Is Absorption Profile of Cyclosporine Really Important for Effective Immunosuppression ?

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The clinical significance of cyclosporine (CsA) concentration 2 h postdose (C_2) monitoring is widely recognized in organ transplantation, because C_2 value is considered to be a predictable surrogate marker of full area under the concentration–time curve (AUC), and/or a peak concentration value exhibits potent inhibition of calcineurin activity. However, the pharmacological advantage of absorption profile (AP) has not been fully elucidated. In a rat skin allotransplantation model, the authors evaluated the efficacy of AP by different dosage regimens (20, 25 or 30 mg/kg/d, once or twice daily) and routes (*p.o.* or *i.v.*), and examined whether high C_2 or AUC_{0-4} is intrinsically valuable for effective immunosuppression. Graft survival was CsA dose-dependent and correlated with full AUC_{0-24} , rather than AP. The difference between the once and twice daily administrations did not influence full AUC_{0-24} or immunosuppressive effect. Continuous intravenous infusion with flat pharmacokinetics also produced adequate immunosuppression as was observed in enteral administration at the same level of total exposure. The impact of high peak concentration in AP on immunosuppressive effect could not be found. It was suggested that AP would not have intrinsic pharmacodynamic value. However, absorption profiling was considered to be clinically useful in that C_2 value is a good surrogate marker of total exposure (AUC_{0-24}).

Key words absorption profile; calcineurins antagonist; cyclosporine; pharmacokinetics; immunosuppression experimental

In organ transplantation, calcineurin inhibitors such as cyclosporine (CsA) and tacrolimus which mainly inhibit helper T cells are now importantly positioned as the cornerstone of immunosuppressive therapy. A calcineurin inhibitor has a narrow therapeutic window between efficacy and toxicity, in that too high a dose would readily induce kidney damage, whereas, too low a dose would cause graft rejection.^{1,2)} Thus, therapeutic drug monitoring (TDM) has been clinically essential to achieve effective immunosuppressive therapy while avoiding toxicity and maintaining tolerability. Monitoring of blood levels of CsA is required to individualize the dose for patients. Although area under the concentration–time curve (AUC), particularly full AUC in 0–12 h of exposure (AUC_{0-12}), has been reported to be a more accurate predictor of clinical outcome.^{3–5)} TDM based on trough levels (C_0) was introduced as a limited sample strategy, and became popular in most transplant units because full profile measurements for AUC require multiple samples and are therefore impractical in patient care. However, C_0 monitoring was not very effective in preventing acute rejection and toxicity in clinical practice because the conventional CsA (Sandimmune; SIM) exhibited considerable inter-individual variability in pharmacokinetics due to an unstable absorption profiling.^{6–8)}

Recently, introduction of the microemulsion formulation of CsA (Neoral; NEO), which has consistent absorption patterns and improved bioavailability, and has thus reduced inter- and intra-individual variability in pharmacokinetics, has enhanced the significance of TDM.^{6–8)} CsA exposure in the first 4 h postdose (AUC_{0-4}) or a single blood concentration measurement at 2 h postdose (C_2) was recommended as an effective monitoring strategy.^{9–11)} The review of the clinical evidence showed the benefits of CsA absorption pro-

filings.¹²⁾ The concept of simple “ C_2 monitoring” has spread rapidly throughout the world. In contrast, continuous intravenous infusion which shows a flattened pharmacokinetic profile has been commonly applied to bone marrow transplantation.^{13,14)}

There are two principal reasons to be considered regarding the importance of the absorption profile. First, is variability in pharmacokinetics during the first 4 h postdose. AUC_{0-4} is closely correlated with total exposure of CsA, which has been proven to be related to major clinical events. Second, is the fact that peak concentration after dosing could be an important parameter that is correlated with the pharmacodynamic measure of CsA activity. The significance of absorption profile would be reaching a high peak level that corresponds to inhibition of calcineurin activity. Thus, in order to clarify the advantage C_2 or AUC_{0-4} monitoring as a surrogate marker of full AUC_{0-12} or an index of potent immunosuppression at the peak CsA concentration, the question that remains to be clarified is whether a peaked pharmacokinetic profile exhibits a more beneficial immunosuppressive effect than a flattened pharmacokinetic profile.

This study therefore aimed to elucidate the significance of absorption profile indicated by C_2 or AUC_{0-4} , and to obtain the instructive information on TDM. We conducted rat skin allotransplantation and compared immunosuppressive effect by dose, frequency of dosing, and route of dosing.

