Original Article

Pharmacokinetic Evaluation Of Tacrolimus Extended-Release Tablets In Rabbit Experimental Model

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Abstract

In this experiment, we compared the pharmacokinetic properties of extended-release tacrolimus tablets to those of a standard medication (Envarsus XR 4 mg). Four healthy rabbits participated in a double-blind, randomised controlled trial. A single dose of 4 mg of each formulation was given to all of the rabbits. There were multiple time points where blood samples were taken (0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 20, 24, 36 and 48 hr). AUC 0-t, AUC 0-∞, and Cmax were the pharmacokinetics parameters computed for the formulations. These bioequivalence criteria are met by the test formulation. The study found that the pharmacokinetic profile of Tacrolimus extended-release tablets was favourable. Comparing the reference and test groups, Tacrolimus AUC_{0-t} and $AUC_{0-\infty}$ were found to be 10973.5 and 10892.2 ng/L×h, respectively. t1/2 and Tmax were 8.5 h and 4.21 h, respectively for tacrolimus extended-release tablets. However, CLz/F and Cmax were 875.1 L/h/kg and 775.8 ng/L, respectively. After testing the drug at a dose of 2000 mg/kg for acute oral toxicity, the drug was found non-toxic. Even with the administration of the drug, no major changes were observed in the aforementioned behavioral, biochemical, or haematological markers. Maximum blood concentration, area under the curve, and half-life values in rabbits were not significantly different between the tacrolimus extended-release tablets and the commercially available Envarsus XR. Consequently, it is anticipated that the produced tablets will be bioequivalent to the commercial medicine, allowing for a once-daily dosage regimen in patients with allogenic rejection. This study suggests that extended-release tablets of tacrolimus may be a viable replacement for the currently used medicine in the role of immune-suppressant.

Keywords: Pharmacokinetics, Tacrolimus, graft rejection, immunosuppressant, organ transplantation, extended release

INTRODUCTION

Clinicians face a significant hurdle when it comes to organ transplantation in the form of graft rejection prevention, and patients are typically forced to adhere to lifelong immunosuppression (Neuberger et al., 2017). Combined with mycophenolate mofetil (MMF), corticosteroids, and either basiliximab induction or no induction, tacrolimus is an immunosuppressive drug used to prevent and treat allograft rejection in recipients of solid organ transplants (Moini et al., 2015; Claeys and Vermeire, 2019; Neuwirt et al., 2019). The macrolide antibiotic family includes tacrolimus, which is one of 23 members. One of the most prescribed medications, it has a limited therapeutic index and high inter- and intra-patient variability. This calls for constant blood-level checks and dosing modifications (Tan and Bunnapradist, 2021).

In the present time, the drug is only available to people who have received a kidney transplant. Liver, heart, and lung transplants have all been examined with the drug, although these uses are not yet approved by the FDA. A once-daily dose schedule offered by an extended-release tacrolimus formulation has the potential to boost patient adherence (Patel et al., 2016). One major cause of transplant rejection and graft loss is patients who fail to take their medications as prescribed. Immunosuppressants are commonly prescribed, and most patients must take several pills every day (Nevins et al., 2017; Banas et al., 2020). Patients' rates of adherence to their treatment plans have been proven to improve when the number of medications they are required to take each day is reduced. In patients who have undergone a liver or kidney transplant for the first time, tacrolimus formulations have shown to have equivalent steady-state systemic exposure (Tremblay et al., 2017; Andrews et al., 2017). Inhibition of interleukin-2 expression and consequent T-lymphocyte activation is how the calcineurin inhibitor tacrolimus produces its immunosuppressive effect (Whitehouse et al., 2017; Ponticelli et al., 2021). This drug is a substrate of P-glycoprotein and is metabolised by cytochrome P4503A enzymes in the liver and small intestine, with varying degrees of oral absorption (Luisa and Alejandro, 2011).

Due to its origin as a fungal entity, tacrolimus shares structural similarities with fungi and other macrolide antibiotics, making it highly metabolised in the pre-systemic route after delivery in the gut wall and the liver. This contributes to its complicated pharmacokinetic (PK) profile. Research shows that African American and Latino patients require larger doses of tacrolimus compared to other ethnic groups (Taber et al., 2015). Due to its variable bioavailability and narrow therapeutic index, therapeutic medication monitoring is critical for optimising outcomes. Because of its association with

total drug exposure (area under the curve from 0 to 24 h; AUC0-24 h) and clinical effectiveness, trough concentrations (Cmin) are the standard monitoring metric (Chavada et al., 2017; Xu et al., 2019).

