INTRODUCTION

It is well known that tacrolimus (FK506) is an outstanding immunosuppressive drug for organ transplantation (Scott et al., 2003); however, there are also several clinical side effects associated with it (Plosker and Foster, 2000; Scott et al., 2003). The principal side effects associated with tacrolimus treatment in the major trials included nephrotoxicity, neurotoxicity, disturbances in glucose metabolism, gastrointestinal disturbance and hypertension (Plosker and Foster, 2000). The mechanism responsible for the side effects has been intensively investigated, and its actions are becoming clear; these effects seem to depend on the pharmacokinetic profile obtained with the type of administration (Abu-Elmagd et al., 1991). Thus, the bulk of the work necessary for minimizing side effects involves finding a better dosing regimen. In clinical organ transplantation, tacrolimus is usually administered orally twice a day, meanwhile continuous intravenous infusion is used only in the perioperative period. We have hypothesized that the maximal blood concentration (Cmax) of tacrolimus may be closely related to its toxicity. In addition, since there is a high correlation between the tacrolimus concentration-time area under the curve (AUC) and its trough concentration, tacrolimus concentration and efficacy may be highly related (Alloway et al., 2005).

ABSTRACT — The aim of this study is to investigate the effect of the pharmacokinetic profile of tacrolimus on its pancreatic toxicity and efficacy in rats. For toxicity evaluation, doses of 0.03, 0.1, or 0.3 mg/kg/day were given once daily for 8 days in the bolus intravenous injection groups. In the continuous intravenous infusion groups, tacrolimus was infused using an Alzet® osmotic mini-pump for 9 days at the same doses. Pancreatic insulin content decreased dose-dependently in both the bolus intravenous injection and continuous intravenous infusion groups, and there was no significant difference between the decreases caused by the two dosing regimens. At 0.03 mg/kg, continuous intravenous infusion did not cause glucose intolerance, but bolus intravenous injection induced significant and dose-dependent glucose intolerance. The pharmacokinetic data indicated that continuous intravenous infusion resulted in a sustained blood drug concentration with an area under the curve (AUC) similar to that obtained with the bolus administration at the same dose. For efficacy evaluation, donor ear grafts were transplanted to the lateral thoraces of recipients. Tacrolimus doses of 0.01, 0.1, or 1 mg/kg/day were administered from day 0 to day 13. Both bolus intramuscular administration and continuous intravenous infusion prolonged skin allograft survival dose-dependently, and there was no significant difference between the median survival times of groups given the same doses. To summarize, the sustained-release of tacrolimus resulted in a steady blood drug concentration with an AUC similar to that of the bolus administration. In rats, it was better tolerated and just as efficacious as the bolus administration without producing a higher maximal blood concentration (Cmax). These results indicate that the sustained-release formulation has the potential to improve the safety of tacrolimus.

Key words: Tacrolimus, Toxicity, Efficacy, AUC, Cmax

Effect of pharmacokinetic profile on the pancreatic toxicity and efficacy of tacrolimus in rats

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Florman et al., 2005; Dansirikul et al., 2006; Masuda and Inui, 2006). If this hypothesis is valid, a sustained-release formulation would be less toxic because the Cmax would be lower. The aim of this study is to demonstrate that a continuous intravenous infusion of tacrolimus is as efficacious as the bolus administration with a comparable AUC, but with a lower Cmax. To elucidate the relationship between the toxicity of tacrolimus and its pharmacokinetic profile, we investigated the effect of tacrolimus on glucose tolerance (pancreatic toxicity) using different administration regimens, such as bolus intravenous injection (high Cmax) and continuous intravenous infusion (constant blood drug concentration), which produced similar AUCs. We also examined the efficacy of continuous intravenous infusion and intermittent intramuscular injection on allograft rejection in rats in order to demonstrate that tacrolimus, which produces a comparable AUC but a different Cmax, provides the same efficacy.

