



Stable bioavailability of cyclosporin A, regardless of food intake, from soft gelatin capsules containing a new self-nanoemulsifying formulation

S.G. Yang, D.D. Kim, S.J. Chung and C.K. Shim

Research Institute of Pharmaceutical Sciences and College of Pharmacy,
Seoul National University, Seoul, Korea

Key words

cyclosporin A – food effects – bioavailability – bioequivalence – micro-emulsion

Abstract. **Aim:** We recently succeeded in preparing soft gelatin capsules containing a new self-nanoemulsifying formulation consisting of cyclosporin A (CsA), triacetin, polyoxyl 40 hydrogenated castor oil, polysorbate 20, medium chain triglycerides and medium chain mono- and diglycerides. The soft capsules containing the new formulation exhibited a significantly improved physical stability in terms of the appearance of the gelatin capsule shells and the composition of the fill mass during long-term storage, compared to commercially available soft capsules containing CsA, in which ethanol was employed as a cosolvent of CsA. In the present study, the influence of a fat-rich meal on the bioavailability of CsA from the soft capsule containing the new formulation (test drug) was evaluated and the results compared to those obtained with a representative soft capsule of CsA. **Volunteers and methods:** A randomized, open-label, 3-way crossover study was performed in the test capsules and reference soft capsules, in a fasted state or after a fat-rich breakfast. 18 healthy male volunteers received a single dose of the reference formulation (Neoral, Novartis Co. AG, Basel, Switzerland) or test formulation (2 capsules each, 200 mg as CsA) with 240 ml of water with a 1-week washout period between the treatments, after a fat-rich (670 kcal, 45 g fat) breakfast (for the test drug, Treatment A; for the reference drug, Treatment B) or a 12-h fasting (for the test drug, Treatment C). Serial blood samples, collected over a 24-h period after the administration, were assayed for blood CsA concentrations using a specific monoclonal radioimmunoassay. **Results:** The differences in bioavailability parameters (i.e., AUC_{0-24h} , $AUC_{0-\infty}$ and C_{max}) between the treatments were within the range of 80 – 125% of the reference treatment. An analysis of variance (ANOVA) revealed no significant differences ($p > 0.05$) between subjects, formulations or periods. The 90% confidence intervals (CI) indicated that the differences be-

tween the treatments (Treatments A and B, Treatments A and C) were also within the criteria. **Conclusion:** These results indicate that the bioavailability of CsA from the test drug is equivalent to reference in the fed state, and is likely to be less influenced by a fat-rich meal. Therefore, the new formulation of CsA using triacetin appears to have an advantage over the commercial soft capsules of CsA using a volatile cosolvent such as ethanol.

Introduction

The introduction of cyclosporin A (CsA) into clinical use in the 1980s resulted in a significantly improved survival rate for transplant patients [Kahan 2004]. CsA still remains the cornerstone of many immunosuppressant regimens in the area of transplantation. However, because of its hydrophobic nature and poor water solubility, the oral bioavailability of CsA is low (30%), with considerable variation between individuals (5 ~ 50%) [Schroeder et al. 1995]. Moreover, the bioavailability of a formulation is often influenced by food intake [Brunner et al. 2000, Gupta et al. 1990, Mueller et al. 1994a]. For example, a fat-rich breakfast increased the bioavailability of CsA from soft gelatin capsules containing an oil-based emulsifying formulation (Sandimmune capsules) by more than 37% [Mueller et al. 1994a], which forms coarse emulsions (mean droplet size, 3.73 μ m) in gastrointestinal fluid [Andrysek 2003]. A similar increase (i.e., 29% increase) as the result of a fat-rich breakfast was also observed for the bioavailability of Cicloral capsules (Hexal AG, Holzkirchen, Germany), a generic formulation of Neoral capsules [Kees et al. 2004]. Fat-rich food

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Correspondence to
C.-K. Shim, PhD
Department of Pharmaceutics, College of Pharmacy, Seoul National University,
San 56-1, Shinlim-dong,
Kwanak-gu, Seoul,
151-742, Korea
shimck@snu.ac.kr

might increase the secretion of bile into the lumen, thereby increasing the bioavailability of CsA via its accelerated dissolution, aided by bile acids. On the contrary, a 15% decrease in the bioavailability of CsA as the result of a fat-rich breakfast was observed for soft gelatin capsules containing a self-nanoemulsifying formulation (Neoral capsules) [Mueller et al. 1994a], which forms nanoemulsions (mean droplet size < 100 nm) in gastrointestinal fluid. The mechanism of this decreased bioavailability is not clearly understood at present.

