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Population Pharmacokinetic Analysis of the Oral Absorption Process of Tacrolimus in Healthy Volunteers

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=Abstract=

Population Pharmacokinetic Analysis of the Oral Absorption Process of Tacrolimus in Healthy Volunteers

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연구배경: 타크로리무스는 이식환자들의 동종이식 거부반응을 예방하기 위해 사용된다.
타크로리무스는 치료 허용 범위가 좁고 개인 간, 개인 내 약동학적 차이가 높으므로 혈중 농도의 모니터링을 이용한 약물 투여 용량 조절이 필수적이다. 현재 타크로리무스의 흡수 기전에 관한 연구가 미흡하여 이번 연구를 통해 건강 피험자 중 타크로리무스의 최적의 흡수 모델을 정의하는 것을 목표로 하였다.
방 법: 31명의 건강한 성인들에게 각각 4 mg의 타크로리무스를 경구투여하였다. 채혈시점은 약물투여 전 및 투여 후 0.5, 1, 1.5, 2, 3, 6, 8, 10, 12, 24, 48, 60 ,72 시간이었으며 혈장 내 타크로리무스의 농도 분석은 HPLC-MS-MS를 이용하였다. 약동학적 분석을 위해 NONMEM을 사용하였고, 영차 흡수, 일차 흡수, 트랜짓 분획 흡수 모델에 2분획적 모델을 적용하여 비교하였다.
결 과: 트랜짓 분획 흡수 모델이 타크로리무스의 약동학적 흡수 과정을 가장 잘 설명하는 것으로 나타났다. 여기서 적용된 3 가지의 흡수 모델 중 트랜짓 분획 흡수 모델에서 OFV와 RMSE가 가장 감소하였고, 각 개인 피험자들의 흡수기를 나타낸 그래프와 산점도 그림에서도 트랜짓 분획 흡수 모델이 일차 흡수 모델보다 더 우수했다.
결 론: 우리는 최근에 고안되어진 트랜짓 분획 모델이 타크로리무스의 불규칙한 약동학적 흡수 과정을 나타내는 것에 유용한 모델임을 알 수 있었다. 타크로리무스의 흡수 과정이 제형에 관계 없이 불규칙적인 것으로 알려져 있으므로, 트랜짓 분획 모델이 흡수 지연 시간을 적용한 약동학적 흡수 과정의 특징을 생리학적으로 보다 잘 나타낼 것으로 기대한다.

키워드: 타크로리무스 트랜짓 분획 약동학 흡수 NONMEM

Introduction

Tacrolimus, a macrolide lactone, is one of the widely used immunosuppressants for transplant patients. The mechanism of tacrolimus is very similar to that of cyclosporine but it is 10- to

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100- fold more potent than cyclosporine[1]. Since functional expression of P-glycoprotein and CYP3A4 in the gut wall and liver determines tacrolimus absorption, its oral bioavailability (BA) is highly variable[2,3]. Because of its narrow therapeutic index and irregular absorption, therapeutic drug monitoring (TDM) of trough blood concentrations for dosage adjustment has been widely accepted. However, the dosage and trough concentration are known to be poorly correlated despite TDM[4]. Thus, a better model is needed to embrace the irregular pharmacokinetic behavior, especially the absorption phase of tacrolimus.

So far, most of the population pharmacokinetic (PK) analyses on the oral absorption process of drugs have adopted zero-order or first-order absorption models. The transit compartment (TC) model has been recently introduced. The absorption delay which can be observed in any oral absorption is a phenomenon with complicated mechanisms. However, most pharmacokinetic models describe the absorption delay using a simple concept of lag time. That is, a drug is assumed to be absorbed in a first or zero-order style after a certain length of time.

The absorption rate is then assumed to increase suddenly from zero to its maximum (in the case of first-order absorption model) or a constant (in the case of zero-order absorption model), which is physiologically not plausible. On the other hand, drug absorption is described using multiple components without time delay in the TC model. It is assumed that drug molecules pass through several catenary TCs until they reach the last absorption compartment[5]. This study was attempted to improve the population PK model for oral tacrolimus by comparing these 3 oral absorption models in healthy subjects.

