FTY720, a Novel Immunomodulator in de Novo Kidney Transplant Patients: Pharmacokinetics and Exposure-Response Relationship

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The pharmacokinetics, safety, and preliminary efficacy of FTY720, a novel immunomodulator, were examined in de novo renal transplant patients. Both noncompartmental and population methods were used to estimate pharmacokinetic estimates in the patients. The steady-state plasma concentrations of FTY720 increased in accordance with maintenance dose level, indicating linearity in clearance and volume of distribution over the 0.25- to 2.5-mg dose range. The pharmacokinetics of FTY720 in de novo renal transplant patients were characterized by the long terminal phase half-life of approximately 200 hours across doses, high volume of distribution (>3000 L), and low clearance (10.8 L/h). The intersubject variation of clearance was 55%, and the intrasubject variation of FTY720 concentrations was 28%. The population analysis revealed significant positive relationships between baseline alkaline phosphatase and clearance, as well as between baseline body weight on apparent volume of distribution. There was no relationship between FTY720 concentrations within a given FTY720 dose cohort and the rate of allograft rejection.

Keywords: *Concentration-efficacy relationship; kidney transplantation; population methods*

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In recent years, a number of new immunosuppressive agents have become available for use in transplantation. These include drugs such as tacrolimus, sirolimus, and mycophenolate mofetil (MMF). These agents elicit their pharmacological responses via antagonism of calcineurin, target of rapamycin, or inosine monophosphate dehydrogenase, respectively.[1,2] One recently discovered drug, which is currently in clinical development, is FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol). FTY720 is the first in a new class of immunomodulators—sphingosine 1-phosphate receptor agonists.[3-5] This novel agent protects transplanted grafts by reducing the recirculation of lymphocytes to tissues and grafts by FTY720-induced sequestration of the lymphocytes into secondary lymphoid tissues.[6-12] Unlike classical immunosuppressive agents, FTY720 does not significantly impair T cell activation or function and thus may not substantially deprive the host of the ability to fight infections.[13] Recently, it was shown that FTY720 is phosphorylated by sphingosine kinase and that this metabolite serves as a potent agonist of sphingosine 1-phosphate receptors.[13] In a dose-finding phase I trial, FTY720 was given to 20 stable renal transplant patients in combination with cyclosporine (CsA).[14] The FTY720 doses ranged from 0.25 to 3.5 mg, and overall, the drug was found to possess a favorable preliminary safety profile in the study subjects. The single-dose versus the area under the blood concentration-time curve (AUC) relationship was linear. The drug was apparently absorbed slowly (t_{max} >12 h) and exhibited log-linear monoexponential decline in the elimination phase. The drug appeared to have a low oral clearance (CL/F) in relation to hepatic blood flow but a very large oral volume of distribution (V/F) of 20 L/kg, which caused it to possess a long terminal phase half-life ($t_{1/2}$)

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of 4 to 5 days.[14] Similar pharmacokinetic results were also measured in a multiple-dose study of FTY720 in stable renal transplant patients.[3]

In this report, we describe the results of a phase II trial involving a larger number of patients than previously studied, thereby permitting an assessment of the safety and preliminary efficacy and pharmacokinetic properties of FTY720 with repeated dosing in de novo renal transplant patients. In addition to noncompartmental pharmacokinetic analysis of the FTY720 blood concentration data, we conducted a population pharmacokinetic analysis to aid in identifying clinical factors that might affect the blood concentrations of the drug.

METHODS

Study Design

Pharmacokinetic assessments of FTY720 were made in patients who had undergone de novo renal transplantation. The trial involved patients from multiple centers in the European Union, Brazil, Canada, and the United States, and the design was randomized, open label, active controlled, and time staggered. In addition to the pharmacokinetic study, measurements of safety, tolerability, and preliminary efficacy were conducted. The study was approved by the institutional review boards within countries that participated in the studies.