The bioavailability of a CsA oral liquid formulation in a bottle, which requires predilution with tap water or apple juice prior to its oral administration, is also influenced by food intake. A SanCya oral solution (SangStat Co.,

Fremont, CA, USA) was recalled from the market because of the lack of bioequivalence when administered with apple juice compared with water as the diluent [Henney 2000]. On the contrary, the bioavailability of a Neoral oral solution was not influenced by dilution [Kovarik et al. 2002]. The reduced absorption of CsA appeared to arise from the characteristics of formulation [Honda et al. 2004, Kovarik et al. 2002].

The above results suggest that the bioavailability of CsA varies with the food depending on the formulation of the drug. Therefore, the effect of food on the bioavailability of CsA needs to be carefully examined before a conclusion on the formulation can be reached.

We recently succeeded in preparing soft gelatin capsules containing a new self-nanoemulsifying formulation which exhibited significantly improved physical stability in terms of the appearance of the gelatin capsule shells and the composition of the fill mass during the long-term storage [Yang 2005]. This formulation consisted of CsA, triacetin, polyoxyl 40 hydrogenated castor oil, polysorbate 20, medium chain triglycerides and medium chain mono- and diglycerides, of which composition was optimized based on an examination of the pseudo-ternary phase diagram. The unique difference in this new formulation is the replacement of volatile cosolvents such as ethanol in commercially available CsA formulations (e.g., Neoral capsules) with triacetin, a nonvolatile cosolvent. As a result of this replacement, the evaporation of the cosolvent

and the migration of water from and to gelatin shells could be avoided [Yang 2005].

Although, the advantage of the new formulation is clear in terms of physical stability, a conclusion on the advantage of a formulation would require information on bioavailability, because the bioavailability of CsA is likely to be sensitively influenced by the intake of food [Brunner et al. 2000, Gupta et al. 1990, Henney 2000, Kees et al. 2004, Mueller et al. 1994a]. In the present study, therefore, the effect of food, especially fat-rich food, on the bioavailability of CsA was investigated for the new formulation in comparison with reference (Neoral) capsules. A 3-way crossover study was conducted for the fed and the fasted states.

Test and reference medications

The test formulation, in a soft gelatin capsule, was composed of CsA, triacetin, polyoxyl 40 hydrogenated castor oil, polysorbate 20, medium chain triglycerides and medium chain mono- and diglycerides, based on the result of a pseudo-ternary phase diagram study. A soft gelatin capsule containing 100 mg of CsA (Neoral, Novartis AG, Basel, Switzerland) was used as the reference drug. Prior to the bioequivalence study, test capsules were confirmed, by an electrophoretic light scattering spectrophotometer (Nicom 370, Particle sizing systems, Santa Barbara, CA, USA), to form nanoemulsions when immersed in saline, pH 1.2 buffer, pH 6.8 buffer and distilled water, with an approximate mean droplet size of 20 ~ 40 nm at 37°C (Figure 1).

Study design and subjects

A single-dose, randomized, open-label, and 3-way crossover study was conducted based on the Food and Drug Administration Guidance entitled "Oral Extended (Controlled) Release Dosage Forms, In Vivo Bioequivalence and In Vitro Dissolution Testing" issued on September 9, 1993 and "Food-Effect Bioavailability and Fed Bioequivalence Studies" issued on December 9, 2002. The study was performed in accordance

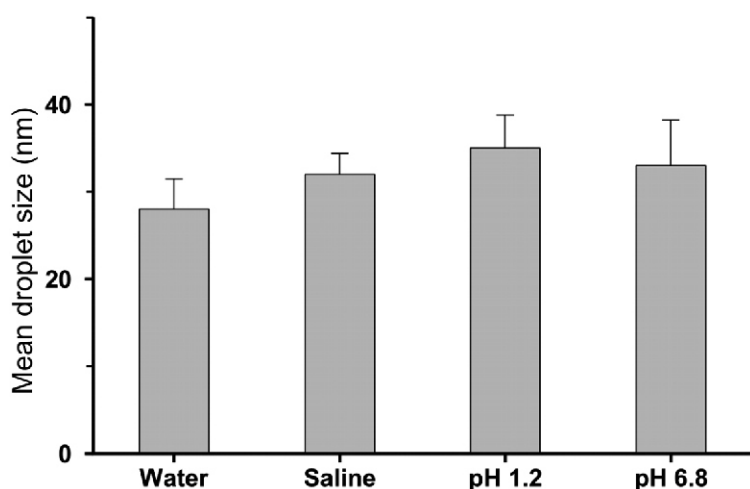


Figure 1. The mean droplet size of CsA micro-emulsions from test formulations prepared with different dispersion media. Each data point represents the mean \pm SD.

