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Pharmacokinetic and pharmacodynamic evaluation of cyclosporin A O/W-emulsion in rats

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Abstract

The pharmacokinetics and pharmacodynamics of the cyclosporin A (CSA) O/W-emulsion were studied after intravenous and oral administration to Sprague–Dawley rats. Two commercial products, CIPOL Inj.® and Sandimmun Neoral®, were used as the reference formulations. CSA concentration and lymphocyte populations in whole blood were measured by TDxFLx® and Coulter STKS®, respectively. The pharmacokinetic and pharmacodynamic parameters were obtained by fitting experimental data to two-compartment model and to indirect pharmacodynamic model, respectively, using WINNONLIN. The area under the concentration–time curve (AUC), terminal half-lives ($T_{1/2}$), total clearance (CL_t) and relative bioavailability (F) after intravenous administration of CSA O/W-emulsion were not significantly different from those of intravenous administration of CIPOL Inj.® ($P > 0.05$). In oral administration, AUC and C_{max} of CSA O/W-emulsion were significantly decreased ($P < 0.05$), while $T_{1/2}$, MRT, T_{max} and F were not significantly different ($P > 0.05$) from those of Sandimmun Neoral®. However, the area between the baseline and effect curves (ABEC) and pharmacodynamic efficiency (EFF) of CSA O/W-emulsion were significantly greater than those of references regardless of routes of administration ($P < 0.05$). The pharmacodynamic availability (F_{PD}) of CSA O/W-emulsion was 1.79- and 2.13-fold higher than that of CIPOL Inj.® and Sandimmun Neroal® ($P < 0.05$), respectively.

Keywords: Cyclosporin A; O/W-emulsion; Pharmacokinetics; Pharmacodynamics

1. Introduction

Cyclosporin A (CSA) is a potent immunosuppressive agent extensively employed to avert graft rejection in kidney, liver and bone marrow transplant patients and to delay or prevent disease progression in patients with autoimmune disease and inflammation (Miller et al., 1992; Tibell and Norrlind, 1994; Noble and Markham, 1995). The immunosuppressive effect of CSA was due to a selective and reversible inhibition of T-lymphocytes (Ferron et al., 1998). CSA selectively inhibits

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interleukin-2 (IL-2) driven proliferation of activated T-lymphocytes. In spite of the great clinical importance of the drug, their extended usage has been often limited by several disadvantages including low bioavailability, narrow therapeutic window, nephrotoxicity and hepatotoxicity (Yee and Salomon, 1992; Gijtenbeek et al., 1999). It has also been reported that Cremophor EL®, a solubilizing agent, present in the commercially available intravenous dosage form of CSA, has a risk of anaphylatic shock and nephrotoxicity (Cavanak and Sucker, 1986; Tibell et al., 1993). Owing to the above-mentioned disadvantages of commercial products, there is a great interest in the development of the alternative dosage forms.

Attempts have been made to reduce the toxicity and to improve the availability of CSA by using emulsions. Several investigators reported that CSA-containing emulsions showed reduced nephrotoxicity (Tibell et al., 1992) and enhanced absorption (Tarr and Yalkowsky, 1989; Corvari et al., 1991; Drewe et al., 1992). In case of intravenous administration, it has been reported that nephorotoxicity caused by CSA or Cremophor EL® could be avoid by using a soybean oil based fat emulsion carrier (Tibell et al., 1993).

In this study, we prepared CSA O/W-emulsion that can be used for both intravenous and oral administration using soybean oil. We also evaluated the pharmacokinetic and pharmacodynamic characteristics of the prepared CSA O/W-emulsion in rats and compared them with those of two commercial products, CIPOL Inj.® for intravenous administration and Sandimmun Neoral® for oral administration.

2. Materials and methods

2.1. Materials

CSA and CIPOL Inj.® were kindly supplied by Chong Kun Dang Pharm. (Seoul, Korea), and Sandimmun Neoral® (Novartis Pharma Schweiz, Basle, Switzerland) was purchased from the local market. Soybean oil (Junsei Chemical, Tokyo, Japan), egg lecithin (Asahi Chemical Industry, Charlotte, NC, USA), ethanol (Oriental Chemical Industries, Seoul, Korea) and glycerin (Sigma, St. Louis, MO, USA) were purchased and used as received. All other reagents used in this experiment were of analytical grade.

