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Intestinal MDR1/ABCB1 level at surgery as a risk factor of acute cellular rejection in living-donor liver transplant patients

Background: Although the prevention of immunologic reactions with sufficient immunosuppression prolongs graft and patient survival rates, the large interindividual variation in tacrolimus pharmacokinetics interferes with treatment. In this study we have examined whether intestinal MDR1 (ABCB1) is a potential biomarker predicting the occurrence of acute cellular rejection, as well as a factor to predict absorption of tacrolimus, after living-donor liver transplantation.

Methods: By use of tissue specimens of intestinal mucosa (n 164) obtained at surgery, the messenger ribonucleic acid (mRNA) expression of intestinal MDR1 and cytochrome P450 (CYP) 3A4 was quantified.

Results: The probability of acute cellular rejection during the first 10 days after surgery was significantly associated with the average trough concentration of tacrolimus between postoperative days 2 and 4 (45.1% for <7 ng/mL versus 22.9% for >7 ng/mL,*P* .0040). High levels of MDR1 were associated with an episode of acute cellular rejection before postoperative day 10 (odds ratio, 2.306 [95% confidence interval, 1.058-5.028]) and with a poor survival rate during the first postoperative year (odds ratio, 7.413 [95% confidence interval, 1.567-36.073]). The mRNA expression level of MDR1 was inversely correlated with the tacrolimus concentration–oral dose ratio during the initial 4 days after surgery in patients with a graft-to-recipient weight ratio greater than 1.5 (*r* 0.6798, *P*< .0001) and those with a graft-to-recipient weight ratio of less than 1.5 (*r* 0.7180, *P*< .0001).

Conclusion: The enterocyte MDR1 mRNA level was suggested to be a risk factor for acute cellular rejection and death after surgery. Therefore obtaining a sufficient tacrolimus blood level via this molecular information–based initial dosage adjustment may enable the episode of acute cellular rejection after liver transplantation to be reduced. (Clin Pharmacol Ther 2006;79:90–102.)

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Living-donor liver transplantation and subsequent immunosuppressive therapy are well acknowledged to provide excellent results and are usually used in coordination with a cadaveric organ transplant program.[1,2] In countries where cadaveric donors are limited, living-donor liver transplantation is often the only treatment option for patients with end-stage liver disease.[3] Because loss of the graft liver will lead to death, postoperative immunosuppressive therapy is essential to protect the grafted liver from immunologic reactions. As acute cellular rejection occurs mostly within 6 weeks of a transplant,[4] high-dose steroid pulse therapy or anti-CD3 monoclonal antibody treatment is required to save the graft liver.[5,6] Subclinical rejection, where cytologic or histologic signs of rejection exist in the absence of clinical dysfunction of the graft, is particularly frequent (incidence of around 59%) between days 5 and 14 after

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liver transplantation.[6] Twenty-five percent of patients with subclinical rejection are treated with high-dose steroid injections. Therefore early exposure to immunosuppressants could reduce the frequency of acute cellular rejection including subclinical rejection. However, these antirejection treatments lead to overimmunosuppression and an infectious state, which are closely associated with death.[7] Although there is a need to protect patients from opportunistic infections including enterobacterium, Epstein-Barr virus, cytomegalovirus, and herpes simplex virus after antirejection treatment, some anti-infectious treatments with antibiotics, antifungal agents, and antiviral drugs are accompanied by drug-induced hepatic and renal dysfunction.[8,9] In addition, high-dose steroid injections should be avoided in patients carrying the hepatitis B or C virus, because steroidal drugs allow the amplification of these viruses in the graft liver and accelerate the recurrence of virus-related hepatitis and cirrhosis.[10,11] Therefore acute cellular rejection should be avoided to prevent further complications, especially immediately after transplantation.

The calcineurin inhibitor tacrolimus (FK-506) has been used as a primary immunosuppressive agent in orthotopic liver transplantation.[12] Therapeutic drug monitoring has facilitated maintaining the blood concentration of tacrolimus within a narrow therapeutic range (between 10 and 20 ng/mL) to prevent side effects such as nephrotoxicity, neurotoxicity, and life-threatening infection.[13,14] However, the bioavailability of orally administered tacrolimus is variable, ranging from 4% to 89% (with a mean value of about 25%),[15,16] and a dosage regimen for the drug immediately after transplantation has yet to be established. This can be attributed to several factors, including poor absorption or extensive first-pass metabolism in the intestine and liver. Therefore a rational dosage regimen for tacrolimus should be determined as early as possible, focusing on its pharmacokinetic interindividual variability.

Tacrolimus is principally metabolized by cytochrome P450 (CYP) 3A subfamilies in the liver. The contribution of active secretion by P-glycoprotein (the product of the *MDR1/ABCB1* gene) and the metabolism by CYP3A expressed in enterocytes are acknowledged as factors influencing the bioavailability of tacrolimus.[17] We reported that the intraindividual variation in the concentration/dose (C/D) ratio of tacrolimus was closely related to the variation in the enterocyte messenger ribonucleic acid (mRNA) expression level of MDR1, but not CYP3A4, in recipients of living-donor small-bowel transplantation.[18,19] Similar results were obtained in patients after living-donor liver transplantation during the initial 7 days after surgery.[20,21] Because of the small number of cases in our past reports, we could not analyze the relationship between the intestinal mRNA expression level of MDR1 and endpoints such as acute cellular rejection. It is necessary to clarify the clinical significance of the intestinal expression level of MDR1 in patients after living-donor liver transplantation to establish the clinical usefulness of adjusting the initial dosage of tacrolimus.

In this study we examined whether the intestinal expression level of MDR1 mRNA could be a molecular marker for acute cellular rejection episodes in patients after living-donor liver transplantation with more enrolled patients, as well as the potential contribution of the molecular information to initial dose setting.

METHODS

Patients and mucosal specimens. The study included 164 patients, having first provided written informed consent, who were enrolled consecutively between November 1998 and December 2004 in whom tissue specimens had been obtained at surgery. The donor was a parent in 119 cases, a spouse in 14, a sibling in 12, an offspring in 12, a grandmother in 3, an uncle in 2, an aunt in 1, and a father-in-law in 1. The demographics of the recipients are listed in Table I. The clinical samples of the upper jejunum were obtained from a part of the Roux-en-Y limb for biliary reconstruction or from a part of the mucosal specimen around the bile drainage tube at living-donor liver transplantation.[22] This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Ethics Committee of Kyoto University, Kyoto, Japan; each adult patient and each parent of small children provided written informed consent.

Dosage regimen of tacrolimus, analysis of blood samples, and criteria for acute cellular rejection. The basic immunosuppression regimen consisted of tacrolimus with low-dose steroids.[23] To cover the immediate postoperative period (the day of living-donor liver transplantation, day 0, and postoperative day 1), induction of immunosuppressive therapy was started from the day before the operation, except in cases of hepatic encephalopathy and severe infection. Tacrolimus was administered orally at a dose of 0.075 mg/kg body weight every 12 hours from the evening of day 1.[13,23] The target for the post-transplantation whole-blood trough concentration of tacrolimus was 10 to 12 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 $mg \cdot kg^{-1} \cdot d^{-1}$ to 0.3 $mg \cdot kg^{-1} \cdot d^{-1}$ during the first 2 weeks after surgery.

Table I. Demographic characteristics of recipients (N 164)

Age (y)	0.3-67 (median, 3.2)
Adults (15 y) (n 54)	15.0-67 (median, 46)
Children (15 years) (n 110)	0.3-13.7 (median, 1.2)
Body weight (kg)	4.3-92.1 (median, 13.5)
Graft-to-recipient weight ratio (%)	0.63-5.6 (median, 1.84)
Gender (male/female)	70/94
Graft lobe (left/right)	113/51
ABO blood group match (identical/compatible/incompatible)	99/37/28
Preoperative condition (home-bound/hospitalized/intensive care unit–bound)	71/85/8
Primary disease*	
Biliary atresia	89 (21)
Cirrhosis	
Hepatitis B virus	9 (1)
Hepatitis C virus	12 (3)
Primary biliary cirrhosis	7 (2)
Unknown	3 (0)
After liver transplantation	11 (3)
Primary sclerosing cholangitis	8 (4)
Fulminant hepatic failure	4 (2)
Other†	21 (6)

*The number of patients with acute cellular rejection episodes during the initial 10 days after surgery is denoted in parentheses.
†The primary disease was Byler disease in 4 cases (2), Alagille syndrome in 3 (0), Wilson disease in 2 (2), hepatoblastoma in 3 (0), polycystic liver disease in 2 (1), biliary dilation in 2 (0), multiple hepatocellular carcinoma in 1 (0), citrullinemia in 1 (1), hypertyrosinemia in 1 (0), Budd-Chiari syndrome in 1 (0), and portal vein deficiency in 1 (0). The number of patients with acute cellular rejection episodes during the initial 10 days after surgery is denoted in parentheses.

The dosage of tacrolimus was adjusted on the basis of whole-blood trough concentrations measured about 12 hours after the evening dosage every day, by use of a semiautomated microparticle enzyme immunoassay (IMX; Dainabot, Tokyo, Japan).[24]

Acute cellular rejection was principally diagnosed with liver biopsy specimens, and the histologic diagnosis was performed according to criteria based on the Banff schema.[25] All episodes of rejection were treated with a high-dose steroid bolus injection.

