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Pharmacokinetic/pharmacodynamic relationships of FTY720 in kidney transplant recipients

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Abstract

FTY720 is a new and effective immunosuppressive agent, which produces peripheral blood lymphopenia through a lymphocyte homing effect. We investigated the relationship between the dose of FTY720 or blood concentration (pharmacokinetics, PK) and peripheral lymphopenia (pharmacodynamics, PD) in 23 kidney transplant recipients randomized to receive FTY720 (0.25-2.5 mg/day) or mofetil mycophenolate (2 mg/day) in combination with cyclosporine and steroids. FTY720 dose, blood concentrations and lymphocyte counts were determined weekly before and 4 to 12 weeks after transplantation. The effect of PD was calculated as the absolute lymphocyte count or its reductions. PK/PD modeling was used to find the best-fit model. Mean FTY720 concentrations were 0.36 ± 0.05 (0.25 mg), 0.73 ± 0.12 (0.5 mg), 3.26 ± 0.51 (1 mg), and 7.15 ± 1.41 ng/ml (2.5 mg) between 4 and 12 weeks after transplantation. FTY720 PK was linear with dose (r^2 = 0.98) and showed low inter- and intra-individual variability. FTY720 produced a dose-dependent increase in mean percent reduction of peripheral lymphocyte counts (38 *vs* 42 *vs* 56 *vs* 77, P < 0.01, respectively). The simple E_{max} model [$E = (E_{max} * C)/(C + EC_{50})$] was the best-fit PK/PD modeling for FTY720 dose (E_{max} = 87.8 ± 5.3% and ED_{50} = 0.48 ± 0.08 mg, r^2 = 0.94) or concentration (E_{max} = 78.3 ± 2.9% and EC_{50} = 0.59 ± 0.09 ng/ml, r^2 = 0.89) *vs* effect (% reduction in peripheral lymphocytes). FTY720 PK/PD is dose dependent and follows an E_{max} model (EC_{50} = 0.5 mg or 0.6 ng/ml). Using lymphopenia as an FTY720 PD surrogate marker, high % reductions (~80%) in peripheral lymphocytes are required to achieve best efficacy to prevent acute allograft rejection.

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- Lymphopenia
- Pharmacokinetics
- Pharmacodynamics
- Immunosuppression
- Renal transplants

Introduction

FTY720 (2-amino-2-[2-(4-octylphenyl) ethyl] propane-1,3 diol hydrochloride) is a novel immunomodulator agent developed by a chemical modification of myriocine (1-3), a metabolite isolated from *Iscaria sinclairii* broth (4). This immunomodulator is effective in protecting solid organ grafts from acute rejection in experimental transplant models (5). FTY720 is effective even in the absence of cyclosporine, and also shows

synergistic interactions with cyclosporine, tacrolimus, sirolimus, or everolimus (6-14). FTY720 is currently undergoing phase III clinical trials in kidney transplant recipients after FTY720 doses and combination therapy were identified in phase II studies.

FTY720 produces peripheral lymphopenia in both animals and humans as a consequence of altered T-cell trafficking and redirection to secondary lymphatic organs (15, 16). At therapeutic concentrations, FTY720 does not interfere with cytokine synthesis or binding, does not inhibit cell proliferation and does not cause cell death by apoptosis (17,18). Lymphopenia is fully reversible upon drug discontinuation (19). FTY720 needs to be phosphorylated by sphingosine phosphatase and the phosphorylated form (FTY720-P) appears to mediate its biological effect through the binding to sphingosine-1-phosphate, G-protein-coupled receptors expressed in the membrane of lymphocytes and endothelial cell of lymph nodes (20-22). The proposed molecular mechanism of action is that lymphocyte homing is altered by FTY720 as a sphingosine-1-phosphate agonist targeting G-protein-coupled receptors expressed in the membrane of lymphocytes and endothelial cell of lymph nodes (23). This binding results in drug-receptor internalization, increasing intrinsic lymphocyte mobility and chemotactic response, accelerating lymphocyte homing, and trapping in secondary lymphoid tissues (21,24).

FTY720 has unique pharmacokinetic properties. Its absorption is slow, reaching peak concentrations 8 to 36 h after oral administration (19). FTY720 has a large volume of distribution (1116-1737 L) and a clearance of about 123 to 383 ml/min, resulting in a long elimination half-life of about 108 h (4.5 days) (19). Since it is given once a day, presumably it takes about 4 weeks to reach steady-state concentration, with 11- to 19-fold accumulation in tissues compared to first dose administration (19).

