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Full Paper

Reduced variability in tacrolimus pharmacokinetics following intramuscular injection compared to oral administration in cynomolgus monkeys: Investigating optimal dosing regimens

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ABSTRACT

Tacrolimus is one of the most commonly used immunosuppressive agents in animal models of transplantation. However, in these models, oral administration is often problematic due to the lowered compliance associated with highly invasive surgery and due to malabsorption in the intestinal tract. Therefore, we carried out a study to determine the pharmacokinetics of tacrolimus after intramuscular (IM) injection and to determine the optimal IM dosing regimens in primate models. Six male cynomolgus monkeys (*Macaca fascicularis*) were used in the study. Doses of 0.1 mg/kg and 5 mg were administered via IM injection and oral administration, respectively, once to determine single-dose pharmacokinetics and once daily for 5 days to determine multiple-dose pharmacokinetics. According to pharmacokinetic model estimates, the inter- and intra-individual variabilities in bioavailability following IM injection were remarkably reduced compared with those following oral administration. Monte Carlo simulations revealed that C_{peak}, C_{trough} and AUC would also have less variability following IM injection compared with oral administration. In this study, we found that the pharmacokinetic characteristics of tacrolimus were more constant following IM injection compared with oral administration. These results suggest that IM injection can be an alternative route of administration fin non-human primate model studies.

1. Introduction

Non-human primates (NHPs) are used in many preclinical studies of organ transplantation because they are anatomically and physiologically similar to human. Tacrolimus is an effective immunosuppressant and a basic component of protocols for immunosuppression in preclinical studies using NHPs as well as in clinical studies.[1–8]

However, few studies have been performed to confirm the therapeutic dose of tacrolimus in NHPs including cynomolgus monkeys (*Macaca fascicularis*).[1,4] In those studies, tacrolimus was orally administered, but no target trough levels were proposed. Intramuscularly (IM) injected tacrolimus has also been used in prior studies.[5,7,9] However, the target trough level used in those studies

Abbreviations: IM, intramuscular; AUC, area under curve; NHP, non-human primate; SC, subcutaneous; LC-MS, liquid chromatography-tandem mass spectrometry; MC-PEM, Monte Carlo Parametric Expectation Maximization; BSV, between subject variability; BOV, between occasion variability; IV, intravenous; EDTA, ethylenediaminetetraacetic acid; HPLC, high performance liquid chromatography; CL, systemic clearance; CLD, distribution clearance; PO, oral administration.

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was not supported by evidence and may have been chosen based on the results observed with orally administered tacrolimus.

In kidney transplantation, it is well known that low tacrolimus trough levels in the early post-transplant period have been associated with a higher rate of acute rejection.[10,11] Moreover, greater intra-individual variability in tacrolimus trough concentration has been associated with decreased renal allograft survival and rejection free survival.[12,13] However, it is challenging to attain and maintain the therapeutic level of tacrolimus when it is orally administered to primates in transplantation studies, both due to poor compliance with oral administration because of the high invasiveness of surgery and due to malabsorption in the intestinal tract.[14] Therefore, alternative ways to administer tacrolimus are necessary in such studies. We have noted that although there are reports on the pharmacokinetics of tacrolimus in cynomolgus monkeys following intravenous and oral administration,[15] no reports on IM injection are available in the literature. Therefore, we initiated this study to compare the pharmacokinetics of IM-injected tacrolimus with that of orally administered tacrolimus in cynomolgus monkeys. Population pharmacokinetic modeling was applied to compare the pharmacokinetic properties of the routes of administration and to determine the optimal dosing regimen for IM-injected tacrolimus.

2. Materials and methods

2.1. Materials

Tacrolimus (Prograf®) was supplied by Astellas Pharma Inc, (Tokyo, Japan). HPLC-grade acetonitrile, methanol, and distilled water were purchased from Burdick & Jackson (Muskegon, MI, USA). Ascomycin (internal standard), zinc sulfate heptahydrate, formic acid and ammonium formate were purchased from Sigma–Aldrich (Darmstadt, Germany).

