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Delayed trough level measurement with the use of prolonged-release tacrolimus

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Summary

Trough concentrations of prolonged-release tacrolimus are usually measured at 24 h after taking the drug in the morning. It is impractical to measure these trough concentrations in patients who visit the outpatient clinic in the afternoon. Trough concentrations obtained in the afternoon may also be suitable for estimating the 24-h exposure. We therefore aimed to assess the usefulness of tacrolimus concentrations measured at 32 h postdose for therapeutic drug monitoring in renal transplant patients who take prolonged-release tacrolimus. We measured tacrolimus pharmacokinetics in 26 patients using prolonged-release tacrolimus. Eleven blood samples were taken during a period of 32 h after ingestion by use of a validated dried blood spot method. Tacrolimus concentrations were measured with HPLC-tandem mass spectrometry. The mean concentrations at 24 and 32 h postdose were 8.3 μg/l (7.5–9.1) and 6.7 μg/l (6.1–7.4), respectively ($P < 0.0001$). The Spearman correlation coefficients between these concentrations and 24-h exposure were 0.83 and 0.82, respectively (both $P < 0.01$). In conclusion, delayed trough level measurement provides lower values and therefore requires adjustment of the target range. However, levels measured until 32 h after ingestion remain strongly correlated with 24-h exposure. This warrants the use of delayed trough level measurement to improve patient convenience.

Introduction

Tacrolimus is an important component of the current immunosuppressive therapy after renal transplantation. While it was developed as an oral twice-daily formulation, a prolonged-release once-daily formulation was introduced on the market in 2007. A recently published systematic review indicated that the efficacy of prolonged-release tacrolimus is comparable to that of the twice-daily formulation [1]. The nature and incidence of adverse effects do not differ either [2]. Kuijpers *et al.* [3] lately demonstrated that the once-daily administration of tacrolimus improves adherence, which might ultimately contribute to a better graft outcome.

Tacrolimus has a narrow therapeutic window and is characterized by a wide interindividual variability in its pharmacokinetics which is partly explained by genetic polymorphisms of the CYP3A isoenzymes and efflux pumps. Moreover, comedication, hematocrit and albumin, patient age and race, gastrointestinal motility, time after transplantation, and liver function can all affect the pharmacokinetics of this drug [4]. Because of the narrow therapeutic window and high interpatient pharmacokinetic variability, therapeutic drug monitoring (TDM) is indicated for

tacrolimus. It is usually based on measurement of the trough level, which is highly correlated with the total exposure as reflected by the area under the concentration–time curve (AUC) [5,6].

According to the instructions of the manufacturer, blood samples for the trough level of prolonged-release tacrolimus should be obtained in the morning, approximately 24 h after the ingestion. As the apparent elimination half-life of the prolonged-release formulation is relatively long [7,8], the interval between ingestion and blood sampling for trough level measurement might be extended beyond 24 h. In daily clinical practice, a widened timeframe for sample collection to measure the trough level would especially be desirable and convenient for patients who visit the outpatient clinic in the afternoon.

The current study aimed to describe the course of the tacrolimus blood concentration between 24 h (C_{24}) and 32 h (C_{32}) after ingestion of prolonged-release tacrolimus. More specifically, we assessed whether the degree of correlation between C_{32} and AUC in a 24-h period ($AUC_{(0–24\ h)}$) equaled that of between C_{24} and $AUC_{(0–24\ h)}$.

Patients and methods

Study design and population

We performed a prospective pharmacokinetic study to measure the tacrolimus concentration during a period of 32 h after ingestion of prolonged-release tacrolimus in the morning.

Adult renal transplant patients with a stable graft function were eligible for enrollment if they used prolonged-release tacrolimus (Advagraf®, Astellas Ireland Co. Ltd., Killorglin, Ireland) of which the dose was not altered during the last visit to the outpatient clinic. Moreover, the two most recently measured trough levels should be within the target range of 5–10 μg/l. Patients were excluded if they were unable to perform the home-based dried blood spot measurements of tacrolimus levels (see below). Patients with diarrhea (more than 3 stools per day) during the preceding 14 days were also excluded, because diarrhea can affect the pharmacokinetics of tacrolimus [9].

The study was approved by the local ethics committee and conducted in accordance with the Helsinki as well as the Istanbul Declaration. All patients gave written informed consent.