MATERIALS AND METHODS

Animals

Male Jcl:SD rats aged 9 weeks were used for the study on pancreatic toxicity. For the study on skin allograft rejection, male Fischer rats and male WKAH rats aged 8 weeks were used as donors and recipients, respectively. Room temperature and relative humidity were set 25°C and 55%, respectively. The room was lighted daily for 12 hr. Jcl:SD rats were purchased from CLEA Japan, Inc., Tokyo, Japan. Fischer rats and WKAH rats were purchased from Japan SLC, Inc., Shizuoka, Japan. All animal experimental procedures were approved by the Animal Experiment Committee of Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan (currently Astellas Pharma Inc., Tokyo, Japan).

Test substance and preparation of dosing solutions

Tacrolimus injectable formulation (Prograf® injection; supplied by Fujisawa Pharmaceutical Co., Ltd. (currently Astellas Pharma Inc.)) was diluted with physiological saline for bolus intravenous injection or bolus intramuscular injection. For continuous intravenous infusion, tacrolimus was dissolved in propylene glycol containing hydrogenated castor oil (HCO-60) and 15% ethanol at a concentration of 30 mg/ml of tacrolimus, and then diluted to make an appropriate concentration of dosing solution. Alzet® mini-pumps (model 2002) were used to administer the dosing solutions. The concentration of tacrolimus solution was calculated based on body weight the day before surgical implantation.

Pancreatic toxicity

The study design is illustrated in Fig. 1. Tacrolimus doses of 0.03, 0.1, or 0.3 mg/5 ml/kg/day were given once a day for 8 consecutive days in bolus intravenous

Animals: SD male rats

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Fig. 1. Experimental protocol (pancreatic toxicity). Dotted bar shows the period of continuous intravenous infusion. Narrow arrows show the bolus intravenous injection and the measurements of body weights. Broad arrows show the glucose tolerance test and the measurement of pancreatic insulin.
injection groups (7 rats/group). On Day 8, the drug was administered prior to commencement of the glucose tolerance test, and the animals were euthanized on Day 9. In the continuous intravenous infusion groups (7 rats/group), tacrolimus doses of 0.03, 0.1, or 0.3 mg/kg/day were administered using Alzet® osmotic mini pumps via a medical polyethylene catheter indwelled in the femoral vein of rats for 9 days, and the following experiments were carried out according to the same schedule as the bolus intravenous injection groups.

The glucose tolerance test was performed in 18-hr-fasted rats that had been given an oral dose of 3 g glucose/kg on day 8. Blood was collected from the tail vein 0, 30, 60, 120, and 240 min after glucose dosing, blood glucose levels were measured using the Glucose B-test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). All rats were fed ad libitum after the glucose tolerance test. On the day after the test, 3 ml of ethanol containing 0.7 N HCl was used to extract the insulin from 100 mg of pancreatic tissue per rat. The insulin in the supernatant of the homogenate (centrifugation; 3,000 rpm, 10 min, 4°C) was then measured using the Glazyme Insulin-EIA Test (Wako Pure Chemical Industries, Ltd.).

For measurement of the tacrolimus trough level, 0.25 ml of blood was taken from the tail vein of each rat through a heparinized polyethylene tube just before dosing for the bolus intravenous injection group. Blood samples were taken at almost the same time for the continuous intravenous infusion group.

For the pharmacokinetic study, a satellite group was used to investigate blood drug concentration-time profiles. Tacrolimus doses of 0.03 and 0.3 mg/kg/day were injected intravenously once daily for the bolus intravenous injection pharmacokinetics group. Blood was then taken from the tail vein of each rat at 5, 15, and 30 min as well as 1, 2, 4, 6, and 24 hr after administration. Tacrolimus doses of 0.03 and 0.3 mg/kg/day were infused into the femoral vein through an osmotic pump for the continuous intravenous infusion group. Blood was taken from the tail vein at 2, 4, 6, and 24 hr on Day 3 after the catheter had been surgically installed.