Pharmacokinetic and pharmacodynamic (PK/PD) modeling has been used increasingly in clinical pharmacology and drug development, not only to speed up the development process but, more importantly, to determine the optimal dosage of new drugs, which will deliver an appropriate effect. The relationship between FTY720 pharmacokinetics and pharmacodynamics has been studied in various experimental transplant models (13). The aim of the present study was to determine the relationship among FTY720 dose, blood concentration and surrogate biological effect in the peripheral blood compartment (lymphopenia). This information will help to choose and possibly individualize drug dose regimens, which will result in best efficacy/toxicity relationships.

Material and Methods

Population

Between June 1st and September 30, 2000, 23 kidney transplant recipients were enrolled at our center as part of an international multicenter prospective, open-label, randomized, dose-finding, and exploratory trial, which included 208 patients. The primary objective was to evaluate the safety, tolerability and preliminary efficacy of increasing doses of FTY720 versus mofetil mycophenolate (MMF, CellCept®, Roche Laboratories, New Jersey, NJ, USA) in combination with a cyclosporine microemulsion and prednisone.

The local Medical Ethics Committee approved the protocol and the study was performed in accordance with the Declaration of Helsinki and US Food and Drug Administration guidelines for good clinical practice. All patients signed an informed consent term after being informed of the details of this study, and were enrolled according to study-specific inclusion and exclusion criteria. The present report describes a retrospective analysis of data obtained from 23 patients enrolled at our transplant center during the clinical trial.

Study design

The study involved 24 weeks of observation divided into 12 weeks of treatment and 12 weeks of follow-up. Patients were randomized 24 h after renal transplantation to one of four FTY720 groups (0.25 mg (N = 4), 0.5 mg (N = 4), 1 mg (N = 5), 2.5 mg (N = 5)) or to one MMF group (N = 5). To be enrolled in the study patients had to be between 18 and 65 years old and to be receiving their first living or cadaver donor allografts. All patients received initial doses of cyclosporine of 8 to 10 mg kg^{-1} day^{-1} twice a day adjusted to achieve therapeutic concentrations of 200-400 ng/ml during the first month, 150-250 ng/ml during the second and third months, and 100-200 ng/ml thereafter. Prednisone was introduced two days after the transplant, with initial doses of 0.5 mg kg^{-1} day^{-1} followed by a gradual reduction to 0.2 mg kg^{-1} day^{-1} at 12 weeks post-transplant. Within 24 h after transplant surgery, a loading dose of 1, 2, 4, and 4 mg was administered to patients randomized to receive fixed maintenance doses of 0.25, 0.5, 1, or 2.5 mg/day of FTY720. Patients randomized to the MMF group received 2 g twice a day. FTY720 treatment lasted 12 weeks with the patients being converted to azathioprine, 2 mg kg^{-1} day^{-1}, or MMF, 2 g/day thereafter at the discretion of the investigator. Drug doses were also adjusted for safety and tolerance.

Pharmacokinetics/pharmacodynamics

There were 14 study visits, namely pre-transplant and at 1, 2, 4 to 12 weeks during the treatment phase and 16, 20 and 24 weeks during the follow-up phase. On the occasion of each study visit, blood samples were obtained to measure blood concentrations of cyclosporine and FTY720 and to perform lymphocyte counts.

Pharmacokinetic analysis. Whole blood cyclosporine concentrations were measured daily during the first week and doses were adjusted until therapeutic concentrations (>200 ng/ml) were achieved. Subsequent monitoring was performed twice a week during the first month, once a week during the second month, and every other week thereafter. Whole blood cyclosporine concentrations were measured by a fluorescence polarization immunoassay (Abbott Laboratories, Chicago, IL, USA) according to the manufacturer instructions. Since FTY720 has a long terminal half-live of about 100 h, steady-state concentrations are achieved only after 4 weeks of treatment. Therefore, blood samples were collected to measure whole blood FTY720 concentrations between 4 and 12 weeks. Blood concentrations of FTY720 were determined using a validated HPLC/mass spectrometry/mass spectrometry method from Novartis (East Hanover, NJ, USA).

Pharmacodynamics study. The surrogate marker of the pharmacodynamic effect of FTY720 used in this study was the peripheral lymphocyte count in the blood compartment. Lymphocyte counts were performed on the occasion of each study visit (pre-transplant and 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, and 24 weeks after transplant) in all 5 groups using the CELL-DYN 3200 automatic counter (Abbott Park, Chicago, IL, USA) whose methods of determinations are based on flow cytometry. Since MMF has no effect on peripheral lymphocytes (25), lymphocyte counts obtained from patients receiving MMF were used to calculate the basal pharmacodynamic effect when FTY720 doses or exposures were equal to zero. The pharmacodynamic effect of FTY720 was determined either as absolute reduction in peripheral lymphocyte counts or as percent reduction compared to the lymphocyte count obtained pre-transplant and before the administration of the first dose of FTY720 or MMF.