Recipients of primary cadaveric or non–human leukocyte antigen (non-HLA) identical living donor kidneys were randomized within 24 hours of transplantation to 1 of 5 groups given a 3-drug regimen consisting of oral CsA microemulsion (Neoral formulation, Novartis, East Hanover, NJ), corticosteroids, and either oral FTY720 or oral MMF. Patients in group 1 received FTY720 as an initial 1-mg dose, followed the next morning by 0.25-mg daily maintenance doses. Patients in group 2 were given an initial dose of 2 mg/kg of FTY720 after renal transplantation, followed by a 0.5-mg daily maintenance dose. Those in groups 3 and 4 received a first dose of 4 mg, followed by daily maintenance doses of either 1.0 mg or 2.5 mg, respectively. To permit a comparison between FTY720 treatment and alternative therapy, patients allocated to group 5 were given daily doses of MMF 2 g in divided doses instead of FTY720. In each patient, the study medication was administered for 12 weeks. Patients were included only after it had been determined that their allografts were functional.

Blood Collection for Determination of FTY720 and Metabolites

Samples of whole blood were drawn into ethylenediaminetetraacetic acid–containing tubes. Blood samples were frozen within 60 minutes of venipuncture and stored at −20°C pending analysis. Samples for analysis of FTY720 whole-blood trough levels were drawn 5 minutes before the initial dose of FTY720, 5 minutes before the administration of the first FTY720 maintenance dose, and 5 minutes before predetermined weekly doses during the 12-week treatment period. During the posttreatment follow-up period, additional blood sampling was performed for those patients who had maintained a consistent 4-week regimen of FTY720 at 2, 6, 10, 24, 48, 72, and 96 hours after the last dose of FTY720. Further samples were obtained at weeks 13, 14, 15, and 16 during the 12-week posttreatment follow-up period or 1, 2, 3, and 4 weeks after discontinuation of study medication for those patients who discontinued FTY720 prior to the conclusion of the 12-week treatment period. After FTY720 was initiated, in a subset of 4 patients, the whole-blood concentrations of the FTY720 metabolites, M2 and M3, were determined once weekly.

In all patients, trough blood samples for measurement of CsA whole-blood concentrations were also collected throughout the treatment period to permit CsA therapeutic drug monitoring and dosage adjustment as necessary. These samples were drawn prior to the morning dose of CsA and study medication at day 2, weekly during the 12-week treatment period, and every 4 weeks thereafter.

Drug Assay

FTY720 and its metabolites were analyzed in whole blood by a validated liquid chromatography method with tandem mass spectrometry (HPLC/MS/MS) in selected reaction monitoring mode using atmospheric pressure chemical ionization (APCI) as an interface.[14] [^{2}H]FTY720 was used as an internal standard. For FTY720, as well as its internal standard and metabolites, the APCI conditions were as follows:

Sheath gas pressure: nitrogen, 30 to 40 psi
Capillary temperature: 175 to 200°C
Vaporizer temperature: 400 to 430°C
Corona discharge: 5 μA

Mass spectrometer conditions:

Manifold temperature: 70°C, selected reaction monitoring
Detection: positive ions
Dynode: 15 kV
Electron multiplier: 1000 to 1630 V
Collision energy: −19 to −23 Ev
Collision gas: argon, 3.0 mTorr
Mass resolution: 0.7 amu
Scan time: 0.2 to 0.5 seconds for FTY720, [^{2}H]FTY720, and M2 and M3 metabolites (FTY720: parent m/z 308.2, daughter m/z 255.0; [^{2}H]FTY720: parent m/z 312.1, daughter m/z 259.2; M2: parent m/z 310.3, daughter m/z 142.9; M3: parent m/z 282.2, daughter m/z 199.1)

Within-study assay validation was performed by analysis of quality control samples together with the study samples. The limit of quantitation for FTY720 was 0.075 ng/mL and 0.3 ng/mL for both metabolites (M2/M3). The method was validated extensively with a mean accuracy and precision for different nominal concentrations of 104% to 109% and 5% to 15%, respectively. Whole-blood concentrations of CsA were measured by radioimmunoassay.

