약제학회지(2009), 제 39 권 제 2 호
J. Kor. Pharm. Sci., Vol. 39, No. 2, 111-115 (2009)

Relative Bioavailability and Pharmacokinetics of Newly Designed Cyclosporin A Self-microemulsifying Formulation after Single and Multiple Doses to Dogs

Su-Geun Yang[1†] and Hee-Jong Shin

CKD Research Institute, Chong Kun Dang Pharm. Co., 15-20 Osackdang-Ri, Seonggeo-Up, Cheonan-Shi, Chungcheongnam-Do 330-830, Korea

[1]Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75390, USA

(Received February 20, 2009 · Revised March 4, 2009 · Accepted March 20, 2009)

ABSTRACT – The pharmacokinetics of cyclosporin A (CsA) after single and multiple oral dosing of new CsA self-microemulsifying drug delivery system (SMEDDS) in dogs were estimated. A single dose study was performed following a two-way crossover design against six dogs with reference SMEDDS. For a multiple dose study, three dogs were allocated for each drug, and 100 mg of drug was administered daily for 6 days. Whole blood concentration of CsA was analyzed by radioimmunoassay. Both drug showed identical blood concentration profiles in both studies, and no statistical difference was detected in pharmacokinetic parameters. The relative bioavailabilities of test SMEDDS were 91.4% and 89.1%, respectively, in the single dose study and the last day of multiple dose study. Especially, multiple dose study proved the good relationship between C-0/C-2 and AUC for reference SMEDDS, which is an indispensable part of therapeutic drug monitoring. These results suggest newly formulated CsA SMEDDS possibly shows identical pharmacokinetics and pharmacodynamic behaviors in clinical trials.

Key words – Cyclosporin A, Single dose, Multiple dose, Microemulsion, SMEDDS (self-microemulsifying drug delivery system), Pharmacokinetics.

After the introduction of cyclosporin A (CsA) in the clinics in the 1980s, the survival rate of transplant patients has significantly improved.[1)] Although new immunosuppressive drugs such as tacrolimus and sirolimus were recently developed and selected as alternative for CsA, CsA still remains as primary immunosuppressive agent.[2)] CsA has many benefits for therapy, without the bone marrow toxicity resulting from other cytotoxic immunosuppressants.[3)] However CsA is also associated with adverse reactions such as renal and hepatic dysfunction, hypertension, peripheral fever and hypertrichosis.[3)] Therefore, maintaining a suitable therapeutic concentration so as to avoid incidence of organ rejection and adverse reactions is one of the major concerns in CsA treatment.[1)] Hence, CsA is classified as a drug with narrow therapeutic index which requires therapeutic drug monitoring (TDM) for patients.[1)]

From a pharmaceutical standpoint, CsA is an intricate drug, hardly introduced into formulations due to its hydrophobic property.[4,5)] CsA product, Sandimmune capsules, characterized by variable and incomplete oral absorption, was introduced in clinical practice two decades ago.[5,6)] Currently, self-microemulsifying drug delivery system (SMEDDS) of CsA, which spontaneously forms microemulsions in the gastrointestinal tract, is the most commonly prescribed drug.[7,8)] The most prominent improvement of this formulation is a significantly reduced intra- and inter- individual variance in oral absorption as well as an improved bioavailability, allowing more reliable dosage regimen and therapy.[5)] There are presently 5 FDA approved CsA generics with a 20% market share in the USA,[9)] irrespective of the several new generic candidates currently under development all over the world.[10,11)] Although development of generic CsA offers a reduced cost alternative with equal efficacy and outcomes, clinical scientists have suggested concerns over the therapeutic equivalence of the generic CsA products.[12,13)] They insist a bioequivalence test which is performed in a selected group of healthy volunteers cannot precisely reflect the complicated clinical situations where patients undergo transplantation of major elimination organs such as the liver or kidney.[14)]

More extensive formulation research and more accurate bioequivalence test are compulsory to address the concerns of generic CsA products. In this study, relative bioavailability and pharmacokinetics of newly developed CsA SMEDDS were evaluated in single and multiple dose studies in dogs with reference SMEDDS. A single dose study was performed following a two-way crossover design with reference SMEDDS.

†본 논문에 관한 문의는 이 저자에게로
Tel : 1-469-531-6818 E-mail : orosyang@gmail.com

For a multiple dose study, the blood concentration at 2 hours (C-2) after dosing and trough concentration (C-0) before dosing were estimated to define a relationship with bioavailability following a general protocol for TDM of CsA in the clinic.

