Effect of Coadministered Lopinavir and Ritonavir (Kaletra) on Tacrolimus Blood Concentration in Liver Transplantation Patients

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With the advent of highly active antiretroviral therapy (HAART), HIV positivity is no longer a contraindication for liver transplantation. Some of the antiretroviral agents, particularly protease inhibitors (e.g., ritonavir, indinavir, and nelfinavir) have been described as potent inhibitors of the metabolism of certain immunosuppressive drugs. In this article we describe a profound interaction between tacrolimus and Kaletra (Abbott Laboratories, Chicago, IL) (a combination of lopinavir and ritonavir) in 3 liver transplantation patients. Patient 1, who was maintained on a 5 mg twice daily dose of tacrolimus with a trough blood concentration around 10.6 ng/mL, required only 0.5 mg of tacrolimus per week after addition of Kaletra to achieve similar tacrolimus blood concentrations, with a half-life of 10.6 days. In patient 2, the area under the blood concentration versus time curve for tacrolimus increased from 31 ng/mL/h to 301 ng/mL/h after addition of Kaletra, with a corresponding half-life of 20 days. When the patient was subsequently switched to nelfinavir, the half-life decreased to 10.3 days. Patient 3, who was maintained with 4 to 8 mg/d of tacrolimus and a corresponding blood concentration of 10 ng/mL before Kaletra, required a tacrolimus dose of 1 mg/wk and tacrolimus concentrations of 5 ng/mL with Kaletra. In conclusion, a combination of lopinavir and ritonavir led to a much more profound increase in tacrolimus blood concentrations than use of single protease inhibitor, nelfinavir. A tacrolimus dose of less than 1 mg/wk may be sufficient to maintain adequate blood tacrolimus concentrations in patients on Kaletra. Patients may not need a further dose of tacrolimus for 3 to 5 weeks depending on liver function when therapy with Kaletra is initiated. Great caution is required in the management of tacrolimus dosage when Kaletra is introduced or withdrawn in HIV-positive patients after liver transplantation, particularly in the presence of hepatic dysfunction. (*Liver Transpl 2003;9:954-960.*)

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With the use of highly active antiretroviral treatment (**HAART**), successful liver and kidney transplantation is possible in HIV positive patients.[1-3] A HAART regimen usually consists of a combination of nucleoside reverse transcriptase inhibitors (**NRTI**), nonnucleoside reverse transcriptase inhibitors (**NNRTI**), and/or protease inhibitors (**PI**). Protease inhibitors are known to inhibit the cytochrome P450 3A (**CYP3A**) enzyme system that is responsible for the metabolism of immunosuppressive drugs such as tacrolimus, sirolimus, and cyclosporine.[4,5] In a previous report, we showed that coadministration of the protease inhibitor nelfinavir significantly increased tacrolimus blood concentrations compared with indinavir, to maintain comparable blood concentrations.[2,3] Kaletra is a combination of two protease inhibitors, ritonavir and lopinavir. The interaction of Kaletra with immunosuppressive drugs such as tacrolimus has not been described. The goal of this study was to examine the interaction between Kaletra and tacrolimus in 3 liver transplantation patients.

Material and Methods

Between September 1997 and December 2002, 18 HIV-positive patients (15 men and 3 women) received liver transplantation at our institution. All of the patients received tacrolimus-based immunosuppression with 1 g of methylprednisone on perfusion of the liver and a total of 600 mg of methylprednisone tapered over the next 6 days. Of the 18 patients, 3 patients died perioperatively and did not receive the HAART regimen. The remaining 15 patients received a combination of NRTI, NNRTI, and/or PI. Protease inhibitors used consisted of nelfinavir (n = 11), indinavir (n = 1), or Kaletra (n = 3). The demographics of the 3 patients on Kaletra are given in Table 1, including HAART treatment. In patient 1, changes in trough blood concentrations of tacrolimus with daily doses were examined when Kaletra was introduced for 18 weeks and then discontinued for suspected hepatotoxicity. In patient 2, the pharmacokinetics of tacrolimus were evaluated over a dosing interval before Kaletra and after Kaletra. Changes in tacrolimus dosage and trough concentration before and after Kaletra, and when Kaletra was discontinued 6 weeks later and nelfinavir was substituted, were

examined. In patient 3, who had a complicated postoperative course with pseudomonas pneumonia, tacrolimus was discontinued between 5 to 10 weeks posttransplantation and Kaletra was introduced during the time interval when the patient was not on tacrolimus. The dose and trough blood concentration of tacrolimus were examined in this patient.