Noncompartmental Pharmacokinetic Analysis of FTY720, Metabolites (M2, M3), and Cyclosporine

Noncompartmental pharmacokinetic parameters were determined using Microsoft Excel (Redmond, Wash) and WinNonlin Pro Version 3.1 (Pharsight, Mountain View, Calif) computer programs. The pharmacokinetic parameters derived from the observed data were trough steady-state blood concentration (C_{ss}), steady-state AUC (AUC_{ss}), CL/F, terminal phase rate constant (k), and $t_{1/2}$. The observed FTY720 blood trough C_{ss} were used to assess dose proportionality and to derive the FTY720 AUC_{ss} ($C_{ss} \times \tau$) and CL/F, which was calculated as

$$\frac{CL}{F} = \frac{Dose}{AUC_{ss}},$$

The time to C_{ss} (t_{ss}) was determined by visual inspection of the mean time course of FTY720 concentrations in each treatment group. The FTY720 blood concentrations from each patient between t_{ss} and week 12 (day 84) were compiled and median steady-state concentration calculated for each patient. The FTY720 blood concentrations in the posttreatment terminal elimination phase were used to determine the terminal phase $t_{1/2}$ of FTY720 in each treatment group. These approaches were used for the pharmacokinetic evaluations of the FTY720 metabolites, M2 and M3.

The observed CsA blood C_{ss} values from each patient were pooled and used to assess the effect of FTY720 treatment on steady-state pharmacokinetics of CsA. Only those CsA concentrations, coinciding with the steady-state FTY720 period (days 42-84), were employed in formal statistical analysis using the linear mixed-effect model (PROC MIXED) implemented in SAS.

Population Pharmacokinetic Modeling of FTY720

Population pharmacokinetic modeling was used to explore the relationship between FTY720 pharmacokinetics and covariates of patient demographics, indices of hepatic and renal function, effects of diabetes, and comedication with beta-blockers. This analysis was specifically used to understand the role of covariates in the pharmacokinetic variability of FTY720 in de novo renal transplant patients.

The pharmacokinetic model used to best describe the FTY720 concentration-time profile[14] was a 1-compartment model with first-order absorption and elimination. The concentration of FTY720 after a single oral administration was estimated by[4]

$$Conc = \frac{Dose \times ka}{V/F \times (ka - k)} [e^{k \times t} - e^{ka \times t}],$$

where F, ka, CL, V, and k (calculated as CL/V) are the fraction of oral dose bioavailability, residual rate constant (assumed to represent absorption rate constant), plasma clearance, central volume of distribution, and terminal rate constant (assumed to represent elimination rate constant) for each patient, respectively. The pharmacokinetic parameters estimated in the pharmacostatistical model were apparent CL/F, apparent V/F, and ka. The covariates included in the analysis were body weight, gender, age, race, baseline creatinine, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, history of diabetes, and coadministration of beta-blockers.

In the model-building process, covariates were added into or removed from the model based on maximum likelihood ratio tests, with a significance level of 1%, and by means of diagnostic plots. The model-building process comprised several steps. Initially, the statistical significance of covariates added to the base model individually was based on changes in the minimum objective function ($\Delta MOF = 3.84$, $P = .05$). In the next step, all significant covariates were then evaluated by adding them in combination on pharmacokinetic parameters of the base model. Comparison of 2 nested models (where 1 model is entirely contained within a second model) was based on change in ΔMOF, agreement between predicted and observed concentrations,

and the magnitude and randomness of residual values. The ΔMOF produced by adding a covariate to the model approximates a chi-square distribution with n degrees of freedom, where n equals the difference in the number of parameters between the models.

To model the intrasubject variability, an error model with both multiplicative (ε1) and additive (ε2) errors was assumed. The model was $Y = [S1] + [S1] \cdot \varepsilon 1 + \varepsilon 2$, where Y and [S1] were the observed and predicted concentrations, respectively, and ε1 and ε2 were 2 independent normal random variates with a common zero mean and unknown variances σ12 and σ22, respectively.

The intersubject variability of CL/F and V/F was modeled by exponential random effects as follows:

$$\text{CL/F intersubject variability} = (\text{typical value of CL/F}) \times \exp(\eta 1),$$

and

$$\text{V/F intersubject variability} = (\text{typical value of V/F}) \times \exp(\eta 2).$$

The random effects (ηs) were assumed to be normally distributed but with arbitrary correlation between ηs. The intersubject variation of ka was not estimated due to the lack of samples prior to attainment of C_{max}; samples were typically taken 12 to 24 hours postdose.

To estimate the effects of continuous covariates on CL/F and V/F, each average parameter value was multiplied by (covariate)$^{\theta}$. For assessing the effect of dichotomous covariates, each average value was multiplied by $\theta^{covariate}$. The magnitude of the covariate effect was determined based on the data, and covariates of the continuous type were typically standardized, divided by their corresponding medians prior to model fitting.