Skin allograft
Skin was grafted according to previously described methods (Inamura et al., 1988). The ears from the donor (male Fischer rats) were grafted to the lateral thoraxes of the recipients (male WKAH rats), and covered with sterile bactericidal gauze. The dressing was removed on Day 5, after which the grafts were inspected daily until rejection. Tacrolimus doses of 0.01, 0.1, and 1 mg/kg/day were given once a day for 14 days to the intramuscular injection group (7 or 8 rats/group), and doses of 0.01, 0.1, and 1 mg/kg/day were infused intravenously using an Alzet® osmotic mini pump, which was removed on Day 14 for the continuous intravenous infusion groups (7 or 8 rats/group). After the final dosing, the skin graft was observed for 28 days after skin transplantation. The graft survival data were expressed as median survival times (MST).

In the independent pharmacokinetic study, the blood concentration of tacrolimus was measured after intramuscular administration and continuous intravenous infusion using an Alzet® pump at doses of 0.01, 0.1, and 1 mg/kg/day for 14 days to male rats. Blood samples were collected via the tail vein of rats just before intramuscular injection on days 2, 3, 8, 10 as well as 14 and 24 hr after the last administration. Blood samples were also collected from the rats in the continuous intravenous infusion at the same time as in the intramuscular injection group.

Measurement of the tacrolimus blood concentration and the calculation of pharmacokinetic parameters
The blood concentrations of tacrolimus were determined using enzyme-linked immunosorbent assay, as described previously (Iwasaki et al., 1995), and pharmacokinetic parameters were calculated with WinNonlin (version 3.1, Pharsight Corp., California, USA).

Statistics
The data were analyzed statistically as follows; homogeneity of variances was analyzed using Bartlett’s test. When a set of variances was homogeneous, multiple comparison was performed using Dunnett’s (when the sample size was equal) or Tukey’s W.S.D. test (when the sample size was not equal). When a set of variances was not homogeneous, the same procedure was performed after rank conversion. The statistical significance of the difference between groups was analyzed using the Peto’s test for MST.

RESULTS

Pancreatic toxicity

Body weight
The body weights in the continuous intravenous infusion groups were lower than those in the bolus intravenous injection groups at control and all dose levels throughout the dosing period. In both the bolus intravenous injection and the continuous intravenous infusion, the body weights were dose-dependently decreased. How-
ever, only in the bolus intravenous injection, a significant decrease in body weights was observed at a dose of 0.3 mg/kg/day (Fig. 2).

**Glucose tolerance test**

**Bolus intravenous injection**

In the 0.1 and 0.3 mg/kg/day groups, a statistically significant elevation in the plasma glucose level was observed 45, 90, and 120 min after glucose loading compared with that in the saline control group. In the 0.03 mg/kg/day group, a significant elevation (compared with that of the saline control) in the plasma glucose level similar to that in the 0.1 and 0.3 mg/kg/day groups was observed 90 min after glucose loading (Fig. 3). The area under the curve for the glucose-time profile (AUC glucose) of these 3 groups was significantly higher than that for the saline control group; the AUC glucose values were 1.20-, 1.35-, and 1.34-fold of that for the saline control group, respectively (Fig. 4).

**Continuous intravenous infusion**

Compared with the vehicle control group, the plasma glucose levels in the 0.03 mg/kg group were not elevated. In the 0.1 and 0.3 mg/kg/day groups, a significant elevation in plasma glucose was observed 45 and 90 min after glucose loading (Fig. 3); the AUC glucose values were 1.32- and 1.40-fold of that for the vehicle control group, respectively. These changes in the AUC glucose for the 0.1 and 0.3 mg/kg/day groups were significant compared with those for the vehicle control group (Fig. 4).