Evaluation of intestinal expression levels of MDR1 and CYP3A4. Biopsy specimens from intestinal mucosa were homogenized in RLT buffer (Qiagen, Hilden, Germany), and total RNA was isolated with MagNA-Pure LC RNA Isolation kit II (Roche) and reverse-transcribed as described previously.[26] The isolated total RNA (500 ng/40 L reaction mixture) was reverse-transcribed by Superscript II reverse transcriptase (Invitrogen, Carlsbad, Calif) with random primers (100 ng/reaction) and digested by RNase H (Invitrogen). After dilution of the single-stranded deoxyribonucleic acid (DNA) mixture with 60 L of sterile water (final volume, 100 L), 5- L aliquots were used for a subsequent real-time polymerase chain reaction (PCR) (final volume, 20 L) with an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster, Calif). The primer/probe set used for glyceraldehyde 3-phosphate dehydrogenase, as an internal control, was predeveloped TaqMan Assay Reagents (Applied Biosystems), and the reaction was performed according to the manufacturer's instructions. The primer/probe set specific for MDR1 and CYP3A4 was as described previously.[26] Each PCR fragment of the target sequences was generated with specific primer/probe sets as described, ligated into the pCR-Script Cloning Vector (Stratagene, La Jolla, Calif), and confirmed to have the exact sequences of the cloned amplicons by the chain-termination method by use of a fluorescence 373A DNA sequencer (Applied Biosystems). After measurement of the concentrations of the purified plasmid DNA by spectrophotometry, the corresponding concentrations (in moles per microliter) were calculated and serial dilutions of respective plasmid DNA were used as standards for calibration curves. The starting mRNA concentration of MDR1 or CYP3A4 was established by determining the fractional PCR threshold cycle number at which a fluorescence signal generated during the replication process passed above a threshold value. The initial amount of target mRNA in each sample was estimated from the experimental fractional PCR threshold cycle value with a standard curve generated by use of known amounts of standard plasmid DNA.

Statistical analyses. Normally distributed values were presented as mean SD. Values that were not

normally distributed were presented as the median and range. Logarithmic transformation of the mRNA levels of MDR1 and CYP3A4 was performed to improve normality before statistical analyses were performed. The nonpaired Student *t* test was used to compare groups with respect to normally distributed variables. If different variances between 2 samples were found with the F test, an unpaired *t* test with Welch correction was performed. The Mann-Whitney *U* test was used to compare groups without normality. The calculated mRNA expression levels of MDR1 and CYP3A4 in each intestinal specimen were categorized as high or low, if the quantified value for the mRNA in question exceeded or fell below the median value for all specimens, respectively. Statistical tests were 2-tailed, and significance was defined as P .05.

The outcome measure studied was immunologic events and survival, defined as the time from living-donor liver transplantation to the first episode of acute cellular rejection during the initial 10 days after surgery and to death during the first year after surgery, respectively. The patients without complications until at least postoperative day 10 were categorized as the event-free group. The patients who were diagnosed with acute cellular rejection by liver biopsy before postoperative day 10 were categorized as the acute cellular rejection group. The probability analysis was performed according to the method of Kaplan and Meier, and the outcome was compared among the subgroups by use of a 2-tailed log-rank test for univariate comparisons. An odds ratio was calculated for the risk. Statistical analyses were performed by use of the statistical software package StatView (version 5.0; Abacus Concepts, Berkeley, Calif).

RESULTS

Patients. Table I shows the demographics and primary diseases of living-donor liver transplant recipients whose mucosal samples we studied. Of the recipients who had acute cellular rejection, 28, 11, and 3 had an ABO blood type that was identical, compatible, and incompatible with that of their donor, respectively. Moreover, 18, 21, and 3 recipients with acute cellular rejection were home-bound, hospitalized, and intensive care unit (ICU)–bound, respectively, before surgery. Of the recipients with acute cellular rejection, 29 and 13 had a graft from the left lobe and right lobe, respectively. Steroid-pulse therapy was used in 32 patients without acute cellular rejection during the first 10 days after surgery, and 11 post–liver transplant patients were treated with immunosuppressants until immediately before the second transplantation. Therefore these 43 patients were excluded from the analyses for the probability of acute cellular rejection but not from the analyses on gene expression and tacrolimus pharmacokinetics, and the analyses for acute cellular rejection were performed with the findings of the other 121 recipients, including 82 event-free patients and 39 acute cellular rejection patients. The survival analysis was performed with these 121 recipients, including 13 patients who died within 1 year after transplantation.

Acute cellular rejection and postoperative tacrolimus trough level. By comparing the daily trough concentration of tacrolimus between the event-free group (n 82) and the acute cellular rejection group (n 39), it was found that the trough concentration at postoperative days 3 (P .0075) and 4 (P .0022) was significantly lower in the acute cellular rejection group (Fig 1). These results suggest that the blood level of tacrolimus immediately after living-donor liver transplantation was associated with the occurrence of acute cellular rejection until postoperative day 10. Then, we examined the relationship between the average trough concentration of tacrolimus between postoperative days 2 and 4 and the complications of patients, because the tacrolimus was usually administered to recipients in the ICU during the first 3 days after liver transplantation. At first, we categorized the patients by the average trough concentrations of tacrolimus between postoperative days 2 and 4. Because a low dosage of tacrolimus was administered to patients at risk of infection or renal impairment from the preoperative status to avoid any further deterioration in condition, the categorization was started from 5 ng/mL, which is considered the lower limit of the initial average concentration of tacrolimus. As shown in Fig 2, the frequency of acute cellular rejection tended to be high in patients with relatively lower tacrolimus blood levels, between 5 and 7 ng/mL. The other complications frequently occurred in the patients whose average tacrolimus trough levels were below 5 ng/mL. The frequency of acute cellular rejection compared with the event-free group tended to be lower in the patients whose average tacrolimus trough levels were maintained above 7 ng/mL. Next, we examined the probability of acute cellular rejection in the recipients dividing the average trough concentration of tacrolimus at 7 ng/mL between postoperative day 2 and 4 (Fig 3). Kaplan-Meier analysis demonstrated that the average trough concentration of tacrolimus immediately after living-donor liver transplantation was significantly associated with acute cellular rejection (P .0040). The resultant odds ratio was 2.772 (95% confidence interval [CI], 1.265-6.075) for the patients whose mean trough level of tacrolimus was

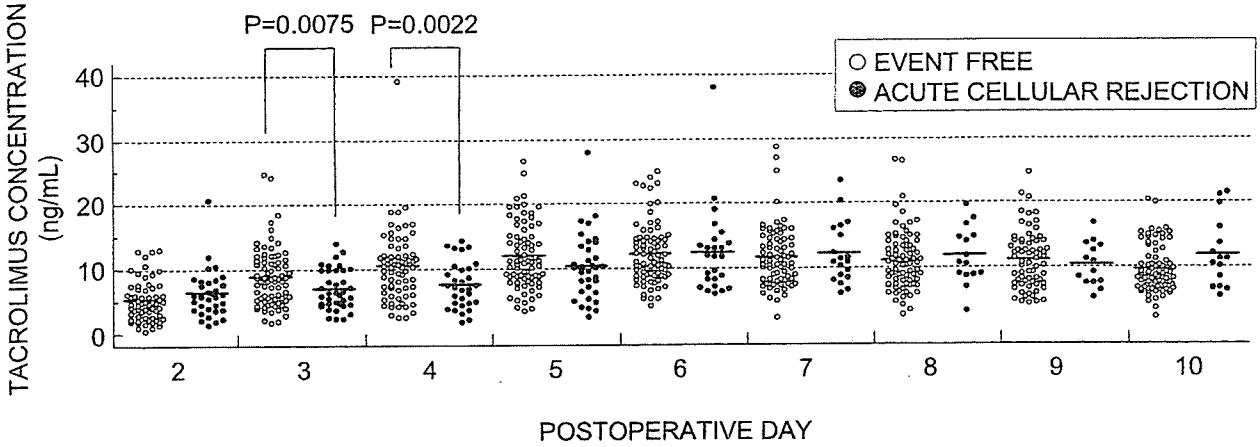

Fig 1. Daily trough levels of tacrolimus in living-donor liver transplant patients. Trough concentrations of tacrolimus in 121 patients receiving de novo living-donor liver transplants are illustrated. The patients are divided into 2 groups: event-free (*open circles*) and acute cellular rejection (*solid circles*). A statistical analysis was performed with the unpaired *t* test after Welch correction. *P* values of less than .05 are shown.

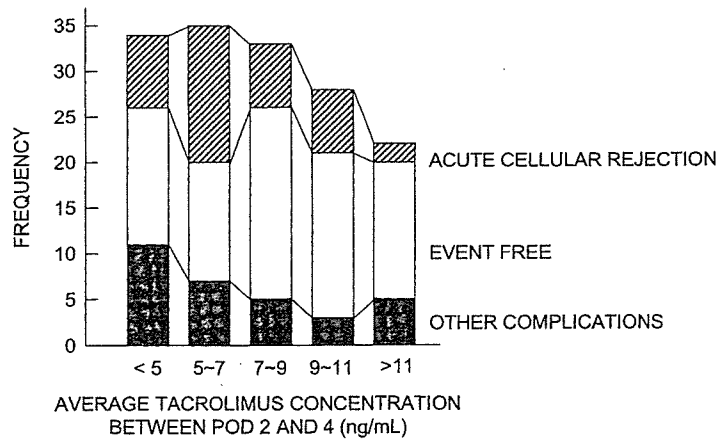

Fig 2. Frequency of complications after living-donor liver transplantation with respect to tacrolimus trough level between postoperative days (POD) 2 and 4. Frequencies of an event-free clinical course, acute cellular rejection, and the need for high-dose steroid treatment for other complications are shown as *open, hatched,* and *solid columns,* respectively. The patients were classified on the basis of the average trough concentration of tacrolimus between postoperative days 2 and 4.

below 7 ng/mL between postoperative days 2 and 4 (Table II).

