Once-a-Day Oral Dosing Regimen of Cyclosporin A: Combined Therapy of Cyclosporin A Premicroemulsion Concentrates and Enteric Coated Solid-State Premicroemulsion Concentrates

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Purpose. To develop once-a-day oral dosing regimen that provides the blood levels of cyclosporin A (CsA) in the therapeutic ranges over 24 hours.

Methods. CsA premicroemulsion concentrates (preME) were formulated from phase diagrams. Enteric-coated solid-state premicroemulsion concentrates (sME) were prepared by coating preME with enteric-coating matrials and solidifying them. CsA was measured using high-performance liquid chromatography or radioimmunoassay.

Results. PreME consisted of CsA, oil, and mixture of surfactants and a cosurfactant. PreME spontaneously formed microemulsions in aqueous medium and showed oral absorption profiles similar to Sandimmune Neoral® in dogs. Dispersion of sME in aqueous medium also formed microemulsions. Release rates of CsA from sME depended on pH and the type of enteric-coating materials and highly correlated with the extent of oral absorption. The co-administration of preME and sME (200 mg CsA) showed the maximum blood level of CsA not significantly different from that of preME (100 mg CsA) and the concentration of CsA close to the minimum therapeutic level at 24 hours.

Conclusions. The combined treatment of preME and sME provided controlled oral absorption of CsA over a 24-hour period. Such once-a-day dosing regimens will lead to increased patient compliance and reduced episodes of organ rejection after transplantation.

KEY WORDS: cyclosporin A; microemulsion; controlled release; enteric coating; oral absorption; once-a-day dosing.

INTRODUCTION

Cyclosporin A (CsA), a highly lipophilic cyclic peptide, is one of the most important immunosuppresive agents in transplantation. CsA has been used as an immunosuppressant of choice because of a lack of myelotoxicity commonly observed in other immunosuppressants (1). However, CsA is one of the drugs that requires careful monitoring of blood levels. The blood levels of CsA above the therapeutic range were related to adverse effects, such as nephrotoxicity and neurotoxicity (2,3). Below the therapeutic levels of CsA, high episodes of organ rejection have been reported (4). The severity of symptoms observed outside the therapeutic window of CsA requires the development of formulations that can control the blood levels of CsA in the therapeutic ranges during the whole dosing interval.

CsA is administered mainly in oral dosage forms (5). However, because of the poor aqueous solubility and the limited absorption in the gut, the bioavailability of CsA administered in general formulation has been low and variable. As an improved oral dosage form, the premicroemulsion concentrate formulation of CsA has been marketed. Unlike emulsions, the premicroemulsion concentrates can spontaneously form oil-in-water microemulsions in the aqueous medium such as gastrointestinal fluids (6). Compared with the crude emulsion formulation of CsA, the premicroemulsion concentrate of CsA has been shown to reduce the variability of oral absorption (7,8) and enhance the predictability of blood levels (9).

Once-a-day oral dosing regimen of CsA would be desirable for patient compliance and therapeutic outcome. Currently marketed CsA premicroemulsion concentrates (Sandimmune Neoral®) usually are administered every 12 hours (10). Given the high episodes of organ rejection observed at the low CsA levels between the dosing intervals (4,11), it might substantially increase the risk of organ rejection for transplanted outpatients not to follow strictly the direction of dosing frequency. Once-a-day dosing would thus not only increase the compliance of outpatients usually taking CsA for a long period but also lessen the episodes of organ rejection in the transplantation patients.

To provide the blood levels of CsA in the therapeutic range for 24 hours, premicroemulsion concentrate formulations *per se* may have limitations because the increase of dose for prolonged residence time in the body would result in the high blood levels accompanying serious toxicity. Accordingly, it might be necessary to develop a controlled release form of a premicroemulsion that keeps the drug within the therapeutic range for a longer period of time. Solid-state emulsions that exist in solid powder but emulsify upon addition of aqueous phase have been studied to modulate the release rates of emulsified compound (12). Until now, few studies have been performed to further control the release rate and absorption pharmacokinetics of CsA by formulating solid dosage forms of premicroemulsion concentrates.