2.2. Preparation of CSA O/W-emulsion

O/W-emulsion was prepared in accordance with the method reported in the literature with some modifications using a microfluidizer (EmulsiFlex-B3, Avestin, Ottawa, Canada; Tibell et al., 1995; Maa and Hsu, 1999). The lipid phase was prepared by dissolving CSA in soybean oil at 70 °C. Purified egg lecithin dissolved in ethanol was slowly added into the lipid phase. The water phase was prepared by mixing water and glycerol at 70 °C. Glycerol (25 mg/ml) was used to make the emulsion isotonic. After removing ethanol from the lipid phase using centrifugal evaporator (CVE-200D, Tokyo Rikakikai, Tokyo, Japan), the water phase and the lipid phase were mixed at 10 000 rpm for 10 min using a homogenizer (Polytron® PT 3100, Kinematica, Littau, Switzerland). A fine emulsion was prepared at 4 °C using a microfluidizer. The final concentration of CSA in the prepared emulsion was 3 mg/ml and the concentrations of egg lecithin and soybean oil were 2 and 10%, respectively. The particle size of the prepared CSA O/W-emulsion was 221.5 ± 12.5 nm as determined by a dynamic light scattering analyzer (Autosizer Lo-C, Malvern Instruments, Worcestershire, UK). The stability test of CSA O/W-emulsion was performed for 4 weeks at 4 °C (Bontempo, 1997). The percentage of CSA in the emulsion ranged from 94.3 to 103.2 as determined every week.

2.3. Animal study

Male Sprague–Dawley rats weighing 280–300 g were obtained from Dae Han Laboratory Animal Research Center (Daejeon, Korea). The experiment was carried out after the approval of the protocols by the ethical-scientific committee of the College of Pharmacy, Chonnam National University. The rats were fed with tap water and food (Cheil Food and Chemical, Icheon, Korea). Before the treatment, the animals were fasted overnight

and had access to water ad libitum. The femoral vein and artery were cannulated with polyethylene tubing (PE-50, Intramedic®, Clay Adams, Parsippany, NJ, USA) under light ether anesthesia. Cannulated rats were kept in restraining cages under normal housing conditions for 1–2 h until they recovered from the anesthesia prior to the experiments. The animals were divided into four groups, and each rat received a 10 mg/kg of CSA in one of the following dosage forms: (1) oral CSA O/W-emulsion, (2) intravenous CSA O/W-emulsion, (3) oral Sandimmun Neoral® and (4) intravenous CIPOL Inj.®. CSA formulations were administered at the same time (09:30 h) to avoid chronopharmacokinetics effects (Malmary et al., 1995). Whole blood sample of 1 ml was withdrawn from the femoral artery at 0 (pretreatment), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36 and 48 h post-administration. Twelve rats were allocated in one group and four samples including 0 time were randomly withdrawn from each rat to avoid excessive loss of the blood from one animal. Samples were collected in sodium EDTA anticoagulant tubes (K3 EDTA Vacutainer®, 13 × 75 mm, Becton Dickinson, Meylan, UK). They were thoroughly mixed and stored at 4 °C until the assay within 24 h.

2.4. Drug analysis

CSA concentrations in whole blood were assayed by the monoclonal antibody fluorescence polarization immunoassay (m-FPIA, TDxFLx®, Abott Laboratories, Abott Park, IL, USA) (Agarwal et al., 1985). Calibration curves (0–1500 ng/ml) were obtained each time a set of samples was analyzed, and the method was evaluated by analyzing the quality control samples provided by the manufacturer. Under these conditions, percentage recovery of CSA in the samples ranged from 95.2 to 102.4% and within-day and between-day coefficients of variation did not exceed 5.0% for the same batch of reagents. Samples exceeding the upper limit of the calibration concentration range were diluted with drug-free murine whole blood and re-assayed.

2.5. Lymphocyte counting

The lymphocyte fraction (LY/WBC), the ratio of lymphocyte population to white blood cell population in whole blood, served as pharmacodynamic marker for CSA since the immunosuppressive activity of CSA is primarily due to its effect on T cells and B cells (Awni, 1992). The total cell counts in whole blood were measured by an automated hemocytometer (Coulter STKS®, Coulter Electronics, Northwell, UK). The whole blood samples were assayed within 24 h after collection to avoid the cell destruction.