2.2. Animals

Six male cynomolgus monkeys, aged 4–5 years and with body weights of 3–4 kg from Cambodia were used in the study. The animals were individually housed indoors on a 12:12-h light:dark cycle and were fed standard macaque biscuits (Harlan Laboratories, Seoul, Korea) and fresh fruit twice daily. Hematology and serum chemistry results were within normal limits for all animals. In addition, the monkeys were negative for tuberculosis, viral serology (herpes B virus, simian T-lymphotropic virus, simian immunodeficiency virus, simian type D retrovirus, and hepatitis B virus), *Salmonella* (*Shigella*), and fecal parasites. The animals were closely monitored with daily physical examinations and measurements of body weight, food consumption, urine output, stool output, and overall activity. The following blood hematological parameters were checked regularly: white blood cell count with differential count, hemoglobin, hematocrit and platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, blood urea nitrogen (BUN), creatinine, albumin, globulin, sodium, potassium, chloride, calcium, inorganic phosphate, cholesterol, triglyceride, amylase, and C-reactive protein (CRP) levels.

This study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Research Council. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at ORIENTBIO, Seongnam, Korea (approval number: ORIENT-IACUC-14194).

2.3. Pharmacokinetics study

Pharmacokinetic studies were carried out according to four-period design with sufficient wash-out period (at least 4 days). Tacrolimus was given to monkeys by multiple IM injections, multiple oral administrations, single IM injection and single oral administration, sequentially. Multiple-dose studies were performed for 5 consecutive days in the first and second periods. The single-dose studies were performed to describe the absorption kinetics of tacrolimus, while the multiple-dose studies were performed to describe its systemic disposition. The dose of tacrolimus were 0.1 mg/kg for IM injections and 5 mg/head for oral administrations. Diluted Prograf® injection with saline was injected into the thigh muscle using 23-gauge needle in IM dosed groups and Prograf® 5 mg capsule was administered by oral gavage in orally dosed groups. The animals were fasted overnight before dosing and food was returned at 1 h after drug administration. Overall process of the animal study is summarized in Fig. 1.

After IM injection or oral administration of tacrolimus, blood samples (1 mL) were collected from the femoral vein into ethylenediaminetetraacetic acid (EDTA) pretreated tubes at predetermined times. The samples were then immediately frozen and stored at −20 °C until analysis.

2.4. Drug analysis

Tacrolimus serum concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The LC-MS/MS instrument comprised an API 4000 mass spectrometer (Applied Biosystems/MDS Sciex, Toronto, Canada) coupled with an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA). The drug was separated from the serum components on a Zorbax SB-C_{18} column 2.1 × 50 mm, i.d., 3.5 μm (Agilent Technologies). Mobile phase A (0.1% formic acid and 2 mM ammonium acetate in water) and mobile phase B (0.1% formic acid in acetonitrile) were conducted with gradient elution. The mass spectrometer was operated using a multiple reaction monitoring model. The transition of the precursors to the product ion was monitored at 821.5 → 768.6 for tacrolimus and 809.6 → 756.5 for the internal standard (ascomycin). The EDTA-whole blood samples were pretreated with the protein precipitation method.

2.5. Population pharmacokinetic modeling

Since the animal studies for determining drug absorption and systemic disposition were performed independently, the pharmacokinetics of tacrolimus was evaluated using population pharmacokinetic modeling. The structural model is depicted in supplementary data.

Tacrolimus in the central compartment (amount, X_1) was assumed to be distributed to the peripheral compartment (amount, X_2) and eliminated from the central compartment. The tacrolimus absorption processes after subcutaneous injection and oral administration were assumed to be transferred into the circulating system from the muscle (X_{muscle}) and gut (X_{gut}) compartments by the respective first-order rate constants ($k_{a,im}$ and $k_{a,po}$) with lag time. The differential mass balance equations were written as follows:

