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New Protocol of Immunosuppression for Liver Transplantation Across ABO Barrier: The Use of Rituximab, Hepatic Arterial Infusion, and Preservation of Spleen

A. Yoshizawa, S. Sakamoto, K. Ogawa, M. Kasahara, K. Uryuhara, F. Oike, M. Ueda, Y. Takada, H. Egawa, and K. Tanaka

ABSTRACT

Introduction. An ABO-incompatible (ABO-I) living donor liver transplantation (LDLT) is a challenge. Until 2000 systemic multidrug immunosuppression and splenectomy was the gold standard with poor results. Application of local administration with prostagrandin E1 (PGE1) and steroids via a portal vein (PV) catheter dramatically improved the survival from 20% to 60% but PV thrombus became a problem (35%). To solve it, an hepatic arterial (HA) catheter was used instead of a PV catheter and splenectomy was omitted. Although the PV thrombus problem was resolved, the ABO antibody titers significantly increased, and two cases of uncontrollable humoral rejection (HR) were experienced. In this study, Rituximab was introduced instead of splenectomy to decrease the antibody. We report the efficacy of prophylaxis with Rituximab for ABO-I LDLT.

Methods. Eight patients received. Rituximab at 2 to 14 days before LDLT. During the operation, the spleen was preserved. Methylpredonisolone and PGE1 were administered via an HA catheter for 2 to 3 weeks after LDLT in addition to an immunosuppressive regimen consisting of tacrolimus and steroids. Antibody titers were measured serially.

Result. There was no clinical HR. Two patients died of complications unrelated to HR. The antibody titer decreased compared to patients without splenectomy/rituximab. B cells (CD19) were depleted from peripheral blood for up to 3 months. Cytomegalovirus infections were decreased compared to patients with splenectomy ($P = .085$).

Conclusion. Rituximab prophylaxis and HA infusion therapy prevented clinical HR, which may provide a breakthrough to overcome the ABO blood-type barrier in liver transplantation.

IN JAPAN, LIVING DONOR transplantation (LDLT) is sometimes the only available option for patients with end-stage liver disease because brain-dead donors are rare. In LDLT donor selection is limited to relatives. When the only available graft is ABO-incompatible (ABO-I), liver transplantation must be performed across the ABO blood-type barrier. ABO-incompatibility has a big impact in living donor LDLT especially among adult recipients (over 16 years old).[1] Until 2000, the gold standard for ABO incompatibility was plasmapheresis, systemic use of multiple immunosuppressive drugs and splenectomy; the 5-year survival was poor (22%). Local administration of prostagrandin E1 (PGE1), steroid, and Gabexate Mesilate via portal vein (PV) catheter, as introduced by Keio University[2] remarkably improved the results (60%). Hepatic arterial (HA) infusion therapy in addition to PV infusion improved the 1-year survival to 85.7%). However, these cases showed frequent PV thrombosis (37.5%), which was assumed to be due to the PV catheter and the splenectomy. To resolve it, HA infusion therapy without a PV catheter or splenectomy was used, but the anti-ABO antibody titer significantly

From the Kyoto University Graduate School of Medicine, Department of Transplantation and Immunology, Kyoto, Japan.

Address reprint requests to Atsushi Yoshizawa, Kyoto University Graduate School of Medicine, Department of Transplantation and Immunology, 54 Kawara-Cho, Shougoin, Sakyo-Ku, KYOTO, Japan 606-8507. E-mail: ayoshi14@kuhp.kyoto-u.ac.jp

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increased and two cases showed uncontrollable total hepatic necrosis. In this study, Rituximab instead of splenectomy was introduced to decrease the antibody. We report the efficacy of prophylaxis with Rituximab for ABO-I LDLT.

MATERIALS AND METHODS

Patients

The eight patients involved in this study were aged 29 to 65 years including seven women. The original diseases were four cirrhosis, three primary biliary cirrhosis, one Wilson's disease. The mean observation period was 3.6 months (range 2 to 7 month).

Protocol

At 2 to 14 days before transplantation, one dose of rituximab (375 mg/m^2) was administered. Before LDLT, plasmapheresis was performed to reduce the anti-ABO antibody to less than 1:8 at transplantation. During the operation, the spleen was preserved, and an HA catheter inserted. After operation, PGE1 (0.01 γ) and methylpredonisolone (125 mg/body) were administrated through the HA catheter for 2 to 3 weeks. Systemic immunosuppression consisted of tacrolimus, methylpredonisolone, and cyclophosphamide (2 mg/kg/d intravenous for 2 weeks, and oral by for another 2 weeks). The antibody titer was serially examined, and a liver biopsy performed when liver function tests were elevated. Statistical analysis was performed by 2 student t or chi-square tests.

RESULT

There was no clinical humoral rejection, hepatic necrosis, or intrahepatic biliary complications. The peak anti-ABO antibody titer after LDLT was 252.6 $\pm$ 356 and 162.6 $\pm$ 175.1 for IgM and IgG, respectively. The peak titer decreased, though at showed no significant difference compared to the titers observed with infusion therapy without splenctomy (580 $\pm$ 1099 vs 375 $\pm$ 693; $P = .31$). Rejection was observed in three cases (38%) with no significant difference between HA infusion therapy with/without splenectomy. Two recipients died of complications, aspiration pneumonia and intestinal perforation, which were not directly related to humoral rejection. The 38% incidence of cytomegalovirus disease was significantly less than that of patients with splenectomy (81%; $P = .018$).

DISCUSSION

Fatal complications attributed to ABO-I liver transplantation are hepatic necrosis and intrahepatic biliary complication, evidences of clinical humoral rejection. This process leads to circulation disturbances due to local DIC caused by an antibody-complement reaction. In this study histological evidence of humoral rejection was defined by signs of periportal edema and necrosis.[3] There was a correlation between clinical/histological humoral rejection and the anti-ABO antibody titer. The ABO-related humoral immune response is activated for 1 to 3 weeks after liver transplantation, and then naturally decreases. It is important to control humoral immune response, that is, to decrease the ABO antibody titers immediately after LDLT. Splenectomy decreases antibody titers but may cause permanent immunodeficiency as well as PV thrombosis. So splenectomy may become a risk factor for infectious diseases such as viral infections. In this regard, prophylactic rituximab is preferable because it is reversible immunosuppression.

After up-regulation of humoral immune response, the use of rituximab rescue was not effective to decrease antibody titers and prevent progression of clinical humoral rejection in our experience. In contrast prophylaxis by rituximab for ABO-I LDLT was not equivalent to splenectomy. We must consider the mode of delivery, timing, any dosage of rituximab, but this protocol successfully prevented clinical humoral rejection. In conclusion, prophylactic rituximab and HA infusion therapy may become a standard protocol for ABO-I LDLT.