All of the patients received three capsules of Kaletra (each capsule contains lopinavir 133.33 mg and ritonavir 33.3 mg) twice per day. Whole blood concentrations of tacrolimus were determined using microparticulate enzyme immunoassay (**MEIA**) using an IMx Analyzer (Abbott Laboratories, Abbott Park, IL). Some of the samples from patient 2 were also analyzed by high-performance liquid chromatography mass spectrometric assay to rule out any potential interference of Kaletra with the MEIA assay.

Results

Patient 1

During the second week after liver transplantation, this patient was on an oral dose 5 mg of tacrolimus twice per day (70 mg/wk) to achieve a 12-hour trough tacrolimus blood concentration of 10.6 ng/mL. Two weeks after liver transplantation and with a stable liver function, Kaletra therapy was initiated at the standard dose and the dose of tacrolimus was reduced to 6 mg/d. Two days later the trough tacrolimus blood concentration increased to 78.5 ng/mL, at which point tacrolimus treatment was discontinued. He experienced neurologic symptoms of somewhat altered mental status for few days without any nephrotoxicity. Twenty-two days later, tacrolimus blood concentration decreased to 6.4 ng/mL, with an apparent half life of 10.6 days. The patient received three doses of 0.5 mg of tacrolimus over the next 2 weeks with a trough tacrolimus blood concentration of 14.7 ng/mL. The tacrolimus dose was further reduced to 0.5 mg once per week (a total reduction in dose by 140 times), with daily tacrolimus blood concentration in the range of 8.9 to 19.7 (mean, 12.9 ng/mL). Twenty weeks after liver transplantation, the patient's liver function deteriorated suddenly and the total bilirubin increased from 0.7 mg/dL to 11.0 mg/dL over a 3-day period because of bile duct obstruction. Kaletra was discontinued for the next 12 days. The patient received a total of 3 mg of tacrolimus during this period, with the tacrolimus blood concentration ranging from 4.5 to 11.2 ng/mL. The patient's bilirubin level came down to 2.0 mg/dL with decompression of the biliary system after 12 days, and Kaletra was reintroduced. The dose of tacrolimus was restored to 0.5 mg per week with a tacrolimus level of 12 ng/mL and a serum bilirubin concentration of 1.2 mg/dL (Fig. 1).

Table 1. Patient Characteristics and Follow-Up

Patient No.	Age	Gender	Diagnosis	Transplant Organ	Follow-Up (mo)	At Last Follow-Up									
						Liver Function					Kidney Function			Tacrolimus	
						Bilirubin (mg/dL)	AST (μ/L)	ALT (μ/L)	ALP (μ/L)	GGT (μ/L)	BUN (mg/dL)	Creatinine (mg/dL)	Tacrolimus Dose (mg)	Tacrolimus Level (ng/mL)	HAART Regimen
1	48	Male	HCV	Liver	6	1.2	34	41	nd	489	18	0.9	0.5/wk	12	Kaletra, lamivudine, zidovudine
2	42	Male	HCV	Liver	3.8	2.7	121	68	258	1894	12	0.6	0.5/wk	6.4	Kaletra (changed to nelfinavir), lamivudine, zidovudine
3	33	Male	Hemophilia, HCV, polycystic kidney	Liver + kidney	5.5	0.5	36	50	183	90	30	1.1	1/wk	4.6	Kaletra, lamivudine, stavudine

Abbreviations: HAART, highly active antiretroviral therapy; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; BUN, blood urea nitrogen; HCV, hepatitis C virus.