$$\frac{dX_{gut}}{dt} = -k_{a,po} \cdot X_{gut}$$

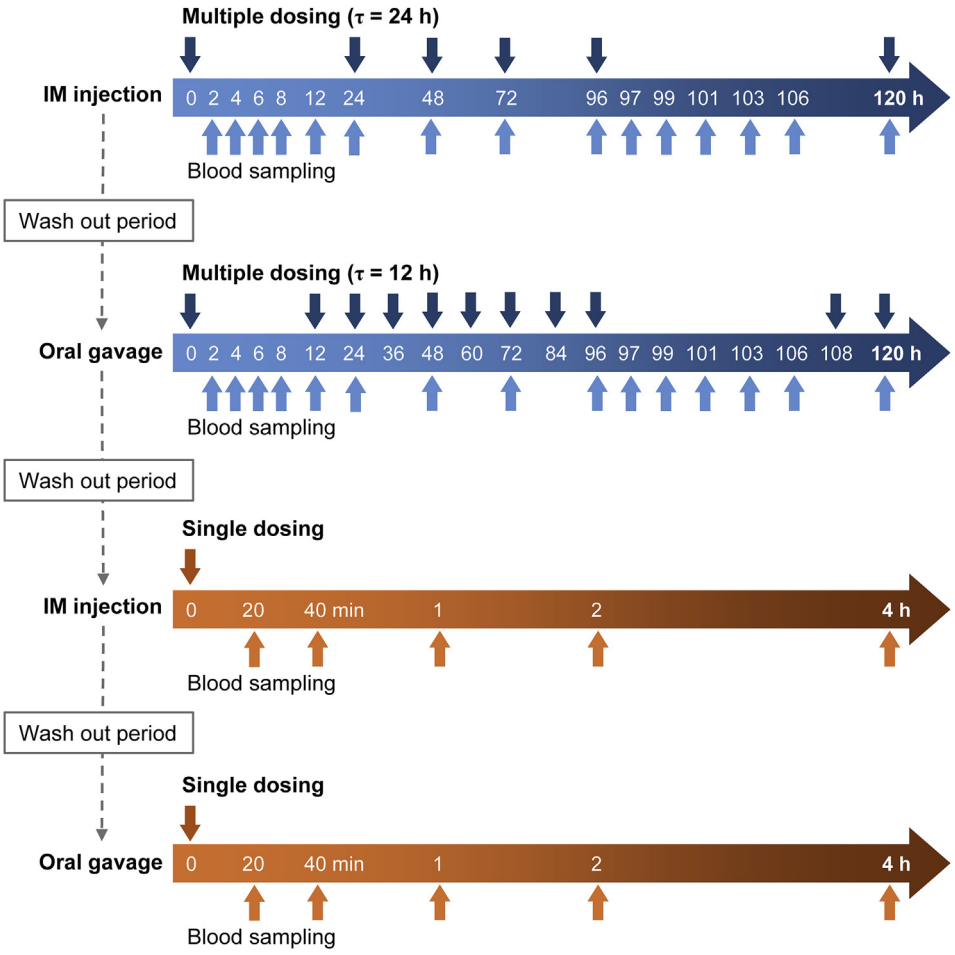

Fig. 1. Overall process of animal studies.

$$\frac{dX_{muscle}}{dt} = -k_{a,im} \cdot X_{muscle}$$

$$\frac{dX_1}{dt} = k_{a,po} \cdot X_{gut} + k_{a,im} \cdot X_{muscle} - CLD \cdot C_1 + CLD \cdot C_2 - CL \cdot C_1$$

$$\frac{dX_2}{dt} = -CLD \cdot C_2 + CLD \cdot C_1$$

C_1 and C_2 represent the tacrolimus concentrations in their respective compartments, while CLD represents the distribution clearance to the peripheral compartment.

