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Rectal and sublingual administration of tacrolimus: a single-dose pharmacokinetic study in healthy volunteers

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- In lung and liver transplant recipients, sublingual administration of tacrolimus has been suggested.
- Systemic exposure to rectally administered tacrolimus has been demonstrated before and is being used in the treatment of refractory distal colitis.
- No well-powered pharmacokinetic and safety data exist on these tacrolimus formulations.

WHAT THIS STUDY ADDS

- True sublingual administration of tacrolimus does not result in systemic exposure.
- Rectal application of tacrolimus gives consistent clinically relevant tacrolimus exposure and may represent an alternative formulation.
- Systemic side-effects should be considered when tacrolimus is administered rectally, as in the treatment of distal colitis.

AIMS

The immunosuppressant tacrolimus is usually administered orally. When this is not feasible, other routes of administration may be useful. Previous research suggested that tacrolimus may be applied sublingually or rectally. Pharmacokinetic data are sparse. The aim of this study was to investigate and compare the pharmacokinetics of these alternative formulations with orally administered tacrolimus.

METHODS

Three single, fixed-dose formulations of tacrolimus were administered in a random sequence in 18 healthy subjects, using a cross-over study design. For sublingual administration, 3 mg of powder obtained from oral capsules was applied under the tongue for a period of 15 min without swallowing, with mouth rinsing afterwards. For rectal administration, a suppository containing 15 mg of the oral powder was used. Oral administration consisted of 7 mg of instant-release tacrolimus capsules (Prograf). Main pharmacokinetic outcome parameters were compared by ANOVA.

RESULTS

Sublingual administration showed no clinically significant exposure, contrary to rectal administration, where all subjects had clinically relevant exposure, with a lower relative bioavailability (78%), a lower maximal blood concentration and a later time of maximal blood concentration compared with oral administration.

CONCLUSIONS

Sublingual administration of a single dose of tacrolimus does not result in systemic exposure if care is taken not to swallow saliva and to rinse the oral cavity afterwards. Rectal administration of tacrolimus results in clinically relevant systemic exposure and might represent an alternative formulation in case oral administration is not feasible. When used as a topical agent, systemic side-effects should be considered.

Introduction

Tacrolimus is a commonly used immunosuppressant in the field of organ transplantation. It is available as a capsule for oral administration. In some clinical conditions (e.g. nausea, vomiting, sedation or intubation), oral administration is less feasible. An intravenous formulation is available; however, intravenous administration has been associated with an increased incidence of adverse drug reactions in comparison to oral administration [1, 2]. Several reasons for this increased toxicity have been proposed. First, there is an increased risk of overexposure owing to unfortunate dose calculations, excessive oral to intravenous conversion rates or inappropriate dilutions for infusion [3]. Second, there may be hypersensitivity to polyoxyethylated hydrogenated castor oil, a constituent of the intravenous solution [4]. Besides, intravenous administration cannot be used for maintenance therapy. In this context, an alternative route of administration would be useful.

Previously, Reams *et al*. measured therapeutic trough concentrations after sublingual tacrolimus administration (0.04 mg kg^{-1}) in lung transplantation recipients suffering from cystic fibrosis [5]. Likewise, a recently published paper reports significant tacrolimus exposure after sublingual administration in six liver transplant recipients [6]. In a pilot study by our group with three renal transplant patients, we could not confirm tacrolimus absorption after sublingual application [7]. Likewise, Romero *et al*. report a case of sublingual tacrolimus administration in a renal transplant patient, in whom the blood concentration–time profile observed suggests absorption in the lower digestive tract rather than the sublingual mucosa [8].

Systemic exposure to tacrolimus has been demonstrated in healthy rats treated with high doses of rectally administered tacrolimus [9]. Previous studies in patients with distal colitis suggest that rectally administered tacrolimus is clinically effective and well tolerated [10, 11]. We previously observed significant blood tacrolimus concentrations after rectal administration of tacrolimus in renal transplant patients [12].