Methods

Ⅰ. The Source of Data

Weused the data on tacrolimus concentration that were obtained from a bioequivalence (BE) study involving 31 healthy male volunteers. The BE study was previously performed at Kangnam St. Mary's Hospital in Seoul in 2005. After the approval of the protocol by the IRB of our hospital, the pharmacokinetic study with a cross-over design was carried out in accordance with the regulation of the Korean government and the Declaration of Helsinki. The demographic profile of the 31 male subjects who participated in the BE study is summarized in Table 1. From the archives of the BE study data, the plasma concentrations obtained after taking the reference formulation (Prograf™ capsules, Fujisawa,

Table 1. Demographics of the 31 male subjects

Characteristics	Median	Range
Age (years)	24	20-34
Bodyweight (kg)	66.2	58-84
Aspartate aminotransferase (U/L)	24	19-29
Alanine aminotransferase (U/L)	24	13-49
Hemoglobin (g/dL)	15.5	13.8-17.8
Hematocrit (%)	45.3	40.6-51.3
Total protein (g/dL)	7.4	6.8-8.7
Albumin (g/dL)	4.7	4.5-5.1

Seoul, Korea) was used to develop the absorption model.

Ⅱ. Pharmacokinetic Study

Four milligram of tacrolimus (four 1-mg Prograf™ capsules, Fujisawa, Seoul, Korea) was given with 240 ml of water and 10 ml of peripheral venous blood was collected at pre-dosing, and 0.5, 1, 1.5, 2, 3, 6, 8, 10, 12, 24, 48, 60 and 72 h after dosing. Samples were analyzed using HPLC-MS-MS according to the previously described methods[6,7]. The lower limit of quantification of the assay was 0.2 ng/ml with the standard curve linear being between 0.2 and 50 ng/ml. The values of interassay variability (coefficient of variation, CV%) of 0.2, 1.0, 10.0 and 50.0 ng/ml were 12.01%, 5.63%, 2.12% and 1.56%, respectively, and the values of intra-assay variability (CV%) with 0.2, 1.0, 10.0 and 50.0 ng/ml were 9.61%, 9.52%, 2.30% and 2.19%, respectively. The mean time-concentration profile of tacrolimus from 0 to 12 h after dosing is given in Fig. 1. Because the aim of this study was to find a better absorption model, the profiles over 12 h were not included.

Ⅲ. Development of a Population Pharmacokinetic Model

The mixed effect method using NONMEM ™ Version 6 (Icon Development Solutions, Ellicott City, MD, USA), was used in all absorption models. Zero-order absorption, first-order absorption and TC models were compared to find the model which best described the absorption of