PK/PD correlations. Peripheral lymphocyte counts were correlated with FTY720

doses and blood concentrations. PK/PD modeling was used to find the best-fit model of the correlation between absolute or percent reduction in peripheral lymphocyte count and increasing doses or blood concentrations of FTY720. For these analysis we used the scientific software WinNonlin (SCI Software) to find the best fit PK/PD model that explains the relationship between FTY720 dose or blood concentration and the chosen pharmacodynamic surrogate marker.

Statistical analysis

Demographic characteristics were analyzed by analysis of variance (ANOVA) for continuous variables and by the chi-square test for categorical variables. Summary statistics were expressed as means ± SD, or frequencies or median and range, respectively. The observed and calculated PK/PD parameters were presented as means ± SD and as dose/response and exposure/response relationships. Linear regression analyses were used to correlate FTY720 dose and blood concentration. Nonlinear regressions were used to correlate the dose and drug concentration in blood with biological response. All statistical analyses were performed using the SPSS 7.5 software (SPSS Inc., Chicago, IL, USA), with the level of significance set at $P < 0.05$.

Results

Demographics and baseline characteristics

Demographic characteristics of the allograft recipients were similar across treatment groups (Table 1). Mean age was 40.1 ± 11.0 years, weight 62.2 ± 8.8 kg, and mean body mass index was 22.8 ± 2.6 kg/m^2. Most patients were males (61%) and white (61%), with 4 (17%) blacks and 5 (22%) with various degrees of miscegenation. The primary etiology leading to renal failure was chronic glomerulonephritis (22%), followed by diabetes (17%) and nephrosclerosis (13%). Mean number of HLA mismatches was 2.9 ± 0.5 and 100% showed no preformed anti-HLA antibodies. All recipients tested negative for hepatitis B and C viruses and 50% of the recipients were positive for cytomegalovirus IgG. No significant differences in demographic characteristics were observed between the 5 groups.

Pharmacokinetics

Cyclosporine doses and whole blood concentrations did not differ statistically between the 5 groups during the 24 weeks of treatment. Mean doses/weight were 9.7 ± 2.5 in the first week, 6.5 ± 1.9 at week 4, 4.7 ± 1.5 at week 12, and 3.8 ± 1.5 mg kg^{-1} day^{-1} at week 24, with no significant differences between groups (Figure 1A). Corresponding mean whole blood cyclosporine concentrations were 271.0 ± 104.1, 351.1 ± 121.4, 213.5 ± 71.3, and 149.1 ± 72.7 ng/ml, respectively (Figure 1B). Also dose-normalized cyclosporine concentrations did not differ significantly between the 5 groups, ranging from 0.50 ± 0.35 at week 1 to 0.63 ± 0.30 ng ml^{-1} mg^{-1} at week 24. Therapeutic concentrations of cyclosporine were achieved in all groups during the study period. Mean prednisone doses were 30.4 ± 3.7 (week 1), 27.3 ± 3.7 (week 4), 13.3 ± 3.7 (week 12), and 9.9 ± 0.5 mg/day (week 24), with no significant differences between the 5 groups.

Mean FTY720 blood concentrations according to dose level and study visit are shown in Figure 2. There were no significant differences in mean blood concentration between study visits (General Linear Model for repeated measurements) within each dose level, confirming that at week 4 steady-state FTY720 concentrations had been achieved (Table 2). Interindividual variability (% coefficients of variation, %CV) increased from 24 to 41% with increasing doses of FTY720 ($P = 0.05$) but no significant differences were observed between study visits. Aver-

Table 1. Demographic characteristics of the transplant population.

