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Original Article

Switch of immunosuppressant therapy from tacrolimus to cyclosporine in 30 liver transplant patients who developed hyperbilirubinemia

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Abstract: In this study, we summarized the efficacy of switching the immunosuppressant treatment for liver transplant patients with hyperbilirubinemia from tacrolimus to cyclosporine and outlined how pharmacogenetics could guide the application and interchangeability of immunosuppressants. We retrospectively reviewed the results of switching immunosuppressant therapy with tacrolimus to that with cyclosporine in patients who developed hyperbilirubinemia after liver transplantation. The method of switching and its effects, and postoperative analysis of the optimal immunosuppressant selection based on the *CYP3A5* genotype are discussed. In a group of 245 liver transplant patients, the main immunosuppressant treatment was switched from tacrolimus to cyclosporine for 30 patients. The reason for the switch was the development of hyperbilirubinemia without biliary complications in those patients. The decrease in bilirubin levels was not significant after an increase in the tacrolimus dosage. The method of switching included discontinuation of tacrolimus administration for 24 h before cyclosporine was infused at a dose of 100-150 mg twice daily. The rest of the conventional immunosuppressant therapy remained unchanged. After the switch, total bilirubin levels in these patients began to decline within 2 weeks. Follow-up examinations six months later revealed that patients did not experience any significant renal damage. We noted that in all 30 cases, either the donor or recipient (or both) had *CYP3A5* genotype AG or AA. The predominance of these genotypes was significantly different from the distribution of donor/recipient genotype combinations in the overall group of liver transplant patients. The widely used tacrolimus-based immunosuppressant treatment after liver transplantation may not be ideal in cases when either the donor or recipient have *CYP3A5* genotype AG or AA. Clinical manifestations in such patients treated with tacrolimus may include persistent hyperbilirubinemia without biliary complications. Therefore, based on the *CYP3A5* genotype for this particular patient population, a switch in the prescribed immunosuppressant may be warranted in order to prevent the development of hyperbilirubinemia and to reduce the adverse effects of tacrolimus.

Keywords: Tacrolimus, cyclosporine, CYP3A5, liver transplantation, hyperbilirubinemia

Introduction

Liver transplantation may be associated with persistent hyperbilirubinemia in the absence of biliary complications. Because of its complex causes and poor sensitivity to treatment, hyperbilirubinemia can reduce the quality of life for patients and is a key problem during any follow-up therapy.

Between January 2011 and June 2015, 245 postoperative liver transplant patients received immunosuppressive treatment with tacrolimus at our hospital. In the clinical follow-up, 30 patients were switched from tacrolimus to cyclosporine treatment because of persistent hyperbilirubinemia without biliary complications. Within 2 to 15 days after the switch, hyperbilirubinemia incidence began to decline. Using our observations of those 30 liver transplant recipients as a case study, we have summarized the data about the consequences of changing the immunosuppression regimen from tacrolimus to cyclosporine. We have also explored the possibility of using pharmacogenetic information for the application and adjustment of treatment with immunosuppressants.

Table 1. The basic information and clinic feature for all 30 cases

Gender (male/female)	23/7
Age (year)	39.4 ± 6.9 (24,52)
Body weight (kg)	69.73 ± 12.63 (45-105)
Graft weight (g)	1327.35 ± 277.69 (880-2190)
Graft-to-recipient weight ratio (%)	1.95 ± 0.43 (1.08-2.88)
Primary disease	
Cirrhosis	30
Cancer	18
Hepatitis C virus infection	2
Hepatitis B virus infection	24
Primary biliary cirrhosis	1
Alcoholic cirrhosis	2
Others (drug induced liver injury)	1

Materials and methods

General information

From January 2012 to June 2015, in the Beijing You An Hospital affiliated with the Capital Medical University, 30 patients were prescribed a switch in the immunosuppressant from tacrolimus to cyclosporine because they presented with persistent hyperbilirubinemia without biliary complications. This group of patients included 23 male and 7 female patients with a mean age of 39.4 ± 6.9 years (range, 24-52 years). The primary causes of disease were primary liver cancer (11 cases), liver cirrhosis (13 cases), autoimmune hepatitis C cirrhosis (4 cases), cirrhosis (1 case), and alcoholic cirrhosis (1 case) (**Table 1**).

