6. Inomata Y, Uemoto S, Asonuma K, et al. Right lobe graft in living donor liver transplantation. Transplantation 2000; 69: 258.
7. Maetani Y, Itoh K, Egawa H, et al. Factors influencing liver regeneration following living-donor liver transplantation of the right hepatic lobe. Transplantation 2003; 75: 97.
8. Qing JO, Robert EH. The role of hepatic veins in liver operations. Surgery 1984; 95: 381.
9. Murata S, Itai Y, Asato M, et al. Effect of temporary occlusion of the hepatic vein on dual blood supply in the liver: Evaluation with spiral CT. Radiology 1995; 197: 351.
10. Kaneko T, Kaneko K, Sugimoto H, et al. Intrahepatic anastomosis formation between the hepatic veins in the graft liver of the living related liver transplantation: Observation by Doppler ultrasonography. Transplantation 2000; 70: 982.

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CALCIUM LEVELS AS A RISK FACTOR FOR DELAYED GRAFT FUNCTION

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Background. **Delayed graft function (DGF) occurs in up to 50% of renal transplants. Hypercalcemia and hyperparathyroidism are associated with impaired renal function. Little is known on the effects of serum calcium levels on DGF. This issue was addressed in the current study.**

Methods. **Patients receiving a cadaveric renal transplant between 1986 and 1996 were studied. Data on calcium metabolism and histologic characteristics of nephrocalcinosis, acute tubular necrosis (ATN), and acute rejection in biopsies taken within the first week were related to the occurrence of DGF.**

Results. **The incidence of DGF in a cohort of 585 cadaveric transplants was 31%. DGF correlated independently with serum calcium levels (odds ratio [OR] 1.14 [95% confidence interval (CI) 1.04–1.26] per 0.1 mmol/L). The use of calcium channel blockers before transplantation protected against DGF (OR 0.5 [95% CI 0.29– 0.87]). In this selected group, we found an association with histologic signs of ATN and DGF. However, most of the biopsies also had features of acute rejection or nephrocalcinosis. Nephrocalcinosis was found in 12 of 71 biopsies and was not associated with serum calcium levels or the occurrence of DGF.**

Conclusions. **In this study, serum calcium levels were independently associated with DGF. This could not be explained by the presence of microscopic nephrocalcinosis. Therefore, DGF is attributed to high intracellular calcium levels. Because calcium supplementation and vitamin D analogues are commonly used in dialysis practice, hypercalcemia influences long-term graft outcome by its effect on DGF. The pretransplant use of calcium channel blockers has a protective effect on the occurrence of DGF.**

The pathogenesis of acute tubular necrosis (ATN) in human kidneys remains enigmatic despite great scientific interest and investigative efforts. The poor correlation between the clinical occurrence of acute renal failure and the morphologic manifestations of ATN in the renal biopsy has hampered progress in this field (*1*). The regular occurrence of delayed graft function (DGF) immediately after transplantation of postmortal kidneys has renewed this interest. However, the circumstances in which DGF occurs in renal allografts differ from the conditions in which acute renal failure develops in native kidneys. Conditions during explantation and implantation of the donor organ as well as calcineurin inhibitor toxicity and rejection episodes immediately after transplantation may be responsible for or contribute to the development of DGF (*2*). The interest in the occurrence of DGF was heightened by the finding that rejection episodes are more likely to occur in association with DGF (*3*, *4*). In combination with rejection episodes, DGF also influences the long-term prognosis of graft function (*4*, *5*).

The study of the morphologic manifestations of DGF in renal allografts is facilitated by the fact that graft biopsies are more readily obtained than biopsies from native kidneys. It is conceivable that the discrepancy between DGF and ATN in allografts is not only explained by factors related to the transplantation procedure, including calcineurin toxicity, but also by elevated serum phosphate or serum calcium levels at the time of transplantation.

The mechanism by which hypercalcemia causes acute renal failure remains largely hypothetical. In dogs and rats, acute renal failure has been reported as a result of experimentally induced hypercalcemia (*6*). In animal models, three types of nephrocalcinosis are distinguished: chemical nephrocalcinosis, microscopic nephrocalcinosis, and macroscopic nephrocalcinosis (*7*). The latter is characterized by gross calcium deposits found on radiographic investigations. Microscopic nephrocalcinosis is characterized by microscopic calcium deposits, mainly located in the lumen of the tubules.

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*CYP3A5*1*-carrying graft liver reduces the concentration/oral dose ratio of tacrolimus in recipients of living-donor liver transplantation

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Objectives Tacrolimus is widely used for immunosuppressive therapy after organ transplantation, but its pharmacokinetics shows such great interindividual variation that control of its blood concentration is difficult. We have previously reported that an intestinal P-glycoprotein (MDR1) contributes to this variation as an absorptive barrier, but the role of hepatic metabolism is not clear.

Methods In this study, we have evaluated the genotypes of *MDR1* and *cytochrome P450 (CYP) 3A* in donor and recipient, and the influence of polymorphisms on mRNA expression and the tacrolimus concentration/dose (C/D) ratio in recipients of living-donor liver transplantation (LDLT).

Results The expression level of MDR1 and tacrolimus C/D ratio were not affected by either *MDR1* C3435T or G2677T/A. The *CYP3A4*1B* genotype was not detected, but the *CYP3A5*3* genotype had an allelic frequency of 76.3%. The mRNA level of CYP3A5 was significantly reduced by the **3/*3* genotype, and the tacrolimus C/D ratio was decreased in recipients engrafted with partial liver carrying *CYP3A5*1/*1* genotype. An analysis of the combination of intestinal MDR1 level and liver *CYP3A5* genotype revealed that the tacrolimus C/D ratio was lower in the group with higher MDR1 levels regardless of *CYP3A5* genotype during postoperative week 1.

Conclusions These results indicate that in recipients of LDLT, the pharmacokinetics of tacrolimus is influenced by flux via P-glycoprotein in the intestine during the first week; after that, it is mostly the hepatic metabolism that contributes to the excretion of tacrolimus, and carriers of the *CYP3A5*1/*1* genotype require a high dose of tacrolimus to achieve the target concentration.
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Introduction

Although the immunosuppressant tacrolimus is widely used in organ transplantations, including living-donor liver transplantation (LDLT), its narrow therapeutic range and variability in pharmacokinetics make difficult the establishment of a dosage regimen of the drug [1,2]. The systemic clearance of tacrolimus is mainly explained by hepatic cytochrome P450 (CYP) 3A-mediated metabolism. Recently, an ATP-driven efflux pump P-glycoprotein (Pgp) was identified as an absorptive barrier to orally administered tacrolimus [3].

We previously reported that the expression level of MDR1 mRNA was a good pharmacokinetic factor for setting the initial oral dosage of tacrolimus in LDLT recipients [4]. However, the interindividual variation in tacrolimus pharmacokinetics could not merely be explained by the expression level of intestinal MDR1, because the grafted liver mass and systemic clearance of tacrolimus increased during the postoperative period. Therefore, the inter- and intraindividual variation in the hepatic metabolism of tacrolimus, as well as intestinal absorption of the drug, needs to be clarified in order to precisely understand the molecular mechanisms behind the variation in the pharmacokinetics of tacrolimus.

Recent advances in pharmacogenetics have revealed that several single nucleotide polymorphisms (SNPs) in transporters and metabolic enzymes affect to the pharmacokinetics of various drugs [5–7]. The clinical relevance of SNPs in *MDR1* and *CYP3A* to post-transplant immunosuppressive therapy has been examined. In the case of renal transplantation, SNPs in *MDR1* did not influence the dose-adjusted trough level of either tacrolimus or cyclosporine, but patients with the

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*CYP3A5**3 polymorphism required significantly less tacrolimus to achieve the target concentration than those with *CYP3A5**1 [8]. We also reported that the 10 *MDR1* SNPs including C3435T and G2677T/A did not influence the intestinal expression level of MDR1 mRNA or the tacrolimus concentration/dose (C/D) ratio in LDLT recipients [9].

In the present study, we have examined the genotypes of recipients for *MDR1*and *CYP3A4* and of grafted liver for *MDR1*, *CYP3A4* and *CYP3A5*, and whether SNPs influence the expression levels of these genes and the tacrolimus CD ratio.

Materials and methods

Patients and biopsy specimens

Between November 1998 and July 2003, 181 recipients and 114 donors were enrolled in this study having first provided a written informed consent. The demographics of the recipients and donors including age, sex, body weight, graft to recipient body weight ratio (GRWR) and primary diseases are summarized in Table 1. Clinical samples of the upper jejunum were obtained from part of the Roux-en-Y limb for biliary reconstruction, and liver samples were obtained from biopsy specimens for pathological testing during the surgery. This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Ethics Committee of Kyoto University.

Table 1 Recipient and donor demographics

	Recipient	Donor
n	181	114
Age		
Range (years)	0.3–70	18–64
Median (years)	10	35
Sex		
Male	84	57
Female	97	57
Body weight		
Weight range (kg)	5–92.1	
Median weight (kg)	25	
GRWR (%)		
Range	0.6–5.6	
Median	1.5	
Primary disease		
Biliary atresia	78	
Cirrhosis	41	
Primary sclerosing cholangitis	10	
Primary biliary cirrhosis	12	
Fulminant hepatic failure	5	
Alagille syndrome	6	
Post LRLT	10	
Others	19*	

GRWR, graft-to-recipient weight ratio.
*The primary disease was Budd–Chiari syndrome, Byler disease, Caroli disease, familial amyloid neuropathy, multiple liver tumors, ornithine transcarbamylase deficiency, Wilson disease, citrullinemia, propionic acidemia, hepatoblastoma, hypertyrosinemia, fibrosis, polycystic liver disease, or billiary ectasia.

Isolation of genomic DNA and genotyping

Genomic DNA was isolated from a homogenate of liver biopsy specimens of the graft and that of intestinal mucosa or peripheral blood from recipients using MagNAPure LC DNA Isolation kit I (Roche, Mannheim, Germany). The isolated DNA was used for genotyping by the PCR–restriction fragment length polymorphism method. The *MDR1* C3435T and G2677T/A polymorphisms were examined based on the method of Cascorbi *et al.* [10]. *CYP3A4**1B and *CYP3A5**3 were examined according to the methods of van Shaik *et al.* [11,12].

Evaluation of liver and intestinal expression levels of MDR1 and CYP3As

Biopsy specimens from liver and intestinal mucosa were homogenized in RLT buffer (QIAGEN, Hilden, Germany), and total RNA was isolated with MagNAPure LC RNA Isolation kit II (Roche) and reverse transcribed as described previously [13]. The mRNA expression level of MDR1, CYP3A4 and CYP3A5 was evaluated by real-time PCR using an ABI prism 7700 sequence detector (Applied Biosystems, Foster, CA). The primer/probe set for MDR1 was as described previously [13]. That for CYP3A4 and for CYP3A5 were as reported by Koch *et al.* [14].

Measurement of drug concentrations

The blood concentration of tacrolimus was determined 12 h after the evening dosage using a semiautomated microparticle enzyme immunoassay (IMX, Dainabot Co., Ltd, Tokyo, Japan). The target whole-blood trough level was set between 5 and 15 ng/ml.

Statistics

Between-group differences were calculated using one-way ANOVA and Kruskal–Wallis test, followed by the Dunnet post hoc test for multiple comparisons. Significance was defined as $P < 0.05$. Statistical analyses were performed using the statistical software package Stat View version 5.0 (Abacus Concepts, Berkeley, CA).