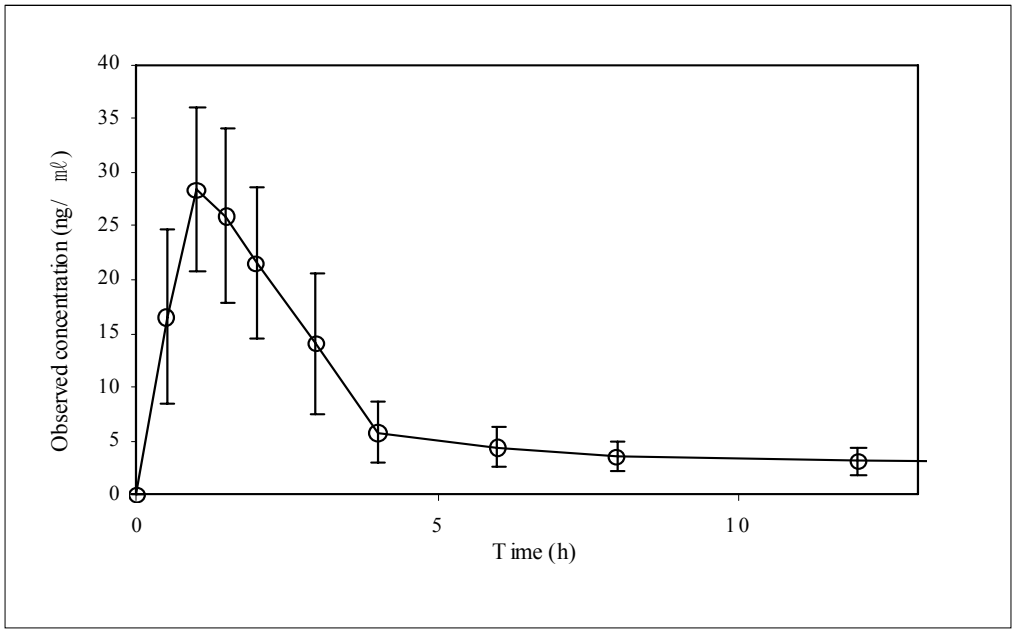

Figure 1. Mean concentration-time profiles of tacrolimus obtained from 31 healthy volunteers. Since we aimed to model the absorption phase, the time-concentration profile shown in this figure was truncated at 12 h.

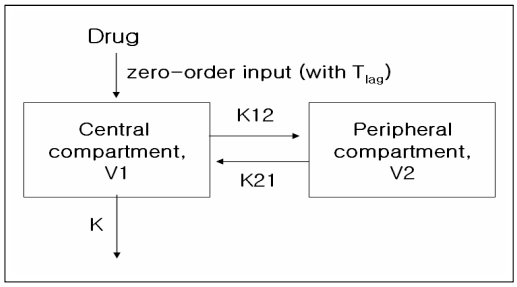

Figure 2. The model of zero-order absorption. T_{lag}, lag time; V1, central compartment volume; V2, peripheral compartment volume; K, elimination rate constant from central compartment; K12, elimination rate constant from central to peripheral compartment; K21, elimination rate constant from peripheral compartment to central compartment.

tacrolimus on the basis of a 2-compartment linear elimination model. First-order conditional estimation with interaction (FOCEI) was chosen as the estimation method.

A. Zero-order absorption

A zero-order input model with lag time was created using ADVAN3 TRANS4 subroutine (Fig. 2). We modeled zero-order input as if it were an intravenous infusion using a parameter, D1. The parameters estimated were lag time (ALAG1) and the duration (D1) of absorption in addition to the distribution and elimination parameters which were also used in other absorption models, including clearance (CL), volume of central compartment (V_c), intercompartmental clearance (Q) and volume of peripheral compartment (V_p). Discrepancy between the individual predicted estimate and the population predicted estimate for the jth individual was expressed as η_j ($\eta \sim N(0, \omega^2)$). The structural model for PK parameters was as follows:

$$ALAG1_j = TVLAG1 * \exp(\eta ALAG1j) \quad (1)$$

$$D1_j = TVD1 * \exp(\eta D1j) \quad (2)$$

$$CL_j = TVCL * \exp(\eta CLj) \quad (3)$$

$$V_{cj} = TVV_c * \exp(\eta Vcj) \quad (4)$$

$$Q_j = TVQ * \exp(\eta Qj) \quad (5)$$

$$V_{pj} = TVV_p * \exp(\eta Vpj) \quad (6)$$

The residual error was expressed as ε_{ij}, (difference between C_{ij} and $C_{pred\ ij}$), which followed Gaussian distribution with mean 0 and variance σ^2. That is,

$$C_{ij} = C_{pred\ ij} * (1+\varepsilon_{ij}) \quad (7)$$

where C_{ij} denotes the ith observed concentration of the jth individual and $C_{pred\ ij}$ denotes the plasma tacrolimus concentration predicted by the individual PK. Typical sources of the residual error include intraindividual variability of PK parameters, assay error and model misspecification. This residual error model was also used in the other 2 absorption models.