Only few data on the pharmacokinetics and safety of sublingual and rectal tacrolimus administration in humans are available [5–11]. We therefore designed a pharmacokinetic study to investigate sublingual and rectal administration of tacrolimus and to compare it with orally administered tacrolimus in healthy volunteers.

Methods

Design

This study was an open-label, single-dose, three-period cross-over study (Table 1). The administration sequence was randomized and balanced. Randomization was done by drawing a random envelope containing the number of the stratum. The study was approved by the local medical ethics committee and was in accordance with the ethical standards of the responsible committee on human experimentation or with the Helsinki Declaration of 1975 (as revised in 1983). The trial was registered in a trial register (Eudra CT, registration no. 2008-005943-40).

Subjects

Subjects between 18 and 65 years of age were recruited from a database of healthy volunteers. Main exclusion criteria were as follows: regular drug use other than oral contraceptives; abnormal liver biochemistry or renal function; blood pressure >160/100 mmHg; and abnormal electrocardiogram. Females with childbearing potential had to ensure effective contraception during the study period. Informed consent was obtained from all subjects.

Treatment

Formulations Subjects received three different tacrolimus formulations: (i) an instant-release capsule (Prograf; Astellas Pharma, Staines, UK) as the oral formulation; (ii) the powder content from the capsule as the sublingual formulation; and (iii) a suppository containing the powder content from capsule. Suppositories were manufactured according to the Good Manufacturing Practice (GMP) by a GMP-certified hospital pharmacy. The suppositories comprised lactose monohydricum as fill-up on the basis of H15 witepsol.

Dose The tacrolimus formulations were administered as a single dose. The oral capsule was given at a dose of 7 mg (corresponding to 0.1 mg kg^{-1}, the usual dose applied in

Table 1

Overview of study design with six different strata of administration sequence

	Sequence 1	Sequence 2	Sequence 3	Sequence 4	Sequence 5	Sequence 6
Period 1	Sublingual	Sublingual	Oral	Oral	Rectal	Rectal
Period 2	Rectal	Oral	Sublingual	Rectal	Sublingual	Oral
Period 3	Oral	Rectal	Rectal	Sublingual	Oral	Sublingual

clinical practice), the sublingual dose consisted of 3 mg (in accordance with the earlier publication by Reams *et al*. [5]), and the suppository dose amounted 15 mg (derived from our pilot study [12]). The rectal dose was higher than used in case reports of patients with colitis, considering the presence of an intact mucosal barrier in healthy volunteers.

Study procedures Each subject received the above-mentioned formulations in a sequence according to their randomization 7 days apart from each occasion at the same time point in the morning after an overnight fast. Consumption of alcohol and nicotine was prohibited during this phase. On each occasion, an indwelling catheter (Braun Vasofix® Safety, Melsungen, Germany) was inserted into a forearm vein for the purpose of repeated venous blood sampling.

After supervised dosing, the subjects had to remain in a semi-recumbent position for 3 h. The subjects received a standardized continental breakfast 1 h after drug administration. Any adverse event during the study period was recorded.

For sublingual application, the powder content of the oral Prograf capsule was placed under the tongue for 15 min, during which period the subjects were not allowed to swallow. To establish a 'true' sublingual route, the subjects had to rinse their mouth with water (and spit this out) after the 15 min period to prevent consecutive swallowing of residual tacrolimus powder. Any act of swallowing before this time point was recorded.

After rectal application, a minimal interval of 3 h had to be maintained between administration of the suppository and possible defaecation (subjects were instructed to defaecate before administration of the drug). In the event of earlier defaecation, this intervention period had to be repeated on a later occasion, at least 1 week after former drug application.