Results

Frequency of *MDR1*, *CYP3A4* and *CYP3A5* SNPs

Table 2 shows the frequency of *MDR1* C3435T and G2677T/A, *CYP3A4**1B, and *CYP3A5**3 SNPs in the recipients and donors of LDLT. The *CYP3A4**1B genotype was not detected. For the *CYP3A5**3 genotype, the *1 allele (A) and *3 allele (G) were found in 23.7% and 76.3% of all grafts, respectively. For the *MDR1* C3435T polymorphism, the C and T allele frequencies were 56.1% and 43.9% in the grafts, and 54.4% and 45.6% in the recipients, with little difference between donors and recipients. For the G2677T/A polymorphism, the G, T and A allele frequencies were 45.1%, 40.7% and 14.2% in the grafts, and 40.6%, 46.4% and 13% in the recipients. In the donor groups,

Table 2 Frequency of *MDR1*, *CYP3A4* and *CYP3A5* polymorphisms

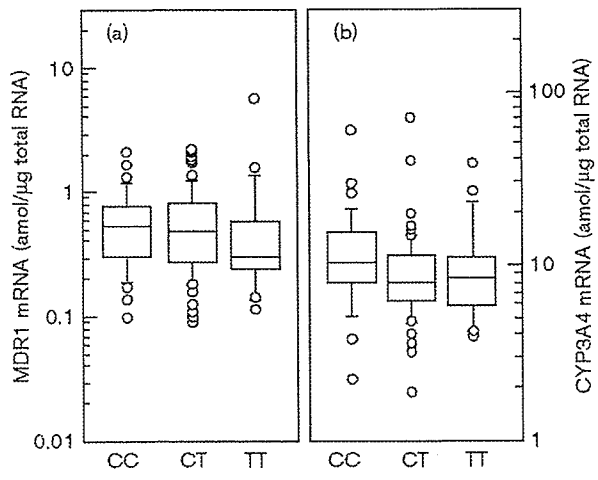
Gene	Position	Genotype	Graft *n*	Graft Frequency (%)	Recipient *n*	Recipient Frequency (%)
MDR1	G2677T/A	GG	23	20.4	30	16.6
		GT	42	37.2	73	40.3
		TA	12	10.6	31	17.1
		GA	14	12.4	14	7.7
		AA	3	2.7	1	0.6
		TT	19	16.8	32	17.7
	C3435T	CC	33	28.9	48	26.5
		CT	62	54.4	101	55.8
		TT	19	16.7	32	17.7
CYP3A4*1B	A(−290)G	AA	113	100.0	111	100.0
		AG	0	0.0	0	0.0
		GG	0	0.0	0	0.0
CYP3A5*3		AA(*1/*1)	7	6.1		
		AG(*1/*3)	40	35.1		
		GG(*3/*3)	67	58.8		

there were three individuals with the AA genotype 2.7% vs 0.6% in the recipient group.

Effect of *MDR1* and *CYP3A5* polymorphisms on MDR1, CYP3A4 and CYP3A5 expression levels

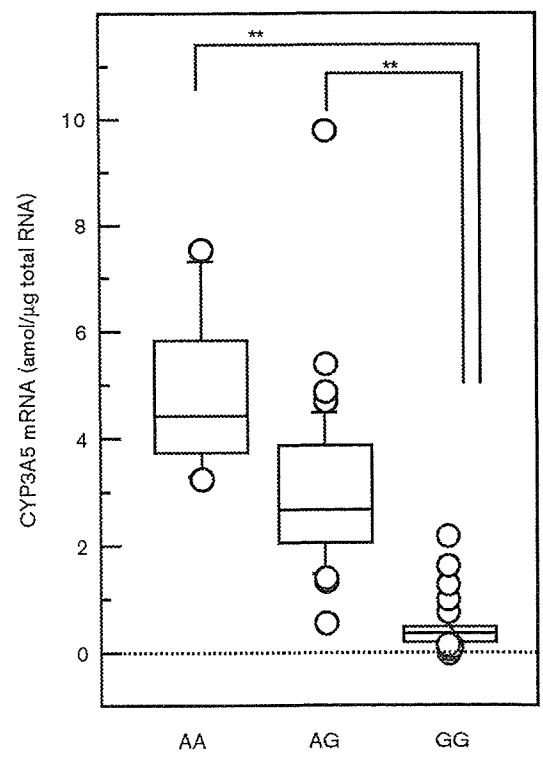
The expression level of MDR1 in the grafted liver was determined by real-time PCR, and the mean was 0.49 (0.08–15.6) amol/ g total RNA. The average MDR1 and CYP3A4 mRNA expression levels in graft liver with the CC, CT and TT genotypes in C3435T polymorphisms of *MDR1* were 0.50, 0.51 and 0.41 amol/ g total RNA and 9.13, 8.90 and 8.83 amol/ g total RNA, respectively, and no significant differences were found between these three groups (Fig. 1). Next, we analyzed the expression level of CYP3A5 in the graft liver. The average for *1/*1, *1/*3, and *3/*3 genotypes was 4.85, 2.99 and 0.41 amol/ g total RNA, respectively ($P < 0.01$; Fig. 2). The probability value between *1/*1 or *1/*3 allele and *3/*3 allele was below 0.01, and the differences were significant.

Fig. 1



Effect of the *MDR1* C3435T polymorphism on mRNA expression of MDR1 and CYP3A4 in liver. mRNA levels of MDR1 (a) and CYP3A4 (b) were determined using the real time PCR method in 112 liver biopsy specimens.

Fig. 2



Effect of the *CYP3A5* polymorphism on mRNA expression of CYP3A5 in liver. The mRNA expression level of CYP3A5 was determined using the real time PCR method in 112 liver biopsy specimens.

Effect of recipient genotype on the tacrolimus C/D ratio

Of the 181 recipients, 143 received immunosuppressive treatment with tacrolimus administered orally. In these patients, the influence of the *MDR1* C3435T and G2677T/A polymorphisms on the tacrolimus C/D ratio was examined. The mean C/D ratio with each genotype for the C3435T and G2677T/A SNPs is shown in Table 3. Neither polymorphism influenced the ratio. Recently, the correlation between MDR1 expression and the tacrolimus C/D ratio was reported to be affected by graft size, that is, the recipients who received relatively large graft had a lower C/D ratio. Therefore, we examined the influence of SNPs on the tacrolimus C/D ratio after dividing the recipients into two groups, those whose GRWR is below 1.7%, those whose GRWR is above 1.7%. There were no significant differences between genotypes, although the overall C/D ratio was higher in those with GRWR < 1.7%.

Effect of graft genotype on C/D ratio in 5 weeks after transplantation

The effect of *CYP3A5* genotype on the tacrolimus C/D ratio was examined for a period after the transplantation (Fig. 3). Since rejection and subsequent high-dose steroid pulse therapy influence the blood concentration of tacrolimus, the period in which liver function worsened was excluded from the analysis. The C/D ratio of tacrolimus decreased with time. For 1–2 weeks after the operation, the genotype had no affect (Fig. 3a, b), but a tendency for the C/D ratio of tacrolimus to decline in groups with the *CYP3A5* *1/*1 and *1/*3 genotype was observed after 3 weeks (Fig. 3c–e). Notably, the patients engrafted with *CYP3A5* *1/*1 genotype liver generally had a lower C/D ratio than the other groups.

Effect of the combination of graft genotype and MDR1 expression in the intestine on the tacrolimus C/D ratio

Next, we examined whether the combination of graft genotype and MDR1 expression level in the intestine influences to the pharmacokinetics of tacrolimus (Fig. 4). Among 38 recipients for whom samples of the intestinal mucosa and grafted liver were obtained to identify mRNA expression and genotype, patients were divided into four groups based on intestinal MDR1 expression, High or Low, and graft genotype, *CYP3A5**1 or *CYP3A5**3/*3. MDR1 High/*CYP3A5**1 carriers had a low C/D ratio in comparison with other groups for the first week after surgery. Notably in the first week, the High MDR1 group had a lower C/D ratio than the Low MDR1 group in *CYP3A5**1 as well as *CYP3A5**3/*3 genotype (Fig. 4a). However, this tendency is not seen after 2 weeks, and no significant differences in the C/D ratio of tacrolimus between the groups were also found (Fig. 4b–e).

Discussion

In the present study, we have examined the genotypes and expression levels of MDR1, CYP3A4 and CYP3A5 in graft livers and recipients of LDLT, and their effects on tacrolimus pharmacokinetics. We demonstrated that in graft liver, the mRNA expression level of CYP3A5 was significantly influenced by *CYP3A5**3-genotype, and the C/D ratio was decreased by *CYP3A5**1/*1 genotype. In addition, the recipients' genotype concerning MDR1 had no influence on the tacrolimus C/D ratio even when the GRWR was taken into consideration.

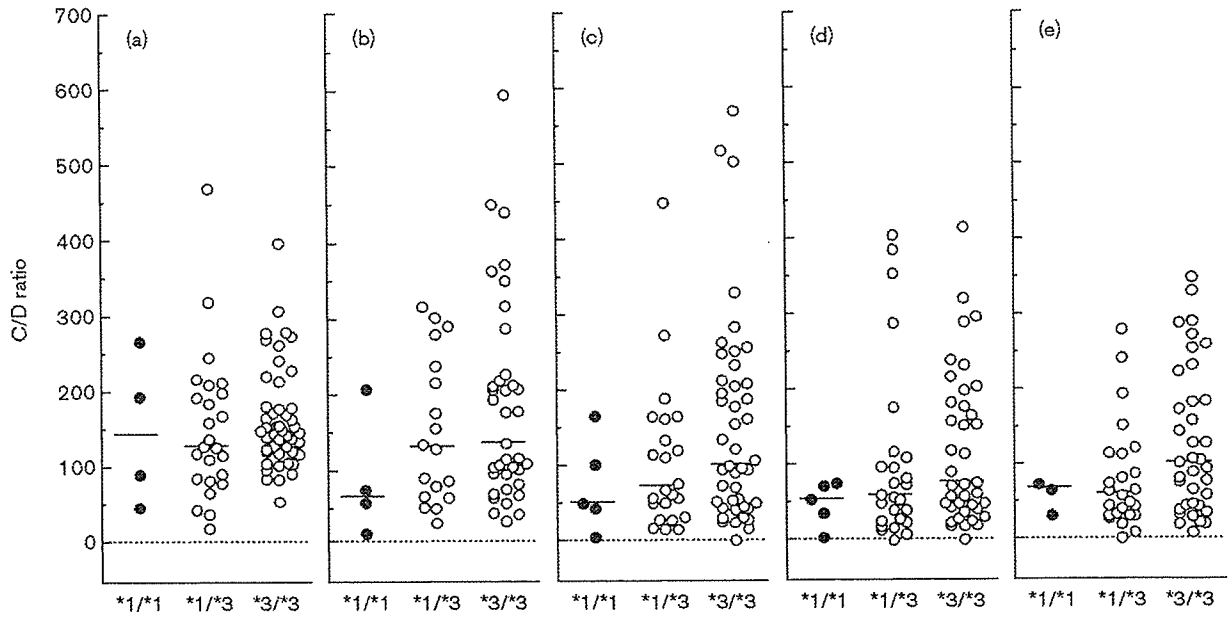
The pharmacokinetics of tacrolimus shows marked inter- and intraindividual variation. Absorption in the intestine and metabolism and excretion in the liver may explain this variation. In the recipients of LDLT, the expression level of P-glycoprotein, was inversely correlated with the tacrolimus C/D ratio for 1 week after transplantation [4], and the total clearance of tacrolimus increased with time [15], probably due to regeneration of the grafted liver. Therefore, the pharmacokinetics of tacrolimus may be influenced by the metabolic activity of the liver, that is, by the expression levels of metabolic enzyme in the graft.