Materials and Methods

Materials

Newly designed SMEDDS soft capsules containing 100 mg of CsA were used for the study.[15] Sandimmune Neoral® 100 mg soft gelatin capsules (Novartis Co, Switzerland) were selected for reference SMEDDS currently available on the market.

Single dose pharmacokinetic study

A single dose study was conducted as a randomized two-way crossover study with a 1-week washout period between treatments. Six male dogs, weighing 12.0~15.0 kg, were randomly divided into two groups. Each group received either test SMEDDS or reference SMEDDS in the first period. After one week of the wash-out period, each group received the other drug during the second period. Dogs were fasted for 12 hours before dosing and for an additional 4 hours after dosing. Both drugs, containing 100 mg of CsA, were orally administered via the pharynx. Approximately 30 mL of water was administered immediately using a syringe. Whole blood samples (3 mL) were collected pre-dose and at 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 hours after dosing. Whole blood samples were obtained from the cephalic vein into plastic tubes containing EDTA and were frozen at −20°C until analysis.

Multiple dose pharmacokinetic study

The multiple dose study was conducted for duration of 6 days. Six dogs were randomized into two groups for administration of test and reference formulations. Dogs were individually housed and allowed access to water and were fed twice daily. The dosing interval was set to 24 hours to avoid any possible acute renal toxicity and pulmonary infection from immune suppression. 100 mg of loading dose was administered 12 hours before the first dosing. A 100 mg of maintenance dose was administered every 24 hours for 6 days. Blood samples (3 mL) were obtained from the cephalic vein. On the first day and the last day of experiment, blood samples were colleted before dosing and at 1, 2, 4, 6, 9 and 24 h after dosing. For the other days, blood samples were obtained prior to dosing (C-0) and 2 hours after dosing (C-2).

Analysis of CsA in the whole blood

The whole blood concentrations of CsA were measured by radioimmunoassay using Cyclo-Trac SP® RIA kit (Diasorin, Stillwater, USA). Briefly, 100 μL of whole blood sample was mixed with 400 μL of methanol on a vortex for 60 sec. It was centrifuged at 1,600×g for 5 minutes. From the supernatant layer containing the methanol extract, 50 μL was removed and placed in a test tube for gamma-counting and added 100 μL of ^{125}I Cyclo-Trac SP® and 1 mL of Anti-Cyclo Trac SP Immunosep®. After fully mixing with a vortex, mixture was left to stand in room temperature for 1 hour and centrifuged at 1,600×g for 20 minutes at room temperature. After centrifuging it, supernatant was discarded and the test tube was left upside down on a filter paper for 1 minute to allow natural drainage of the remaining fluid. The radioactivity of the precipitate left in the bottom of the test tube was measured with a gamma counter (1470 WIZARD, Perkin Elmer, CA). A calibration curve was prepared using the supplied kit calibrators in the concentration range of 20, 61, 154, 379 and 1099 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.

Data analysis

Whole blood concentration–time data of CsA was analyzed by conventional non-compartmental pharmacokinetic techniques using WinNonlin (Pharsight Inc., Mountain View, CA). Pharmacokinetic parameters were expressed in terms of mean ± standard deviation. The estimated pharmacokinetic parameters of each treatment were statistically compared using the student's t-test, with significance at $P \leq 0.05$.

Results and Discussion

Pharmacokinetic parameters of CsA SMEDDS in single dose study

The whole blood CsA concentration-time profile is shown in Figure 1. The whole blood CsA concentration-time profiles of two formulations were almost identical each other. The pharmacokinetic parameters after oral administration of test SMEDDS were: $AUC_{0\rightarrow24h}$ = 3983.3 ± 693.0 ng·h/mL, C_{max} = 928.4 ± 134.0 ng/ml, T_{max} = 1.83 ± 0.41 h, V_{dss} = 265.75 ± 77.07 L, Cl = 23.65 ± 3.95 L/h and MRT = 6.02 ± 0.59 as shown in Table I. The relative bioavailability $AUC_{0\rightarrow24h}$ and C_{max} of test SMEDDS were 91.47% and 90.63%, respectively, compared to those of reference SMEDDS. There were no significant differences in pharmacokinetic parameters between both formulations after single dose treatment ($p > 0.05$).