The blood concentration-time data obtained after oral administration and IM injection were simultaneously fitted to the population pharmacokinetic model. The model fitting was conducted using the Monte Carlo Parametric Expectation Maximization (MC-PEM) algorithm in parallelized S-ADAPT (version 1.57). An important sampling MC-PEM method (pmethod = 4 in S-ADAPT) was used for the population pharmacokinetic parameter estimation. Relative standard errors describing the uncertainty of each model parameter were calculated from a formula implemented in S-ADAPT (proper type = 8). Between-subject variability (BSV) was estimated using an exponential parameter variability model. Between-occasion variability (BOV) was considered for the bioavailability and absorption rate constant. Models were assessed with a full, block diagonal, or major diagonal variance-covariance matrix. The goodness-of-fit for population modeling was assessed using the objective function (−1 log-likelihood), plausibility of parameter estimates, visual inspection of the observed and fitted concentrations, and standard diagnostic plots. The predictive performance of the population model was evaluated by calculating visual predictive checks. Simulations were carried out using Berkeley Madonna (version 8.3.18).

3. Results

3.1. Toxicity

Liver enzyme levels, including AST, ALT, and albumin levels, did not change after tacrolimus administration with either the oral or the IM routes. BUN and creatinine levels were also stable after administration with either route. General conditions including body weight, food intake, urine output, stool output, and overall activity did not change. No signs of infection or serum CRP elevation were detected.

3.2. Population pharmacokinetic modeling

The average blood concentration vs. time profiles of tacrolimus obtained after oral administration and IM injection are shown in Fig. 2. The absorption and disposition profiles of tacrolimus were obtained by single dose studies and multiple dose studies, respectively. To determine these profiles, one or two distribution compartment models with first- or mixed- order rate absorption

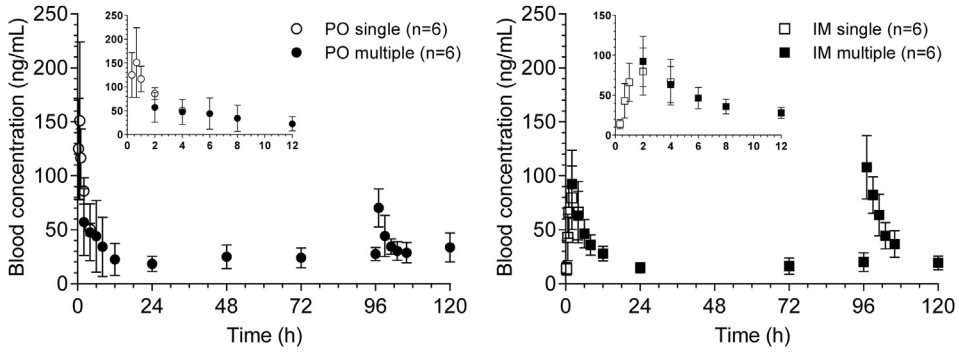

Fig. 2. Average blood concentration vs. time profiles obtained after the oral administration (left panel) and IM injection (right panel) of tacrolimus in cynomolgus monkeys.

from gut and muscle compartment models were tested. Based on the objective function value and simplicity of the model structure, we chose a two-compartment model with first-order drug absorption as a final model.

The final population pharmacokinetic parameter estimates are presented in Table 1. The full blood concentration-time profiles and the plots of the observed vs. fitted values (Fig. 3) indicated that the model appropriately described the observed data. Following the Monte Carlo simulation, visual predictive checks for evaluating the predictive performance of the model were carried out by comparing 10th, 25th, 50th, 75th and 90th percentile-predicted concentration time profiles to the observed data (Fig. 4). As evidenced by these checks, the final model adequately predicted the median concentration-time profiles after the administration of tacrolimus.

The relationship of the administration route with the pharmacokinetics of tacrolimus was evaluated using population pharmacokinetic modeling. The mean oral bioavailability of tacrolimus relative to the IM injection was estimated be 4.8% while mean relative bioavailability of IM injection was assumed to be 100% (Table 1).

Variabilities in the relative bioavailability were evaluated by between subject variability (BSV) and between occasion variability (BOV). Both BSV and BOV were predicted to be higher for oral administration compared with IM injection by 1.79- and 3.65-fold, respectively (Table 1). Despite the low bioavailability, the absorption rate constant following oral administration was 9.81-fold higher than that following IM injection.