In this study, to design once-a-day oral dosing regimen, we formulated new CsA premicroemulsion concentrates (preME) and developed a novel technology for enteric coated solid-state premicroemulsion concentrates of CsA (sME). Here, we report that the co-administration of preME and sME (200 mg CsA) provided the blood levels of CsA above the minimum therapeutic level for about 24 hours with a peak level comparable to that of preME (100 mg).

MATERIALS AND METHODS

Materials

Cyclosporin A (CsA) was supplied from Chong Kun Dang Corporation (Seoul, South Korea). Polyoxyl 40 hydrogenated castor oil 40 (Cremophor RH 40®, Cre) was pur-

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chased from BASF (Ludwigshafen, Germany). Mono- and di-glyceride (Gly) and poloxamer 124 (Pol) were from ICI (Wilmington, Germany). Propylene carbonate (ProC) was from Sigma (St. Louis, MO). Eudragit L 100 (EuD) was from Rohm Pharma (Kirschenallee, Germany). Cellulose acetate phthalate (CAP) was from Eastman Fine Chemicals (Kingsport, TN). Sodium alginate (AL) was from Hayashi Pure Chemical Industry (Osaka, Japan).

Construction of Pseudo-Ternary Phase Diagrams

The pseudo-ternary phase diagrams were constructed with water, oil (medium chain triglyceride), and a mixture of surfactants and a cosurfactant (Smix) in each axis. The surfactants consisted of Cre, Gly, and Pol (weight ratio 5:1:1). Smix contained the surfactants and the cosurfactant (ProC) at the weight ratio of 2.5:1. The mixtures of oil and Smix were titrated with water and kept at 25°C to reach equilibrium. The phase of each mixture was visually determined to be clear isotropic microemulsion (L), gel (G), crude o/w emulsion (E1), or crude w/o emulsion (E2) region (13).

Preparation of Enteric-Coated sME

Enteric-coated sME were prepared by coating preME with enteric carrier polymers, such as AL, EuD, and CAP, in organic solvents and pulverizing the dried enteric coated-preME film. PreME consisted of CsA (10% w/w), medium chain triglyceride (18.5% w/w), the surfactants (51% w/w), and cosurfactant (20.5% w/w). The addition of preME into water of various volumes produced o/w microemulsion upon mild agitation. The droplet sizes of microemulsions were measured using a laser particle size analyzer (Autodilute 370, Nicomp particle sizing systems, USA).

For EuD-coated sME and CAP-coated sME, EuD and CAP dissolved in acetone were homogeneously mixed with preME at the ratio of 1:1 (w/w) and 2:1 (w/w), respectively. Acetone was slowly evaporated at 40—50°C until a film was formed. The film was completely dried at 40°C and powdered using a mortar, then passed through a 20-mesh screen. For AL-coated sME, AL (2%) dissolved in acetone was mixed with polyethylene oxide (1%) dissolved in acetone, then dropped to preME with a mixing ratio of 1:5 and emulsified. The emulsion was dropped into 0.2 M $CaCl_2$ and cured. The resulting AL-coated sME were collected and dried.

Preparation of Solid Dispersion and Solid-State Dispersion of Emulsion

Solid dispersion was made of using EuD as a dispersion base. EuD (1 g) dissolved in sufficient acetone was added to the mixture containing CsA (100 mg) and Cre (350 mg). The resulting mixture was homogenized and then acetone was slowly evaporated at 40–50°C until a brittle film was produced. Solid-state dispersion of emulsion was done by dissolving 1 g of EuD in acetone and mixing the EuD solution with the crude emulsion content of Sandimmune® containing 100 mg of CsA. The mixture was then dried and powdered.

In Vitro Release Study

CsA release from solid dosage forms was preformed according to USP XXIII dissolution procedure using a dissolution apparatus II. Solid dosage forms containing 100 mg of CsA were filled into hard gelatin capsules (capsule No. 00). The capsules were added into 500 ml of simulated gastric juice without pepsin (pH 1.2, first fluid) at 37 ± 0.5°C with a paddle speed of 100 rpm. Each sample (4 ml) was withdrawn at a 30-minute time interval, and the same volume of simulated gastric juice was compensated. Two hours after incubation in first fluid, 400 ml of 0.235 M sodium phosphate monobasic solution was added into the vessel to adjust the pH of the medium to 6.8. Aliquots of the samples were taken every 10 minutes and simulated intestinal fluid without pancreatin (pH 6.8, second fluid) was added to compensate the volume. The amounts of CsA were determined by high-performance liquid chromatography (HPLC) (Hewlett Packard, USA) using a Capcellpak C8 column (Shiseido, Japan). A mobile phase consisted of 65% (v/v) acetonitrile, 5% (v/v) methanol, and 30% (v/v) phosphate buffer. The flow rate was 2 ml/min, and the effluents were monitored at 215 nm. The limit of detection and the limit of quantitation were 2.57 μg/ml and 7.81 μg/ml, respectively. CV value was 55.27%.