Measurements

A validated dried blood spot method for sampling and analysis of tacrolimus was used, which allowed participants to take their own blood samples at home [10]. Using this method, capillary blood is obtained by a finger prick with an automatic lancet by the patients themselves. Subsequently, the drop of blood is applied to the sampling paper. After drying and transport of the paper to the laboratory, a disk from the blood spot is punched out, extracted and analyzed by high-performance liquid chromatography–tandem mass spectrometry. Values obtained with the dried blood spot technique show an excellent correlation ($r^2 = 0.96$) with those obtained from venous blood sampling, with a limited difference between both methods [11]. Participants received thorough training in using the dried blood spot method prior to performing the pharmacokinetic measurements. Each pharmacokinetic profile started with measurement of the whole blood tacrolimus concentration (C_0) at 24 h after the previous morning ingestion of prolonged-release tacrolimus and after overnight fasting. Subsequently, prolonged-release tacrolimus was taken and blood samples were collected at 1, 2, 4, 8, 12, 24, 26, 28, 30, and 32 h after the ingestion. The participants took prolonged-release tacrolimus on an empty stomach and refrained from food intake until 2 h after drug ingestion because it has been reported that the tacrolimus concentration profile can be influenced by meal consumption [12].

Pharmacokinetic parameters were assessed with non-compartmental methods. C_0 and concentrations at 24, 26, 28, 30, and 32 h postdose were read directly from the pharmacokinetic profile. The $AUC_{(0–24\ h)}$ was calculated by the linear–log trapezoidal rule.

Statistical analysis

Pharmacokinetic parameters and concentrations are described with a geometric mean and 95% confidence interval. T_{max}, daily tacrolimus dose, and time after ingestion are presented as median and range. The other data are shown as mean with standard deviation (SD).

We primarily focused on the correlation between blood concentrations obtained at various time points on the one hand and total exposure as reflected by $AUC_{(0–24\ h)}$ on the other hand. Correlations between blood concentrations measured at 24, 26, 28, 30, or 32 h after drug intake and $AUC_{(0–24\ h)}$ values were assessed with the nonparametric Spearman's rank correlation coefficient (rho). In addition, we analyzed the difference between blood levels measured at 24 and 32 h after taking tacrolimus, using the paired-samples t-test on log-transformed concentration data. A univariate analysis was carried out to identify variables affecting the relative difference between C_{24} and C_{32} concentrations, with the aim to perform a multiple linear regression analysis to evaluate the effect of variables with a P value of less than 0.1 in univariate analysis.

Because of the explorative character of the study, it was difficult to determine the sample size. We intended to include approximately 25 patients because this is an

acceptable and customary number of patients for a descriptive pharmacokinetic study.

All statistical analyses were performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Of the thirty patients who were recruited for the study, 26 patients completed a full pharmacokinetic profile. All 26 participants had an isolated kidney transplantation and were of Caucasian ancestry. Other patient characteristics are shown in Table 1.

The median dose of prolonged-release tacrolimus was 4.0 mg (range 1.5–10.0).

The geometric mean of $AUC_{(0–24\ h)}$ was 288.1 μg h/l (261.6–317.3). Geometric means of C_{24} and C_{32} were 8.3 (7.5–9.1) μg/l and 6.7 (6.1–7.4) μg/l, respectively ($P < 0.0001$). Other pharmacokinetic parameters and geometric mean concentrations at time points 26, 28, and 30 h postdose are summarized in Table 2.

The Spearman's rank correlation coefficients between single blood levels and $AUC_{(0–24\ h)}$ were 0.83, 0.83, 0.84, 0.88, and 0.82 for blood levels taken at 24, 26, 28, 30, and 32 h postdose, respectively ($P < 0.01$ for each time point). The relationship between AUC and blood levels obtained after 24 and 32 h in individual subjects is depicted in Fig. 1. The coefficients of variation of the ratios between $AUC_{(0–24\ h)}$ and C_{24} or C_{32} were 13.2% and 12.1%, respectively.

The median relative difference between the blood levels obtained after 24 and 32 h was −18.7% (range −35.4% to 14.8%). The median relative differences between blood concentrations at 24 h postdose and at 26, 28, and 30 h postdose were −6.7% (−26.1% to 29.5%), −9.5% (−28.3% to 39.4%), and −14.1% (−28.3% to 0.0%), respectively. The relative change in concentration between 24 and 32 h postdose showed no correlation with the absolute mean trough level (Fig. 2). The relative difference between C_{24} and C_{32} was also not significantly associated with age, weight, time after transplantation, hematocrit, albumin, creatinine, estimated glomerular filtration rate, or use of steroids and calcium channel blockers. As univariate analyses revealed no possible predictors of the percent difference between C_{24} and C_{32}, no multivariate analysis was performed.

Table 1. Patient characteristics ($n = 26$).

Male (%)	69
Age (years)	46.5 ± 13.4
Weight (kg)	80.0 ± 15.4
Time after transplantation (years)	5.6 ± 3.9
eGFR (ml/min/1.73 m^2)	49.9 ± 14.5
Hematocrit	0.39 ± 0.05
Albumin (g/l)	38.6 ± 3.2
Use of calcium channel blockers (%)	58
Use of steroids (%)	77

Data are shown as mean with standard deviation.

