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RESEARCH PAPER

Evaluation of Oil/Water-Type Cyclosporine Gel Ointment with Commercially Available Oral Solution

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ABSTRACT

Oil/water-type cyclosporine (CyA) hydrogel ointment was evaluated as a candidate for the percutaneous application of CyA. The physical properties and the permeation profiles of 2% w/w CyA gel ointment were compared with other CyA ointments. All ointments used in this study were prepared with commercially available CyA (Sandimmune) oral solution, unlike the ointment reported in the publication of by Mizoguchi et al. The gel ointment required a surfactant corresponding to 5–7% w/w to obtain fine uniform particles. Mean diameter of oily particles in the gel ointment was 8.75 μm. The permeation of CyA from the ointments was investigated by using the abdominal skin of hairless rats in vitro. The percutaneous permeation of CyA was observed to be influenced by a variety of ointment bases used and by the presence of a stratum corneum which plays a role as the main barrier. In intact skin, the extent of permeation from the gel ointment was almost equivalent to that from Mizoguchi's ointment, which used the raw CyA. No permeation was observed in ointment bases with either white petrolatum or hydrophilic petrolatum, indicating values under the limit of detection (78 ng/ml) of the high-performance liquid chromatographic method used in this study. On the other hand, in stripped skin, differences in flux value of each ointment were shown. Those values increased in the following order: Mizoguchi's ointment, white petrolatum, hydrogel, hydrophilic petrolatum. From these results, hydrogel ointment seemed to be applicable for various skin diseases which respond to CyA. Of the physical properties, spreadability and consistency showed that gel ointment was superior

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to Mizoguchi's ointment. In stability tests, the gel ointment was stable with regard to particle size and residual CyA for 4 weeks. These results suggested that the oil/water-type CyA hydrogel ointment prepared in a combination with hydrogel and commercially available oral solution was useful for practical hospital preparations, with good physical properties, permeability, and stability.

INTRODUCTION

Cyclosporine A (CyA), a nonpolar cyclic oligopeptide consisting of 11 amino acids and a potent immunosuppressive agent (1), has been primarily used for the prevention and treatment of organ transplant rejection (2). Recently, it has shown remarkable efficacy in psoriasis (3) and has shown potential usefulness in other dermatological disease (4). CyA is a systemically administered orally and by intravenous infusion (5). However, clinical use of CyA is limited because of its significant side effects such as nephrotoxicity (6). Therefore, effective topical preparations without systemic absorption are now being researched in the field of dermatology. Although there are many reports of topical CyA showing effectiveness for alopecia areata (7), nickel contact sensitivity (8), psoriasis (9), and atopic dermatitis (10), most of these studies unfortunately have used a raw CyA which the pharmaceutical company is not able to supply for clinical use. In spite of many reports, it appears that there is little published information on those preparations. Meanwhile, gel bases have been known to be useful for pharmaceutical preparations over the past years. In the present investigation, the usefulness of an oil/water-type hydrogel ointment prepared with commercially available CyA oral solution was evaluated for possible development of practical hospital preparations.

EXPERIMENTAL

Materials

Commercially available Cyclosporine (CyA) oral solution containing 100 mg of an active ingredient per milliliter as a labeled amount, was purchased from Sandoz Pharma Ltd. (Basel, Switzerland). CyA (Lot No. 92286 01) and cyclosporine D were generously supplied by Sandoz Pharma Ltd., and they were used without further purification. The carboxyvinyl polymer marketed as Hiviswako 104 was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). White petrolatum and hydrophilic petrolatum were of Japanese Pharmacopoeia grade. All other chemicals were of pure reagent grade.

Preparation of Ointments

Four kinds of 2% w/w CyA ointment were prepared as follows. Composition in the hydrogel ointment (HGO) is shown in Table 1. With HGO, immediate coagulation or coalescence of particles has been shown in simple homogenization. Therefore, a surfactant which had been already included in CyA oral solution, polyoxyethylated glycerides (5), was added as an emulsifier to the composition of HGO. Nikkol TMGS-5 (Nikko Chemicals Co., Ltd., Tokyo, Japan) was used as the emulsifier in this study. Hiviswako 104 was dissolved in a mixture of propylene glycol and an adequate amount of purified water in a water bath at 50°C. Another mixture of the oral solution and various amounts of TMGS-5 (0–10% w/w), an oily composition, was thoroughly mixed at 50°C and poured into the above viscous fluid. The resultant mixture was then emulsified at 7000 rpm for 10 min with a homogenizer (ACE AM-11; Nihonseiki Kaisha Ltd., Tokyo, Japan) to obtain fine oily particles. Finally, the residue of purified water, including di-isopropanolamine as a neutralizing agent, was added to the emulsifion to adjust the pH value to 7.0, then mixed thoroughly in a mortar. The quantity of purified water was regulated according to TMGS-5 concentrations. The ointment which has been studied by Mizoguchi et al. (PUO) (10) was prepared with a raw CyA according to the patent specification