2.6. Pharmacokinetic analysis

Pharmacokinetic parameters associated with each group were estimated by compartmental method using WINNONLIN (Version 1.1, Scientific Consulting Inc., NC, USA; Gabrielsson and Weiner, 1997). The lowest Akaike's number which indicates the goodness of fit was obtained when the CSA concentration–time profiles were fitted to a two-compartment open model with bolus intravenous input for intravenous administration and first-order absorption input for oral administration, and first-order output from the central compartment. The CSA pharmacokinetic parameters, such as total clearance (CL_t), mean residence time (MRT), and biological half-life ($T_{1/2}$) were calculated from standard equations using WINNONLIN (Gibaldi and Perrier, 1982). The area under the whole blood concentration–time curve (AUC) and area under the first moment curve (AUMC) were calculated using trapezoidal rule. The MRT was calculated as AUMC/AUC. Maximum whole blood concentration (C_{max}) and time for maximum whole blood concentration (T_{max}) were obtained directly from the experimental data. The relative bioavailability F (%) for each formulation was calculated from (AUC for 'test formulation'/AUC for CIPOL Inj.®) × 100.

2.7. Pharmacodynamic analysis

The indirect pharmacodynamic response model was used to describe the whole blood CSA concentration–pharmacodynamic activity rela-

tionship (Dayneka et al., 1993; Gobburu and Jusko, 1998). The mathematical expression that describes the relationship between the lymphocyte fraction as the pharmacological response and the CSA concentration is:

$$\frac{dR}{dt} = k_{in}\left\{1 - \frac{C}{C + IC_{50}}\right\} - k_{out}R \quad (1)$$

where R is the observed response, i.e., lymphocyte fraction (LY/WBC, %); k_{in}, zero-order rate constant for the production of the response and k_{out}, the first-order rate constant for loss of the response; IC_{50}, the whole blood CSA concentration that leads to 50% of maximum inhibition, and C is the whole blood CSA concentration at the time of the observed response. When no CSA is present, this model yields the following relation for the response production rate constant (k_{in}) at steady-state:

$$k_{in} = k_{out}R_0 \quad (2)$$

where R_0 is a no-dose baseline of the lymphocyte fraction at steady-state. Therefore, the rate of change of CSA pharmacological response can be described by following equation:

$$\frac{dR}{dt} = k_{out}\left\{\frac{R_0}{1 + (C/IC_{50})} - R\right\}. \quad (3)$$

The area between the baseline and effect curves (ABEC) was calculated using the trapezoidal rule. Two different metrics, pharmacodynamic efficacy (EFF) and pharmacodynamic availability (F_{PD}), were used to evaluate pharmacological response of each formulation (Gobburu and Jusko, 1998). The EFF (response–concentration units) was calculated as ABEC/AUC, and F_{PD} was determined as.

$$F_{PD} = \frac{ABEC_{test}}{ABEC_{ref}}\frac{AUC_{ref}}{AUC_{test}} = \frac{EFF_{test}}{EFF_{ref}} \quad (4)$$

The EFF provides an overall measure of effect per unit dose and the F_{PD} provides a dimensionless quantitation of relative efficiency of test formulation to that of reference formulation. In a linear pharmacokinetic system, an F_{PD} value less than 1.0 indicates lower pharmacodynamic availability and a value greater than 1.0 indicates that the test formulation is more efficient than the reference formulation.

The pharmacodynamic model parameters were estimated by fitting lymphocyte fraction and whole blood CSA concentration to the above model using WINNONLIN.

2.8. Statistical evaluation

The data were analyzed for statistical significance by Student's t-test ($P < 0.05$). All calculated values were expressed as their mean ± S.E.

3. Results and discussion

3.1. Pharmacokinetics

The mean whole blood concentration–time profiles of CSA after intravenous administration (10 mg/kg) of CSA O/W-emulsion and CIPOL Inj.®, and oral administration (10 mg/kg) of CSA O/W-emulsion and Sandimmun Neoral® are shown in Figs. 1 and 2, respectively. The pharmacokinetic parameters that were obtained by fitting experimental data to two-compartment model using WINNONLIN are listed in Table 1. An adequate data fit was evidenced by the high correlation ($r^2 > 0.99$) between computer-calculated and experimental CSA blood concentrations. The whole blood concentration–time profile of CSA has also been described by a two-compartment model in the literature (Fahr et al., 1995). The parameter values of CIPOL Inj.® were similar to the other investigators' (Vadiei et al., 1989; Molpeceres et al., 2000).

