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Dig Dis Sci
DOI 10.1007/s10620-009-0773-9

ORIGINAL ARTICLE

Risk Factors for Recurrence of Primary Sclerosing Cholangitis After Living Donor Liver Transplantation: A Single Center Experience

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Received: 4 January 2009 / Accepted: 11 February 2009
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Abstract We retrospectively reviewed our 10-year experience with living donor liver transplantation (LDLT) in 30 consecutive patients with end-stage primary sclerosing cholangitis (PSC) to determine long-term patient and graft survival and risk factors for recurrence of PSC. For strict diagnosis of recurrence, patients with hepatic artery thrombosis ($n = 2$), ABO blood type incompatible transplantation ($n = 3$), and postoperative survival shorter than 1 year ($n = 5$) were excluded from the study, leaving 20 patients for analysis. Recurrence was diagnosed in 11 patients 26–71 months after transplantation. Multivariate analysis showed that cytomegalovirus diseases within 3 months after transplantation and related donors were independent risk factors for recurrence. When the effects on recurrence were compared among donor-recipient relationships, there were significant differences, especially between nonrelated donors and parents. Multivariate analysis showed that age was an independent risk factor for time to graft loss. Cytomegalovirus prophylaxis and avoidance of related donors are important in reducing PSC recurrence, although this is a preliminary report with limitations due to the small number of patients. LDLT for young patients with PSC using grafts from their parents might have to be avoided where deceased donor liver transplantation is available.

Keywords Primary sclerosing cholangitis · Living donor liver transplantation · Cytomegalovirus · Recurrence · Risk factor

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Abbreviations

PSC	Primary sclerosing cholangitis
LDLT	Living donor liver transplantation
CMV	Cytomegalovirus
ALP	Alkaline phosphatase
γ-GTP	Gamma-glutamyl transferase
MELD	Model for end-stage liver disease
HLA	Human leukocyte antigen
HR	Hazard ratio
CI	Confidence interval

Introduction

Primary sclerosing cholangitis (PSC) recurs in 20–30% of patients within 5 years after liver transplantation, and the

Published online: 07 March 2009

incidence of recurrence appears to increase with the time following transplantation [1]. Recurrent PSC is usually first apparent more than 1 year after transplantation with selective elevation of alkaline phosphatase (ALP) and gamma-glutamyl transferase (γ-GTP) [1]. Symptoms of ascending cholangitis and biliary cirrhosis can develop, but patient and allograft survival are not adversely influenced up to 5 years after deceased donor liver transplantation [1]. In a review by Gordon, eight of 13 peer-reviewed publications from 1995 through 2005 found no clinical variables to be associated with an increased risk of PSC recurrence [2]. The remaining studies showed cytomegalovirus (CMV) infection, intact colon, male sex, OKT3 treatment, gender mismatch, and corticosteroid-resistant rejection to be risk factors for recurrent PSC [2]. Gautam et al. failed to analyze risk factors for recurrence in their meta-analysis [3]. Although both Demetris and Gordon mentioned in their reviews that living donor liver transplantation (LDLT) could be a risk for PSC recurrence, there have been no large-scale studies of recurrent PSC after LDLT.

In this report, we reviewed our 10-year experience with LDLT in 30 patients with end-stage PSC, such as intractable pruritus, bacterial cholangitis, gastrointestinal bleeding, ascites, jaundice, and hepatic encephalopathy. An emphasis was placed on examining long-term patient and graft survival, and the incidence and outcome of recurrent PSC. Risk factors for recurrence and graft loss were also evaluated. Based on this experience, we propose a strategy to improve the outcome of LDLT for PSC.

Patients and Methods

Patients

Thirty patients with PSC (14 male and 16 female) underwent primary LDLT, including three domino transplantations, from July 1996 through August 2005. Ages ranged from 5 to 58 years, with a median age of 29 years. Seventeen patients also had ulcerative colitis, and one patient had early cholangiocarcinoma. Preoperative status was "at home" in 11 patients and "hospitalized" in 19 patients. The model for end-stage liver disease (MELD) score ranged from 8 to 36, with a median of 22 in the 28 adult patients. The New Mayo score for PSC ranged from 1.557 to 35.403, with a median of 8.916. The donors were parents in 16 cases, siblings in seven cases, sons in two cases, husbands in two cases, and domino donors of familial amyloid polyneuropathy in three cases. The graft type was the whole liver in two cases, the right lobe in 21 cases, the left lobe in five cases, and the left lateral segment in two cases. The blood type combination was identical in 21 cases, compatible in six cases, and incompatible in three cases. The follow-up period ranged from 1 to 133 months, with a median length of 63 months.

Written informed consent was obtained from each patient and donor, and the study was approved by the Ethics Committee of Kyoto University Hospital according to the Declaration of Helsinki of 1975 as revised in 1996.

Donor Selection

Donors were selected from parents, grandparents, siblings, offspring, and spouses of recipients for standard LDLT. In domino transplantation, the donors were recipients of LDLT for familial amyloid polyneuropathy. Donors were fully informed of the risks and benefits of LDLT and confirmed their voluntary decision to become a live donor, and consent was obtained from each. Our institutional donor age limit is 65 years. Preoperative evaluations for estimating graft and remnant liver volume in the donor were performed using three-dimensional reconstructed images of the hepatic vascular anatomy.

Definition of Recurrence

Recurrence of PSC was strictly defined using both positive and negative criteria according to Graziadei et al. [4]. Criteria included a confirmed diagnosis of PSC before transplantation and intrahepatic multiple biliary strictures confirmed by cholangiography occurring more than 90 days after transplantation, or biopsy findings showing fibrous cholangitis and/or fibro-obliterative lesions with or without ductopenia, biliary fibrosis, or biliary cirrhosis. All of the above findings occurred in the absence of hepatic artery thrombosis/stenosis, established chronic (ductopenic) rejection, anastomotic strictures alone, nonanastomotic strictures occurring before post-transplantation day 90, and ABO blood group incompatibility between donor and recipient. When clinical signs (fever, abdominal pain, jaundice, pruritus) or laboratory signs (hepatic enzyme levels or serum bilirubin levels at least 1.5 times greater than the normal upper range) indicated cholangitis, a liver biopsy was performed percutaneously. Pathological diagnosis was made by a pathologist (H-H.) [5]. Cholangiography was performed by occasional percutaneous transhepatic cholangiography, or endoscopic retrograde cholangiography, or recently magnetic resonance cholangiography.

In our 1,350-patient experience with LDLT, none of the patients developed multiple intrahepatic biliary strictures without gallstones or biliary casts, other than after ABO incompatible transplantation, after hepatic artery thrombosis/stenosis, or with the original disease of PSC.

Surgery and Immunosuppression Regimen

Twenty-seven patients underwent standard LDLT, and three underwent domino LDLT. The standard LDLT

procedures for both donors and recipients were performed according to our previously reported methods [6, 7], and we have also reported our method of domino transplantation [8].

Briefly, all grafts for standard LDLT were flushed and preserved with a histidine-tryptophan-ketoglutarate solution (Dr. Franz Köhler Chemie, Alsbach-Hähnlein, Germany), and three grafts from domino donors were flushed with University of Wisconsin solution. The grafts were revascularized by reconstruction first of the portal vein and then of the hepatic artery. Regarding biliary reconstruction, the biliary system was reconstructed with a Roux-en-Y choledochojejunostomy if inflammation was noted in routine frozen section biopsy specimens of the common bile duct. If atypia or inflammation was present in the frozen section biopsy specimen, the entire common bile duct was resected to the level of the pancreas.

Methylprednisolone (10 mg/kg) was administered just before the start of graft reperfusion, followed by 1 mg/kg of intravenous methylprednisolone for 3 days and 0.5 mg/kg of intravenous methylprednisolone for a further 3 days. Oral prednisolone (0.3 mg/kg) was continued. Tacrolimus was administered on the postoperative day 1 according to our standard procedure [9]. Antimetabolites (azathioprine or mycophenolate mophetil) were added in patients with refractory rejection or by decision of the attending physicians during long-term follow-up. If acute cellular rejection was confirmed with Banff criteria [10], patients received a 3- to 5-day course of intravenous bolus therapy (methylprednisolone, 10 mg/kg).

Tissue typing was performed in patients and donors for human leukocyte antigen (HLA)-A, HLA-B (Bw), HLA-C, HLA-DR, and HLA-DQ for class I and II loci according to Terasaki methods. We found that HLA-DR 15 was most frequent (15/30; 50%) in 30 patients of PSC and that the reported incidence of DR 15 was 14.8% in Japanese population [11].

Prophylaxis for Viral Infection

Oral acyclovir (10 mg/kg/day) was administered on postoperative day 7 and continued for 3 months as prophylaxis generally for herpes virus family. Preemptive treatment for CMV was performed instead of prophylaxis. Gancyclovir was administered when weekly CMV antigenemia was detected over two cells in 20,000 cells or CMV diseases were diagnosed. CMV diseases were diagnosed when clinical manifestations concomitant with positive CMV antigenemia developed or when positive findings, such as microabscess in the liver and inclusion body in the intestinal wall, were observed on pathological examination of biopsy specimens.

Statistical Analysis

Overall survival curves were calculated with the Kaplan–Meier method. The univariate log-rank test was used to evaluate the effects of characteristics on recurrence and graft loss. Multivariate Cox regression analysis was used to evaluate the association between time to recurrence or time to graft loss and patient characteristics and to estimate hazard ratios (HRs). To assess the effect of PSC recurrence on graft loss, we performed Cox regression analysis with recurrence as the time-dependent covariate. Graft loss was defined as death or retransplantation. The software program SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. All statistic analysis was done by S.T.

Results

Analysis of Risk Factors for Recurrence and Graft Loss

Three patients with ABO incompatible transplantation and two patients with hepatic artery thrombosis were excluded from analysis for recurrence according to Graziadei et al. [4]. To evaluate long-term effects of characteristics on recurrence and graft loss, five patients who died within 1 year after transplantation were excluded. These five patients did not have recurrence of PSC until death. The remaining 20 patients were enrolled in the statistic analysis.

