약제학회지(2008), 제 38 권 제 5 호
J. Kor. Pharm. Sci., Vol. 38, No. 5, 343-348 (2008)

Bioequivalence of Cyclosporine A 100 mg Soft Capsules (Cipol-N® *vs.* Sandimmun Neoral®) in Healthy Korean Volunteers

Yong Ho Huh[1], Euna Park[1], Youn Bok Chung[2], Hee soo Pyo[1] and Oh Seung Kwon[1†]

[1]*Toxicology Lab., Bioanalysis and Biotransformation Research Center, Korea Institute of Science and Technology, Seoul 136-790, Korea*

[2]*College of Pharmacy, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea*

(Received September 22, 2008 · Revised October 6, 2008 · Accepted October 8, 2008)

ABSTRACT – The bioequivalence of two cyclosporine A (CyA) 100 mg soft capsules (Chong Kun Dang's Cipol-N® as the test drug; Korea Novartis' Sandimmun Neoral® as the reference drug) was assessed in healthy male Korean volunteers after oral administration of 200 mg CyA according to a randomized crossover design. The whole blood samples were analyzed for the determination of parent CyA in the blood by using a validated HPLC/diode array detector method. The mean AUC_t values for reference and test products were 4095.3 ± 1397.2 and 3958.3 ± 1138.2 ng·hr/mL, respectively. The mean C_{max} values were 1135.9 ± 293.2 ng/mL for the reference product, and 985.0 ± 207.9 ng/mL for the test product. T_{max} was 1.6 ± 0.4 hr for the reference and 1.8 ± 0.5 hr for the test product. The differences of AUC_t, C_{max} and T_{max} were −3.35, −13.28 and +10.63%, respectively. The point estimates and 90% confidence intervals for AUC_t and C_{max} were 0.981 (0.9171 to 1.0514) and 0.876 (0.8229 to 0.9336), respectively. Based on the pharmacokinetic and statistical data, we conclude that these two products are bioequivalent and can be considered interchangeable in the medical practice.

Key words – Cyclosporine A, Bioequivalence, Pharmacokinetics, HPLC, Chong Kun Dang Cipol-N®, Korea Novartis Sandimmun Neoral®

Cyclosporine A (CyA), a cyclic peptide consisting of 11 amino acids, has been widely used as a selective immunosuppressive agent that is potentially active against proliferating T-lymphocytes.[1)] Among the cyclosporines, CyA is a major immunosuppressive agent that is used primarily in combination with prednisolone to sustain renal, hepatic and cardiac transplants.[2-4)] Renal toxicity is a major adverse effect of chronic CyA administration and neurotoxicity is the second major problem especially in children, characterized by convulsion and sudden alteration of mental function that are reversible upon drug withdrawal.[5,6)]

CyA is highly bound to plasma proteins, mostly to lipoprotein and extensively distributed to adipose tissue, adrenal gland and liver. CyA is also extensively metabolized by hepatic cytochrome P450 enzymes.[7)]

Therefore, the large inter-individual and intra-individual variabilities in the pharamacokinetic behaviors of patients can be important factors to be considered at the time of CyA therapy. For instance, African American patients with renal transplants are reported to have a significantly lower bioavailability than White patients,[8,9)] suggesting that CyA treatment requires a careful drug therapeutic monitoring step due to the variation of bioavailability between racial population. It can be summarized that treatment of patients with appropriate drug dosages is essentially important to protect patients from toxicities, as well as therapeutic monitoring of blood CyA concentrations.

In the present work a simple and sensitive HPLC/diode array detector method was used for the quantitation of cyclosporine 100 mg soft capsules (Chong Kun Dang's Cipol-N® as the test drug; Korea Novartis' Sandimmun Neoral® as the reference drug) in the whole blood of volunteers and applied to the bioequivalence test of CyA in healthy Korean volunteers.