Table 2. Pharmacokinetic data for tacrolimus administered once-daily in a prolonged-release formulation ($n = 26$).

$AUC_{(0–24\ h)}$ (μg h/l)	288.1 (261.6–317.3)
C_0 (μg/l)	7.7 (7.0–8.5)
C_{max} (μg/l)	22.1 (19.4–25.2)
T_{max} (h)	2.0 (1.0–4.1)
C_{24} (μg/l)	8.3 (7.5–9.1)
C_{26} (μg/l)	7.8 (7.1–8.6)
C_{28} (μg/l)	7.5 (6.8–8.3)
C_{30} (μg/l)	7.1 (6.4–7.9)
C_{32} (μg/l)	6.7 (6.1–7.4)
$T_{½}$ (h)	29.4 (26.3–33.0)

Data are shown as geometric mean (95% confidence interval) except in the case of T_{max}, which is shown as median and range. AUC (0–24 h): area under the curve in a 24-h period; C0, predose trough level; C_{max}, maximal concentration; T_{max}, time for reaching C_{max}; C24, level after 24 h; C26, level after 26 h; C28, level after 28 h; C30, level after 30 h; C32, level after 32 h; $T_{½}$, elimination half-time.

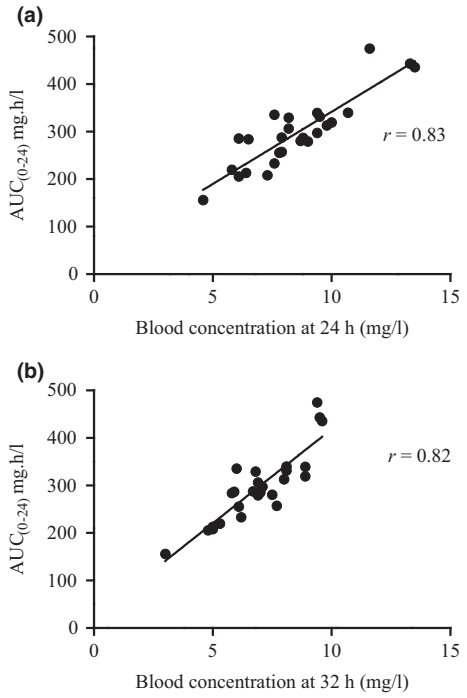

Figure 1 Correlation between 24-h area under the curve ($AUC_{0–24\ h}$) and tacrolimus blood concentration. Correlation coefficients relate to Spearman's rho. (a) tacrolimus blood concentration at 24 h after ingestion. (b) tacrolimus blood concentration at 32 h after ingestion.

Discussion

To our knowledge, this is the first prospective study to assess the pharmacokinetics of prolonged-release

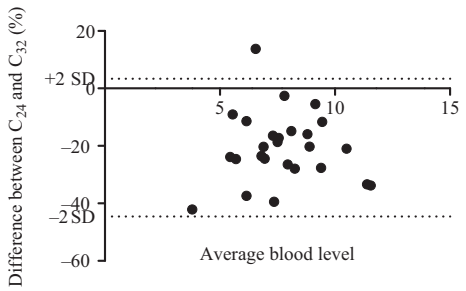

Figure 2 Bland–Altman plot of percent difference between tacrolimus concentrations at 24 h and 32 h postdose versus the mean blood concentration.

tacrolimus beyond 24 h after its intake in solid organ transplant recipients. We showed that $AUC_{(0–24\ h)}$ was strongly correlated to the blood concentration at all time points measured between 24 and 32 h postdose. The collection of the blood sample to measure the trough level can therefore be postponed until 32 h after ingestion for the purpose of TDM of prolonged-release tacrolimus. For daily clinical practice, this provides the opportunity for ambulatory patients to visit the outpatient clinic, with measurement of the trough level, in the afternoon. However, adjustment of the target range is required if C_{32} is used for TDM as there was a significant difference between blood levels obtained at 24 and 32 h after ingestion. The average concentration at 32 h postdose was approximately 19% lower than at 24 h.

Prolonged-release tacrolimus was developed by adding a combination of ethylcellulose, hypromellose, and lactose monohydrate to tacrolimus [2]. Of these, ethylcellulose changes drug release by controlling water penetration and hypromellose affects drug release by forming a protective polymer gel layer around the drug. Consequently, tacrolimus once-daily is slowly released along the gastrointestinal tract which has been shown to delay T_{max} and reduce C_{max} in stable renal transplant recipients [2,6].

