

Brief Communication

Immunosuppressant Pharmacokinetics and Dosing Modifications in HIV-1 Infected Liver and Kidney Transplant Recipients

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Solid organ transplantation in human immunodeficiency virus (HIV)-infected individuals requiring concomitant use of immunosuppressants (IS) (e.g. cyclosporine [CsA], sirolimus [SrL], tacrolimus [FK]) and antiretrovirals (ARVs) (e.g. protease inhibitors [PIs] and/or nonnucleoside reverse transcriptase inhibitors [NNRTIs]) is complicated by significant drug interactions. To assist in appropriate clinical management, we describe the pharmacokinetics and dosing modifications in 35 patients (20 kidney, 13 liver and two kidney-liver HIV-infected subjects with end-stage kidney or liver disease), on both IS and NNRTIs, PIs, and combined NNRTIs + PIs, in studies done at weeks 2–4 and/or 12 weeks after transplantation or after a change in IS or ARV drug regimen (n = 97 studies). CsA, SrL and FK concentrations were measured in whole blood by LC/MS. HIV-infected transplant recipients using PIs with IS had marked increases in CsA, FK or SrL trough levels compared to those on NNRTIs alone or to patients not on ARVs, necessitating either a reduction in dose or an increase in dosing interval. Subjects on efavirenz (EFV) and CsA required much higher doses of CsA than those using any other ARV. Changes in antiretroviral therapy should be carefully managed to avoid insufficient immunosuppression or toxicity due to drug interactions.

Key words: Dose modification, HIV, immunosuppressant pharmacokinetics, kidney transplantation, liver transplantation

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Introduction

With increasing experience with transplantation in human immunodeficiency virus (HIV)-infected subjects with end-stage liver and kidney disease, we and others have shown that HIV protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) can alter the pharmacokinetics and dosing requirements for calcineurin inhibitors and other medications with similar metabolism pathways (1–7). Immunosuppressants (IS) such as cyclosporine (CsA) or sirolimus (SrL) are crucial to the prevention of rejection in transplant patients. These IS and the HIV PIs are both substrates and inhibitors of the cytochrome P450 metabolizing enzyme CYP3A4 (CYP3A4) and tend to increase systemic blood levels of CYP3A4 substrates, while NNRTIs such as efavirenz (EFV) are often CYP3A4 inducers (8,9) that increase drug metabolism and decrease blood levels of CYP3A4 substrates. In addition, many of these drugs are also substrates and inhibitors of P-glycoprotein (10), a transporter found on the apical membranes of intestinal epithelial and hepatic cells, whose function is to decrease absorption and increase excretion of its substrates (11). In intestinal cells, P-glycoprotein and CYP3A4 together act as a 'gauntlet', inhibiting intestinal drug absorption and increasing intestinal drug metabolism (11,12). Thus, concomitant administration of inhibitors with substrates of both P-glycoprotein and CYP3A4, such as ISs with PIs, would be expected to increase both IS and PI uptake and systemic blood levels.

However, the extent of these drug interactions is not well characterized. As both types of medications have narrow therapeutic windows, understanding the degree of interaction would help clinicians correctly dose ISs and antiretroviral medications (ARVs). We report IS dosing modifications in 35 subjects studied at 2 and 12 weeks after transplantation or after a change in drug regimens.

Subjects and Methods

Study design and subjects

This was an observational study of kidney and/or liver transplantation in HIV-infected patients transplanted at UCSF (initially during a pilot study and then during an NIH-sponsored multicenter trial). To be eligible for transplantation, HIV-infected kidney transplant candidates had to have an undetectable plasma HIV RNA level and CD4⁺ T-cell count greater than 200/mm³, while

liver transplant candidates had to have a CD4⁺ T-cell count greater than 100/mm³. Subjects were usually taking three or more ARVs prior to and following transplantation, including nucleoside reverse transcriptase inhibitors, which were not measured in this study. Those subjects on NNRTIs or PIs plus an IS underwent pharmacokinetic studies. This protocol was approved by the UCSF Institutional Review Board, and all study subjects gave signed informed consent.