Association between intestinal mRNA level of MDR1 or CYP3A4 and acute cellular rejection. We previously reported that patients with high levels of enterocyte MDR1, but not CYP3A4, required about 2-fold higher oral dosages of tacrolimus than patients with low levels of MDR1.[20] On the basis of the previous findings, we have re-examined the expression profile of the intestinal mRNA level of MDR1 and CYP3A4 to re-evaluate the influences of these factors on the risk for acute cellular rejection, as well as the interindividual variation of postoperative tacrolimus pharmacokinetics. In Fig 4, *A* and *B*, the logarithmically transformed distribution of the intestinal expression level of MDR1 and CYP3A4 at living-donor liver transplantation is shown. The median value of MDR1 and CYP3A4 was 0.242 amol/ g (range, 0.01-6.51

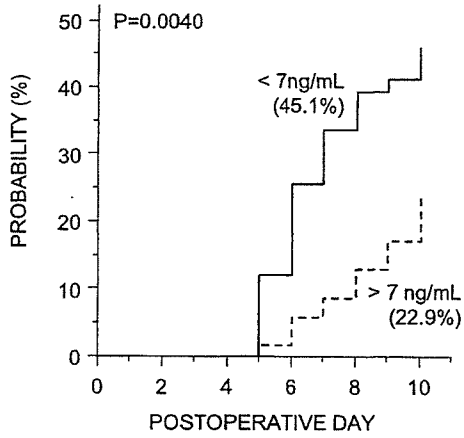

Fig 3. Probability of acute cellular rejection episodes in initial 10 days after living-donor liver transplantation. Kaplan-Meier curves show the probability of acute cellular rejection with respect to the average trough concentration of tacrolimus between postoperative days 2 and 4 (7 ng/mL or 7 ng/mL). *P* values were determined with the log-rank test.

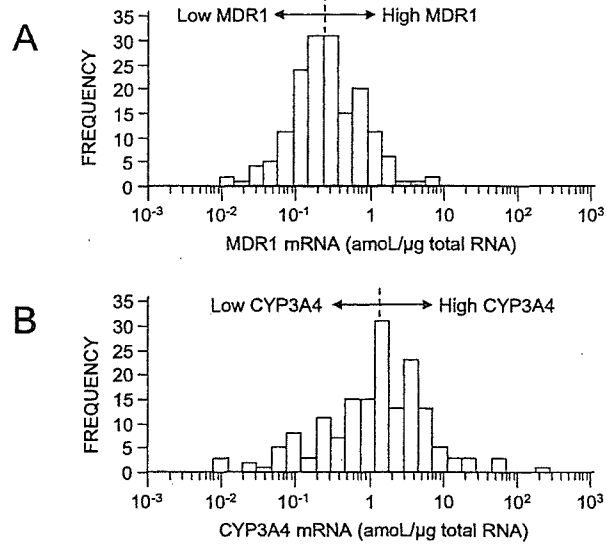

Fig 4. Histograms of messenger ribonucleic acid (mRNA) expression of intestinal MDR1 and CYP3A4 at living-donor liver transplantation. Distribution of mRNA expression levels of MDR1 (**A**) and CYP3A4 (**B**) in intestinal mucosa, both logarithmically transformed to improve normality, are illustrated as histograms for 164 recipients after living-donor liver transplantation. The *dotted lines* denote the median value. RNA, Ribonucleic acid.

amol/ g) of total RNA and 1.278 amol/ g (range, 0.002-185.5 amol/ g) of total RNA, respectively. After dividing the samples by each median value, we examined the probability of acute cellular rejection based on the expression of MDR1 or CYP3A4 (high or low). As illustrated in Fig 5, *A*, a high level of intestinal MDR1 expression was associated with the probability of acute cellular rejection (42.1% in high-MDR1 group versus 23.4% in low-MDR1 group, *P* .0265). The resultant odds ratio was 2.376 (95% CI, 1.087-5.191) for the patients with a high level of intestinal MDR1 mRNA at living-donor liver transplantation (Table II). However, there was no significant association between the intestinal CYP3A4 mRNA level and the probability of acute cellular rejection (*P* .9211) (Fig 5, *B*). The odds ratio showed that a high level of CYP3A4 mRNA at liver transplantation was not a risk factor for the occurrence of postoperative acute cellular rejection (Table II). Moreover, the mRNA expression level of mucosal MDR1 in the patients with acute cellular rejection was weakly but significantly higher compared with those in the event-free group (*P* .0476) (Fig 5, *C*).

Furthermore, the impact of mRNA expression levels of absorptive barriers on patient survival was also examined. According to the method of Kaplan-Meier and subsequent log-rank statistics, the high-level expression of both MDR1 mRNA (Fig 6, *A*) and CYP3A4 (Fig 6, *B*) was significantly associated with patient survival. The odds ratio of the intestinal expression level of MDR1 mRNA at surgery was 7.413 (95% CI, 1.567-36.073), whereas that of CYP3A4 was 3.590 (95% CI, 0.936-13.769).

Table II. Risk factors associated with acute cellular rejection until postoperative day 10

Factors	*Odds ratio*	*95% CI*
Mean trough level of tacrolimus 7 ng/mL between postoperative days 2 and 4	2.772	1.265-6.075
High level of intestinal MDR1 mRNA at surgery	2.376	1.087-5.191
High level of intestinal CYP3A4 mRNA at surgery	1.026	0.485-2.168

CI, Confidence interval; mRNA, messenger ribonucleic acid.

Dosage adjustment based on expression level of intestinal MDR1. To obtain more information about the effect of the intestinal expression level of MDR1 on the pharmacokinetics of tacrolimus, as well as the risk of acute cellular rejection, we compared the daily oral dosage and trough level of tacrolimus between the high- and low-MDR1 groups (Fig 7). The oral dosages

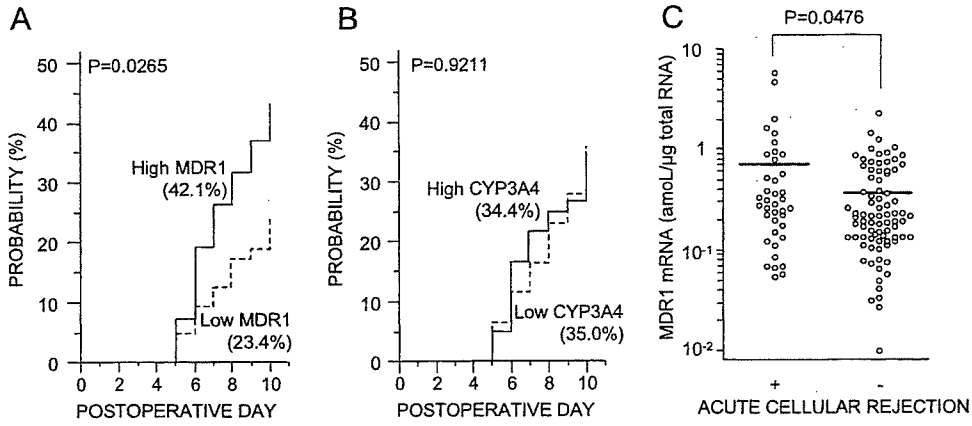

Fig 5. mRNA expression levels of MDR1 and CYP3A4 and acute cellular rejection episodes in 121 recipients of living-donor liver transplantation. The mRNA expression levels of MDR1 (**A**) and CYP3A4 (**B**) in mucosa derived from living-donor liver transplant recipients were determined by a real-time polymerase chain reaction (PCR) analysis, as described in the Methods section. High and low indicate whether the expression level of MDR1 mRNA and CYP3A4 mRNA in individual mucosa was higher or lower than the median value for all intestinal samples, respectively. *P* values were determined with the log-rank test. **C**, The mRNA expression levels of MDR1 in mucosa were shown with ( ) or without ( ) acute cellular rejection during 10 days postoperatively. The *P* value was determined with the Mann-Whitney *U* test.

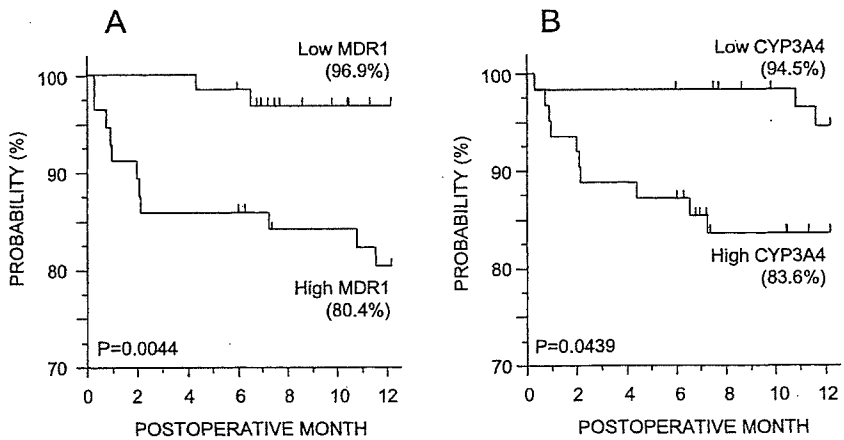

Fig 6. mRNA expression levels of MDR1 and CYP3A4 and cumulative survival rate in 121 recipients of living-donor liver transplantation. The mRNA expression levels of MDR1 (**A**) and CYP3A4 (**B**) in mucosa derived from living-donor liver transplant recipients were determined by a real-time PCR analysis, as described in the Methods section. High and low indicate whether the expression level of MDR1 mRNA and CYP3A4 mRNA in individual mucosa was higher or lower than the median value for all intestinal samples, respectively. *P* values were determined with the log-rank test. *Tick marks* indicate the length of follow-up of individual patients who survived.

of tacrolimus were significantly higher in patients categorized in the high-MDR1 group than those in the low-MDR1 group from postoperative day 3 (Fig 7, *A*). However, the daily trough levels of tacrolimus between postoperative days 2 and 10 were comparable between the 2 groups (Fig 7, *B*). The odds ratio was 2.283 (95% CI, 1.058-4.926) for patients whose average trough concentration of tacrolimus between postoperative days