The age of the remaining 20 patients analyzed in this study ranged from 5 to 58 years, and the median age was 32 years. The characteristics of donors and recipients are shown in Table 1. The 5- and 10-year graft survival was 68.6 and 39.7%, respectively, and the 5- and 10-year patient survival was 81.4 and 81.4%, respectively (Fig. 1). The patients were divided into three groups: under 18 years, 18–29 years, and 30 years or older. Recurrence was diagnosed in 11 patients at 26–71 months after transplantation. In all 11 patients, the recurrence was diagnosed by liver biopsy specimens and five patients by PTC and six patients by MRCP. Recurrence developed in six patients within 36 months after transplantation, and in three patients between 36 month and 48 month, and two patients thereafter. Recurrence developed in a patient receiving a graft from her husband 71 months after transplantation. Two patients became pregnant before recurrence.

Each clinical course of 11 patients with recurrence is shown in Fig. 2. The interval from recurrence to retransplantation ranged from 9 to 93 months. Four of the patients received second grafts from the other parent, two from siblings, one from her husband, and one from a nonrelated

Table 1 Characteristics and outcome according to patient characteristics ($n = 20$)

Characteristics	Number (%)	Recurrence		Graft loss	
		Incidence (%)	*P*-value	Incidence (%)	*P*-value
Age					
Under 18 years old	3 (10)	67	0.016	100	0.001
18–29 years old	5 (25)	80		80	
30 years old -	12 (60)	42		17	
MELD score					
−21	10 (56)	40	0.042	30	0.257
22−	8 (44)	75		50	
Unknown	2				
New Mayo					
<9.0	10 (50)	60	0.422	50	0.498
9.0≤	10 (50)	50		40	
Ulcerative colitis					
Without	7 (35)	43	0.642	29	0.835
With	13 (65)	62		54	
Number of immunosuppressants at 1 year after transplant					
1	3 (15)	100	0.728	33	0.376
2	12 (60)	50		58	
3	5 (25)	40		20	
Biliary reconstruction					
Choledocho–choledochostomy	4 (20)	75	0.852	50	0.779
Choledocho–jejunostomy	16 (80)	50		44	
Number of biliary anastomosis					
1	13 (65)	69	0.142	54	0.596
2 or more	7 (35)	29		29	
Biliary anastomotic complications within 1 year					
Without	10 (50)	40	0.464	40	0.454
With	10 (50)	70		50	
Corticosteroid pulse for ACR within 3 months					
No	9 (45)	56	0.160	44	0.923
1 time	7 (35)	86		57	
2 times	4 (20)	0		25	
CMV diseases within 3 months					
Without	14 (70)	43	0.007	36	0.085
With	6 (30)	83		67	
Donor					
Nonrelated	5 (25)	20	0.008	20	0.152
Related	15 (75)	67		53	
Sex (recipient)					
Male	8 (40)	25	0.080	50	0.912
Female	12 (60)	75		42	
Sex (donor)					
Male	16 (80)	44	0.106	44	0.945
Female	4 (20)	100		50	
Gender mismatch					
Match	10 (50)	40	0.419	40	0.745
Mismatch	10 (50)	70		50	
Graft type					
Left lobe	5 (25)	80	0.083	80	0.696

Table 1 continued

Characteristics	Number (%)	Recurrence		Graft loss	
		Incidence (%)	*P*-value	Incidence (%)	*P*-value
Right lobe	13 (65)	54		31	
Whole liver	2 (10)	0		50	
HLA-DR15 (recipient)					
Without	11 (55)	55	0.861	55	0.293
With	9 (45)	56		33	
HLA-DR15 (donor)					
Without	13 (65)	31	0.025	38	0.504
With	7 (35)	100		57	
HLA-DR15 (recipient and donor)					
Without	15 (75)	40	0.068	40	0.633
With	5 (25)	100		60	
HLA-DR15 (either donor or recipient)					
Without	9 (45)	44	0.456	56	0.188
With	11 (55)	64		36	
HLA-DR1501/1502 (recipient and donor)					
Without	16 (80)	44	0.252	44	0.420
With	4 (20)	100		50	

P-value: log rank test

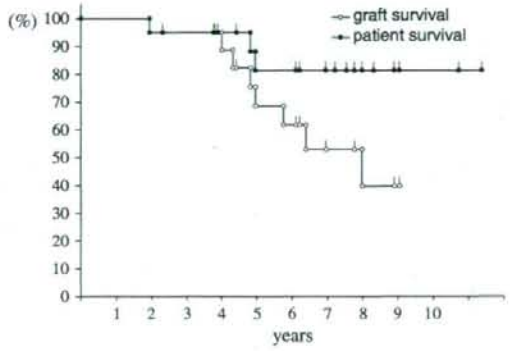

Fig. 1 Patient and graft survival after LDLT for PSC of 20 patients

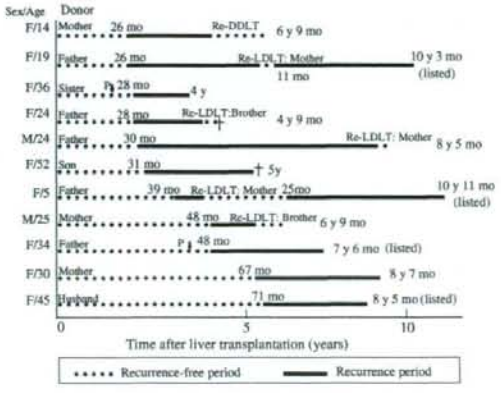

Fig. 2 Clinical courses of 11 patients with PSC recurrence. Sex/age: sex and age (years) of recipients, *Re-DDLT* retransplantation with deceased donor liver transplantation, *Re-LDLT* retransplantation with LDLT, *listed* on waiting list for retransplantation, *P* pregnancy, *y* years, and *mo* months

deceased donor. One patient died of surgical complications 1 month after retransplantation. In two patients who received the second grafts from the other parent, PSC recurred at 11 and 25 months after retransplantation, respectively. The interval between a second recurrence and retransplantation was shorter than the interval between the first transplantation and the first recurrence (the second recurrence interval versus the first recurrence interval in each patient: 11 months versus 28 months, and 25 months versus 39 months). Four patients are on waiting lists to undergo deceased donor liver transplantation because of graft failure due to PSC recurrence. Two patients are waiting for second grafts, and two patients are waiting for third grafts.

Five nonrelated donors were two husbands and three domino-donors. Fifteen related donors were eight fathers, two mothers, two brothers, one sister and two sons. Recurrence of PSC developed in eight of ten patients (80%) receiving grafts from parents, including one of two (50%) from children, one of three (33%) from siblings, and one of five (20%) from non-related donors.

The hepatic chemistries at the time of recurrence were shown in Fig. 3. The levels of ALP and γ-GTP were

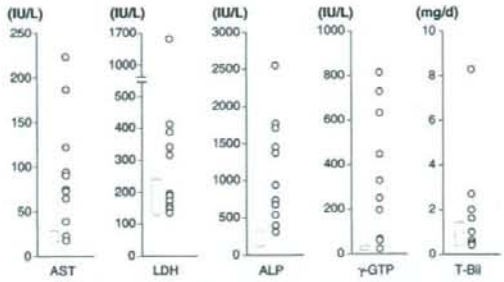

Fig. 3 Hepatic chemistries at diagnosis of PSC recurrence. The *shaded bars* indicate the normal range for each hepatic chemistry

increased to higher than the normal ranges in ten of 11 patients with recurrence.

Four patients with PSC recurrence and three without recurrence had CMV diseases within 3 months after transplantation. The CMV diseases were viral syndrome on postoperative day (POD) 25, hepatitis on POD 26, peritonitis on POD 26, colitis 3 months after transplantation in the four patients with the recurrence, and viral syndrome on PODs 27 and 31, and diarrhea on POD 28 in the three patients without recurrence. All CMV diseases were treated successfully with ganciclovir and decrease of immunosuppression. Ten patients had biliary anastomotic complications. Six of them had anastomotic stenosis, two had anastomotic leakages, and two had both leakage and stenosis.

Risk Factors for Recurrence

Univariate log-rank test of the risk factors for recurrence (Table 1) showed that younger age ($P = 0.016$), MELD score >21 ($P = 0.042$), CMV diseases within 3 months after transplantation ($P = 0.007$), a related donor ($P = 0.008$), and a donor with HLA-DR15 ($P = 0.0025$) were significantly associated with a greater incidence of recurrence after transplant. Multivariate Cox regression analysis showed that independent risk factors for recurrence were CMV diseases within 3 months after transplantation (HR, 4.53; 95%CI, 1.17–17.6; $P = 0.029$), and a related donor (HR, 7.98; 95%CI, 0.96–66.1; $P = 0.054$) (Table 2). When the effects on recurrence were compared among parents, siblings, sons, and nonrelated donors, there were significant differences, especially between nonrelated donors and parents (Table 3).

Risk Factors for Graft Loss

Seven of the 11 patients (64%) with recurrence and two of the nine patients (22%) without recurrence had graft loss. However, recurrence of PSC was not a statistically significant risk factor according to score tests based on the Cox regression model with recurrence as a time-dependent covariate ($P = 0.076$). The univariate log-rank test of the risk factors for graft loss showed that younger age ($P = 0.001$) was significantly associated with a greater incidence of graft loss (Table 1). In multivariate analysis using Cox regression analysis, only age per year was an independent risk factor for time to graft loss (HR, 0.87; 95% CI, 0.79–0.96; $P = 0.006$).

Table 2 Cox regression analysis for time to relapse in PSC patients ($n = 20$)

Factors	Hazard ratio	95%CI	*P*-value
CMV diseases within 3 months			
Without	1	–	–
With	4.53	1.17–17.6	0.029
Donor			
Nonrelated	1	–	–
Related	7.98	0.96–66.1	0.054

CI confidence interval

Table 3 Impact of related donors on time to relapse in PSC patients ($n = 20$)

Characteristics	Number (%)	Relapse	
		Incidence (%)	*P*-value*
Donor			
Nonrelated donors	5 (25)	20	0.034
Parents	10 (50)	80	
Sons	2 (10)	50	
Siblings	3 (15)	33	
Factors	Hazard ratio	95%CI	*P*-value
Donor			
Nonrelated donors	1	–	–
Parents	17.9	1.48–218	0.024
Sons	11.5	0.48–276	0.131
Siblings	3.3	0.20–52.6	0.402

* Log rank test

CI confidence interval

Discussion

The recurrence of PSC in this study was strictly defined according to the criteria of Graziadei et al. [4]. One of two patients with hepatic artery thrombosis and one of three patients undergoing ABO-incompatible transplantation were judged to have PSC recurrence rather than ischemic cholangitis or humoral rejection on the basis of clinical courses and histological examination of biopsy specimens. All five patients were excluded from the statistic analysis.