Animal Experiments

Animal experiments were performed according to the Seoul National University guideline for experimental animal case. Male beagle dogs weighing 15 ± 2 kg were fasted overnight before oral administration of CsA and dosed independently. For oral administration, various dosage forms with 100 mg of CsA were filled into gelatin capsules. Soft gelatin capsules were used for preME and hard gelatin capsules for solid dosage forms. Immediately after the dosing, 30 ml of water was orally supplied with a syringe. Blood (3 ml) was collected from the cephalic vein over 24 hours and frozen under –20°C until analysis. The concentrations of CsA in whole blood were measured using CYCLO-Trac™ SP-whole blood® radioimmunoassay kit (Incstar Corp., USA).

Statistical Analysis

Data are expressed as means ± standard deviation ($n \leq 3$). Statistical differences were evaluated using the unpaired Student's *t*-test or ANOVA. Results were termed significant at $P < 0.05$. Duncan's multiple range test was used as a post-hoc test.

RESULTS AND DISCUSSION

Formulation of preME

To develop an optimum formulation of preME spontaneously forming o/w microemulsions upon addition of water, the pseudo-ternary phase diagram was constructed (Fig. 1). Medium-chain triglycerides were selected as oil because they have been reported to improve the intestinal absorption of various active compounds (14). ProC was used as a cosurfactant to solubilize CsA. The solubility of CsA was 27.51 ± 2.12 mg/g in ProC whereas it was 3.66 ± 0.4 mg/g in medium-chain triglyceride and 3.84 ± 0.6 mg/g in the surfactants which consisted of Cre, Gly, and Pol (5:1:1 weight ratio). Cre was chosen as a major surfactant because its activity is less dependent on pH and ionic strength. In addition, Cre was reported to enhance the intestinal permeability of drugs (15). As minor surfactants, Pol (HLB 12-18) and Gly (HLB 1-3) were added in equal amounts to improve the stability of microemulsions (6).

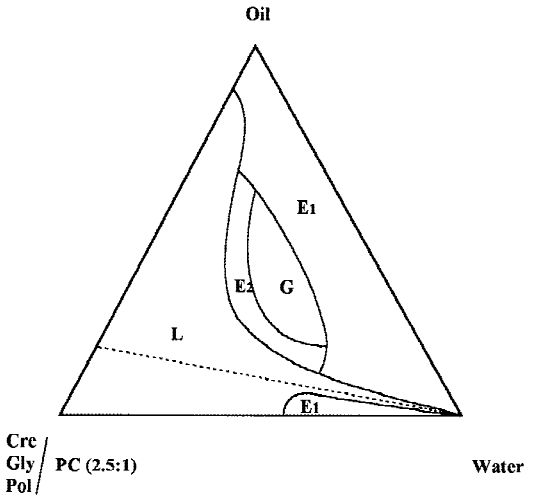

Fig. 1. Pseudo-ternary phase diagram. Mixtures were composed of CsA (10% w/w), oil (medium chain triglyceride), water, and Smix containing surfactants and a cosurfactant (ProC). The surfactants consisted of Cre, Gly, and Pol (weight ratio 5:1:1). Each phase was described as L (isotropic microemulsion), G (gel), E1 (crude o/w emulsion), and E2 (w/o emulsion).

