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Pharmacokinetics and organ distribution of cyclosporin A incorporated in liposomes and mixed micelles

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Abstract

The commercially available intravenous dosage form of cyclosporin A (C-CsA) contains a solubilizing agent, polyoxyethylated castor oil, which has been reported to be toxic. To replace the toxic solubilizing agent present in C-CsA, liposomal and mixed micellar preparations were made to solubilize CsA by the proliposome method and characterized. Furthermore, pharmacokinetics and organ distributions of these preparations were evaluated in comparison to C-CsA, which is micellar. The mean size of liposomal preparation (L-CsA) composed of DPPC/PA (molar ratio 3/1) and CsA was 43.6 nm and that of mixed micellar preparation (M-CsA) composed of DMPC/DSPE-PEG (molar ratio 95/5) and CsA was 6.5 nm. The solubilization of CsA was 2-fold greater in mixed micellar solution than in liposomes (0.06 vs 0.03 mg of CsA/mg of lipid). L-CsA, M-CsA and C-CsA were intravenously administered into rats via the femoral vein to analyze pharmacokinetics and organ distribution of CsA. M-CsA was not significantly different from C-CsA in every pharmacokinetic parameter studied. However, L-CsA resulted in 30% decrease in AUC and 55% increase in Cl_t compared with C-CsA ($P < 0.05$), without any significant differences in MRT, V_{dss} and $t_{1/2}$. In addition, the distributions of M-CsA and L-CsA in different organs were not significantly different from those of C-CsA ($P > 0.05$), except for a 51% decrease of M-CsA in the spleen at 4 h and a 33% increase of L-CsA in the liver at 4 h ($P < 0.05$). These findings demonstrate that the liposomal preparation composed of DPPC/PA and CsA shows slightly different pharmacokinetics and organ distribution patterns from C-CsA, whereas the mixed micellar preparation composed of DMPC/DSPE-PEG and CsA exhibits similar patterns to C-CsA, as expected. Furthermore, these results suggest that those mixed micellar and liposomal preparations can replace C-CsA containing the toxic solubilizing agent, thus providing useful alternative dosage forms for intravenous administration of CsA.

Keywords: Cyclosporin A; Liposome; Mixed micelle; Parenteral delivery; Pharmacokinetics; Proliposome

1. Introduction

Cyclosporin A (CsA), a poorly water-soluble cyclic peptide comprising 11 amino acids, has been utilized clinically for immunomodulation,

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such as the prevention of rejection following transplantation of kidney, liver, bone marrow and pancreas (Matzke and Luke, 1988). The use of CsA has been limited due to a broad toxicity profile, including nephrotoxicity and hepatotoxicity. Nephrotoxicity may appear acutely, associated with reversible hemodynamic changes (Curtis and Laskow, 1988), or as a result of interstitial fibrosis after chronic dosing (Humes et al., 1985). It has also been reported that Cremophor EL®, a solubilizing agent, present in the commercially available intravenous dosage form of cyclosporin A (C-CsA), was nephrotoxic and hemolytic (Thiel et al., 1986; Tibell et al., 1993).

Attempts have been made to reduce the toxicity of CsA during intravenous therapy by using liposomes. Several investigators reported that CsA-containing liposomes showed reduced nephrotoxicity (Smeesters et al., 1988; Freise et al., 1994) and various dispositions (Vadiei et al., 1991; Freise et al., 1994). Many of those products were usually made by the thin lipid film hydration method or by a modification of the method (Vadiei et al., 1989). The size of those products is so large for intravenous administration that the administered liposomes might block the microvasculature of lungs and other organs. Hence, the size distribution of liposomes for intravenous administration has to be carefully controlled.

In addition, aqueous dispersions of liposomal drugs have the following problems during the storage period: phospholipid hydrolysis, decomposition of encapsulated drug, separation of drug from liposome, and sedimentation, aggregation and fusion of liposomes. Payne et al. (1986a, 1986b) introduced drug-loaded proliposomes, a dry free-flowing granular product, which is hydrated immediately before use in order to overcome the above-mentioned problems of liposomes. Drug-loaded proliposomes are composed of water-soluble porous powder as a carrier to load phospholipid and drugs. Proliposomal drugs can be stored and sterilized in a dry state, and dispersed/dissolved to form an isotonic multilamellar liposomal suspension suitable for administration either intravenously or by other routes after the addition of water just prior to use.