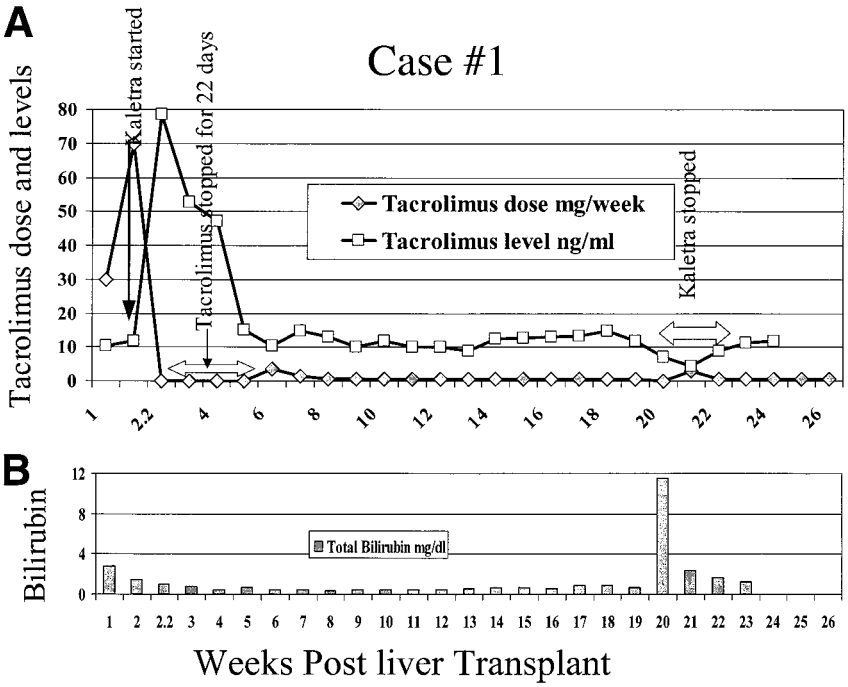

Figure 1. Patient 1.
(A) Changes in weekly tacrolimus dosage (mg) ◇ with the introduction of Kaletra and the discontinuation of Kaletra.

(B) Serum bilirubin (mg/dL) at the same time point.

Patient 2

This patient was on a stable oral dose of 4 mg of tacrolimus twice per day with trough tacrolimus blood concentrations between 8.2 to 20.9 ng/mL. In this patient, we measured the concentration of tacrolimus over a dosing interval on the sixth postoperative day. Venous blood samples were collected at 0 (before tacrolimus dose), 1, 2, 3, 4, 6, 8, and 12 hours after tacrolimus dose. Kaletra was started in the evening (three tablets twice daily) after the 12-hour sample, and he received only 2 mg of tacrolimus that night (instead of the usual 4 mg) and none for the next 2 days. After the fifth dose of Kaletra, the patient received a 1-mg oral dose of tacrolimus and multiple blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 12,16, 20, and 24 hours after Kaletra and tacrolimus. The 24-hour trough blood concentration of tacrolimus was 49 ng/mL. The concentration at 60 hours was 20.6 ng/mL without any additional dose of tacrolimus. He did not require any further dose of tacrolimus for 38 days, and had a blood tacrolimus concentration of 7.2 ng/mL. The half-life of tacrolimus was 20.6 days. At this point, the patient became jaundiced and the total serum bilirubin concentration increased to 5.0 mg/dL. Liver biopsy showed hepatitis activity index of 7 of 18, a fibrosis score 0 of 6, and a rejection activity index of 2 of 9. Hepatitis C virus RNA viral load was 30, 100,000 IU/mL. Clinically Kaletra toxicity with recurrent hepatitis C was suspected, and Kaletra was discontinued and nelfinavir was initiated 17 days later. During these 17 days, the patient received 46 mg of tacrolimus (about 20 mg/wk). After starting nelfinavir, the tacrolimus dose was discontinued for 30 days. The trough blood tacrolimus concentration ranged from 3.4 to 10.9 ng/mL during this time (Fig. 2). The half-life of tacrolimus was 10.3 days. Tacrolimus was reintroduced at a dose of 0.5 mg/wk. The patient had a symptom of severe headache when tacrolimus concentration was high, but fortunately renal function remained stable.

Tacrolimus blood concentrations over the 24-hour time period with a 1-mg dose of tacrolimus while on Kaletra showed sustained high concentrations with multiple peak levels at 1, 3, 8, 20, and 24 hours (Fig. 3). The 12-hour area under the curve increased to 300.6 ng/mL/h per milligram dose of tacrolimus after Kaletra compared with 31 ng/mL/h per milligram of tacrolimus before Kaletra. The morning venous blood samples collected on the third and fourth posttransplantation week were simultaneously analyzed by the MEIA and high performance liquid chromatography mass spectrometry (**HPLC-MS**) methods. The correlation between the two methods was 0.912 (Table 2).