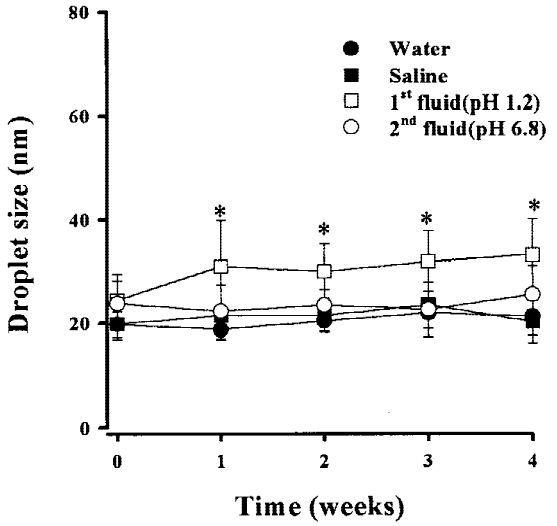

Fig. 2. Physical stability of CsA microemulsion in the aqueous medium. After dispersion of preME in aqueous media, the droplet sizes of microemulsions were measured by a laser particle size analyzer. *First fluid group significantly different from others ($P < 0.05$).

The composition of CsA (10%)/oil (18.5%)/Smix (71.5%) expressed as a running line (Fig. 1) formed microemulsion (L phase) regardless of the amount of water. *In vivo* preME might diffuse into variable volumes of gastrointestinal fluids to produce microemulsions. In this respect, the composition of preME forming microemulsion in any volumes of aqueous medium would be the most suitable. Thus, the mixture of CsA:medium chain triglyceride: Smix (10:18.5: 71.5) was chosen as the formulation of preME for further experiments.

PreME spontaneously formed microemulsions in various aqueous media regardless of pH and ionic strength. The mean droplet sizes of preME dispersion in water, saline, simulated gastric fluid without pepsin (pH 1.2), and intestinal fluid without pancreatin (pH 6.8) did not significantly differ and were in 18–33 nm (Fig. 2), satisfying the size range (5–200 nm) of microemulsions (16,17). The droplet sizes did not significantly change until after at least 4 weeks of storage at room temperature, confirming that the dispersions of preME in the aqueous media are thermodynamically stable microemulsions.

pH-Dependent Release of CsA from Enteric-Coated sME

To design controlled release dosage forms that rapidly release CsA in the absorption window, the upper small intestine (18), we formulated enteric-coated preME and tested the release rates of CsA in two different pH, first fluid (pH 1.2) and second fluid (pH 6.8). Table I shows that the enteric-coated solid dosage forms worked as premicroemulsion concentrates and formed transparent microemulsions in distilled water with droplet sizes less than 100 nm. Of enteric carriers, CAP-coated sME formed larger microemulsion with variation of sizes higher than other sME. Because the higher variation of microemulsion size is known to decrease the stability of the system (19), EuD and AL showing smaller size variations appear to be more suitable coating materials than CAP.

The release profiles of CsA depended on pH and the type of coating materials. All enteric-coated sME almost completely released CsA within 20 min in the second fluid (pH 6.8) (Fig. 3A). However, the initial rates of pH-dependent release varied. EuD-coated sME released 97% of CsA at 10 min whereas AL-coated sME released 53% of CsA. The rapid release of CsA from EuD-coated sME might be attributed by the stronger pH-dependent solubility of EuD.

The release profiles of CsA also varied among solid dosage forms. EuD-coated sME showed much higher rate and extent of pH-dependent release in simulated intestinal fluid than did other solid dosage forms (Fig. 3B). EuD-coated solid dispersion of CsA released 64% of CsA 10 minutes after the change of medium pH, then the amounts of CsA release did not significantly increase with time. EuD-coated solid state emulsions, prepared by coating the crude emulsion contents of Sandimmune®, showed slower and gradual release patterns of CsA. The mechanism by which sME showed more rapid release of CsA appears to result from the smaller droplet sizes of microemulsions that might offer the thinner diffusion barriers against CsA release.