We compared the pharmacokinetics of CSA O/W-emulsion with that of the two commercial products, CIPOL Inj.® and Sandimmun Neoral®. Two commercial products are microemulsion system consisting of surfactants and oil phase. They can form emulsion droplets with the size of only about 30 nm upon contact with water, which are advantageous for the absorption of CSA, a poorly soluble drug (Zhang et al., 2000). The AUC and $T_{1/2}$ after intravenous administration of CSA O/W-emulsion (100 106 ± 27 895 ng/ml/h and 31.16 ± 15.92 h for AUC and $T_{1/2}$, respectively)

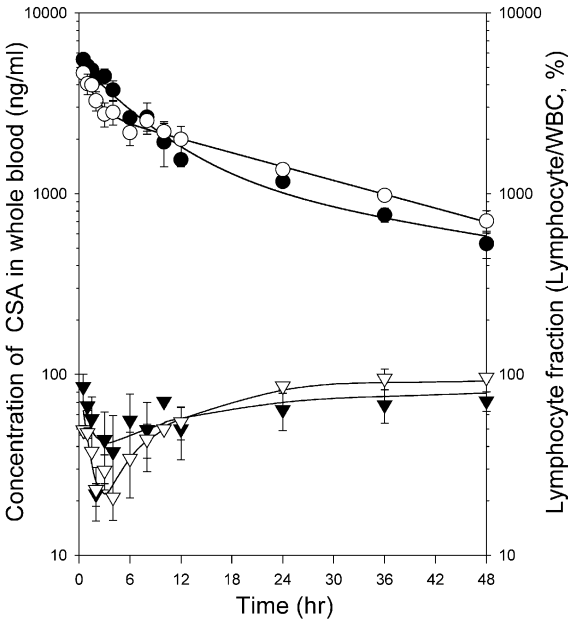

Fig. 1. Mean whole blood concentration of CSA (● for CSA O/W-emulsion and ○ for CIPOL Inj.®) and lymphocyte fraction (▼ for CSA O/W-emulsion and ▽ for CIPOL Inj.®)—time profiles after intravenous administration (10 mg/kg) of CSA O/W-emulsion and CIPOL Inj.® to rats. The points are experimental data (mean ± S.E., $n = 4$) and the lines are pharmacokinetic–pharmacodynamic model fitted curves in whole blood.

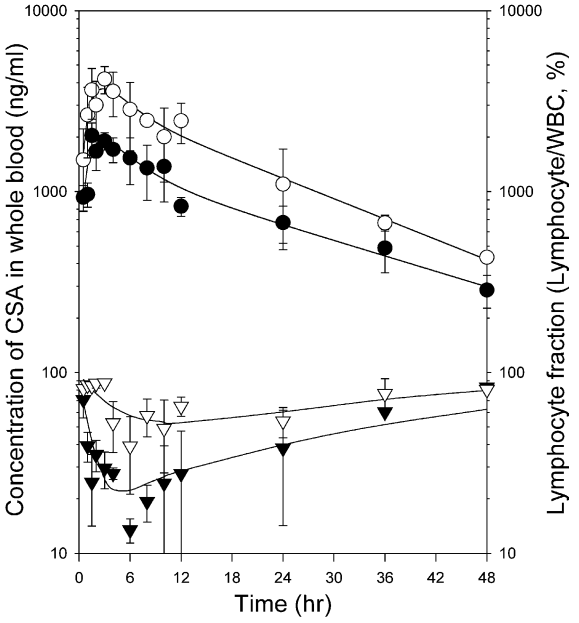

Fig. 2. Mean blood concentration of CSA (● for CSA O/W-emulsion and ○ for Sandimmun Neoral®) and lymphocyte fraction (▼ for CSA O/W-emulsion and ▽ for Sandimmun Neoral®)—time profiles after oral administration (10 mg/kg) of CSA O/W-emulsion and Sandimmun Neoral® to rats. The points are experimental data (mean ± S.E., $n = 4$) and the lines are pharmacokinetic–pharmacodynamic model fitted curves in whole blood.

to rats were not significantly different from those of CIPOL Inj.® (103 837 ± 824 ng/ml/h and 28.60 ± 5.17 h for AUC and $T_{1/2}$, respectively). CSA O/W-emulsion after oral administration resulted in about 26.8 and 49.2% decrease in AUC and C_{max}, respectively, compared with Sandimmun Neoral® ($P < 0.05$). However, there were no significant differences in $T_{1/2}$, MRT, T_{max}, and F between CSA O/W-emulsion and Sandimmun Neoral® oral administration ($P > 0.05$).