The C_{peak}, C_{trough}, and partial AUC following 5-day repeated dosing of tacrolimus were predicted by Monte-Carlo simulations. As shown in Table 2, the pharmacokinetic parameters of the 90th percentile divided by those of the 10th percentile, representing the distribution (combined BSV and BOV), were higher in the orally administered group for all three parameters.

3.3. Simulations for selection of the IM injection dosing regimen

In a previous report, graft survival rate was significantly increased when 2 mg/kg of tacrolimus was orally administered once a day in cynomolgus monkeys, and the mean trough blood concentration was observed to be 8.9 ng/mL.[4] Using the population pharmacokinetic model developed in the current study, we simulated various dosing regimens of tacrolimus to find out which would result in a comparable trough blood concentration.

The simulated median blood concentration-time profiles and pharmacokinetic parameters are presented in Fig. 5 and Table 3, respectively. Following oral administration at 2 mg/kg once daily, the predicted C_{trough} value was 17.3 ng/mL, which was higher than the previously reported C_{trough} value. The predicted AUC_{0-24h} on day 5 after repeated dosing of 2 mg/kg of tacrolimus once daily was 1005.8 ng·h/mL, and comparable AUC_{0-24h} values were predicted after IM injection of 0.1 mg/kg once daily (1045.4 ng·h/mL) and 0.05 mg/kg twice daily (1034.1 ng·h/mL). After once-daily oral administration of 1 mg/kg, the predicted C_{trough} value on day 5 was comparable with the reported value (8.6 ng/mL vs. 8.9 ng/mL). IM doses of 0.05 mg/kg once daily and 0.025 mg/kg twice daily showed predicted AUC_{0-24} values on day 5 that were comparable with 1 mg/kg once daily oral dosing.

4. Discussion

Tacrolimus is one of the most commonly used immunosuppressive agents in studies of animal models of transplantation. Although tacrolimus is widely used as an immunosuppressant, it has several drawbacks and requires careful attention due to its narrow therapeutic window and large inter- and intra-individual variability.[16–18] The low therapeutic index of tacrolimus necessitates an optimized dosing regimen to minimize adverse effects and the likelihood of graft rejection, but choosing such a regimen is

Table 1
Parameter estimates of the population pharmacokinetic model for tacrolimus.

Parameter	Symbol	Unit	Population mean	BSV	BOV
Volume of the central compartment	V_1	L	2.08	0.146	–
Volume of the peripheral compartment	V_2	L	2.94	0.466	–
Systemic clearance	CL	L/h	0.31	0.074	–
Distribution clearance	CLD	L/h	0.63	0.395	–
Relative oral bioavailability[a]	F_{Oral}	–	0.048	0.245	0.427
IM injection bioavailability[a]	F_{IM}	–	1	0.137	0.117
Rate constant for drug absorption from gut	$k_{a,po}$	1/h	10.1	0.257	0.314
Rate constant for drug absorption from muscle	$k_{a,im}$	1/h	1.03	0.260	0.307
Lag time for absorption from gut	$T_{Lag,po}$	h	0.12	0.567	–
Lag time for absorption from muscle	$T_{Lag,im}$	h	0.22	0.049	–

[a] Bioavailability of the oral administration relative to the intramuscular injection.