	Total	FTY720 (0.25 mg)	FTY720 (0.5 mg)	FTY720 (1 mg)	FTY720 (2.5 mg)	MMF (2 g)
N	23	4	4	5	5	5
Age (years)	40.1 ± 11.0 (23.0-59.0)	45.5 ± 5.0 (40.0-52.0)	38.3 ± 14.0 (23.0-52.0)	40.4 ± 14.3 (24.0-59.0)	38.6 ± 11.8 (24.0-57.0)	38.6 ± 10.7 (25.0-52.0)
Gender (male/female)	14/9	3/1	3/1	0/5	4/1	4/1
Weight (kg)	62.2 ± 8.8 (47.4-83.6)	68.6 ± 10.1 (62.3-83.6)	60.4 ± 5.6 (53.1-65.9)	55.6 ± 7.5 (47.4-61.8)	61.8 ± 10.4 (53.8-79.7)	65.4 ± 7.2 (57.6-76.0)
BMI (kg/m^2)	22.8 ± 2.6 (19.2-27.0)	24.7 ± 2.2 (22.3-27.0)	22.1 ± 1.7 (20.5-24.2)	23.9 ± 3.8 (19.2-27.0)	21.5 ± 2.0 (20.1-24.9)	22.0 ± 2.5 (19.7-26.0)
Ethnicity (white/black/mulatto/others)	14/4/2/3	3/0/0/1	4/0/0/0	2/1/1/1	3/1/0/1	2/2/1/0
HLA mismatches	2.9 ± 0.5	3.3 ± 0.5	3.0 ± 0.0	2.6 ± 0.5	2.8 ± 0.4	2.9 ± 0.5
Cause of ESRD						
Chronic glomerulonephritis	5 (21.7%)	2	1	0	1	1
Nephrosclerosis	3 (13.0%)	0	0	1	1	1
Polycystic kidney disease	2 (8.9%)	0	0	0	1	1
Diabetic nephropathy	4 (17.4%)	1	1	1	0	1
Unknown	9 (39.1%)	1	2	3	2	1
Time on dialysis (months)	17.7 ± 10.2 (2.0-36.0)	22.3 ± 12.9 (6.0-36.0)	18.5 ± 4.9 (12.0-24.0)	20.0 ± 13.6 (2.0-36.0)	16.2 ± 11.3 (7.0-36.0)	12.6 ± 6.8 (5.0-23.0)
Panel reactive antibody <5/≥5 (%)	23/0	4/0	4/0	5/0	5/0	5/0
Viral status						
Anti-HCV positive (IgG)	0	0	0	0	0	0
HbsAg positive	0	0	0	0	0	0
CMV positive (IgG)	12	3	1	2	3	3

Data are reported as means ± SD (range). MMF = mofetil mycophenolate; BMI = body mass index; HLA = human leukocyte antigen; ESRD = end-stage renal disease; HCV = hepatitis C virus; CMV = cytomegalovirus. Panel reactive antibody between donor and recipients greater or less than 5%.

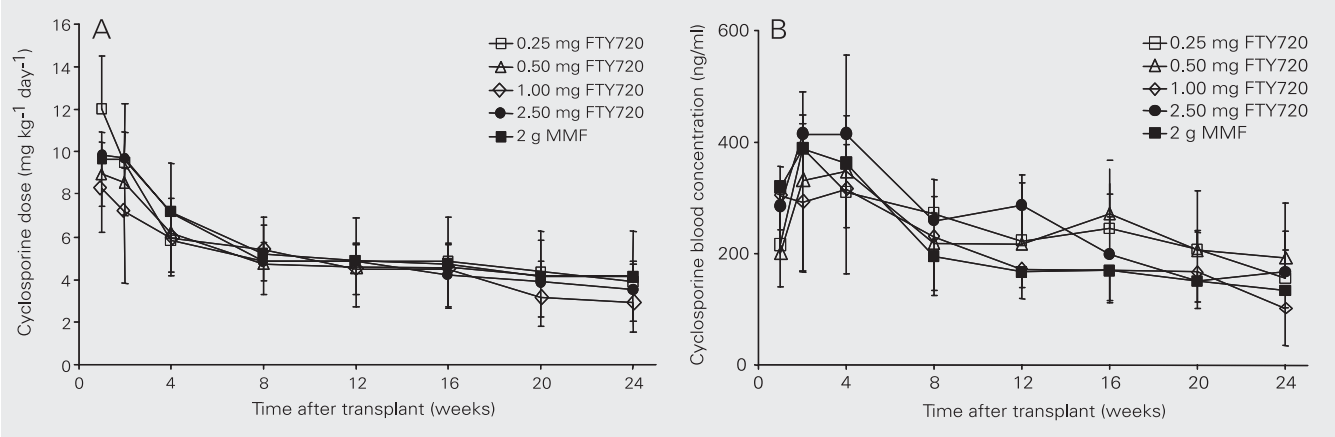

Figure 1. Time course of mean cyclosporine doses (A) and blood concentrations (B). There were no significant differences for mean cyclosporine doses or blood concentrations among study groups at each visit (independent Student *t*-test). MMF = mofetil mycophenolate.

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