Treatment regimen before immunosuppressant switch

According to the departmental regulations, tacrolimus, mycophenolate mofetil, and prednisone were administered postoperatively within the triple immunosuppression regimen. Tacrolimus (Prograf, Astellas) was given at an average initial dose of 4 mg/day. The regimen was adjusted based on the clinical condition of the patient, adverse reactions, plasma concentration, and types of other concomitant medications in order to maintain the plasma drug concentration at a level of 3-8 ng/mL. Prednisone (1000 mg) was administered intravenously during the surgery. Its dose was subsequently reduced to 240 mg/day, and gradually withdrawn completely within three months after the surgery. In addition, according to the patient's condition, mycophenolate mofetil was administered daily at a dose of 1000 mg. Regular check-ups were scheduled after the surgery at an interval of 1-3 months. The check-up included checking for clinical symptoms, liver and kidney function, blood concentration, and if necessary, liver biopsy.

Reasons for immunosuppressant switch

Thirty patients with persistent postoperative hyperbilirubinemia had an average plasma bilirubin concentration of 49.97 ± 13.17 (range 32-88) μmol/L. Anastomotic stenosis, hilar bile duct structure, diffuse intrahepatic biliary structure, and other biliary complications were ruled out. Of the 30 patients, 23 had elevated plasma concentration of transaminases and exhibited organ rejection, as confirmed by liver biopsy, whereas 7 patients had normal levels of alanine transaminase and cholangiolar cholestasis indicated by liver biopsy. The dose of tacrolimus was increased during clinical follow-up examinations, reaching 6.3 ± 0.9 (range 5-8) mg daily. Plasma concentration of tacrolimus fluctuated between 1.9 and 6.7 ng/mL and was 3.75 ± 1.32 ng/mL on average. Patients were observed for 2-4 weeks following the increase in tacrolimus dosage, but the rejection could not be controlled and hyperbilirubinemia persisted. In some patients, the necessary therapeutic plasma concentration of tacrolimus was not reached even after the increase in dosage. In addition, 18 patients developed tremors, trembling jaw, and other neurotoxic effects. Furthermore, nine patients showed a significant increase in their blood glucose level.

Drug switch method

Cyclosporine was administered in 24 h after the discontinuation of tacrolimus. The initial dose of cyclosporine was 100-150 mg b.i.d. The regimen was adjusted based on the clinical condition of the patient, adverse reactions, plasma drug concentration, and the types of other concomitant medications in order to ensure that the plasma C0 value reached 100-150 ng/mL in 1-3 months after the surgery and

Table 2. Conditions and effects of switching the immunosuppressant treatment in 30 liver transplant patients from tacrolimus to cyclosporine