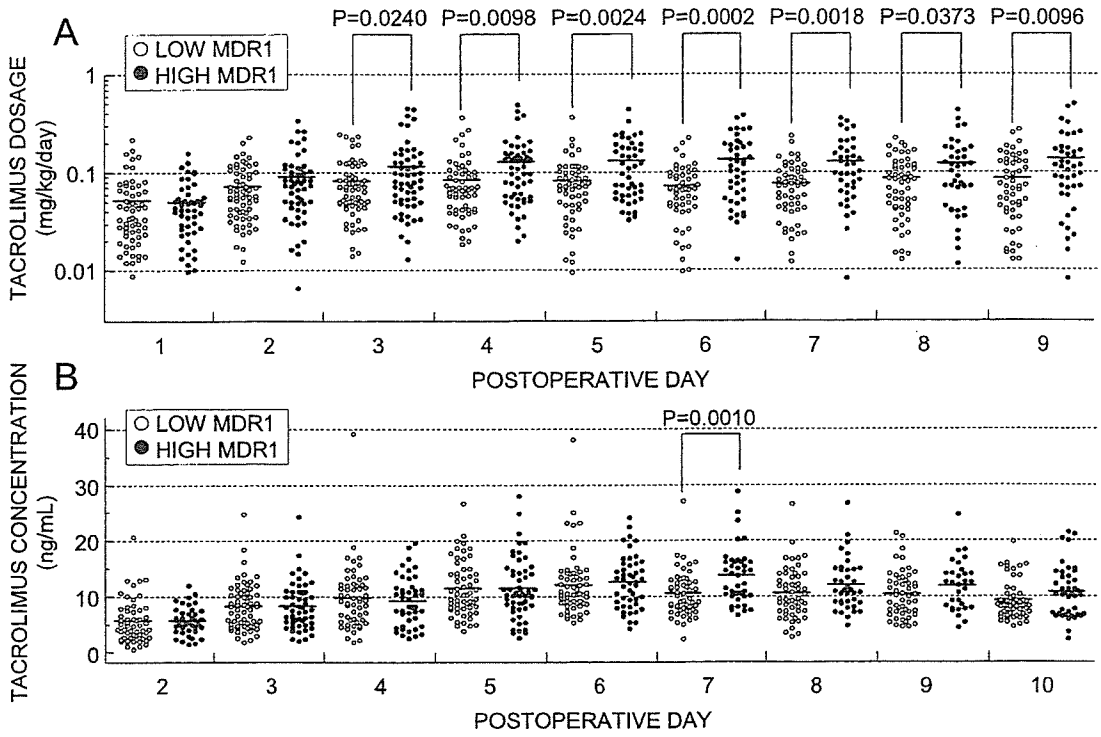

Fig 7. Postoperative oral dosage and trough concentration of tacrolimus in recipients after living-donor liver transplantation. Daily oral dosages (**A**) and trough concentrations (**B**) of tacrolimus for 121 patients receiving de novo living-donor liver transplants are illustrated. The patients are divided into 2 groups: low MDR1 (*open circles*) and high MDR1 (*solid circles*). Low MDR1 and high MDR1 indicate whether the expression level of MDR1 mRNA in individual mucosa was lower or higher than the median value for all intestinal samples. Statistical analysis was performed by use of the unpaired *t* test after Welch correction. *P* values of less than .05 were shown.

2 and 4 was under 7 ng/mL and 2.306 (95% CI, 1.058-5.028) for patients whose intestinal expression level of MDR1 at surgery was greater than 0.242 (Table II).

Correlation between mRNA level of MDR1 and tacrolimus C/D ratio. In this study the average trough concentration of tacrolimus immediately after living-donor liver transplantation and the intestinal expression level of MDR1 were identified as factors useful for predicting the risk of acute cellular rejection immediately after transplantation (Figs 3 and 5 and Table II). If the mRNA level of MDR1 at operation is a potential pharmacokinetic factor, control of the tacrolimus blood concentration will be easier, and the frequency of episodes of acute cellular rejection may be reduced. On the basis of this hypothesis, we performed a correlation analysis of the molecular data on intestinal absorptive barriers and tacrolimus pharmacokinetics to examine whether the MDR1 mRNA level at operation could be a pharmacokinetic factor for individualized initial dosage adjustment. As shown in Fig 8, the mRNA expression level of MDR1 (r 0.5672, P .0001), but not of CYP3A4 (r 0.0490, P .5466), was inversely correlated with the C/D ratio of tacrolimus between postoperative days 2 and 4. Although the mass of graft liver from the living donor was limited in the adult patients, the graft liver was relatively sufficient or large in the pediatric patients. Therefore it is also important to evaluate the engrafted liver mass as the graft-to-recipient weight ratio (Graft liver mass [in kilograms]/Recipient body weight [in kilograms] at surgery 100 [percent]).[2] Furthermore, when the patients were divided into 2 groups based on the graft-to-recipient weight ratio (1.5) (Fig 9, *A*), the coefficient of the correlation between the intestinal mRNA level of MDR1 and tacrolimus C/D ratio improved to 0.6798 (P .0001) and 0.7180 (P

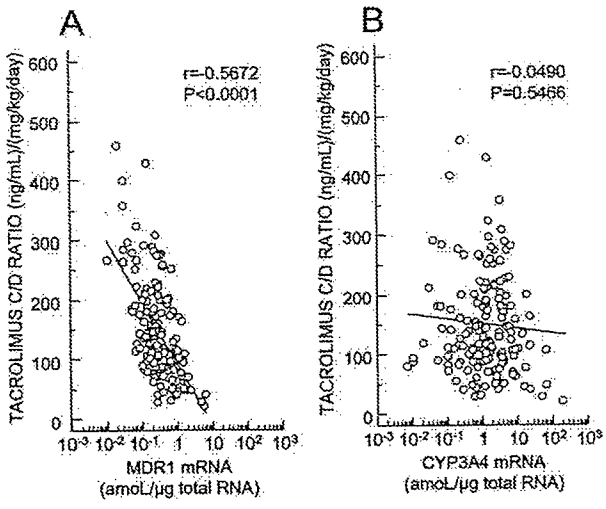

Fig 8. Concentration/dose (C/D) ratio as a function of mRNA expression levels of MDR1 and CYP3A4 in 164 recipients of living-donor liver transplantation. The average C/D ratio in the first 4 days after surgery is compared with the logarithmically transformed mRNA levels of MDR1 (**A**) and CYP3A4 (**B**).

.0001) for the patients whose graft-to-recipient weight ratio was over 1.5 and those whose ratio was under 1.5, respectively (Fig 9, *B* and *C*). However, the coefficient of the correlation between the intestinal MDR1 mRNA level at surgery and the tacrolimus C/D ratio after postoperative day 5 gradually decreased (Table III).

DISCUSSION

Acute cellular rejection, an early complication of orthotopic liver transplantation, occurs in more than 50% of patients[27-29] and can be diagnosed only by means of a liver biopsy.[25] Although it has minimal impact on either death or late graft function, acute cellular rejection should be avoided to reduce the adverse effects of antirejection treatment. The use of high-dose steroids is the first step in both the induction of immunosuppression and the treatment of acute cellular rejection despite side effects such as osteoporosis, recurrent viral hepatitis, and infections.[30-32] The direct association between acute cellular rejection and patient mortality rate is weak,[7] but this episode would be a trigger for other severe complications such as infections, drug-induced renal injury and neurotoxicity, and recurrence of hepatitis with viral amplification in patients receiving the antirejection treatment. In our study the patients categorized in the high-MDR1 group showed a higher frequency of acute cellular rejection until postoperative day 10 and poor survival within 1 year after surgery (Figs 5 and 6). Therefore the individual patients' clinical history should be explored because "acute cellular rejection" might be hidden behind the diagnosis at death. The incidence of acute cellular rejection in our series was 25.6% (42/164 cases) until postoperative day 10 and 32.2% overall (39/121 cases, excluding 32 patients treated with high-dose steroids for other reasons and 11 cases of post-transplant graft liver failure) (Table I). Of 13 patients who died within 1 year after transplantation, 11 were categorized in the high-MDR1 group. The mortality rate of high-MDR1 patients who had acute cellular rejection early on was 25% (6/24 cases), whereas all 15 patients in the low-MDR1 group were alive despite an episode of acute cellular rejection. In addition, the mortality rate of event-free patients was 15% (5/33 cases) in the high-MDR1 group and 4% (2/49 cases) in the low-MDR1 group. Focusing on the patients with acute cellular rejection during the first 10 days after surgery, the high-level expression of intestinal MDR1 was suggested to be associated with poor survival by ^{2} statistics (6/24 cases in high-MDR1 group versus 0/15 cases in low-MDR1 group, P .0352). Although our results were derived from a relatively small number of cases, the intestinal expression level of MDR1 at surgery could be a prognostic factor in patients with acute cellular rejection at an early phase. If the occurrence of acute cellular rejection can be avoided in the high-MDR1 patients, the mortality rate may be decreased to a level comparable to that in the low-MDR1 group. To reduce the frequency of acute cellular rejection early on, the average trough concentration of tacrolimus during the initial 4 days after surgery should be kept above 7 ng/mL, with an initial dosage adjustment that takes into consideration the intestinal expression level of MDR1 at surgery. In addition, extensive exposure to tacrolimus at an early phase may reduce the mortality rate of patients categorized in the high-MDR1 group.