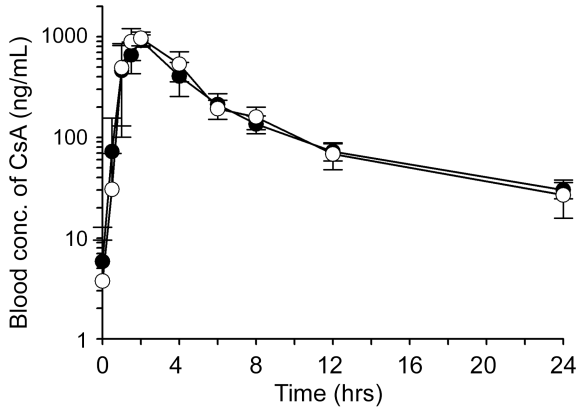

Figure 1–Whole-blood concentration profiles of CsA following single oral dosing of test (○) and reference SMEDDS (●) in dogs at a 100 mg dose of CsA (n=6, mean ± S.D.).

Table I–*Pharmacokinetic Parameters of CsA after Single Oral Administration of Test SMEDDS and Reference SMEDDS in Dogs*

	Reference SMEDDS	Test SMEDDS	P value
$AUC_{0\to24h}$ (ng·h/mL)	4354.63±584.78	3983.30±693.04	0.34
$AUC_{0\to\infty}$ (ng·h/mL)	4630.18±647.86	4318.98±651.87	0.43
C_{max} (ng/mL)	1024.36±210.15	928.43±134.01	0.36
T_{max} (h)	1.83±0.26	1.83±0.41	1.00
$T_{1/2}$ (h)	6.75±1.66	7.67±0.99	0.27
V_{dss} (L)	212.31±54.65	265.75±77.07	0.16
Cl (L/h)	21.94±3.01	23.65±3.95	0.42
MRT (h)	5.56±0.57	6.02±0.59	0.26

Pharmacokinetic parameters of CsA SMEDDS in multiple dose study

The pharmacokinetic parameters and the whole blood CsA concentration-time profile of CsA during multiple dose treatment of both formulations are shown in Table II and Figure 2, respectively. No significant differences were detected between pharmacokinetic parameters in both formulations during the multiple dose treatments ($p > 0.05$).

The pharmacokinetic parameters between the singe dose study and the first day of the multiple dose study cannot be exactly comparable due to a different time point of blood sampling and a loading dose for the multiples dose study. However, AUC and C_{max} of the single dose are much higher than those of the first day of multiple treatments (Table I and II). The decreased absorption of CsA (AUC and C_{max}) can be explained by a food effect. Although the advantage of self-microemulsifying formulation is apparent in terms of absorption enhancement, food generally decreases the absorption of CsA.[16,17] Muller et al. reported a fat-rich meal decreased AUC and C_{max} of CsA by 15 and 26%, respectively, in healthy humans.[16]

By comparison, pharmacokinetic parameters between the first day and the last day of treatment showed that the AUC of the last day in each formulation increased after the multiple dose treatment (3835.22 ± 779.50 vs 5315.45 ± 1047.53 ng·h/mL for test SMEDDS and 3962.10 ± 688.32 vs 4736.08 ±

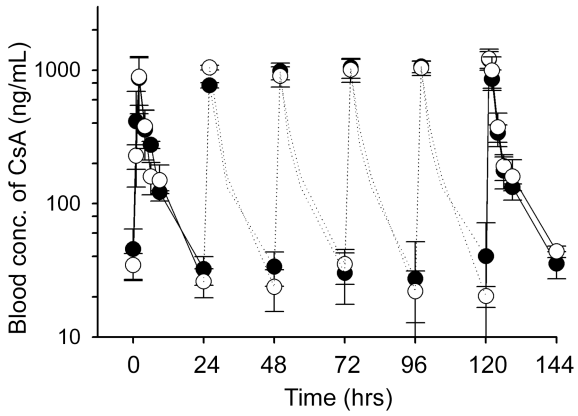

Figure 2–Whole-blood concentration profiles of CsA following multiple oral dosing of test (○) and reference SMEDDS (●) in dogs at a 100 mg dose of CsA per day. Dotted blood concentration profiles from 2nd to 5th day are hypothetical blood concentration (n=3, mean ± S.D.).