Table 1

Composition of the Oil/Water-Type Hydrogel Ointment Using a Commercially Available Cyclosporine Oral Solution

Cyclosporine A oral solution	20.0 ml
Carboxyvinyl polymer	1.0 g
Propylene glycol	12.0 g
Diisopropanolamine	1.2 g
Polyoxyethylene glyceryl monostearate	0.0–10.0 g
Purified water	ad. 100.0 g

(11). The ointment with white petrolatum (WPO) or hydrophilic petrolatum (HPO) was prepared by sufficient mixing with the oral solution in a mortar. All ointments were stored at 20°C under light-resistant conditions until use.

Physical Properties

Particle Size

Particle size in oil/water-type emulsified ointments was observed with an optical light microscope (VANOX AHB-LB; Olympus Optical Co. Ltd., Tokyo, Japan), having a magnification of ×100. Mean value of the diameter of 200 particles in each ointment was measured after confirming a logarithmic normal distribution.

Spreadability

Spreadability in each ointment was evaluated with a spread meter having a 115.5-g glass plate (Rigosha & Co. Ltd., Tokyo, Japan). Diameters of the samples were measured at 1 min after beginning at 20°C.

Consistency

Consistency in each ointment was evaluated with a penetrometer having a 49-g needle with a disk tip (diameter: 1 cm)—Rigosha & Co. Ltd., Tokyo, Japan). Depth of penetration of the samples was measured at 5 sec after beginning at 20°C.

In Vitro Permeation Study

A modification of the method used by P. Schmook et al. (12) was employed. Intact or stripped abdominal skin which had been excised from male hairless rats, WBN, each weighing about 200 g, was mounted in static Franz-type diffusion cells (exposed area: 3.14 cm^2—Ishii Co. Ltd., Tokyo, Japan) with a water jacket (32°C). As pretreatment, skin was immersed in saline for 12 hr at 5°C prior to the study, then dried by an ambient air stream. For the stripped skin, the stratum corneum was removed by 15 strippings with adhesive tape (Scotch 810; Sumitomo 3M Co. Ltd., Tokyo, Japan); this was confirmed by histology. The receptor chamber (volume: 16.0 ml) was filled with a mixture of saline and methanol (3:1, v/v) and stirred by a magnetic stirrer during the study. Each 0.5 g of ointment was placed on the skin, then covered firmly with a bell-shaped cap. Samples, 500 μl, were withdrawn from the receptor chamber at 1-hr intervals and immediately replaced with an equal volume of the flesh mixture. Samples were analyzed as described below. All experiments were performed three times up to 12 hr after start; mean values and standard deviations were plotted. All experimental procedures described above were performed according to the rules set by the Committee on Ethics in the Care and Use of Laboratory Animals in Hoshi University.

Stability

As an index of stability for the emulsified ointment which had been stored at 20°C under light-resistant condition, the measurement of particle size and CyA content analysis described below were performed at 1-week intervals for 4 weeks.

Determination of CyA

CyA was assayed by means of a high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. For CyA in ointments, the United States Pharmacopoeia method for cyclosporine oral solution was modified. Each 0.5 g of ointment was mixed with 10 ml of tetrahydrofuran in 50 ml light-resistant centrifugal tubes, and sonicated for 10 min (UK-20; Sakura seiki Co. Ltd., Tokyo, Japan) to dissolve the ointments completely. Then 20 ml of the mobile phase described below was added to the tubes and shaken for 30 min with a mechanical shaker (SC-D; Kayagaki Irikakogyo Co. Ltd., Tokyo, Japan). After centrifugation at 2330 × g for 15 min (Universal 5800; Kubota Co. Ltd., Tokyo, Japan), the liquid phase was filtered with paper (Advantec No. 6; Toyo Roshi Kaisha Ltd., Tokyo, Japan) and then poured into a 100-ml volumetric flask. This procedure was repeated three times in the same manner for the residue. Finally, the flask was filled with the mobile phase and mixed thoroughly. Aliquots of 100 μl, filtered through a membrane filter (pore size: 0.2 μm, Sumplep LCR4(T)-LG; Nihon Millipore Ltd., Tokyo, Japan), were injected into the HPLC system described below.