The intestinal adenosine triphosphate–driven efflux pump MDR1 is considered to play an important role in drug pharmacokinetics.[33] This drug transporter prevents the luminal entry of orally administered drugs such as tacrolimus, cyclosporine (INN, ciclosporin), and sirolimus at apical membranes. Since the report by Hoffmeyer et al,[34] several single-nucleotide polymorphisms (SNPs) in *MDR1* affecting expression or function (or both) have been reported. Notably, C3435T and G2677T/A are detected at a relatively high frequency and have been examined for influences on the drug pharmacokinetics and expression level of the gene product.[34,35] We previously found that these SNPs did

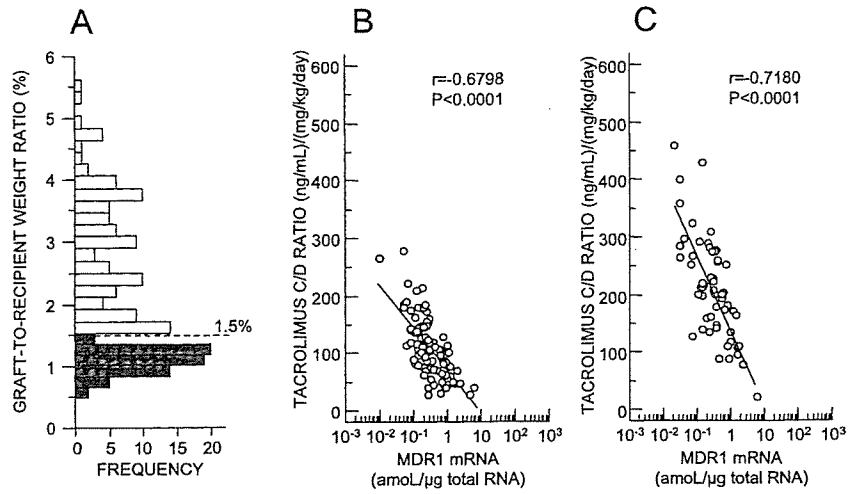

Fig 9. Histogram of graft-to-recipient weight ratio and C/D ratio as a function of mRNA expression levels of MDR1 in 164 recipients of living-donor liver transplantation. A histogram of graft-versus-recipient body weight ratio is shown (**A**). The *dotted line* denotes a graft-to-recipient weight ratio of 1.5. The average C/D ratio in the first 4 days after surgery is compared with the logarithmically transformed mRNA levels of MDR1 in the recipients of living-donor liver transplantation with a graft-to-recipient weight ratio above 1.5 (**B**) and below 1.5 (**C**).

Table III. Coefficient of correlation between intestinal mRNA expression level of MDR1 and tacrolimus concentration/dose ratio in living-donor liver transplantation patients

Postoperative days	*Graft-to-recipient weight ratio 1.5*		*Graft-to-recipient weight ratio 1.5*	
	*r**	*P value*	*r*	*P value*
Between 2 and 4	0.6798 and (n 99)	.0001	0.7180 and (n 55)	.0001
Between 5 and 7	0.3721 and (n 90)	.0003	0.4188 and (n 51)	.0020
Between 8 and 14	0.1598 and (n 98)	.1163	0.0241 and (n 52)	.8660

*Data for patients treated with high-dose steroids including the days of administration and 3 days after withdrawal were excluded.

not affect the intestinal expression of *MDR1* and the tacrolimus C/D ratio in living-donor liver transplant recipients.[21,26] In recipients of renal transplantation, the trough concentrations of cyclosporine and tacrolimus were not influenced by the C3435T SNP.[36,37] In addition, examinations 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 P-gp or transport activity of drugs such as digoxin and cyclosporine.[38,39] However, there is little consensus concerning the effect on drug pharmacokinetics by SNPs in *MDR1*.[40] Recently, a meta-analysis by Chowbay et al[41] revealed no significant effect of the *MDR1 C3435T* SNP on either the pharmacokinetics of digoxin or the intestinal expression level of P-glycoprotein. In our study preoperative complications such as cholangitis, hyperbilirubinemia, intestinal congestion, pulmonary hypertension, and renal dysfunction may have affected the expression levels of MDR1, and therefore the direct expression level rather than SNPs of the gene would provide more significant information on liver transplant recipients. On the other hand, CYP3A4 is also expressed in the upper intestinal epithelium and mediates the detoxification of these immunosuppressants at the intestinal wall. Therefore MDR1 and CYP3A4 are considered to provide an "absorptive barrier." We previously demonstrated that the intestinal expression level of MDR1, but not of CYP3A4, was inversely correlated with the tacrolimus C/D ratio in small bowel transplant recipients, as well as patients after living-donor liver transplantation.[18-21] In addition, enhanced expression of MDR1 was associated with a reduction in the bioavailability of cyclosporine in an adult case of living-

donor liver transplantation.[42] In our study we have refined the correlation between the intestinal expression level of MDR1 and tacrolimus C/D ratio during the first 4 days after surgery by taking into consideration the graft-to-recipient weight ratio (Figs 8 and 9). The trough concentration of tacrolimus at postoperative days 3 and 4 was significantly lower in the patients categorized in the acute cellular rejection group than in those in the event-free group (Fig 1). In addition, keeping the initial trough concentration of tacrolimus above 7 ng/mL was suggested to reduce the risk of acute cellular rejection (Fig 3). Therefore the initial adjustment of dosage based on the intestinal MDR1 mRNA level may provide sufficient immunosuppression mediated by tacrolimus with a rapid increase in the blood concentration to around the target range (7 ng/mL) and help to reduce the frequency of acute cellular rejection.

Some risk factors for acute cellular rejection after liver transplantation such as primary disease, Child's classification, and polymorphisms of several cytokines have been postulated.[43,44] These were considered to be congenital factors for patients receiving living-donor liver transplantation, and there is no individualized treatment to reduce the occurrence of acute cellular rejection in patients categorized in the high-risk group. In this study we have found that both the postoperative blood concentration of tacrolimus and the intestinal mRNA level of MDR1 at surgery are significant risk factors for acute cellular rejection early on (Figs 3 and 5 and Table II). However, these risk factors are relatively acquired issues and can be overcome by maintaining the trough concentration of tacrolimus above 7 ng/mL for at least the first 4 days after surgery. In addition, the intestinal expression level of MDR1 at surgery would be a simple pharmacokinetic marker with which to adjust the initial dosage of tacrolimus after living-donor liver transplantation. Therefore quantification of mucosal MDR1 expression may provide for individualization of the dosage regimen of tacrolimus, especially the initial dosage. In this study most of the tacrolimus concentrations were below 10 ng/mL, especially in the acute cellular rejection group (Figs 1 and 7, *B*). To avoid adverse reactions, doctors might be reluctant to raise the dose of tacrolimus in patients with acute cellular rejection. If we can obtain jejunal biopsy specimens for the quantification of mucosal MDR1, the postoperative immunosuppressant dosage regimen could be established before liver transplantation, enabling the tacrolimus trough level to be reached earlier in patients with or without a high level of intestinal MDR1. Therefore a pretherapeutic determination of the intestinal MDR1 mRNA level was suggested to be useful to predict the initial dosage of tacrolimus required in individual patients and thus reduce the frequency of acute cellular rejection immediately after liver transplantation.

In this study we have confirmed that a high expression level of intestinal MDR1 is a prognostic factor for recipients of living-donor liver transplantation (Fig 6, *A*). Although that of CYP3A4 was also associated with poor survival, the odds ratio was not statistically significant. Therefore it was suggested that the intestinal expression level of CYP3A4 was a prognostic factor resulting from some secondary or unknown mechanism. The intestinal expression level of MDR1 was clearly related to the oral clearance of tacrolimus until postoperative day 4 and the occurrence of acute cellular rejection up to postoperative day 10 (Figs 5, *A*, 8, and 9 and Tables II and III). The significant association between the high level of intestinal MDR1 and the 1-year patient survival rate might be explained at least partly by the prognostic significance of early exposure to immunosuppressive therapy after liver transplantation. Therefore medication during ICU care may be critical to survival, as well as the occurrence of acute cellular rejection. The molecular and immunologic mechanism(s) behind these phenomena should be clarified.

The grafted liver mass gradually regenerated after surgery. Fukudo et al[16] demonstrated kinetically that the clearance of orally administered tacrolimus improved or increased (or both) in the postoperative period. In our study the coefficient of the correlation between the intestinal MDR1 level and the C/D ratio of tacrolimus decreased from postoperative day 5 (Table III). This background and our results suggested that hepatic function and the interindividual variation in the rate of graft liver regeneration were associated at least in part with the reduced contribution of the intestinal MDR1 or large intraindividual variation in the pharmacokinetics of tacrolimus after surgery. Surrogate markers relating to the enzymatic activity associated with the interindividual and intraindividual variation in graft liver function after living-donor liver transplantation are needed.

In conclusion, we have advanced our previous finding that the enterocyte mRNA expression level of MDR1 was a simple and useful pharmacokinetic factor for tacrolimus, especially for adjusting the initial dosage in living-donor liver transplant patients. In addition, the average trough concentration of tacrolimus immediately after living-donor liver transplantation should be maintained above 7 ng/mL

for at least 4 days after surgery to prevent acute cellular rejection. Therefore initial dosage adjustment with consideration of the expression level of MDR1 in the small intestine at living-donor liver transplantation may reduce the frequency of acute cellular rejection.

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Biliary Reconstruction in Right Lobe Living-Donor Liver Transplantation

Comparison of Different Techniques in 321 Recipients

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Objective: To assess the incidence of biliary complications after right lobe living-donor liver transplantation (LDLT) in patients undergoing duct-to-duct choledochocholedochostomy or Roux-en-Y choledochojejunostomy reconstruction.

Summary Background Data: Biliary tract complications remain one of the most serious morbidities following liver transplantation. No large series has yet been carried out to compare the 2 techniques in LDLT. This study undertook a retrospective assessment of the relation between the method of biliary reconstruction used and the complications reported.

Methods: Between February 1998 and June 2004, 321 patients received right lobe LDLT. Biliary reconstruction was achieved with Roux-en-Y choledochojejunostomy in 121 patients, duct-to-duct choledochocholedochostomy in 192 patients, and combined Roux-en-Y and duct-to-duct choledochocholedochostomy in 8 patients. The number of graft bile duct and anastomosis, mode of anastomosis, use of stent tube, and management of biliary complications were analyzed.

Results: The overall incidence of biliary complications was 24.0%. Univariate analysis revealed that hepatic artery complications, cytomegalovirus infections, and blood type incompatibility were significant risk factors for biliary complications. The respective incidence of biliary leakage and stricture were 12.4% and 8.3% for Roux-en-Y, and 4.7% and 26.6% for duct-to-duct reconstruction. Duct-to-duct choledochocholedochostomy showed a significantly lower incidence of leakage and a higher incidence of stricture; however, 74.5% of the stricture was managed with endoscopic treatment.

Conclusions: The authors found an increase in the biliary stricture rate in the duct-to-duct choledochocholedochostomy group. Because of greater physiologic bilioenteric continuity, less incidence of leakage, and easy endoscopic access, duct-to-duct reconstruction represents a feasible technique in right lobe LDLT.