with the revised Declaration of Helsinki for biomedical research involving human subjects [Howard-Jones 1982] and the rules of Good Clinical Practice [Allen and Vandenburg 1992]. The protocol for the study was approved by Ethical Committee of the Engineering Research Center (ERC), Seoul National University (Seoul, Korea). 18 healthy non-smoking male volunteers, ranging in age from 21 – 23 years (22 ± 0.5 years) and in weight from 60 – 75 kg (67 ± 2.1 kg) completed the study. The volunteers were selected after passing a clinical screening procedure including a physical examination and laboratory tests (blood analysis, hemoglobin, hematocrit, WBC, platelet, differential counting of WBC, blood urea nitrogen, total bilirubin, total cholesterol, total protein, albumin, alkaline phosphatase, glucose fasting, sGOT and sGPT; and a urinalysis, specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC and cast). Volunteers were excluded if they were possibly sensitive to this type of medication, had a history of any hepatic, or renal, or cardiovascular system illnesses, or had previously taken alcohol or other medication for an extended long period of time. All participants signed a written informed consent after they had been informed of the nature and details of the study in accordance with the Korean Guidelines for Bioequivalence Test (KGBT 1998). All subjects avoided the use of other drugs for at least 1 week prior to the study and until after its com-

pletion. They also refrained from alcoholic beverages and xanthine-containing foods and beverages 48 hours prior to each dosing and until the collection of the last blood sample.

Each volunteer received 2 reference capsules (i.e., Neoral 200 mg dose of CsA, Lot no. F47MFD0501) and 2 capsules of the test formulation (i.e., 200 mg dose of CsA in soft gelatin capsules, Lot no. SNU001) in a standard 3×3 crossover model (single dose, randomized, 3-treatment, 3-period crossover design). There was a 1-week washout period among the doses. The subjects were hospitalized (Samsung Hospital, Seoul, Korea) at 9:00 p.m. 1 day before the study and fasted 12 hours before and 4 hours after the administration of each drug. At 4 hours after an oral administration, all subjects were given standardized meals.

For each study period, the subjects were randomly assigned to receive test capsules with or without a high-fat breakfast (Treatments A and C) and reference capsules with a high-fat breakfast (670 kcal, 45 g fat) (Treatment B). At 8:00 a.m., the median cubital vein of the subject was cannulated (D&B-CATH, Seoul, Korea). In Treatments A and C, each drug was administered with 240 ml of tap water 30 minutes after the start of the high-fat breakfast at 9:00 a.m. In Treatment B, each drug was administered in a similar manner under fasting conditions.

An approximately 7 ml aliquot of blood was collected via the cannula in tubes containing EDTA (BD Vacutainer, BD Diagnosis, Franklin Lakes, NJ, USA) at the following predose (control), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours after administration. A heparinized normal saline injectable solution, 1 ml, was flushed after each blood sampling and 2 ml of blood was discarded in each sampling. Subjects were not allowed to remain in a supine position or to sleep during the whole blood collection period. Vital signs and adverse events were repeatedly monitored during the hospitalization phase. The blood samples were stored frozen at -20°C until analysis.

Analysis of CsA in blood CsA

The concentrations of CsA in blood samples were analyzed using a Cyclo-Trac SP[®]