MATERIALS AND METHODS

Animals Inbred strains of male Dark Agouti (DA) and Lewis (LEW) rats weighing 220 to 250 g were purchased from Japan SLC (Hamamatsu, Shizuoka, Japan). DA and LEW rats were used as donors and recipients respectively.

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All animals were housed and maintained under standard conditions in our animal laboratory. All animals used in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health. Experimental protocols were approved by the Committee on Research Animal Care, Institute for Laboratory Animal Research, Nagoya University School of Medicine.

Experimental Design First of all, we examined the feasibility and efficacy of direct administration of the drug *via* a gastrostomy tube with the tip inserted into the duodenum, because of the following concerns: i) frequent dosing of twice daily by conventional gavage over a 3 week period might cause mechanical damage to the recipient rats, and ii) the pharmacokinetics could be easily influenced by gastric emptying time in case of oral gavage. We compared the pharmacokinetics of CsA when 25 mg/kg was administered by oral gavage and *via* a gastrostomy tube ($n=4$ each). The CsA microemulsion, NEO (Novartis Pharma Ltd., Basel, Switzerland) was dissolved in 2 ml of water and adjusted to the designated dosage each day.

Fifty two recipient rats were divided into the following 7 groups by dose and frequency of daily dosing. Group A ($n=4$) received no treatment as a control. Groups B-1, C-1 and D-1 ($n=8$ each) received CsA at the daily dose of 20, 25, and 30 mg/kg, once daily at 12 noon, respectively. Groups B-2, C-2 and D-2 ($n=8$ each) received CsA at the same daily dose as groups B-1, C-1 and D-1, but twice daily at 12 noon and at 12 midnight. CsA was administered *via* a gastrostomy tube with the tip inserted into the duodenum. A pharmacokinetic study was conducted in 4 rats without skin transplantation in each group with the exception of Group A (control). In the twice daily administration, the pharmacokinetic study was separately performed after administration at 12 noon and at 12 midnight, to clarify the difference between daytime and nighttime pharmacokinetics, and to calculate AUC_{0-24} accurately.

Three more groups, namely, Groups E, F and G ($n=4$ each), were used to investigate continuous intravenous infusion of CsA at the doses of 20, 25, and 30 mg/kg. CsA for intravenous use, SIM was diluted with 3 ml of 5% glucose solution containing 60 U/ml of heparin and administered at the rate of 125 μ l/h by infusion pump.

Surgical Preparation The following procedure was performed to directly administer CsA into the duodenum of the rats: three days prior to skin transplantation (on Day -3), the abdomen was opened by a midline incision and a SP50 polyethylene tube (Natume Seisakusho Co., Ltd., Tokyo, Japan) was inserted from the stomach into the duodenum through the pyloric sphincter. This gastrostomy tube was tunneled subcutaneously and brought out to the back of the head in order to facilitate the administration of CsA.

For the pharmacokinetic study, the rat's jugular vein was cannulated with a PE 45 polyethylene tube (Natume Seisakusho Co., Ltd., Tokyo, Japan) on Day -3. The catheter was also tunneled subcutaneously and brought out onto the back of the head in order to facilitate blood sampling. The pharmacokinetic study was performed on Day 0 without skin transplantation.

For the continuous intravenous infusion study, the

Tsumura Free Moving System (Tsumura, Tokyo, Japan) was applied to the recipient rats using a modified version of the technique that was previously described.¹⁵⁾ The intravenous catheter, which was brought out to the back of the head, was connected to the end of the swivel joint through a leather harness, which allowed free movement of the rats during the continuous vascular access for as long as 3 weeks.

Skin Transplantation Model Full-thickness skin grafting was performed using the fully allogeneic model of DA donors and LEW recipients, as described previously.¹⁶⁾ Four skin grafts of full thickness (round pieces of approximately 1 cm $\times$ 1 cm) were prepared from the excised tail and placed into 0.9% physiological saline solution until required. The recipient was anesthetized by administering intraperitoneal sodium pentobarbital at a dose of 50 mg/kg, and its back was disinfected with 75% alcohol. Each side of the back had two graft beds with a distance of 4 to 5 cm between them. The graft bed was prepared by removing an area of epidermis and dermis down to the panniculus carnosus slightly larger than the graft. The skin grafts were transplanted onto the prepared graft bed and sutured with 5-0 nylon. After coverage with sterile bactericidal gauze, the entire chest was wrapped with an elastic bandage. The dressings were removed on Day 5 and the grafts were inspected daily until Day 21. The rejection was defined as more than 90% necrosis of the graft epithelium. CsA administration started 3 d before transplantation. In this study, all the rats tolerated the treatment well. The recipient rats were intramuscularly injected with 60 mg/kg of cefazolin. The follow up period was determined to be 21 d (3 weeks) post-transplant.