We also assessed the risks of characteristics for recurrence or graft loss. Our statistic methods using time-to-event analysis can estimate both risks for event of recurrence and risks for time to recurrence. Because the number of enrolled patients was only 20, the top two independent risk factors for early recurrence were obtained in multivariate analysis. These sophisticated statistical analysis procedures showed that CMV diseases within 3 months after transplant and related donors were independent risk factors for the recurrence.

CMV infection has been reported as a risk factor for biliary anastomotic complications, although the mechanisms have not been clarified yet [7, 12, 13]. Our results suggested that CMV prophylaxis might be important to reduce the recurrence of PSC. Our current protocol for CMV is not satisfactory for PSC patients. Valganciclovir, an oral pro-drug of gancyclovir, is an attractive agent for both antiviral prophylaxis and the preemptive treatment of CMV viremia [14].

Related donor is an issue specific to LDLT. We found that a related donor was also an independent risk factor for recurrence, and grafts transplanted from parents had a higher incidence of recurrence. The incidence of recurrence in recipients with grafts from nonrelated donors was similar to reports of deceased donor liver transplantation [2]. Siblings seem to be more suitable donors in related donors. HLA-DR8 was reported as one of the risks for recurrence in the Caucasian population [15, 16]. Although six patients had HLA-DR8 (20%) in our series, five of them were excluded from statistic analysis due to their clinical courses. Tamura et al. [17] reported that two of nine PSC patients had HLA-DR8 (18%) and there was no significance in recurrence in Japanese patients. On the other hand, the incidence of HLA-DR15 was very high in PSC recipients in our series (45%) and Tamura's series (four in nine, 44%). Tamura et al. [17] reported that three in four patients with HLA-DR15 had recurrence (75%). In our series, a donor with HLA-DR15 was found to be a significant risk with the log rank test, although not with Cox regression analysis. We separately analyzed the significance of HLA-DR1501 and HLA-DR1502 on recurrence and found no statistic significance. However, these results suggested that some genetic factors play significant roles in the recurrence of PSC.

Although not significant by multivariate Cox regression analysis, MELD score was one of the significant risk factors for recurrence by univariate log-rank test. In other words, the more advanced the disease, the higher the rate of recurrence after LT, which would suggest earlier LT for PSC patients to prevent recurrence.

The number of immunosuppressions 1 year after transplantation had no significant impact on recurrence, although recurrence rate was lower in patients with two or three immunosuppressions than patients with one. "One" means only calcineuline inhibitors (CI), "two" means CI and steroid or antimetabolites, and "three" means CI, steroid, and antimetabolites. Among antimetabolites, mycophenolate mophetil is major in our hospital. Rapamycine also seems to be promising, but there is no evidence yet to support this. This result suggested that current immunosuppressive regimens could not prevent recurrence of PSC.

In duct-to-duct anastomosis in liver transplantation for patients with PSC, cholangiocarcinoma in the remnant bile duct is a concern. Goss et al. [20] reported that they choose choledochojejunostomy when inflammation was present in frozen-section biopsy specimens of the common bile duct. Otherwise, they choose duct-to-duct anastomosis. We had followed their policy but we encountered a case in which sclerosing cholangitis developed in the remnant duct 2 years after transplantation. The remnant duct was resected, and the duct was reconstructed by means of choledochojejunostomy. There was no malignancy in the resected bile duct. However, PSC recurred in the bile duct of the graft 2 years after revision. After this experience, choledochojejunostomy became our first choice for biliary reconstruction for patients with PSC, although the present study did not show a significant effect of biliary reconstruction on recurrence. The incidence of biliary complication was very high on PSC patients compared to patients with other original diseases. In our past series, the overall incidence of anastomotic biliary complications ranged from 18 to 24% [7, 18]. We might find that PSC itself is a risk for anastomotic complication when the number increases in the future.

Reports of pediatric PSC are rare. Debray reported their 56 children of PSC [19]. The prognosis was poor, but liver transplantation should be considered. Fifteen patients underwent liver transplantation and 11 of them were alive without recurrence longer than 6 months. In our series, two of three children had recurrence and adult patients under 30 were likely to have early recurrence. There were no children of 5-year graft-loss-free. In multivariate analysis using Cox regression analysis, only age per year was an independent risk factor for time-to-graft loss in this study. Goss et al. [20] have reported that PSC recurrence has no effect on patient or graft survival in deceased donor liver transplantation. Although PSC recurrence was not a significant risk for graft loss in this study, six of 11 patients with PSC recurrence underwent retransplantation for graft failure, one patient died of graft failure, two patients are on waiting lists for retransplantation, and only two patients are alive with acceptable liver function.

Whether the donor is a relative or not, one might hypothesize that preoperative liver biopsy for the donor candidate might be effective in decreasing PSC recurrence in LDLT. In our hospital, liver biopsy is performed in

donor candidates with normal liver function and possible steatosis by imaging studies for safety of recipients and donors, anti-mitochondrial antibody-positive donor candidates with normal liver functions for safety of recipients and donors, parents of ornithion transcarbamylase deficiency to measure enzyme activity, and parents of children of Alagille syndrome to avoid a graft without extrahepatic bile duct. In our PSC series, no living donors for PSC patients had abnormal pathological findings in liver specimens obtained during donor surgery. Routine studies on liver specimens by preoperative liver biopsy of donor candidates cannot prevent PSC recurrence, unless a biomarker specific to PSC is available.

Although we need to study more patients to show firm conclusions, this study suggested that deceased donor liver transplantation using a graft from non-related donors is preferable for a young patient with PSC to LDLT using a graft from a parent in countries where deceased donor liver transplantation is available.

Acknowledgments This work was funded by a Health Science Research Grant on a Specific Disease (Study of Intractable Liver and Biliary Diseases), The Ministry of Health, Labor and Welfare of Japan, 2005 to 2007.

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A Retrospective Analysis of Vancomycin Pharmacokinetics in Japanese Cancer and Non-cancer Patients Based on Routine Trough Monitoring Data

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Received June 13, 2008; accepted October 24, 2008; published online October 28, 2008

The pharmacokinetics of vancomycin was retrospectively examined based on trough concentrations obtained during routine therapeutic drug monitoring to examine possible pharmacokinetic differences between adult Japanese cancer and non-cancer patients with various degrees of renal function. A total of 231 data points from 65 cancer patients and 41 non-cancer patients were collected, and patients' background, vancomycin dose, and vancomycin clearance estimated by an empirical Bayesian method were summarized. Regarding the patients' characteristics and clinical laboratory test data, no clear differences were found between the two groups. The relationship between vancomycin clearance and creatinine clearance were similar between the groups, suggesting little effect of malignancy on vancomycin clearance. After the sub-group comparisons regarding fluid retention and cancer type, no clear differences were found in the vancomycin clearance *versus* creatinine clearance relationship. We conclude that the initial dose of vancomycin should not necessarily be adjusted for cancer patients. For individualized vancomycin-based therapy, dose adjustment at the appropriate time is important according to information from routine therapeutic drug monitoring and clinical laboratory tests, and to observations of the efficacy, nephrotoxicity, and other conditions in each patient.

Key words vancomycin; pharmacokinetics; cancer patient; therapeutic drug monitoring; malignant; methicillin-resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is known to cause diseases especially in individuals with less resistance to infectious bacteria, such as elderly, immunosuppressive, or postoperative patients, or patients with total parenteral nutrition (TPN), with trachea catheter or any passage in the trachea, premature infants and newborns, and wound patients. In cancer patients, the risk rate for MRSA infection is generally high because of depressed immunocompetence, a side effect of anticancer agents, and nosocomial infections by MRSA have been reported.[1)]

Vancomycin, a glycopeptide antibiotic, has been used to treat MRSA infections, and it requires effective therapeutic drug monitoring (TDM) during treatment period especially to reduce the incidences of nephrotoxicity by controlling its serum concentration within the therapeutic range.[2—4)] The pharmacokinetics of vancomycin in MRSA-infected patients has been studied,[5—10)] and population pharmacokinetic parameters were reported in adult Japanese patients.[11)] More recently, an empirical Bayesian method has been widely applied to TDM data,[12)] and the predictability of the Bayesian forecasting methodology for vancomycin with the population pharmacokinetic parameters in Japanese patients was examined.[13,14)]

Vancomycin is mainly eliminated into urine, and most of the pharmacokinetic variability can be explained by the degree of renal function.[15)] However, there have been several reports of a difference in vancomycin pharmacokinetics between malignant and non-malignant adult[7,10)] and pediatric[16—18)] patients, suggesting malignant state to be a covariate of the pharmacokinetic variability. Le Normand *et al.*[7)] reported a larger distribution volume and shorter elimination half-life in neutropenic adult patients and they suggested the need of new therapeutic regimen. Fernández de Gatta *et al.*[10)] reported a significant effect of malignancy on clearance in patients with hematologic malignancies and proposed a nomogram for dose setting in cancer patients. Teramachi *et al.*[19)] reported that the clearance was significantly greater in the malignancy group (0.077±0.029 l/h/kg) than non-malignancy group (0.056±0.018 l/h/kg) in Japanese patients. They also found a significant difference in distribution volume at steady state (1.29±0.41 l/kg in malignancy group, 1.05±0.34 l/kg in non-malignancy group). However, it is also true that in their report many patients in the malignancy group showed similar values of clearance and distribution volume to those in the non-malignancy group. Based on these findings, it seems that the strategy for dose setting in cancer patients seems confusing for vancomycin, and even if the mean differences in the pharmacokinetic parameters exist, we considered that dosage individualization based on observed drug concentration data should be much more important as Fernández de Gatta *et al.*[10)] commented, because the inter-individual variability of vancomycin pharmacokinetics is still extensive. Thus in this report, we aimed to clarify the possible impact of such pharmacokinetic differences on the routine vancomycin therapy and performed a retrospective study regarding the pharmacokinetics of vancomycin based on clinical data which were taken in the routine therapeutic drug monitoring in Kyoto University Hospital.