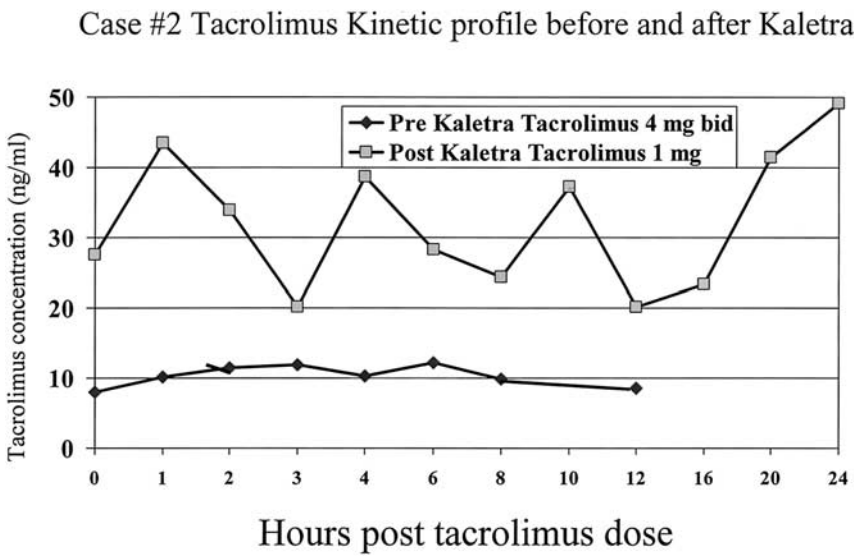

Figure 2. Patient 2. ◇, Concentration of tacrolimus over time before Kaletra (tacrolimus, 4 mg). ■, After Kaletra (tacrolimus, 1 mg).

Patient 3

This patient received combined liver and kidney transplantation from the same donor. This patient had a complicated postoperative course and developed pseudomonas pneumonia with suspected Nocardia infection. He was on 2 mg of tacrolimus twice per day (28 mg/wk) with trough concentrations ranging from 9.5 to 11.9 mg/mL. His tacrolimus was discontinued from week 6 to 11 after liver transplantation. He had undetectable blood concentrations of tacrolimus 1 week after stopping tacrolimus. Kaletra was started while he was off of tacrolimus. Tacrolimus was started 1 week after starting Kaletra; the currant dose is 1 mg/wk with daily blood concentrations between 2.9 to 5.4 ng/mL (Fig. 4).

All 3 patients had undetectable HIV RNA (<50 copies/mL) before transplantation and remained negative throughout the posttransplantation period with HAART regimen.

Discussion

Tacrolimus is a more potent immunosuppressive drug than cyclosporine and is being increasingly used in liver transplantation patients all over the world. However, the drug has a narrow therapeutic window, and frequent monitoring of the drug concentration is necessary to optimize tacrolimus therapy.[6-9] Although lower concentrations can induce rejection, high concentrations can lead to toxicity aside from overimmunosuppression-related complications, such as infection. The latter is particularly significant in HIV-positive patients, who already have depleted CD4 counts.

Tacrolimus is primarily metabolized in the liver and the gut by the CYP3A enzyme system. Tacrolimus is also a substrate for p-glycoprotein.[10] CYP3A activity in the gut and liver and p-glycoprotein activity in the gut primarily determine the bioavailability of tacrolimus. Tacrolimus blood concentrations are increased by inhibitors of CYP3A (ketoconazole, itraconazole, fluconazole, verapamil, diltiazem, erythromycin) and decreased by inducers of CYP3A.[8,11-13] Recently, profound interactions between tacrolimus and sirolimus with various protease inhibitors have been reported.[2-4,12,14-16] Protease inhibitors are known inhibitors of CYP3A and p-glycoprotein.[17] The initial HAART regimen consisted of a combination of NRTI and NNRTI without or with one PI. Recently, a combination of two PIs (lopinavir and ritonavir), both of which are metabolized by the CYP3A enzyme system, has been increasingly used. The interaction that was observed in the patients reported here was of a higher magnitude than what has been observed with single a PI.