Materials and Methods

Study products and reagents

Cipol-N® soft capsules containing 100 mg CyA as a test formulation are products from Chong Kun Dang (Seoul, Korea) and this product was identified with the lot number of FC005 and the expiration date of Sep-24-2006. Sandimmun Neoral® soft capsules (100 mg CyA) as a reference product were obtained from Korea Novartis (Seoul, Korea) and its lot number was SK0700 and the expiration date was Oct-2006.

Authentic cyclosporine A (CyA) was purchased from Sigma (St. Louis, MO, USA). Cyclosporine D (purity, 99%) used as

†본 논문에 관한 문의는 이 저자에게로
Tel : 02)958-5184, E-mail : oskwon@kist.re.kr

internal standard was gifted from Novartis Pharma AG (Basel, Switzerland). Zinc sulfate was obtained from Sigma. Acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other agents were of analytical grade.

Medical examination of volunteers

According to the planed proposal, healthy male volunteers were selected by a medical doctor. Twenty-four volunteers were selected based on clinical examination including seropathological and serochemical data (hemoglobin, hematocrit, WBC, platelets, blood urea nitrogen, creatinine, total protein, albumin, SGOT, SGPT, total bilirubin, cholesterol, glucose fasting, alkaline phosphate), and urological data (specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC). The volunteers were not taken any medicine for at least 1 week prior to and during study period. They were fasted for at least 12 hr before administration of drugs. Lunch and dinner were allowed, respectively, 4 and 12 hr after drug intake. Physical and biological examinations were carried out after completion of the study.

Oral administration of cyclosporine A soft capsules to volunteers

A 21-gauge scalp-vein set was established on the arm vein of each volunteer and 8 mL of the blood before drug intake were collected for using as a blank sample. Two soft capsules were orally administered to each volunteer of the randomly designed control and test groups (12 volunteers/group) with 240 mL of drinking water. These two groups were taken the formulations by the randomized Latin square crossover design after a 1-week washing-out period. Blood was collected into heparin-treated tubes (Vacutainer, Becton Dikinson, Rutherford, NJ, USA) before administration and at 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 8, 12 and 24 hr after oral administration of 200 mg CyA (2 soft capsules). The blood was stored to a refrigerator at −20°C until analyzed.

HPLC equipment

CyA concentrations in the whole blood were measured by using a HPLC system (HP 1090M, Hewlett Packard, DE, USA) equipped with DR5 solvent delivery system and an auto liquid sampler. The diode array detector (HP 1090 series) at 207 nm was used. The separation of cyclosporines was made by using XTerra® RP18 column (2.1 mm × 150 mm, 5 μm pore size; Waters, Milford, MA, USA) and the temperature of column oven set to 60°C. Mobile phase was consisted of acetonitrile/water (80 : 20, v/v%). The flow rate of the column was 0.25 mL/min.

Clean-up procedure for the calibration curve of cyclosporine A

The clean-up procedure for CyA determination in whole blood was developed with a modification as described in Brozmanova *et. al.*[10] and this method has been reported (KFDA, 2002).[11] The stock solutions of cyclosporine A and D (1000 μg/mL) were prepared in methanol and were serially diluted to prepare 1, 10 and 100 μg/mL solutions. One mL of cyclosporine-free whole blood was added to Corning disposable tubes and standard solutions of CyA were added to prepare 0, 25, 50, 100, 250, 500, 1000 and 2000 ng/mL. Cyclosporine D (10 μg/mL × 50 μL) was added as internal standard and the tube was agitated on a vortex-mixer (Maxi-Mix II, Thermolyne, Dubuque, IA, USA). For precipitation of protein in the blood, 2 mL of 10% $ZnSO_4 \cdot 7H_2O$/acetonitrile/methanol (50 : 20 : 30, v/v/v%) was added and the tubes were shaken on a shaker (SM-25, Edmund Buhler, Germany) and centrifuged (HA-1000-3, Hanil Industrial Co., Seoul, Korea) at 3000 rpm. The upper layer was transferred to another tube and evaporated under nitrogen gas flow at 40°C. The residue was reconstituted in 100 μL of methanol and the solution was filtered through membrane (Ultrafree-MC centrifugal filter units, 0.22 μm; Milipore, Canton, MA, USA) by centrifugation at 2000 rpm (Hm-150IV, Hanil Co., Seoul, Korea) for 5 min. Ten μL of the solution was injected to the HPLC. The calibration curve for CyA determination was prepared by ratios of the CyA peak area to the internal standard. This method was validated by determining inter- and intra-day precisions and accuracies from 5 repeated experiments.