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MATERIALS AND METHODS

Data Source and Patients Characteristics The data from patients treated with vancomycin and monitored as to its serum concentration during hospitalization in Kyoto University Hospital (Kyoto, Japan) were collected retrospectively. The investigation period was from November 2005 to September 2006. Patients under 14 years old or patients with hemodialysis were excluded. The total number of patients was 106, including 65 cancer patients and 41 non-cancer patients. This study was conducted in accordance with the Declaration of Helsinki and its amendments, and the protocol was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine.

All data were retrospectively collected from the electronic charts, and patient characteristics and results of clinical laboratory tests such as serum creatinine (Scr), blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum albumin (ALB) levels were summarized. Main diagnosis, vancomycin dose, dosing interval, and trough concentration data at steady state were also collected. Serum concentrations were measured by fluorescence polarization immunoassay (FPIA) using a TDxFLx® analyzer (Abbott Japan, Tokyo, Japan) in the Department of Pharmacy, Kyoto University Hospital. Only the trough data at more than 3 d after the initiation of vancomycin therapy were used for the data at steady state. In addition, a steady state condition was confirmed for each patient by visually checking the simulated concentration–time profiles by the Bayesian method described below. In some patients, more than two data points were obtained on different dosing days, and finally, the number of trough concentration values was 145 in cancer patients and 86 in non-cancer patients. In some patients, doses were also changed during treatment, and so we picked the dose and concentration data at typical sampling times, *i.e.* the sampling time when the steady state trough concentration was first measured (referred to as the 'first sampling point') and the sampling time when the concentration data were last measured ('last sampling point'). For daily dose and trough concentration, data were summarized at each of these sampling points. For clinical laboratory tests, values at only the last sampling point were used for further comparison.

Data Analysis The relationships of the observed trough concentration with dose, values of clinical laboratory tests, and patients' other characteristics were examined, and statistical comparisons between the cancer and non-cancer patients were performed accordingly for these observed values. Especially for relationships of the trough concentrations with daily dose, dosing interval, calculated creatinine clearance (CLcr) by the Cockcroft–Gault method,[20] and ALT and ALB were examined by multiple linear regression analysis.

Using the observed trough concentration data, an empirical Bayesian method[21] was applied to obtain the individual estimates of vancomycin clearance (CL). In this study, we obtained the Bayesian estimates for distribution volume but we did not use them for further discussion because only the trough concentration data were available for most patients. Bayesian estimation was carried out using the software package VCM-TDM (Ver. 2.03, Shionogi & Co., Ltd., Osaka, Japan)[22] based on a linear two-compartment model. A population pharmacokinetic parameter set for Japanese adult patients[11] was adopted, where calculated creatinine clearance (CLcr) by the Cockcroft–Gault method[20] was used. That is, in case the calculated CLcr is greater than 85 ml/min, the population average clearance was set to 3.51 l/h, otherwise an equation CL (l/h)=0.0478×CLcr (ml/min) was adopted. All the available trough concentration data in each patient were simultaneously used for the Bayesian estimation. In the patients whose Scr changed more than 0.5 mg/dl, the Bayesian estimates obtained from only the data before the Scr change were used for the following evaluation.

Bayesian predictability was evaluated by a correlation analysis of the observed and predicted values of vancomycin concentration, and mean prediction error (ME) and root mean squared error (RMSE) were used as indices of bias and prediction, respectively[23];

$$\mathrm{ME} = \frac{1}{n}\sum_{i=1}^{n}(C_{\mathrm{pred}} - C_{\mathrm{obs}}) \tag{1}$$

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(C_{\mathrm{pred}} - C_{\mathrm{obs}})^2} \tag{2}$$

In Eqs. 1 and 2, n is number of concentration data, C_{pred} is predicted drug concentration and C_{obs} is observed drug concentration.

The cancer patients were divided into sub-groups according to the presence of fluid retention or cancer type, and comparisons of vancomycin clearance were made based on statistical analyses or visual inspection. Type of fluid retention such as ascites and pleural effusion was diagnosed by physicians based on the imaging test, and edema was also diagnosed by physicians by referencing increase of body weight.

Statistical Analysis Mean difference of the patients' characteristics including the results of clinical laboratory tests, vancomycin dose, observed trough concentration, and estimated clearance were tested between the cancer and non-cancer patients. Statistical comparisons were made for the sub-group data, if appropriate, by Student's t-test or Welch's t-test according to results of an F-test for equal variance, with a 5% level of significance. Statistical tests and regression analyses were carried out with Microsoft Excel® (Microsoft, WA, U.S.A.) or Dr. SPSS (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Comparison of Patients' Characteristics The patients' characteristics are summarized in Table 1. No significant differences were found between the two groups for age, body weight, Scr, BUN, AST, ALT, ALB and CLcr. The cancer type and clinical stage in the cancer patients are summarized in Table 2. Most patients were in stage III or IV.

Comparison of Vancomycin Dose and Observed Trough Concentration A summary of the vancomycin dose and observed trough concentration is given in Table 1. In some patients, doses were changed during the treatment, and thus dose and trough concentration at both the first and the last sampling point as defined in the previous section were compared. In the case of the first sampling point, the

Table 1. Summary of Patients' Characteristics

	Cancer	Non-cancer	
Number of patients	65 (male 43, female 22)	41 (male 24, female 17)	
Age (years old)	57.4±14.4 (24—81)	62.6±15.6 (27—92)	N.S.
Body weight (kg)	52.6±11.3 (35.3—94.8)	53.1±10.5 (31.4—78.7)	N.S.
Scr (mg/dl)	0.8±0.8 (0.3—6.1)	0.8±0.4 (0.3—2.2)	N.S.
BUN (mg/dl)	15.8±12.8 (3—76)	18.3±13.6 (3—55)	N.S.
AST (IU/l)	27.9±21.6 (6—129)	35.0±33.1 (8—182)	N.S.
ALT (IU/l)	34.0±32.2 (3—194)	34.5±41.0 (6—245)	N.S.
ALB (g/dl)	3.1±0.5 (1.8—4.5)	3.2±0.6 (1.8—4.4)	N.S.
CLcr (ml/min/kg)[a]	1.69±0.75 (0.21—3.61)	1.62±0.83 (0.42—3.70)	N.S.
CLcr (ml/min)[a]	88.1±40.3 (8.7—181.2)	86.0±46.5 (17.8—203.8)	N.S.
Number of trough measurements	145	86	
Daily dose (mg/kg)[b]	35.5±11.9 (10.5—78.5)	28.5±8.9 (12.2—46.7)	$p<0.01$
Daily dose (mg/kg)[c]	31.4±12.5 (11.8—78.5)	30.4±11.1 (12.2—53.4)	N.S.
Vancomycin conc. (μg/ml)[b]	11.5±5.4 (2.4—29.7)	10.8±5.2 (2.1—24.2)	N.S.
Vancomycin conc. (μg/ml)[c]	11.5±5.0 (2.1—29.7)	12.8±6.4 (2.1—36.6)	N.S.

Values are the mean±S.D. (minimum—maximum). N.S.: Not significantly different. *a*) Calculated by Cockcroft-Gault formula. *b*) At the first sampling point. *c*) At the last sampling point.

Table 2. Cancer Type and Number of Patients in Each Clinical Stage

Cancer type	Stage				Total
	I	II	III	IV	
Acute myelocytic leukemia		Not defined			9
Lung cancer			5	3	8
Esophageal cancer	1		3	2	6
Bladder cancer	2	1		3	6
Malignant lymphoma	1	1	1	2	5
Lymphocytic leukemia		Not defined			4
Pharyngeal cancer		1	1	1	3
Hepatocellular carcinoma				3	3
Soft tissue tumor		Not defined			3
Cervical cancer	1			1	2
Tongue cancer	1		1		2
Multiple myeloma		1	1		2
Pancreatic cancer			1	1	2
Osteosarcoma		Not defined			2
Testicular cancer			1		1
Duodenal neoplasm				1	1
Gastric cancer				1	1
Gallbladder cancer				1	1
Brain tumor		Not defined			1
Adult T-cell leukemia		Not defined			1
Chronic myelocytic leukemia		Not defined			1
Myelodysplastic syndrome		Not defined			1

Not defined: clinical stage is not defined because of non-solid cancer.

dose was 35.5±11.9 mg/kg (mean±S.D.) and 28.5±8.9 mg/kg in the cancer and non-cancer patients, respectively, which were significantly different ($p<0.01$), but the trough concentration was not significantly different (11.5±5.4 μg/ml in the cancer patients and 10.8±5.2 μg/ml in the non-cancer patients). In the case of the last sampling point, no significant differences were found in dose (31.4±12.5 mg/kg *vs.* 30.4±11.1 mg/kg) or trough concentration (11.5±5.0 μg/ml *vs.* 12.8±6.4 μg/ml).

Figures 1 and 2 show the relationship of trough concentration with daily dose (Fig. 1), CLcr (Fig. 2a), ALT as a marker of hepatic function (Fig. 2b), and ALB (Fig. 2c). Multiple linear regression analysis with a stepwise method showed that the trough concentration depends on daily dose ($p<0.001$) and CLcr ($p<0.001$), and was independent of dosing interval, ALT, ALB and cancer type.

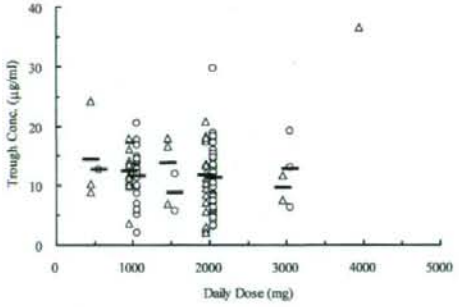

Fig. 1. Relationship of Trough Concentration with Daily Dose

Open circles: cancer patients, open triangles: non-cancer patients. Mean values for each dose are plotted with horizontal bars.

Comparison of Bayesian Estimated Vancomycin Clearance Figure 3 shows a correlation between the observed and the Bayesian predicted vancomycin trough concentrations. The indices ME and RMSE were 0.49 and 2.91, respectively for the cancer patients, and 0.37 and 2.00 for the non-cancer patients. According to these results, Bayesian predictability seems generally acceptable. The accuracy of Bayesian estimation depends on sampling times,[24—26] and it is reported that the estimation error for distribution volume is large but that for clearance is relatively small in case using only trough data.[26] Therefore, we did not compare distribution volume by the Bayesian method between the cancer and non-cancer patients as only trough concentration data were available.