Micelles can be thought in a general sense as aggregates with a liquid-like core and with their ionic or polar nonionic moieties exposed to water. Most amphiphiles in biological membranes, including phospholipids, cholesterol and membrane proteins, are insoluble. The components of membrane can be solubilized in the form of mixed micelles with soluble amphiphiles (Lichtenberg et al., 1983). In a phospholipid–surfactant mixture, the formation of aggregates at equilibrium depends on the composition of the mixture.

In the present study, we prepared and characterized liposomal and mixed micellar formulations for the solubilization of CsA by preparing proliposomes to replace the toxic solubilizing agent present in C-CsA. Furthermore, we compared the pharmacokinetics and organ distribution of these preparations with those of the currently available intravenous dosage forms of CsA, using rats as an animal model.

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (EPC), dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC) and phosphatidic acid (PA) were purchased from Sigma (St. Louis, MO, USA). Distearoyl phosphoethanolamine–polyethylene glycol 2000 (DSPE-PEG) was a product of Avanti Polar Lipids (Birmingham, AL, USA). Sorbitol was purchased from Yakuri Pure (Osaka, Japan). CsA was kindly supplied as a courtesy of Ryun Pharmaceutical (Seoul, South Korea). All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of CsA-containing mixed micellar solution and liposomes

The liposomal and mixed micellar formulations composed of DPPC/PA (molar ratio, 3/1) and DMPC/DSPE-PEG (molar ratio, 95/5), respectively, were prepared by the method for the preparation of proliposomes (Payne et al., 1986b; Park et al., 1994). A modified rotary evaporation unit

was used for coating the inert core substrate, sorbitol, with the organic solutions of drug and lipids. The modification involved inserting a polyethylene tube into the unit via the vacuum line, which was connected with a stopcock and 2-ml syringe. The organic solution of CsA and lipids in the syringe was introduced automatically via the polyethylene tube running through the condenser unit into the sorbitol in a round-bottomed flask under reduced pressure. The sorbitol, passed through a 500-μm sieve and remained on a 298-μm stainless-steel sieve, was dried overnight at 35–45°C under reduced pressure (360 mmHg). The CsA and lipids were dissolved in a mixture of chloroform and methanol (2.5:1, v/v) to give a final drug concentration of 1 mg/ml. About 1 ml of the solution of CsA and lipids was added into the tumbling sorbitol via the feed line running through the condenser unit. Evaporation was allowed to proceed until the powder bed flowed absolutely freely, at which point a second aliquot of the CsA–lipid solution was introduced. Evaporation continued until the powder bed was absolutely dried after the addition of the final aliquot. At that point, the material was removed from the flask and lyophilized for at least 4 h. When dry, the material, passed through a 500-μm sieve, was stored in a sealed container at 4°C. Just prior to use, the material was hydrated with water-for-injection above the phase transition temperature of lipids, shaking occasionally for 30 min. In the case of liposomes, the trace of unentrapped CsA was occasionally removed by centrifugation at $12\,000 \times g$ for 20 min and ultrafiltration using 0.45-μm polycarbonate filter.

2.3. Determination of particle size of mixed micelle and liposomes

The particle size distributions of formulations were measured by photon correlation spectroscopy (Kim et al., 1999; Park et al., 1999). A light scattering spectrophotometer (LPA-3100, Otsuka Electronics, Osaka, Japan) equipped with a data-processing unit (LPA-3000, Otsuka Electronics) was used for characterizing the particle size using the dynamic light scattering method. For measuring the particle size by photon correlation spectroscopy, the hydrated formulations were diluted with water-for-injection to the concentration giving a ratemeter count of 8000–12 000 counts per second (cps). The water-for-injection did not exhibit light scattering.

2.4. Amount of solubilized CsA

To determine the amount of drug entrapped after hydration, the liposomal suspension was centrifuged at $12\,000 \times g$ for 20 min and then the supernatant was filtered through a 0.45-μm polycarbonate filter. The amounts of CsA in liposome and mixed micellar solution were determined by the HPLC method (Kim et al., 1997; Gao et al., 1998). The HPLC system (Hitachi, Tokyo, Japan) consisted of a solvent delivery system, a column oven (75°C), a variable wavelength detector, an integrator and a reversed-phase column (Nucleosil R C18, ET 300/8/4; particle size, 10 μm). Mobile phase was water–acetonitrile (3:7, v/v) filtered through a 0.45-μm membrane filter and delivered at a flow rate of 1.0 ml/min. Effluents were monitored at a wavelength of 210 nm. The phospholipid content in the filtered solution were determined by Stewart assay (New, 1990).