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Living-Donor Liver Transplantation for Hepatoblastoma

Mureo Kasahara[a,*], Mikiko Ueda[b], Hironori Haga[a], Hidefumi Hiramatsu[c], Michihiro Kobayashi[c], Souichi Adachi[c], Seisuke Sakamoto[b], Fumitaka Oike[b], Hiroto Egawa[a], Yasutsugu Takada[b] and Koichi Tanaka[a,b]

[a] *Organ Transplant Unit, Department of Transplant Surgery, Kyoto University Hospital, Kyoto, Japan*
[b] *Department of Transplant Surgery, Kyoto University Hospital, Kyoto, Japan*
[c] *Department of Pediatrics, Kyoto University Hospital, Kyoto, Japan*
* *Corresponding author: Mureo Kasahara, mureo@kuhp.kyoto-u.ac.jp*

Hepatoblastoma is the most common malignant liver tumor in children. Recently, liver transplantation has been indicated for unresectable hepatoblastoma. We retrospectively reviewed 14 children with a diagnosis of hepatoblastoma who had undergone living-donor liver transplantation (LDLT) at Kyoto University Hospital. During the period from June 1990 to December 2004, 607 children underwent LDLT. Of these interventions, 2.3% were performed for hepatoblastoma. Based on radiological findings, the pre-treatment extent of disease (PRETEXT) grouping was used for pre-treatment staging of the tumor. There were grade III in seven patients and grade IV in seven patients. Thirteen patients received chemotherapy, and seven underwent hepatectomy 11 times. Immunosuppressive treatment consisted of tacrolimus monotherapy in 11 patients. Actuarial 1- and 5-year graft and patient survival rates were 78.6% and 65.5%. The poor prognostic factors were macroscopic venous invasion and extrahepatic involvement with 1-year and 5-year survival rates of 33.0% and 0%. Pediatric patients without these factors showed an acceptable 5-year survival rate of 90.9%. LDLT provides a valuable alternative with excellent results in children with hepatoblastoma because it allows optimal timing of the liver transplantation, given the absence of delay between the completion of chemotherapy and planned liver transplantation.

Key words: Chemotherapy, hepatectomy, hepatoblastoma, living donor, liver transplantation

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Introduction

Hepatoblastoma is the most common malignant liver tumor in children, and is seen mostly in patients aged 2 years and under. Congenital anomalies are present in 5.5% of patients with hepatoblastoma (1). While a variety of histological patterns are noted, prognosis is primarily dependent on the surgical resectability of the tumor, with an overall survival rate of approximately 60%. Prognosis is better with the fetal-predominant type than with other types (2). Through combined improvements in imaging, surgical resection and systemic chemotherapy, higher survival rates have been achieved. While disease-free patient survival rates have been dramatically improved by cisplatin chemotherapy regimens, liver transplantation remains an alternative curative treatment for patients whose liver tumor is unresectable following systemic chemotherapy or radical hepatectomy (3,4). Initial studies of liver transplantation in children with unresectable hepatoblastoma report a 50% survival rate. While tumor recurrence was the most common cause of patient death, most of these patients had not received adjuvant chemotherapy (5,6).

Over the last decade, living-donor liver transplantation (LDLT) has become the primary therapeutic modality for end-stage liver disease in children (7). LDLT provides a valuable alternative in children with hepatoblastoma because it allows optimal timing of the liver transplantation, given that there is no delay between the completion of chemotherapy and the planned liver transplantation. We report here the long-term outcome of 14 children with hepatoblastoma who underwent LDLT at Kyoto University Hospital, Kyoto, Japan.

Patients and Methods

During the period from June 1990 to December 2004, 607 children (aged under 15 years) underwent LDLT at Kyoto University Hospital. Of these children, 14 (2.3%) underwent transplantation due to hepatoblastoma. All children (10 boys and four girls; age range: 9 months–12 years) underwent serial contrast-enhanced computed tomography (CT), magnetic resonance imaging and bone scanning to assess both response to treatment and extrahepatic disease. Ten children underwent liver biopsy at the time of presentation. Two congenital anomalies were presented: biliary atresia (case 4) and absence of inferior vena cava (IVC) (case 7). All patients had elevated alpha-fetoprotein (AFP) levels at the time of diagnosis, which ranged from 2619–2 700 000 ng/mL (normal range: <15 ng/mL). The overall follow-up

period ranged from 6 to 81 months, and the median follow-up for the survivors was 42 months.

Thirteen children, except for a child with biliary atresia, received preoperative chemotherapy. Each child received from 2 to 17 cycles of chemotherapy (median: six cycles) before LDLT in an attempt to shrink the primary tumor and eliminate micrometastatic disease (Table 1). Preoperative chemotherapy protocol using a combination of cisplatin (CDDP) and tetrahydropyranyl-doxorubicin (Adriamycin®) [(THP-ADR); Japanese study group for pediatric liver tumor protocol-1 (JPLT-1)] was indicated as an initial protocol in 13 cases (8,9). Eight children (cases 2, 3, 7, 8, 11, 12, 13 and 14) showed poor response to JPLT-1 chemotherapy. An additional protocol using a combination of ifosfamide (IFO), carboplatin (CBDCA), THP-ADR and etoposide (VP-16®) [JPLT-2] was initiated in five patients (cases 1, 11, 12, 13 and 14). Three children (cases 5, 7 and 12) underwent autologous peripheral blood stem cell transplantation after undergoing high-dose chemotherapy using a combination of IFO, etoposide, CBDCA and melphalan (L-PAM) (Hi-MEC protocol) or IFO, etoposide, L-PAM and thiotepa (Hi-MT protocol) proposed by the JPLT group (9,10). If the patient showed sufficient response and no dose-dependent side effects to preoperative chemotherapy, the same preoperative chemotherapy regimen was adapted postoperatively. If not, irinotecan (CPT-11) was used as a postoperative chemotherapy.

Before referral to Kyoto University Hospital, seven children underwent hepatectomy 11 times after systemic chemotherapy. Liver resections performed included three right hepatectomies; two right trisectionectomies; two left hepatectomies and four nonanatomical tumor nucleations. Seven children had disease recurrence in the liver after prior resection, and further resection was not possible. Case 4 with biliary atresia had undergone Kasai's porto-enterostomy at the age of 47 days (11). The patient presented with liver failure, pathological fractures and growth retardation. The liver tumor was diagnosed by CT scan just before LDLT. Three children (cases 3, 13 and 14) showed the tumor invasion to all four sectors of the liver after chemotherapy, and two children (cases 6 and 9) showed close proximity to hepatic vessels. These children remained to have unresectable hepatoblastoma. In summary, seven children underwent 'primary' liver transplantation and seven underwent 'rescue' liver transplantation.