Preparation of whole blood samples

The blood samples were thawed at room temperature, and 1 mL of the sample was used to determine CyA concentrations. Cyclosporine D (10 μg/mL × 50 μL) was added as internal standard and the tube was agitated on a vortex-mixer. All other procedures were the same as described above.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined from the time-blood concentrations of CyA by non-compartmental analysis by using WinNonlin software (Scientific Consulting Inc., Cary, NC, USA). The highest concentration (C_{max}) and the time to reach the highest concentration (T_{max}) were read directly from the time-blood concentration curves of CyA. The area under the curve of time-blood concentrations of CyA from 0 to the infinitive time ($AUC_{0\rightarrow inf}$) was determined by the equation of

$AUC_{0\rightarrow inf}=AUC_{0\rightarrow last}+C_{last}/\beta$, where β is the slope of the terminal phase of the time-log blood concentration curve and C_{last} is the concentration at the last sampling time.[12]

Statistics

Data are presented as mean ± standard deviation. K-BE test® software (KFDA, 2002) was used for the determination of 90% confidence limit.[13] Analysis of variance (ANOVA) was done by the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA) to determine *F*-values and probability. Two formulations are considered to be bioequivalent when 90% confidence limits of logarithmically transformed C_{max} and AUC_t ranged from log 0.8 to log 1.25 according to the revised KFDA guideline (KFDA, 2005), which is finally identical to European Community and FDA guidelines.[12,14,16]

Results and Discussion

Dissolution tests of CyA soft capsules

The dissolution tests for two products were carried out in 4 different kinds of dissolution media of pH 1.2, pH 4.0, pH 6.8 buffer solutions and distilled deionized water. Dissolution patterns are very similar between two products at dissolution media used.

Validation of analytical methods of CyA in the human whole blood

HPLC chromatograms showing CyA and internal standard (cyclosporine D) were presented in Figure 1. Retention times