3.2. Pharmadynamics

The mean lymphocyte fraction–time curves in rat whole blood following intravenous (10 mg/kg) and oral (10 mg/kg) administration of CSA formulations are shown in Figs. 1 and 2, respectively. The pharmacodynamic parameters of the effects on lymphocyte fraction in whole blood were calculated by Eq. (3) using predicted CSA concentration from two-compartment model and are listed in Table 2. We applied the experimental data to the E_{max} model and the sigmoidal E_{max} model including Hill's coefficient, and discriminated between them using statistical methods such as Akaike's information criterion (AIC) and runs in the residuals using WINNONLIN. The AIC of the E_{max} model was lower than that of the sigmoidal E_{max} model. For the E_{max} model, the amplitude of the scatter was larger and deviating trend was more obvious when the absolute residual was plotted against the concentration. The Hill's coefficients of all formulations were not significantly different from 1. They were 1.05 ± 0.40, 1.59 ± 1.20 and 0.99 ± 0.28, 1.38 ± 0.45 (mean ± S.E.) for intravenously administered CSA O/W-emulsion, CIPOL Inj.® and orally administered CSA O/W-emulsion, Sandimmun Neoral®, respectively. Therefore, we applied the lymphocyte

Table 1
Pharmacokinetic parameters of CSA O/W-emulsion and commercial products in rats (mean ± S.E., $n = 4$)

Parameters	Intravenous administration		Oral administration	
	O/W-emulsion	CIPOL Inj.®	O/W-emulsion	Sandimmun Neoral®
AUC (ng/ml/h)	100106 ± 27895	103837 ± 824	52317 ± 14235[d]	71436 ± 25576
$T_{1/2}$ (h)	31.16 ± 15.92	28.60 ± 5.17	24.18 ± 13.81	23.66 ± 11.59
CL_t (ml/h/kg)	106.48 ± 25.65	75.22 ± 14.58	Nd[a]	Nd[a]
MRT (h)	24.35 ± 3.48[c]	35.71 ± 5.45	26.89 ± 5.18	26.23 ± 6.25
T_{max} (h)	Nd[a]	Nd[a]	2.94 ± 1.29	3.11 ± 0.63
C_{max} (ng/ml)	Nd[a]	Nd[a]	1952.7 ± 410.4[d]	3842.9 ± 1158.9
F[b] (%)	96.33 ± 26.55	100	50.50 ± 13.83	68.74 ± 24.52

[a] Not determined.
[b] Relative bioavailability based on AUC of CIPOL Inj.® in rats.
[c] $P < 0.05$ from intravenous administration of CIPOL Inj.®.
[d] $P < 0.05$ from oral administration of Sandimmun Neoral®.

fraction data to the E_{max} pharmacodynamic model using WINNONLIN.

The ABEC and EFF of CSA O/W-emulsion were significantly increased regardless of routes of administration. In case of oral administration, IC_{50} and $E_{1/2}$ were significantly decreased, and k_{out} and F_{PD} were significantly increased when compared with Sandimmun Neoral® ($P < 0.05$). The F_{PD} of CSA O/W-emulsion administered intravenously was 1.79-fold higher than that of CIPOL Inj®. In case of oral administration, the F_{PD} of CSA O/W-emulsion (5.23 ± 1.07) was significantly greater than that of Sandimmun Neoral® (2.45 ± 1.42) ($P < 0.05$).

The immunosuppressive effect of CSA has been reported using several pharmacodynamic markers including lymphokine (interleukin-2, interleukin-6 and tumor necrosis factor and so on), calcineurin and lymphocyte proliferation assay (Awni, 1992; Kawaguchi-Miyashita et al., 1997; Berg et al., 1998; Ferron and Jusko, 1998). However, the analytical methods of these pharmacodynamic markers in biological fluid have disadvantage of complicated and expensive procedures due to a specific monoclonal antibody or radioisotope required. Miller has investigated the peripheral white blood cell components (% lymphocytes, leukocytes, neutrophil and monocytes) to compare

Table 2
Pharmacodynamic parameters of CSA O/W-emulsion and commercial products in rats (mean ± S.E., $n = 4$)