The most common indication for liver transplantation in HIV-positive infection is hepatitis C virus–related end-stage liver disease. The disease is known to recur after liver transplantation,[18-22] resulting in hepatic dysfunction, which can further impair the metabolism of tacrolimus.[23,24] In this event, extreme caution is necessary to avoid overimmunosuppression. It is clear that after the introduction of Kaletra, the patients may not need a further tacrolimus dose for up to 3 weeks, even with normal hepatic function (patient 1). If there is associated hepatic dysfunction, 1 mg of tacrolimus may provide a therapeutic level of tacrolimus for up to 5

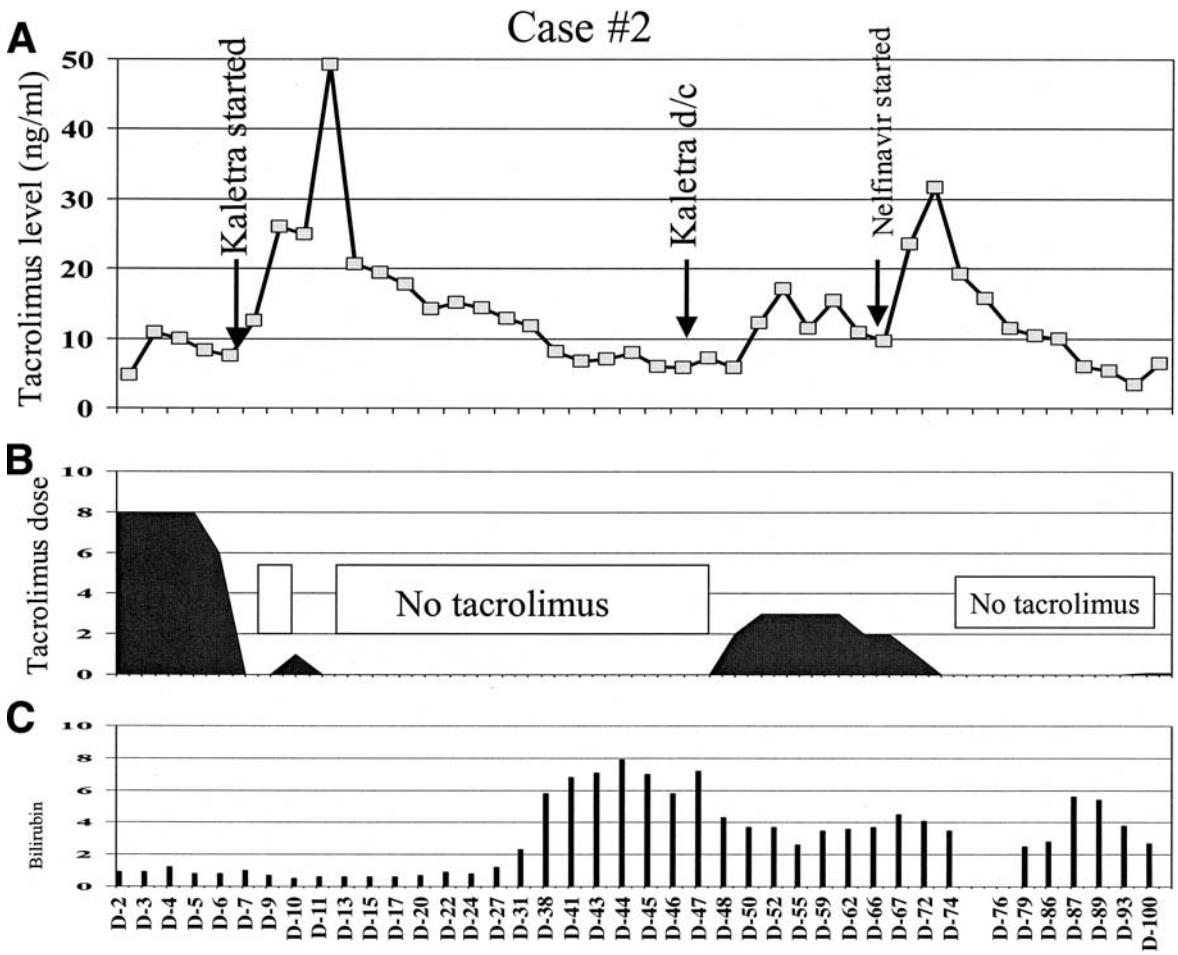

Figure 3. Patient 2. (A) Daily concentration of tacrolimus before the introduction of Kaletra, after Kaletra, after the discontinuation of Kaletra, and with the introduction of nelfinavir. (B) Tacrolimus dosage (mg/day) at the same time point as in A. (C) Changes in serum bilirubin (mg/dL) for the same time point as in A.