Pharmacokinetics, Blood Tests, and Histopathology Blood samples were collected immediately before, and at 1, 2, 4, 6, 12 h after CsA administration to evaluate the pharmacokinetic parameters in rats. In the continuous intravenous infusion study (Groups E, F and G), blood was collected from the recipient rats twice a week to measure CsA concentration.

Blood cell count, and serum biochemical and electrolyte values were measured at 3 weeks post-transplant. Whole blood levels of CsA were measured using a fluorescence polarization immunoassay method with a TDX analyzer (Abbott Laboratories, Chicago, IL, U.S.A.). After the study was completed, all the animals were sacrificed by a lethal dose of sodium pentobarbital. Immediately after sacrifice, kidneys of the recipient rats were excised and fixed in formalin for histopathological study.

Statistical Evaluation and Pharmacokinetic Analysis The values of area under the concentration-time curve from 0 to 4 h (AUC_{0-4}) and from 0 to 24 h (AUC_{0-24}) were calculated by the linear trapezoidal rule. Mann-Whitney *U* test, Pearson correlation test, and log-rank test were used for the statistical analysis. *p* values <0.05 were considered significant.

RESULTS

Comparison of CsA Pharmacokinetics Following Administration by Oral Gavage and *via* a Gastrostomy Tube The pharmacokinetic study revealed that T_{max} is reached earlier following administration *via* a gastrostomy tube than by oral gavage (Fig. 1).

C_2 and AUC_{0-4} were significantly higher in the administration *via* a gastrostomy tube than by oral gavage (C_2 : 6300 vs. 4700, $p=0.021$; AUC_{0-4} : 22100 vs. 17400, $p=0.021$), while the values for full AUC_{0-24} were comparable (AUC_{0-24} : 99700 vs. 94200). Administration into the duodenum *via* a gastrostomy tube produced a higher bioavailability in the absorption profile than by oral gavage. In rat studies, the pharmacokinetic profile following administration *via* a gastrostomy tube was closer to that observed in a clinical setting than that following administration by conventional oral gavage. CsA administration through a gastrostomy tube was considered to be useful for human models.

Pharmacokinetics and Skin Transplantation The pharmacokinetic study showed that the values for nighttime AUC_{0-4} and AUC_{0-12} (administration at 12 midnight) were considerably higher (9 to 18%) than the corresponding daytime values (at 12 noon) (Fig. 2). The value for AUC_{0-24} in the twice daily administration was calculated as a sum of the daytime and nighttime AUC_{0-12} values. The values of AUC_{0-4} in the once daily administration were considerably higher than those in the twice daily administration because double the dose was administered in the once daily administration. On the other hand, the values of AUC_{0-24} were almost equivalent, and increased in a dose-dependent manner. Daily dose of CsA exhibited a strong correlation with AUC_{0-24} (Fig. 3). AUC_{0-4} and C_2 values in each group of the once and twice daily administrations (daytime and nighttime) closely correlated with the AUC_{0-24} values (Figs. 4, 5).

Fig. 1. Comparison of CsA Pharmacokinetics Following Administration at the Dose of 25 mg/kg by Oral Gavage and *via* a Gastrostomy Tube

Pharmacokinetic profile of CsA following administration *via* a gastrostomy tube (closed circle, solid line) showed significantly higher C_2 (6300 vs. 4700, $p=0.021$) and AUC_{0-4} (22100 vs. 17400, $p=0.021$) than that by oral gavage (closed square, dotted line). No significant difference of AUC_{0-24} was observed.

Fig. 3. Correlation between CsA Dose and AUC_{0-24} in Once Daily Administration (A) and in Twice Daily Administration (B)

The daily dose of CsA showed a strong correlation with AUC_{0-24} in once daily administration (A) and in twice daily administration (B). Pearson correlation coefficients were $r=0.805$ ($p<0.01$) (A) and $r=0.893$ ($p<0.01$) (B).