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Biliary tract complication remains one of the most serious morbidities following liver transplantation, with an incidence of 10% to 30% in deceased liver transplantation.[1–4] It has been reported that the pathogenesis of biliary leakage and stricture in deceased liver transplantation were related to preoperative patient condition, blood type incompatibility, ischemic time, hepatic artery complications, and cytomegalovirus (CMV) infection.[5–9] The published data have also suggested that the frequency of biliary complications is higher in post-living donor liver transplantation (LDLT) compared with deceased liver transplantation.[10,11] Major concerns are early leakage and late stricture at the anastomotic site, which are associated with technical, anatomic, or microcirculatory considerations. Particularly in the recipient with "small-for-size" graft or deteriorated preoperative status, early biliary complications readily result in a fatal outcome, and these conditions themselves may increase the risk of complications.

There remains considerable disparity in the reported cases with regard to the incidence of biliary complications after right lobe LDLT, with reported rates ranging from 24% to 60%.[11,12–15] In right liver graft, current controversy focuses on the selection between Roux-en-Y hepaticojejunostomy and duct-to-duct choledochocholedochostomy. Many technical issues, such as the method of dissection, selection of suture and mode, and the use of stenting tube, are still under discussion. Duct-to-duct is currently our standard technique of choice for biliary reconstruction in right lobe LDLT, with the following advantages over Roux-en-Y choledochojejunostomy: 1) no need for intestinal manipulation, serving as an

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anatomic barrier to reflux of enteric contents into the biliary tract, and it may theoretically decrease the risk of ascending cholangitis and the morbidity is reduced even when early anastomotic leakage occurs; 2) technically faster and easier than Roux-en-Y; and 3) the physiologic bilioenteric continuity enables good endoscopic access postoperatively.[16,17]

This report describes surgical trials for biliary reconstruction in 321 consecutive right lobe LDLT, focusing on technical considerations regarding the biliary anatomy on graft, suture mode and stent tube in duct-to-duct/Roux-en-Y biliary reconstructions during long-term follow-up.

PATIENTS AND METHODS

Between June 1990 and June 2004, 953 patients underwent 1000 LDLTs at Kyoto University Hospital, Kyoto, Japan. Right lobe LDLT was first carried out at our institution in February 1998, and we have since performed 346 right lobe LDLTs. Of these, 25 patients died within 3 months of LDLT and are thus excluded from this study. A total of 321 patients were the subjects of the present study.

The patients were 164 males and 157 females, with a median age of 43.4 years (range, 15.5–70.3 years), and a median weight of 59.9 kg (range, 34.6–99.5 kg). Median model for end-stage liver disease[18] score was 18.0. The indication for liver transplantation was hepatocellular carcinoma in 86 patients, followed by viral hepatitis (n = 57), cholestatic liver disease (n = 57), fulminant hepatic failure (n = 39), biliary atresia (n = 34), metabolic liver disease (n = 9), metastatic liver tumor (n = 3), retransplantation (n = 16), and others (n = 20). Forty patients (12.5%) received blood type incompatible grafts. Thirty patients (9.3%) received right lobe with middle hepatic vein graft. A total of 121 patients (37.7%) received biliary reconstruction using Roux-en-Y and 192 (59.8%) had duct-to-duct anastomosis, while 8 (2.5%) patients underwent combined Roux-en-Y and duct-to-duct anastomosis. After the introduction of duct-to-duct anastomosis in July 1999, patients who had liver disease without extrahepatic biliary tract involvement were candidates for duct-to-duct anastomosis. The median follow-up period was 60 months (range, 7–80 months) in Roux-en-Y choledochojejunostomy and 34 months (range, 7–64 months) in duct-to-duct anastomosis ($P < 0.01$). There were no significant differences in patient characteristics between either group, except for the follow-up period and the patient age. Because of the patient population with biliary atresia in the Roux-en-Y group, the patient age was significantly younger in the Roux-en-Y group ($P < 0.01$) (Table 1).

Immunosuppression consisted of tacrolimus and low-dose steroids.[19] Patients who received blood type incompatible transplants had preoperative plasma exchange or double filtration plasmapheresis to reduce the anti-ABH antibody titer. Prostaglandin E1, cyclophosphamide, and additional steroids were administered from the portal vein or hepatic artery postoperatively.[20,21]

Statistical analysis was performed using the generalized Wilcoxon test. Actuarial survival rate was calculated with the nonparametric Kaplan-Meier method and was compared with the Wilcoxon test throughout the study. − values of less than 0.01 were considered significant.

The study was approved by the international review board and informed consent was obtained in all the cases.

TABLE 1. Patient Characteristics

Characteristic	Roux-en-Y (n = 121)	DD (n = 192)	P
Age (yr)	35.2 ± 13.5	48.8 ± 11.3	<0.01
MELD score	16.9 ± 11.4	18.4 ± 10.5	NS
Donor age (yr)	43.1 ± 10.9	41.4 ± 11.9	NS
ABO incompatibility (%)	11.6	13.5	NS
GRWR (%)	1.19 ± 0.29	1.13 ± 0.26	NS
Cold ischemic time (min)	123 ± 103	97 ± 77	NS
Warm ischemic time (min)	42 ± 13	49 ± 17	NS
Operation time (min)	789 ± 192	693 ± 173	NS
Blood loss (g)	7855 ± 10582	6060 ± 8082	NS
Median follow-up period (mo)	60.0	34.0	<0.01

DD indicates duct-to-duct choledochocholedochostomy; GRWR, graft-to-recipient weight ratio; NS, not significant.

Donor Operation

Standard right lobe technique was previously described.[12,22,23] Before parenchymal transection, the right lobe was mobilized and the sizeable (>5 mm) right inferior hepatic vein was preserved with a caval cuff for reconstruction. After careful definition of biliary anatomy in the hepatic hilum using intraoperative cholangiography, the right hepatic duct was transected 2 to 3 mm away from the bifurcation. Minimal dissection of pericholedochal tissue was required at this point to maintain blood supply around the hepatic duct, and the hepatic duct was separated with fine scissors. Right-sided liver has a higher incidence of vascular and biliary variant. Overall, 39.6% of grafts had multiple bile ducts in our right lobe LDLT series, which poses a further difficulty in reconstruction.[16,24] The right portal vein and the right hepatic artery were temporally clamped to clarify the parenchymal transection line. An 8-mm Penrose drain was passed between the right hepatic vein superiorly and the portal bifurcation inferiorly to maintain the cutting plane during parenchymal dissection. The end of remnant hepatic ducts were closed with a continuous suture using 6–0 polydioxanone absorbable monofilament, and cholangiogram was performed to ensure that there was no leakage or stricture.

Recipient Operation

Hilar dissection was carefully performed to preserve adequate blood supply of the epicholedodochal arterial plexus and the 2 distinct intramural arteries (3 and 9 o'clock arteries),[25,26] and the bile duct was divided above the hilar bifurcation. Biliary anastomosis was performed with 6–0 polydioxanone absorbable monofilament suture after completion of vascular anastomosis. The graft hepatic duct was anastomosed to Roux-en-Y limb and/or bile duct. When bile ducts in a graft were far apart, they were anastomosed separately. In 8 grafts, the bile ducts were so far apart that both duct-to-duct and Roux-en-Y reconstructions were indi-

cated. If the blood supply of the recipient cystic duct was sufficient and the recipient cystic duct was a better size match, the recipient cystic duct was used for the posterior duct reconstruction. Variations in technical preference remain, and modifications may be necessary to meet anatomic variants. The anastomosis was started at the posterior wall with interrupted or continuous suture, after which the anterior anastomosis was completed. A 4-French polyethylene tube was inserted for anastomotic decompression in some cases.

For internal stent in Roux-en-Y, 2 cm of 18G silicon vascular catheter was placed in the anastomosis. For external stent in Roux-en-Y, the 4-French tube was inserted through the jejunum and the tip was placed through the anastomosis. The stent tube was removed 8 weeks after transplantation.[27]

Biliary complications were diagnosed clinically and radiologically. Biliary leakage was defined by bilirubin level in the bilious ascites higher than the serum level, and stricture was diagnosed by dilated intrahepatic bile ducts with ultrasonography, hepatobiliary scan with Tc-99m Sn-*N*-pyridoxyl-5-methyltryptophan (^{99m}Tc-PMT), and radiologic intervention in all cases.[28]

RESULTS

Overall Incidence of Biliary Complication and Risk Factor

Of 321 right lobe LDLTs, 77 patients (24.0%) experienced 87 biliary complications (leakage: n = 27, 8.4%; stenosis: n = 60, 18.7%). There were no significant differences between the patient with or without biliary complication (n = 77 versus n = 244, respectively) in model for end-stage liver disease score (18.3 ± 9.3 versus 18.0 ± 11.3); donor age (40.9 ± 11.6 years versus 42.5 ± 11.5 years); percentage of blood type incompatibility (16.9% versus 12.3%); graft-to-recipient weight ratio (1.11 ± 0.24% versus 1.16 ± 0.29%); cold ischemic time (112 ± 92 minutes versus 87 ± 67 minutes); and warm ischemic time (48 ± 26 minutes versus 48 ± 16 minutes). However, the respective incidence of hepatic artery complications (28.6% versus 0.4%) and CMV infection (39.0% versus 22.5%) was significantly higher in the patients with biliary complications ($P < 0.01$) (Table 2). Blood type incompatibility was not a significant risk factor in overall right lobe LDLT series.

Overall incidence of biliary leakage and stricture were 12.4% and 8.3% in Roux-en-Y (n = 121), 4.7% and 26.6% in duct-to-duct (n = 192), and 0% in combined Roux-en-Y and duct-to-duct (n = 8), respectively. Duct-to-duct anastomosis showed significantly lower incidence of leakage and a higher incidence of stricture ($P < 0.01$). The onset of biliary leakage and stricture were 19.0 ± 7.7 days (range, 8–35 days; median, 17.5 days) and 12.3 ± 12.2 months (range, 2–36 months; median, 7.5 months) in Roux-en-Y, and 26.5 ± 26.1 day (range, 2–90 days; median, 20 days) and 8.7 ± 5.4 months (range, 2–35 months; median, 8 months) in duct-to-duct (P = not significant), respectively.