Class II biopharmaceutical (BCS) drugs have a narrow therapeutic index (NTI) and require careful dosing monitoring to prevent adverse effects and ensure patient safety due to individual differences in response (Tamargo et al., 2015; Papich and Martinez, 2015). We developed a method using the UPLC-MS/MS technique to quantify tacrolimus in human plasma in the present investigation. Hypromellose is used in the modified release formulation of extended-release tacrolimus to slow the drug's absorption in the intestines. An alternative to the twice-daily administration required by the immediate-release form of tacrolimus is a once-daily morning dose, as provided by the prolonged-release form.

MATERIALS AND METHODS

Drugs and Chemicals:

Tacrolimus extended-release tablets reference product (Envarsus XR 4 mg) obtained from retail medical stores. The laboratory chemicals used in this study were from analytical reagent grade.

Tacrolimus extended-release tablets formulation

To create the extended-release tablet of tacrolimus, we used a melt granulation technique, with glyceryl behenate in a quick mixer granulator, in the presence of polyethylene glycol, and Magnesium alumino metasilicate as an adsorbent to impart blend flow. Granules that had been absorbed were crushed, screened, and mixed with other granules like hypromellose (a hydrophilic polymer), lactose monohydrate, and magnesium stearate. Tablets were formed from granules using oval dies.

PHARMACOKINETIC STUDY

Experimental model

Experiment described in this paper was approved by the institution's animal ethics committee (IAEC). We used animals from the National Center for Laboratory Animal Science (NCLAS), located in Hyderabad, India. These were four White New Zealand rabbits, each of which was between 2.5 and 3.5 kilogram in weight and 6-12 months old. Animals were transported from NCLAS to CIP Raipur in accordance with CPCSEA regulations. Animals were acclimated in the animal house at CIP, Raipur, where they were kept in conventional polypropylene cages and subjected to laboratory conditions, including a constant temperature of 27°2°C and a relative humidity of 55 to 60%. The ideal lighting schedule consists of 12 hours of light followed by 12 hours of darkness. Animals were provided with a healthy feed and enough of water during the duration of the trial. The study's experimental procedure has been sanctioned, and strict adherence to the directions is maintained throughout the examinations.

Experimental design

To the experiment, white New Zealand rabbits were randomly divided in two groups (each group consisting of four animals). Group 1 received a single dose of Tacrolimus ER tablets, whereas Group 2 received a reference product (Envarsus XR tab 4 mg). It was found that 0.5% sodium carboxymethyl cellulose (CMC-Na) in a solution for oral gavage worked well. Food and water were supplied to the rabbits at the prescribed times.

Drug administration and sample collection

When treating animals, 10 mg per kilogramme of Tacrolimus extended-release tablets is a common dosage. Granules were used to deliver the testdrug. The adsorbent magnesium alumino metasilicate was added to the molten tacrolimus in a quick mixer granulator after the granules were melted with glyceryl behenate and polyethylene glycol. Additional granular ingredients such hypromellose, lactose monohydrate, and magnesium stearate were ground, filtered, and combined with the absorbed granules. Approximately 2 ml of blood was drawn from the marginal ear vein at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 20, 24, 36, and 48 hours after medication delivery, and centrifuged at 3,500 rpm for 10 minutes in a tube containing EDTA. Centrifuging the blood to isolate plasma allowed for UPLC-MS/MS analysis.

Apparatus and conditions

The UPLC-MS/MS setup consisted of an API 5500 triple-quadrupole mass spectrometer and a Waters BEH C18 column (2.1 mm x 100 mm x 1.7 m) (Sciex Co., Ltd., Framingham, MA, USA). The optimal procedure called for a mobile phase B of acetonitrile and a mobile phase A of water containing 0.1% formic acid. To follow the prescribed gradient, Phase B started at 40%, increased linearly to 75% over 4 minutes, and finally increased to 85% over 0.8 minutes. At a flow rate of 0.25 mL/min, this took 1 minute. The UPLC-MS/MS system received the prepared sample (3 L). The temperature of the auto-sampler was set at 4, and the temperature of the column was set at 40°C. An ESI source is built inside the mass spectrometer. When studying Tacrolimus, we monitored ion transitions from 462.2 to 191.0m/z and 451.4 to 71.0 m/z using a declustering potential of 66.5 and 66 V and a collision energy of 35.1 eV (internal standard, IS).