Allografts in the untreated recipients (Group A) survived for a mean of 7 d (Table 1). Administration of CsA was effective in preventing acute skin allograft rejection (Fig. 6). Graft survival was extended significantly in proportion to daily CsA dose (log-rank test, $p<0.01$). Graft survival significantly correlated with AUC_{0-24} (regression test, $r_2=0.816$, $p=0.0008$), rather than AUC_{0-4} ($r_2=0.499$, $p=0.0333$). There was no significant difference in graft survival between the once and twice daily administrations in the same daily dosage group.

The continuous intravenous infusion study also showed similar results, depending on the amount of full AUC_{0-24} . In this study, blood concentration was measured twice a week in a recipient rat with skin allograft. AUC_{0-24} was calculated by multiplying mean blood level by 24 h. Continuous intravenous infusion with flat pharmacokinetics also produced adequate immunosuppression as was observed in enteral administration at the same level of total exposure.

Fig. 2. Pharmacokinetic Profiles Following Once and Twice Daily Administration *via* a Gastrostomy Tube at the Daily Dose of 20, 25 and 30 mg/kg

CsA was administered *via* a gastrostomy tube with the tip inserted into the duodenum. Once daily administration groups (B-1, C-1 and D-1) received CsA at 12 noon as indicated by 0 h (daytime). Twice daily groups (B-2, C-2 and D-2) received CsA both at 12 noon (daytime) and at 12 midnight CsA as indicated by 0 h (nighttime). Groups B-1 (open circle, dotted line), C-1 (open triangle, dotted line) and D-1 (open square, dotted line) received CsA at the daily dose of 20 mg/kg, 25 mg/kg, and 30 mg/kg once daily, respectively. Groups B-2 (closed circle, solid line), C-2 (closed triangle, solid line) and D-2 (closed square, solid line) received CsA at the daily dose of 20 mg/kg, 25 mg/kg, and 30 mg/kg twice daily, respectively. The values of AUC_{0-4} in once daily administration were considerably higher than those in twice daily administration. AUC_{0-4} and AUC_{0-12} at nighttime in twice daily administration were considerably higher (9 to 18%) than those at daytime.

Fig. 4. Correlation between AUC_{0-4} and AUC_{0-24} in Once Daily Administration (A) and in Twice Daily Administration (at Daytime (B) and at Nighttime (C))

The values of AUC_{0-4} significantly correlated with AUC_{0-24} . Pearson correlation coefficients were $r=0.969$ ($p<0.01$) (A), $r=0.979$ ($p<0.01$) (B) and $r=0.904$ ($p<0.01$) (C).

Fig. 5. Correlation between C_2 and AUC_{0-24} in Once Daily Administration (A) and in Twice Daily Administration (at Daytime (B) and at Nighttime (C))

The values of C_2 significantly correlated with AUC_{0-24} . Pearson correlation coefficients were $r=0.906$ ($p<0.01$) (A), $r=0.968$ ($p<0.01$) (B) and $r=0.833$ ($p<0.01$) (C).

Among the recipient rats that received once or twice daily administration, or continuous intravenous infusion, outcome of skin graft correlated with full AUC_{0-24} but not with AUC_{0-4} in the absorption profile. Recipients rats that had a value >100000 ($\text{ng} \cdot \text{h/ml}$) for full AUC_{0-24} showed $\geq 75\%$ of graft survival. In contrast, a value <85000 ($\text{ng} \cdot \text{h/ml}$) for full AUC_{0-24} showed $\leq 12.5\%$ of graft survival.

Blood tests and histopathological studies of the kidneys at 3 weeks post-transplant revealed minimum level of nephrotoxicity in rats treated with 30 mg/kg of CsA, however, there was no difference between the once daily, twice daily, or continuous administration regarding extent (data not shown).

DISCUSSION

Full AUC has long been reported to be correlated with major clinical events.^{3,4,11} However, as a precise indicator of total drug exposure, AUC_{0-24} monitoring requires multiple blood samples, which actually limits its use in clinical practice because it is a time-consuming and expensive method.