Solubilization of CsA was represented by a ratio of solubilized CsA to lipid, mg of CsA/mg of lipid.

2.5. Animals

Male Sprague–Dawley rats weighing 280–370 g were obtained from the Laboratory Animal Center, Seoul National University (Seoul, South Korea). The rats were fed with tap water and food (Cheil Food and Chemical, I-Cheon, South Korea) ad libitum.

2.6. Pharmacokinetic studies

The femoral artery and the femoral vein were catheterized with polyethylene tubing (PE-50, Clay Adams, Becton-Dickinson, NJ, USA) under light ether anesthetization. Each rat was allowed for 1–2 h to recover from anesthesia before the experiment. About 0.1 ml of EDTA solution (1% EDTA in normal saline) was used for flushing the

cannula to prevent blood clotting. C-CsA for intravenous injection was diluted with saline to the final concentration of 1 mg CsA/ml. All for-mulations were prepared just prior to use. L-CsA, M-CsA and C-CsA equivalent to 1 mg of CsA per kg of body weight were administered intravenously via the femoral vein. Approximately 100 μl of blood samples were collected via the femoral artery into the tube containing 5 μl of EDTA as an anticoagulant just prior to dosing and at designated time intervals after drug administration. The whole blood samples were mixed thoroughly on a Vortex mixer and stored at 4°C until analysis.

The non-compartmental pharmacokinetic parameters, such as area under the drug concentration–time curve (AUC), mean residence time (MRT), total clearance (Cl_t), apparent volume of distribution at steady state (V_{dss}) and biological half-life ($t_{1/2}$) were calculated based on the reported method (Gibaldi and Perrier, 1982). The data were analyzed for statistical significance by the t-test ($P < 0.05$). All results were expressed as the mean ± standard deviation (S.D.).

2.7. Organ distribution studies

L-CsA, M-CsA and C-CsA, equivalent to 1 mg of CsA/kg, were injected through the femoral vein to rats. At 1, 4 and 8 h after intravenous injection of L-CsA, M-CsA and C-CsA, as much blood as possible was collected through the carotid artery and each rat was exsanguinated. Then, liver, spleen, kidney and lung were immediately removed. Total weights of each organ were measured. Approximately 1 g of tissue slices was excised, rinsed, minced and homogenized with 4-fold volume of saline in a tissue homogenizer and centrifuged immediately. Aliquots (100 μl) of the supernatant of tissue homogenates were collected and measured by radioimmunoassay (RIA) which was selective for the parent drug.

2.8. Quantification of CsA in whole blood

CsA concentration in the whole blood was measured by the RIA method using the CYCLO-Trac™ SP-Whole Blood RIA kit (INCSTAR Corporation, Stillwater, MN, USA). The RIA is based on a double-antibody competitive-binding assay. The standards and controls were provided in the kit, and the whole blood samples were extracted with methanol, vortex-mixed for 15 s and centrifuged at 1600 × g for 5 min at 20–25°C. A total of 50 μl of the methanolic supernatant was withdrawn into a 12 × 75-mm test tube and incubated with 100 μl of ^{125}I-labelled ligand and 1 ml of pre-mixed antibody (first and second antibodies) solution for 1 h. The mixture was then centrifuged at 1600 × g for 20 min at 20–25°C. The supernatant was immediately decanted from the tube, and any drops remaining in the tube were removed with absorbent paper. The precipitate in each tube was counted for 1 min using a well type γ-scintillation counter.

3. Results and discussion

3.1. Characterization of CsA-containing mixed micellar solution and liposomes

To solubilize CsA, we used the liposomal and mixed micellar formulations consisting of DPPC/PA (molar ratio, 3/1) (L-CsA) and DMPC/DSPE-PEG (molar ratio, 95/5) (M-CsA) and the method for the preparation of proliposomes, respectively. Previously, L-CsA and M-CsA have been observed to show typical shape of micelles and liposomes, respectively, above the phase transition temperature of lipids under transmission electron micrograph after hydration (Choi, 1995). The method used in this study to prepare L-CsA and M-CsA is very simple compared to the conventional one. Only the simple hydration of the dried product to prepare M-CsA was needed without any further operations (e.g. sonication). When the dried M-CsA composed of DMPC/DSPE-PEG and CsA was hydrated with water-for-injection, a transparent solution with slight opalescent fluorescence was obtained. In addition, the hydrated solution of M-CsA could be stored at room temperature for longer than 20 days without any change in the external appearance (data not shown). However, in the case of L-CsA, we occasionally removed the trace of unentrapped CsA by centrifugation and ultrafiltration due to the incomplete solubilization

of CsA under our conditions. The mean size of M-CsA was 6.5 nm (Table 1), which was within the range of micelles. The mean size of L-CsA was 43.6 nm (Table 1), which was somewhat smaller than those of the conventional liposomes.