Parameters[c]	Intravenous administration		Oral administration	
	O/W-emulsion	CIPOL Inj.®	O/W-emulsion	Sandimmun Neoral®
ABEC	1539 ± 242[a]	953 ± 36	2427 ± 472[b]	1385 ± 296
IC_{50} (ng/ml)	3009.5 ± 1464.6[a]	2318.6 ± 127.6	977.5 ± 349.3[b]	2394.6 ± 891.7
k_{out} (per h)	0.99 ± 0.42	0.80 ± 0.14	0.64 ± 0.18[b]	0.19 ± 0.06
$E_{1/2}$ (h)	0.71 ± 0.29	0.87 ± 0.16	1.07 ± 0.27[b]	3.85 ± 1.30
EFF	0.02 ± 0.01[a]	0.01 ± 0.00	0.05 ± 0.01[b]	0.02 ± 0.02
F_{PD}	1.79 ± 0.64	1	5.23 ± 1.07[b]	2.45 ± 1.42

[a] $P < 0.05$ from intravenous administration of CIPOL Inj.®.
[b] $P < 0.05$ from oral administration of Sandimmun Neroal®.
[c] Definitions of the parameters: area between the baseline and effect curves, ABEC (%lymphocyte fraction h); whole blood CSA concentration that lead to 50% of maximum inhibition, IC_{50} (ng/ml); first-order rate constant for the loss of the response, k_{out} (per h); half-life of pharmacodynamic effect, $E_{1/2}$ (h); pharmacodynamic efficacy, EFF (%lymphocyte fraction ml/ng); pharmacodynamic availability based on EFF of CIPOL Inj., Fpd I.

the T-cell dependence between wild type and athymic rats, and reported that lymphocyte fraction (%) showed the T-cell dependence (Miller et al., 1992). The studies on the change in lymphocyte proliferation used to investigate the immunosuppressive effect of CSA were mostly performed in vitro (Berg et al., 1998; Ferron and Jusko, 1998). Therefore, to develop an economical and convenient method to evaluate pharmacodynamic effect of CSA, lymphocyte fraction was chosen as a pharmacodynamic marker. Pharmacodynamic evaluation was performed with the predicted CSA concentration in whole blood using WINNONLIN. The experimental data and the respective fitted data of lymphocyte fraction in whole blood showed good correlation ($r^2 > 0.94$). These results suggested that the pharmacodynamic model of lymphocyte fraction in whole blood developed in this study could be used in describing lymphocyte population resulting from CSA concentration in whole blood.

In conclusion, the prepared CSA O/W-emulsion could be used for both oral and intravenous administration, and the oral route was more effective than the intravenous route. The lymphocyte fraction was found to be a useful pharmacodynamic marker that might be used to evaluate the effect of CSA in whole blood.

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References

Agarwal, R.P., McPherson, R.A., Threatte, G.A., 1985. Assessment of cyclosporin A in whole blood and plasma in five different hematocrits. Ther. Drug Monit. 7, 61–65.

Awni, W.M., 1992. Pharmacodynamic monitoring of cyclosporin. Clin. Pharmcokinet. 23, 428–448.

Berg, A.P., Twilhaar, W.N., Mesander, G., Son, W.J., Bij, W., Klompmaker, I.J., Slooff, M.J.H., The, T.H., Leij, L.H.F.M.L., 1998. Quantitation of immunosuppression by flow cytometric measurement of the capacity of T cells for interleukin-2 production. Transplantation 65, 1066–1071.

Bontempo, J.A., 1997. Development of Biopharmaceutical Parenteral Dosage Forms. Marcel Dekker, New York.

Cavanak, T., Sucker, H., 1986. Formulation of dosage forms. Prog. Alleragy 38, 65–72.

Corvari, S.J., Hollenbeck, R.G., Leslie, J., Plaisance, K.I., Young, D., 1991. Absorption and disposition of colloidal drug delivery systems: high-performance liquid chromatographic (HPLC) analysis of a cyclosporin emulsion. Pharm. Res. 8, 40–42.

Dayneka, N.L., Garg, V., Jusko, W.J., 1993. Comparison of four basic models of indirect pharmacodynamic response. J. Pharmacokinet. Biopharm. 21, 457–478.

Drewe, J., Meier, R., Vonderscher, J., Kiss, D., Posanski, U., Kissel, T., Gyr, K., 1992. Enhancement of the oral absorption of cyclosporin in men. Br. J. Clin. Pharmacol. 34, 60–64.