Table II–*Pharmacokinetic Parameters of CsA after Multiple Oral Administrations of Test SMEDDS and Reference SMEDDS in Dogs*

	Test SMEDDS		Reference SMEDDS	
	1st day	6th day	1st day	6th day
$AUC_{0\to24h}$ (ng·h/mL)	3835.22 ± 779.50	5315.45 ± 1047.53	3962.10 ± 688.32	4736.08 ± 683.10
C_{max} (ng/mL)	892.06 ± 347.43	1218.61 ± 307.71	868.91 ± 398.32	1203.30 ± 472.52
T_{max} (h)	2.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.00
$T_{1/2}$	6.84 ± 1.44	8.72 ± 1.43	8.18 ± 2.06	7.97 ± 0.88
V_{dss} (L)	263.70 ± 20.16	249.59 ± 98.56	295.72 ± 32.15	247.65 ± 54.86
Cl (L/h)	26.88 ± 5.99	19.38 ± 4.31	25.81 ± 4.93	21.39 ± 2.86
MRT (h)	8.17 ± 0.70	8.62 ± 1.77	8.84 ± 0.34	5.13 ± 0.70

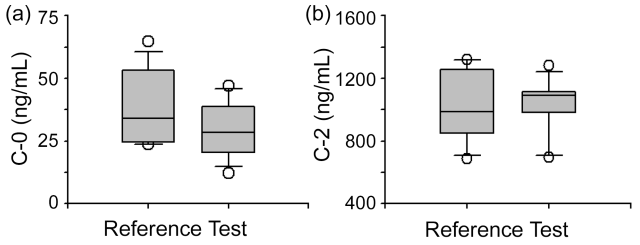

Figure 3–Distribution of C-0 (a) and C-2 (b) during the multiple treatments of test and reference SMEDDS.

Table III–*Pearson's Correlation Coefficient between Blood Concentrations (C-0, C-2) and AUC of Test SMEDDS and Reference SMEDDS*

	Test SMEDDS		Reference SMEDDS	
	Pearson's correlation coefficient	P value	Pearson's correlation coefficient	P value
C-0	0.794	0.059	0.828	0.083
C-2	0.699	0.136	0.619	0.266

683.10 ng·h/mL for reference SMEDDS). C_{max} also increased after the multiple dose treatment (892.06 ± 347.43 vs 1218.61 ± 307.71 ng/mL for test SMEDDS and 868.91 ± 398.32 vs 1203.30 ± 472.52 ng·h/mL for reference SMEDDS). The increased AUC and C_{max} may stem from an accumulation of CsA in body and a decreased clearance (Table II).

Therapeutic drug monitoring is an integral part of CsA treatment for organ recipients. Normally, trough concentration (C-0) of CsA has been considered to accurately reflect systemic exposure of CsA (AUC) to the patients and is used as an index to set up the next dose scheme.[1,18] But recent clinical studies performed with a self-microemulsifying formulation of CsA proved that blood concentration at 2 hrs after dosing (C-2) was much more accurate in extrapolation of AUC with a reduced incidence of organ rejection.[19,20] Figure 3 shows distribution of C-2 and C-0 of both formulations during the multiple dose treatment. Test SMEDDS showed lower range of C-0 than those form reference SMEDDS with statistical difference ($p = 0.046$). However, C-2 of test SMEDDS showed narrower distribution than those of reference SMEDDS with no statistical difference ($P = 0.945$). Pearson's correlation coefficients between blood concentrations (C-0, C-2) and AUC were estimated and shown in Table III. Generally, Pearson's correlation coefficients between C-2 and AUC were much higher than the value between C-0 and AUC, suggesting C-2 is a more reliable index for TDM. Additionally, test SMEDDS also showed a relatively good relationship between C-2 and AUC like reference SMEDDS. These data suggest CsA SMEDDS may be utilized in a human bioequivalence study and in clinical pharmacodynamic studies with a high degree of reliability. Additionally, more precise observation will be required to practice TDM with test SMEDDS based on a trough concentration (C-0).

Conclusion

The relative bioavailability and pharmacokinetic parameters of newly formulated CsA SMEDDS was compared with Sandimmune Neoral in a single and multiple dose study in dogs. The pharmacokinetic parameters of both formulations showed no significant difference among each other after single and multiple dose treatments, suggesting that the newly formulated SMEDDS possibly demonstrates identical pharmacokinetic and pharmacodynamic behavior with reference SMEDDS in clinical trials.

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD). (KRF-2006-214-E00039).