weeks (patient 2). The most surprising finding of the study is that the concentration of tacrolimus may increase for the next few days with the introduction of Kaletra without any additional dose of tacrolimus, an observation that was unexpected. We postulate that Kaletra may almost completely inhibit CYP3A-mediated metabolism and p-glycoprotein–mediated efflux of tacrolimus and thereby increase the amount of parent tacrolimus excreted in the bile. This in turn leads to reabsorption from the gut, establishing the enterohepatic circulation, which is not normally observed in the absence of Kaletra, because of extensive hepatic and gut metabolism or p-glycoprotein efflux of tacrolimus. This may explain multiple peak concentrations of tacrolimus over a 24-hour period in patient 2 with a sustained concentration of tacrolimus for several weeks without any additional dose of tacrolimus. The effect of Kaletra on the CYP3A enzyme system and p-glycoprotein is reversible, as seen in patient 2. In this patient, when Kaletra was discontinued for 17 days, 46 mg of tacrolimus was required in spite of impaired liver function at

Table 2. MEIA and HPLC, Patient 2

Posttransplantation Day	MEIA (ng/mL)	HPLC (ng/mL)
17	17.8	18.3
20	14.2	12.6
22	15.2	11.8
24	14.3	12.9
Mean	15.375	13.9

Abbreviations: MEIA, microparticulate enzyme immunoassay; HPLC, high-performance liquid chromatography.