Time after the surgery (months)	Before switch						After switch			*CYP3A5* genotype	
	Tacrolimus dose (mg/day)	Tacrolimus concentration (ng/dL)	TBil (μmol/L)	High blood glucose	Adverse neurological symptoms	Period of decrease for TBil (d)	1 month later TBil (μmol/L)	Suitable CSA dose (mg/day)	Creatinine level after 6 months (μmol/L)	Donor	Recipient
3 m	6	3.2	67		+	5	28	300	63	GG	AG
7 m	6	3.2	51			10	24	250	52	GG	AG
8 m	7	4.9	55	+	+	8	17	250	66	AG	AA
6 m	6	2.7	77	+		7	12	250	82	GG	AG
16 m	5	3.6	62	+		12	28	200	63	GG	AG
3 m	6	2.2	33		+	4	24	300	52	GG	AG
11 m	6	5.9	56	+	+	14	17	200	66	AG	AA
3 m	6	2.7	56		+	3	12	300	72	GG	AG
3 m	5	4.3	58			3	19	300	64	AG	GG
2 m	5	3.9	48			3	22	300	56	AG	GG
3 m	6	6.7	42	+		14	19	300	99	AG	GG
9 m	6	5.1	45		+	10	16	250	120	AG	GG
12 m	5	3.2	32	+		14	14	200	99	AG	GG
15 m	7	6.2	44	+	+	14	29	200	82	AG	AG
7 m	6	3.2	47	+	+	7	25	250	52	AG	AG
8 m	6	4.1	39			7	19	250	92	GG	AG
5 m	6	4.2	49			7	19	250	62	GG	AG
3 m	8	4.1	88		+	2	33	300	69	AG	AG
14 m	6	2.1	64	+		14	15	200	88	AG	AA
10 m	8	2.8	47	+	+	14	22	200	92	AG	AG
8 m	7	3.9	33		+	5	12	250	96	GG	AG
9 m	7	2.2	38		+	5	15	250	102	AG	AG
12 m	7	1.9	39		+	4	13	250	58	AG	AG
3 m	7	3.5	42		+	3	11	300	72	AA	GG
7 m	5	3.7	36			2	14	250	95	AG	GG
10 m	7	3.2	52		+	7	15	250	65	AG	GG
6 m	7	2.4	58		+	2	17	250	74	AG	AG
18 m	5	3.9	60			3	16	200	66	AG	AG
19 m	7	2.1	44		+	4	14	200	80	AG	AG
6 m	8	3.2	37		+	2	16	250	71	AG	AG

TBil: total bilirubin; CSA: Cyclosporin A.

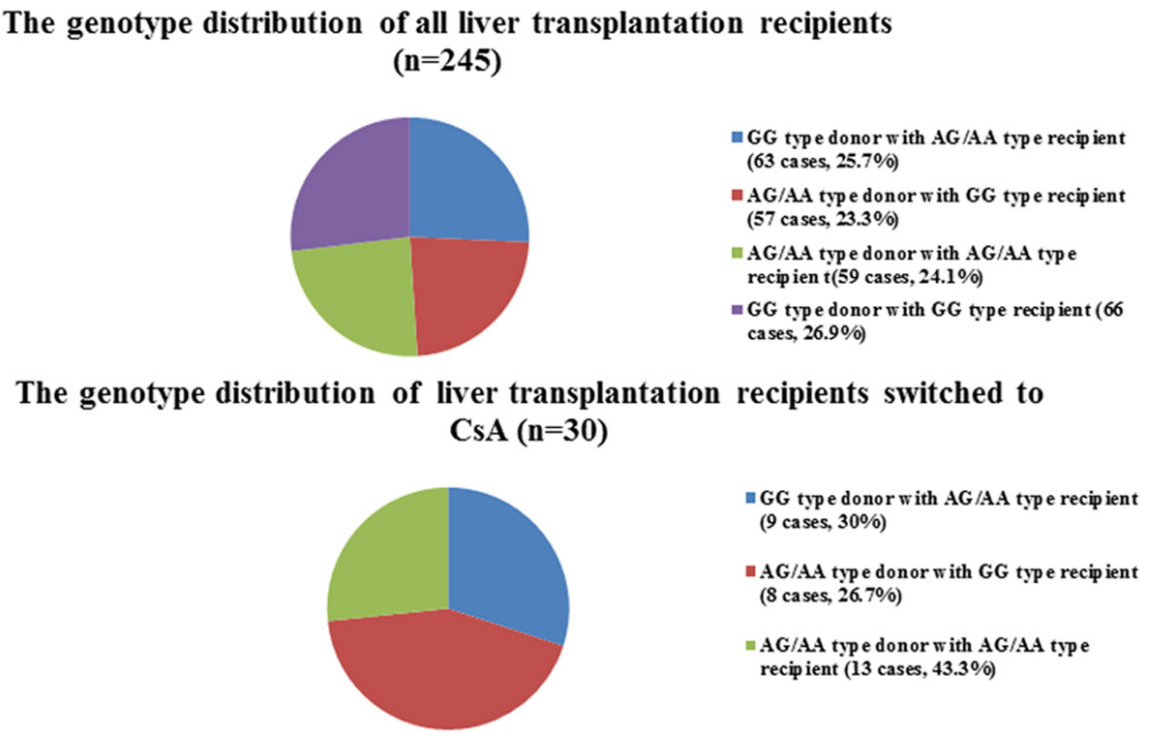

Figure 1. The distribution of *CYP3A5* genotypes in all 245 liver transplantation patients (top) and in the 30 patients who were prescribed a switch from tacrolimus to cyclosporine.