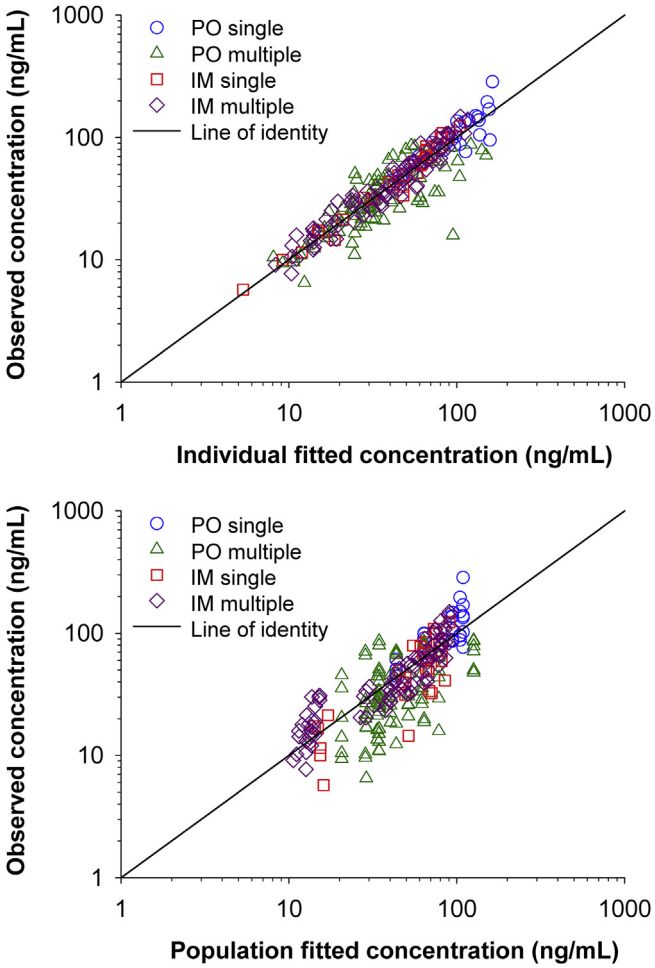

Fig. 3. Standard diagnostic plots of fit for the model. Observed vs. fitted blood concentrations of tacrolimus after intramuscular and oral administration.

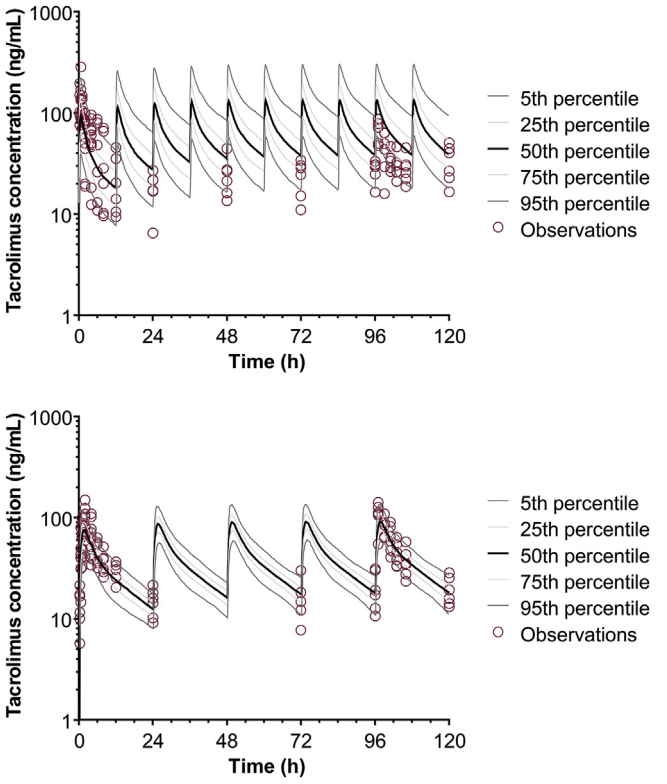

Fig. 4. Visual predictive check plots of the pharmacokinetic model. The symbols represent the observed blood concentration following oral administration (upper panel) and IM injection (lower panel) and the lines present the predicted profiles by Monte-Carlo simulations.

complicated by the large variability in pharmacokinetics.[19] This variability can be attributed to various factors including poor solubility, low bioavailability, food effects and disease state.[19–21] These limitations coupled with potential drug–drug interactions result in the need for therapeutic monitoring of the drug.[16,18] Furthermore, there have been efforts to reduce this variability by improving solubility,[20] and designing sustained release systems,[22] and pharmacogenetics-based approaches.[17] However, the studies for establishing optimal dosing regimens of tacrolimus have been limited to the oral administration route in animals.[1,4,8] In this study, we characterized the pharmacokinetics of tacrolimus after oral administration and IM injection in cynomolgus monkeys using population pharmacokinetic modeling in order to suggest IM injection as an alternative dosing route.