RIA kit (Diasorin, Stillwater, MN, USA). In a typical experiment, blood samples (100 μ l) were mixed vigorously with methanol (400 μ l) and centrifuged. 50 μ l of the methanolic supernatant was withdrawn, incubated in a glass tube with 100 μ l of 125 I Cyclo-Trac SP and 1 ml of Anticyclo-Trac SP Immunosep solution for 1 h. After centrifugation, the supernatant was discarded and the radioactivity of the precipitate in each tube was counted for 1 min using a well type γ -scintillation counter (1470 WIZARD, Perkin Elmer, Wellesley, CA, USA). A calibration curve was prepared using the supplied kit calibrators in the concentration range of 20, 61, 154, 379 and 1,099 ng/ml. The accuracy was 5.4, 2.1, 9.8, 1.3 and 3.8%, and the intra-assay coefficient of variation was 12.1, 6.4, 7.5, 5.5 and 4.7%, and the inter-assay coefficient of variation was 10.1, 8.4, 4.7, 6.9 and 8.1%, respectively for each concentration. The overall quantification limit was 20 ng/ml.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic characteristics were derived by standard methods, using the WinNonlin program (Standard version 3.1, Pharsight Inc., Mountain View, CA, USA), when necessary. The maximum blood concentration (C_{\max}), time to reach C_{\max} (t_{\max}) and the concentration at the last measured time (C_{24h}) were compiled from concentration-time data. The area under the blood CsA concentration-time curve (AUC) extrapolated to infinity ($AUC_{0-\infty}$) was calculated in 2 steps, using a log-linear trapezoidal formula for up to the last measured time and extrapolation to time infinity. The AUC from time zero to the last point (AUC_{0-24h}) was calculated using a log-linear trapezoidal formula. The AUC from the last determined time (i.e., 24 h) to infinity was calculated by C_{24h}/λ , where λ , the apparent rate constant for elimination, was estimated by linear regression (weighting $1/C_{\text{estimated}}$) of the log-transformed plasma concentrations during the terminal log-linear decline phase. The apparent terminal elimination half-life ($t_{1/2}$) was calculated by $0.693/\lambda$.

Statistical analysis of data

Statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA), using the general linear model (GLM). Analyses were performed on log-transformed AUC_{0-24h} , $AUC_{0-\infty}$ and C_{\max} and on untransformed t_{\max} and $t_{1/2}$. AUC_{0-24h} , $AUC_{0-\infty}$ and C_{\max} were used to assess the relative bioavailability between treatment. t_{\max} and $t_{1/2}$ were tested for sequence and treatment effects using an analysis of variance (ANOVA).

Treatments A and B were compared to assess the bioequivalence between the test capsules and reference capsules in the fed state. The point estimate (i.e., ratio of the mean parameters) between treatments was calculated. All parameters were tested for carry-over effect using crossover analysis techniques in the GLM. The primary method used to compare the relative bioavailability parameters (AUC_{0-24h} , $AUC_{0-\infty}$ and C_{\max}) was 90% CI computed from the two 1-sided t-test method with $\alpha = 0.05$ on ratios of the geometric means for the treatments [Schuirmann 1987].

To assess the effect of food on the bioavailability of test capsules, the pharmacokinetic parameters from Treatments A and C were analyzed using the same methods. Statistical comparisons of pharmacokinetic parameters (t_{\max} and $t_{1/2}$) between the fasted and fed states were performed using ANOVA. The effect of food was assessed using criteria in which 90% CI for the ratio between fasted and fed states were within the acceptance criteria proposed by the Center for Drug Evaluation and Research, Department of Health and Human Services, US Food and Drug Administration (C_{\max} 90% CI, 80 – 125; $AUC_{0-\infty}$ 90% CI, 80 – 125).

Results and discussion

Clinical observations

No serious adverse events were found during the study period. Beginning from 30 min to 3 h, a slight fever and hot sensation or rubefaction of the face, extremities, and sometimes generalized, was observed in most of the subjects for both drugs. The symptoms spontaneously subsided with time and no medical management was required.

Table 1. Pharmacokinetic parameters for CsA following a single oral administration of 200 mg of the reference formulation (Sandimmune Neoral) and the test formulation under fasted and fed states to 18 healthy male volunteers.

Parameters		AUC _{0-24h} (ng × h/ml)	AUC _{0-∞} (ng × h/ml)	C _{max} (ng/ml)	t _{max} (h)	t _{1/2} (h)
Test capsule in fed state (Treatment A)	Arithmetic mean	4,602.1	4,897.2	1,066.9	2.0	6.4
	SD*	921.3	1,052.0	152.5	0.5	1.4
	Geometric mean	4,508.7	4,783.3	1,056.7	1.9	6.3
	e ^{mean-SD}	3,642.8	3,807.2	916.0	1.5	4.9
	e ^{mean+SD}	5,580.4	6,009.7	1,218.9	2.4	8.0
Neoral capsules in fed state (Treatment B)	Arithmetic mean	4,706.6	5,069.9	1,094.2	1.7	6.9
	SD	914.8	1,026.6	163.3	0.5	1.4
	Geometric mean	4,627.96	4,877.4	1,082.7	1.7	6.7
	e ^{mean-SD}	3,839.3	4,092.6	931.8	1.2	5.4
	e ^{mean+SD}	5,578.5	6,053.4	1,258.1	2.2	8.5
Test capsule in fasted state (Treatment C)	Arithmetic mean	5060.0	5465.7	1,140.1	1.5	7.2
	SD	1102.6	1273.5	185.7	0.4	1.8
	Geometric mean	4948.5	5332.1	1,124.3	1.5	6.9
	e ^{mean-SD}	3978.6	4243.8	941.6	1.2	5.2
	e ^{mean+SD}	6154.8	6699.4	1,342.5	1.9	9.3