80-120 ng/mL after 4-6 months. C2 value can reach 800-1200 ng/mL after 1-3 months and 600-800 ng/mL after 4-6 months. The suitable daily dose of cyclosporine was 252.17 ± 38.37 (range 200-300) mg.

Effect of the drug switch

Elevated total bilirubin levels in patients began to decline in 8.1 ± 4.3 (range 2-14) days after switching the immunosuppressant, and within a month, plasma bilirubin levels returned to normal (19.74 ± 6.05 μmol/L, range 12-33). After six months of follow-up, the patients had an average creatinine level of 75.67 ± 17.45 μmol/L (range 52-120). One patient had an elevated but stable creatinine level of 120 μmol/L, whereas 22 patients had normal levels of creatinine and stable blood glucose levels. The adverse neurological effects of tacrolimus disappeared. The conditions of the switch and its results are illustrated in **Table 2**.

CYP3A5 genotypes of the 30 patients

We explored the distribution of *CYP3A5* genotypes in the 30 patients with hyperbilirubinemia and compared it to that in the overall cohort of the treated 245 patients with a liver transplant.

CYP3A5 genotype detection method

DNA was extracted from the samples of patients with liver diseases and postsurgical paraffin-embedded liver biopsy samples. *CYP3A5* gene polymorphisms were detected by the polymerase chain reaction-restriction fragment length polymorphism method. The PCR primers used were as follows: forward primer 5'-GAA GCA AGT GGG AGA AAG-3', reverse primer 5'-TGA TGA AGG GTA ATG TGG-3'. The pre-denaturing PCR condition was 94°C for 4 min. Denaturation (94°C for 30 s), annealing (58°C for 30 s), and extension (72°C for 30 s) stages were repeated for 35 cycles. The PCR-amplified products were separated by 2% agarose gel electrophoresis, visualized using ethidium bromide staining, and observed using a gel imaging system, which captured the image. The 427-bp PCR-amplified product was sequenced. The genotypes were classified as follows: 6986A represented *CYP3A5* AA genotype, 6986AG represented *CYP3A5* AG genotype, and 6986G represented *CYP3A5* GG genotype.

Results

Distribution of CYP3A5 genotypes among liver transplant recipients

In the set of the investigated 245 liver transplantations, donor-recipient combinations were as follows: a GG type donor and an AG/AA type recipient, 63 cases (25.7%); an AG/AA donor and a GG recipient, 57 cases (23.3%); an AG/AA donor and an AG/AA recipient, 59 cases (24.1%); and a GG donor and a GG recipient, 66 cases (26.9%). In the 30 patients in which immunosuppressant was switched, liver transplantations involved the following donor-recipient combinations: a GG donor and an AG/AA recipient, 9 cases (30.0%); AG/AA donor with GG recipient, 8 cases (26.6%); and an AG/AA donor and an AG/AA recipient, 13 cases (43.3%). In none of these 30 cases, there was a combination of a GG donor and a GG recipient. The distribution of the genotypes is shown in **Figure 1**. It is clear that the distribution of *CYP3A5* genotypes in patients that developed hyperbilirubinemia was different from that in the overall cohort of patients.

Discussion

Tacrolimus is an immunosuppressant drug, which is widely used following organ transplantation. Its pharmacokinetics is influenced by the activity of the CYP3A5 enzyme. Patients with the *CYP3A5* AA or AG genotype or patients with other genotypes, which receive liver transplants from donors with those genotypes, require a higher dose of tacrolimus to achieve the same immunosuppressive effects [1, 2].