Study procedures

Medication regimens: All subjects initiated IS posttransplantation on day 0 (liver recipients), or as clinically indicated when the serum creatinine improved (kidney recipients) as per UCSF's transplant protocol. Initial IS dosing was estimated as previously described (1). Subsequent changes in IS doses were made in response to IS trough levels obtained just prior to the morning dose of IS and measured at the UCSF clinical laboratory. In general, drug dosing was adjusted to maintain CsA trough concentrations between 75–150 ng/mL and SrL and FK troughs between 4–9 ng/mL. With the addition of SrL to a CsA-containing regimen, both trough concentrations were adjusted to about half their previous levels. The study protocol did not mandate a specific ARV regimen; thus, in addition to nucleoside reverse transcriptase inhibitors, some subjects were on PIs, some were on NNRTIs and some were using drugs from both classes. Standard posttransplantation management included tapering steroids, mycophenolate mofetil, anti-CMV and antifungal (weekly fluconazole) prophylaxis. Treatment for rejection included pulse steroids and antilymphocyte antibodies.

Pharmacokinetic (PK) studies: Subjects had protocol-driven pharmacokinetic studies at weeks 2–4 and 12 after transplantation and after a change in either IS, PI or NNRTI agents. We have previously reported on an 18 patient subset (1,13). Drug regimens were modified in response to drug side effects, low IS drug levels or rejection episodes. A change in drug regimen required reinitiating the cycle of PK studies.

Subjects were admitted to the UCSF Clinical Research Center for the pharmacokinetic studies. Each study started at 8 a.m. after an overnight fast. An indwelling catheter was inserted into a forearm vein and kept patent using a normal saline flush. After drawing trough blood samples, subjects took their antiretroviral therapy and their IS dose. Blood was sampled from the indwelling catheter at different time intervals, depending on the drug regimen: for medications dosed on an every 12-h schedule, blood was sampled at 0, 0.5, 1, 2, 3, 4, 5, 6, 8 and 12 h and for drugs dosed every 24 h, blood was sampled at times 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12 and 24 h. For EFV, additional blood was sampled at 15 and 19 h. No food was allowed for the first 3 h after the study was started, and all subjects ate a 30% fat meal at the same times relative to taking their medication. No grapefruit or grapefruit juice was allowed. Medications known to be strong CYP3A and P-glycoprotein inhibitors (e.g. ketoconazole, erythromycin) were not given until after the pharmacokinetic study had been completed.

Analysis of blood samples

Blood samples were frozen at –70°C until analyzed. Whole blood samples were analyzed for CsA, FK and SrL by a validated HPLC/MS assay in combination with automated online sample preparation (LC/LC-MS) (Hewlett-Packard; Palo Alto, CA). Method validation has been described in detail by Christians et al. (14).

Pharmacokinetic analysis

The following PK parameters were calculated after oral administration of the IS: C_{max}—the maximum observed blood concentration, C_{min}—the lowest observed blood concentration and AUC—the area under the blood concentration-time curve from 0 to T_{last} (T_{last} is the time of the last measur-

able concentration in the dosing interval), computed using linear trapezoidal summation. The PK analysis program was written by one of the authors (A. Cheng).

Statistical analysis

Doses and dosing intervals for each subject were measured at each visit and averaged for each antiretroviral group. Data are reported as the mean ± standard deviation or median and interquartile range (IQR). Comparisons between groups were done using ANOVA or *t*-tests with alpha = 0.05. All statistical analyses were done using Sigmapstat (Jandel Scientific, San Rafael, CA).

Results

Subject characteristics

Thirty-five HIV-infected subjects with end-stage renal (K) or liver (L) disease were transplanted (20 K, 13 L, 2 K-L). Thirty-four were men and the median (IQR) age was 47 years (42–52) at the time of transplantation (Table 1). Twenty were Caucasian, 13 were African-American and two were Asian. One subject initially had a liver transplant, and then required a combined liver-kidney transplant when the first graft failed, and one subject got a combined liver-kidney transplant; all other transplants were single organ grafts.