Intestinal P-glycoprotein functions as an absorptive barrier against various orally administered drugs, and its functional and expressional variation may affect the bioavailability of these drugs [16]. Several SNPs in *MDR1* affecting expression and/or function have been reported. Among these SNPs, C3435T and G2677T/A

Table 3 Effect of *MDR1* C3435T and G2677TA polymorphisms on tacrolimus C/D ratio

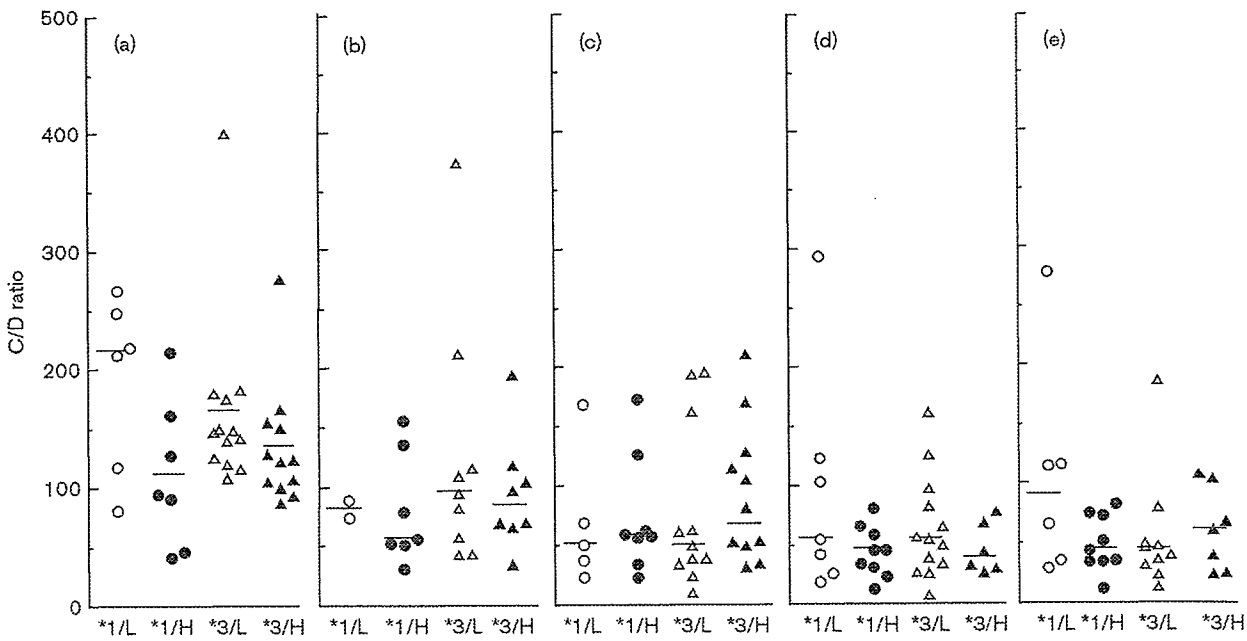
Genotype	All patients			GRWR < 1.7%			GRWR > 1.7%		
C3435T	*n*			*n*			*n*		
CC	44	158.5	77.9	25	187.9	70.3	15	109.4	65.7
CT	76	160.2	94.5	33	226.9	102.9	43	110.2	42.7
TT	27	147.4	81.2	13	188.4	94.2	14	109.3	41.9
G2677TA	*n*			*n*			*n*		
GG	25	147.1	74.1	13	184.8	69.4	12	106.2	56.2
GA	10	156.7	71.5	5	190.6	76.1	5	122.7	53.2
GT	55	171.9	102.7	29	228.4	111.0	26	108.9	34.1
TA	24	145.0	78.8	8	217.8	46.4	16	108.6	65.3
TT	29	150.7	78.4	16	182.2	88.5	13	112.0	40.3

Fig. 3



Effect of graft liver *CYP3A5* genotype on tacrolimus C/D ratio in LDLT recipients over 5 weeks. The mean tacrolimus C/D ratio for the period of 1–7 (a), 8–14 (b), 15–21 (c), 22–28 (d), and 29–35 (e) days post-transplantation was compared based on *CYP3A5* genotype.

Fig. 4



Effect of the combination of intestinal MDR1 expression level and graft liver *CYP3A5* genotype on tacrolimus C/D ratio in LDLT recipients over 5 weeks. The 38 cases for which data on both MDR1 mRNA expression in the intestine and graft genotype were available, were devided into four groups based on MDR1 expression (High or Low) and graft genotype (*CYP3A5**1 carriers or *CYP3A5**3/*3 carriers), that is, *CYP3A5**1/MDR1 High (*1/H), *CYP3A5**1/MDR1 Low (*1/L), *CYP3A5**3/MDR1 High (*3/H) and CYP3A5*3/MDR1 Low (*3/L). In each group, the C/D ratio was compared for five weeks, over the period 1–7 (a), 8–14 (b), 15–21 (c), 22–28 (d), 29–35 (e) post-transplantation days.

are detected at a relatively high frequency, and several groups have examined their influence on gene expression and function [17,18]. Previously, we reported that these SNPs affected neither the intestinal expression of MDR1 nor tacrolimus C/D ratio in 46 patients of LDLT [9]. In the present study, we reanalyzed the cases of 132 recipients using intestinal biopsy specimens, and again found no effect of C3435T and G2677T/A on the mRNA level of MDR1 (data not shown). Furthermore, no correlation between MDR1 genotype and tacrolimus C/D ratio was obtained in 143 recipients (Table 3). Because there is extensive variation in the GRWR of LDLT recipients, the influence of genotype might be more clearly observed by considering the GRWR. However, even when taking GRWR into consideration, no significance difference among genotypes was observed (Table 3). The trough concentrations of the immunosuppressant cyclosporine and tacrolimus were not influenced by the polymorphism C3435T in renal transplant recipients [8,19]. However, there is little consensus concerning the affect on drug pharmacokinetics by SNPs in *MDR1* [20]. The polymorphism G2677T/A was also reported to affect the pharmacokinetics of digoxin and fexofenadin [18,21]. However, results obtained from *in vitro* with a vaccinia virus expression system and mammalian expression system using LLC-PK1 cells indicated that the SNPs in *MDR1* do not affect the membrane expression of Pgp or transport activity of drugs such as digoxin and cyclosporine A [22,23]. As there are discrepancies between results of *in vitro* and *in vivo* examinations, the effect of these SNPs on expression and function might contribute little to the pharmacokinetics of Pgp-substrates.

Pgp is expressed in various other organs including the liver and kidney [24]. The effect of *MDR1* SNPs in the kidney is controversial. The renal expression level of MDR1 was decreased in the 3435TT genotype in comparison with the 3435CC genotype in Caucasians, but not in Japanese [13,25]. In liver, there were no significant differences in MDR1 levels in either of the C3435T genotypes (Fig. 1a).

We have previously reported that the C3435T SNP in *MDR1* reduces the expression of CYP3A4 in the small intestine [9]. In the present study, we examined the relationship between this SNP and CYP3A4 mRNA expression in the liver (Fig. 1b). The expression tended to be decreased in the patients carrying C3435T, but did not show significant differences. Because of the different regulatory mechanism for the constitutive expression of CYP3A4 mRNA in liver and intestine, the effect of the C3435T polymorphism in *MDR1* on CYP3A4 expression could not be observed in the liver.

CYP3A5, a homolog of CYP3A4 with overlapping substrate specificity [26], shows significant racial differences in expression. Recently Kuehl *et al.* [27] demonstrated that a single nucleotide polymorphism in intron 3 causes a splicing variant of CYP3A5 mRNA, and this splicing error results in a defect of protein synthesis. The *CYP3A5**3 allele, which causes the splicing error, was frequently detected in Caucasian subjects, 60–90% of whom did not express CYP3A5. In contrast, much more than 50% of African subjects have at least one *CYP3A5**1 allele, and express CYP3A5 protein. Andrews *et al.* [28] reported that black recipients of renal transplants needed twice the dose of tacrolimus as white recipients to achieve the target blood concentration. In an attempt to explain this difference, the *CYP3A5**3 genotype has been examined in several groups. Hesselink *et al.* [8] demonstrated that in 62 renal transplant recipients, *CYP3A5**3/*3 carriers required a lower dose of tacrolimus to achieve the target concentration, and the trough C/D ratio was statistically higher than in *CYP3A5**1 carriers. Macphee *et al.* [29] made similar findings in renal transplant recipients on analyzing the genotype of the *CYP3AP1* G-44 polymorphism which was considered to be linked to *CYP3A5**3 allele [30,31]. We have analyzed the *CYP3A5**3 polymorphism in 114 Japanese and found the frequency of the *1 allele to be 27.3% (Table 2), much lower than in black subjects. The level of CYP3A5 mRNA in patients with the *CYP3A5**3/*3 genotype was significantly low (Fig. 2). For 2 weeks after the transplantation, there was extensive variation in the C/D ratio for all genotypes, and no clear differences among genotypes were observed (Fig. 3a, b). After 3 weeks, a low tacrolimus C/D ratio was observed in those with the *CYP3A5**1/*1 genotype, and *CYP3A5**1 carriers showed a lower C/D ratio after that (Fig. 3 c–e). The *CYP3A5**3/*3 carriers showed great variation in the C/D ratio, so no significant change was observed even in the postoperative period. In the present study, the genotype–phenotype relationship was examined in the hospitalized patients, and it was found that the C/D ratio in *CYP3A5**1/*1 carriers gradually decreased with time after transplantation. In addition, the difference in the tacrolimus C/D ratio with *CYP3A5**3 genotype may become clearer after discharge from the hospital.

Kiuchi *et al.* [32] demonstrated that the size of the liver graft, expressed as GRWR, influenced the outcome of LDLT including survival, and suggested that it affected the pharmacokinetic variation in postoperative tacrolimus therapy. When recipients were divided into two groups based on the median of GRWR, the effect of *CYP3A5* genotype was not clear during the first 2 weeks. After three weeks, the C/D ratio of tacrolimus tended to be low in the graft with the *CYP3A5**1/*1 genotype and the large GRWR group (> 1.8%), but was not improved in the small GRWR group (< 1.8%;

data not shown). However, the classification by GRWR did not reveal significant differences between the three *CYP3A5* genotypes. The LDLT recipients engrafted with a liver having the *CYP3A5**1/*1 genotype showed a low C/D ratio after three weeks (Fig. 3). In contrast, the recipients with the *CYP3A5**1/*3 or *3/*3 genotype liver showed extensive variation in the C/D ratio. The genetic variation responsible for the large variability in systemic clearance mediated by CYP3A4 is still unknown. The hepatic expression levels and molecular mechanisms of inter-individual variation of other CYP3A isoforms as well as CYP3A5 should be examined to understand the *in vivo* drug pharmacokinetics.

In LDLT, the genotype of the recipient (intestine) and donor (liver) could be important to understanding the variation in postoperative tacrolimus pharmacokinetics. We examined the recipients' *MDR1* C3435T or G2677T/A genotype and graft *CYP3A5* genotype. Despite the tendency for recipients with at least one *CYP3A5**1 to have a low C/D ratio, little effect of *MDR1* genotype was detected even in the first week. At first, C3435T or G2677T/A SNPs had no effect on the mRNA expression of enterocyte MDR1 and tacrolimus C/D ratio, and the combination of recipients and graft genotype did not show significant differences (data not shown). As *MDR1* genotype has no effect on the tacrolimus C/D ratio, we conducted analyses using the MDR1 mRNA expression level. When recipients were grouped based on intestinal MDR1 mRNA expression, high or low, the tacrolimus C/D ratio was found to be the lowest in the MDR1/High and *CYP3A5**1 group. When comparing *CYP3A5**1 or *CYP3A5**3, the MDR1/High group had a lower C/D ratio than the MDR1/Low group in the first week (Fig. 4A). But the trend of a low C/D ratio in the MDR1/High group diminished after 2 weeks, and the trend of a low C/D ratio in CYP3A5*1 carriers was not observed after that. In this study, among 38 recipients only two had *CYP3A5**1/*1-graft liver. The *CYP3A5**1/*3 carriers also exhibited variation in the C/D ratio, so such a low C/D ratio as in carriers might not be observed. Therefore, it was suggested that in LDLT recipients, MDR1 in the intestine contributed to the trough level of tacrolimus for the first week following the transplantation, after which the main organ of influence was the liver, *CYP3A5**1/*1 genotype caused the tacrolimus C/D ratio to decrease. In the first week after the transplantation, the trough level is affected by intestinal MDR1 expression, but no SNP which closely affects MDR1 expression has been discovered. To achieve a target concentration of tacrolimus quickly, SNPs that explain the variation in MDR1 expression must be identified.