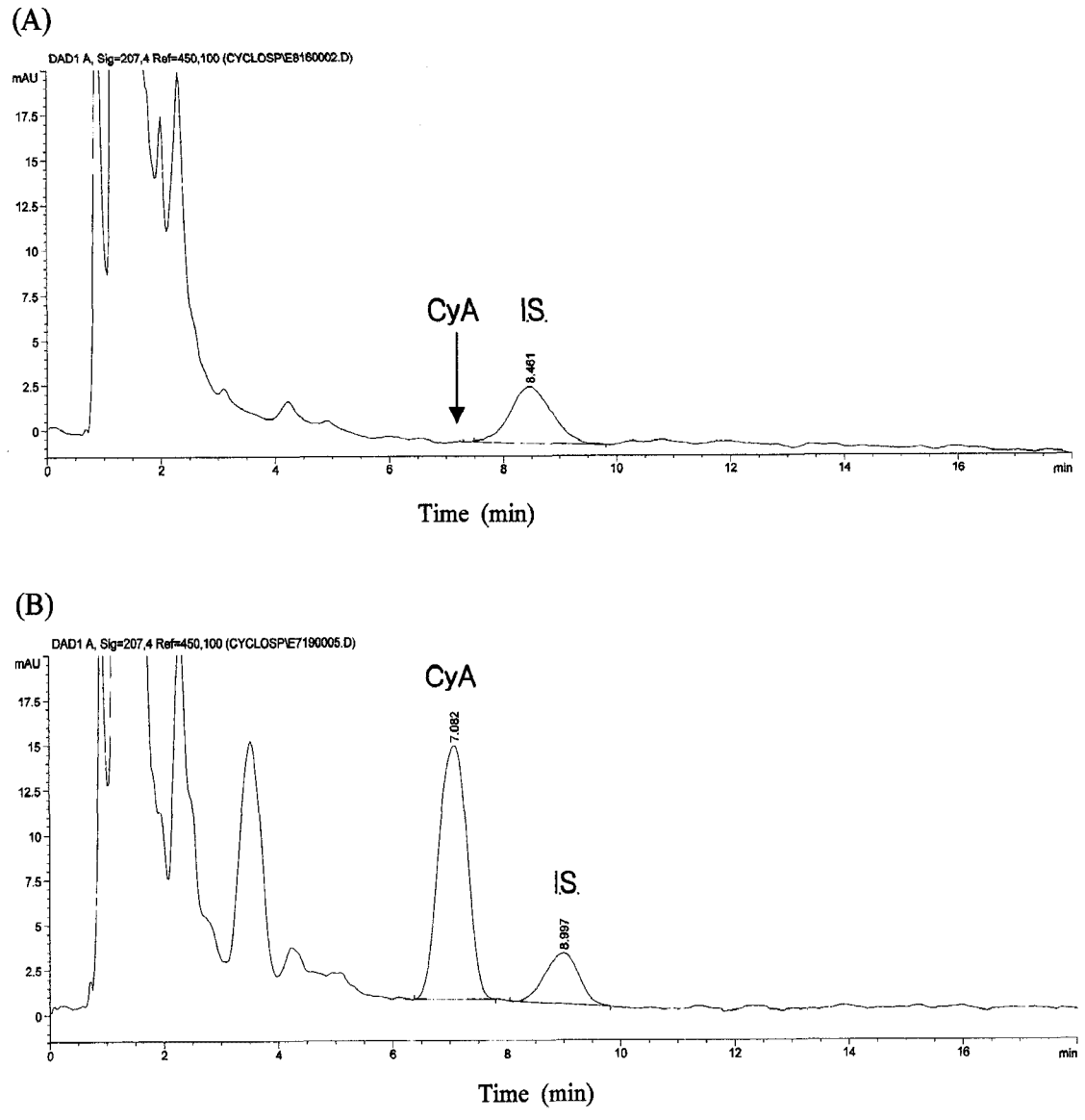

Figure 1–Typical HPLC chromatograms of cyclosporine A. (A) Blank and (B) the whole blood sample from a volunteer 2 hr after oral administration of 200 mg cyclosporine A (CyA, 7.08 min; I.S., 8.99 min).

Table I–*Precision and Accuracy in Intra-day and Inter-day for the Determination of Cyclosporine A in the Human Blood*

Concentrations (ng/mL)	Precision (C.V. %)		Accuracy %	
	Intra-day	Inter-day	Intra-day	Inter-day
25.0 (LLOQ)	4.61	6.77	−4.37	−20.00
50.0	10.28	6.65	12.92	1.99
100.0	9.98	11.77	0.45	2.35
250.0	6.94	9.91	0.33	3.81
500.0	6.03	10.08	1.61	0.17
1000.0	5.97	10.65	−1.55	1.03
2000.0	4.22	5.61	−0.63	−1.12

Table II–*Bioavailability Parameters in Normal and Logarithmic Scales Obtained after Oral Administration of Cipol-N® or Sandimmun Neoral® Soft Capsules Containing 100 mg Cyclosporine A (as Total 200 mg) to Healthy Volunteers*