Blood sampling and assay Three millilitres of venous blood was drawn into an EDTA vacuum tube (Becton and Dickinson, Franklin Lakes, NJ, USA). Blood was sampled predose and during the first hour after drug administration at 5, 10, 20, 30 and 60 min. Thereafter, samples were taken at 2, 4, 6, 8, 10, 12 and 24 h postdose. In the case of rectal administration, samples were also taken at 36 and 48 h after dosing.

The blood samples were stored at 4°C until further analysis between 3 days and 3 weeks later. Tacrolimus concentrations were determined by high-performance chromatography–tandem mass spectroscopy (HPLC/MS/MS) as previously described [13]. The routine assay in venous blood is linear from 1 to 300 $\mu g\ l^{-1}$. Intra-assay precision and accuracy were 3.4, 2.2 and 3.0% and 102, 94 and 94%, respectively at 3.04, 6.23 and 13.0 $\mu g\ l^{-1}$ ($n = 6$). Interassay precision and accuracy were 8.2, 5.2 and 4.6% and 102, 94 and 93% ($n = 9$), respectively. The limit of quantification was 1.0 $\mu g\ l^{-1}$. The laboratory successfully participates in the international tacrolimus proficiency testing scheme (http://www.bioanalytics.co.uk).

Genotyping Each subject had a venous blood sample taken at the screening visit for genetic analysis for cytochrome P450 3A5 polymorphisms. For this purpose, genomic DNA was extracted from all subjects according to the manufacturer's instructions (Qiagen, Leusden, The Netherlands). Real-time polymerase chain reaction (PCR) fluorescence resonance transfer (FRET) assays were used for genotyping with the LightCycler (Roche Diagnostics, Almere, The Netherlands).

Pharmacokinetic outcome parameters

For all formulations, the pharmacokinetic outcome parameters were time to reach maximal concentration (T_{max}), maximal blood concentration (C_{max}) and area under the blood concentration–time curve (AUC) from 0 time to 24 h (AUC_{0-24}). For rectal administration, we measured the AUC from time 0 to 48 h (AUC_{0-48}). The C_{max} and T_{max} were obtained from the raw data. The AUC was measured by the linear trapezoidal rule. The C_{max} and AUC were 'dose normalized' (Dn) by dividing the measured parameter by the respective dose. The relative bioavailability of the rectal formulation was estimated using the rectal-to-oral ratio of the natural log-transformed values for AUC and DnAUC. The intersubject variability was quantified as the coefficient of variation (CV%) according to:

$$CV\% = 100\sqrt{e^{S_{BR}^2} - 1}$$

where S_{BR} is the between-subject standard deviation of the natural log-transformed values.

Statistical analysis

The study was powered to meet the European Medicines Agency regulatory criteria for assessment of bioequivalence [14]. According to these guidelines, the number of evaluable subjects should not be fewer than 12. Considering possible drop-out and the three-period, six-sequence cross-over design, we included 18 (i.e. 6×3) subjects in our study. Demographic data are reported as the means and their standard deviations. Pharmacokinetic data are presented as the geometric means and their 90% confidence intervals (CIs). Data were natural log-transformed prior to statistical analysis and transformed back to the original scale for display of the results. The pharmacokinetic parameters under consideration were compared using ANOVA. The terms used in the ANOVA model were sequence, period and formulation. A P value <0.05 was considered to be statistically significant. Statistical analysis was performed using SPSS 16.0 for Windows, release 16.0.1 (SPSS Inc., Chicago, IL, USA).