The predicted vancomycin clearance was (mean±S.D., minimum—maximum) 3.73±1.39 (0.912—5.97) l/h for the cancer patients, and 3.48±1.56 (0.545—6.87) l/h for the non-cancer patients, and when adjusted for body weight, clearance was 0.072±0.028 (0.022—0.140) l/h/kg for the cancer patients, and 0.065±0.026 (0.017—0.127) l/h/kg for the non-cancer patients. Although average clearance did not

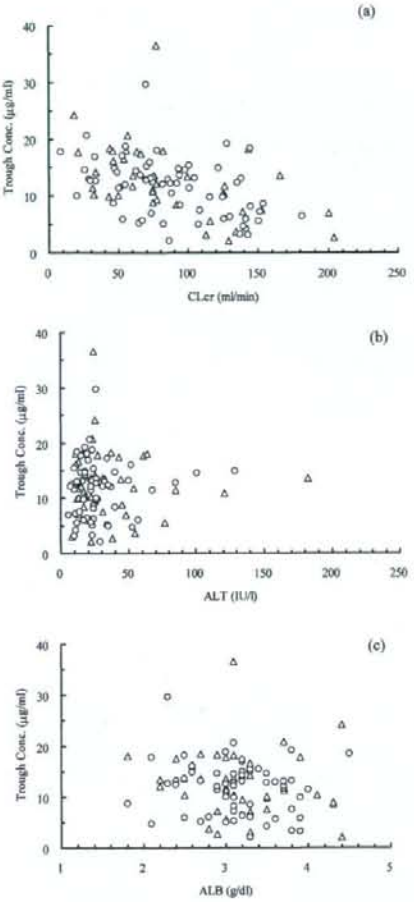

Fig. 2. Relationship of Trough Concentration with (a) Creatinine Clearance (CLcr), (b) Alanine Aminotransferase (ALT), and (c) Serum Albumin (ALB)

Open circles: cancer patients, open triangles: non-cancer patients.

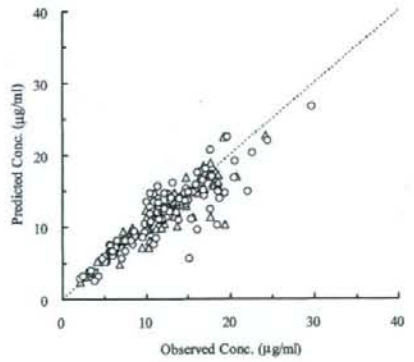

Fig. 3. Correlation Plot for Observed and Predicted Trough Concentrations

Dotted line represents a unit line. Open circles: cancer patients, open triangles: non-cancer patients.

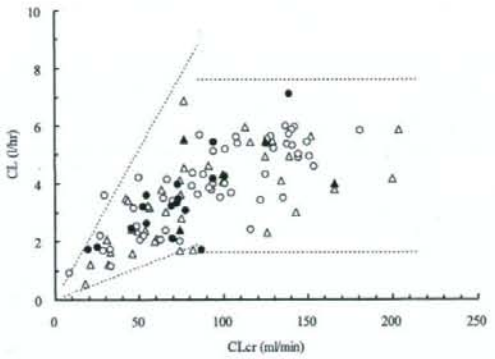

Fig. 4. Correlation of Bayesian Estimated Clearance (CL) and Creatinine Clearance (CLcr)

Circles: data from cancer patients, Triangles: data from non-cancer patients. Data from patients with fluid retention are plotted with closed circles (cancer) or closed triangles (non-cancer). The predictive range given by the mean±2×S.D. of inter-individual variability of the population parameters was given by dotted lines. The lines are discontinuous because the original population profile is discontinuous.

differ significantly between the groups, this does not suggest that there is no effect of malignancy on vancomycin clearance because clearance depends on creatinine clearance, and creatinine clearance varies between patients in the present data set. Therefore visual checking and a comparison of the correlation plots of vancomycin clearance and creatinine clearance in both groups would be a better approach. Figure 4 shows the correlation plots in both groups. The scatters of the data in both groups were similar, suggesting no difference in elimination processes. The similarity of the current data with the previous population data set can be confirmed by comparing the data with a predictive range of CL *vs.* CLcr relationship calculated by 'population mean±2×S.D. of inter-individual variability' as shown in Fig. 4.

Vancomycin distributes abdominal dropsy,[27] and thus we examined the possible difference of vancomycin clearance in patients with fluid retention. A scatter plot of the clearance against creatinine clearance for each sub-group is given in Fig. 4 with closed symbols for those with fluid retention. By visual inspection of Fig. 4, vancomycin clearance in patients with fluid retention was within the range of that in patients without fluid retention.

Estimates of vancomycin clearance were compared among different cancer types. The relationship between clearance (l/h) and creatinine clearance (ml/min) were plotted for each cancer type in Fig. 5. Data for different cancer types with more than 3 patients were plotted separately. Regarding the patients backgrounds, no statistical comparisons were performed because we considered that the numbers of data sets were too small to be used for multiple comparisons, however, mean values for age, body weight, and results of clinical laboratory tests were similar among the cancer types. As shown in Fig. 5, no clear pattern showing the difference by cancer type was found.

DISCUSSION

In this study, the pharmacokinetics of vancomycin was retrospectively examined based on steady state trough concen-

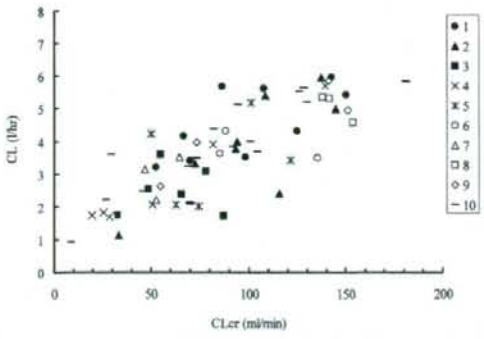

Fig. 5. Correlation of Bayesian Estimated Clearance (CL) and Creatinine Clearance (CLcr) in Patients with Different Cancer Types

Cancer types in the cancer patients are; 1: acute myelocytic leukemia, 2: lung cancer, 3: esophageal cancer, 4: bladder cancer, 5: malignant lymphoma, 6: lymphocytic leukemia, 7: pharyngeal cancer, 8: soft tissue tumor, 9: hepatocellular carcinoma, and 10: others.

trations in routine therapeutic drug monitoring. Although some patients had values outside the recommended concentration range, the trough levels were generally well controlled. As shown in Table 1, average trough concentrations were around 12 μg/ml and were not significantly different between the cancer and non-cancer patients for either the first or last sampling points. Regarding the body weight-normalized dose, the cancer group showed significantly higher values at the first sampling point, however, the doses were not significantly different at the last sampling point. There was one patient with a soft tissue tumor who received a relatively high dose per body weight (38.2 kg body weight, 62 years old, daily dose: 3000 mg/d, 78.5 mg/kg/d). If we exclude this patient, doses did not differ between the two groups. In this patient, body weight was extremely low because of weight loss (15 kg was lost) due to excision of the femoral region and anorexia as a side effect of radiation therapy, and such low body weight resulted in high calculated daily dose per body weight. Based on our present data, vancomycin doses as well as trough concentrations at steady state in routine therapy did not differ between the cancer and non-cancer patients.

From the results of the statistical comparison (Table 1) and visual check of the plot of clearance *versus* creatinine clearance (Fig. 4), there was no clear difference in the estimated clearance between the cancer and non-cancer patients, although there are some reports of a significant pharmacokinetic difference between cancer and non-cancer patients.[7,10,16—18] In the report by Teramachi *et al.*,[19] they selected patients with normal renal function, and they did not have to consider the effect of renal function on their data. In such a well controlled data set, they found some patients that showed greater clearance and distribution volume, however in their report, some individual plots showed similar values of clearance and distribution volume between the malignancy group and non-malignancy group. Based on these findings, we consider that pharmacokinetics of vancomycin may change in some cancer patients but dosage should be individualized based on other covariates such as patients' condition and on observed drug concentration data considering that the inter-individual variability of vancomycin pharmacokinetics is still extensive. In this work, we could not discuss the possible changes in distribution volume because we used only trough concentration data, but as complementary information, we found fluid retention did not affect the vancomycin clearance, and the results suggested no need of dose adjustment for patients with fluid retention to control trough concentration.

Regarding the initial dose adjustment for cancer patients, it is also reported that vancomycin dose should be higher in pediatric cancer patients due to changes of pharmacokinetic parameters.[16—18] However, malignancy state is not the sole covariate affecting vancomycin pharmacokinetics,[10] and also considering a large inter-individual variability in clearance after correcting by each patient's renal function, we should not discuss dosage adjustment in cancer patients based on only average differences between cancer and non-cancer patients. We would suggest that initial dose should not necessarily be increased in cancer patients, but we still should be careful of the possibility that vancomycin concentrations might be lower than expected in cancer patients due to an increase in clearance and/or distribution volume. We consider that peak concentration monitoring is not necessary in routine therapeutic drug monitoring because the side effect can be monitored based on trough levels, and efficacy is related to a ratio of the area under the concentration *vs.* time curve to minimum inhibitory concentration (*AUC/MIC*),[6,28] where *AUC* is a function of clearance that can be calculated by the Bayesian method using only trough data.

There are a few reports regarding the effects of cancer type on vancomycin pharmacokinetics in Japanese patients. Teramachi *et al.*[19] reported significant increase in clearance and distribution volume in patients with breast cancer and Hodgkin's lymphoma (n=3 or 4). In our study, we have no data for breast cancer and 5 patients with malignant lymphoma. For the latter, vancomycin dose and clearance showed similar values as shown in Fig. 5 although the numbers of cases are small. In other cancer groups, although it is difficult to compare the pharmacokinetics precisely, by visually checking the plots, no clear dependence on cancer type was found for clearance.

In our present analysis, we found no clear difference in vancomycin clearance between the cancer and non-cancer patients, and therefore we conclude that initial dose should not necessarily be adjusted (increased) for cancer patients. In dosage adjustment in routine therapeutic drug monitoring according to a Bayesian prediction, one trough concentration is usually enough to establish an individualized dosage.[14,29] Of course in all patients, dose adjustment at the appropriate time is important according to information from routine therapeutic drug monitoring and clinical laboratory tests, and should be based also on observations of efficacy, nephrotoxicity and other conditions in each patient.