Tacrolimus trough levels are commonly used as marker for the exposure because it is not practically feasible in daily clinical practice to perform a pharmacokinetic profile. The correlation between trough level and $AUC_{(0–24\ h)}$ is high and similar for the prolonged-release and the regular formulation of tacrolimus [5,6]. In this study, we showed that also beyond 24 h after the intake of prolonged-release tacrolimus, the correlation between trough level and $AUC_{(0–24\ h)}$ remains equally high. Moreover, the coefficients of variation of the ratios between $AUC_{(0–24\ h)}$ and C_{24} and between $AUC_{(0–24\ h)}$ and C_{32} are comparable. An average decline in tacrolimus concentrations occurred from 24 to 32 h postdose, but differences between tacrolimus concentrations at 24 and 32 h postdose varied from a rise of 15% to a decline of 35% in individual patients. These differences show that despite a high correlation between blood concentrations at 24 h or 32 h postdose and $AUC_{(0–24\ h)}$, wide interpatient variability of the shape of the tacrolimus concentration time curve exists. Hence, similar concentrations measured at 24 h or 32 h represent a range of $AUC_{(0–24\ h)}$ in individual patients, and this variability should be considered if these blood concentrations are assessed.

According to the manufacturer, the elimination half-life of prolonged-release tacrolimus in healthy volunteers is approximately 43 h. In our study, we found an average apparent elimination half-life of 29.4 h, much less than 43 h. It should be noted that the observation period of 32 h is too short for accurate estimation of such a long elimination half-life, yet the difference with the elimination half-life data found in healthy volunteers appeared to be rather large. This discrepancy between healthy volunteers and renal transplant patients has also been observed in users of tacrolimus twice-daily [4]. Potential explanations for this difference are enzyme induction by use of steroids and a lower hematocrit and albumin concentration, resulting in a larger free fraction of tacrolimus, in renal transplant recipients [4,13].

Parameters that can influence the pharmacokinetics of the tacrolimus twice-daily formulation include comedication, hematocrit and albumin, patient age and race, gastrointestinal motility, time after transplantation, and genetic polymorphisms of the CYP3A isoenzymes and P-glycoprotein pump [4]. The same parameters could also affect the pharmacokinetics of the prolonged-release formulation because the active constituent of both preparations is identical. Nevertheless, we found no significant effect of age, weight, time after transplantation, hematocrit, albumin, and use of steroids and calcium channel blockers on the decrease in tacrolimus blood levels between 24 and 32 h after ingestion.

The TDM for prolonged-release tacrolimus is usually performed by measuring the trough level as a reflection of total exposure. In agreement with several other studies, we showed a strong correlation between trough level and $AUC_{(0–24\ h)}$ in renal transplant patients ($r > 0.80$) [6,14].

Using the C_{32} (or another concentration measured beyond 24 h after the dose) for TDM offers a practical solution for ambulatory patients who visit the outpatient clinic in the afternoon. This strategy implies that on the day of examination, patients take their prolonged-release tacrolimus dose in the afternoon (after collection of the blood sample). The occasional prolongation of the dosing interval with 8 h appears to be safe because the C_{32} is on average only 19% lower than the C_{24}. Clearly, the target range requires adjustment for late tacrolimus measurements and this adjustment can be calculated straightforward based on the average decline of tacrolimus concentrations beyond 24 h after the dose (Table 2).

Alternatively, the data from this study (i.e., population pharmacokinetic data, Table 2) can be used to build a Bayesian forecasting model. In such a model, the *a priori* population pharmacokinetic parameters, the patient's drug dosing information and measured blood concentrations are used to assess *a posteriori* pharmacokinetic parameters. Use of the Bayesian approach offers more flexibility regarding time point of measurement, but it requires expertise with specific software. A pharmacist or clinical pharmacologist has to be involved when using the Bayesian approach. Another alternative option for patients, who visit the outpatient clinic in the afternoon, is to use the dried blood spot test for sampling in the morning at home. However, this technique requires careful training and is not suitable for every patient. Finally, delay of the intake of prolonged-release tacrolimus at the day before the office visit to 24 h before blood sampling is not a good alternative because tacrolimus pharmacokinetics is affected by diurnal variations. In healthy volunteers, administration of prolonged-release tacrolimus in the evening reduced the $AUC_{(0–24\ h)}$ by 35% as compared by intake in the morning [5].

In conclusion, delayed trough level measurement during use of prolonged-release tacrolimus formulation provides a good estimate of the $AUC_{(0–24\ h),}$ although the target range has to be lowered. This allows planning visits to the outpatient clinic with blood sampling for measuring the tacrolimus trough level in the morning as well as in the afternoon.

Authorship

GvB: designed the study, performed the research, analyzed the data, and wrote the manuscript. RA: analyzed the data and wrote the manuscript. KH: performed the research and wrote the manuscript. TH: performed the research and wrote the manuscript. LH: designed the study, analyzed the data, and wrote the manuscript.

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