Preparation of standard and quality control samples

Stock methanol solution of 1 mg/mL Tacrolimus was prepared. Tacrolimus solution was produced by diluting stock solutions with methanol. Calibration standards of 50, 200, 1,000, 5,000, 10,000, 15,000, 20,000, and 30,000 ng/mL of Tacrolimus were prepared by spiked plasma/tissue homogenates and sample extraction. Qualitative threshold values of

5, 10, and 2,400 ng/mL were used as low, medium, and high, respectively.

Preparation of sample

Liquid-liquid extraction (LLE) removed Tacrolimus from plasma. Separating 50 L of plasma, 5 L of IS, and 200 L of MTBE took 1 minute of vortexing and 10 minutes of centrifugation at 12,000 rpm. The supernatant was dried in a nitrogen stream. 50 L of the reconstituted, centrifuged for 2 minutes at 12,000 rpm residual solution was aliquoted into sample vials.

Acute toxicity studies

Tacrolimus prolonged-release tablets' acute toxicity was evaluated using OECD 425 criteria. New Zealand white Rabbits (weighing 2500-3500 gm of both sexes) were chosen for the study, and they were observed every 30 minutes for the first four hours and then once per hour for the following 24 hours after the medicine was administered. Behavioral, biochemical, and haematological markers were tracked during the duration of the 14-day study. The effective oral dose was 2 gram per kilogram of body weight.

Behavioral, Biochemical, and Hematological Parameters

Animals were compared to healthy controls in terms of skin tone, body mass index, respiratory rate, muscle spasm, range of motion, and lacrimation. To conclude the study, a patient's heart was punctured and their blood was combined with an anticoagulant. Centrifuged blood sample for five minutes at 4°C and 2000 rpm (SIGMA 1-15K, Germany). Before the serum could be analysed, it was frozen. Hematological and biochemical characteristics were evaluated in the samples. The animals blood was analysed for various haematological parameters, such as bleeding time, hematocrit, clotting time, white blood cells, red blood cells, monocytes, neutrophils, granulocytes, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration and mass. All haematological values were obtained using an AcT diff2 Hematology Analyzer (Beckman Coulter India, Ltd., Mumbai).

Statistics

For the acquisition, we utilised Analyst TF 1.7.1 and Analyst software (AB Sciex, Redwood City, CA, USA). The pharmacokinetic parameters were analysed using the non-compartmental methodology available in DAS 2.1.1. The data was presented as a mean standard deviation. Statistical significance was determined using the Student's t-test and a nonparametric rank-sum test (*$p<0.05$). (SPSS 25.0 software, SPSS Inc., Chicago, IL, USA).

RESULTS

The results demonstrated that SDs manufactured with magnesium aluminometasilicate as a suitable carrier, in addition to excipients including glyceryl behenate, magnesium alumino, and metasilicate, increased the dissolving rate and bioavailability of Tacrolimus. FTIR, DSC, XRD, and SEM studies all backed up the findings, showing that the particles went from crystalline to amorphous (a more soluble state), improved in wettability, reduced in size, and aggregated less.

Pharmacokinetic study

Following an oral gavage administration of tacrolimus to rabbits, plasma levels was measured. Average plasma concentration vs. time curves for a single dosage of Tacrolimus (Figure 1) and a seven-day dosing schedule (Figure 2) are shown. Key pharmacokinetic parameters were determined using a non-compartmental model, the results of which are displayed in Table 1 and analysed using DAS 2.1.1 software. Maximum blood concentration, area under the curve, and half-life values in rabbits were not significantly different between the tacrolimus extended-release tablets and the commercially available Envarsus XR. The area under curve (AUC0-t) for tacrolimus was 10973.5 ng/Lh, while the area under curve (AUC0-∞) for tacrolimus was 10892.2 ng/Lh. Tablets of extended-release tacrolimus had a half-life of 8.5 hours and a maximum concentration after 4.21 hours. The maximum concentration (Cmax) was 775.8 ng/L, while the clearance (CLz/F) was 875.1 L/h/kg.