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0090-9556/09/3704-821–826$20.00
DRUG METABOLISM AND DISPOSITION
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DMD 37:821–826, 2009

Vol. 37, No. 4
25833/3449361
Printed in U.S.A.

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Impact of Intestinal *CYP2C19* Genotypes on the Interaction between Tacrolimus and Omeprazole, but Not Lansoprazole, in Adult Living-Donor Liver Transplant Patients

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Received November 24, 2008; accepted January 8, 2009

ABSTRACT:

To assess the effects of intestinal cytochrome P450 2C19 on the interaction between tacrolimus and proton pump inhibitors, we examined the concentration/dose ratio [(ng/ml)/(mg/day)] of tacrolimus coadministered with omeprazole (20 mg) or lansoprazole (30 mg) to 89 adult living-donor liver transplant patients on postoperative days 22 to 28, considering the *CYP2C19* genotypes of the native intestine and the graft liver, separately. The concentration/dose ratio of tacrolimus coadministered with omeprazole was significantly higher in patients with two variants (*2 or *3) for intestinal CYP2C19 (median, 6.38; range, 1.55–22.9) than intestinal wild-type homozygotes (median, 2.11; range, 1.04–2.54) and heterozygotes (median, 2.11; range, 0.52–4.33) ($P = 0.010$), but the extent of the increase was attenuated by carrying the wild-type allele in the graft liver even when patients were *CYP3A5*1* noncarriers. Conversely, the *CYP2C19* polymorphisms both in the native intestine and in the graft liver little influenced the interaction between tacrolimus and lansoprazole, but *CYP3A5*1* noncarriers showed higher tacrolimus concentration/dose ratio than *CYP3A5*1* carriers. Furthermore, our experiments in vitro revealed that lansoprazole had a stronger inhibitory effect on the CYP3A5-mediated metabolism of tacrolimus than omeprazole, although not significantly (IC_{50} = 19.9 ± 13.8 μM for lansoprazole, 53.7 ± 6.1 μM for omeprazole). Our findings suggest that intestinal and graft liver CYP2C19 plays a relatively greater role in the metabolism of omeprazole than it does for lansoprazole, so that the effects of CYP3A5 on the metabolism of tacrolimus might be masked by the interaction with omeprazole associated with the *CYP2C19* genotype.

The immunosuppressant tacrolimus is characterized by a narrow therapeutic index and remarkable intraindividual and interindividual variability in its pharmacokinetics (Venkataramanan et al., 1995; Kahan et al., 2002). This variability can be attributed to factors such as poor absorption (Masuda and Inui, 2006), extensive first-pass metabolism (Lampen et al., 1995; Wilkinson, 2005), and drug-drug interactions (Christians et al., 2002). Tacrolimus is mainly metabolized by cytochrome P450 (P450) 3A4 and CYP3A5 in the small intestine and liver (Shiraga et al., 1994; Hesselink et al., 2003). In particular, CYP3A5 plays a key role in the pharmacokinetics of tacrolimus (Kamdem et al., 2005; Dai et al., 2006). Several studies of heart (Zheng et al., 2003), lung (Wang et al., 2006), kidney (Haufroid et al., 2004, 2006; Macphee et al., 2005; Kuypers et al., 2007), and liver (Goto et al., 2004; Masuda et al., 2006; Uesugi et al., 2006; Fukudo et al., 2008) transplant patients treated with tacrolimus have shown a significant association between the *CYP3A5* polymorphisms and tacrolimus dose-adjusted trough blood levels.

Clinically relevant drug-drug interactions have been observed between tacrolimus and proton pump inhibitors (PPIs) in those with *CYP2C19* gene variants, poor metabolizers (PMs) and intermediate metabolizers (IMs), compared with those with no variants, extensive metabolizers (EMs) (Itagaki et al., 2004; Miura et al., 2007). Because CYP2C19 and CYP3A4/5 are mainly responsible for the metabolism of PPIs (Andersson, 1996), PPIs themselves inhibit the metabolism of tacrolimus via CYP3A4/5 in patients carrying variant alleles of *CYP2C19*, thereby increasing the blood concentrations of tacrolimus. Furthermore, the magnitude of CYP2C19-mediated metabolism of omeprazole is greater than that of lansoprazole. We have recently reported the interaction between tacrolimus and lansoprazole in a living-donor liver transplant (LDLT) patient with the *CYP2C19*2/*3* and *CYP3A5*3/*3* genotypes both in the native intestine and in the graft liver (Hosohata et al., 2008). In liver transplantation, the genetic backgrounds of the native intestine (recipient) and the graft liver (donor) are different in many cases. Furthermore, several studies have assessed the expression or catalytic activity of intestinal CYP2C19

This work was supported in part by the 21st Century Center of Excellence (COE) Program "Knowledge Information Infrastructure for Genome Science"; a grant-in-aid from the Japan Health Sciences Foundation for "Research on Health Sciences Focusing on Drug Innovation"; and a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.

doi:10.1124/dmd.108.025833.

ABBREVIATIONS: P450, cytochrome P450; PPI, proton pump inhibitor; PM, poor metabolizer; IM, intermediate metabolizer; EM, extensive metabolizer; LDLT, living-donor liver transplantation; C/D, concentration/dose; M-I, 13-O-demethyl tacrolimus.

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TABLE 1

Characteristics of patients (n = 89)

Data are expressed as number or mean ± S.D.

Variables	Whole (*n* = 89)	Treatment with PPIs	
		Omeprazole (*n* = 35)	Lansoprazole (*n* = 54)
Age, years	51.9 ± 10.3	52.4 ± 8.8	51.6 ± 11.2
Gender (male/female), *n*	46/43	19/16	27/27
Body weight, kg	60.0 ± 10.0	59.8 ± 10.2	60.1 ± 9.9
Graft-to-recipient weight ratio, %	1.14 ± 0.30	1.15 ± 0.29	1.13 ± 0.30
ABO blood group match (identical/compatible/incompatible), *n*	60/12/17	24/4/7	36/8/10
Primary disease, *n*			
Cirrhosis	70	30	40
Primary biliary cirrhosis	7	2	5
Fulminant hepatic failure	3	1	2
Others	9[a]	2	7
Donor age, years	45.0 ± 13.2	46.0 ± 12.6	44.3 ± 13.6
Donor gender (male/female), *n*	50/39	18/17	32/22

[a] The primary disease was biliary atresia, Budd-Chiari syndrome, nonalcoholic steatohepatitis, and primary sclerosing cholangitis.

(Obach et al., 2001; Lapple et al., 2003; Paine et al., 2006). Based on these backgrounds, we hypothesized that intestinal CYP2C19 would affect the interaction between tacrolimus and PPIs in liver transplant patients.

In the present study, we examined the impact of the *CYP3A5* and *CYP2C19* genotypes in the small intestine on the interaction between tacrolimus and PPIs in LDLT patients, considering the genotypes of the native intestine and the graft liver, separately. Furthermore, the inhibitory effects of the PPIs on the CYP3A5-mediated metabolism of tacrolimus were examined using recombinant microsomal preparations.

Materials and Methods

Patients. Between February 2004 and January 2008, 89 de novo adult LDLT patients and their 89 corresponding donors were enrolled in this study, having first provided their written informed consent. Patients (all Japanese) who were receiving tacrolimus with either omeprazole ($n = 35$) (Omepral; AstraZeneca Co. Ltd., Osaka, Japan) at 20 mg/day or lansoprazole ($n = 54$) (Takepron; Takeda Pharmaceutical Co. Ltd., Osaka, Japan) at 30 mg/day were studied on days 22 to 28 post-transplantation (Table 1). This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee.

Dosage Regimen of Tacrolimus and Measurement of Tacrolimus Concentrations. The basic immunosuppression regimen consisted of tacrolimus with low-dose steroids (Inomata et al., 1996). Tacrolimus was administered orally at a dose of 0.075 mg/kg every 12 h from the evening of postoperative day 1 (Yasuhara et al., 1995; Inomata et al., 1996). The target of the whole-blood trough concentration of tacrolimus was set at between 10 and 15 ng/ml during the first 2 weeks. Steroid treatment was started at graft reperfusion at a dose of 10 mg/kg, with a gradual reduction from 2 to 0.3 mg/kg/day during the first 2 weeks after surgery. The dosage of tacrolimus was adjusted based on whole-blood trough concentrations measured approximately 12 h after the evening dosage every day using a semiautomated microparticle enzyme immunoassay (Imx; Abbott, Tokyo, Japan) (Yasuhara et al., 1995).

Evaluation of Drug Interactions between Tacrolimus and PPIs. Because the oral administration of PPIs started approximately 2 weeks after surgery, we evaluated data on postoperative days 22 to 28. The clinical course of all the patients enrolled in this study was stable. The average of dose-normalized blood concentration of tacrolimus during this observation period then was assessed as concentration/dose (C/D) ratio [(ng/ml)/(mg/day)] of tacrolimus for each patient and used for the analysis. We excluded data obtained during treatment with a temporal high-dose steroid injection against acute cellular rejection because of induction of the intestinal expression of CYP3A4 (Masuda et al., 2004).

Genotyping. Genomic DNA was extracted from the peripheral blood of transplant patients or donors with a Wizard Genomic DNA Purification kit (Promega, Madison, WI). Because two *CYP2C19* variant alleles, *CYP2C19*2* and *CYP2C19*3*, account for the poor metabolizer phenotype in Japanese subjects (De Morais et al., 1994a), the detection of the wild-type allele (**1*) and these two variant alleles was performed using a polymerase chain reaction-restriction fragment length polymorphism method (De Morais et al., 1994a,b). The genotyping of *CYP3A5* was performed as described previously (Goto et al., 2004; Uesugi et al., 2006; Fukudo et al., 2008).

Classification of Patients. The patients themselves and their corresponding donors were separately classified into three groups based on the *CYP2C19* genotype as follows: *CYP2C19*1/*1* (EMs), *CYP2C19*1/*2* or *CYP2C19*1/*3* (IMs), and *CYP2C19*2/*2*, *CYP2C19*3/*3*, or *CYP2C19*2/*3* (PMs) (Itagaki et al., 2004). As for the *CYP3A5* genotype, patients were allocated to two groups as follows: *CYP3A5*1/*1* or *CYP3A5*1/*3* (**1* carriers) and *CYP3A5*3/*3* (**1* noncarriers).