In summary, in recipients of LDLT, the trough concentration of tacrolimus is mainly affected by the intestinal expression level of MDR1 during the first week, but after 3 weeks, recipients engrafted with a *CYP3A5**1/*1 genotype liver show a lower C/D ratio even taking into consideration factors such as graft size and CYP3A4 expression level. These results indicated that at the *CYP3A5**1/*1 genotype could be used to predict the maintenance dose of tacrolimus, but for carriers of the *CYP3A5**1/*3 or *3/*3 genotype, the quantification of CYP3A4 expression would be helpful. To establish an appropriate tacrolimus dosage regimen, it is important to know the expression level of MDR1 in the intestine and CYP3A4 in the liver. The finding of a genotype explaining such factors would be able to realize the medication for individuals.

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References

1 Denton MD, Magee CC, Sayegh MH. Immunosuppressive strategies in transplantation. *Lancet* 1999; **353**:1083–1091.
2 Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, *et al.* Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet* 1995; **29**:404–430.
3 Hebert MF. Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. *Adv Drug Deliv Rev* 1997; **27**:201–214.
4 Hashida T, Masuda S, Uemoto S, Saito H, Tanaka K, Inui K. Pharmacokinetic and prognostic significance of intestinal MDR1 expression in recipients of living-donor liver transplantation. *Clin Pharmacol Ther* 2001; **69**:308–316.
5 Evans WE, McLeod HL, Johnson JA, Relling MV. Pharmacogenomics – drug disposition, drug targets, and side effects *N Engl J Med* 2003; **348**:538–549.
6 Schwab M, Eichelbaum M, Fromm MF. Genetic polymorphisms of the human MDR1 drug transporter. *Annu Rev Pharmacol Toxicol* 2003; **43**:285–307.
7 Pirmohamed M, Park BK. Cytochrome P450 enzyme polymorphisms and adverse drug reactions. *Toxicology* 2003; **192**:23–32.
8 Hesselink DA, van Schaik RH, van der Heiden IP, van der Werf M, Gregoor PJ, Lindemans J, *et al.* Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* 2003; **74**:245–254.
9 Goto M, Masuda S, Saito H, Uemoto S, Kiuchi T, Tanaka K, Inui K. C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation. *Pharmacogenetics* 2002; **12**:451–457.
10 Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, *et al.* Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin Pharmacol Ther* 2001; **69**:169–174.
11 van Schaik RH, de Wildt SN, van Iperen NM, Uitterlinden AG, van den Anker JN, Lindemans J. CYP3A4-V polymorphism detection by PCR-restriction fragment length polymorphism analysis and its allelic frequency among 199 Dutch Caucasians. *Clin Chem* 2000; **46**:1834–1836.
12 van Schaik RH, van der Heiden IP, van den Anker JN, Lindemans J.

CYP3A5 variant allele frequencies in Dutch Caucasians. *Clin Chem* 2002; **48**:1668–1671.

13 Uwai Y, Masuda S, Goto M, Motohashi H, Saito H, Okuda M, *et al.* Common single nucleotide polymorphism of MDR1 gene have no influence on its mRNA expression level of normal kidney cortex and renal cell carcinoma in Japanese. *J Hum Genet* 2004; **49**:40–45.

14 Koch I, Weil R, Wolbold R, Brockmoller J, Hustert E, Burk O, *et al.* Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. *Drug Metab Dispos* 2002; **30**:1108–1114.

15 Fukatsu S, Yano I, Igarashi T, Hashida T, Takayanagi K, Saito H, *et al.* Population pharmacokinetics of tacrolimus in adult recipients receiving living-donor liver transplantation. *Eur J Clin Pharmacol* 2001; **57**:479–484.

16 Fromm MF. The influence of MDR1 polymorphisms on P-glycoprotein expression and function in humans. *Adv Drug Deliv Rev* 2002; **54**:1295–1310.

17 Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, *et al.* Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc Natl Acad Sci U S A* 2000; **97**:3473–3478.

18 Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, *et al.* Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001; **70**:189–199.

19 von Ahsen N, Richter M, Grupp C, Ringe B, Oellerich M, Armstrong VW. No influence of the MDR-1 C3435T polymorphism or a CYP3A4 promoter polymorphism (CYP3A4-V allele) on dose-adjusted cyclosporin A trough concentrations or rejection incidence in stable renal transplant recipients. *Clin Chem* 2001; **47**:1048–1052.

20 Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM. P-glycoprotein: from genomics to mechanism. *Oncogene* 2003; **22**:7468–7485.

21 Kurata Y, Ieiri I, Kimura M, Morita T, Irie S, Urae A, *et al.* Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. *Clin Pharmacol Ther* 2002; **72**:209–219.

22 Kimchi-Sarfaty C, Gribar JJ, Gottesman MM. Functional characterization of coding polymorphisms in the human MDR1 gene using a vaccinia virus expression system. *Mol Pharmacol* 2002; **62**:1–6.

23 Morita N, Yasumori T, Nakayama K. Human MDR1 polymorphism: G2677T/A and C3435T have no effect on MDR1 transport activities. *Biochem Pharmacol* 2003; **65**:1843–1852.

24 Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *J Histochem Cytochem* 1990; **38**:1277–1287.

25 Siegsmund M, Brinkmann U, Schaffeler E, Weirich G, Schwab M, Eichelbaum M, *et al.* Association of the P-glycoprotein transporter MDR1(C3435T) polymorphism with the susceptibility to renal epithelial tumors. *J Am Soc Nephrol* 2002; **13**:1847–1854.

26 Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* 2002; **54**:1271–1294.

27 Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, *et al.* Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001; **27**:383–391.

28 Andrews PA, Sen M, Chang RW. Racial variation in dosage requirements of tacrolimus. *Lancet* 1996; **348**:1446.

29 Macphee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, *et al.* Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome p4503A5 and P-glycoprotein correlate with dose requirement. *Transplantation* 2002; **74**:1486–1489.

30 Paulussen A, Lavrijsen K, Bohets H, Hendrickx J, Verhasselt P, Luyten W, *et al.* Two linked mutations in transcriptional regulatory elements of the CYP3A5 gene constitute the major genetic determinant of polymorphic activity in humans. *Pharmacogenetics* 2000; **10**:415–424.

31 Hustert E, Haberl M, Burk O, Wolbold R, He YQ, Klein K, *et al.* The genetic determinants of the CYP3A5 polymorphism. *Pharmacogenetics* 2001; **11**:773–779.

32 Kiuchi T, Kasahara M, Uryuhara K, Inomata Y, Uemoto S, Asonuma K, *et al.* Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. *Transplantation* 1999; **67**:321–327.

The reason why the number of living donor transplantations is so di erent between countries, has certainly to do with the experience available in certain transplant centers. Another major factor is the increased risks for the donor in terms of mortality and morbidity. Several donor deaths have been reported with this procedure. The outcome of the total group of living donor liver transplantation at 1 yr was 80%. At 3 yr this was 67%, which is borderline significant ($p = 0.07$) from the results obtained with split cadaveric liver transplants (61% at 3 yr).

Conclusion: In view of organ donor shortage and considering the good success rates, the source of living related and unrelated donors should certainly be considered more frequently in countries with lower than average cadaveric donor transplants. All measurements which might stimulate this, should be initiated and stressed.

INV-106

Socio-economoic issues in living donor transplantation – economic and judicial aspects: the Asian perspective

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The biggest reason of earlier spread of living donor liver transplantation in Asia is definitely a highly limited access to cadaveric graft. Contrary to the speculation of the majority, a major obstacle in the evolution of cadaveric transplantation in Asia seems not cultural or religious but organizational including social indi erence, public distrust for medicine, and economical problem. If organization for cadaveric transplantation is premature, much less can be expected for the organization of living donor transplantation? The precise data to answer this question is unfortunately missing due to the lack of trans-Asia registry. Not only surgery-related morbidity and mortality, but also the results of long-term quality of life (QOL) survey in living donors all underline the importance of organized system for donor evaluation, selection, and protection in consistency with a fundamental policy of medicine: first, do no harm. The discussion seems to reach its peak in living liver donation especially for adult patients than any other types of living donation. The system is expected to cover all the process of donor selection, safety, recovery and post-donation psychosocial survey with an appropriate care. This is emphasized again in New York State Guideline on Quality Improvement in Living Liver Donation recently prepared after a mortality. It should always be confirmed that a donor candidate is competent, willing to donate, free from coercion, medically and psychosocially suitable, fully informed of the risks and benefits in donor and recipient, and of any alternative treatments available to the recipient. The organization of Independent Donor Advocate Team supporting the process of informed choice with a period of reflection and giving care for the ambivalence between motivation and anxiety is strongly recommended. Acceptable social limit of lower age and relationship to the recipient still leave considerable controversies. Acceptable range of medical and/or surgical risk factors, including upper age limit, latent viral infections as hepatitis B core-antibody positivity, and mild hepatic/extra-hepatic diseases, is left to institutional and case-by-case decision. On the other hand, accumulated experiences in the use of right liver graft for adult recipients has brought controversies on acceptable graft/remnant liver size and anatomical variants to a rough consensus, which, however, still depends on the accessibility to cadaveric graft in each country. In addition to the highest quality of care given to living donors during donation and recovery process, recent survey emphasizes the importance of organized system for long-term follow-up of donors for their lifetime. This would include not only medical/surgical morbidities directly related with donation, but also psychosocial issues of financial and occupational concerns and family conflict, *i.e.* whole impact on the donor's life style. Trials of QOL survey in agreement with institutional ethics committee are just started in limited number of programs with a large experience. For example, more than 2600 cases of living donor liver transplantation were done in Japan in the last 14 yr, which is definitely a majority of all Asian cases. In the same period only 24 cadaveric liver grafts were transplanted. A total of 48 hospitals did at least one case. Public trust in this field of medicine is in surprising contrast to public indi erence or distrust for the process of brain death diagnosis and donation in Japan. On the other hand, socioeconomic support is still sub-optimal. Public insurance coverage has been extended to fulminant hepatic failure and decompensated cirrhosis with/without early hepatocellular carcinoma in adults only at the beginning of 2004. Advanced hepatocellular carcinoma beyond Milan criteria and that not accompanied by cirrhosis are still outside coverage despite their potential curability. Most insurance companies do not regard living donation as subject of reimbursement. Regarding medical and/or surgical selection criteria of potential donors in Japan, a rough domestic consensus is established owing to active exchange of information via academic society. However, lack of judicial regulation leaves not a few social and medical decisions to each program and local ethics committee, which are often variable by program. Furthermore, the quality of psychosocial evaluation, protection and care, the process of informed choice, and long-term follow-up and care system are all left to a self-regulation of each program. Long-term QOL survey seems just prepared in programs with a large experience. Due to a reduced accessibility to cadaveric graft and due to unique epidemiology of liver diseases, living donor liver transplantation has flourished much faster in Asian countries. However, statistical information on donor evaluation/selection is quite limited due to the absence of registry covering the entire Asia. Except recent reports on donor morbidity from Japan and major Asian centers, even a total number of cases remain under speculation. In addition, varieties in social, cultural, economical, and religious background in Asian countries may deeply a ect the process of informed choice, selection, safety measure, and psychosocial protection of donors and their families. A questionnaire inquiring these issues is being sent to representative Asian programs. A rough status quo in medical and psychosocial approach will be presented in the conference.