Subjects	Reference drug (Sandimmun Neoral®)					Test drug (Cypol-N®)				
	AUC (ng·hr/mL)	Ln AUC	C_{max} (ng/mL)	Ln C_{max}	T_{max} (hr)	AUC (ng·hr/mL)	Ln AUC	C_{max} (ng/mL)	Ln C_{max}	T_{max} (hr)
A1	6781.51	3.83	1581.62	3.20	2.0	7673.37	3.89	1488.97	3.17	1.5
A2	3946.12	3.60	1171.41	3.07	1.5	3982.78	3.60	1249.49	3.10	1.5
A3	5330.47	3.73	1406.65	3.15	1.5	4066.17	3.61	946.01	2.98	1.0
A4	7530.97	3.88	1857.56	3.27	2.0	5319.26	3.73	1252.95	3.10	1.5
A5	5538.07	3.75	1374.55	3.14	1.5	5802.02	3.76	1191.80	3.08	1.5
A6	2724.41	3.44	707.38	2.85	1.5	2471.62	3.39	654.38	2.82	1.5
A7	6652.86	3.82	1186.32	3.07	3.0	3736.50	3.57	938.65	2.97	2.5
A8	3716.87	3.57	1060.54	3.03	2.0	3974.75	3.60	1102.60	3.00	1.5
A9	2665.00	3.43	1113.33	3.05	1.5	2334.56	3.37	935.34	2.97	1.5
A10	2696.20	3.41	898.41	2.95	1.5	3335.15	3.52	610.98	2.79	3.0
A11	2434.39	3.39	975.65	2.99	1.5	2859.18	3.46	946.77	2.98	1.5
A12	4183.84	3.62	1378.37	3.14	1.0	3948.27	3.60	749.56	2.88	2.5
B1	4985.26	3.70	1539.81	3.19	1.0	3438.88	3.54	976.49	2.99	2.0
B2	4002.91	3.60	1162.86	3.07	1.5	4030.25	3.61	973.90	2.99	1.5
B3	3862.13	3.59	1019.84	3.01	2.0	4860.65	3.69	917.15	2.96	2.5
B4	3853.53	3.59	1193.95	3.08	1.0	4265.44	3.63	1130.31	3.05	2.0
B5	4080.94	3.61	1266.88	3.10	1.5	4487.13	3.65	1283.32	3.11	1.5
B6	3423.74	3.54	967.55	2.99	1.5	3419.33	3.53	971.32	2.99	2.0
B7	3170.32	3.50	920.94	2.96	1.5	3395.08	3.53	892.07	2.95	2.0
B8	2830.83	3.45	1011.50	3.01	1.5	2964.95	3.47	929.99	2.97	1.0
B9	2576.40	3.41	716.98	2.86	1.5	3105.71	3.49	847.39	2.93	2.0
B10	3243.27	3.51	658.59	2.82	1.5	3533.76	3.55	719.00	2.86	1.5
B11	3832.52	3.58	1251.51	3.10	1.5	3629.26	3.56	1126.66	3.05	1.5
B12	4225.30	3.63	839.01	2.92	2.0	4364.59	3.64	905.16	2.96	2.0
Mean	4095.33	3.59	1135.88	3.04	1.60	3958.28	3.58	985.01	2.98	1.77
SD	1397.52	0.14	293.18	0.11	0.42	1138.20	0.11	207.88	0.09	0.49

of CyA and internal standard were observed to be about 7.08 and 8.90 min, respectively. Intra- and inter-day precisions and accuracies for CyA determination in the whole blood were evaluated and indicated in Table I. Intra-day precision was below 10.3% and inter-day precision was less than 11.8%. At the lowest concentration of 25 ng/mL CyA, intra- and inter-day precisions were 4.6 and 6.8%, respectively. Accuracy bias % for intra- and inter-days were below 12.9 and 20%, respectively. The lowest limit of quantitication for determination of CyA in the blood samples was decided to be 25 ng/mL. The

Table III–*Statistics for Pharmacokinetics and Bioequivalence Parameters of Cyclosporine A*