Table 2

Overview of pharmacokinetic outcome parameters

Parameter	Oral Geometric mean (90% CI)	Oral CV%	Rectal Geometric mean (90% CI)	Rectal CV%	Ratio rectal/oral (90% CI)
T_{max} (h)	1.58 (1.37–1.80)	44.7	4.23 (3.35–5.10)*	52.3	3.23 (2.28–4.17)
C_{max} (μg l^{-1})	34.5 (30.10–39.50)	34.1	23.1 (19.8–26.9)*	38.7	0.67 (0.57–0.79)
DnC_{max} (μg l^{-1} mg^{-1})	4.9 (4.3–5.6)	34.1	1.5 (1.3–1.8)*	38.7	0.31 (0.27–0.37)
$AUC_{0–24}$ (μg h l^{-1})	194 (170–222)	33.0	292 (233–365)*	59.5	1.5 (1.22–1.85)
$DnAUC_{0–48}$ (μg h l^{-1} mg^{-1})	28 (24–32)	33.0	19 (16–24)*	59.5	0.70 (0.57–0.86)
$AUC_{0–48}$ (μg h l^{-1})	–	–	394 (309–504)	65.2	NA
$DnAUC_{0–48}$ (μg h l^{-1} mg^{-1})	–	–	26 (21–34)	65.2	NA
$T_{1/2}$ (h)	24.0 (19.5–28.5)	36.85	22.8 (21.6–24.0)	27.23	NA

Abbreviations are as follows: AUC, area under the blood concentration-time curve; CI, confidence interval; C_{max}, maximal blood concentration; CV%, between-subject coefficient of variation; Dn, dose-normalized; NA, not applicable; T_{max}, time to reach maximal concentration; $T_{1/2}$, drug concentration half-life. *$P < 0.05$ for oral *vs.* rectal administration, ANOVA.

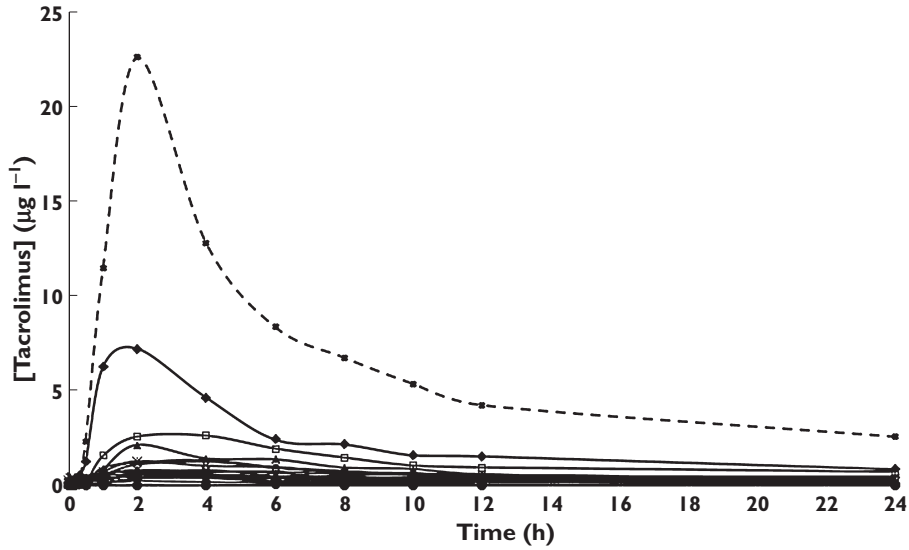

Figure 1

Individual blood concentration–time curves of tacrolimus after sublingual application (raw data, including patients with observed act of swallowing; see main text)

Results

Study population

Eighteen healthy Caucasian subjects (six male and 12 female; age 39 ± 16 years, body mass index 27.3 ± 5.5 kg m^{-2}) were recruited and completed the study. Six female subjects used oral contraception during the study.

Pharmacokinetics

Regarding the single-dose, three-period cross-over design, there was neither a confounding influence of the period nor of the sequence to which the subjects were randomized, confirming the absence of carry-over effect between treatment periods (see Supporting information Table S1). A summary of the pharmacokinetic parameters for the oral and rectal formulations is given in Table 2, while Figure 1 shows the individual blood concentration–time profiles after sublingual tacrolimus administration to all individual subjects.