INV-107

Socio-economic issues in living donor transplantation: the American perspective

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Solid organ transplantation has progressed from an innovative and experimental therapy o ered as a last resort to patients who had exhausted all other therapeutic avenues to a standardized therapeutic option for many patients with irreversible end-organ dysfunction. Consequently, transplantation has become a standard procedure and the indications for its application have continued to expand. Unfortunately, with expansion of the indications for transplantation comes the realization that the number of transplantable organs is relatively fixed and insu cient to meet the ever expanding demand. Only through expansion of deceased donor inclusion criteria: acceptance of more donor organ pathology and inclusion of live donors as a source of transplantable organs does the community have any hope of serving even a minority of the many patients listed and awaiting transplantation. Although the limited access to such a successful but scarce and expensive therapy

Surgical Site Infection in Living-Donor Liver Transplant Recipients: A Prospective Study

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Background. Infection is a constant threat for the living-donor liver transplantation (LDLT) recipients, although little information is available on the occurrence of infection in such patients.

Methods. One hundred and thirteen consecutive LDLT recipients were prospectively followed for the presence of surgical site infections (SSIs) defined by CDC from April 2001 to March 2002. Risk factors for SSIs were evaluated by univariate and multivariate analysis.

Results. Of the 113 LDLT recipients, 42 (37%) developed 57 episodes of SSIs (21 intraabdominal abscess, 20 peritonitis, 8 cholangitis, and 9 wound). Of the 57 episodes, 29 (51%) had secondary bacteremia in 19 patients. Causative pathogens, including 17 episodes of polymicrobial infections, were 37 gram-positive cocci (17 *Staphylococcus aureus*, 16 *Enterococcus* spp., and 4 others), 40 gram-negative rods (25 Enterobacteriaceae, 13 *Pseudomonas aeruginosa*, and 4 others), and 2 *Candida albicans*. Univariate analysis revealed that ABO incompatibility and repeat surgery were associated with the development of SSIs. Also, univariate analysis revealed that adult recipients, ABO incompatibility, total operation duration, repeat surgery, and NNIS risk index were associated with secondary bacteremia. Multivariate analysis revealed that ABO incompatibility (OR: 14.0; 95% CI, 2.52–77.2) and repeat surgery (OR: 9.29; 95% CI, 2.00–43.1) were independently associated with secondary bacteremia. Eleven of the 42 cases (26%) who developed SSIs died. Of these 11 cases, 5 (45%) developed secondary bacteremia within 30 days before death.

Conclusion. SSIs occurred in 37% of LDLT recipients. ABO incompatibility and repeat surgery increased the risk of developing SSIs with secondary bacteremia, which correlated with poor prognosis.

Keywords: Clinical transplantation, Liver, Complications of clinical transplantation, Infection, Surgical.

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The development of living-donor liver transplantation (LDLT) in Asia over the past 12 years has been driven by a critical shortage of cadaveric grafts in the region, and cadaveric organ donation remains less than five per 1 million per year (*1*). The major indications have expanded from biliary atresia and other pediatric liver diseases to a variety of adult liver diseases, particularly hepatitis virus-related liver disease with or without hepatocellular carcinoma (HCC). LDLT activity in Asia has increased rapidly as growing multidisciplinary transplant expertise and public awareness of this novel therapeutic option have increased (*2*).

However, infection remains a constant threat for all liver transplant recipients, and the incidence of infection after liver transplantation is generally higher than that after other types of solid-organ transplantation (*3,4*). This increased incidence is likely related to the technical complexity of the liver-transplantation procedure itself, its performance in a potentially contaminated environment within the abdominal cavity, and the extremely poor medical condition of many recipients; bacterial infections remains an important cause of death after liver transplantation (*5*).

At present, infectious complications have been reported in 35% to 68% of liver transplantation (*6–9*). Most infections occur within the first 30 days after transplantation and are primarily surgical complications, nosocomial in origin, or, rarely, reactivation of latent infections in the recipient (*4*). Although several reports from the United States and European centers include data on infection after cadaveric liver, only a few small series have been reported in LDLT. The purpose of this 1-year, prospective study was to determine the incidence, timing, sites of, and risk factors for surgical site infections (SSIs) after LDLT.

PATIENTS AND METHODS

Patients

The prospective study population included 115 consecutive patients undergoing liver transplantation at the Department of Transplantation Surgery, Kyoto University Hospital, from April 2001 to March 2002. Two patients each who initially had undergone cadaveric donor liver transplantation and who had an uncontrollable infection perioperatively were excluded from the study. Thus 113 LDLT procedures performed in 111 patients were included in this study. All of 39 pediatric recipients aged from 5 months to 7 years and weighing approximately 20 kg or less received left lateral segment or lobe grafts, whereas all of 74 adult recipients aged from 14 to 69 years and weighing 30 kg or more received right lobe grafts (*10*). Therefore, adults were defined as individuals aged 14 years or more. All infections from the time of surgery until hospital discharge were recorded. The median follow-up for living patients was 62 days (range: 5–252 days).

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Immunosuppression

The details of donor evaluation, donor surgery protocols, surgical techniques, and perioperative management of recipients in our hospital have been described elsewhere (*11*). The basic immunosuppression regimen consisted of tacrolimus and low-dose corticosteroid. Methylprednisolone was administered intraoperatively immediately after reperfusion, tapered, and changed to oral prednisolone (0.5 mg/kg per day) 7 days after transplantation. Patients were weaned off steroids 3 to 6 months postoperatively if liver function was stable. Acute rejection was initially treated by increasing the dose of tacrolimus. If liver function did not respond to raising the trough level, high-dose methylprednisolone was used as steroid pulse therapy. When mild liver dysfunction persisted after steroid therapy, azathiopurine was added to the basic regimen until liver function again stabilized. If rejection was refractory to these treatments, OKT3 was used as the last resort.

Antimicrobial Prophylaxis

Perioperative prophylaxis consisted of flomoxef, an oxacefem antibiotic, for 72 hr. Trimethoprim and sulfamethoxazole once daily was administered as prophylaxis against *Pneumocystis*. Selective bowel decontamination (SBD) was performed for 3 days before transplantation using oral kanamycin. Miconazole was administered for 7 days after transplantation as antifungal prophylaxis.

Definitions of Infections

SSI was defined in accordance with the Centers for Disease Control and Prevention criteria. Major surgical wound-related infections included intra-abdominal abscess, peritonitis, cholangitis, and wound infections. An abscess was defined as a collection of fluid, drained surgically or aspirated under ultrasound guidance, in which microscopy showed pus cells, and culture yielded one or more organisms. Peritonitis was diagnosed if the ascitic fluid neutrophil count was greater than 250 cells/mm^3 and if a pathogen was isolated. In all cases, intraabdominal abscesses were excluded by ultrasound scanning. Cholangitis was defined when there was one or more clinical indicators of infection (temperature 38°C or a white blood cell count 15 10^9/L) with otherwise unexplained elevation of liver function tests concomitant with the repeated isolation of an organism in pure culture from T-tube bile. Secondary bacteremia related to SSIs was defined as isolation of the pathogen responsible for the SSI from at least one set of blood cultures.

Risk Factors for Surgical Site Infections

The following variables were assessed as risk factors for SSIs: (i) pretransplant variables, including adult recipient (as representative risk factor of age, body weight, and type of grafts), gender, previous Roux-en-Y anastomosis, encephalopathy, ABO incompatibility, serum albumin concentration, serum bilirubin concentration, pretransplantation intensive care unit stay, moderate or massive ascites, and Child-Pugh score; and (ii) operative and posttransplant variables, including duration of transplant surgery, intraoperative packed red blood cell transfusion, type of biliary reconstruction, and repeat intraabdominal or intrathoracic surgery. Using the National Nosocomial Infections Surveillance (NNIS) SSI risk index, each operation was scored 0 to 3 by counting the number of risk factors present in patients; an American Society of Anesthesiologists preoperative assessment score of 3, 4, or 5; an operation classified as either contaminated or dirty-infected; an operation with a duration of 13 hr, which was the 75th percentile of the duration of 95 consecutive LD-LTs that were performed at our hospital in 2000.

Statistical Analysis

Continuous variables were compared using the Student *t* test or when a normal distribution could not be assumed, the Mann-Whitney test. Categorical data were compared using the chi-square or Fisher exact test. A logistic regression model was used to evaluate variables found to be associated with SSI by univariate analysis (P 0.1). All of the analyses were performed with computer software, Statview version 5.0 (SAS Institute, Cary, NC). Differences of P 0.05 were considered statistically significant.

RESULTS

Characteristics of the Study Population

One hundred and eleven patients underwent 113 LD-LTs in the study period. Two patients required a second transplant for chronic rejection. Fifty-two patients (46.8%) were male, and the age range was 0.2 to 69 years (median: 30 years). The predominant underlying liver diseases were biliary atresia (33.3%, 37 patients), HCC (17.1%, 19 patients), and fulminant hepatic failure and metabolic liver disease (8.1% each, 9 patients) (Table 1).

Focus of Surgical Site Infections

Forty-two transplantations in 42 patients were complicated by 57 episodes of SSIs. The risk of developing at least one SSI was 38% (42/111) per patient and 37% (42/113) per transplantation. The average number of SSIs was 0.51 epi-

TABLE 1. Demographic and clinical characteristics of study patients

Patients, n	111
Median age (range), y	30 (0.2–69)
Gender, male/female	52/59
Adults[a], n (%)	74 (66.7%)
Underlying liver disease, n (%)	
Biliary atresia	37 (33.3%)
HCC[b]	19 (17.1%)
Fulminant hepatic failure	9 (8.1%)
Metabolic liver disease	9 (8.1%)
Primary biliary cirrhosis	8 (7.2%)
Primary sclerosing cholangitis	8 (7.2%)
Hepatitis C	8 (7.2%)
Hepatitis B	3 (2.7%)
Neoplastic liver disease other than HCC	3 (2.7%)
Other	7
Child-Pugh score (mean SD)	11.3 2.2

[a] Age 14 years.
[b] HCC, hepatocellular carcinoma.

TABLE 2. Site of infection after living-donor liver transplantation

Infection site	No. of episodes (no. of patients)	No. of episodes with secondary bacteremia (%)
Intra-abdominal abscess[a]	21 (16)	12 (57.1%)
Peritonitis	20[b] (20)	10[b] (50.0%)
Cholangitis	8[b] (8)	5[b] (62.5%)
Wound	9 (9)	1 (11.1%)

[a] Including subphrenic, liver, and subhepatic abscess.
[b] One patient had both peritonitis and cholangitis.

sodes per patient and 0.5 episodes per transplantation. Twenty-six episodes (46%) were associated with secondary bacteremia in 18 patients (0.23 episodes per patient). The predominant infection sites were intraabdominal abscess and peritonitis (Table 2). Secondary bacteremia occurred in between 50.0% and 62.5% of cases of intraabdominal abscess, peritonitis and cholangitis.

Time of Occurrence

SSIs occurred most commonly in the first 2 weeks after surgery (Fig. 1). The incidence declined over 3 weeks but increased moderately again after 4 weeks. Of the SSIs, 56% (32/57) occurred within 2 weeks after surgery, and 21% (12/57) occurred after 5 weeks. The timing of secondary bacteremia was more or less in parallel.

Pathogens

Seventy-nine organisms were recovered from 57 episodes of SSIs, so 17 episodes were polymicrobial infections. Thirty-seven Gram-positive cocci, 40 Gram-negative rods, and 2 *Candida albicans* were recovered (Table 3). Thirty-one of 37 (83.8%) Gram-positive cocci and 24 of 40 (60%) Gram-negative rods were resistant to flomoxef. *Staphylococcus aureus*, *Enterococcus* spp., and *Pseudomonas aeruginosa* were the predominant pathogens. Methicillin resistance was common (*S. aureus* isolates, 75.5%; coagulase-negative staphylococci [CNS], 66.7%). Infections due to *Staphylococcus* spp. occurred soon after transplantation. Thirteen of 17 (76.5%) *S. aureus* and 15 of 20 (75%) all staphylococcal infections occurred within 14 days. None of the enterococci isolates were vancomycin resistant. The type of SSI did not correlate with the pathogen.