Subcutaneous (SC) or IV injections also could be considered as ways to administer tacrolimus. However, we observed skin necrosis after SC injection of tacrolimus (0.1 mg/kg) in our preliminary experiments. In addition for daily IV injection of tacrolimus, the use of an indwelling venous catheter is necessary, which can increase the risk of infection and limit the activity of the monkeys. Therefore, we decided to deliver tacrolimus through IM injection. In this study, there was no sign of local infection or other symptoms relating to IM injection. IM injection was not inducing renal and hepatic toxicity those could be related to local toxicity following IM injections.[23,24]

The final population pharmacokinetic model was developed to measure BSV and BOV in the absorption process associated with the oral and IM routes. The variability in AUC was lower following IM injection, as was demonstrated by the BSV and BOV in Table 1. The lower BSV and BOV for IM injection support its use as a dosing route to replace oral administration. The Monte-Carlo simulation for multiple dosing generated predictions with the combined effects of BSV and BOV (Table 2). The results showed that the variability of the parameters of AUC, C_{trough} and C_{peak} would be reduced by IM injection. It is noteworthy that the AUC, the parameter that best represents tacrolimus drug exposure,[16] showed the least variability with IM injection. The C_{trough} value, which is the most frequently used parameter in the clinic for its practicality, also predicted that IM injections would more reliably deliver tacrolimus within the narrow therapeutic window of the drug.

Kinugasa et al reported that cynomolgus monkeys who received daily oral administration of tacrolimus 2.0 mg/kg had significantly longer graft survival compared to a group that was administered 1 or 0.5 mg/kg. In that study, the mean trough blood level was 8.9 ± 3.72 ng/mL when a dose of 2.0 mg/kg was administered.[4]

However, according to the model developed in our study, a comparable median C_{trough} was associated with daily oral administration of 1 mg/kg. Since C_{trough} may vary with factors associated with the absorption process, we set therapeutic daily doses at 1 and 2 mg/kg. According to the Monte-Carlo simulations, the C_{trough} and AUC_{0-24h} values on day 5 of IM injection at 0.05 and 0.1 mg/kg once daily were comparable to those of oral administration at 1 and 2 mg/kg once daily, respectively. The AUC_{0-24h} values on day 5 following IM injections at 0.025 and 0.05 mg/kg twice daily were also predicted to be comparable with those after oral administration of 1 and 2 mg/kg once daily, respectively.

Table 2
Predicted pharmacokinetic parameters of tacrolimus on day 5 following repeated oral administrations and IM injections of tacrolimus at doses of 5 mg and 0.1 mg/kg, respectively.

Percentile	PO (5 mg)			IM (0.1 mg/kg)		
	C_{peak} on day 5 (ng/mL)	C_{trough} on day 5 (ng/mL)	AUC_{0-24h} on day 5 (ng·h/mL)	C_{peak} on day 5 (ng/mL)	C_{trough} on day 5 (ng/mL)	AUC_{0-24h} on day 5 (ng·h/mL)
10%	52.19	20.96	857.23	22.29	12.59	742.36
25%	68.20	28.50	1129.33	26.36	14.77	850.00
50%	96.50	38.71	1571.56	30.50	17.97	972.93
75%	134.82	54.08	2193.78	35.15	21.59	1105.01
90%	176.47	80.09	3000.95	39.79	24.49	1241.07
P90/P10	3.38	3.82	3.50	1.79	1.94	1.67