CyA contents of each ointment were calculated with correction in an absolute calibration curve. With CyA in the samples of permeation study, the method by Schmook et al. (12) was employed: 100 μl of an internal standard solution (10 μg/ml as cyclosporine D in acetonitrile) was added to 500 μl of the samples and mixed thoroughly. Aliquots of 300 μl, filtered through a 0.2-μm membrane filter, were injected into the HPLC

system described below.

The HPLC system consisted of a pump (LC-3A; Shimadzu Co., Kyoto, Japan), a UV detector (SPD-2A; Shimadzu Co.), an integrator (C-R1A; Shimadzu Co.), and an autoinjector (SIL-10A; Shimadzu Co.). A STR ODS-II column (4.6 mm ID × 150 mm L, 5 μm, 120 Å—Shimadzu Techno-Research, Inc., Kyoto, Japan) was equipped with a precolumn [Shim-pack CLC G-ODS (4); Shimadzu Co.]. CyA in the ointments and in the samples of permeation study was assayed using a mobile phase consisting of acetonitrile, water, methanol, and phosphoric acid (550:400:50:0.5, v/v); and one consisting of acetonitrile and 0.01 M aqueous ammonium sulfate, pH 6.0 (60:40, v/v), respectively. The column temperatures were kept at 50° and 70°C, respectively. The column effluents were monitored at 210 and 215 nm, respectively. Both flow rates were kept at 2.0 ml/min.

RESULTS AND DISCUSSION

Evaluation of Hydrogel Ointments

The emulsified ointments were evaluated by measuring the particle size. Milky ointments were easily prepared by homogenizing with and without surfactants. However, immediate coagulation or coalescence of particles was observed without the surfactant (Fig. 1). Therefore, the surfactants as emulsifiers, TMGS-5 corresponding to 1–10% w/w, were added to the composition of HGO to make fine, uniform particles. Assuming a logarithmic normal distribution of particle sizes, mean value of the particle diameter in each HGO was estimated. Results are given in Table 2. The particle size had a tendency to decrease with an increase in TMGS-5 concentration up to 7% w/w. Considering the standard deviation in a logarithmic normal distribution of particle size, fine, uniform particles could not be obtained with an addition of less than 3% w/w. It was found that the emulsifier 5–7% w/w was required for HGO preparation. Consequently, TMGS-5 corresponding to 5% w/w was used in this study. Figure 2 shows a photograph of typical oily particles dispersed in a sufficient quantity of purified water. The diameter of each particle was in the range of 2.7–39.2 μm. The mean diameter was 8.75 μm.

Table 2

Particle Size of the Oil/Water-Type Emulsified Ointments[a]

Surfactant (%w/w)	Diameter (μm)	*SD* (μm)
Free	19.59	2.20
1.0	18.57	2.84
3.0	7.83	2.35
5.0	8.75	1.55
7.0	7.73	1.60
10.0	10.68	1.60

[a]Each value is given as the mean of 200 particles.

Physical Properties

Table 3 shows the physical properties of various ointments. Content in each ointment was almost equivalent. If a general requirement for the content of CyA oral

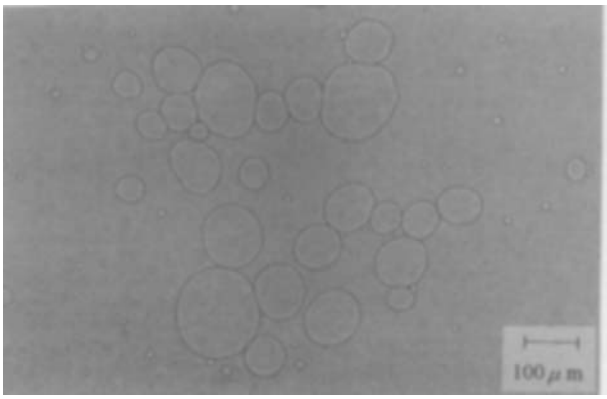

Figure 1. Photograph of particles in HGO without TMGS-5.