The indication for transplantation was unresectable liver tumor in 12 patients, acute liver failure after systemic chemotherapy in one patient (case 1) and biliary cirrhosis associated with extrahepatic biliary atresia in another patient. Case 1 received six cycles of chemotherapy, thereafter the patient showed hyperbilirubinemia, hyperammonemia and coagulopathy. Despite the conventional treatment with blood exchange, the patient's condition deteriorated and LDLT was indicated for acute liver failure. The PRETEXT system based on radiological findings was used for clinical grouping of hepatoblastoma (12). According to PRETEXT staging system, a tumor occupying 1, 2, 3 or 4 adjacent liver sector is defined as PRETEXT I, II, III or IV, respectively. The PRETEXT grouping was III and IV in seven patients and in seven patients, respectively. All the children were judged to have an unresectable tumor after laparotomy. Case 2 showed a major portal vein tumor thrombus, case 3 revealed suspected tumor involvement of the stomach and case 11 showed suspected hilar lymph node swelling and direct IVC invasion, which was completely resectable together with total hepatectomy. The median interval between onset of hepatoblastoma and LDLT was 12 months (range: 2–59 months). The median interval between the last cycle of chemotherapy and LDLT was 23 days (range: 15–44 days).

The potential live donor candidates were evaluated through the use of liver function tests; determination of blood type; human leucocyte antigen typing and determination of anatomical variation and graft size using 3D-CT volumetry. There were four blood-type compatible and 10 identical grafts. Graft type was selected according to the graft-to-recipient weight ratio

Table 1: Clinical data on 14 children undergoing living-donor liver transplantation for hepatoblastoma

Patient	Age/Sex	Serum AFP at diagnosis	PRETEXT group	Pre-LDLT operation	Pre-LDLT initial chemotherapy	Serum AFP at LDLT	Donor age (years)	Relation	Graft type	GRWR (%)
1	9 m/M	677 400	III	None	CDDP+THP-ADR	4390	35	Father	LLS	6.83
2	7 y/M	529 000	IV	Right hepatectomy	CDDP+THP-ADR	54 700	37	Mother	Left	1.52
3	2 y/M	590 000	IV	None	CDDP+THP-ADR	5749	27	Father	LLS	2.50
4	4 y/F	12 900	III	Kasai	None	12 924	30	Father	LLS	2.32
5	10 y/M	2600	IV	Right trisectionectomy	CDDP+THP-ADR#	383	47	Father	Left	1.22
6	3 y/F	1 500 000	IV	None	CDDP+THP-ADR	10	40	Father	LLS	1.50
7	5 y/M	15 000	III	Right hepatectomy	CDDP+THP-ADR+VP-16#	37	29	Mother	LLS	1.63
8	6 y/M	2 700 000	IV	Right trisectionectomy, S3	CDDP+THP-ADR	1411	32	Mother	Left	1.32
9	4 y/M	266 000	IV	None	CDDP+THP-ADR	7040	25	Father	LLS	1.07
10	12 y/M	36 200	III	Left hepatectomy	CDDP+THP-ADR	113	40	Mother	Left	0.96
11	3 y/F	887 800	IV	Right lo hepatectomy, S3, S4	CDDP+THP-ADR	170 910	31	Father	LLS	1.65
12	9 y/M	3800	III	Left hepatectomy, S8	CDDP+THP-ADR+VP-16#	12	44	Father	LLS	2.29
13	11 m/F	1 000 000	III	None	CDDP+THP-ADR	7008	41	Mother	Mono	3.08
14	4 y/M	1 880 000	III	None	CDDP+THP-ADR	1 175 690	29	Mother	LLS	1.60

LDLT, living-donor liver transplantation; AFP, alpha-fetoprotein; PRETEXT, pre-treatment extent of disease based on radiological findings; GRWR, graft-to-recipient weight ratio; CDDP, cisplatin; THP-ADR, tetrahydropyranyl-doxorubicin; LLS, left lateral segment; VP-16, etoposide; #with blood stem cell transplantation.

(GRWR) (13). The graft types were one monosegmental graft (segment III), nine left lateral segments and four left lobe grafts (Table 1). All the donors were discharged from the hospital within 12 days after donation without complications.

Immunosuppression consisted of tacrolimus monotherapy in 11 patients, and tacrolimus and low-dose steroid therapy in three. Methylprednisolone was only given at the time of graft reperfusion intraoperatively (10 mg/kg). Low-dose steroid therapy was tapered off on postoperative days 19, 45 and 40 in cases 4, 5 and 9, respectively. Tacrolimus administration was started from 1 day before transplantation, except for one patient with acute liver failure (case 1). Target whole blood trough level of tacrolimus was 10–12 ng/mL for the first 2 weeks, approximately 10 ng/mL for the following 2 weeks and 5–10 ng/mL thereafter.

This study was approved by the institutional review board and informed consent was obtained in all the cases.

Results

Serum AFP level at LDLT were in the range of 9.5–1 175 690 ng/mL (median: 5070 ng/mL). All patients underwent LDLT using a standard procedure (14). Biliary reconstruction was achieved using Roux-en-Y hepaticojejunostomy in 11 patients and duct-to-duct anastomosis in three. The duct-to-duct biliary anastomosis was started at the posterior wall with continuous suture, after which the interrupted anterior anastomosis was completed with magnificent glasses. All patients received hilar lymph node dissection, and the hilar lymph node was positive in case 11. Hilar vascular dissection was performed before mobilization of the native liver to prevent the spread of malignant cells.

At the time of transplantation, case 3 had an exophytic extension of the primary tumor involving the stomach. This child underwent complete resection of the extrahepatic extension including resection of the body of stomach at the time of transplantation. Case 11 showed direct IVC invasion and hilar lymph node metastasis. The liver and involved IVC were removed and the graft hepatic vein was directly anastomosed with the suprahepatic IVC in end-to-end fashion, given that the IVC was not reconstructed. Case 7 showed congenital absence of IVC. The left and middle hepatic vein was directly drained to right atrium. The left hepatic vein of graft to left and middle hepatic vein anastomosis was made. Veno-venous bypass or portocaval shunt was not used in any of the cases. Duration and blood loss of the recipient operation was 691.8 ± 178.3 min (range: 426–1086 min) and 851.7 ± 445.4 g (range: 210–1870 g). Cold and warm ischemic times were 50 ± 31 min and 39.3 ± 7.3 min, respectively.

Histopathological examination of the explanted liver is listed in Table 2. There were six embryonal, four fetal, three embryonal and fetal, and one microtrabecular pattern. Twelve patients (85.7%) showed vascular invasions. Two children showed macrovascular invasion: case 2 in segment III portal vein, and case 11 in the IVC.