In Vivo Pharmacokinetics of CsA in preME and sME

CsA given in preME and various sME formulations showed significantly different pharmacokinetic profiles. PreME showed oral absorption profiles similar to Sandimmune Neoral®. The area under the curve $(AUC)_{0-24\ hours}$ values were 3274.7 ± 221.1 ng · h/ml in preME and 3238.3 ± 466.9 ng · h/ml in Sandimmune Neoral® (Fig. 4). They also

Table I. Droplet Sizes of Microemulsion Formed after Dispersion of Enteric Coated sME in Distilled Water

Enteric coated sME	Droplet size (nm)
EuD-coated sME	44.83 ± 8.40
AL-coated sME	45.70 ± 10.08
CAP-coated sME	61.67 ± 26.80

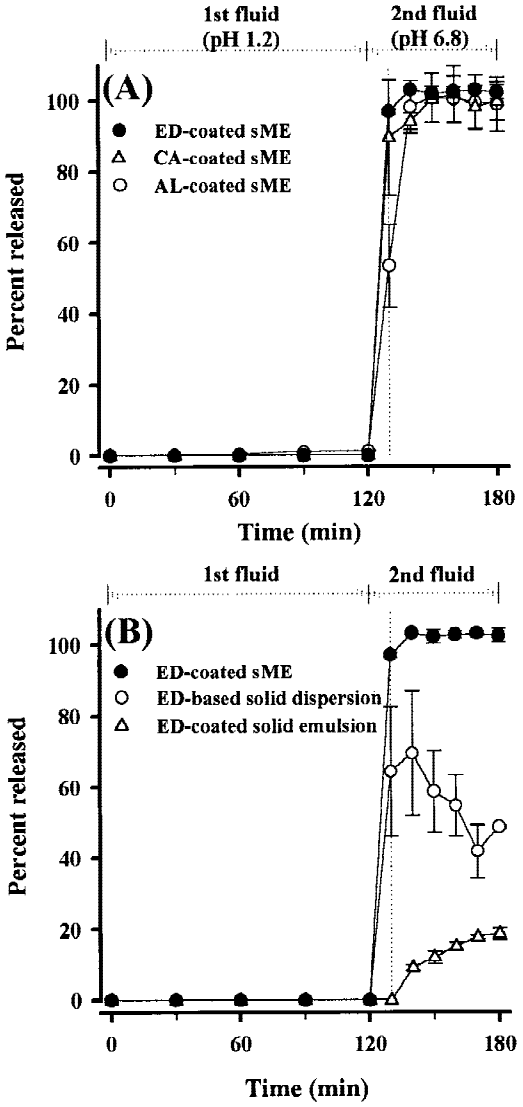

Fig. 3. *In vitro* release of CsA from various solid dosage forms. Released amounts of CsA from various sME (A) and solid dosage forms (B) were determined by HPLC using a Capsellpak C8 column. EuD was used as a dispersion base of solid dispersions or a coating material in solid-state emulsions. Solid dosage forms were incubated in first fluid (pH 1.2) for 60 minutes, then the medium was changed to second fluid (pH 6.8).

showed similar rates of absorption with T_{max} at 2 hours. The similar extents and rates of oral absorption in the two formulations suggest that preME might be bioequivalent to Sandimmune Neoral®.

Sandimmune Neoral® and preME showed the blood levels of CsA lower than 100 ng/ml, the minimum therapeutic level (11), within 8 hours after oral administration. In human studies, the blood levels of Sandimmune Neoral®-treated groups decreased to lower than 100 ng/ml within 8 hours (7). Based on the pharmacokinetics, we can not rule out the risk of organ rejection resulting from to the ineffectively low blood levels of CsA. Actually, the episodes of acute organ rejections were observed at 45% of transplantation patients taking Sandimmune Neoral®, although they were significantly lower compared with Sandimmune® group (60.5%) (20). The high episodes of low blood level-related organ rejection indicate the need for controlled release dosage forms and improved dosing regimens of CsA that can provide the blood levels of CsA above the minimum effective concentration for the whole dosing interval without increasing C_{max} to toxic levels.

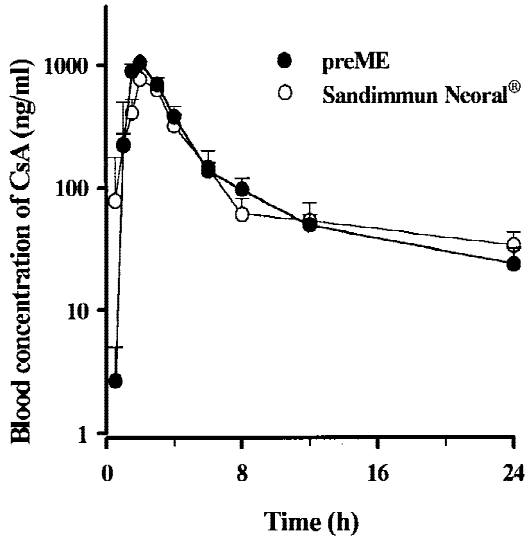

Fig. 4. Pharmacokinetics of CsA administered in preME and Sandimmune Neoral®. CsA (100 mg) in either dosage form was administered orally to a dog. The blood levels of CsA were measured by radioimmunoassay.