Parameters in all investigated population models were estimated using double-precision NONMEM Version V, Level 1.1 (NONMEM Project Group, San Francisco, Calif). The first-order conditional method with the interaction option (METHOD = 1 INTERACTION) was used in all calculations.

Prior to evaluation of covariate effects, the following dose-(CL/F) relationship was added to the base model to test for dose proportionality:

$$\text{Typical value of CL/F} = (\text{average CL/F}) \times (\text{Dose})^{\theta}.$$

Linear mixed-effect models were used to evaluate the dose proportionality and time dependency of FTY720 pharmacokinetics by comparing C_{ss} at 7 different visits (days 42, 49, 56, 63, 70, 77, and 84).

The concordance between the observed steady-state FTY720 concentrations and population model–derived average C_{ss} was tested to complement the model validation using a bootstrap procedure. The possible influence of FTY720 coadministration on the C_{ss} of CsA was evaluated using linear mixed-effect models.

Relationship Between Efficacy and FTY720 Steady-State Concentrations

The first occurrence of biopsy-confirmed rejection within 12 weeks of administration of the initial dose of FTY720 was matched to the FTY720 blood concentration most proximal to the time of rejection. The median steady-state FTY720 concentrations from each subject were pooled and summarized using median, 25th, and 75th percentile statistics. The statistics were classified according to the efficacy outcome of the presence or absence of biopsy-confirmed rejection.

For each patient, the Kruskal-Wallis test was used to determine the relationship between the first biopsy-confirmed rejection in the initial 12 weeks of FTY720 administration and the median steady-state FTY720 blood concentration.

Hematological, clinical biochemical, and cardiovascular parameters and other indicators of drug safety were monitored and recorded throughout the course of the study.

RESULTS

Pharmacokinetic Results

Demographic and baseline data for the study subjects are shown in Table I. Sample makeup by race of patients given FTY720 was 73% Caucasian, 11% African American, 3% Asian, and 12% Other. Based on the observed time course of trough FTY720 blood concentrations during the 12-week dosing period, a plateau was reached by day 42 (week 6), giving rise to sustained blood levels for the remainder of the dosing period (Figure 1). Therefore, t_{ss} was judged to occur on week 7 (Figure 1), and 155 patients who successfully completed 7 weeks of therapy were included in the noncompartmental pharmacokinetic analysis of FTY720. This included 40, 40, 38, and 37 patients in each of groups 1 through 4, respectively. A total of 163 subjects were included in the population pharmacokinetic analysis of FTY720 (Table I). A total of 196 patients were used in pharmacokinetic calculations of CsA, of which 42, 41, 39, 37, and 37 patients were allocated to groups 1 through 5, respectively. Among the study sample, a medical history related to diabetes was recorded in 31 patients, and 91 subjects received treatment with beta-blockers.

Table I Baseline Demographic Statistics of Patients Given FTY720

	Mean ± SD	(Range)
Gender	91 M, 72 F	
Age, y	46.3 ± 11.8	(19-69)
Weight, kg	70.9 ± 15.6	(40-115)
Albumin, g/L	34.0 ± 4.4	(25-46)
Alkaline phosphatase, U/L	64.1 ± 29.4	(18-192)
Bilirubin, μmol/L	7.8 ± 4.3	(1-39)
Creatinine, μmol/L[a]	471 ± 225	(114-1432)
Aspartate aminotransferase, U/L	24.6 ± 26.6	(7-283)
Alanine aminotransferase, U/L	22.3 ± 35.3	(3-373)

After completion of FTY720 dosing, terminal phase $t_{1/2}$ was determined in most of the study subjects. Extended values were observed, with mean values generally exceeding 200 hours across all 4-dose regimens of FTY720 (Table II). The pharmacokinetic results derived from noncompartmental analysis indicated that CL/F is low (Table II) in relation to average hepatic blood flow.