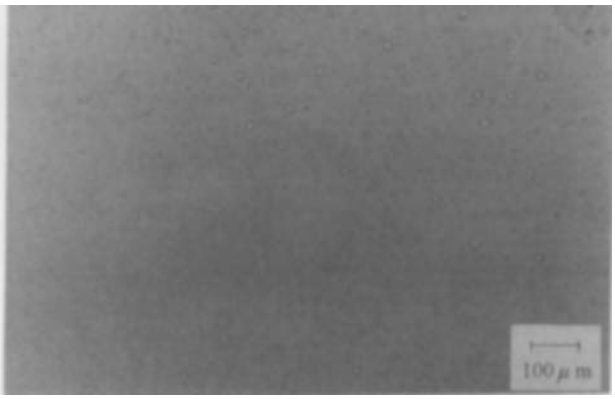

Figure 2. Photograph of particles in HGO containing 5% w/w TMGS-5.

Table 3

Physical Properties of Various Ointments Containing CyA[a]

Ointment	Mean Content ± *SD* (mg/g)	Spreadability (mm)	Consistency (mm)
HGO	20.20 ± 0.31	34	13.2
PUO	19.81 ± 0.30	24	1.4
WPO	19.26 ± 0.37	40	8.0
HPO	19.81 ± 0.12	30	7.8

[a]Each value is given as the mean of three experiments.

solution (90.0–110.0% of a labeled amount) is imposed on the ointments, all ointments conform to the pharmacopeial requirement. Little deviation was obtained in each ointment. These results suggest that CyA homogeneity is acceptable for all 2% w/w CyA ointments prepared in this study. The spreadability and the consistency are important factors in therapy and they are shown as indexes of ease of application. In these tests, it was indicated that the other ointments—unlike PUO—were soft and suitable for sensitive skin disorders such as atopic dermatitis and psoriasis. In addition, judging patient compliance, HGO seems to be superior to the other ointments because it is not sticky and is easily washed off.

In Vitro Permeation Study

Figures 3 and 4 show the in vitro permeation profiles of CyA from various ointments. In intact skin, it seems that the amount of permeation from HGO is almost equivalent to that from PUO. CyA in WPO and HPO could not be detected using the HPLC method, indicating values under the limit of detection (78 ng/ml). Although the reason for these results is not clear, the high lipophilicity of CyA might inhibit the partitioning from ointments to the skin due to its high affinity to an oily base such as petrolatum in WPO and HPO. Mizoguchi et al. have reported that myristic acid isopropyl ester formulated in PUO acts as an effective penetration enhancer for the skin permeation of CyA (10). Although the so-called penetration enhancers were not included in HGO at all, the preparation indicated the comparable enhancement with PUO. On the other hand, as shown in Fig. 4, the permeability of each ointment was remarkably increased in stripped skin, and various profiles were shown depending on the ointments. This suggests that a stratum corneum acts as the main barrier for drugs, as others have also reported (13), and a variety of bases influence these diffusion behaviors in dermis.

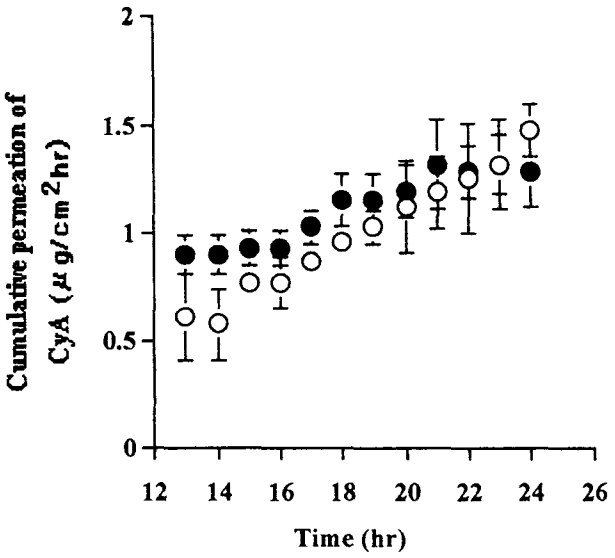

Figure 3. Cumulative amounts of CyA permeation from various ointments through the intact skin in vitro: ○, HGO; ●, PUO. Each point represents the mean ± *SD* of three experiments. WPO and HPO were under the limit of detection (78 ng/ml).