Sublingual administration of 3 mg of tacrolimus gave no detectable tacrolimus concentrations (i.e. >1 μg l^{-1}) in 11 of the 18 subjects. In seven subjects, tacrolimus concentrations were detected. In three of these seven volunteers, a swallowing act was documented; these subjects had a C_{max} ranging between 1.3 and 22 μg l^{-1} and a T_{max} of 2 h. In the four cases without documented swallowing, the C_{max} ranged from 1.3 to 7.2 μg l^{-1}, also with a T_{max} of 2 h. All subjects reported to have difficulties with keeping the powder content in their mouth without swallowing for the

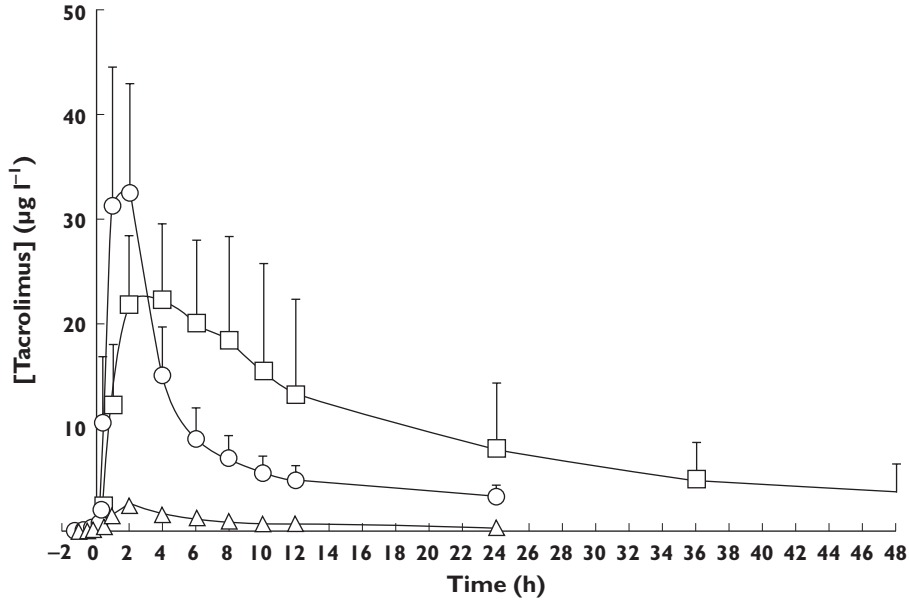

Figure 2

Average (and SD) blood concentration–time curves of the three different formulations. -○-, oral; -□-, rectal; -△-, sublingual

required 15 min. Given that tacrolimus concentrations after 'true' sublingual application (i.e. with the precautions taken to prevent gastrointestinal absorption) were not consistent, no further statistical analysis was performed for the sublingual formulation.

Rectal application of tacrolimus showed clinically relevant systemic exposure in all volunteers (Figure 2). As listed in Table 2, dose-corrected AUCs were numerically lower for the suppository, with a relative bioavailability of ~70% (90% CI 57–86%) after 24 h in comparison to the oral formulation, with a significantly lower DnC_{max} [1.5 µg l^{-1} mg^{-1} (CI 1.3–1.8 µg l^{-1} mg^{-1}) *vs.* 4.9 µg l^{-1} mg^{-1} (CI 4.3–5.6 µg l^{-1} mg^{-1}); $P < 0.001$] and a prolonged T_{max} [4.2 h (CI 3.3–5.1 h) *vs.* 1.58 h (CI 1.4–1.80 h); $P < 0.001$]. After 48 h, the AUC for the rectal formulation amounted to 394 µg h l^{-1} (CI 309–504 µg h l^{-1}), with a trough level of 3.7 µg l^{-1}. Eight of the 18 subjects had a biphasic absorption pattern, as shown in Figure 3 for one representative subject. Volunteers with a biphasic absorption had a numerically higher C_{max} (26.8 *vs.* 22.5 µg l^{-1}) and a statistically significantly higher (Dn)AUC_{0-24} (27 *vs.* 15 µg h l^{-1} mg^{-1}; $P = 0.02$). Rectal application of tacrolimus had a higher intersubject coefficient of variation (CV) of drug exposure compared with the oral reference (Table 2). Volunteers with a biphasic absorption pattern had a 13–27% lower CV with regard to the different pharmacokinetic parameters in comparison to the subjects without a second concentration peak ($P < 0.001$).

