3.96 μg/ml, respectively (Guo et al., 2000). Liposomal materials in the present study have enhanced its aqueous solubility to 120 μg/ml. It may be noted that the drug in the filtrate following the passage of lipid vesicle formulation through 0.025 μm filter was considered as soluble drug based on the assumption that the contribution of lipid vesicles of size less than 0.025 μm to solubility is negligible as the mean ± S.D. size of the lipid vesicles was 88.1 ± 5 nm. Aqueous solution with 20% ethanol was demonstrated to be the most appropriate considering the sink conditions and skin physiological activity and was employed as the receiver fluid in the present study (Guo et al., 2000). The results from the current study have revealed that permeation of CsA across human epidermis can be enhanced using lecithin vesicles and anodal iontophoresis. Although both the microemulsion and lecithin vesicles were negatively charged, cathodal iontophoresis has resulted in the lowest amount of CsA permeability. This could be because of the presence of competing negatively charged cholate and chloride ions in relatively large amounts in the donor in case of lecithin vesicles. Such a possibility cannot be ruled out for microemulsion, as its excipient composition is not known. Even anodal iontophoresis of CsA suspension has resulted in permeation of detectable amounts of CsA and significantly higher permeation of CsA was achieved during and after anodal iontophoresis of microemulsion and lipid vesicles, respectively, in the present study. Cyclosporin A is a neutral, lipophilic undecapeptide and is not known to exist as a zwitterion. Thus, the electrophoretic force, component of iontophoretic delivery, may not be expected to influence the permeation of soluble CsA to a significant extent. Further, in the present study, major part of CsA (96.3%) was incorporated into lipid vesicles that are negatively charged and thus electrophoretic force under anodal iontophoresis should not contribute to the flux. However, iontophoresis might provide a significant flux enhancement due to electroosmotic flow of the soluble CsA (120 μg/ml) and increased skin permeability mechanisms. Decrease in the trans-epidermal flux of CsA during 1.5–5 h anodal iontophoresis could be due to the decreased electroosmotic flow as a function of time under the influence of electric current. Rapid increase in the flux to reach a steady state following iontophoresis of lipid vesicles clearly indicated that the skin permeability is increased under the influence of electric current. Although, the intensity of charge on lipid vesicles and microemulsion droplets differ, both were negatively charged and it is interesting to note that the trans-epidermal flux decreased during anodal iontophoresis in case of lipid vesicles while it increased with time in case of NEORAL® microemulsion. This could be because of the size differences between the lecithin vesicles (mean size 88 nm) and the emulsion droplets (mean size 27.5 nm). Electroosmosis has been reported to dominate the mechanisms of flux for larger molecules/ions while ionic or Nernst–Planck effect is important for small ions (Pikal, 2001). Apart from electroosmosis, the flexibility imparted by sodium cholate to lipid vesicles appears to have facilitated their permeation following iontophoresis as the flux increased and remained much higher for lipid vesicles after passage of current was stopped despite their larger size when compared to microemulsion droplets (Guo et al., 2000; Paul et al., 1998; Cevc et al., 1998; Paul et al., 1995). To conclude, electroosmosis of soluble CsA and increased permeability of epidermis due to passage of electric current resulting in facilitated transfer of lecithin vesicles, appear to have contributed for the enhanced delivery of CsA across epidermis. If the results of the present study are verified in vivo psoriasis models and in patients, the strategy might help to achieve site-specific immunosuppression using CsA.

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