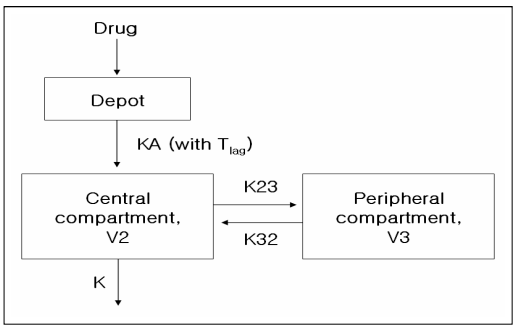

Figure 3. The model of first-order absorption. T_{lag}, lag time; KA, absorption rate constant; V2, central compartment volume; V3, peripheral compartment volume; K, elimination rate constant from central compartment; K23, elimination rate constant from central to peripheral compartment; K32, elimination rate constant from peripheral to central compartment.

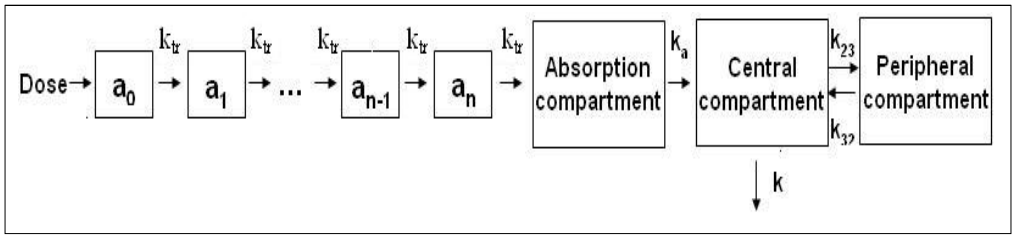

Figure 4. Schematic view of the drug flow though the chain of transit compartment. k_{tr}, transfer rate constant; a_n, drug amount in nth compartment; K_a,absorption rate constant; K, elimination rate constant from central compartment; K23, elimination rate constant from central compartment to peripheral compartment; K32, elimination rate constant from peripheral compartment to central compartment (adapted from Savic *et al.*[5]).

B. First-order absorption

A first-order input model with lag time was created using the ADVAN4 TRANS4 subroutine (Fig. 3). The absorption parameters estimated in this model were the absorption rate constant (KA) and the lag time (ALAG1). Between-subject variability (BSV) was modeled using the same structure employed in the zero-order absorption model as follows:

$$KA_j = TVKA^{*}\exp(\eta KAj) \quad (8)$$

$$ALAG1_j = TVLAG1^{*}\exp(\eta_{ALAG1j}) \quad (9)$$

C. Transit compartment absorption

The TC absorption is based upon the assumption that the lag time phenomenon is related to the number of compartments existing between the gut and the central compartment. In other words, the drug absorption may appear delayed because of the time needed topass many intervening compartments despite initiation of absorption immediately after the bolus dosing (swallowing) into the depot compartment (Fig. 4). The details of equations and their approximate solutions used herein are described in the report by Savic *et al*[5]. The TC model assumes the existence of several TCs before the absorption compartment. The transfer rate constant (k_{tr}) which determines the passage rate from a TC to the next is identical throughout the chains of TCs.

TC absorption was estimated on the basis of a 2-compartment model using the ADVAN6 subroutine. Equation 10 demonstrates changes in A_a (amount in the absorption compartment) by time. N (number of TCs) and MTT (mean transit time) were the 2 TC parameters estimated using NONMEM, while k_{tr} was derived through the relationship given in the Equation 11. However, the N may not necessarily be an integer.

$$\frac{dAa}{dt} = Dose \cdot F \cdot k_{tr} \cdot \frac{(k_{tr} \cdot t)^{N} \cdot e^{-k_{tr} \cdot t}}{\sqrt{2\pi} \cdot N^{(N+0.5)} \cdot e^{-N}} - k_a \cdot A_a \quad (10)$$

$$k_{tr} = \frac{N+1}{MTT} \quad (11)$$

The absorption rate constant (KA) was also estimated. The mean transit time indicates the average time of drug molecules transferring from the first TC to the absorption compartment.