The estimated elimination rate constant (K_e) was 0.03 for both the oral and the rectal formulations, with respective corresponding drug elimination half-lives of 24 h (CI 19.5–28.5 h) and 22.8 h (CI 21.6–24.0 h) (see Supporting information Appendix S1 for the calculation of elimination half-life).

Safety

In total, 32 adverse events were reported during the study, none being serious. Most events were classified as 'mild' and five as 'moderate' (headache $n = 3$, 'migraine' $n = 2$). The most often reported adverse events were headache ($n = 10$) and paraesthesia at the application site ($n = 7$). Headache was mostly reported after rectal administration ($n = 6$). Paraesthesia at the application site occurred after sublingual ($n = 5$) and after rectal ($n = 2$) administration (for details, see Supporting information Table S2).

Discussion

Sublingual tacrolimus

To our knowledge, this is the first 'adequately powered' pharmacokinetic study of sublingual and rectal formulations of tacrolimus with direct comparison of these formulations to the pharmacokinetics of the oral formulation in healthy volunteers. We first confirmed our earlier report that, contrary to earlier papers [5, 6], a single 'true' sublingual application (i.e. with the precautions taken to prevent gastrointestinal absorption) of tacrolimus at a dose of 3 mg (being comparable to the dose of 0.04 mg kg^{-1} applied in the study of Reams *et al.* [5]) fails to produce consistent systemic exposure in healthy volunteers.

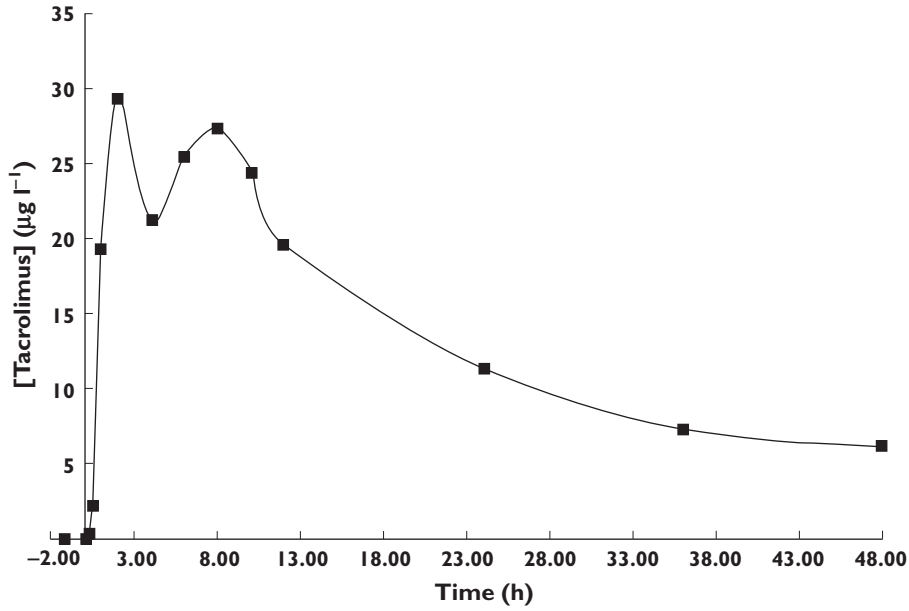

Figure 3

Example bloodconcentration–time curve of an individual after rectal administration of tacrolimus, with a biphasic absorption pattern

In accordance with the findings of Zhang *et al*. [15] and Romero *et al*. [8], the single subject in our series with clinically relevant exposure after sublingual dosing showed a concentration–time profile with a late C_{max} comparable to that of oral dosing, rather than to an early C_{max} as expected after sublingual absorption. The concentration–time profiles in Nasiri-Toosi's study show a similar pattern [6].