3.2. Solubilization of CsA

CsA was solubilized 2-fold greater in mixed micellar solution than that in liposomes (0.06 vs. 0.03 mg/mg of lipid, Table 2). Moreover, the value of 0.06 mg of CsA/mg of lipid in the mixed micellar solution was found not to be the saturation value and CsA could be solubilized up to about 0.12 mg of CsA/mg of lipid (data not shown). The entrapping efficiency of CsA in liposome was 46.9% (Table 2). DSPE-PEG is a synthetic lipid made of DSPE and PEG 2000, having two fatty acyl chains and a very long hydrophilic domain, respectively. We think that DSPE-PEG, a component of M-CsA used in the present study, acted as a good solubilizing agent since only 5% of DSPE-PEG could make the DMPC/DSPE-PEG system a micellar solution, whereas 100% DMPC without DSPE-PEG could hardly solubilize CsA. In addition, sorbitol used in our system might act as a cosurfactant, as observed by others (Ktistis, 1990; Attwood et al., 1992). Among the liposomal formulations examined, DPPC/PA liposome could solubilize CsA most effectively under our experimental conditions (data not shown).

Table 1
The size of CsA-containing liposomes and mixed micelles[a]

Lipid composition (molar ratio)	Mean size (nm)
Liposomes: DPPC/PA (3:1) with CsA	43.6 ± 3.2
Mixed micelles: DMPC/DSPE-PEG (95:5) with CsA	6.5 ± 1.4

[a] Each value represents the mean $\pm$ S.D. ($n = 5$).

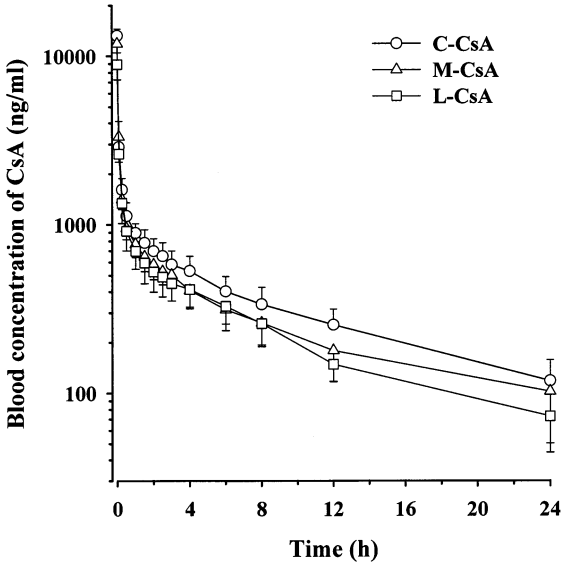

Fig. 1. CsA concentration–time profiles in whole blood after intravenous injection. Each point represents the mean $\pm$ S.D. ($n = 5$).

3.3. Pharmacokinetics and organ distributions after intravenous administration

The mean plasma concentration–time curves of CsA after intravenous administration of L-CsA, M-CsA and C-CsA to rats showed that the elimination of CsA from the blood was rapid up to 30 min followed by the slow elimination phase in all three preparations (Fig. 1). The pharmacokinetic parameters of L-CsA, M-CsA and C-CsA are listed in Table 3. M-CsA was not significantly different from C-CsA in every pharmacokinetic parameter studied ($P < 0.05$). However, L-CsA resulted in 30% decrease in AUC and 55% increase in Cl_t compared with C-CsA ($P < 0.05$). In addition, MRT and $t_{1/2}$ of L-CsA were shorter than those of two other formulations, although statistically not significant ($P > 0.05$).

The distributions of CsA in each organ at 1, 4 and 8 h after intravenous injections of L-CsA,

Table 2
Solubilization and entrapping efficiency of CsA in liposome and mixed micelle[a]

Lipid composition (molar ratio)	CsA/lipid (mg/mg)	Entrapping efficiency (%)
Liposomes: DPPC/PA(3:1) with CsA	0.030 ± 0.002	46.9 ± 5.4
Mixed micelles: DMPC/DSPE-PEG(95:5) with CsA	0.060 ± 0.005	

[a] Each value represents the mean $\pm$ S.D. ($n = 5$).