In Vitro Inhibition of CYP3A4/5-Dependent Tacrolimus Metabolism by PPIs. To evaluate the inhibitory effects of omeprazole and lansoprazole on the metabolism of tacrolimus, we performed experiments in vitro using recombinant microsomes, as reported previously with slight modifications (Li et al., 2004; Dai et al., 2006). In brief, omeprazole and lansoprazole were serially diluted with methanol to yield final concentrations ranging from 1 to 300 μM and 1 to 200 μM, respectively. The reaction was started with 50 μl of 1.5 mM NADP (Nacalai Tesque, Kyoto, Japan) and was stopped with 1 ml of 6.25% $ZnSO_4$. The reaction mixture consisted of 400 nM tacrolimus, 12 mM glucose 6-phosphate, 0.25 IU of glucose 6-phosphate dehydrogenase, 6 mM $MgCl_2$, and 0.07 mg/ml microsomal protein for P450 (heterologous baculovirus-insect cell-expressed human CYP3A4 or human CYP3A5 in P450 reductase and cytochrome b_5) (BD Gentest, Woburn, MA) in 100 mM potassium phosphate buffer, pH 7.4, with or without PPIs, in a final volume of 500 μl. The final methanol concentration in the incubation mixture was less than 1%, with minimal impact on enzymatic activity (Li et al., 2004). The incubation conditions for CYP3A4 and CYP3A5 activities were 5 min at 37°C. The concentration of 13-*O*-demethyl tacrolimus (M-I) (a gift from Astellas Pharma Inc., Tokyo, Japan), the primary metabolite, was quantified by liquid chromatography/tandem mass spectrometry method (Shimomura et al., 2008). IC_{50} values were estimated using a nonlinear regression analysis of competition curves with one compartment, and the following equation: $V = (100 \times IC_{50})/(IC_{50} + [I]) + A$, where V is production of M-I, which accounts for most of the metabolic clearance of tacrolimus (% of control), [I] is the concentration of each PPI, and A is the nonspecific metabolism of tacrolimus (% of control).

Statistical Analysis. The C/D ratio of tacrolimus coadministered with PPIs was compared using the *U* test for two genotype groups or the Kruskal-Wallis test, followed by the Dunn post hoc test for multiple comparisons for more than two genotype groups. Data are expressed as the median and range or mean ± S.D., depending on data type. For all the analyses, two-tailed $P < 0.05$ was considered statistically significant. All the statistical analyses were conducted using GraphPad Prism, version 4 (GraphPad Software Inc., San Diego, CA).

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Effects of intestinal and graft liver CYP2C19 genotypes on the C/D ratio of tacrolimus coadministered with omeprazole (n = 35) or lansoprazole (n = 54)

Data are expressed as median (range). *CYP3A5*1* noncarriers, *CYP3A5*3/*3*. *P* values are for the differences among the genotype groups, using the Kruskal-Wallis test, followed by the Dunn post hoc test for multiple comparisons.

PPI	Variables	*CYP2C19* Genotype (*CYP3A5*1* Noncarriers)			*P*
		EMs	IMs	PMs	
Omeprazole	Native intestine				
	n	11 (9)	15 (9)	9 (9)	
	Tacrolimus C/D ratio	2.11 (1.04–2.54)	2.11 (0.52–4.33)	6.38*† (1.55–22.9)	0.010
	Graft liver				
	n	10 (6)	13 (8)	12 (7)	
	Tacrolimus C/D ratio	1.83 (1.04–6.38)	2.31 (0.52–7.10)	3.27* (1.9–22.9)	0.022
Lansoprazole	Native intestine				
	n	16 (11)	27 (21)	11 (4)	
	Tacrolimus C/D ratio	2.34 (1.16–12.8)	2.83 (0.72–13.4)	2.27 (0.98–11.7)	0.52
	Graft liver				
	n	15 (9)	30 (22)	9 (7)	
	Tacrolimus C/D ratio	2.47 (1.13–10.6)	2.76 (1.10–12.8)	2.20 (0.72–13.4)	0.82

* $P < 0.05$, EMs versus PMs; † $P < 0.05$, IMs versus PMs.

Results

Effects of Intestinal and Graft Liver *CYP2C19* Genotypes on the Interaction between Tacrolimus and PPIs. For the *CYP2C19* genotype, **1*, **2*, and **3* alleles were found in 52.2, 32.6, and 15.2% in the graft liver and 53.9, 35.4, and 10.7% in the native intestine, respectively. The EMs (**1/*1*), IMs (**1/*2* and **1/*3*), and PMs (**2/*2*, **2/*3*, and **3/*3*) of CYP2C19 then were found in 28.1% ($n = 25$), 48.3% ($n = 43$), and 23.6% ($n = 21$) in the graft liver and 30.3% ($n = 27$), 47.2% ($n = 42$), and 22.5% ($n = 20$) in the native intestine, respectively. For the *CYP3A5* genotype, **1* and **3* alleles were found in 19.1 and 80.9% in the graft liver and 20.8 and 79.2% in the native intestine, respectively. Then, the frequencies of *CYP3A5*1* carriers (**1/*1* and **1/*3*) and *CYP3A5*1* noncarriers (**3/*3*) were 33.7% ($n = 30$) and 66.3% ($n = 59$) in the graft liver and 32.6% ($n = 29$) and 67.4% ($n = 60$) in the native intestine, respectively.

To investigate whether intestinal *CYP2C19* polymorphisms affected the interaction between tacrolimus and PPIs, patients were divided based on the *CYP2C19* genotype of transplant recipients (Table 2). The C/D ratio of tacrolimus coadministered with omeprazole was significantly higher in patients with two variant alleles for intestinal *CYP2C19* than those with the wild-type homozygote (*CYP2C19*1/*1*) or heterozygote (*CYP2C19*1/*2* or *CYP2C19*1/*3*) ($P = 0.010$). Likewise, patients with an engrafted liver carrying two variant alleles for *CYP2C19* showed significantly higher C/D ratio than the other groups ($P = 0.022$). Furthermore, the distribution of *CYP3A5*1* noncarriers did not vary between the different *CYP2C19* genotype groups (Table 2). In contrast, the C/D ratio of tacrolimus coadministered with lansoprazole was not associated with *CYP2C19* polymorphisms in the native intestine ($P = 0.52$) or the graft liver ($P = 0.82$).

The tacrolimus C/D ratio between CYP2C19 EMs and IMs was found comparable (Table 2), so that the analyses were carried out between the EMs/IMs versus PMs. In patients receiving omeprazole, the C/D ratio of tacrolimus was significantly increased in PMs compared with EMs/IMs ($P = 0.005$ for native intestine, $P = 0.018$ for graft liver).

Effects of Intestinal and Graft Liver *CYP3A5* Genotypes on the Interaction between Tacrolimus and PPIs. Because we have reported that the *CYP3A5* genotypes of both recipients and donors are important for the oral clearance of tacrolimus in liver transplant recipients (Goto et al., 2004; Fukudo et al., 2006, 2008; Uesugi et al., 2006; Hosohata et al., 2008), we examined the effects of CYP3A5 on the interaction between tacrolimus and PPIs (Table 3). In patients receiving omeprazole, the C/D ratio of tacrolimus was significantly higher in patients with an engrafted liver carrying the *CYP3A5*3/*3* genotype (**1* noncarriers) than the other group (**1* carriers) ($P = 0.034$). Similar trends were observed, although not statistically significant, for the intestinal *CYP3A5* genotype ($P = 0.47$). In patients receiving lansoprazole, **1* noncarriers conferred significantly higher C/D ratio of tacrolimus than **1* carriers ($P = 0.015$ for native intestine, $P = 0.049$ for graft liver).

Effects of the Combination of Intestinal and Graft Liver Genotypes on the Interaction between Tacrolimus and PPIs. As a feature of liver transplantation, the genotypes of recipients (native intestine) are different from those of donors (graft liver) in many cases. Focusing on this feature, we assessed the effects of the combination of intestinal and graft liver *CYP2C19* genotypes on the interaction between tacrolimus and PPIs.

As shown in Fig. 1A, carriers of at least one *CYP2C19* wild-type allele in both the native intestine and the graft liver (EMs/IMs for native intestine and graft liver) showed the lowest values for the tacrolimus C/D ratio (reference group). Compared with the reference group, carriers of at least one *CYP2C19* wild-type allele either in the native intestine or in the graft liver showed almost the same values for the C/D ratio of tacrolimus. However, two *CYP2C19* variant alleles both in the native intestine and in the graft liver (PMs for native intestine and graft liver) conferred a significantly higher (6.9-fold) C/D ratio of tacrolimus than the other groups ($P = 0.0032$). Furthermore, within *CYP3A5*1* noncarriers both in the native intestine and in the graft liver (closed circles, $n = 18$), the C/D ratio was significantly higher in those who also carried two *CYP2C19* variant alleles than any other group ($P = 0.017$), whereas there was no difference among the genotypes carrying at least one *CYP2C19* wild-type allele either in the native intestine or in the graft liver. Conversely, the combination of intestinal and graft liver *CYP2C19* genotypes little affected the C/D ratio of tacrolimus in patients receiving lansoprazole (Fig. 1B).

Experiments in Vitro. Next, we examined the inhibitory effects of PPIs on the metabolism of tacrolimus by CYP3A4 and CYP3A5 using recombinant microsomal preparations. Figure 2 shows representative data among three separate experiments. The apparent inhibition constant (IC_{50}) values (mean ± S.D.) for omeprazole and lansoprazole were 51.9 ± 15.9 and 44.5 ± 18.0 μM for CYP3A4 and 53.7 ± 6.1 and 19.9 ± 13.8 μM for CYP3A5, respectively ($P > 0.05$, omeprazole versus lansoprazole for CYP3A4 and CYP3A5).

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TABLE 3

Effects of intestinal and graft liver CYP3A5 genotypes on the C/D ratio of tacrolimus coadministered with omeprazole (n = 35) or lansoprazole (n = 54)

Data are expressed as median (range). **1* carriers, *CYP3A5*1/*1* or**1/*3*; **1* noncarriers, *CYP3A5*3/*3*.