Theoretically, the 15 min sublingual administration might not have been long enough to allow for substantial absorption. However, 40% of the drug is dissolved within 15 min, and we would expect fast absorption if true sublingual absorption had taken place with the current formulation with relevant tacrolimus levels [15, 16]. In clinical practice, we assume that it would not be feasible to keep the powder content under the tongue for longer than 15 min. All subjects declared that keeping the drug content sublingual for 15 min without swallowing was very difficult, and indeed, in several subjects swallowing was observed.

Hence we postulate that in Reams' and Nasiri-Toosi's studies normal gastrointestinal absorption occurred after swallowing; the studies do not mention precautions that were taken to prevent this. Subjects in our study were instructed not to swallow during the first 15 min and to rinse their mouth afterwards, with spitting the rinse solution under strict supervision. In addition, a substantial proportion of the subjects mentioned local paraesthesia as a side-effect, underscoring the fact that tacrolimus (although in a small amount) had some local (side-)effect. Thus, in conclusion, we believe that single-dose sublingual tacrolimus application does not result in 'true' sublingual absorption, but that systemic exposure is probably a result of gastrointestinal absorption. Whether repetitive or higher doses might result in sublingual tacrolimus absorption remains unanswered, so far.

Rectal administration

The second goal of our study was to perform an adequately powered pharmacokinetic study of single-dose rectal tacrolimus application through a suppository. All subjects had a clinically relevant systemic tacrolimus exposure. This has two consequences. First, this might be an alternative route for adequate systemic tacrolimus exposure (e.g. in solid organ transplant recipients). Second, rectal administration of tacrolimus with the goal of inducing a local therapeutic effect in patients suffering from colitis may have systemic (side-)effects.

Van Dieren *et al*. reported local efficacy and detectable tacrolimus trough levels (maximum 5 µg ml^{-1}) after repeated rectal administration of 2–4 mg tacrolimus in patients with active colitis [11], which implies systemic therapeutic levels with potential side-effects. It should be considered that patients suffering from inflammatory bowel disease have a diminished mucosal barrier function, resulting in increased and less predictable drug exposure in comparison to healthy volunteers.

Drug exposure after rectal application appeared to be more variable, with almost twice as high intersubject CV for the rectal formulation compared with the oral formulation. We assume that this increased intersubject variability could be explained by the presence of two distinct subpopulations with different absorption patterns, i.e. a

monophasic and a biphasic absorption. The reason for these different patterns is not completely understood. It could be that late defaecation led to incomplete absorption, resulting in a higher variability. By protocol, no subject defaecated within the first 3 h after dosing, but it is not documented whether the subjects did so on a later occasion during their stay at the study site. Another explanation for the second absorption peak could be saturation of the mucosal transport, combined with insufficient delivery of tacrolimus molecules due to poor dissolution (absence of water in the rectum) [17], and subsequent drug absorption in more proximal bowel segments. Mucosal saturation may also be responsible for the later T_{max} and the consistently elevated concentrations after rectal application (Figure 2), which indicates a prolonged absorption phase due to sustained transport over the mucosal barrier and slow release of tacrolimus from the rectal mucosal cells into the circulation. In clinical practice, this could allow for less frequent dosing, which may facilitate drug administration for people who are unable or unwilling (e.g. children) to take the drug via the normal oral route.

As we cannot exclude the possibility that the mucosal surface is a limitation to absorption of a large amount of drug, it might be that if the healthy volunteers were to have received a substantially lower rectal dose, this might have resulted in better bioavailability. However, in our view 15 mg is a relatively small amount of drug in comparison to other lipophilic drugs, such as 500 mg of acetaminophen, which is almost completely absorbed after rectal administration. The extent of absorption also depends on the surface over which the drug can spread after melting of the suppository, and a large suppository will deliver a larger amount of drug than a small one [18]. In this context, we do not assume that the absorption of 15 mg of tacrolimus is limited by its amount or the compartmental size, but by other mechanisms such as mucosal presystemic metabolism of tacrolimus.