The total number of pharmacokinetic studies done was 97. Drug regimen alterations that required repeating the protocol PK studies occurred in 22 patients; the

Table 1: Baseline characteristics of HIV-infected transplant recipients

Characteristic	Liver recipients ¹ (n = 14)	Kidney recipients ² (n = 21)
Age, years (median [IQR])	43 [39,49]	48 [44,53]
Male sex, no. (%)	15 (100)	19 (97)
Race/ethnicity, no. (%)		
White	10 (72)	10 (48)
African-American	2 (14)	11 (52)
Asian	2 (14)	0
Donor, no. (%)		
Deceased	11 (78)	13 (62)
Living	3 (22)	8 (38)
PK studies (n = 42)		(n = 55)
ARVs, no. (%)		
PIs	16 (38)	21 (38)
NNRTIs	12 (29)	24 (44)
PI + NNRTI	14 (33)	10 (18)
IS, no. (%) ³		
CsA	26 (62)	43 (73)
FK	10 (24)	11 (20)
SrL	6 (14)	6 (7)

¹Total of 38 organs; includes two subjects who had combined liver-kidney transplants, one subject had a liver graft that failed and was retransplanted with a combined liver-kidney graft.

²One subject with a failed renal allograft had a second renal transplant.

³Includes five studies on combination CsA and SrL.

Table 2: Combined weeks 2 and 12 IS doses, dosing intervals and trough blood concentrations of HIV-infected transplant patients compared with non-HIV-infected transplant patients

Antiretroviral regimen		CsA + SrL				
		CsA	CsA	SrL	SrL	FK
NNRTI	N	31	3	3	0	7
	Dose (mg)	215 ± 95 ^{b,c}	133 ± 76	2.0 ± 1.4		3.1 ± 1.4 ^{b,c}
	Interval (h)	12 ± 0 ^{b,c}	12 ± 0	24 ± 0	ND	12 ± 0 ^b
	Trough (ng/mL)	113 ± 57	61 ± 59	1.3 ± 1.5		6.4 ± 2.9
PI	N	20	1	1	3	10
	Dose (mg)	51 ± 43 ^a	25	1.0	1.0 ± 0.0	0.7 ± 0.5 ^a
	Interval (h)	19 ± 11 ^a	24	84	78 ± 7	80 ± 54 ^{a,c}
	Trough (ng/mL)	176 ± 167	59	5.0	6.0 ± 3.4	6.6 ± 6.6
NNRTI + PI	N	13	1	1	4	4
	Dose (mg)	40 ± 28 ^a	25	1.0	1.0 ± 0.0	1.0 ± 0.0 ^a
	Interval (h)	21 ± 13 ^a	36	84	92 ± 72	12 ± 0 ^b
	Trough (ng/mL)	142 ± 58	104	13.0	10.3 ± 9.3	8.8 ± 6.4
No ARVs ¹	Dose (mg)	334 ± 122			8.2 ± 4.2	7–9
	Interval (h)	12			24	12
	Trough (ng/mL)	251 ± 116			23.3 ± 5.0	3–30

^avs. NNRTI.^bvs. PI.^cvs. NNRTI + PI.^dp < 0.05.

N = number of PK studies done; ND = not done.

¹Non-HIV-infected subjects on typical IS doses at 3–4 months posttransplant (15)

majority of these were changes in immunosuppressive regimens, usually due to treatment of rejection episodes. The rest had ARV regimens changed due to intolerance to medications. Subjects had no intercurrent illness when studied.

Effects of antiretrovirals on IS pharmacokinetics

For the 97 PK studies, just over one third of patients were on PIs alone, approximately one third were on NNRTIs and just under one third were on combinations of PIs and NNRTIs (Table 1). Initially after transplantation, the majority of the subjects were placed on CsA. Table 2 and Figure 1 summarize the effects of NNRTIs, PIs and the combination of NNRTIs + PIs on CsA alone, CsA when given with SrL, SrL alone and FK alone. In Figure 1 which reports only CsA data, the NNRTIs are divided into EFV or NVP, and the PIs are divided into a ritonavir (RTV)-boosted regimen or not. As there were no significant differences in doses or C_{min} between weeks 2 and 12, the data were combined for the analyses. Thus, in Table 2, all IS data with NNRTIs and PIs were averaged together; that is, CsA with nevirapine (NVP) was not separated from CsA with EFV, etc. Within each IS regimen, there were no significant differences in trough levels between ARV groups.