Median FTY720 C_{ss} were 1.0, 1.9, 4.0, and 8.8 ng/mL in groups 1 through 4, respectively. In general, the FTY720 AUC_{ss} and C_{ss} rose in proportion to the maintenance dose (Table II), and no significant departure from dose proportionality was observed. FTY720 has 2 predominant, inactive metabolites, M2 and M3.[5] In the examination of FTY720 metabolite levels (Figure 1, Table III), the majority of the blood samples yielded undetectable M2 blood concentrations. However, at the highest dose of FTY720 (2.5 mg daily), the M2 metabolite C_{ss} was measured in all 4 patients and attained approximately 6% of the levels of corresponding circulating FTY720 (corrected for molecular weight [MW]). In contrast to M2, the M3 metabolite was detected in all patients from the 4 treatment groups. The observed M3 steady-state concentrations increased proportionally with the increasing dose of FTY720. Relative to FTY720, M3 C_{ss} corresponded to approximately 60% of circulating FTY720. The $t_{1/2}$ of both metabolites was similar to those values observed for FTY720 (Tables II and III).

The effect of baseline ALP (Table IV) and body weight, both of which were positively and significantly associated with CL/F and V/F, respectively, were the only covariates retained in the final pharmacostatistical model (Table V). Other covariates, including age, gender, indices of hepatic and renal function, and history of diabetes, had no significant effect on FTY720 CL/F (Table IV). The population determinations of CL/F, V/F, and $t_{1/2}$ were 10.8 L/h, 3280 L, and 210 h, respectively (Table V), and were in good agreement with the results from noncompartmental analysis (Table II). The predicted C_{ss} derived from CL/F estimates in the population analysis were in good agreement with the observed FTY720 C_{ss} (Figure 2). The intersubject variation of CL/F was 55%, and the intrasubject variation of FTY720 concentrations was 28%. The mean and median values from bootstrapping agreed well with the NONMEM estimates.

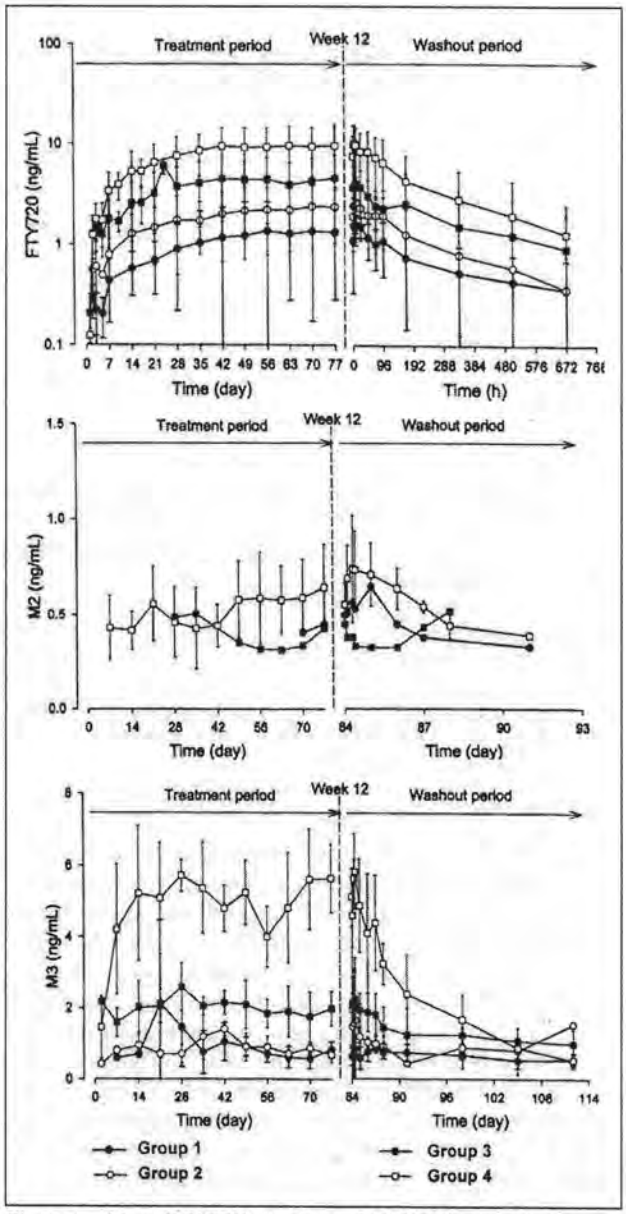

Figure 1. Mean (SD) FTY720, M2, and M3 blood concentration versus time profiles.

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