After the introduction of the CsA microemulsion formulation, which improved intra-individual variations in pharmacokinetics,^{6,8} AUC_{0-4} monitoring has been advocated for characterizing CsA absorption profiles due to the excellent correlation between AUC_{0-4} and full AUC that has been shown.^{10,11} Since C_2 has been proven to be the best single-point predictor of AUC_{0-4} , C_2 monitoring of CsA is widely employed in most transplant institutes.^{10,11,17-20} A large

Table 1. Graft Survival, AUC_{0-4} and AUC_{0-24} Levels in Rat Skin Allotransplantation Model

Group	Dose	Administration	Graft survival (d)	AUC_{0-4} (ng·h/ml) Mean±S.D.	AUC_{0-24} (ng·h/ml) Mean±S.D.	
A	0		7, 7, 7, 8	—	—	
B-1	20 mg/kg/d	Once a day, <i>p.o.</i>	13, 13, 13, 14, 15, 16, 16, 21	—	—	
			—	19300±2400 (<i>n</i> =4)	81400±12500 (<i>n</i> =4)	
B-2	20 mg/kg/d	Twice a day, <i>p.o.</i>	10, 13, 15, 16, 16, 16, 17, 21	—	—	<i>p</i> =0.772
			—	13600±2600 (<i>n</i> =4)	78300±9900 (<i>n</i> =4)	<i>p</i> =0.043
			—	16400±2100 (<i>n</i> =4)	—	<i>p</i> =0.083
C-1	25 mg/kg/d	Once a day, <i>p.o.</i>	15, 18, 18, >21×5	—	—	
			—	22100±1200 (<i>n</i> =4)	99700±10800 (<i>n</i> =4)	
C-2	25 mg/kg/d	Twice a day, <i>p.o.</i>	16, 16, >21×6	—	—	<i>p</i> =0.386
			—	17600±1700 (<i>n</i> =4)	95500±3600 (<i>n</i> =4)	<i>p</i> =0.021
			—	20900±1700 (<i>n</i> =4)	—	<i>p</i> =0.386
D-1	30 mg/kg/d	Once a day, <i>p.o.</i>	16, >21×7	—	—	
			—	27900±4200 (<i>n</i> =4)	125800±20700 (<i>n</i> =4)	
D-2	30 mg/kg/d	Twice a day, <i>p.o.</i>	>21×8	—	—	<i>p</i> =0.564
			—	22100±500 (<i>n</i> =4)	123300±6000 (<i>n</i> =4)	<i>p</i> =0.021
			—	22900±2300 (<i>n</i> =4)	—	<i>p</i> =0.083
E	20 mg/kg/d	Continuous i.v.	13, 14, 14, 16	14000±300	84000±1600	
F	25 mg/kg/d	Continuous i.v.	18, 18, >21×2	16100±400	96500±2300	
G	30 mg/kg/d	Continuous i.v.	17, >21×3	17400±900	104500±5600	

p.o.: administration via a gastrostomy tube. i.v.: intravenous infusion. Mann Whitney *U* test (once a day vs. twice a day).

Fig. 6. Graft Survivals in Rats Treated with Once and Twice Daily Administration, and Continuous Intravenous Infusion at the Daily Dose of 20, 25 and 30 mg/kg

The values of AUC_{0-24} and AUC_{0-4} are shown in parenthesis. Groups B-1, C-1 and D-1 received CsA at the daily dose of 20, 25, and 30 mg/kg, once a day, respectively. Groups B-2, C-2 and D-2 received CsA at the same daily dose as groups B-1, C-1 and D-1, but twice a day. Groups E, F, G received continuous intravenous infusion of CsA at the daily dose of 20, 25, and 30 mg/kg, respectively.

number of papers have reported the usefulness and the feasibility of C_2 monitoring in clinical renal, liver, heart and lung transplantation.^{10,12,21–24}) It is reasonable to suggest that absorption profiling, namely, AUC_{0-4} or C_2 would be an important as a surrogate marker of full AUC_{0-24} , and that will result in a good correlation with clinical events.

On the other hand, it is also suggested that the value of CsA absorption profiling should be placed on the potent immunosuppressive effect that is achieved when there is an adequate amount of CsA exposure in the early post-dose period. Detailed analysis of the pharmacokinetics and clinical results in renal transplant patients revealed that AUC_{0-4} is more significantly correlated with acute rejection and nephrotoxicity than full AUC_{0-12} , which is the total exposure over a 12-h dosing period.¹¹) Most intra-individual variability in pharmacokinetics occurred during absorption profiling,^{1,8}) and a close correlation was observed between CsA blood concentration and inhibition of calcineurin activity or interleukin-2 (IL-2) production.^{25,26}) These findings indicate the possibility that early exposure to an adequately high peak level of CsA as expressed by AUC_{0-4} or C_2 might be more important for immunosuppression than total exposure as expressed by full AUC .