In recent years, many research centers have begun administering tacrolimus based on pharmacogenetic guidelines and achieved positive clinical results [3-5]. Since 2011, when our center started to adjust the initial tacrolimus treatment regimen based on the *CYP3A5* genotype, the treatment has been sequentially administered in 245 cases of first-time adult liver transplants. Patients were treated in accordance with the donor and recipient *CYP3A5* genotype and scheduled for a follow-up six months later. The incidence rates of acute rejection and drug toxicity (such as neurotoxicity, high cholesterol, and high blood sugar) significantly decreased in the first six months following the transplantation [6].

However, the 23 patients included in this study were abnormal in the liver function recovery process after liver transplation, that we thought they were unsuited for tacrolius therapy. They did not demonstrate expected improvement of their condition and manifested uncontrollable rejection of the transplant, and hyperbilirubinemia after the established *CYP3A5* genotype-based tacrolimus treatment despite multiple adjustments to their initial treatment regimen. We have shown that in all these 23 cases, either the donor or recipient tissue (or both-in 13 cases) had *CYP3A5* AA or AG genotype, indicating the need for a higher dose of tacrolimus. However, the therapeutic window of tacrolimus is quite small, and potential side effects drastically limited the maximum dose that could be used in these patients. Therefore, we chose to switch their basic immunosuppressant treatment from tacrolimus to cyclosporine. The dose of cyclosporine was 300 mg/day during the first 6 months, 250 mg/day between 6 and 12 months, and 200 mg/day after 12 months following the switch. The regimen was adjusted based on the clinical condition of the patient, adverse reactions, plasma concentration of the drug, and the types of other concomitant medications. Within 2-14 days of the immunosuppressant switch, bilirubin levels of these patients began to decline, and their rejection reactions were under control.

Cyclosporine is mainly metabolized by CYP3A4 in the body; therefore, it is not affected by the *CYP3A5* gene polymorphisms [7]. Studies have shown that the *CYP3A4*1B* variant is associated with cyclosporine metabolism, but the frequency of *CYP3A4* mutations is very low among the Chinese; therefore, the effect of individual variation on the activity of this enzyme is very small [8].

A limited number of studies have been published abroad about observations in patients who were prescribed a switch from tacrolimus to cyclosporine. In one such study of a group of 203 liver transplant patients who were administered tacrolimus, 37 patients (18%) were switched to cyclosporine. The reasons for the switch included neurotoxicity (41%), diabetes (16%), hematopoietic system disorders (16%), and severe gastrointestinal reactions (11%). Two months after the switch, symptoms improved in 35 of those patients (94%) [9].

Although we suspected that a change in the immunosuppressant regimen might aggravate renal injury, follow-up results showed that the switch from tacrolimus to cyclosporine after transplantation did not result in a significant increase of creatinine levels compared with those observed in the rest of the patients at the same period. A study by Yang et al. [10] showed that in 51 cases, when patients were switched from tacrolimus to cyclosporine, the levels of serum creatinine, blood urea nitrogen, and blood sugar significantly decreased, confirming the safety of immunosuppressant switching.

The cause of hyperbilirubinemia that occurred in the absence of biliary complications following liver transplantation may be very complex [11]. In some cases, in the early postoperative period, hyperbilirubinemia may be related to ischemic bile duct injury [12] and can be alleviated by administering sirolimus [13]. The group of 23 patients in the current study did not develop hyperbilirubinemia in the early postoperative period, suggesting that it was unrelated to reperfusion injury. We suspect that it could be a result of immunopathological damage caused by the improper use of immunosuppressants.

In this report, we identified a group of liver transplant recipients with fast CYP3A5-mediated metabolism, who were not sufficiently sensitive to tacrolimus treatment. We managed to improve therapeutic efficacy by switching their main immunosuppressant from tacrolimus to cyclosporine. Furthermore, we highlighted the value of pharmacogenetics in guiding the application of personalized immunosuppressant therapy in post-transplant patients. Nonetheless, long-term follow-up studies are required to further confirm the safety and rationale for the switch of the conventional immunosuppressant treatment for specific groups of patients.

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Disclosure of conflict of interest

None.

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