Table 2: Outcomes of 14 children receiving living-donor liver transplantation for hepatoblastoma

Patient	Histology Type	Vascular invasion	Complications	Adjuvant chemotherapy	Follow-up time (months)	Latest serum AFP	Current status
1	Microtrabecular	Hepatic vein	None	CPM+5-FU	9	43	Died on POD 280 (recurrence)
2	Embryonal	Hepatic and portal vein	None	CPM+CBDCA+VP-16+L-PAM#	32	10.4	Died on POD 960 (recurrence)
3	Embryonal	Hepatic and portal vein	None	CBDCA+VP-16+5-FU	11	81	Died on POD 330 (recurrence)
4	Embryonal	Portal vein	None	CBDCA+THP-ADR	81	3.5	ANED
5	Fetal	None	None	CBDCA+THP-ADR	794	3.9	ANED
6	Fetal	None	None	CDDP+THP-ADR	67	3	ANED
7	Embryonal and fetal	Portal vein	Intra abdominal bleeding	CPM	55	3	ANED
8	Embryonal	Portal vein	Biliary stricture	-	42	3	ANED
9	Embryonal	Hepatic and portal vein	Biliary stricture	CDDP+THP-ADR	36	3	ANED
10	Fetal	Hepatic and portal vein	Biliary stricture	CBDCA+VP-16	21	3	ANED
11	Embryonal	Hepatic and portal vein	Intestinal perforation	CPT-11	6	300	Died on POD 202 (recurrence)
12	Fetal	Portal vein	Biliary leakage	CPT-11	17	4	ANED
13	Embryonal and fetal	Portal vein	Biliary stricture	CPT-11	8	4.5	ANED
14	Embryonal and fetal	Hepatic and portal vein	None	CPT-11	6	8.1	ANED

AFP, alpha-fetoprotein; CPM, cyclophosphamide; 5-FU, fluorouracil; POD, postoperative day; CBDCA, carboplatin; VP-16, etoposide; L-PAM, melphalan; #with blood stem cell transplantation and partial hepatectomy; THP-ADR, tetrahydropyranyl-doxorubicin; ANED, alive with no evidence of disease; CDDP, cisplatin; CPT-11, irinotecan.

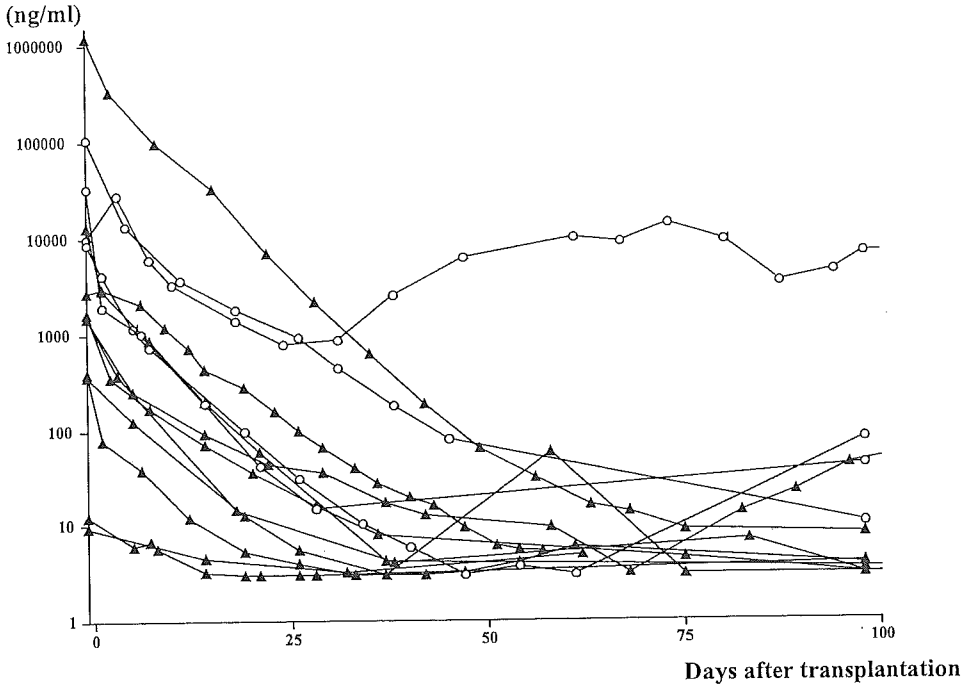

Figure 1: Serum alpha-feto level afte-fetoprotein levels after living-donor liver transplantation. Open circle: Child with tumor recurrence, Closed triangle: Child without tumor recurrence.

The post-LDLT course was uneventful in seven children. Two children required a laparotomy for intra-abdominal bleeding on day 6 (case 7) and intestinal perforation on day 6 (case 11), but then made a complete recovery. Five children (35.7%) had biliary complications. Three children with duct-to-duct biliary reconstruction showed biliary stricture (cases 9 and 10) and leakage (case 12). Two of them (cases 9 and 12) required conversion to Roux-en-Y biliary anastomosis on days 350 and 29, respectively. A further three patients with biliary stricture (cases 8, 10 and 13) were successfully managed with radiological intervention.

Five children (35.7%) experienced histologically proven acute cellular rejection, and this was successfully treated with steroid bolus injection therapy. Thereafter, no further maintenance doses of steroids were given in these cases. Cases 5 and 7 had achieved withdrawal from immunosuppressive therapy at postoperative years 5 and 3, respectively.

Postoperative adjuvant chemotherapy was performed in 13 patients (Table 2). CDDP (or CBDCA) and THP-ADR was used in four cases, CBDCA and etoposide in three, cyclophosphamide in two and irinotecan (CPT-11) in the remaining four. Postoperative chemotherapy was started on median day 33 (range: 0–150 days); this was dependent on the postoperative condition of the patient. Four patients (cases 1, 2, 3 and 11) died from tumor recurrence on days 280, 960, 330 and 202 after LDLT, respectively. Case 2, who showed tumor recurrence in the graft liver, received partial hepatectomy after high-dose chemotherapy and stem cell transplantation 16 months after LDLT. Cases 1, 3 and 11 showed multiple lung metastasis at postoperative months 6, 2 and 2, respectively.

Ten children are doing well, showing normal graft function and no evidence of tumor recurrence was found at a median follow-up after LDLT of 42 months. Postoperative AFP levels are shown in Figure 1. Serum AFP level was decreased in all of our cases just after LDLT. Consequently, actuarial 1- and 5-year graft and patient survival rates were 78.6% and 65.5%, respectively (Figure 2). The better patient survival was seen in the fetal type than the other types (100% vs. 50.0% in 5-year survival), however, no statistical difference was found among the histological types. There was no significant difference in the patient survival regarding PRETEXT grouping, history of previous hepatectomy, response to preoperative chemotherapy and serum AFP levels. However, children with macroscopic vascular extension and extrahepatic organ involvement were shown to have significantly worse patient 1- and 5-year survival rate: 33.3% vs. 90.9%, and 0% vs. 90.9% ($p < 0.01$), respectively.

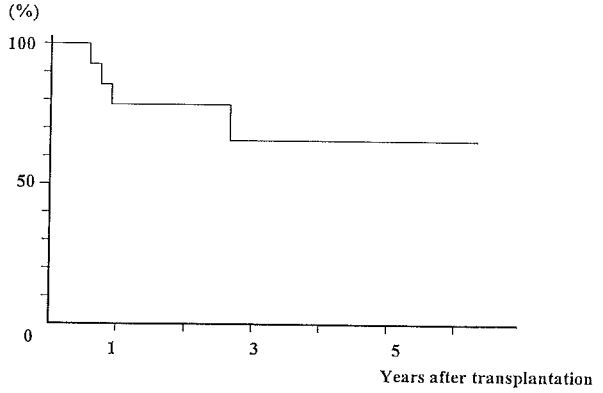

Figure 2: Patient survival after living-donor liver transplantation.