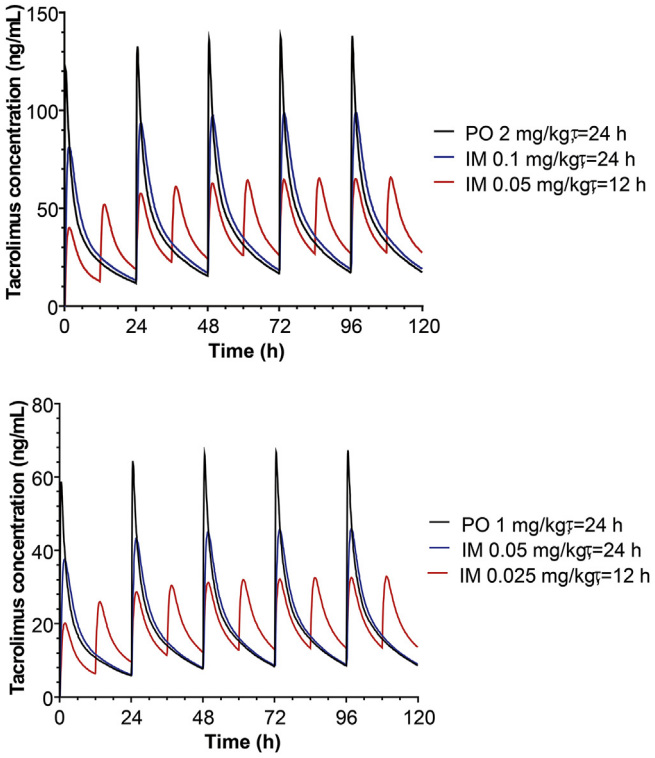

Fig. 5. Predicted median blood concentration vs. time profiles of tacrolimus following various dosing regimens.

The reduced variability in tacrolimus pharmacokinetics following IM injection is an interesting result, as IM injection is not necessarily associated with decreased variability compared to oral administration. Studies reporting head-to-head pharmacokinetic comparisons between IM injections and oral administration of methotrexate,[25] artemether,[26] lorazepam,[27] diazepam[28] and ketoprofen[29] showed that across the various drugs, the parameters varied in different ways between the two routes of administration. These results may mean that the variability of each parameter across various routes of administration depends on the characteristics of the administered drug. In the case of tacrolimus, we have shown that IM injections reduce the variability for parameters that have importance in the therapeutic exposure of the drug. Furthermore, this study indicates that long-acting IM injections could prove useful for tacrolimus. The advantages of reduced variability combined with sustained release shown in this study for the IM injection of tacrolimus could warrant a new strategy for administering tacrolimus.

Table 3
Predicted median pharmacokinetic parameters of tacrolimus on day 5 following various dosing regimens.

Route	Dose	C_{peak} on day 5 (ng/mL)	C_{trough} on day 5 (ng/mL)	AUC_{0-24h} on day 5 (ng·h/mL)
PO	2 mg/kg, $\tau = 24$ h	142.5	17.3	1005.8
IM	0.1 mg/kg, $\tau = 24$ h	101.6	19.0	1045.4
IM	0.05 mg/kg, $\tau = 12$ h	66.6	27.2	1034.1
PO	1 mg/kg, $\tau = 24$ h	69.1	8.6	489.9
IM	0.05 mg/kg, $\tau = 24$ h	47.0	8.9	483.7
IM	0.025 mg/kg, $\tau = 12$ h	33.3	13.5	519.6

5. Conclusion

In the present study, we compared the pharmacokinetics of tacrolimus following oral administration and IM injection in cynomolgus monkeys. While both routes of administration were well tolerated, the overall variability in pharmacokinetics including C_{trough} and AUC at steady state was significantly decreased in the IM-injected group. This suggests that IM injection can be an alternative administration route for reliable systemic delivery of tacrolimus in NHP models of transplantation.

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Conflict of interest

The authors have no conflicts of interest to declare.

Authorship

Kyo Won Lee participated in research design, conducting of the research, and the writing of the paper.

Tae Hwan Kim participated in research design, the writing of the paper and data analysis.

Jong Bong Lee participated in the writing of the paper and data analysis.

Kyeong Sik Kim participated in conducting of the research.

Jae Berm Park participated in research design.

Pavel Gershkovich participated in data analysis.

Sun Dong Yoo participated in research design.

Soyoung Shin participated in research design.

Beom Soo Shin participated in research design.

Sung Joo Kim participated in research design and data analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphs.2018.05.013.

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