Mucosal enzymes such as cytochrome-P450, subfamily 3a5 (Cyp3a5) and P-glycoprotein (P-gp) are major contributors to the presystemic metabolism of tacrolimus [19]. Three of the 18 subjects were heterozygous carriers of the wild-type *Cyp3A5*1* genotype. However, these subjects did not show consistently identical absorption patterns, so that we do not assume that the *Cyp3A5*3* genotype plays a major role. The Cyp3a5 enzyme appears to be localized mainly in the proximal digestive tract [19–21], with decreasing catalytic activity downstream [22]. This makes a contribution of this enzyme with regard to presystemic tacrolimus metabolism limited in the case of rectal application.

The P-gp may play a more important role for active extrusion of the absorbed tacrolimus in the rectum, because P-gp mRNA levels are reported to increase longitudinally along the intestine, with the highest level in the colon [21, 23]. Single-nucleotide polymorphisms of the P-gp may be associated with a decreased protein expression [24]. We were not able to confirm this hypothesis, because we did not determine P-gp single-nucleotide polymorphisms.

Limitations

We achieved our goals of determining whether rectal tacrolimus administration results in systemic exposure and of comparing the pharmacokinetics with orally administered tacrolimus. However, at our latest sample time point (48 h) tacrolimus blood concentrations were still 3.7 $\mu g\ l^{-1}$ after rectal administration; therefore, sampling in future pharmacokinetic studies should be prolonged up to at least 72 h for better evaluation of the elimination phase and bioavailability.

We are aware of the fact that only the relative bioavailability compared with the oral formulation can be given. This is due to the nature of the study; on the one hand, it was meant to provide us, for the first time, with firm pharmacokinetic data on alternative tacrolimus formulations, but on the other hand, it was meant to prove the concept of whether or not these formulations result in significant exposure, at all. In this light, we argued that comparison with the oral formulation would suffice. Given that we found a clinically relevant outcome with potential use of rectal tacrolimus formulations, future studies should provide us with data on the absolute bioavailability, and thus, an intravenous arm will have to be added.

Another issue is the different doses used for each formulation. Due to these different doses, blood concentrations had to be dose corrected, making direct comparison less clear. Nevertheless, these limitations do not undermine our conclusions.

Conclusion and future directions

In conclusion, single sublingual dosing of 3 mg tacrolimus in healthy volunteers does not result in consistent clinically relevant systemic exposure, provided swallowing of the tacrolimus powder (with subsequent gastrointestinal resorption) is prevented. It is not precluded, however, that repetitive and/or escalated dosing or other formulations of tacrolimus (e.g. intravenous solution or solid dispersions [25]) could result in significant drug exposure after sublingual exposure. This is in line with the finding that one in three subjects experienced local paraesthesia after sublingual dosing. This has to be explored further.

On the contrary, rectal application of a single dose of tacrolimus by means of a suppository gives consistent clinically relevant tacrolimus exposure and, therefore, may represent an alternative formulation in circumstances where normal gastrointestinal absorption is not feasible. Future bioequivalence and phase II studies with repetitive ascending dosing should establish the optimal doses and dosing frequencies, which would also supply us with more data on dose proportionality. These studies should then also be carried out in relevant patient populations [e.g.

solid organ transplant recipients or patients with (distal) colitis]. Until then, when applying tacrolimus by suppository, one has to be aware of significant exposure including systemic (side-)effects apart from the local/topical efficacy.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; MC reports grants from Astellas Pharma, outside the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1
Influence of sequence, period and formulation on pharmacokinetic outcome parameters (ANOVA, *P* values)
Table S2
Overview of adverse events
Appendix S1
Calculation of elimination half-life