Discussion

The aim of this study was to evaluate the outcome of children who underwent LDLT as a curative treatment for hepatoblastoma at Kyoto University. To the best of our knowledge, this is the first report on long-term follow-up of LDLT for hepatoblastoma.

The administration of an optimal chemotherapy regimen such as the JPLT protocol in this study has substantially improved the outcome of children with hepatoblastoma with a 6-year survival rate of 73.4% (9). Although adjuvant chemotherapy makes the primary tumor resectable in 75–85% of the cases, factors still remain which limit the possibility of undergoing radical resection: invasion of main vascular trunks; extension of the tumor to all hepatic lobes; poor response to chemotherapy and intrahepatic recurrence (15). Based on the findings of a previous study that the multifocal hepatoblastoma may arise from multiple foci of genetically altered embryonal liver cells, total hepatectomy with liver transplantation might be an attractive treatment modality for unresectable hepatoblastoma (16).

In this study, LDLT was indicated in 14 children with unresectable hepatoblastoma with or without extrahepatic involvement after several courses of adjuvant chemotherapy. The actual 1- and 5-year survival rates are 78.6% and 65.5%, respectively, which are comparable with survival rates following both radical resection and deceased liver transplantation (6,9,15–18). Four children (28.6%) died from tumor recurrence after LDLT.

Patient selection might be crucial in determining the efficacy of liver transplantation for hepatoblastoma. PRETEXT grouping, history of previous hepatectomy, response to preoperative chemotherapy, histology of hepatoblastoma and serum AFP levels were not a prognostic factor in the present series. Contrary to the previous report from the Pittsburgh experience, macroscopic venous invasion (cases 2 and 11) and extrahepatic involvements (cases 3 and 11) were shown to be poor prognostic factors, with 1- and 5-year survival rates of 33.0% and 0%, respectively (17). The children without these factors showed an acceptable 5-year survival rate of 90.9%. Although the number of patients included in this study was limited and the follow-up period was too short to enable any valid conclusions to be drawn, these results suggest that macroscopic venous invasion and extrahepatic involvement at the time of transplantation might be considered as relative contraindications for liver transplantation in hepatoblastoma.

Postoperative biliary complications were observed in five children (35.7%), which is higher than the incidence in children undergoing LDLT for other indications (19). In this study, all of the children with duct-to-duct biliary reconstruction had biliary complications. Because of radical hilar lymph node dissection during transplant surgery, an insufficient blood supply to the native common bile duct may have induced an increased incidence of biliary complications in pediatric hepatoblastoma patients with duct-to-duct anastomosis. Our current preference is to use the Roux-en-Y hepaticojejunostomy for children with hepatoblastoma.

Postoperative immunosuppression consisted of tacrolimus monotherapy or tacrolimus with early steroid withdrawal in this study. Steroid-free immunosuppression may reduce opportunistic infections and malignancies in already immunocompromised children with hepatoblastoma due to reiterated preoperative chemotherapy (20,21). There is no difference among the type of immunosuppression received in post-transplant tumor recurrence, and the potential benefits of steroid-free immunosuppression on hepatoblastoma recurrence require further evaluation.

The necessity of post-transplant chemotherapy remains an open debate. It was reported that 30% of children with localized hepatoblastoma developed recurrent disease after complete resection (22). In the present series, 85.7% of the children had vascular invasion, and it is possible that manipulating of the malignant cells when handling the native liver during transplantation provoked micrometastasis. In this respect, post-transplant chemotherapy should be done in all patients. It is sometimes difficult to adapt the preoperative chemotherapy regimen postoperatively, because of side effects such as cardiac, renal and hearing impairment. Recently, the efficiency of irinotecan (CPT-11) for hepatoblastoma with significant anti-tumor effects was reported (23–26). We used novel protocol with irinotecan (CPT-11) in four patients, a patient who showed poor response to the preoperative chemotherapy regimen. The following irinotecan (CPT-11) regimen was used: irinotecan (CPT-11) infusion of 20 mg/m^2/day on days 1–5, repeated every 7 days for 2 weeks. A minimum of three cycles of chemotherapy was given post-transplantation. Whereas myelosuppression and diarrhea have been the

dose-limiting toxicities, all four children in the present series tolerated therapy, even when immunosuppressive therapy was administered (27).

Concern existed that survival was highly unlikely in the present children without liver transplantation, and that additional time without chemotherapy while waiting for the tumor to become evident would allow for the further dissemination of the tumor, which might then contraindicate transplant surgery. LDLT provides adequate timing of transplant surgery: in this study, the median interval between the last cycle of chemotherapy and LDLT was 23 days. Chemotherapy may play a role to prevent regrowth of the tumor while the child is awaiting transplantation (28).

Recently, Otte et al. reported the world experience of liver transplantation for hepatoblastoma, and demonstrated that the survival of 'primary' liver transplantation is superior to 'rescue' liver transplantation. Liver transplantation should be considered for every child presenting with unresectable disease (29). We agree with this suggestion that heroic attempts at partial hepatectomy surgery should be avoided. However, it was reported that children with recurrent hepatoblastoma could sometimes be treated with aggressive hepatectomy, and that high-dose chemotherapy with peripheral blood stem cell transplantation provided curative potential in some patients (9,30). The present study demonstrated that history of previous hepatectomy ('rescue' liver transplantation) was not a prognostic factor after LDLT. The results seen here suggest that aggressive hepatectomy following chemotherapy with or without peripheral blood stem cell transplantation might be one potential treatment modality prior to liver transplantation. The JPLT-2 protocol study using high-dose chemotherapy with stem cell rescue is currently underway to draw a definite conclusion about this treatment modality (9). Priority is given to aggressive hepatectomy combined with chemotherapy; accordingly, when conventional resection seems too difficult, the patient should be proposed for transplantation, as recommended by Pimpalwar et al. (15).

In conclusion, LDLT was indicated in 14 children with hepatoblastoma, but better results after LDLT are desirable. Macroscopic venous invasion and extrahepatic involvements at the time of transplantation might be considered as relative contraindications for liver transplantation in hepatoblastoma. Extensive investigations are essential to understand the factors leading to tumor recurrence after LDLT and, more importantly, to identify prognostic factors in children with hepatoblastoma.

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PHARMACODYNAMICS AND DRUG ACTION

Pharmacodynamic analysis of tacrolimus and cyclosporine in living-donor liver transplant patients

Background: The calcineurin inhibitors tacrolimus and cyclosporine (INN, ciclosporin) have been widely used to prevent allograft rejection after transplantation. We investigated pharmacodynamic properties of the 2 drugs and their clinical relevance in liver transplantation.