Table 1: Effects of Tacrolimusextended release tablets on the pharmacokinetic parameters in rabbit plasma

Parameters	Reference drug	Test drug
AUC 0-t (ng/L×h)	10734.3±1944.3	10973.5±3742.07
AUC 0-∞ (ng/L×h)	10836±2041.81	10892.2±3642.19
MRT (h)	12.8±1.11	13.9±1.12
t1/2z (h)	8.2±1.21	8.5±1.64
Tmax (h)	6.21±1.23	6.22±1.48
Vz/F (L/kg)	11734.34±212.58	8226.8±1942.62
CLz/F (L/h/kg)	964.4±168.23	875.1±147.43
Cmax (ng/L)	752.5±179.22	775.8±124.3

Data are represented as mean ±standard deviation (n=4), statistically significant at *P<0.05 compared with the single-day Tacrolimus administration group. AUC0–t, area under the curve of 0 to t; AUC0–∞, area under the curve of 0 to infinity; CLz/F, clearance divided by absorption fraction; Cmax, peak concentration; MRT, mean residence time of 0 to infinity; t1/2z, half time; Tmax, peak time; Vz/F, apparent volume of distribution divided by absorption fraction

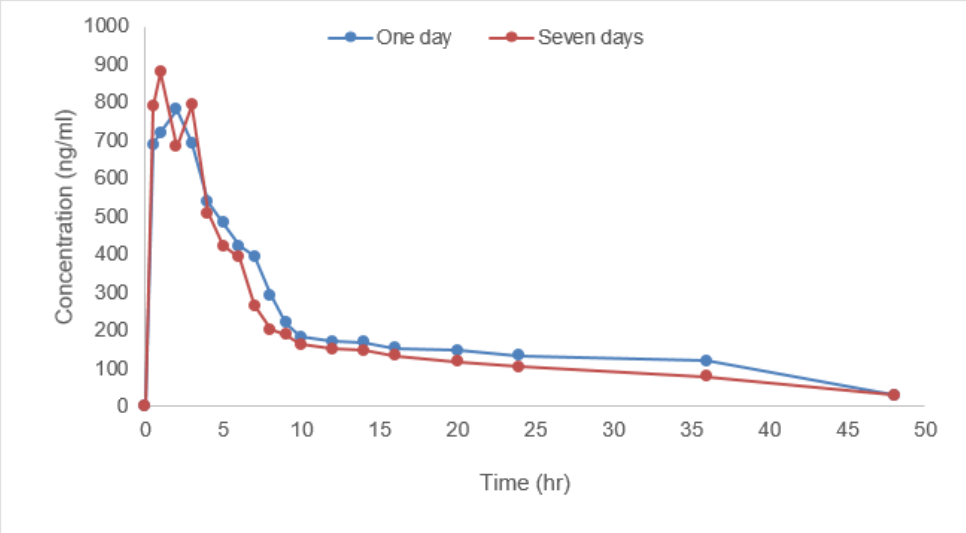

Figure 1: Tacrolimus ER tablets (test product) mean plasma concentration vs. time curves following single day and seven days of dosing. The data (n=4) is presented as a mean± standard deviation.

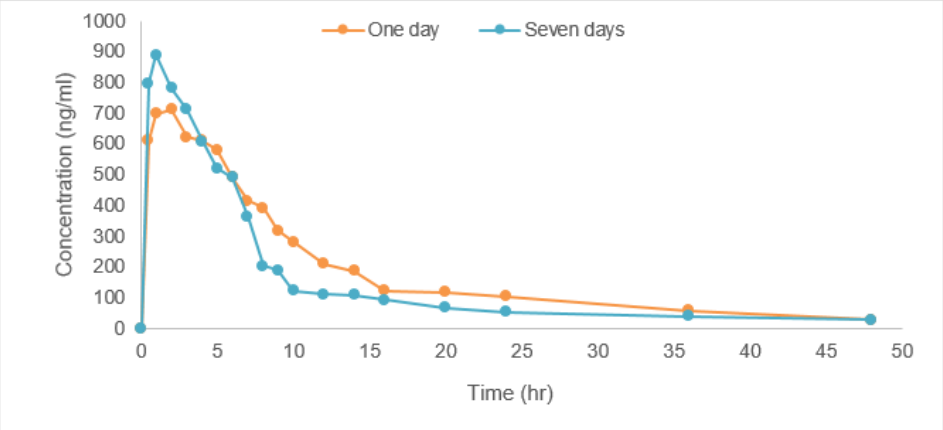

Figure 2: Envarsus XR tablets (Reference product) mean plasma concentration vs. time curve for test drug. The data (n=4) is presented as a mean±standard deviation.

Acute toxicity study

Mortality and clinical outcomes were not affected by the oral administration of Tacrolimus tablets to rabbit for 14 days at doses up to 2000 mg/kg. That led to the determination of an LD50 of over 2,000 mg/kg. Tacrolimus tablets were chosen as an anti-allergy and anti-anaphylaxis treatment based on acute toxicity data from doses of 250 and 500 mg/kg. No noticeable shifts were found in any of the biochemical, haematological, or behavioural measures (Tables 2, 3, and 4).