Table 3
Noncompartmental pharmacokinetic parameters of CsA after intravenous administration of L-CsA, M-CsA and C-CsA to rats[a]

	L-CsA	M-CsA	C-CsA
AUC (ng h ml^{-1})	7806.5 ± 1644.1*	9559.1 ± 2754.1	11 091.0 ± 1927.3
AUMC (ng h^2 ml^{-1})	96 053 ± 44 771	152 830 ± 78 738	175 270 ± 95 407
MRT (h)	11.94 ± 3.19	14.84 ± 4.62	15.25 ± 6.62
Cl_t (l h^{-1} kg^{-1})	0.14 ± 0.03*	0.11 ± 0.03	0.09 ± 0.02
Vdss (l kg^{-1})	1.57 ± 0.41	1.55 ± 0.22	1.37 ± 0.51
$t_{1/2}$ (h)	11.66 ± 2.83	13.46 ± 3.60	12.56 ± 5.48

[a] Each value represents the mean ± S.D. ($n = 5$).
* Significantly different from C-CsA ($P < 0.05$).

M-CsA and C-CsA are shown in Table 4. CsA was initially taken up to a considerable extent by the liver and the kidney compared with other organs. The distributions of L-CsA and M-CsA in different organs were not significantly different from those of C-CsA ($P > 0.05$), except for a 51% decrease of M-CsA in the spleen at 4 h and 33% increase of L-CsA in the liver at 4 h ($P < 0.05$). The similarity in pharmacokinetic parameters and organ distribution patterns between M-CsA and C-CsA was expected since both are micelles. The increased localization of L-CsA in the liver at 4 h, together with the decreased AUC and the increased Cl_t (Table 3) suggests the rapid uptake of L-CsA by the liver after intravenous administration, possibly via the reticuloendothelial system (Kim et al., 1994; Kim and Jeong, 1995).

Table 4
The concentrations of CsA in blood and various organs after intravenous administration of L-CsA, M-CsA and C-CsA[a]

	L-CsA	M-CsA	C-CsA
Blood (ng/ml)			
1 h	833.5 ± 41.7	710.0 ± 67.4	828.4 ± 102.4
4 h	497.9 ± 36.6	379.1 ± 53.2	447.3 ± 71.1
8 h	332.2 ± 43.0	220.2 ± 19.1	274.9 ± 30.3
Liver (ng/g)			
1 h	7434.0 ± 1838.0	7034.0 ± 633.3	7626.0 ± 1530.0
4 h	7587.0 ± 305.9*	5665.0 ± 445.6	5686.0 ± 1314.0
8 h	3360.0 ± 516.1	3602.0 ± 609.8	4094.0 ± 1230.0
Spleen (ng/g)			
1 h	4010.0 ± 1162.0	4580.0 ± 1003.0	4672.0 ± 192.3
4 h	4584.0 ± 821.1	3160.0 ± 254.8*	6397.0 ± 1126.0
8 h	3962.0 ± 1312.0	3269.0 ± 782.5	3918.0 ± 406.2
Kidney (ng/g)			
1 h	6488.0 ± 451.2	5274.0 ± 1568.0	5776.0 ± 776.0
4 h	5010.0 ± 1393.0	2748.0 ± 481.2	4505.0 ± 1238.0
8 h	3179.0 ± 611.7	2573.0 ± 289.0	3780.0 ± 987.5
Lung (ng/g)			
1 h	3921.0 ± 80.8	3387.0 ± 1664.0	3797.0 ± 435.4
4 h	2322.0 ± 709.5	1656.0 ± 30.0	2461.0 ± 1004.0
8 h	1789.0 ± 57.7	1448.0 ± 762.5	1733.0 ± 140.1

[a] Each value represents the mean ± S.D. ($n = 5$).
* Significantly different from C-CsA ($P < 0.05$).

4. Conclusion

In conclusion, this study indicates that liposomes containing CsA show slightly different pharmacokinetics and tissue distribution patterns from C-CsA, while the mixed micelles containing CsA exhibit similar patterns to C-CsA. Furthermore, these results suggest that those mixed micellar and liposomal preparations may serve as suitable intravenous dosage forms, replacing C-CsA, which contains the toxic solubilizing agent. Further studies should be performed to ascertain whether these liposomal and mixed micellar preparations can reduce nephrotoxicity.

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