To estimate the difference between the two dosing regimens, statistical analysis of the differences in the plasma glucose levels between the bolus intravenous injection and continuous intravenous infusion groups were carried out for all time points. At a dose of 0.03 mg/kg/day, the AUC glucose of the bolus intravenous injection group was significantly higher than that of the continuous intravenous infusion group. The plasma glucose levels of the two controls (saline and vehicle) were similar for both dosing routes (Fig. 4).

**Pancreatic insulin content**

Pancreatic insulin content decreased in an approximately dose-dependent manner in both administration groups. Insulin content in the 0.03, 0.1, and 0.3 mg/kg/day groups were 52, 30, 37% (bolus intravenous injection) and 73, 45, 47% (continuous intravenous infusion) of that in each control group, respectively. There were no noteworthy differences in the magnitude of decrease in the insulin content between the bolus intravenous injection and the continuous intravenous infusion groups (Fig. 5).

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**Fig. 2.** Time course of body weight in the bolus intravenous injection and continuous intravenous infusion groups. Data represent the means ± S.D. of 4-7 rats. Tacrolimus or Control (○) was administered intravenously as bolus injection or infusion at doses of 0.03 (■), 0.1 (▲), or 0.3 (●) mg/kg.

*: P < 0.05 vs each Control (Dunnett’s test or Tukey’s W.S.D. test)
Plasma was taken from non-fasted rats the day after

WKHJOXFRVHWROHUDQFHWHVW$VLJQL¿FDQWHOHYDWLRQLQSODV-

ma glucose only was noted for both routes in the 0.3 mg/

kg/day groups; the increase in the glucose level was com-

parable for the two routes (data not shown).

Blood was sampled on Days 2 (before dosing) and 9

day of euthanasia) to determine the trough levels of tac-

rolimus in whole blood (Table 1). The trough levels in

the 0.03, 0.1, and 0.3 mg/kg/day bolus intravenous injec-

tion groups on Day 9 were 0.203, 1.375, and 2.761 ng/ml,

respectively. The levels in the 0.03, 0.1, and 0.3 mg/kg/

day continuous intravenous infusion groups were 1.441,

3.941, and 10.675 ng/ml, respectively.

Blood drug concentrations in the continuous intrave-

nous infusion group at doses of 0.03 and 0.3 mg/kg/day

were 0.941-1.128 and 10.65-12.31 ng/ml, respective-

ly; the blood level remained almost constant at all time

points (Fig. 6). The mean blood drug concentration at 0

hr (just after dosing) in the bolus intravenous injection

group at doses of 0.03 and 0.3 mg/kg/day were estimat-
	ed to be 17.2 and 88.69 ng/ml, respectively; these levels

Plasma was taken from non-fasted rats the day after

the glucose tolerance test. A significant elevation in plasma

glucose only was noted for both routes in the 0.3 mg/

kg/day groups; the increase in the glucose level was com-

parable for the two routes (data not shown).

Whole blood concentration of tacrolimus

Blood was sampled on Days 2 (before dosing) and 9

day of euthanasia) to determine the trough levels of tac-

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hr (just after dosing) in the bolus intravenous injection

group at doses of 0.03 and 0.3 mg/kg/day were estimat-
	ed to be 17.2 and 88.69 ng/ml, respectively; these levels

Fig. 3. Time course of plasma glucose in the bolus intravenous injection and continuous intravenous infusion groups. Data repre-

sent the means ± S.D. of 4-7 rats. Tacrolimus or Control (○) was administered intravenously as bolus injection or infusion at
doses of 0.03 (■), 0.1 (▲), or 0.3 (●) mg/kg.

*: P < 0.05, **: P < 0.01 vs each Control (Dunnett’s test or Tukey’s W.S.D. test)

Fig. 4. AUC of plasma glucose in the bolus intravenous injec-
tion and continuous intravenous infusion groups. Data repre-
sent the means ± S.D. of 4-7 rats. Tacrolimus or Control was administered intravenously as bolus injec-
tion (open column) or infusion (closed column) at doses of 0.03, 0.1, or 0.3 mg/kg.