Because some individual subjects had the

number of TC (N) less than 1 when BSV (η) term was tested, we did not apply BSV to N. BSV was not applied to KA either because NONMEM could not kick off the iteration process with it. Thus, only MTT had BSV.

$$KA_j = TVKA \quad (12)$$

$$MTT_j = TVMTT^{*}\exp(\eta_{MTTj}) \quad (13)$$

$$N_j = TVN \quad (14)$$

D. Covariate Selection

Covariates, such as weight (kg), hemoglobin (g/dL), hematocrit (%), ALT (U/L), AST (U/L), albumin (g/dL) andtotal protein (g/dL), were tested for their contribution to all PK parameters in the 3 aforementioned models. The cut-off value of the OFV change which determined the inclusion or exclusion of a covariate in the model was -3.84, which is approximately equivalent to a p-value of 0.05 in a chi-square distribution with d.f. =1.

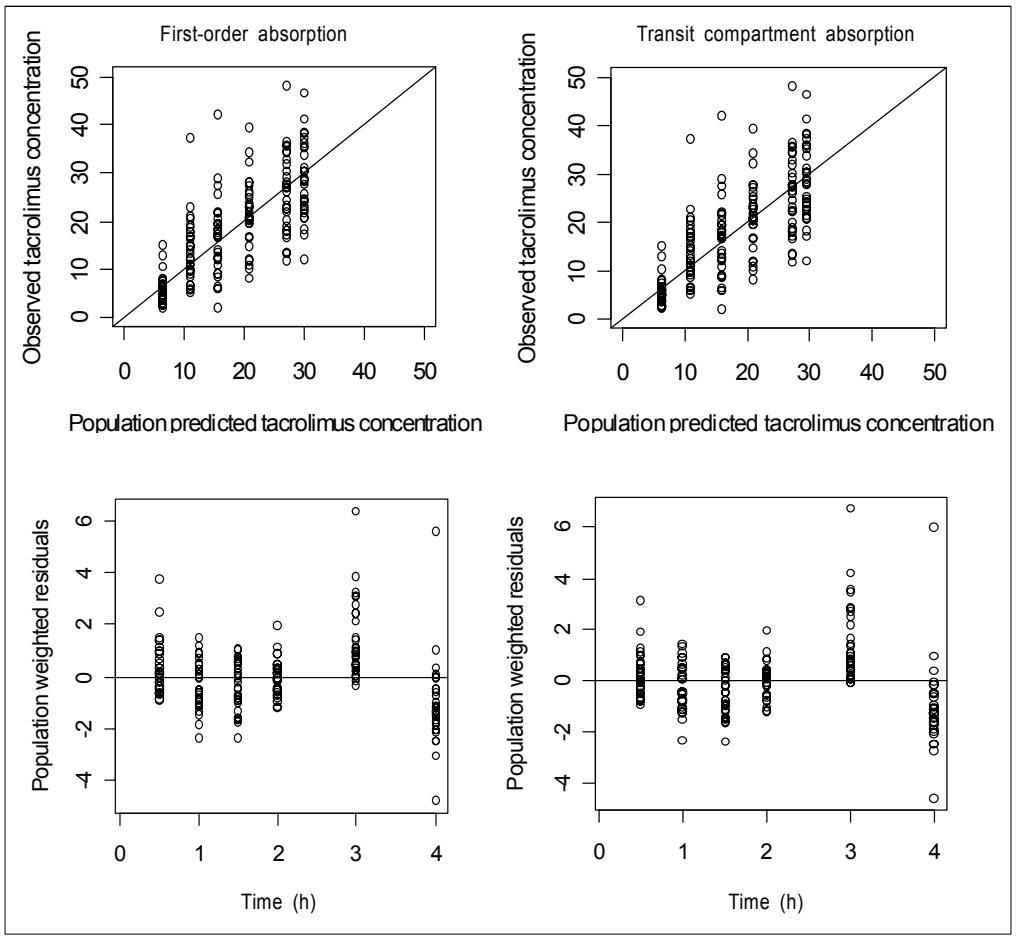

Figure 5. Scatter plots and weighted residuals from the first-order (left) and the transit compartment (right) absorption models for concentrations from 0 to 4 h after dosing.