Fahr, A., Holz, M., Fricker, G., 1995. Liposomal formulations of cyclosporin A: influence of lipid type and dose on pharmacokinetics. Pharm. Res. 12, 1189–7798.

Ferron, G.M., Jusko, W.J., 1998. Species- and gender-related differences in cyclosporine–prednisolone–sirolimus interactions in whole blood lymphocyte proliferation assays. J. Pharmacol. Exp. Ther. 286, 191–200.

Ferron, G.M., Pyszczynsk, N.A., Jusko, W.J., 1998. Gender-related assessment of cyclosporine–prednisolone–sirolimus interactions in three human lymphocyte proliferation assays. Transplantation 65, 1203–1209.

Gabrielsson, J., Weiner, D., 1997. Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Application, second ed.. Swedish Pharmaceutical Press, Stockholm.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics, second ed.. Marcel Dekker, New York.

Gijtenbeek, J.M.M., Van den Bent, M.J., Vecht, C.J., 1999. Cyclosporine neurotoxicity: a review. J. Neurol. 246, 339–346.

Gobburu, J.V.S., Jusko, W.J., 1998. Role of dosage regimen in controlling indirect pharmacodynamic response. Adv. Drug Del. Rev. 33, 221–223.

Kawaguchi-Miyashita, M., Nanno, M., Shimada, S., Nagaoka, N., Okada, Y., Matsumoto, S., 1997. A step-wise expansion of intestinal intraepithelial T lymphocytes in association with microbial colonization is defined by sensitivity to cyclosporin A. Immunology 91, 628–634.

Maa, Y., Hsu, C.C., 1999. Performance of sonication and microfluidization for liquid–liquid emulsification. Pharm. Dev. Technol. 4, 233–240.

Malmary, M.F., Houti, I., Labat, C., Batalla, A., Moussamih, S., Bouguettaya, D., Oustrin, J., Houin, G., 1995. Chronopharmacokinetics of cyclosporine A following a single i.v. dose in the Wistar rat. Eur. J. Pharm. Sci. 3, 49–56.

Miller, T.E., Findon, G., Ormrod, D.J., 1992. Suppression of inflammation by cyclosporin A is mediated via a T lymphocyte-independent process. Pharm. Res. 9, 1252–1255.

Molpeceres, J., Chacon, M., Guzman, M., Aberturas, M.R., Berges, L., 2000. Dependency of cyclosporine tissue distribution and metabolism on the age and gender of rats after a single intravenous dose. Int. J. Pharm. 197, 129–141.

Noble, S., Markham, A., 1995. Cyclosporin-A review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation. Drugs 50, 924–941.

Tarr, B.D., Yalkowsky, S.H., 1989. Enhanced intestinal absorption of cyclosporine in rats though the reduction of emulsion droplet size. Pharm. Res. 6, 40–43.

Tibell, A., Norrlind, B., 1994. Cyclosporin A in fat emulsion carriers: studies on the immunosuppressive potential, using the heterotopic heart transplant model in rats. Trans. Int. 7, 438–441.

Tibell, A., Ringden, O., Sundberg, B., 1992. Cyclosporine A in fat emulsion carrier: immunosuppressive effect in vitro. Scand. J. Immunol. 35, 231–236.

Tibell, A., Larsson, M., Alvestrand, A., 1993. Dissolving intravenous cyclosporin A in a fat emulsion carrier prevents acute renal side effects in the rats. Trans. Int. 6, 69–72.

Tibell, A., Lindholm, A., Säwe, J., Chen, G., Norrlind, B., 1995. Cyclosporin A in fat emulsion carriers: experimental studies on pharmacokinetics and tissue distribution. Pharmacol. Toxicol. 76, 115–121.

Vadiei, K., Perez-Soler, R., Lopez-Berestein, G., Luke, D.R., 1989. Pharmacokinetic and pharmacodynamic evaluation of liposomal cyclosporine. Int. J. Pharm. 57, 125–131.

Yee, G.C., Salomon, D.R., 1992. Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring, third ed.. Applied Therapeutics, Vancouver.

Zhang, Q., Yie, G., Li, Y., Yang, Q., Nagai, T., 2000. Studies on the cyclosporin A loaded stearic acid nanoparticles. Int. J. Pharm. 200, 153–159.