*: P < 0.05, **: P < 0.01 vs each Control (Dunnett’s test or Tukey’s W.S.D. test)
were 17- and 8-fold of that in the continuous intravenous infusion groups, respectively (Table 2).

Skin allograft

MST of the skin graft

The MST for the vehicle control was 5 or 6 days when administered intramuscularly or infused intravenously, respectively (Table 3). The intramuscular administration of tacrolimus prolonged skin graft survival significantly and dose-dependently at doses of 0.1 and 1 mg/kg/day with an MST of 10 to 20 days (p < 0.01), respectively; however, it had no effect at 0.01 mg/kg/day. Intravenous infusion of tacrolimus also caused a similar prolongation of skin graft survival at doses of 0.1 and 1 mg/kg/day with an MST of 10 (p < 0.05) to 22 days (p < 0.01), respectively; however, it had no effect at 0.01 mg/kg/day. There was no significant difference between the MST of those groups at the same dosage.

Whole blood concentration of tacrolimus

The trough levels after intramuscular injection on 14th day with 0.01, 0.1, and 1 mg/kg were 0.317 ± 0.065, 1.259 ± 0.148, and 5.047 ± 1.154 ng/ml, respectively, and those after continuous intravenous infusion on 14th day with 0.01, 0.1, and 1 mg/kg were 0.451 ± 0.225, 4.768 ± 0.408, and 22.25 ± 11.88 ng/ml, respectively, suggesting that both intramuscular injection and continuous intravenous infusion resulted in a dose-dependent increase in the blood drug level. After continuous intravenous infusion

Table 1. Trough concentration of tacrolimus after repeated bolus intravenous injection and continuous intravenous infusion to rats

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<th>Dosing route</th>
<th>Dose (mg/kg)</th>
<th>Trough concentration of tacrolimus (ng/ml)</th>
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<tr>
<td></td>
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<td>Day 2</td>
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<tr>
<td>i.v. bolus</td>
<td>0.03</td>
<td>0.099 ± 0.170</td>
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<td></td>
<td>0.1</td>
<td>0.981 ± 0.269</td>
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<td></td>
<td>0.3</td>
<td>3.040 ± 0.655</td>
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<tr>
<td>i.v. infusion</td>
<td>0.03</td>
<td>1.489 ± 0.885</td>
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<td></td>
<td>0.1</td>
<td>3.829 ± 1.520</td>
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<td>0.3</td>
<td>10.215 ± 2.515</td>
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Data represent the mean ± S.D. of 7 animals.

Fig. 5. Pancreatic insulin content in the bolus intravenous injection and continuous intravenous infusion groups. Data represent the means ± S.D. of 4-7 rats. Tacrolimus or Control was administered intravenously as bolus injection or infusion at doses of 0.03, 0.1, or 0.3 mg/kg.

*: P < 0.05, **: P < 0.01 vs each Control (Dunnett’s test or Tukey’s W.S.D. test)
for 14 days with 1 mg/kg, the blood drug level was within the range of 22.25 to 36.26 ng/ml, whereas the trough blood drug concentration was within the range of 5.047 to 11.26 ng/ml after repeated intramuscular injection with 1 mg/kg.

**DISCUSSION**

Tacrolimus is a commonly used immunosuppressant for the prevention and treatment of organ rejection (Scott et al., 2003). However, tacrolimus is known to have a variety of side effects, the reduction of which is an important issue (Plosker and Foster, 2000; Scott et al., 2003). One of the possible solutions is to flatten out the peak blood level by developing a sustained-release formulation; however, depending on when the Cmax occurs, toxicity is a possibility. In the present study, we investigated whether flattening of the peak blood level resulted in a reduction of tacrolimus-induced toxicity or not. Since pancreatic toxicity (glucose intolerance) is a characteristic side effect of tacrolimus, it was selected as the parameter by which to test this hypothesis. Intravenous bolus injection and intravenous infusion were employed as the dosing methods, which resulted in a daily peak blood level and a constant blood level, respectively.