TABLE 2. Potential Risk Factor for Biliary Complication in 321 Consequent Right Lobe Living Donor Liver Transplantations

	Biliary Complications		
	Yes (n = 77)	No (n = 244)	*P*
MELD score	18.3 ± 9.3	18.0 ± 11.3	NS
Donor age (yr)	40.9 ± 11.6	42.5 ± 11.5	NS
Blood type incompatibility (%)	13 (16.9%)	30 (12.3%)	NS
GRWR (%)	1.11 ± 0.24	1.16 ± 0.29	NS
Cold ischemic time (min)	112 ± 92	87 ± 67	NS
Warm ischemic time (min)	48 ± 26	48 ± 16	NS
Hepatic artery stenosis/ thrombosis (%)	22 (28.6%)	1 (0.4%)	<0.01
CMV infection (%)	30 (39.0%)	55 (22.5%)	<0.01

GRWR indicates graft-to-recipient weight ratio; CMV, cytomegalovirus; NS, not significant.

Analysis of Biliary Complication According to the Type of Anastomosis

A total of 121 patients received Roux-en-Y biliary reconstruction (Table 3). There was no significant difference in biliary complications among the number of bile ducts in the graft and mode of anastomotic suture (P = not significant). There was a high incidence of biliary complications in the graft with 3 ducts. There was a trend toward a lower incidence of leakage and a higher incidence of stricture in continuous suture, but no significant difference was found among the groups. The patients with external stent (n = 103) showed lower incidence of biliary leakage compared with those with internal stent (n = 5), but this observation did not achieve statistical significance. The incidence of biliary stricture in the patients with external stent was significantly lower than in the patients without stent (n = 13) ($P < 0.01$).

TABLE 3. Biliary Complication in Roux-en-Y Choledochojejunostomy (n = 121)

	n	Leakage (%)	Stricture (%)
No. of graft bile ducts and anastomosis			
1 duct/1 anastomosis	66	7 (10.6)	4 (6.1)
2 ducts/2 anastomoses	64	7 (10.9)	5 (7.8)
3 ducts/1 anastomosis	1	1 (100)	1 (100)
Mode of anastomosis suture			
Interrupted	68	10 (14.7)	5 (7.4)
Continuous	48	4 (8.3)	5 (10.4)
Posterior: continuous/anterior: interrupted	5	1 (20.0)	0 (0.0)
Stent use for biliary reconstruction			
No stent	13	3 (23.1)	3 (23.1)
Internal stent	5	3 (60.0)	2 (40.0)
External stent	103	9 (8.7)	5 (4.9)*

*$P < 0.01$.

TABLE 4. Biliary Complication in Duct-to-Duct Choledochocholedochostomy (n = 192)

	n	Leakage (%)	Stricture (%)
No. of graft bile ducts and anastomosis			
1 duct/1 anastomosis	117	8 (6.8)	38 (32.4)
2 ducts/1 anastomosis	32	0 (0.0)	5 (15.6)
2 ducts/2 anastomoses	41	0 (0.0)	7 (17.0)
3 ducts/1 anastomosis	1	1 (100)	1 (100)
3 ducts/2 anastomoses	1	1 (100)	0 (0.0)
Mode of anastomosis suture			
Interrupted	25	2 (8.0)	9 (36.0)
Continuous	148	7 (4.7)	37 (25.0)
Posterior: continuous/anterior: interrupted	19	1 (5.3)	5 (26.3)
Stent type for biliary reconstruction (12)			
No stent	6	1 (16.7)	2 (33.3)
Cystic drainage	16	2 (12.5)	6 (37.5)
Cystic stent	9	0 (0.0)	2 (22.2)
External stent	163	7 (4.3)	41 (25.1)

Duct-to-duct biliary reconstruction was achieved in 192 cases (Table 4). If we focus on blood type incompatibility in biliary complication with duct-to-duct reconstruction, leakage and stricture was observed in 11.5% and 38.5% of the patients with blood type incompatibility; the incidence of biliary complications was significantly higher in duct-to-duct patients with blood+ type incompatibility ($P < 0.01$).

In 117 recipients (60.9%) of duct-to-duct anastomosis, the common bile duct was used to perform the reconstruction with a single right bile duct. In 11 of 117 patients with single duct-to-duct anastomosis (9.4%) and 8 of 41 patients with 2 anastomoses for 2 ducts (19.5%), a small incision (1–2 mm) in the anterior wall of the graft bile duct was made to accommodate the size mismatch. In 5 patients with one anastomosis for 2 ducts (15.6%), a ductoplasty was performed to enable a single anastomosis to the recipient common bile duct. In 6 of 41 patients with 2 anastomoses for 2 ducts (14.6%), the recipient cystic duct was used to perform the posterior duct reconstruction for better size matching. Two of them (33.3%) had biliary stricture at 2 and 6 months after transplantation. In another case with 2 ducts, the ducts were anastomosed to the recipient left and right hepatic ducts. Totally, there was no significant difference in biliary complications among the number of bile ducts in the graft and mode of anastomosis suture in duct-to-duct reconstruction. However, if the graft had 3 ducts, there was a high incidence of biliary complication.

In 188 cases, the biliary stent tube was inserted for anastomotic decompression in duct-to-duct anastomosis. For cystic drainage (n = 16), the stent was inserted through the remaining cystic duct and pushed downward into the recipient common bile duct. For cystic stent (n = 9), the tube was inserted through the remaining cystic duct and was placed through the anastomosis as a splint. For external stent (n = 163), the tube was placed through the anastomosis and was pulled out through the common bile duct.[16] There was no significant difference in biliary complications according to the type of biliary stent in duct-to-duct reconstruction. If we compare the incidence of anastomotic complication in single duct-to-duct reconstruction (n = 117), the incidence of biliary leakage and stricture was 10.0% and 40% in interrupted suture, 7.2% and 31.3% in continuous suture, 0% and 28.6% in combined interrupted and continuous suture (P = not significant), respectively. Also, the use of the stent tube did not reduce biliary complications in single duct-to-duct anastomosis.

Clinical Outcome of Patients After Biliary Complication in Roux-en-Y Hepaticojejunostomy

The clinical outcome of the patients with biliary complications in Roux-en-Y reconstruction is summarized in Figure 1. Two patients with bile leaks and one with biliary stricture died of sepsis. Biliary leakage was first treated with percutaneous drainage. When the amylase level of aspirated fluid was high or the patient's condition was critical, relaparotomy was indicated. Because the anastomosis appeared to be too fragile for revision, we put drains and carried out a Roux-en-Y enterostomy to isolate/rest the biliary anastomosis (Roux-en-Y diversion). Five patients received Roux-en-Y diversion. The enterostomy was removed after the leak had

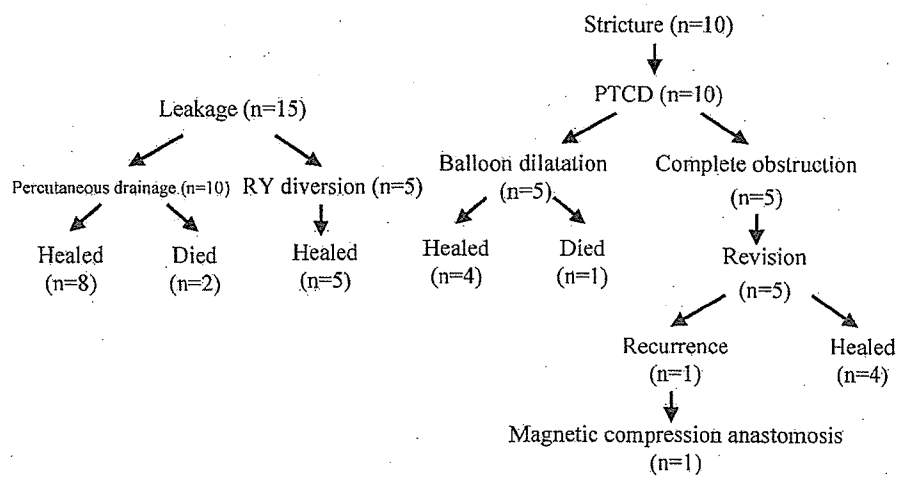

FIGURE 1. Summary of clinical outcome after biliary complications in Roux-en-Y choledochojejunostomy. PTCD, percutaneous transhepatic cholangiography and drainage.

been successfully treated. Two patients died of septic complication after biliary leakage at 14 and 3 months after transplantation.

Four of the 10 biliary strictures were secondary to biliary leakage. Anastomotic stricture was initially managed with percutaneous transhepatic cholangiodrainage (PTCD). Five patients were successfully treated with balloon dilatation. Five patients (50%) with complete anastomotic obstruction required surgical revision. One patient developed biliary stricture after surgical revision and was treated with magnetic compression anastomosis between the hepatic duct and Roux-en-Y loop, as proposed by Yamanouchi et al.[29] One patient with biliary stricture died of sepsis after several courses of PTCD and balloon dilatation 11 months after transplantation. One patient who underwent revision surgery for biliary stricture died of recurrence of hepatocellular carcinoma 30 months after transplantation.

Clinical Outcome of Patients After Biliary Complication in Duct-to-Duct Choledochocholedochostomy

Figure 2 shows clinical outcome of the patients with biliary complications in duct-to-duct reconstruction. In patients with biliary leakage, endoscopic retrograde nasobiliary drainage (ENBD) was indicated as an initial treatment. Four of the 10 patients with biliary leakage required conversion to Roux-en-Y (n = 3) or reoperation with duct-to-duct reconstruction (n = 1). One patient with a blood type incompatible graft died of sepsis 11 months after transplantation. Five of 10 patients were successfully treated with ENBD.