	Parameters		
	AUC_t	C_{max}	T_{max}
Difference %	−3.35	−13.28	+10.63
Test/Reference point estimate	0.981	0.876	1.097
F_{Group} [a)]	0.683	0.784	0.225
90% Confidence limit[b)]	$0.9171 \leq \delta \leq 1.0514$	$0.8229 \leq \delta \leq 0.9336$	$0.9675 \leq \delta \leq 1.2442$

Log-transfprmed values of AUC_t and C_{max} were used for statistics of bioequivalence evaluation. The statistical results of T_{max} value were obtained without the log-transformation.
a) a=0.05, F(1,22)=4.301.
b) a=0.05

linearity of CyA calibration curve was good (R^2=0.9999) with the linear equation of y=0.0026x+0.0442. This data suggest that the method is suitable to determine CyA concentrations in the whole blood and can be applied to the pharmacokinetics and bioequivalence studies of CyA.

Pharmacokinetic parameters and the statistical consideration of CyA in healthy volunteers

The principal pharmacokinetic parameters were determined from the time-blood CyA concentrations (Figure 2), and individual pharmacokinetic parameters were indicated in Table II. The mean AUC_t for reference and test products were 4095.3 ± 1397.2 and 3958.3 ± 1138.2 ng·hr/mL, respectively. The mean C_{max} values were 1135.9 ± 293.2 ng/mL for the reference product, and 985.0 ± 207.9 ng/mL for the test product. T_{max} was 1.6 ± 0.4 hr for reference and 1.8 ± 0.5 hr for test products. As shown in Table III, the differences of AUC_t, C_{max} and T_{max} were −3.35, −13.3 and +10.6%, respectively. These data indicate that the values for the difference of the mean AUC_t and C_{max} satisfy the criteria that these two products are accepted to be bioequivalent if the differences of mean AUC_t and C_{max} values are ranged within ±20%.

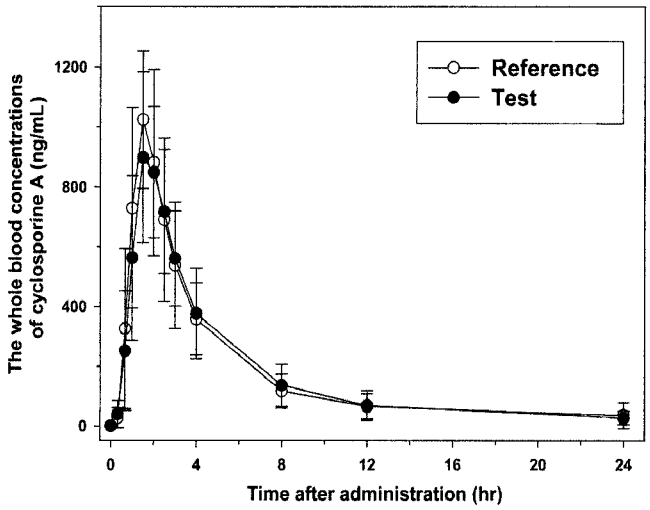

Figure 2–Cyclosporine A concentrations in the time-blood curves after oral administration of 2 capsules (total 200 mg). Each point represents the mean ± S.D. of 24 human volunteers.

Analysis of variance (ANOVA) was carried out using logarithmically transformed AUC_t, C_{max} and untransformed T_{max}. There were no significant differences between the formulations in AUC_t and C_{max}. The point estimates and 90% confidence intervals for AUC_t and C_{max} were 0.981 (0.9171 to 1.0514) and 0.876 (0.8229 to 0.9336), respectively, showing that these values satisfy the criteria of KFDA guideline[14,15] and these results, concomitantly, satisfied the bioeqivalence criteria of European Community and the United State FDA guidelines.[12,16]

Taken together, the statistical comparison of AUC_t and C_{max} indicated no significant difference in the two formulations of CyA 100 mg soft capsules. 90% confidence intervals of AUC_t and C_{max} were satisfied the criteria as suggested in the Korea or United State FDA guidelines. Based on the pharmacokinetic and statistical data, we conclude that these two products (Chong Kun Dang's Cipol-N® as the test drug; Korea Novartis' Sandimmun Neoral® as the reference drug) are bioequivalent and can be considered interchangeable in the medical practice.