The present study demonstrated that the onset of pancreatic toxicity depends on the dosing regimen at 0.03 mg/kg/day. In the 0.03 mg/kg/day continuous intravenous infusion group, pancreatic insulin content tended to decrease, and glucose intolerance was not significant. In contrast, in the bolus intravenous injection groups, although pancreatic insulin content also decreased to a level close to that of the continuous intravenous infusion group, significant glucose intolerance was induced at doses of 0.03 mg/kg/day or higher (Figs. 4 and 5). The total daily exposure to tacrolimus was similar for both dosing regimens, but the pattern of blood drug concentration was obviously different (Fig. 6). In the 0.03 mg/kg/day group, the concentration just after dosing was estimated to be 17.2 ng/ml in the bolus intravenous injection group, but that in the continuous intravenous infusion group remained almost constant, at approximately 1 ng/ml. This suggests that the AUC after intravenous infusion is around 24 ng·hr/ml, which is similar to the AUC after intravenous bolus dosing (Table 2). These results indicate that glucose tolerance was less affected by tacrolimus at a constant blood concentration than that with a high Cmax under similar conditions of daily exposure (AUC). The tacrolimus-induced side effects on glucose metabolism may be reduced by a sustained-release formulation or other dosing regimen that reduces the peak blood level.

This study also showed that a significant decrease in body weights was observed at a dose of 0.3 mg/kg/day only in the bolus intravenous injection, although, the body weights in both the bolus intravenous injection and the continuous intravenous infusion were dose-dependently decreased (Fig. 2). These results suggest that the bolus intravenous injection may cause a stronger toxic effect on the whole body than the continuous intravenous infusion. It is consistent with the results of pancreatic toxic-
ty. On the other hand, it is shown that the body weights in the continuous intravenous infusion groups were lower than those in the bolus intravenous injection groups at control and all dose levels throughout the dosing period (Fig. 2). These lower values are considered to be due to physical insult by the installation of the osmotic pump.

Tacrolimus-induced glucose intolerance in rats may be due to the transcriptional inhibition of insulin production in pancreatic beta cells (Tamura et al., 1995). Two-week oral administration of tacrolimus at 10 mg/kg/day to rats decreased pancreatic insulin content and insulin mRNA, but had no effect on glucagon content in pancreatic alpha cells. After stopping tacrolimus, insulin production returned to normal level (Tamura et al., 1995).

The binding of insulin to the erythrocytes of rats dosed with tacrolimus was similar to that to the placebo control. Scatchard analysis confirmed that tacrolimus did not cause damage to the insulin receptor of the erythrocytes (Hirano et al., 1994). It is also known that Nuclear Factor of Activated T cells (NFAT) and calcineurin are involved in insulin gene transcription induced by glucose or glucagon-like peptide-1 (Lawrence et al., 2002). These findings agree with our data, which indicated that the administration of tacrolimus decreased pancreatic insulin content dose-dependently.

This study also demonstrated that continuous intravenous infusion of tacrolimus has the same efficacy as bolus intramuscular administration on skin allograft survival in rats. In the pharmacokinetic study, it was shown that intramuscular administration of tacrolimus (1 mg/kg/day) produced a trough concentration of 5-11 ng/ml and a Cmax of about 60 ng/ml, and that continuous intravenous infusion (1 mg/kg/day) yielded a concentration of 22-36 ng/ml. The concentration of continuous intravenous infusion is the intermediate value between the Cmax and the trough concentration of intramuscular administration, which suggests that the AUCs of both dosing regimens are similar. Therefore, these results indicate that the efficacy of tacrolimus on skin graft survival depends on total exposure rather than Cmax.