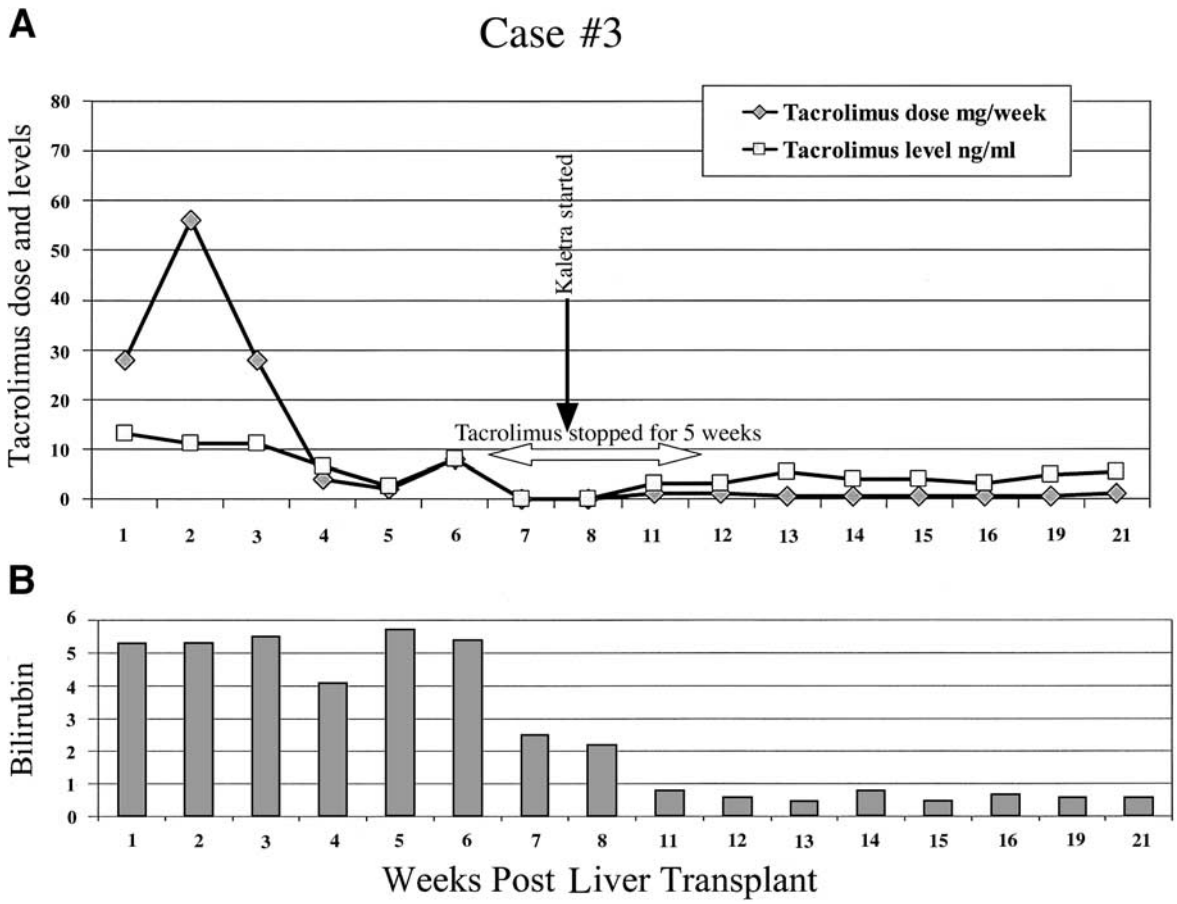

Figure 4. Patient 3. (A) ◇, weekly dosage of tacrolimus with tacrolimus concentration (ng/mL). (B) ■, serum bilirubin (mg/dL) at the same time point.

this time, as compared with 1 mg of tacrolimus, which was adequate for up to 38 days when the patient was on Kaletra. Based on our experience, we think that Kaletra has a more profound interaction with tacrolimus compared with the reported interaction between tacrolimus and nelfinavir.[3] We recommend a preemptive decrease in tacrolimus dose by at least 50% 1 day before starting therapy with Kaletra and not administering any further dose of tacrolimus for the next few days. Tacrolimus therapy must be guided by measuring tacrolimus blood concentrations in these patients. The usual dose of tacrolimus while a patient is on Kaletra is expected to be about 0.5 mg to 1 mg per week. Further caution is necessary if the patient has an associated hepatic dysfunction or is on other drugs, which may interfere with CYP3A enzyme systems or a p-glycoprotein efflux pump. A large variability in tacrolimus concentration with introduction or discontinuation of Kaletra may affect the clinical course of the patient in terms of tacrolimus-related drug toxicity worsening hepatitis or inducing rejection. Additional pharmacokinetics studies in patients on Kaletra are needed to further understand such a profound influence and to optimize tacrolimus therapy in these patients.

Conclusion

Kaletra, a combination of two protease inhibitors, has an even more profound interaction with tacrolimus compared with the effect of a single protease inhibitor such as nelfinavir. The usual requirement of tacrolimus is less than 1 mg/wk to maintain therapeutic tacrolimus blood concentrations when Kaletra is used in liver transplantation patients with normal liver function. The tacrolimus dose must be reduced further with even slight hepatic dysfunction, and great caution is warranted to avoid over immunosuppression in HIV-positive liver transplantation patients. Further kinetic studies are needed to determine the impact of drug interaction between Kaletra and tacrolimus.