Enteric-coated sME showed delayed oral absorption rates compared with preME. Moreover, the oral absorption of CsA given in enteric-coated sME depended on the nature of enteric coating polymers (Fig. 5A). EuD-coated sME showed T_{max} at 4 hours whereas preME showed T_{max} at 2 hours after administration. Among the polymers, EuD showed the highest $AUC_{0-24\ hours}$ (2506.44 ± 670.95 ng · h/ml) and C_{max} (475.88 ± 130.39 ng/ml). $AUC_{0-24\ hours}$ values decreased in the order of EuD-coated sME, CAP-coated sME, solid dispersion, and AL-coated sME. The AUC values showed a high correlation with the amounts of CsA released for 10 minutes after change of pH in the medium (Fig. 5B). AL-coated sME with the lowest pH-dependent release of CsA *in vitro* (Fig. 4A) showed the lowest $AUC_{0-24\ hours}$. In contrast, EuD-coated sME releasing more than 95% of CsA *in vitro,* showed the highest $AUC_{0-24\ hours}$. This result supports that the rapid release rates of CsA in the second fluid would be important to utilize the absorption window in the full extent. It is speculated that much of the dose in AL-coated sME were released beyond the absorption window and resulted in the low $AUC_{0-24\ hours}$. The higher $AUC_{0-24\ hours}$ observed in EuD-coated sME suggests that the novel dosage form might be well applied to increase the oral absorption of other drugs such as amoxicillin (21) whose absorption window is confined to the small intestine.

Once-a-Day Dosing Regimen for Controlled Oral Absorption of CsA

As a once-a-day dosing regimen, preME (100 mg CsA) was orally co-administered with sME (100 mg CsA). Double peaks were observed at 2.5 hours and 6 hours (Fig. 6). The first peak level (1262.46 ± 314.27 ng/ml) seems to be contrib-

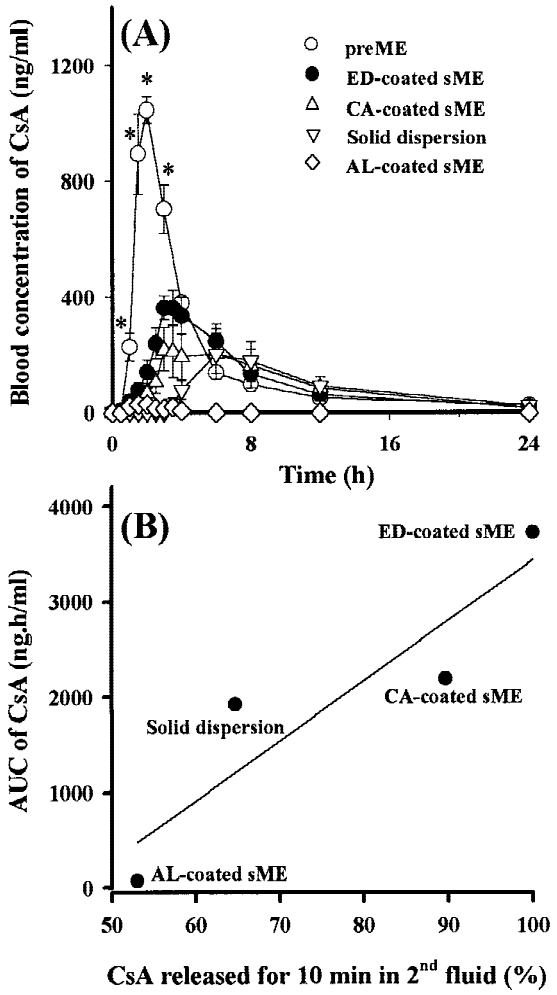

Fig. 5. Effects of solid dosage forms on oral absorption kinetics of CsA (A) and correlation between AUC and amounts of release *in vitro* (B). CsA (100 mg) in either dosage form was filled into hard gelatin capsules and administered orally to a dog. The amounts of CsA in the blood were measured by radioimmunoassay. *preME group significantly different from others. (B) The amounts of CsA released *in vitro* at 10 minutes after pH change were determined by HPLC. AUC were calculated by trapezoidal methods.