In clinical organ transplantation, tacrolimus is usually administered orally twice a day, meanwhile continuous intravenous infusion is used only in the perioperative period. Oral administration might be a better way for investigating the toxicity and efficacy of tacrolimus in different pharmacokinetic profiles. However, the bioavailability of tacrolimus in rats is lower than that in human, and the interindividual difference of AUC in oral administration is large (Iwasaki et al., 1991). Therefore, we selected a parenteral administration in this study for making stable experimental conditions. We used bolus intravenous injection for pancreatic toxicity experiments and bolus intramuscular administration for efficacy experiments because intravenous administration and intramuscular administration are the most common methods in pancreatic toxicity studies and transplantation studies, respectively, in rats.

The relationship between the AUC/trough and its efficacy/toxicity has been reported while the relationship between Cmax and rejection has not been demonstrated (Takahara, 1993; Yasuhara et al., 1995; Kershner and Fitzsimmons, 1996; Undre et al., 1999; Venkataramanan, 2001). The AUCs of tacrolimus are known to correlate highly with trough concentrations after dosing of a standard twice-daily formulation, it can easily be speculated that the AUC of tacrolimus may be associated with its efficacy (Alloway et al., 2005; Florman et al., 2005; Dansirikul et al., 2006; Masuda and Inui, 2006). Because AUC measurement for tacrolimus is complicated and graft rejection is a multi-factorial reaction, it is difficult to elucidate significant and direct correlations between its

| Table 3. Effect of tacrolimus on survival time of skin allograft |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Dosing route**            | **Dose** (mg/kg) | **No. of animals** | **Skin graft survival time** (day) | **Median survival time** (day) |
| i.m. bolus                  |                 |                 |                             |                             |
| Control                     | 0.01           | 7               | 5,5,5,6,6,6,6              | 5                           |
|                             | 0.1            | 8               | 7,7,7,10,10,10,10          | 10                          |
|                             | 1              | 8               | 19,19,20,20,20,20,21,21    | 20**                        |
| i.v. infusion               |                 |                 |                             |                             |
| Control                     | 0.01           | 7               | 6,6,7,7,7,7,7             | 7                           |
|                             | 0.1            | 8               | 10,10,10,10,10,10,10,12,12| 10*                         |
|                             | 1              | 8               | 17,20,21,22,22,22,22,22,22| 22**                        |

*: P < 0.05, **: P < 0.01 vs each Control (Peto’s test)
AUC and efficacy. However, the present study demonstrates that administration of the same dose via different administration routes results in the same efficacy for skin allograft survival. The sustained-release formulation of tacrolimus also enables once-daily dosing that may improve drug compliance. A significant contributing factor to the incidence of transplant rejection is patient noncompliance with immunosuppressive drug therapies (Siegal and Greenstein, 1997; Didlake et al., 1988). Compliance issues are of a particular concern with children and adolescents. On average, 50% of children with chronic illnesses do not comply with treatment regimens (Fennell et al., 1994). Weng and colleagues found a statistically significant association between adherence to medication regimens and once-daily dosing versus twice-daily dosing in a prospective cohort study of adult recipients of deceased donor renal transplants (Weng et al., 2005). This suggests that compliance could be improved with a once-daily regimen. Recently, a once-daily formulation of tacrolimus (Advagraf<sup>®</sup> once-daily regimen. Recently, a once-daily formulation of tacrolimus (Advagraf<sup>®</sup>) was developed that had an efficacy and safety similar to that of the standard twice-daily formulation. Pharmacokinetic studies have shown that the steady-state daily exposure tacrolimus with the once-daily formulation is equivalent to that with the standard formulation. Furthermore, the new formulation is associated with a lower Cmax (Heffron et al., 2007). Although the clinical significance of the lower Cmax remains to be clarified, the once-daily formulation is expected to reduce the side effects of tacrolimus.

In conclusion, the sustained blood concentration of tacrolimus with an AUC similar to that of the bolus administration was better tolerated and had the same efficacy as the bolus administration, which produces a higher Cmax in rats. These results indicate that the sustained-release formulation of tacrolimus has the potential to improve the safety of tacrolimus.

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