Subjects on NVP required ISs at doses and intervals close to non-HIV-infected transplant subjects on the same IS; CsA trough/dose ratios were approximately the same (~0.75 ng/mL/mg CsA) for non-HIV-infected transplant subjects (15; Table 2) as for patients receiving NVP plus CsA (Figure 1). Compared to subjects on NVP, using EFV increased the CsA dosing requirements from 189 ± 44

to 275 ± 129 mg bid at week 2 (p < 0.05) and from 147 ± 52 to 279 ± 123 mg bid at week 12 (p < 0.001; Figure 1). Trough levels averaged about 30% lower with EFV (50–60% lower on a dose-adjusted basis). For example, at week 2, CsA C_{min} was 130 ± 61 ng/mL (0.7 ± 0.3 ng/mL/mg CsA) with NVP compared to 91 ± 57 ng/mL (0.3 ± 0.1 ng/mL/mg CsA) with EFV (p < 0.05).

Subjects on any PI-containing regimen required a four- to five-fold lower CsA dose (p < 0.05) and ~50% increase in dosing interval (p < 0.05) compared to those on CsA with an NNRTI. At week 2, adding PIs to CsA significantly decreased the dosing requirements to 57 ± 31 mg (p < 0.001), and increased the dosing interval to 21 ± 14 h (p < 0.05) if the PI regimen did not contain RTV. For PI regimens with RTV, the dosing requirement was decreased further to 25 ± 0 mg (p < 0.001), with an even greater dosing interval of 33 ± 18 h (p < 0.01), (Figure 1; Table 2). The FK dose with PIs decreased ~80% to 0.7 ± 0.5 mg (p < 0.05) and the dosing interval increased ~7-fold to every 80 ± 54 h (p < 0.05). Similar trends were seen with those subjects on SrL, but no patients received SrL plus NNRTI for statistical comparison (Table 2).

Adding an NNRTI to a PI or PI + RTV did not significantly change CsA doses at either weeks 2 or 12 (Figure 1), but decreased the dosing interval, for example, from 33 ± 18 h for PI regimens with RTV to 16 ± 7 h (p < 0.05) for an NNRTI + PI regimen with RTV (data not shown). FK doses increased ~40% to 1.0 ± 0.0 mg (p < 0.05) when an NNRTI was added to a regimen with a PI, while dosing interval dropped ~70% to 12 h.

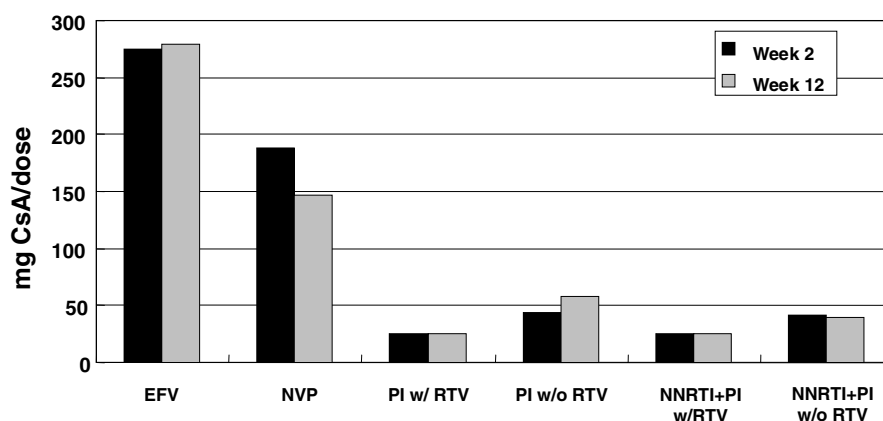


Figure 1: Mean CsA dose and trough levels at weeks 2 and 12 for 6 ARV drug regimens: EFV, NVP, PI/no RTV, PI+RTV, NNRTI+PI/noRTV, NNRTI+PI+RTV. There were no significant differences in CsA dose or Cmin between weeks 2 and 12.