Acknowledgement

This study was supported from Chong Kun Dang Co., Ltd. (Seoul, Korea). Authors also express deep gratitude to Novartis Pharma AG (Basel, Switzerland) for providing cyclosporine D used as internal standard.

References

1) White DJ, Plumb AM, Pawelec G and Brons G, Cyclosporine A, an immunosuppressive agent preferentially active against proliferating T cells, *Transplantation*, **27**, 55-58 (1979).

2) Fowler MB and Schroeder JS, Current status of cardiac transplantation, *Mod. Concepts Cardiovasc. Dis.*, **55**, 37-41 (1986).
3) Kahan BD, Mickey R, Flechner SM, Lorber MI, Wideman CA, Kerman RH, Tersaki P and van Buren CT, Multivariate analysis of risk factors impacting on immediate and eventual cadaver allograft survival in cyclosporine-treated recipients, *Transplantation*, **43**, 65-70 (1987).
4) O'Grady JG, Forbes A, Rolles K, Calne RY and William R, An analysis of cyclosporine efficacy and toxicity after liver transplantation, *Transplantation*, **45**, 575-579 (1988).
5) Magnasco A, Rossi A, Cartasi P, Gusmano R, Ginevri F, Perfumo F and Ghiggeri GM, Cyclosporine and organ specific toxicity: clinical aspects, pharmacogenetics and perspectives, *Curr. Clin. Pharamcol.*, **3**, 166-173 (2008).
6) Garcia-Escriq M, Martinez J, Fernandez-Ponsati J, Diaz J and Soto O, Severe central nervous system toxicity after chronic treatment with cyclosporine, *Clin. Neuropharmacol.*, **17**, 298-302 (1994).
7) Kronbach T, Fischer V and Mever UA, Cyclosporine metabolism in human liver: identification of cytochrome P450III gene family as the major cyclosporine-metabolizing enzyme explains interaction of cyclosporine with other drugs, *Clin. Pharmacol. Ther.*, **43**, 630-635 (1988).
8) Min DI, Lee M, Ru YM and Flanigan M, Gender-dependent racial difference in disposition of cyclosporine among healthy African, African American and White volunteers. *Clin. Pharmacol. Ther.*, **68**, 478- (2000).
9) Stein CM, Sadeque AJ, Murray JJ, Wandel C, Kim RB and Wood AJJ, Cyclosporine pharmacokinetics and pharmacodynamics in African American and White subjects, *Clin. Pharmacol. Ther.*, **69**, 317- (2001).
10) Brozmanova B., Grundmann M, Safarcik K and Jegorov A, High-performance liquid chromatographic method for therapeutic drug monitoring of cyclosporine A and its two metabolites in renal transplant patients, *J. Chromatogr. B.*, **749**, 93-100 (2000).
11) KFDA, 18. Cyclosporine, In "*The Standard Protocols of Bioequivalence Tests*", 94-98 (2002).
12) EC, European Community, Investigation of Bioavailability and Bioequivalence, *The Rules Governing Medicinal Products in European Community*, Vol. III, 149-169, Addendum 2 (1991).
13) KFDA, *K-BE test for Window*, Lee YJ, Chung SJ, and Shim CK, Version 1.2.1 (2002).
14) KFDA, Notification No. 2005-31, *The Standard Protocol of Bioequivalence Test* (June 07, 2005).
15) KFDA, *Guidance for Industry. Statistical Approaches to Establishing Bioequivalence*, Bioequivalence Division, Pharmacology Department, National Institute of Toxicology Department, http://www.kfda.go.kr (2005).
16) FDA, *Guidance for Industry. Statistical Approaches to Establishing Bioequivalence*, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and research (CDER). Office of Training and Communication, Division of Communications Management, Rockville, MD (January 2001).