Risk Factors

Risk factors for SSIs including ten pretransplant variables and four operative and posttransplant variables, and the NNIS risk index was examined by univariate analysis. The only factors that were associated with the development of SSI were ABO incompatibility (P 0.011) and repeat intraabdominal or intrathoracic surgery (P 0.0017). Multivariate analysis using a logistic regression model based upon the variables identified by univariate analysis at the P 0.1 level revealed that only repeat surgery (odds ratio, 4.27; 95% CI, 1.6–11.4) was independently associated with the development of SSI.

The same risk factors for SSI with secondary bacteremia were examined by univariate analysis. Factors that were associated with SSI with secondary bacteremia included adult recipients (P 0.003), ABO incompatibility (P 0.0001), total operation duration (P 0.0008), repeat intraabdominal or intrathoracic surgery (P 0.0001), and NNIS risk index (P 0.011) (Table 4). Multivariate analysis using a logistic regression model based upon variables identified by univariate analysis at the P 0.1 level revealed that only ABO incompatibility (odds ratio, 14.0; 95% CI, 2.52–77.2) and repeat surgery (odds ratio, 9.30; 95% CI, 2.00–43.1) were independently associated with the development of SSIs with secondary bacteremia (Table 5).

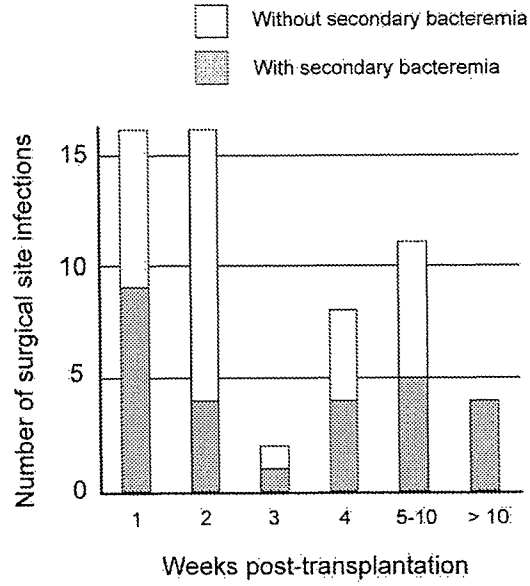

FIGURE 1. Time of onset of surgical site infections after living donor liver transplantation.

Prognosis

Eleven of 42 patients (26%) who had an SSI at posttransplantation died, whereas only 9 of 71 patients (13%) without an SSI died (P 0.19). Among patients who died, SSIs with secondary bacteremia occurred in 5 of 11 patients (45%) within 30 days of death. Poor prognosis did not correlate with the pathogen.

DISCUSSION

This is the first prospective study detailing SSI after LDLT. Abdominal infections, including wound infections, intraabdominal abscesses, peritonitis, and biliary infections, are the most common bacterial infections after liver transplantation (*3,4*). Therefore, we focused upon these infections as surgical site infections in this study.

Previous studies have reported that 35% to 68% of liver transplant recipients experience at least one episode of bacterial infection, with the overall incidence ranging between 0.52 to 1.46 episodes per patient (*6–9,12–16*). In these reports, SSIs accounted for 35% to 54% of all bacterial infections. In one report, SSIs occurred in 37.5% of patients, for an incidence of 0.91 per patient (*17*). Therefore, the result of this prospective study in an LDLT setting showed that the incidence of SSIs (38% of patients and 0.51 episodes per patient) is almost consistent with the cadaveric setting.

We found that most SSIs occurred in the first 2 weeks, which was consistent with previous reports (*9,12,13*). Recent

TABLE 3. Pathogens isolated in 57 episodes of surgical site infection after living-donor liver transplantation and their susceptibility to flomoxef

Pathogen	No. of episodes[a]	% Resistant to flomoxef[b]
Gram-positive cocci		
Staphylococcus aureus (% Methicillin resistance)	17 (76.5%)	76.5
Enterococcus spp.[c]	16	100
Coagulase negative staphylococci (% Methicillin resistance)	3 (66.7%)	66.7
Streptococcus intermedius	1	0
Gram-negative rods		
Pseudomonas aeruginosa	13	100
Enterobacter spp.	9	66.7
Klebsiella spp.	6	0
Escherichia coli	4	0
Serratia spp.	4	50
Others[d]	4	75
Fungi		
Candida albicans	2	—

[a] Including 17 episodes of polymicrobial infection.
[b] Drug susceptibility testings were performed according to NCCLS standards. Resistance breakpoint for flomoxef was determined by the breakpoint of cefmetazole in NCCLS standards.
[c] Vancomycin resistance was not isolated.
[d] Including one isolate each of *Acinetobacter baumannii, Aeromonas caviae, Chryseobacterium indologenes, Stenotrophomonas maltophilia.*

studies have reported that bacteremia occurs in 17% to 38% of liver transplant recipients (*9,13,14,18*), which is higher than the incidence of 17% (19/111) in this study. This difference may be related to the exclusion in this study of intravascular catheter-related infection, which is the most common source of bacteremia (*9,12,13,18*).

Thirty-seven Gram-positive cocci, 40 Gram-negative rods, and 2 *Candida albicans* were the causal pathogens in this study. Methicillin-resistant staphylococci isolates were common. However, no glycopeptide-resistant enterococci were isolated. The recent Japanese Nosocomial Infection Surveillance Report found that 67% and 80% of the *S. aureus* and CNS isolates, respectively, were methicillin resistant, although no vancomycin-resistant enteroccocal isolates were identified (http://www2.medis.or.jp/janis/). Among the causal pathogens, enterococci, almost all gram-negative rods, and candida can reside as intestinal flora, which may emanate from intestine to cause infection. On the other hand, *Staphylococcus* spp. usually resides as normal skin flora, which may emanate from skin through large surgical wounds or multiple drains that breach the integrity of the skin and cause infection. Liver transplant recipients have been shown to have a high incidence of staphylococcal nasal carriage before transplantation (*19*). Further, patients with prior nasal carriage tended to develop methicillin-resistant *Staphylococcus aureus* (MRSA) infections sooner after transplantation than did those without nasal carriage. The high incidence and early occurrence of *S. aureus* infections in this study may suggest a potentially high incidence rate of nasal carriage of *S. aureus*. On the other hand, 31 of 37 (83.8%) Gram-positive cocci and 24 of 40 (60%) Gram-negative rods were resistant to flomoxef, which was used as perioperative prophylaxis in this study. Broad-spectrum cephalosporin plus ampicillin, which was recommended in the guideline (*20*), should be considered as a more favorable prophylactic regimen to prevent prophylaxis failure.

Fungal infection by *Candida albicans* occurred in only two patients (2%). The incidence of invasive candidiasis in solid organ transplant recipients is the highest among liver transplant recipients, and *Candida* species account for greater than 50% of invasive fungal infections in liver transplantation (*4,21,22*), with an incidence of 1% to 26% (*21*). Invasive candidiasis after liver transplantation usually presents as an intraabdominal abscess, peritonitis, catheter-related fungemia, or fungemia of unknown source (*4*), and most fungal infections caused by *Candida* spp. occur within 2 months of transplantation. In this study, *Candida* infection caused one abdominal abscess and one wound infection, 5 and 9 days after surgery. A number of approaches toward antifungal prophylaxis have been proposed (*4*). The efficacy of oral nystatin and amphorericin-B has never been demonstrated conclusively (*23*). Two randomized, controlled studies have shown the efficacy of oral fluconazole in the prevention of invasive fungal infection caused by Candida species (*24,25*). The relatively lower incidence of candidiasis in this study may be related to the use of miconazole as antifungal prophylaxis and the exclusion of catheter-related fungemia or fungemia of unknown source.

Many risk factors for bacterial infections in liver transplant recipients have been reported (*3,4*), including prolonged duration of surgery, a greater than normal number of intraoperative transfusions, additional immunosuppression, repeat intraabdominal or intrathoracic surgery, length of postoperative intensive unit stay, cytomegalovirus infection, and portal vein thrombosis. In contrast with other reports, the rate of the infection was not affected by the time of oper-

ation or transfusion volume, but it was affected by ABO incompatibility and repeat intraabdominal or intrathoracic surgery. Moreover, adult recipients who received right lobe grafts, ABO incompatibility, operation duration, repeat surgery, and NNIS index were shown to be significant risk factors for SSIs with secondary bacteremia. The type of graft, which was specific for LDLT setting, may be related to the occurrence of SSIs. However, only ABO incompatibility and repeat surgery were also independent risk factors for SSIs with secondary bacteremia. ABO incompatibility has been shown to be a significant risk factor for biliary anastomotic complications (*11*), which is a risk factor contributing to infection after liver transplantation. Even though donors for LDLT are restricted to relatives, the indication for transplantation may need to be more restrictive when the donor candidate is ABO incompatible.

Mortality in bacteremic liver transplant recipients ranges between 21% and 36% (*5,6,9,12,13,18,26–28*). Bacteremia has been a predictor of mortality in some, but not all, series of liver transplant recipients (*9,15,27*). The high mortality rate (45%) associated with bacteremia in this study may be caused by the exclusion of intravascular catheter related bacteremia, which has emerged as a leading cause of bacteremia and carries a better prognosis (*29*).

CONCLUSION

This prospective study showed that the incidence of SSI and time of occurrence in LDLT recipients in Japan were comparable with those in Western studies. Methicillin-resistant *Staphylococcus aureus* was the most common multi-drug resistant bacteria, but no isolates of vancomycin-resistant enterococi were identified. ABO incompatibility is a risk factor

TABLE 4. Univariate analysis of risk factors for SSIs with secondary bacteremia in 113 living-donor liver transplants[a]

Variables	SSIs with secondary bacteremia (n 19)	All others (n 94)	*P* values
Pretransplant variables			
Adult, n (%)	18 (95)	56 (60)	0.003
Gender, male/female	12/7	40/54	0.10
Previous Roux-en-Y, n/n (%)	9/18 (47)	38/91 (42)	0.65
Dialysis, n/n (%)	5/19 (26)	11/86 (13)	0.14
ABO incompatibility, n (%)	9 (47)	8 (9)	0.0001
Serum albumin concentration 2.9 g/dL, n (%)	9 (47)	29 (31)	0.16
Serum bilirubin concentration 12 mg/dL, n (%)	8 (42)	42 (45)	0.84
Pretransplantation ICU care, n (%)	3 (16)	14 (15)	0.53
Ascites, n/n (%)	12/19 (63)	61/90 (68)	0.70
Child-Pugh score class C	14/19 (74)	70/87 (80)	0.51
Operative and post-transplant variables			
Total operation duration (min SD), min	788 181	656 145	0.0008
Intraoperative packed RBC transfusion (min SD), mL/kg	106 133	59 96	0.075
Biliary reconstruction, Roux-en-Y %: end-to-end %	58:42	56:44	0.88
Repeat intra-abdominal or intrathoracic surgery, n (%)	12 (63)	13 (14)	0.0001
NNIS risk index			
0, n (%)	2 (11)	12 (13)	0.011
1, n (%)	6 (32)	62 (66)	
2, n (%)	10 (53)	19 (20)	
3, n (%)	1 (5)	1 (1)	

[a] SSI, surgical site infection; NNIS, National Nosocomial Infection Surveillance.

TABLE 5. Multivariate analysis of risk factors for SSIs with secondary bacteremia in 113 living-donor liver transplants[a]

Variables	*P* value	Odds ratio	95% CI
Adult	0.37	3.03	0.27–33.9
Gender, male	0.20	2.77	0.59–13.0
ABO incompatibility	0.0025	14.0	2.5–77.2
Total operation duration, min	0.33	1.00	0.997–1.01
Intraoperative packed RBC transfusion, mL/kg	0.93	1.00	0.99–1.01
Repeat intra-abdominal or intrathoracic surgery	0.0044	9.29	2.00–43.1
NNIS risk index (3 or 4)	0.32	2.42	0.43–13.6

[a] SSI, surgical site infection; NNIS, National Nosocomial Infection Surveillance; CI, confidence interval; RBC, red blood cell.

for SSI with secondary bacteremia and carries a high mortality rate. The indications may need to be more restrictive when the donor candidate is ABO-incompatible.