In the present study, the outcome of skin transplantation was dependent on CsA dosage, and was closely related to full AUC_{0-24} , regardless of a once or twice daily administration of CsA. The difference between the once and twice daily administrations certainly changed the level of absorption profile, but did not influence the immunosuppressive effect. Furthermore, continuous intravenous infusion which provided constant exposure without a peak concentration, also showed that skin graft survival was well correlated with full AUC_{0-24} . The minimum levels for effective prevention of acute rejection in continuous intravenous infusion were almost similar to those in once or twice daily administration via a gastrostomy tube. Graft survival did not seem to be directly related to AUC_{0-4} .

Absorption profile is considered to be a surrogate marker for predicting total CsA exposure. Single-point C_2 monitoring in particular would be very useful in medical practice when a good and stable absorption is expected post-transplant. However, during the early post liver transplant period, poor intestinal motility and insufficient graft function resulted in an unstable absorption profile.^{27,28}) Because of delayed gut absorption due to laparotomy, impaired bile production, and poor metabolism in the grafted liver, the pharmacokinetics became readily changeable. Thus, AUC_{0-4} or C_2 cannot accurately reflect total exposure, especially when there is malabsorption. Adjustment of the CsA dose by C_2 monitoring alone is susceptible to overexposure, which would cause adverse effects such as nephrotoxicity.^{27,28}) The measurement of additional time point would be necessary in poor or delayed absorbers, as indicated by Nashan *et al.*¹²) Continuous intravenous infusion, with which there is constant CsA exposure, and full AUC readily increases, might be desirable in overcoming the changeable pharmacokinetics.

A definitive conclusion has not been reached regarding the use of once or twice daily administration. The prospective and randomized trial designed to compare once and twice daily administrations of the same dose of CsA has been conducted in renal transplantation.²⁹) The once daily administra-

tion exhibited a lower C_0 but a higher C_2 and AUC_{0-4} than the twice-daily administration. No significant difference was observed in acute rejection, graft survival or adverse effect. Evidence on increased peak blood concentration being advantageous in absorption profile was not obtained,²⁹) which corresponds with our result.

In this study, nighttime AUC_{0-12} increased by 9 to 18% compared to daytime AUC_{0-12} . Considering that rats are usually active at nighttime, our results corresponded with the other investigations on humans which showed that though not significantly, daytime AUC is somewhat higher than nighttime AUC , because humans are more active during the daytime.^{30–32}) This finding will provide useful information in determining once daily administration in the morning or in the evening.

The intravenous dose is generally thought to be about one third of the oral dose in humans. However, to obtain a comparable level of total exposure (AUC_{0-24}), rats treated with continuous intravenous infusion required the same daily dose of CsA as treated with enteral administration. One of the reasons we can consider is adsorption of CsA into the polyethylene tube. Indeed, additional study indicated that nearly 20% of the CsA were lost in the 30 cm polyethylene tube (data not shown). As for another possibility, CsA bioavailability could be improved by inhibition of CYP3A and p-glycoprotein function in enteral administration. However, as the relation between pharmacokinetic profile and graft outcome was directly examined, the disparity of dosage would not exert a great influence on the data analysis.

One of the limitations of this study is that CsA-induced adverse effects were not adequately analyzed. Most rats tolerated the treatment throughout this study and did not show any serious adverse effects. Blood tests and histopathology of the kidneys of the recipient rats did not show typical nephrotoxicity. As rats appear to be very unsusceptible to the nephrotoxic effect of CsA,³³) we could not fully assess the relationship between pharmacokinetic profiles and adverse effects. Finding the factor among full AUC , C_{max} , C_0 , and drug interval that most influences adverse effects is very important. Clinically, the optimal dose and method of administration should be determined by balancing between immunosuppressive effect and toxicity.

The result of this study cannot be directly applied to a clinical setting. The required dose of CsA in rats was approximately 3-fold that in humans, as reported previously.^{34,35}) The effective AUC_{0-24} for inhibiting acute rejection reached 100000 ng·h/ml, which is more than 5-fold the clinical therapeutic range (*i.e.* 9500–11500 for AUC_{0-12}) in humans.^{10,11})

The findings obtained in organ transplantation such as kidney, heart and liver may differ from those in our experiments of skin transplantation. This study is preliminary, but would provide the basic information for the advanced large animal experiments.

In conclusion, the impact of absorption profile itself, namely, a peaked pharmacokinetic profile, on immunosuppressive effect could not be found in a rat skin transplantation model, compared with a flattened pharmacokinetic profile. The absorption profile would not have intrinsic pharmacodynamic value, but merely reflect total exposure (AUC_{0-24}). A comprehensive study that includes adverse ef-

fects will be required in clinical settings.

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