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References

1. Ragni MV, Dodson SF, Hunt SC, Bontempo FA, Fung JJ. Liver transplantation in a hemophilia patient with acquired immunodeficiency syndrome. Blood 1999;93:1113-1114.
2. Jain AK, Venkataramanan R, Fridell JA, Gadomski M, Shaw LM, Ragni M, et al. Nelfinavir, a protease inhibitor, increases sirolimus levels in a liver transplantation patient: a case report. Liver Transpl 2002;8:838-840.
3. Jain AB, Venkataramanan R, Shapiro R, Scantlebury V, Potdar S, Bonham A, Ragni M, Fung JJ. The interaction between antiretroviral agents and tacrolimus in liver and kidney transplant patients. Liver Transpl 2002;8:841-845.
4. Furlan V, Taburet AM. Drug interactions with antiretroviral agents [in French]. Therapie 2001;56:267-271.
5. Vincent SH, Karanam BV, Painter SK, Chiu SH. In vitro metabolism of FK-506 in rat, rabbit, and human liver microsomes: Identification of a major metabolite and of cytochrome P450 3A as the major enzymes responsible for its metabolism. Arch Biochem Biophys 1992;294:454-460.
6. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. The US Multicenter FK506 Liver Study Group. N Engl J Med 1994;331: 1110-1115.
7. Randomised trial comparing tacrolimus (FK506) and cyclosporin in prevention of liver allograft rejection. European FK506 Multicentre Liver Study Group. Lancet 1994;344:423-428.
8. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet 1995;29:404-430.
9. Jain A, Reyes J, Kashyap R, Dodson SF, Demetris AJ, Ruppert K, et al. Long-term survival after liver transplantation in 4,000 consecutive patients at a single center. Ann Surg 2000;232:490-500.
10. Saeki T, Ueda K, Tanigawara Y, Hori R, Komano T. Human P-glycoprotein transports cyclosporin A and FK506. J Biol Chem 1993;268:6077-6080.
11. Manez R, Martin M, Raman D, Silverman D, Jain A, Warty V, et al. Fluconazole therapy in transplant recipients receiving FK506. Transplantation 1994;57:1521-1523.
12. Iwasaki K, Matsuda H, Nagase K, Shiraga T, Tokuma Y, Uchida K. Effects of twenty-three drugs on the metabolism of FK506 by human liver microsomes. Res Commun Chem Pathol Pharmacol 1993;82:209-216.
13. Venkataramanan R, Shaw LM, Sarkozi L, Mullins R, Pirsch J, MacFarlane G, et al. Clinical utility of monitoring tacrolimus blood concentrations in liver transplant patients. J Clin Pharmacol 2001;41:542-551.
14. Schvarcz R, Rudbeck G, Soderdahl G, Stahle L. Interaction between nelfinavir and tacrolimus after orthoptic liver transplantation in a patient coinfected with HIV and hepatitis C virus (HCV). Transplantation 2000;69:2194-2195.
15. Sheikh AM, Wolf DC, Lebovics E, Goldberg R, Horowitz HW. Concomitant human immunodeficiency virus protease inhibitor therapy markedly reduces tacrolimus metabolism and increases blood levels. Transplantation 1999;68:307-309.
16. Hesse LM, von Moltke LL, Shader RI, Greenblatt DJ. Ritonavir, efavirenz, and nelfinavir inhibit CYP2B6 activity in vitro: Potential drug interactions with bupropion. Drug Metab Dispos 2001; 29:100-102.
17. Washington CB, Duran GE, Man MC, Sikic BI, Blaschke TF. Interaction of anti-HIV protease inhibitors with the multidrug transporter P-glycoprotein (P-gp) in human cultured cells. J Acquir Immune Defic Syndr Hum Retrovirol 1998;19:203-209.
18. Feurer ID, Wright JK, Payne JL, Kain AC, Wise PE, Hale P, et al. Effects of hepatitis C virus infection and its recurrence after liver transplantation on functional performance and health-related quality of life. J Gastrointest Surg 2002;6:108-115.
19. Sanchez-Fueyo A, Restrepo JC, Quinto L, Bruguera M, Grande L, Sanchez-Tapias, et al. Impact of the recurrence of hepatitis C virus infection after liver transplantation on the long-term viability of the graft. Transplantation 2002;73:56-63.
20. Weinstein JS, Poterucha JJ, Zein N, Wiesner RH, Persing DH, Rakela J. Epidemiology and natural history of hepatitis C infections in liver transplant recipients. J Hepatol 1995;22(suppl): 154-159.
21. Guerrero RB, Batts KP, Burgart LJ, Barrett SL, Germer JJ, Poterucha JJ, et al. Early detection of hepatitis C allograft reinfection after orthotopic liver transplantation: A molecular and histologic study. Mod Pathol 2000;13:229-237.
22. Narayanan Menon KV, Poterucha JJ, El-Amin OM, Burgart LJ, Kremers WK, Rosen CB, et al. Treatment of posttransplantation recurrence of hepatitis C with interferon and ribavirin: lessons on tolerability and efficacy. Liver Transpl 2002;8:623-629.
23. Jain AB, Venkataramanan R, Cadoff E, Fung JJ, Todo S, Krajack A, Starzl TE. Effect of hepatic dysfunction and T tube clamping on FK 506 pharmacokinetics and trough concentrations. Transplant Proc 1990;22:57-59.
24. Abu-Elmagd K, Fung JJ, Alessiani M, Jain A, Venkataramanan R, Warty VS, et al. The effect of graft function on FK506 plasma levels, dosages, and renal function, with particular reference to the liver. Transplantation 1991;52:71-77.