REFERENCES

1. De Villa VH, Lo CM, Chen CL. Ethics and rationale of living-donor liver transplantation in Asia. *Transplantation* 2003; 75(suppl): 2.
2. Chen CL, Fan ST, Lee SG, et al. Living-donor liver transplantation: 12 years of experience in Asia. *Transplantation* 2003; 75(suppl): 6.
3. Winston DJ, Emmanouilides C, Busuttil RW. Infections in liver transplant recipients. *Clin Infect Dis* 1995; 21: 1077.
4. Singh N. Infectious diseases in the liver transplant recipient. *Semin Gastrointest Dis* 1998; 9: 136.
5. Singh N, Gayowski T, Wagener M, et al. Infectious complications in liver transplant recipients on tacrolimus. Prospective analysis of 88 consecutive liver transplants. *Transplantation* 1994; 58: 774.
6. Kusne S, Dummer JS, Singh N, et al. Infections after liver transplantation. An analysis of 101 consecutive cases. *Medicine* 1988; 67: 132.
7. Colonna JO, 2nd, Winston DJ, Brill JE, et al. Infectious complications in liver transplantation. *Arch Surg* 1988; 123: 360.
8. Paya CV, Hermans PE, Washington JA 2nd, et al. Incidence, distribution, and outcome of episodes of infection in 100 orthotopic liver transplantations. *Mayo Clin Proc* 1989; 64: 555.
9. Wade JJ, Rolando N, Hayllar K, et al. Bacterial and fungal infections after liver transplantation: An analysis of 284 patients. *Hepatology* 1995; 21: 1328.
10. Inomata Y, Uemoto S, Asanuma K, et al. Right robe graft in living donor liver transplantation. *Transplantation* 2000; 69: 258.
11. Egawa H, Inomata Y, Uemoto S, et al. Biliary anastomotic complications in 400 living related liver transplantations. *World J Surg* 2001; 25: 1300.
12. George DL, Arnow PM, Fox AS, et al. Bacterial infection as a complication of liver transplantation: Epidemiology and risk factors. *Rev Infect Dis* 1991; 13: 387.
13. Lumbreras C, Lizasoain M, Moreno E, et al. Major bacterial infections following liver transplantation: A prospective study. *Hepatogastroenterology* 1992; 39: 362.
14. Barkholt L, Ericzon BG, Tollemar J, et al. Infections in human liver recipients: Different patterns early and late after transplantation. *Transpl Int* 1993; 6: 77.
15. Singh N, Gayowski T, Wagener MM, et al. Predictors and outcome of early- versus late-onset major bacterial infections in liver transplant recipients receiving tacrolimus (FK506) as primary immunosuppression. *Eur J Clin Microbiol Infect Dis* 1997; 16: 821.
16. Garcia S, Roque J, Ruza F, et al. Infection and associated risk factors in the immediate postoperative period of pediatric liver transplantation: A study of 176 transplants. *Clin Transplant* 1998; 12: 190.
17. Hollenbeak CS, Alfrey EJ, Souba WW. The effect of surgical site infections on outcomes and resource utilization after liver transplantation. *Surgery* 2001; 130: 388.
18. Singh N, Gayowski T, Wagener MM, et al. Bloodstream infections in liver transplant recipients receiving tacrolimus. *Clin Transplant* 1997; 11: 275.
19. Singh N, Paterson DL, Chang FY, et al. Methicillin-resistant *Staphylococcus aureus*: The other emerging resistant gram-positive coccus among liver transplant recipients. *Clin Infect Dis* 2000; 30: 322.
20. American Society of Health-System Pharmacits. ASHP therapeutic guideline on antimicrobial prophylaxis in surgery. *Am J Health-Syst Pharm* 1999; 56: 1839.
21. Patel R, Paya CV. Infections in solid-organ transplant recipients. *Clin Microbiol Rev* 1997; 10: 86.
22. Fishman JA, Rubin RH. Infection in organ-transplant recipients. *N Engl J Med* 1998; 338: 1741.
23. Paya CV. Prevention of fungal and hepatitis virus infections in liver transplantation. *Clin Infect Dis* 2001; 33(suppl 1): 47.
24. Lumbreras C, Cuervas-Mons V, Jara P, et al. Randomized trial of fluconazole versus nystatin for the prophylaxis of *Candida* infection following liver transplantation. *J Infect Dis* 1996; 174: 583.
25. Winston DJ, Pakrasi A, Busuttil RW. Prophylactic fluconazole in liver transplant recipients. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1999; 131: 729.
26. Wagener MM, Yu VL. Bacteremia in transplant recipients: A prospective study of demographics, etiologic agents, risk factors, and outcomes. *Am J Infect Control* 1992; 20: 239.
27. Singh N, Paterson DL, Gayowski T, et al. Predicting bacteremia and bacteremic mortality in liver transplant recipients. *Liver Transpl* 2000; 6: 54.
28. Torre-Cisneros J, Herrero C, Canas E, et al. High mortality related with *Staphylococcus aureus* bacteremia after liver transplantation. *Eur J Clin Microbiol Infect Dis* 2002; 21: 385.
29. Mermel LA. Prevention of intravascular catheter-related infections. *Ann Intern Med* 2000; 132: 391.

ELSEVIER

Critical Progressive Small-Graft Injury Caused by Intrasinusoidal Pressure Elevation Following Living Donor Liver Transplantation

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ABSTRACT

In adult-to-adult living liver transplantation, small-for-size graft syndrome sometimes occurs. The relationship between the hemodynamic changes and histologic findings has not been studied in patients with failure of small-for-size grafts. We analyzed the relationship between the postoperative hemodynamic changes and pathologic findings in patients with small-for-size grafts that ended in graft failure. From March 1999 to December 2002, adult-to-adult living-donor liver transplantation with small-size grafts (graft volume/standard liver volume less than 40%) was performed in eight patients. Three patients died from graft failure caused by overperfusion, which was diagnosed from pathologic findings. We analyzed the relation between hepatic hemodynamic parameters, such as portal venous blood velocity or splenic arterial pulsatility index, and histologic changes in patients with graft failure. Severe portal hyperperfusion (90 cm/sec at the umbilical portion) was observed on postoperative day 1. Among patients with graft failure, critical hemodynamic changes, such as sudden onset of extremely deteriorated portal venous blood flow, occurred during the early postoperative period (postoperative day 5, 3, 6, respectively). Histologic examination revealed vacuolar changes in the cytoplasm of hepatocytes, and submassive necrosis indicated intrasinusoidal pressure elevation. These changes were not observed in the biopsy obtained soon after reperfusion. In conclusion, critically decreased vascular beds may cause intrasinusoidal pressure elevation and sinusoidal circulatory disturbances.

LIVING-DONOR liver transplantation (LDLT) or split-liver transplantation for adult recipients are now accepted procedures because of the shortage of transplantable livers. However, they may sometimes yield small-for-size grafts, which are known to induce postoperative hyperbilirubinemia and liver injury in recipients, resulting in liver-regeneration failure.[1–3]

In a series of LDLTs, Kiuchi et al[4] demonstrated that transplantation of a liver mass corresponding to less than 1% of the recipient's body weight (graft-to-weight ratio) leads to increased parenchymal cell injury and poor recipient outcomes. The mechanisms for this failure, however, remain unclear, although the existence of postoperative portal hypertension in recipients may promote the liver injury.

Although postoperative hemodynamic changes in LDLT are important, only a few reports have been published on quantitative hemodynamic analysis in a clinical setting.[5,6] Further, although the clinical signs in small-for-size graft, including prolonged hyperbilirubinemia, massive ascites, and insufficiency of protein synthesis sometimes occur, most patients can recover from this syndrome.[3] The difference between survivors and those with graft failure is unknown. There are few reports of hemodynamic analyses of patients with graft failure.

A detailed elucidation of the portal hemodynamic changes and the mechanism of injury in small-for-size grafts may provide helpful information for transplant surgeons to develop new strategies to minimize the injury and improve the prognosis of liver transplantation using small-for-size grafts.

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To elucidate the possible mechanism of small-for-size graft injury, in the present report we describe the time course of the hemodynamics of the portal vein and the hepatic artery in patients with small-for-size grafts, and analyze the relationship between the hemodynamic changes and pathologic findings.

PATIENTS AND METHODS

Patients

From March 1999 to December 2002, adult-to-adult LDLT was performed in 12 patients with the approval of the Ethics Committee. Among them four cases were excluded: one patient received an auxiliary transplantation; two, a right lobe graft; and one patient's hemodynamic parameters could not be measured by Doppler ultrasonography due to poor insonation. All eight patients included in this study underwent a left lobe (with middle hepatic vein) LDLT, with a small-for-size graft of volumes less than 40% of the standard liver volume. Data on the recipients are shown in Table 1. No donors had severe microvesicular steatosis.

Surgical Technique

Operations on donors and recipients were performed as described elsewhere.[4] Briefly, hepatic graft resection in donors was performed without any blood inflow occlusion at the hepatic hilum. After parenchymal transection, hepatic grafts were flushed in situ via the portal vein and preserved in a cold University of Wisconsin solution.

In recipient operations, the native liver was resected preserving the inferior vena cava, and the graft was flushed via the portal vein with 4.4% cold albumin solution (PPF; Baxter, Glendale, Calif, USA) during the hepatic venous anastomosis. After the reinstitution of portal venous inflow, the hepatic artery was anastomosed under a surgical microscope. The biliary tract was reconstructed by hepaticojejunostomy with a Roux-en-Y jejunal limb. If the graft was too small, the remnant falciform ligament of the graft was fixed to the diaphragm to prevent graft dislocation.

Doppler Measurements

Postoperative Doppler ultrasonography was performed daily for the first 14 days after LDLT. All Doppler studies were performed with color and pulsed Doppler units using a 2- to 5-MHz convex probe (HDI5000; Philips, Bothell, Wash, USA). We measured the portal venous peak velocity (PVPV; cm/sec) and the hepatic arterial peak systolic velocity (HAPSV; cm/sec) in the umbilical portion. The hepatic venous blood flow was analyzed by the waveform. The splenic arterial pulsatility index (SAPI) was based on flow in the splenic artery at the splenic hilum, with identification of the branches of the splenic artery by color Doppler ultrasonography. The axial size of the sample volume was kept in the 2- to 3-mm range. The angle between the Doppler beam and the long axis of the vessel was kept at less than 60 degrees. PVPV, HAPSV, and SAPI were automatically determined for samples of the Doppler signal. The pulsatility index was automatically calculated as (peak systolic velocity peak end diastolic velocity)/mean velocity.

The so-called impedance indices evaluate the waveform of arterial blood flow velocity and describe the relationship between diastolic and systolic blood flow velocity in arterial vessels. The usefulness of these measures is based on the supposition that, although systolic velocity is mainly determined by cardiac systole, diastolic blood velocity is affected by outflow resistance. Therefore, these parameters are considered indicative of outflow resistance from the site evaluated. A deceleration of diastolic velocity results in an increase in these indices. Bolognesi et al[7–9] found that SAPI increased in patients with cirrhosis and portal hypertension. SAPI not only reflects resistance in the arterial and capillary bed of the spleen, but also likely reflects the sum of downstream resistance, including the splenic arterial and capillary bed as well as the splenic and portal venous and hepatic vascular resistance. These investigators reported that the rapid and steady improvement in splenic pulsatility index after liver transplantation underscored the resolution of splenic congestion after liver transplantation.[9]

A change in hepatic venous waveform from triphasic to monophasic due to stiffening of the liver parenchyma has been documented in patients with cirrhosis.[10] In liver transplantation, changes of hepatic venous flow pattern from triphasic to monophasic occur as the result of a decreased liver compliance because of lymphocytic infiltration during rejection.[11,12]

We have reported that simultaneous quantitative Doppler measurement of portal venous blood velocity, hepatic arterial blood velocity, and the splenic arterial pulsatility index are useful to assess hepatic circulation.[13,14] Moreover, we also have reported the usefulness of these measurements to detect post-

Table 1. Recipients' Backgrounds

	All (*n* 8)	Patients With Graft Failure (*n* 3)	Patients Without Graft Failure (*n* 5)	*P* Value
Diagnosis				
Fulminant hepatic failure	3	0	3	
PBC	3	2	1	
PSC	1	0	1	
Cirrhosis (HBV)	1	1	0	
Cirrhosis/noncirrhosis	4/4	3/0	1/4	
Age (ys)	39 10	50 1	34 8	.03
Donor age (ys)	39 10	46 7	35 10	.23
Graft weight (g)	336 27	351 3	336 27	.65
GW/SLV (%)	31.6 4.6	33.6 2.4	32.1 4.5	.65
Cold ischemic time (min)	56.5 26.6	60.7 5.1	54.0 34.8	.88
Warm ischemic time (min)	49.8 16.0	47.0 10.6	51.4 19.6	.76

Abbreviations: PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; HBV, hepatitis B virus; GW, graft weight; SLV, standard liver volume; *P* value, Mann-Whitney *U* test.

operative complications in LDLT.[15,16] PVPV and HAPSV reflect hepatic blood flow, SAPI reflects portal pressure, and HV waveform shows liver compliance. Thus, hepatic perfusion and graft condition can be estimated from simultaneous Doppler measurements.