* Standard deviation.

Table 2. Summary of statistical analysis for the effect of food on the bioavailability of CsA formulations in the fed and fasted state.

A) Treatments A and B^a (i.e., test and reference capsules in the fed state)

	AUC _{0-24h} (ng × h/ml)	AUC _{0-∞} (ng × h/ml)	C _{max} (ng/ml)	t _{max} (h)	t _{1/2} (h)
Point estimate ^b	0.97	0.98	0.97	1.16	0.93
90% CI ^c or p ^d	91.0 ≤ ≥ 104.3	89.5 ≤ ≥ 103.2	92.7 ≤ ≥ 102.8	p = 0.198	ND ^e

^a Reference capsules in the fed state (i.e., Treatment B) served as reference. ^b Geometric mean ratios of parameters between treatments. ^c Confidence intervals for the difference between the treatments. ^d Analysis of variance at significant level of 0.05. ^e Not determined.

B) Treatment A and Treatment C^f (i.e., test capsules in fed and fasted states)

	AUC _{0-24h} (ng × h/ml)	AUC _{0-∞} (ng × h/ml)	C _{max} (ng/ml)	t _{max} (h)	t _{1/2} (h)
Point estimate	0.91	0.89	0.94	1.29	0.90
90% CI or p	85.1 ≤ ≥ 97.5	83.5 ≤ ≥ 96.4	89.2 ≤ ≥ 99.0	p = 0.009	ND

^f Test capsules in the fasted state (i.e., Treatment C) served as reference.

Bioavailability parameters

The mean blood CsA concentration-time data from the 3 treatments are shown in Figure 2 and the relevant bioavailability parameters are summarized in Table 1. In the fed

state, blood concentration-time profiles of CsA were almost nearly between the test (Treatment A) and reference capsules (Treatment B) (Figure 2A). As a result, the bioavailability parameters (i.e., AUC_{0-24h}, AUC_{0-∞}, and C_{max}) of the test medication were within

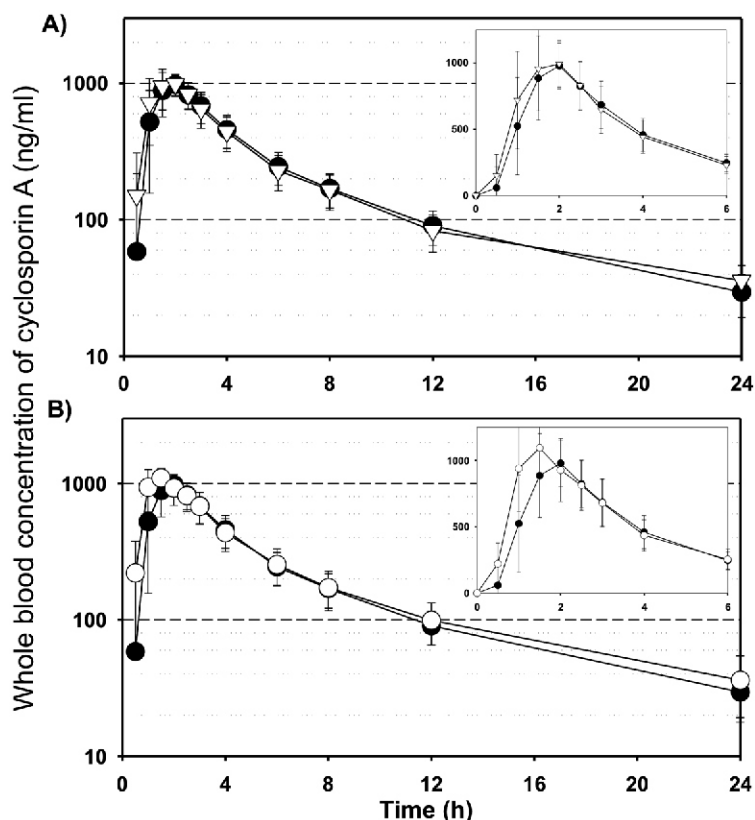


Figure 2. The mean blood CsA concentration-time profiles following the oral administration of CsA soft capsules to 18 healthy volunteers at a 200 mg dose of CsA (i.e., 2 capsules), A) test (-●-) and reference (-▽-) capsules in fed state, B) test capsules in fed (-●-) and fasted (-○-) states. Each data point represents the mean \pm SD.