References

1) Kahan, B. D., Therapeutic drug monitoring of cyclosporine: 20 years of progress, *Transplant. Proc.*, **36**, 378S-391S (2004).
2) Hardinger, K.L., Koch, M.J. and Brennan, D.C., Current and future immunosuppressive strategies in renal transplantation, *Pharmacotherapy*, **24**, 1159-1176 (2004).
3) Magnasco, A., Rossi, A., Catarsi, P., Gusmano, R., Ginevri, F., Perfumo, F. and Ghiggeri, G.M., Cyclosporin and organ specific toxicity: clinical aspects, pharmacogenetics and perspectives, *Curr. Clin. Pharmacol.*, **3**, 166-173 (2008).
4) Watts, A.B. and Williams, R.O., 3rd, Peters JI, Recent developments in drug delivery to prolong allograft survival in lung transplant patients, *Drug Dev. Ind. Pharm.*, 1-13 (2008).
5) Ritschel, W.A., Microemulsion technology in the reformulation of cyclosporine: the reason behind the pharmacokinetic properties of Neoral, *Clin. Transplant.*, **10**, 364-373 (1996).
6) Andrysek, T., Impact of physical properties of formulations on bioavailability of active substance: current and novel drugs with cyclosporine, *Mol. Immunol.*, **39**, 1061-1065 (2003).
7) Lawrence, M.J. and Rees, G.D., Microemulsion-based media as novel drug delivery systems, *Adv. Drug. Deliv. Rev.*, **45**, 89-121 (2000).
8) Constantinides, P.P., Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects, *Pharm. Res.*, **12**, 1561-1572 (1995).
9) Masri, M., The generics in transplantation and the rules on their use, *Exp. Clin. Transplant.*, **1**, 65-68 (2003).
10) Sharma, A., Shekhar, C., Heer, M. and Minz, M., Comparison

of generic cyclosporine microemulsion versus neoral in de novo renal transplant recipients managed by 2-hour postdose monitoring, *Transplant. Proc.*, **38**, 2051-2053 (2006).
11) Al Wakeel, J.S., Shaheen, F.A., Mathew, M.C., Abouzeinab, H.M., Al Alfi, A., Tarif, N.M., Al Mousawi, M.S., Mahmoud, T.S., Alorrayed, A.S., Fagir, E.A., Dham, R.S. and Shaker, D.S., Therapeutic equivalence and mg:mg switch ability of a generic cyclosporine microemulsion formulation (Sigmasporin Microral) in stable renal transplant patients maintained on Sandimmun Neoral, *Transplant. Proc.*, **40**, 2252-2257 (2008).
12) Cattaneo, D., Perico, N. and Remuzzi, G., Generic cyclosporine formulations: more open questions than answers, *Transpl. Int.*, **18**, 371-378 (2005).
13) Ponticelli, C., Generic cyclosporine: a word of caution, *J. Nephrol.*, **17 Suppl 8**, S20-24 (2004).
14) Johnston, A., Belitsky, P., Frei, U., Horvath, J., Hoyer, P., Helderman, J.H., Oellerich, M., Pollard, S., Riad, H., Rigotti, P., Keown, P. and Nashan, B., Potential clinical implications of substitution of generic cyclosporine formulations for cyclosporine microemulsion (Neoral) in transplant recipients, *Eur. J. Clin. Pharmacol.*, **60**, 389-395 (2004).
15) Hong, C.I., Kim, J.W., Choi, N.H., Shin, H.J. and Yang, S.G., Cyclosporin-containing microemulsion preconcentrate composition, *United State Patent*, **6063762**, (2000).
16) Mueller, E.A., Kovarik, J.M., Van Bree, J.B., Grevel, J., Lucker, P.W. and Kutz, K., Influence of a fat-rich meal on the pharmacokinetics of a new oral formulation of cyclosporine in a crossover comparison with the market formulation, *Pharm. Res.*, **11**, 151-155 (1994).
17) Yang, S.G, Kim, D.D., Chung, S.J. and Shim, C.K., Stable bioavailability of cyclosporin A, regardless of food intake, from soft gelatin capsules containing a new self-nanoemulsifying formulation, *Int. J. Clin. Pharmacol. Ther.*, **44**, 233-239 (2006).
18) Billaud, E.M., C2 versus C0 cyclosporine monitoring: still not the end, *Transplantation*, **80**, 542-544 (2005).
19) Marin, J.G, Levine, M. and Ensom, M.H., Is C2 monitoring or another limited sampling strategy superior to C0 monitoring in improving clinical outcomes in adult liver transplant recipients?, *Ther. Drug. Monit.*, **28**, 637-642 (2006).
20) Midtvedt, K., Therapeutic drug monitoring of cyclosporine, *Transplant. Proc.*, **36**, 430S-433S (2004).