RESULTS

There were four cases of hospital mortality among recipients in this study. Three of these patients died from graft failure caused by overperfusion, which was diagnosed based on pathologic findings. Another patient died from gastrointestinal bleeding. The recipients were divided into two groups, those with (group A) and without (group B) graft failure caused by overperfusion.

Figure 1 shows the postoperative hepatic hemodynamic changes between group A and B. In group A, the PVPV decreased dramatically in the early postoperative period. The PVPV on postoperative day 1 was especially higher, at over 90 cm/sec. One patient in group B whose PVPV also exceeded 90 cm/sec died in the hospital. Moreover, in group A, HAPSV reciprocally increased. Conversely, in group B, portal and HAPSV were more stable than in group A in the early postoperative period.

Figure 2 shows the hemodynamic and laboratory findings for each patient in group A. In group A, deteriorated Doppler parameters, such as sudden onset of extremely deteriorated PVPV, reciprocal high HAPSV, high SAPI indicating portal hypertension, and continuous HV waveform indicating impaired graft compliance, were seen before the change in abnormal laboratory findings. We defined the critical hepatic circulatory disturbance point as when these hemodynamic changes were seen simultaneously. The liver parenchymal enzymes rapidly increased after the critical hepatic circulatory disturbance. The critical

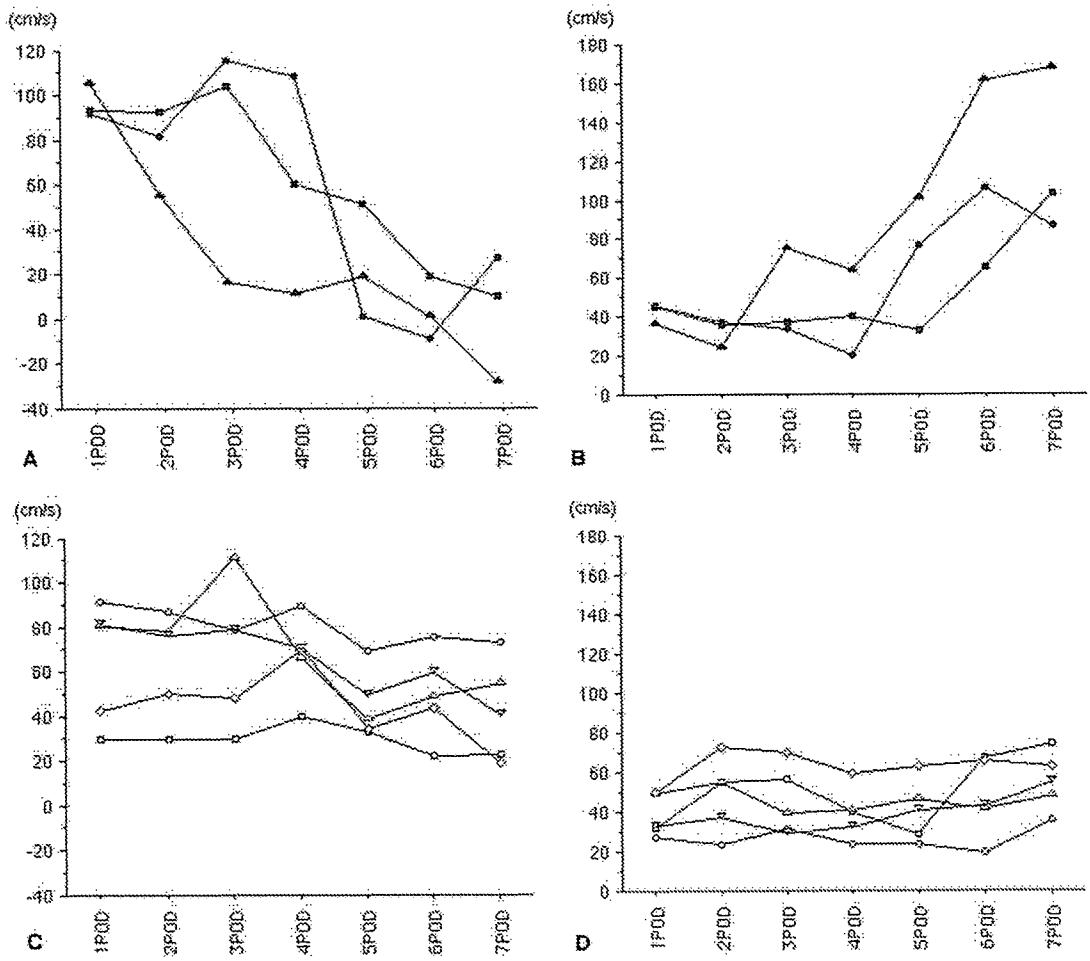

Fig 1a–d. Postoperative hepatic hemodynamic changes between patients with and without graft failure caused by overperfusion. In the graft failure group, portal blood velocity decreased dramatically in the early postoperative period. The hepatic arterial blood velocity reciprocally increased. Conversely, stable hepatic hemodynamics were seen in the non-graft failure group. (Symbols indicate each patient.) **a.** PVPV in patients with graft failure. **b.** HAPSV in patients with graft failure. **c.** PVPV in patients without graft failure. **d.** HAPSV in patients without graft failure.

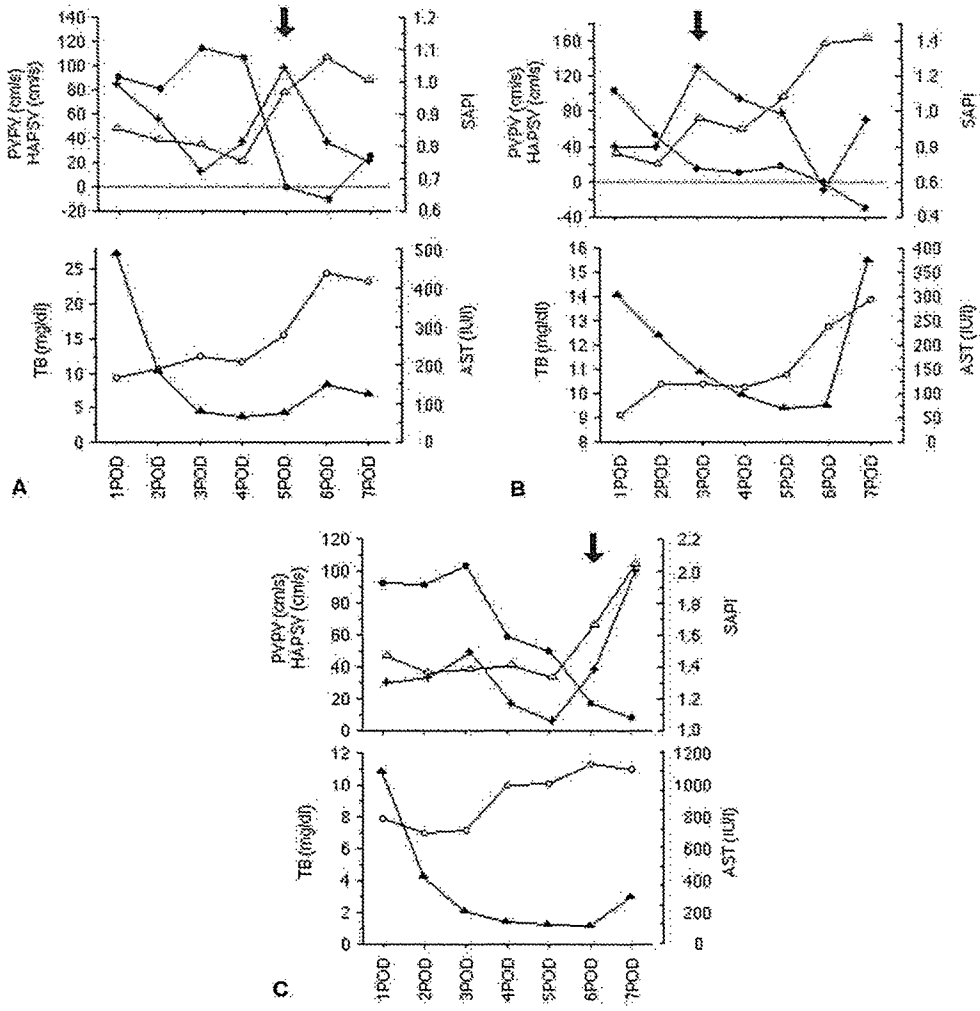

Fig 2a–c. The relation between hemodynamic changes and laboratory findings in each patient with graft failure. **a.** Patient 1. **b.** Patient 2. **c.** Patient 3. Closed circle, portal venous peak velocity (PVPV; cm/sec); open triangle, hepatic arterial peak systolic velocity (HAPSV; cm/sec); cross, splenic arterial pulsatility index (SAPI); open circle, serum total bilirubin (TB; mg/dL); closed triangle, aspartate aminotransferase (AST; IU/L); arrow, the day when the critical hepatic hemodynamic disturbance occurred (see text).

hepatic circulatory disturbance occurred in the early postoperative period (postoperative day 5, 3, 6, respectively). Although sequential hepatofugal portal flow was seen in all patients in group A, patient 1 regained a hepatopetal flow on day 7. However, hepatic failure was prolonged and patient 1 died on postoperative day 125. In patients 2 and 3, hepatofugal portal flow did not improve, and total liver necrosis occurred 4 days after hepatofugal portal flow was seen.

Biopsies were performed to diagnose the liver injury on days 6, 8, 8 in patients 1, 2, 3, respectively. In patients 1 and 2, the biopsies were performed because hepatofugal portal flow was seen and liver parenchymal enzymes were elevated. In patient 3, the biopsy was performed because critical hemodynamic disturbances were seen by Doppler ultrasound. Portal pressure was simultaneously measured in this patient. The portal pressure was extremely high (40 cm H_2O). A 16-gauge catheter was placed into the portal vein to inject prostaglandin E_1 continuously. However, 1 day after biopsy, the portal flow was found to be hepatofugal.

Figure 3 shows the pathologic findings. There were nearly normal findings in biopsies 3 hours after reperfusion. In patient 1, relaparotomy was performed because of intra-abdominal bleeding on postoperative day 1. The biopsy on day 1 also revealed normal findings, except for mild cholestasis. However, when a critical hepatic circulatory disturbance occurred, there were vacuolar changes and cholestasis in the cytoplasm of hepatocytes and submassive necrosis in some parts of the biopsy specimen. There were no signs of acute rejection in the portal areas. The same findings were observed in patient 3 when the critical hepatic circulatory disturbance occurred. However, in patient 2, significant congestive necrosis was seen because the biopsy was performed too late after the critical hepatic hemodynamic change.