Goodness of fit (GOF) for the 3 absorption models was also assessed using graphical exploration of scatter plots and individual time-concentration plots. Additionally, the root mean squared error (RMSE) from 0 h to 4 h after dosing was compared. The RMSE was defined as follows:

$$RMSE = \left[\frac{1}{n}\sum_{k=1}^{n}(C_K - \hat{C}_K)^2\right]^{1/2} \quad (15)$$

where C_K and $\hat{C}_K$ denote the observed and the individual predicted tacrolimus concentrations sampled from 0 to 4 h, respectively. The sum of square was divided by n, the total number of the 0 to 4 h concentrations.

Results

PK data from 31 healthy male volunteers were used for modeling. Their ages ranged from 20 to 34 years (median, 24 years). The demographic profile including laboratory test results is shown in Table 1.

In the covariate selection step, we could not find any covariates that contributed to PK parameters in the 3 absorption models. Such a lack of contributing covariates seems to be attributed to the homogeneity of our study population which had a rather narrow range of demographics.

Table 2 summarizes the population PK parameters obtained from the 3 absorption models. Although the scatter plots of the zero-order absorption and first-order absorption models were not significantly different (data not shown), we found that the first-order absorption model was better than the zero-order absorption model. The OFV and RMSE were smaller by 21.416 and 0.3, respectively, in the first-order absorption model than those of the zero-order absorption model. The TC absorption model had the smallest OFV and RMSE in the three models. However, the population predicted versus the observed tacrolimus concentrations and weighted residuals from the first-order

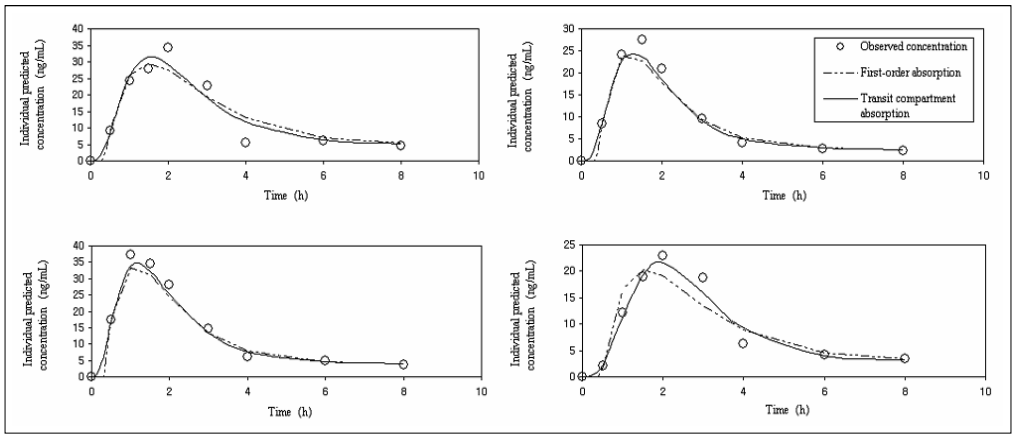

Figure 6. Individual predicted concentrations from the first-order (dotted line) and the transit compartment (straight line) absorption models in 4 representative subjects (Truncated at 8 h).

absorption and TC absorption models (Fig. 5) were almost identical.

Overlay plots of individual predictions from the first-order absorption (dotted line) and TC absorption (straight line) models during the first 8 h in 4 representative subjects are illustrated in Fig. 6. The data over 8 h after dosing were truncated because our interest was to look into the absorption phase.