Six of the 51 biliary strictures (15.7%) were secondary to biliary leakage. Initially, anastomotic stricture was referred for endoscopic retrograde cholangiography (ERC). Thirteen of 51 patients (25.5%) could not receive endoscopic treatment because of the difficulty in accessing the papilla of Vater and the difficulty of passing a guidewire through the tight anastomotic stricture. All of them required PTCD. Consequently, 5 patients underwent revision surgery with Roux-en-Y reconstruction to repair the stricture. Two patients with tight anastomotic stricture were closely observed for a week with PTCD for anastomotic decompression, and were successfully treated with endoscopic retrograde biliary drainage (ERBD). Eight patients were treated with ERC balloon dilatation without placing inside stents. One patient died of sepsis secondary to chronic cholangitis 5 months after transplantation. The remaining 27 of 51 patients (52.9%) were treated by placing inside stents endoscopically above the sphincter of Oddi. One patient with a blood type incompatible graft underwent conversion to Roux-en-Y after ERBD because of acute cholangitis and hemobilia. As shown in Figure 2, 9 of 51 patients (17.6%) with duct-to-duct anastomotic stricture required surgical revision. The need for surgical revision due to biliary stricture tended to be lower in the duct-to-duct group compared with the Roux-en-Y group (50.0%), but this did not reach statistical significance ($P = 0.03$).

DISCUSSION

Right-lobe LDLT can provide an adequate graft size to compensate for the metabolic demands in most adult recipients, and the clinical outcome has improved in our series.[22] Among the controversies in right lobe LDLT, techniques of biliary reconstruction remain an open question. Right-sided liver has a higher incidence of vascular and biliary variants, this was explained by the relative consistency between the left umbilical vein and the liver. Multiple biliary orifices are encountered in 26.0 to 39.6% of the cases, which presents a further difficulty in reconstruction in right lobe LDLT.[16,24,30] For safe biliary reconstruction, precise evaluation of the biliary anatomy is essential.

The method for preoperative or intraoperative biliary duct evaluation remains a controversial topic for discussion. We have performed preoperative biliary duct evaluation with three-dimensional drip infusion cholangiographic computed tomography (CT) or magnetic resonance (MR) cholangiography in the evaluation of the potential donor. Although it provides adequate anatomic information of the biliary system, adaptation of these valuable methods for potential donor candidates is not always possible because of the risk of allergic reaction to contrast medium and the cost. In our experience, intraoperative cholangiography is an adequate and convenient way to evaluate the donor biliary tree.

The blood supply for biliary anastomosis is a major concern in LDLT. The arterial blood supply of the biliary system has been described by several investigators. A previous study using fine casts showed that 60% of the arterial

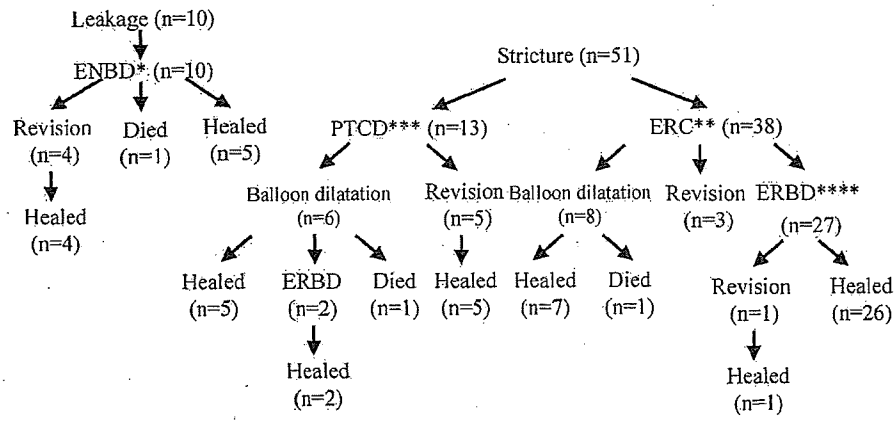

FIGURE 2. Summary of clinical outcome after biliary complications in duct-to-duct choledochocholedochostomy. ENBD, endoscopic nasobiliary drainage; ERC, endoscopic retrograde cholangiography; PTCD, percutaneous transhepatic cholangiography and drainage; ERBD, endoscopic retrograde biliary drainage.

supply for the bile duct comes from the caudal side through periduodenal arteries, 38% from the cranial side and only 2% from the hepatic artery itself. The 3 o'clock, 9 o'clock, and retroportal arteries give rise to multiple arteriolar branches, which form a free anastomosis within the wall of the bile duct.[26] In the absence of any attachments in transplanted liver recipients, the blood supply to the graft bile duct is derived solely from the hepatic artery. Histologic examination of disrupted duct-to-duct reconstruction often shows the loss of 3 o'clock and 9 o'clock intramural arteries on the recipient side (Fig. 3). Preservation of periductal microcirculation in the recipient duct and excellent hepatic artery reconstruction might be a key factor for successful duct-to-duct anastomosis.

Our current study confirmed that arterial complications, CMV infection, and blood type incompatibility were significant and important etiologic variables in biliary complications.[16,27] We do not use prophylactic administration of ganciclovir. However, the results of this study underline the importance of prophylaxis. In our LDLT program, a blood type incompatible graft was unavoidable in 12% of the recipients.[21] Despite the application of preoperative plasma exchange, splenectomy, and enhanced immunosuppression, the 5-year survival rate in adult patients was less than 50% and nearly 70% of the adult patients had biliary complications. We started the intrahepatic arterial immunosuppression protocol from December 2001 and the preconditioning regimen with anti-CD20 monoclonal antibody infusion from April 2004. Although it is still a tentative trial, these protocols have dramatically improved the outcome, with a 1-year graft survival rate of 85.7% and a biliary complication rate of 38.8%.[31]

There is still no consensus among transplant surgeons with regard to the type of biliary reconstruction in right lobe LDLT. Recently, the use of duct-to-duct reconstruction has been increasingly reported in LDLT.[12,14,16,32] We have reported our initial experience of 51 cases of duct-to-duct biliary reconstruction and concluded that it represents a useful technique for right lobe LDLT.[16] In July 1999, duct-to-duct reconstruction became the first choice for biliary reconstruction in our institution. In the series reported here, duct-to-duct technique had a lower incidence of biliary leakage. In cases of biliary leakage with duct-to-duct, peritoneal contamination from intestinal contents was minimized. In addition to the physiologic bilioenteric continuity and later good access by endoscopy, duct-to-duct reconstruction has an advantage over Roux-en-Y that the morbidity is reduced even when early anastomotic leakage occurs.

Biliary stricture was encountered in 26.6% of the patients with duct-to-duct reconstruction in this series, which was significantly higher than the Roux-en-Y group (4.7%). Although strictures seemed to develop more frequently in the duct-to-duct group, the requirement for surgical revision tended to be lower in that group. Because of easy access and imaging through endoscopy, 38 of 51 patients (74.5%) could be treated with ERC. Once ERBD was initiated, 26 of 27 patients (96.3%) were successfully treated. Recently, Gondolesi et al reported the largest Western experience with biliary complications in right lobe LDLT, and demonstrated that duct-to-duct reconstruction had higher incidence of stricture (31.7%) and lower incidence of leakage (16.3%), while the opposite was true following Roux-en-Y reconstruction (7.3% and 18.2%). Also, they recommended early and aggressive use of interventional treatment of biliary complications.[32] We agree with this suggestion that early interventional treatment could avoid further operative intervention. Endoscopic biliary intervention is useful for most anastomotic strictures. Unless the anastomotic site is completely necrotic, insertion of a long-term short stent is very effective in securing bile drainage without increased risk of ascending cholangitis.[17]

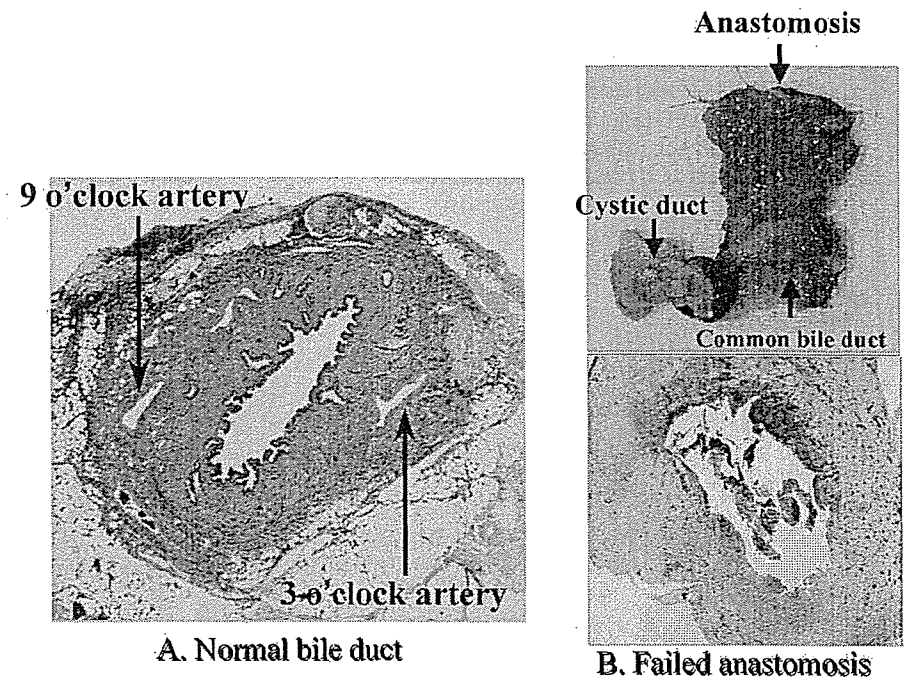

FIGURE 3. Histologic examination of the failed duct-to-duct choledocho-choledochostomy often shows the loss of the 3 and 9 o'clock arteries on the recipient side. Preservation of periductal microcirculation on the recipient side is a key factor for successful anastomosis. A, Normal common bile duct with patent 3 and 9 o'clock intramural arteries. B, Failed duct-to-duct choledochocholedochostomy with loss of 3 and 9 o'clock intramural arteries.