the criteria of bioequivalence to the reference medication (Tables 1 and 2A). No significant differences were observed between the 2 medications in terms of period, formulation or sequence, as evidenced by *p* values greater than 0.05 (ANOVA, data not shown). The 90% CI (%) of the difference ranged from 91.0 ~ 104.3, 89.5 ~ 103.2 and 92.7 ~ 102.8 for the AUC_{0-24h} , $AUC_{0-\infty}$ and C_{max} , respectively (Table 2A). The above data indicate that the test and reference capsules are bioequivalent in the fed state when estimated based on FDA and KFDA guidelines.

The effect of a fat-rich breakfast on the bioavailability of CsA in the test medication was investigated in Treatments A and C. In fed state, the t_{max} was significantly retarded from 1.5 – 2.0 h in arithmetic mean, and the C_{max} was decreased from 4,948.5 to 4,508.7 ng/ml in geometric mean (Tables 1 and 2). The AUC of the test capsule was also decreased about 10% by the fat-rich meal. How-

ever, the mean ratios of parameters (AUC_{0-24h} , $AUC_{0-\infty}$ and C_{max}) between treatments were within the range of 80 – 125% (Table 1), exhibiting no significant difference in terms of period, formulation or sequence (i.e., *p* values > 0.05 by ANOVA, data not shown), and the 90% CI of the difference were in the range of 85.1 ~ 97.5, 83.5 ~ 96.4 and 89.2 ~ 99.0 for AUC_{0-24h} , $AUC_{0-\infty}$ and C_{max} , respectively (Table 2B). The 90% CI were also within the criteria.

It is generally known that the effect of food intake on the bioavailability of highly soluble and highly permeable drugs (Class I of bioclassification system, BCS) in immediate-release formulations is minimal [Martinez et al. 2002, Wu and Benet 2005], but is often significant for BCS Class II (i.e., drugs with low solubility and high permeability) or Class III (i.e., drugs with a high solubility and low permeability) drugs [Martinez et al. 2002, Wu and Benet 2005]. Indeed, the absorption of CsA, a Class II drug [Chiu et al. 2003], from oil-based emulsifying formulations (e.g., Sandimmune capsule), was highly variable and greatly increased by a fat-rich meal [Honcharik 1991]. The effect of food could be significantly reduced by employing microemulsifying formulations (e.g., Neoral capsule) [Kahan et al. 1995, Mueller et al. 1994a, 1994b]. The reduction can be attributed to the spontaneous and rapid formation of micro- or nanoemulsions from these formulations [Pouton 2000].

In our previous study, a gelatin soft capsule containing a new self-nanoemulsifying formulation was developed by employing triacetin, as a cosolvent of CsA, instead of ethanol in commercial emulsion products. By this replacement, the physical stability of the soft capsule (e.g., appearance of gelatin shell and composition of the fill mass of the soft capsule) during long-term storage could be substantially improved [Yang 2005], compared to commercial products which contain ethanol [Duplay 2005]. The bioavailability of CsA from the soft capsule containing the new formulation was shown to be equivalent to a reference (Neoral) capsule in the fasted state for healthy Korean volunteers [Yang 2005].

In the present study, the effect of fat-rich food on the bioavailability of CsA, a typical problem associated with CsA formulations, was tested, and compared to the case for refer-

ence capsules. The results obtained in the present study indicate that the bioavailability of CsA from our formulation containing triacetin (test drug) is equivalent to reference capsule (reference drug), and is less influenced by a fat-rich breakfast. Therefore, we conclude that our formulation (i.e., triacetin containing self-nanoemulsifying formulation of CsA in soft gelatin capsules) has advantages over the classical self-nanoemulsifying formulation of CsA, in which volatile cosolvents of CsA (e.g., ethanol and polyethylene glycol) are used, in terms of physical stability of the dosage form during the storage and consistency of the bioavailability of CsA regardless of food intake.

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