Methods: Forty de novo living-donor liver transplant patients participated in this study, and they were treated with either tacrolimus (N = 30) or cyclosporine (N = 10). We simultaneously measured blood drug concentrations and calcineurin phosphatase activity in peripheral blood mononuclear cells during the first 14 postoperative days. Nephrotoxicity and acute rejection were also examined in relation to the blood drug concentrations and calcineurin activity.

Results: Calcineurin activity was only partially inhibited by tacrolimus concentrations greater than 20 ng/mL, although it could be almost completely inhibited by cyclosporine concentrations greater than 700 ng/mL. According to a maximum effect model, the population mean estimates of the EC_{50} (blood concentration that yields a half-maximal effect) for tacrolimus and cyclosporine were 26.4 ng/mL (95% confidence interval [CI], 15.7-37.1 ng/mL) and 200 ng/mL (95% CI, 127-274 ng/mL), respectively. Patients with nephrotoxicity in both groups had significantly higher trough concentrations compared with those without this adverse event. In addition, patients with acute rejection in the tacrolimus group had significantly lower trough concentrations and higher calcineurin activity than those without a rejection episode.

Conclusions: The inhibitory effects on calcineurin activity in peripheral blood mononuclear cells differed between tacrolimus and cyclosporine in living-donor liver transplant patients. Pharmacodynamic assessment in combination with blood concentration monitoring may be useful for determining the individual therapeutic range of tacrolimus and cyclosporine. (Clin Pharmacol Ther 2005;78:168-81.)

Masahide Fukudo, MS, Ikuko Yano, PhD, Satohiro Masuda, PhD, Sachio Fukatsu, BS, Toshiya Katsura, PhD, Yasuhiro Ogura, MD, Fumitaka Oike, MD, Yasutsugu Takada, MD, Koichi Tanaka, MD, and Ken-ichi Inui, PhD *Kyoto, Japan*

Living-donor liver transplantation is now acknowledged as a life-saving therapy for patients with end-stage liver failure. Tacrolimus and cyclosporine (INN, ciclosporin) have been cornerstone immunosuppressants in the prevention of acute rejections after liver transplantation.[1,2] They have a similar mechanism of action involving the formation of a complex with their

From the Department of Pharmacy, Kyoto University Hospital, and Department of Transplantation and Immunology, Graduate School of Medicine, Kyoto University.

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Reprint requests: Ken-ichi Inui, PhD, Department of Pharmacy, Kyoto University Hospital, Sakyo-ku, Kyoto 606-8507, Japan.

E-mail: *inui@kuhp.kyoto-u.ac.jp*

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respective binding proteins, immunophilins—FK506-binding protein for tacrolimus and cyclophilin for cyclosporine. Subsequently, the drug-immunophilin complexes bind to and inhibit the activity of the Ca^{++}- and calmodulin-dependent protein phosphatase calcineurin, which is a key enzyme of the rate-limiting step in the activation of T lymphocytes.[3-5]

Because tacrolimus and cyclosporine have a narrow therapeutic range and show large interindividual and intraindividual pharmacokinetic variability, therapeutic drug monitoring of trough blood concentrations (C_0) is necessary to avoid adverse effects.[6,7] Despite C_0 levels within therapeutic range, acute rejection or infections still occur in some patients. Recently, a new monitoring strategy based on blood concentrations 2 hours after dosing (C_2) of cyclosporine has been clinically validated in liver transplant patients and has been suggested to be more effective for predicting cyclosporine exposure and risk of rejection than the traditional C_0 monitoring.[8,9] More recently, the LIS2T study (Liver Investigational Study of Neoral C2 vs Tacrolimus) comparing cyclosporine with C_2 monitoring and tacrolimus with C_0 monitoring has demonstrated that both drugs are effective primary immunosuppressants in liver transplantation.[10] However, it is essentially difficult to determine the optimal therapeutic range of these drugs. Therefore pharmacodynamic assessment in combination with the classical blood concentration monitoring may be useful in defining an effective and safe therapeutic range for an individual patient treated with a calcineurin inhibitor.

The strategy for evaluating the pharmacologic effects of tacrolimus and cyclosporine includes measuring calcineurin phosphatase activity in circulating blood.[11] We have recently clarified that the properties of calcineurin inhibition in whole blood differ between tacrolimus and cyclosporine in rats.[12] Batiuk et al[13]and Halloran et al[14] have extensively examined the pharmacodynamics of cyclosporine in peripheral blood leukocytes and suggested that calcineurin activity is closely related to blood cyclosporine concentrations in kidney transplant patients. However, limited information is available on the relationship between blood tacrolimus concentrations and calcineurin activity in transplant recipients. It has been reported that blood tacrolimus concentrations did not correlate well with calcineurin activity in whole blood in renal transplant patients.[15] In contrast, Blanchet et al[16] showed a good correlation between calcineurin activity in lymphocytes and blood tacrolimus concentrations measured at 2 hours after dosing in liver transplant patients. Moreover, little is known about the degree of interindividual and intraindividual variability in calcineurin inhibition by tacrolimus and cyclosporine.

In this study we investigated the relationship between calcineurin phosphatase activity in peripheral blood mononuclear cells (PBMCs) and blood drug concentrations of tacrolimus and cyclosporine in living-donor liver transplant patients to compare the pharmacodynamic properties of the 2 drugs. Furthermore, nephrotoxicity and acute rejection after suboptimal treatment with tacrolimus or cyclosporine were examined in relation to the pharmacokinetics and pharmacodynamics of each drug.

METHODS

Patients and immunosuppressive therapy

Forty de novo living-donor liver transplant patients were enrolled in this study, and they were treated with either tacrolimus (N = 30) or cyclosporine (N = 10). All of the patients underwent living-donor liver transplantation between September 2003 and September 2004 at the Department of Transplantation and Immunology, Kyoto University Hospital, Kyoto, Japan. This study was performed in accordance with the Declaration of Helsinki and its amendments and was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee. Written informed consent was obtained from each patient, and the study was conducted as part of treatment.