Permeation parameters in stripped skin are compared in Table 4. The lag times were almost the same, shortened to approximately 2 hr in each ointment. The flux values increased in the following order: PUO, WPO, HGO, and HPO, indicating 1.2, 2.4, 3.4, and 4.2 μg/cm², respectively. Emulsified preparations, HGO (O/W type) and HPO (W/O type), were superior to the other ointments in permeability. There was no significant difference in the flux value between HGO and HPO. It is impossible to determine whether the bases used influenced a coefficient of distribution and/or of diffusion because it is difficult to determine the solubility of CyA in the ointments. However, these results suggest that an emulsified ointment is useful as a carrier vehicle for

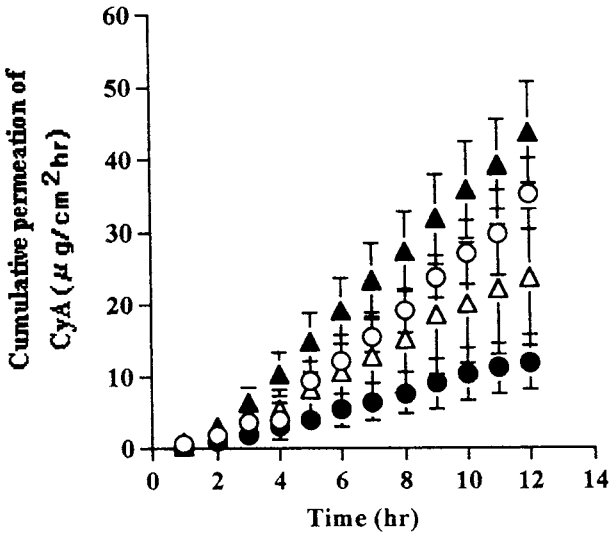

Figure 4. Cumulative amounts of CyA permeation from various ointments through the stripped skin in vitro: ○, HGO; ●, PUO; △, WPO; ▲, HPO. Each point represents the mean ± *SD* of three experiments.

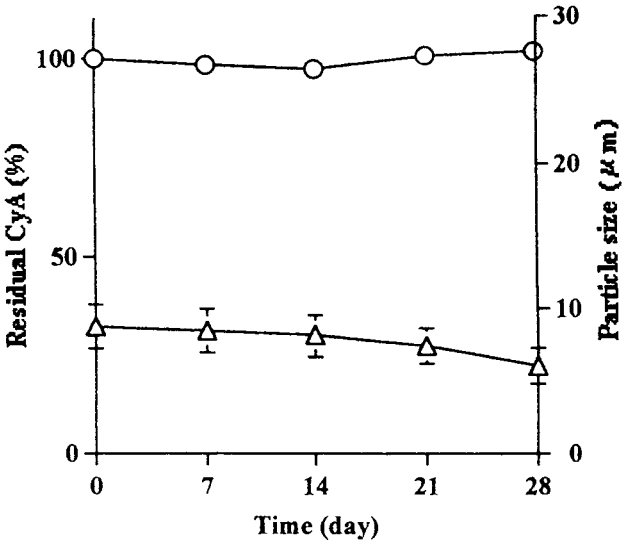

Figure 5. Stability of HGO stored at 20°C: ○, residual amounts; △, particle size. Each point represents the mean ± *SD* of three experiments. The *SD* of content stability means within a symbol.

CyA. Especially, considering dermatological diseases such as atopic dermatitis, in which the stratum corneum is partially broken, or alopecia areata, in which it is normal, HGO is expected to be useful for various skin diseases.

Stability Tests

Although there are some reports indicating that CyA is stable in intravenous fluids such as fat emulsion or lipid emulsion (14), there are no reports about ointments. Results of stability tests are shown in Fig. 5. The residual amounts of CyA in HGO are expressed as a percentage against the initial values. Change in particle size in HGO was also followed. CyA in HGO was stable and there were no significant changes in particle size for 4 weeks. These results suggest that the oil/water-type hydrogel ointment is sufficient for hospital preparations.

Table 4

Permeation Parameters of CyA from Various Ointments Through Stripped Skin In Vitro[a]

Ointments	Lag Time (hr)	Flux (μg/cm²hr)
HGO	2.1 ± 0.4	3.39 ± 0.60
PUO	1.6 ± 0.7	1.19 ± 0.32
WPO	1.8 ± 0.4	2.43 ± 1.00
HPO	1.5 ± 0.4	4.21 ± 0.62

[a]Each value is given as the mean ± *SD* of three experiments.

CONCLUSION

Oil/water emulsified-type hydrogel ointments containing commercially available CyA oral solution were easily prepared; and were superior in permeability, better to spread, washable, and stable. The use of a hydrogel for topical CyA application enabled the ointments to be prepared from commercially available oral solution. The hydrogel ointments prepared in this study are useful candidates from the point of view of practical hospital preparations.

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