Table 2: Behavioral parameters of animals after administration of Tacrolimus extended release Tablet

Parameters		Results
Body Weight	:	No significant change
Skin Color	:	Normal
Salivation	:	Normal
Lacrimation	:	Normal
Respiration	:	Normal
Motor activity	:	Normal
Muscle spasm	:	Negative

Table 3: Hematological profile of animals after administration of Tacrolimus extended release Tablets

Parameters	Control	Reference group	Test group
BT(sec)	71±2.21	71.35±1.35	71.40±1.35
CT(sec)	54.3±1.23	565.3±1.24	53.6±1.22
Hemoglobin (g/dL)	12.4±1.11	10.85±1.56	11.72±1.44
Hematocrit (%)	39.2±1.64	38.4±1.22	40.43±1.42
RBC (mill/mcl)	5.42±0.22	5.92±0.62	5.54±1.47
WBC (Thous/mcl)	12.3±1.74	7.05±1.33	9.79±1.11
Neurtophils (%)	44.1±1.24	49±1.77	45.44±1.15
Monocyte (%)	3.2±1.03	2.95±1.54	3.12±1.5
Granulocyte (%)	2.0±0.22	1.65±0.74	2.6±1.55
MCV(fl)	52.1±1.32	51.65±1.5	55.3±2.61
MCH (g)	17.81±1.44	19.01±1.51	18.55±0.51
MCHC (g/dl)	32.3±1.54	30.5±1.34	30.05±1.53

Data are expressed as mean ± SEM.

Table 4: Biochemical profile of animals after administration of Tacrolimus extended release Tablets

Parameters	Control	Reference group	Test group
Glucose(mg/dl**)**	85±1.45	81.45±2.44	84.16±1.44
Cholesterol (mg/dl)	54.5±1.3	50.58±2.13	61.35±1.55
Urea (mg/dl)	18.44±1.58	18.54±1.35	17.45±1.45
Triglycerides(mg/dl)	140.22±1.46	145±1.43	141.42±1.44
SAP (U/l)	136±2.35	136.33±1.25	146.41±2.53
GGT (U/l)	29.44±1.34	34.52±1.4	37.51±1.06
SGOT (U/l)	67.2±1.45	63.59±1.57	61.55±1.58
SGPT (U/l)	45.2±1.58	58.32±2.45	62.2±2.47
LDH (U/l)	143.4±2.57	161.4±3.55	171.53±3.4

Data are expressed as mean ± SEM.

DISCUSSION

End-stage organ disease is best treated with a transplant since it significantly improves survival rates and quality of life (Tonelli et al., 2011; Liem and Weimar, 2009; Schnuelle et al., 1998). Allograft rejection can be prevented with maintenance immunosuppression, but transplant patients typically need to take it for the rest of their lives (Dharnidharka et al., 2016). In the United States, most immunosuppressive regimens revolve around the calcineurin inhibitor tacrolimus. It is an extremely effective exposure-dependent T-cell inhibitor, far more so than other immunosuppressive drugs (Matas et al., 2015). Because of its complicated pharmacokinetic (PK) profile, however, tacrolimus has a narrow therapeutic index with highly variable exposure.

In order to determine the pharmacokinetic profile of extended-release tacrolimus tablets in rabbits, the present study's procedure was developed. The results showed that the produced formulation had a high plasma concentration and a long half-life in the blood. In animals, the extended-release version of tacrolimus had the same Cmax and AUC as the commercially available drug. In addition, the test drug's Tmax and T1/2 values were similar to those of the commercial product, suggesting that the innovative extended-release tablets using magnesium alumino metasilicate as a suitable carrier are bioequivalent to the commercial tablets. The once-daily dose schedule of the commercial product may be equivalent to that of the extended-release tablets of tacrolimus from a pharmacokinetic perspective.

CONCLUSION

In a rabbit pharmacokinetic investigation, the drug's blood concentration was found to gradually increase (Tmax = 6.21 h) and then decrease over time (48 h), with T1/2 values for elimination that were prolonged by 8.5 hours. The pharmacokinetic properties of tacrolimus extended-release tablets were similar to those of the commercially available drug. We conclude that in patients with allogenic rejection, the cellulose derivative used in the extended-release tablets of tacrolimus may be bioequivalent to the commercial Envarsus XR 4 mg.

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