uted by preME and the second peak (1044.25 ± 123.79 ng/ml) by sME. Notably the peak levels after the combined administration of 200 mg CsA did not significantly differ from the peak level of preME (100 mg CsA) single administration. No significant increase of C_{max}in the combined treatment at the doubled dose indicates that the once-a-day dosing regimen (200 mg CsA) might be as tolerable as preME single dosing (100 mg CsA), almost bioequivalent to Sandimmune Neoral®.

Figure 6 shows that the combined dosing regimen provided the blood levels over the minimum therapeutic level for prolonged period. The blood levels of CsA declined to lower than the minimum therapeutic level within 8 hours after single administration of preME (Fig. 6). In contrast, at 24 hours after the combined administration of preME and sME, the concentration of CsA was 82.51 ± 28.28 ng/ml, close to the minimum therapeutic level. It appears that enteric-coated sME showing delayed oral absorption of CsA played a major role to maintain the blood levels over the minimum therapeutic level for about 24 hours while mitigating the peak levels in tolerable ranges at the doubled dose.

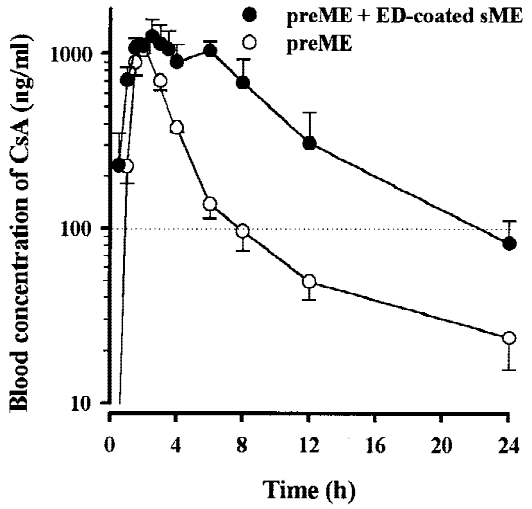

Fig. 6. Oral absorption profiles of CsA after co-administration of preME and sME. Overnight-fasted dogs were orally administered with preME (100 mg CsA) alone or with preME (100 mg CsA) and EuD-coated sME (100 mg CsA).

CONCLUSION

To our knowledge, it is the first report that dry powders of enteric-coated sME spontaneously formed microemulsions in the aqueous medium, releasing the microemulsified compound in a pH-dependent manner. Our results also suggest that the once a day dosing regimen (200 mg CsA) by combined treatment of preME and sME offered the blood levels of CsA in the therapeutic ranges for about 24 hours while retaining a C_{max} level similar to preME (100 mg). Although our preclinical results need to be further investigated in the clinical setting, the once-a-day dosing regimen shows the potential of reducing the episodes of organ rejection after transplantation and increasing compliance of outpatients in CsA treatment.

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REFERENCES

1. J. P. Scott and T. W. Higenbottam. Adverse reactions and interactions of cyclosporin. *Med. Toxicol. Adverse Drug Exp.* **3**:107–127 (1988).
2. H. Zachariae. Renal toxicity of long-term cyclosporin. *Scand. J. Rheumatol.* **28:**65–68 (1999).
3. J. M. Gijtenbeek, M. J. van den Bent, and C. J. Vecht. Cyclosporine neurotoxicity: A review. *J. Neurol.* **246**:339–346 (1999).
4. B. J. Nankivell, M. Hibbins, and J. R. Chapman. Diagnostic utility of whole blood cyclosporine measurements in renal transplantation using triple therapy *Transplantation* **58**:989–996 (1994).
5. R. M. Ferguson and R. Fidelus-Gort. The in vitro assessment of the immunosuppressive effect of fractionated total lymphoid irradiation in renal allotransplantation. *Transplant Proc.* **14**:2350–2356 (1983).
6. P. P. Constantinides. Lipid microemulsions for improving drug