PPI	Variables	*CYP3A5* Genotype		*P*
		**1* Carriers	**1* Noncarriers	
Omeprazole	Native intestine			
	n	11	24	
	Tacrolimus C/D ratio	1.96 (1.04–7.10)	2.34 (0.52–22.9)	0.47
	Graft liver			
	n	14	21	
	Tacrolimus C/D ratio	1.93 (0.52–7.10)	2.54* (1.55–22.9)	0.034
Lansoprazole	Native intestine			
	n	18	36	
	Tacrolimus C/D ratio	1.97 (0.98–11.7)	3.85* (0.72–13.4)	0.015
	Graft liver			
	n	16	38	
	Tacrolimus C/D ratio	1.73 (0.72–11.1)	2.85* (0.98–113.4)	0.049

* $P < 0.05$, *1 carriers versus *1 noncarriers (*U* test).

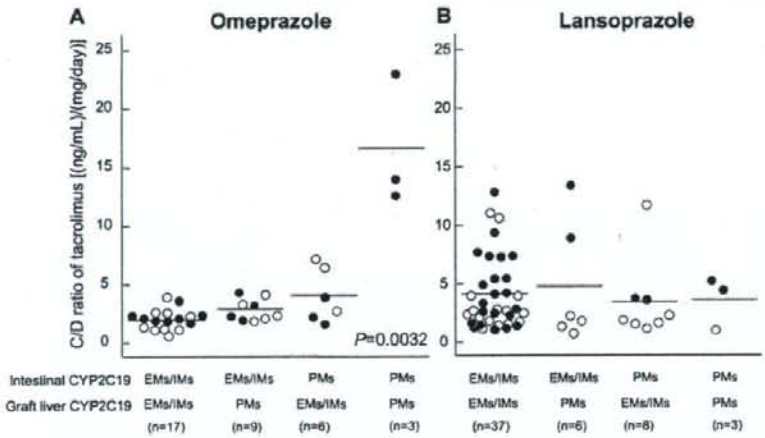

FIG. 1. Effects of the combination of intestinal and graft liver *CYP2C19* genotypes on the C/D ratio of tacrolimus coadministered with omeprazole (A) or lansoprazole (B). Patients were categorized based on the intestinal and graft liver *CYP2C19* genotypes (EMs, *CYP2C19*1/*1*, **1/*2*, and **1/*3*; IMs, *CYP2C19*1/*2* and **1/*3*; PMs, *CYP2C19*2/*2*, **2/*3*, and **3/*3*). The closed circles indicate the *CYP3A5*1* noncarriers (*CYP3A5*3/*3*) both in the native intestine and in the graft liver, and open circles indicate the *CYP3A5*1* carriers (*CYP3A5*1/*1* or **1/*3*). Each bar indicates the median values. *P* values were determined by the Kruskal-Wallis test, followed by the Dunn post hoc test for multiple comparisons.

Discussion

There has been growing recognition that intestinal and hepatic metabolism of orally administered drugs can play a significant role in the first-pass effects (Floren et al., 1997; Hebert, 1997). Despite evaluation of the expressions or catalytic activity of intestinal CYP2C19 (Obach et al., 2001; Lapple et al., 2003; Paine et al., 2006), no studies have assessed the effects of intestinal CYP2C19 on the oral clearance of drugs. The interaction between tacrolimus and PPIs in CYP2C19 PMs are based on indirect effects of PPIs as CYP3A4/5 inhibitors because the main metabolic pathway of PPIs is CYP3A4/5 in CYP2C19 PMs. Furthermore, the direct effects of CYP2C19 on tacrolimus biotransformation can be negligible because tacrolimus never interacts with any P450 drug metabolism enzymes in addition to CYP3A4/5 (Lecointre et al., 2002). Therefore, we focused on the indirect interaction between tacrolimus and PPIs via CYP3A4/5 in patients with the *CYP2C19* polymorphisms, considering the *CYP2C19* genotypes of the native intestine and the graft liver, separately. Our results revealed that LDLT patients with two *CYP2C19* variants in the native intestine and graft liver seemed susceptible to the inhibitory effects of omeprazole, rather than lansoprazole, on the metabolism of tacrolimus (Table 2; Fig. 1). This varying degree of interaction of omeprazole and lansoprazole with tacrolimus in CYP2C19 PMs compared with EMs might be partly because of the different magnitudes of CYP2C19-mediated metabolism among PPIs. The contribution of CYP2C19 in the metabolism of omeprazole is greater than that of lansoprazole (Ishizaki and Horai, 1999). To the best of our knowledge, this is the first study indicating the pharmacokinetic significance of intestinal CYP2C19 in humans in vivo.

In liver transplantation, the genotypes of drug metabolism enzymes between recipients (native intestine) and donors (graft liver) are generally different. Thus, the different genotypes of intestinal and hepatic P450s could regulate the clearance of tacrolimus. In the present study, the C/D ratio of tacrolimus coadministered with omeprazole was significantly high only in cases in which the recipients themselves and their corresponding donors had two variant alleles for *CYP2C19* (Fig. 1A). However, the extent of the increase was attenuated by carrying at least one *CYP2C19* wild-type allele either in the native intestine or in the graft liver, indicating that intestinal and hepatic CYP2C19 could compensate for the functional loss caused by the *CYP2C19* variants in the interaction between tacrolimus and omeprazole in LDLT patients. Considering the relatively high frequency of CYP2C19 PMs in the Japanese population (approximately 20%) (De Morais et al., 1994a), the interaction between tacrolimus and omeprazole is more relevant in Japanese than Caucasian populations. However, this study has clarified that there is a low probability of a strong tacrolimus-omeprazole interaction in liver transplant pa-

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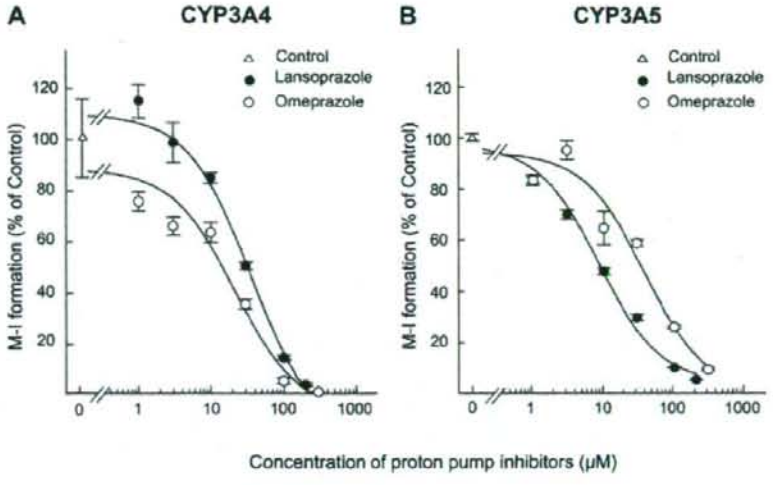

FIG. 2. Concentration-dependent inhibition of the formation of M-I by omeprazole and lansoprazole for CYP3A4 (A) and CYP3A5 (B). Each symbol represents the mean ± S.E. for three independent experiments. The apparent inhibition constant (IC_{50}) values for omeprazole and lansoprazole were 51.9 ± 15.9 and 44.5 ± 18.0 μM for CYP3A4 and 53.7 ± 6.1 and 19.9 ± 13.8 μM for CYP3A5, respectively.

tients because of the extremely small concordance rate of PM genotypes of the recipients with those of their corresponding donors.

We previously reported that intestinal CYP3A5 was significantly associated with the oral clearance of tacrolimus in liver transplant patients (Uesugi et al., 2006). In the present study, CYP3A5 also affected the interaction between tacrolimus and PPIs (Table 3), which is consistent with our experiments in vitro using recombinant microsomes, showing that omeprazole and lansoprazole inhibited the metabolism of tacrolimus via CYP3A4 and CYP3A5 (Fig. 2). Our findings suggest that intestinal and hepatic CYP3A5 is responsible for the interaction between tacrolimus and PPIs. However, even if patients had the *CYP3A5*3/*3* genotype both in the native intestine and in the graft liver (Fig. 1A, closed circles), the C/D ratio of tacrolimus coadministered with omeprazole showed low values when carrying at least one *CYP2C19* wild-type allele either in the native intestine or in the graft liver. These results suggest that the CYP2C19 has a greater effect on overall metabolism of omeprazole than that of CYP3A5. Therefore, in patients receiving omeprazole, carriers of two *CYP2C19* variant alleles both in the native intestine and in the graft liver (PMs/PMs) showed the greatest increased C/D ratio of tacrolimus, and the variability in the metabolism of omeprazole caused by *CYP2C19* polymorphisms is more likely to mask the effects of the *CYP3A5* genotype on the metabolism of tacrolimus. Conversely, in patients receiving lansoprazole, there was no significant difference among the *CYP2C19* genotypes (Table 2), but the *CYP3A5*1* noncarriers conferred a higher tacrolimus C/D ratio than *CYP3A5*1* carriers (Table 3). Our experiments in vitro showed that lansoprazole had a stronger inhibitory effect on the CYP3A5-mediated metabolism of tacrolimus than omeprazole, although not significantly (IC_{50} = 19.9 ± 13.8 μM for lansoprazole, 53.7 ± 6.1 μM for omeprazole) (Fig. 2). The present results are in good agreement with the previous reports that the relative contribution of CYP2C19 against CYP3A4/5 in omeprazole is greater than that in lansoprazole (Ishizaki and Horai, 1999). In addition, our data are also consistent with lansoprazole being a poorer inhibitor of CYP3A4, as suggested by Li et al. (2004).

The present study must be interpreted within the context of its potential limitations. First, we did not have a control group (patients treated with tacrolimus not receiving PPIs). Second, medications including inducers or inhibitors of CYP3A4 were not strictly controlled in both transplant patients and their corresponding donors.

In conclusion, we first showed that the *CYP2C19* defective genotype in the native intestine affected the interaction between tacrolimus and omeprazole in LDLT patients, but the effect was attenuated by the wild-type genotype in the graft liver even when patients had the *CYP3A5*3/*3* genotype in both the native intestine and the graft liver. On the other hand, CYP3A5 rather than CYP2C19 was associated with the interaction between tacrolimus and lansoprazole in liver transplantation. The present findings suggest that the genotyping of *CYP2C19* and *CYP3A5* both in the native intestine and in the graft liver might contribute to safer dosing and monitoring of tacrolimus coadministered with omeprazole and lansoprazole early on after liver transplantation.

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