Comedication(s):	EFV	NVP	PI w/ RTV	PI w/o RTV	NNRTI+PI+RTV	NNRTI+PI-RTV	
N	20	11	13	7	8	5	
CsA dose (mg/dose)	Wk 2	275 ± 129	189 ± 44	25 ± 0	57 ± 31	25 ± 0	42 ± 30
	Wk 12	279 ± 123	147 ± 52	25 ± 0	75 ± 63	25 ± 0	39 ± 28
CsA Cmin (ng/ml)	Wk 2	91 ± 57	130 ± 61	156 ± 67	128 ± 45	168 ± 77	173 ± 25
	Wk 12	84 ± 60	116 ± 57	111 ± 77	185 ± 177	104 ± 61	154 ± 21

Pharmacokinetic parameters such as IS doses, trough levels or AUCs did not differ between kidney transplant subjects and liver transplant subjects (data not shown).

Discussion

Tacrolimus (1,3,5,7) and CsA (1,2,4,6) have been previously reported to require dosing adjustments in both liver and kidney HIV-infected transplant patients. We report here on 97 pharmacokinetic studies conducted in 35 HIV-infected subjects at 2 and 12 weeks after kidney or liver transplantation. These results expand on previous reports of drug interactions between subjects on ARVs and IS (1–7), both in terms of the number of studies done and the IS–ARV combinations evaluated.

To achieve similar trough levels, individuals on PIs took lower IS doses at longer dosing intervals than patients not on PIs. Subjects on NNRTIs + PIs took similar doses as subjects on PIs with similar dosing intervals for CsA, but required shorter dosing intervals for SrL and FK. We have, however, previously shown that when CsA is given with unboosted PI regimens over a 24-month interval, CsA bioavailability increases, so that dose- and weight-adjusted CsA AUCs increased nearly three-fold while CsA dose decreased by 85% ($p < 0.01$) (1).

Doses and dosing intervals with CsA and NVP in HIV-infected transplant recipients were slightly lower than those used with CsA alone in non–HIV-infected transplant patients (15,16). EFV is a potent CYP3A4 inducer (10), increasing the metabolism rate of all of the ISs used, and

significantly increased the doses of CsA required (Table 2; Figure 1). This can be seen by comparing the ratios of CsA trough/dose for both NNRTIs from the table in Figure 1. The trough/dose ratio for EFV is less than half that of NVP. However, because of the limited number of patients available for study, we combined both NNRTI drugs in Table 2 to emphasize the difference between PIs and NNRTIs on immunosuppressant dosing.

There were several limitations of this study. Although some subjects in this study are now more than 5 years post-transplant, the number of patients at each time point is small, and the drug regimens are not uniform. However, despite the small sample size, there was a clear difference in pharmacokinetic behavior of ISs in subjects taking a PI-containing regimen compared with those taking NNRTIs. These differences were similar across IS groups.

In subjects with multiple medical problems and on multiple drug regimens, drug absorption and drug interactions have to be considered (17). Some of the subjects were on other medications that could have interfered or interacted with either P–glycoprotein or cytochrome P4503A4, such as fluconazole or proton pump inhibitors. To avoid these problems, subjects were studied when they had no intercurrent illnesses and concomitant use of interacting medications during the pharmacokinetic studies was avoided. Prior studies have shown that taking two drugs that are P-glycoprotein and CYP3A4 inhibitors a few hours apart can decrease the degree of interaction between the drugs (18). Food can also alter the absorption of ARV medication, which could potentially increase or decrease the degree of

interaction observed when subjects take their medication on a daily basis with food (19,20).

Avoiding insufficient immunosuppression or toxicity due to drug interactions required ongoing IS therapeutic drug monitoring and dose adjustments depending on the ARV regimen. Larger numbers of PK studies are needed on some drug regimens (e.g. SrL) than what is reported here to determine if the data trends remain the same. In addition, longer studies on FK and SrL are needed to see what kind of effects occur due to ongoing changes in drug metabolism that can produce an increase in CsA bioavailability over time (1,17).

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