Within 24 hours after liver transplantation, immunosuppression was started with a combination of tacrolimus or cyclosporine and low-dose corticosteroids. Because tacrolimus has been a primary immunosuppressant in our living-donor liver transplant program, it was more likely to be used than cyclosporine in this study. Tacrolimus (Prograf; Fujisawa Pharmaceutical, Osaka, Japan) was orally administered at a dose of 0.05 $mg \cdot kg^{-1} \cdot d^{-1}$ twice daily (at 9 AM and 9 PM). According to the physicians' decision, cyclosporine (Neoral; Novartis Pharma KK, Tokyo, Japan) was orally administered at a dose of 8 $mg \cdot kg^{-1} \cdot d^{-1}$ twice daily (at 9 AM and 9 PM) for patients undergoing liver transplantation before July 2004 (n = 6) and was given once daily (at 9 AM) to improve the absorption profile for those undergoing liver transplantation thereafter (n = 4). Blood samples for the daily C_0 monitoring of tacrolimus and cyclosporine were drawn into ethylenediaminetetraacetic acid–containing tubes before the morning dose (at 8 AM) starting on postoperative day 2. We routinely monitored C_2 levels of cyclosporine in the morning to evaluate drug absorption. The dosage of calcineurin inhibitors was adjusted to achieve target blood drug concentrations. The target C_0 level of ta-

Table I. Demographics and clinical data of living-donor liver transplant patients receiving tacrolimus or cyclosporine

	Tacrolimus (N = 30)	*Cyclosporine (N = 10)*
Demographics		
Gender (male/female)	17:13	6:4
Age (y)	48 ± 14	46 ± 13
Body weight (kg)	64.4 ± 15.4	59.1 ± 9.3
Grafted liver weight (g)	718 ± 132	599 ± 148*
GRWR (%)	1.13 ± 0.27	1.01 ± 0.19
ABO blood group match		
Identical	17	6
Compatible	4	3
Incompatible	9	1
Primary disease		
Hepatitis virus infection (HBV/HCV)	20 (9:11)	7 (4:3)
Biliary atresia	1	1
Primary sclerosing cholangitis	3	0
Primary biliary cirrhosis	1	2
Fulminant hepatic failure	1	0
Other	4	0
Clinical laboratory data at baseline		
Albumin (g/dL)	3.1 (2.2-4.6)	3.6 (2.8-6.1)
Total bilirubin (mg/dL)	4.8 (0.5-29.6)	2.5 (0.6-10.2)
AST (IU/L)	92 (21-597)	42 (28-219)
ALT (IU/L)	44 (12-811)	28 (12-220)
γ-Glutamyl transpeptidase (IU/L)	41 (18-199)	40 (15-463)
Serum creatinine (mg/dL)	0.8 (0.3-1.3)	0.6 (0.5-3.0)
Creatinine clearance (mL/min)	67 (28-118)	70 (55-142)
Blood glucose (mg/dL)	117 (49-265)	86 (78-235)
Pharmacokinetic data		
No. of blood concentration measurements	385	198 (114/84)†
C_0 level (ng/mL)	9.6 ± 4.7	277 ± 145
C_2 level (ng/mL)	NA	603 ± 349
Pharmacodynamic data		
No. of calcineurin activity measurements	406	201 (118/83)†
Baseline calcineurin activity (pmol · min^{-1} · mg $protein^{-1}$)	61.9 ± 13.5	64.4 ± 10.9

Data are expressed as mean ± SD or median and range, depending on data type.
GRWR, Graft-to-recipient weight ratio; HBV/HCV, hepatitis B virus/hepatitis C virus; C_0, trough blood concentration; C_2, blood concentration 2 hours after dosing; NA, not applicable.
*$P < .05$, significantly different from tacrolimus arm (Mann-Whitney U test).
†The left and right numbers in parentheses denote the number of measurements at the trough time point and at 2 hours after dosing of cyclosporine, respectively.

crolimus was set between 5 and 15 ng/mL during the first month. The target C_2 level of cyclosporine was set between 600 and 1000 ng/mL for the first month. When the C_0 level of cyclosporine exceeded 300 ng/mL, the cyclosporine dosage was appropriately reduced. For corticosteroid administration during the first month, the initial dose of intravenous methylprednisolone was 1 mg · kg^{-1} · d^{-1} on postoperative days 1 to 3 and the dosage was reduced to 0.5 mg · kg^{-1} · d^{-1} on postoperative days 4 to 6 and to 0.3 mg · kg^{-1} · d^{-1} on postoperative day 7. Thereafter, oral prednisolone was administered at a dose of 0.3 mg · kg^{-1} · d^{-1} on postoperative days 8 to 28. Clinical laboratory test markers were also measured daily in the morning after liver transplantation. The patients' demographics and clinical data in both treatment arms are summarized in Table I.

Calcineurin phosphatase activity in PBMCs was measured as an index of the pharmacologic effects of tacrolimus and cyclosporine. On the day of transplantation, we determined the baseline activity before administration of each drug. On the basis of the fact that most first acute rejections occurred within 2 weeks of transplantation,[17] the enzyme activity was measured in

parallel with the therapeutic drug monitoring everyday during the first 2 weeks after transplantation. When a limited volume of blood sample was obtained from patients, calcineurin activity could not be determined with the sample.

Measurement of drug concentrations and calcineurin phosphatase activity

The concentrations of tacrolimus and cyclosporine in whole blood were measured with a microparticle enzyme immunoassay method by use of an IMx analyzer (Abbott Japan, Tokyo, Japan) and with a fluorescence polarization immunoassay method by use of a TDx analyzer (Abbott Japan), respectively. All samples were assayed on the day of blood collection. The remnant (approximately 2 mL) was subsequently diluted with the same volume of phosphate-buffered saline solution to isolate PBMCs by Ficoll-Paque Plus (Amersham Biosciences, Uppsala, Sweden). The contaminating red blood cells were removed with red blood cell lysis buffer (Roche Diagnostics KK, Tokyo, Japan). All isolation procedures were performed at room temperature within 12 hours after blood sampling. The collected mononuclear cells were lysed with ice-cold lysis buffer containing protease inhibitors as described previously.[12] After centrifugation at 10,000*g* for 10 minutes at 4°C, the resulting supernatants were used for the measurement of calcineurin phosphatase activity.

The assay was performed by use of [γ–phosphorus 32] regulatory subunit type II (RII) phosphopeptide, consisting of 19 amino acids (Asp-Leu-Asp-Val-Pro-Ile-Pro-Gly-Arg-Phe-Asp-Arg-Arg-Val-Ser-Val-Ala-Ala-Glu), as a substrate, according to a procedure described previously.[12] In brief, total phosphatase activity was measured in a Ca^{++} assay buffer containing 2.5-mmol/L calcium chloride and 500-nmol/L okadaic acid to inhibit protein phosphatase types 1 and 2A. The radioactivity of ^{32}P released during a 20-minute incubation was determined by liquid scintillation counting, and the phosphatase activity was expressed as picomoles of phosphate released per minute per milligram protein. Background activity resulting from protein phosphatase type 2C, measured in a Ca^{++}-free assay buffer containing 5.0-mmol/L EGTA (ethylene glycol-O,O′-*bis*-[2-amino-ethyl]-*N*,*N*,*N*′,*N*′-tetraacetic acid) instead of calcium chloride and 500-nmol/L okadaic acid under the same assay conditions as for the Ca^{++} assay buffer, was subtracted from the total phosphatase activity. The Ca^{++}-sensitive phosphatase activity was taken as calcineurin phosphatase activity.