dissolution and oral absorption: Physical and biopharmaceutical aspects. *Pharm. Res.* **12**:1561–1572 (1995).
7. J. M. Kovarik, E. A. Mueller, J. B. Van Bree, W. Tetzloff, and K. Kutz. Reduced inter- and intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation. *J. Pharm. Sci.* **83**:444–446 (1994).
8. C. K. Kim, E. J. Lee, M. K. Lee, J. K. Park, H. J. Shin, H. G. Choi, S. W. Lee, S. J. Lim, Z. G. Gao, and I. S. Kim. Bioequivalence of cyclosporin A hard capsules. *J. Applied Pharmacol.* **6**:296–302 (1998).
9. Z. G. Gao, H. G. Choi, H. J. Shin, K. M. Park, S. J. Lim, K. J. Hwang, and C. K. Kim. Physicochemical characterization and evaluation of a microemulsion system for oral delivery of cyclosporin A. *Int. J. Pharm.* **161**:75–86 (1998).
10. P. Keown, D. Landsberg, P. Halloran, A. Shoker, D. Rush, J. Jeffery, D. Russell, C. Stiller, N. Muirhead, E. Cole, L. Paul, J. Zaltzman, R. Loertscher, P. Daloze, R. Dandavino, A. Boucher, P. Handa, J. Lawen, P. Belitsky, and P. Parfrey. A randomized, prospective multicenter pharmacoepidemiologic study of cyclosporine microemulsion in stable renal graft recipients. Report of the Canadian neoral renal transplantation study group. *Transplantation* **62**:1744–1752 (1996).
11. B. D. Kahan and J. Grevel. Optimization of cyclosporine therapy in renal transplantation by a pharmacokinetic strategy. *Transplantation* **46**:631–644 (1988).
12. S. L. Myers and M. L. Shively. Preparation and characterization of emulsifiable glasses: Oil-in-water and water-in-oil-in-water emulsions. *J. Colloid Interface Sci.* **149**:271–278 (1992).
13. C. K. Kim, S. A. Ryuu, K. M. Park, S. J. Lim, and S. J. Hwang. Preparation and physicochemical characterization of phase inverted water/oil microemulsion containing cyclosporin A. *Int. J. Pharm.* **147**:131–134 (1997).
14. E. S. Swenson and W. J. Curatolo. Intestinal permeability enhancement for proteins, peptides and other polar drugs—Mechanisms and potential toxicity. 2. *Adv. Drug. Deliv. Rev.* **8**:39–92 (1992).
15. M. M. Nerurkar, P. S. Burton, and R. T. Borchardt. The use of surfactants to enhance the permeability of peptides through Caco-2 cells by inhibition of an apically polarized efflux system. *Pharm. Res.* **13**:528–534 (1996).
16. K. M. Park and C. K. Kim. Preparation and evaluation of flurbiprofen-loaded microemulsion for parental delivery. *Int. J. Pharm.* **181**:173–179 (1999).
17. K. M. Park, M. K. Lee, K. J. Hwang, and C. K. Kim, Phospholipid-based microemulsions of flurbiprofen by the spontaneous emulsification process. *Int. J. Pharm.* **183**:145–154 (1999).
18. J. Drewe, C. Beglinger, and T. Kissel, The absorption site of cyclosporin in the human gastrointestinal tract. *Br. J. Clin. Pharmacol.* **33**:39–43 (1992).
19. D. Attwood. Microemulsions. In J. Kreuter (ed.), *Colloidal Drug Delivery Systems*, Marcel Dekker Inc., New York, 1994 pp. 31–65.
20. P. Keown and D. Niese, Cyclosporine microemulsion increases drug exposure and reduces acute rejection without incremental toxicity in de novo renal transplantation. InternationalSandimmun Neoral Study Group. *Kidney Int.* **54**:938–944 (1998).
21. A. Hoffman, H. D. Danenberg, I. Katzhendler, R. Shuval, D. Gilhar, and M. Friedman. Pharmacodynamic and pharmacokinetic rationales for the development of an oral controlled-release amoxicillin dosage form. *J. Control. Release* **54**:29–37 (1998).