Discussion

The aim of this study was to compare the 3 models used for the description of the absorption process of tacrolimus. We found that the TC absorption model gave a significantly lower OFV than the zero-order and first-order absorption model. Although the TC absorption model has the advantage of not using a nonphysiological parameter of lag time, its clinical application has

Table 2. Final parameter estimates of zero-order absorption, first-order absorption and transit compartment absorption models

Parameters	Unit	zero-order absorption		first-order absorption		transit compartment absorption	
		Estimate	CV (%)	Estimate	CV (%)	Estimate	CV (%)
OFV	—	458.25	—	436.834	—	429.161	—
CL/F	L/h	18.8	2.13	18.9	2.12	19.7	2.03
V_C/F	L	79.2	0.25	35.8	1.23	39.7	0.50
Q	h^{-1}	40.8	0.66	45.0	0.6	47.3	0.56
V_P/F	L	498	0.05	528	0.05	544	0.05
KA	h^{-1}	—*	—	0.999	12.8	1.09	—
T_{lag}	h	0.11	550	0.30	54.6	—*	—
D1	h	1.09	29.0	—*	—	—*	—
MTT	h	—*	—	—*	—	0.31	169.6
N†	—	—*	—	—*	—	1.81	—
ω_{cl}	%	40.1	—	40.2	—	40.0	—
ω_{Vc}	%	19.9	—	44.1	—	20.1	—
ω_{Q}	%	27.0	—	27.2	—	26.6	—
ω_{Vp}	%	27.1	—	28.6	—	27.8	—
ω_{ka}	%	—*	—	12.8	—	—‡	—
ω_{Tlag}	%	60.5	—	16.4	—	—*	—
ω_{D1}	%	31.7	—	—*	—	—*	—
ω_{mtt}	%	—*	—	—*	—	52.6	—
ω_{n}	%	—*	—	—*	—	—‡	—
σ_{prop}	%	15.8	—	15.5	—	15.1	—
RMSE§	%	2.76	—	2.46	—	2.37	—

*Parameter is not used
†The number of transit compartment
‡Fixed to zero as estimation of this parameter
§RMSE from 0 h to 4 h after dosing
C_k: the observed tacrolimus concentration
$\hat{C}_k$: the individual predicted tacrolimus concentration

$$RMSE = \left[\frac{1}{n}\sum_{k=1}^{n}(C_K - \hat{C}_K)^2\right]^{1/2}$$

not been extensive. In fact, there are many other models that have been developed to accommodate the concentration changes in the absorption phase. Higaki *et al*[8] compared 7 different absorption models devised based upon Fick's first law. They pointed out that the assumption of the first-order absorption rate constant (ka) remaining unchanged throughout the absorption phase cannot be correct. The reason is that the ka is dependent upon the amount of the drug and the fluid volume at the absorption site which may change according to time. For example, if the area of the surface where the drug is absorbed increases by time through passing different parts of the gastrointestinal lumen, the ka will also increase according to Fick's first law. Moreover, fluid volume in the lumen, which determines the luminal drug concentration and thereby ka, may always fluctuate according to the balance between secretion and re-absorption[8]. Therefore, modelers do not necessarily have to use the first-order absorption model despite its popularity.

Unlike the zero-order absorption and first-order absorption models, the TC absorption model has the advantage of allowing the absorption rate to increase gradually. The mean transit time (MTT), one of the parameters used in the TC absorption model, is equivalent to lag time used in the conventional absorption models. In our TC model, the number of TCs was the same (N=1.81) in all subjects. However, the BSV given to MTT in our model allows for the estimation of the lag time difference between subjects. Although no significant difference between the first-order absorption and TC absorption models was observed graphically, the BSV and the intraindividual variability for the parameters of the TC absorption model as well as the OFV and the RMSE were smaller than those of the other 2 models (Table 2).

In this study, the usefulness of a TC absorption model was assessed by comparing it with conventional absorption models. The results of this study may provide basic information improving the therapeutic strategy of tacrolimus which has a narrow therapeutic window with irregular absorption.

Acknowledgements

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