Evaluation of drug effects in vitro

To evaluate the dose-dependent inhibition of tacrolimus and cyclosporine on calcineurin phosphatase activity in PBMCs, we performed experiments in vitro using blood samples from a healthy volunteer. In brief, injection solutions of tacrolimus (Prograf, 5 mg/mL) and cyclosporine (Sandimmun [Novartis Pharma KK], 50 mg/mL) were serially diluted with saline solution to yield final concentrations of 1, 10, 30, 100, and 1000 ng/mL for tacrolimus and 10, 100, 300, 1000, and 10,000 ng/mL for cyclosporine. Then 100 μL of saline solution containing tacrolimus or cyclosporine was added to 900 μL of ethylenediaminetetraacetic acid–containing whole blood (final concentration of ethanol, 0.1%). The same volume of saline solution containing ethanol alone was added to control blood to yield a final concentration of 0.1%. After a 1-hour incubation at 37°C with gentle shaking, calcineurin phosphatase activity in PBMCs was measured as described earlier. The concentration causing 50% inhibition (IC_{50}) was determined by nonlinear regression analysis.

Pharmacodynamic analysis

Because we could not investigate whether hysteresis was detectable in the pharmacologic effects of tacrolimus and cyclosporine during the sampling of C_0 or C_2 levels, we assumed that the pharmacologic effects were directly related to the blood drug concentrations. For tacrolimus, data from all patients (N = 30), measured on the day of transplantation and at the trough time point during the first 14 postoperative days, were examined. For cyclosporine, data from all patients (N= 10), measured on the day of transplantation and at the trough time point, as well as at 2 hours after dosing during the first 14 postoperative days, were examined. The relationship between the blood concentration of tacrolimus or cyclosporine and calcineurin phosphatase activity in PBMCs was analyzed with the following maximum effect (E_{max}) model, by use of the nonlinear mixed-effect modeling program NONMEM, by the first-order conditional estimation (FOCE) method[18]:

$$CaN = CaN_0 - (E_{max} \cdot C_b)/(EC_{50} + C_b)$$

where CaN is calcineurin activity at blood concentration C_b, CaN_0 is the baseline activity measured on the day of transplantation before drug administration, E_{max} is the maximum inhibitory effect attributable to the drug, and EC_{50} is the C_b value that yields a half-maximal effect. For the error model, the interindividual variability for pharmacodynamic parameters (E_{max} and EC_{50}) and residual variability were assumed to be log-normally and normally distributed, respectively. The mag-

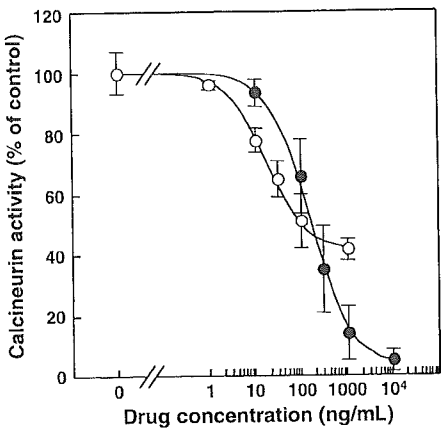

Fig 1. Concentration-dependent inhibition of calcineurin phosphatase activity in peripheral blood mononuclear cells (PBMCs) by tacrolimus and cyclosporine. Whole blood taken from a healthy volunteer was incubated with saline solution containing various concentrations of tacrolimus (*open circles*) or cyclosporine (*solid circles*) at 37°C with gentle shaking. After a 1-hour incubation, PBMCs were isolated from the blood samples by Ficoll-Hypaque gradient centrifugation (Ficoll-Paque Plus). Calcineurin phosphatase activity in PBMCs was measured by use of [γ–phosphorus 32] regulatory subunit type II (RII) phosphopeptide as a substrate. Data are shown as a percentage of the control activity measured in blood samples that had been treated with saline solution containing vehicle alone. Each *circle* represents the mean (±SD) of 3 independent experiments.

nitude of interindividual and residual variability in the error model was expressed as the percent coefficient of variation (% CV) and SD of enzyme activity (in picomoles per minute per milligram protein), respectively.

Clinical outcome analysis

Safety and efficacy of tacrolimus and cyclosporine were investigated in relation to blood drug concentrations and calcineurin phosphatase activity in PBMCs during the first 2 weeks after transplantation. Safety was evaluated by the adverse event of nephrotoxicity, which was defined as an initial increase in serum creatinine concentration of 0.5 mg/dL or greater above baseline. Efficacy was evaluated by acute rejection episode, which was defined as clinically and biochemically suspected rejections that were initially treated with steroid pulse therapy. To avoid the potential influence of preoperative renal dysfunction and mismatching of ABO blood group on these outcomes, we excluded 1 patient who had renal dialysis before the transplant in the cyclosporine treatment arm and 10 patients receiving ABO-incompatible transplantation (9 in the tacrolimus group and 1 in the cyclosporine group). For patients who had an episode of nephrotoxicity or acute rejection, data regarding blood drug concentrations and calcineurin activity measured for 3 days immediately before the onset of each event were used to calculate the individual average values. For control patients without an episode of nephrotoxicity or acute rejection, data regarding blood drug concentrations and calcineurin activity available during 3 days immediately before the mean postoperative day of the onset of each event were used to calculate the individual average values.

Statistical analysis

Data were presented as mean ± SD or median and range, depending on data type. The differences in mean values between 2 groups were statistically examined with the unpaired t test. The statistical significance of differences in nonparametric values between 2 groups was analyzed with the Mann-Whitney U test or the Wilcoxon test. The Fisher exact probability test was used to compare the proportion of patients with a given characteristic between 2 groups. $P < .05$ was considered statistically significant. For the estimation of pharmacodynamic parameters, the statistical significance of the parameters was evaluated with the likelihood ratio test. A difference in the objective function (−2 log-likelihood difference [−2 LLD]) of more than 7.88, with 1 *df*, was considered statistically significant ($P < .005$).

RESULTS

In vitro effects of tacrolimus and cyclosporine on calcineurin phosphatase activity

Specific calcineurin phosphatase activity was measured in PBMCs from a healthy volunteer (105 ± 7 pmol · min^{-1} · mg protein^{-1} [mean ± SD], n = 3). Background activity resulting from protein phosphatase type 2C was almost negligible compared with basal calcineurin activity in the healthy volunteer (4 ± 3 pmol · min^{-1} · mg protein^{-1}, n = 3). No remarkable change in enzyme activity was observed for up to 48 hours when the blood samples were stored at room temperature after being collected. The values for inter-assay and intra-assay variability were 2.6% and 1.2% (% CV), respectively. Both tacrolimus and cyclosporine inhibited enzyme activity in a concentration–dependent manner under in vitro conditions (Fig 1). The IC_{50} values for blood concentrations of tacrolimus and cyclosporine were calculated as 18.9 ± 4.6 ng/mL and 181 ± 74 ng/mL (mean ± SD), respectively. At high drug concentrations in whole blood (≥1000 ng/

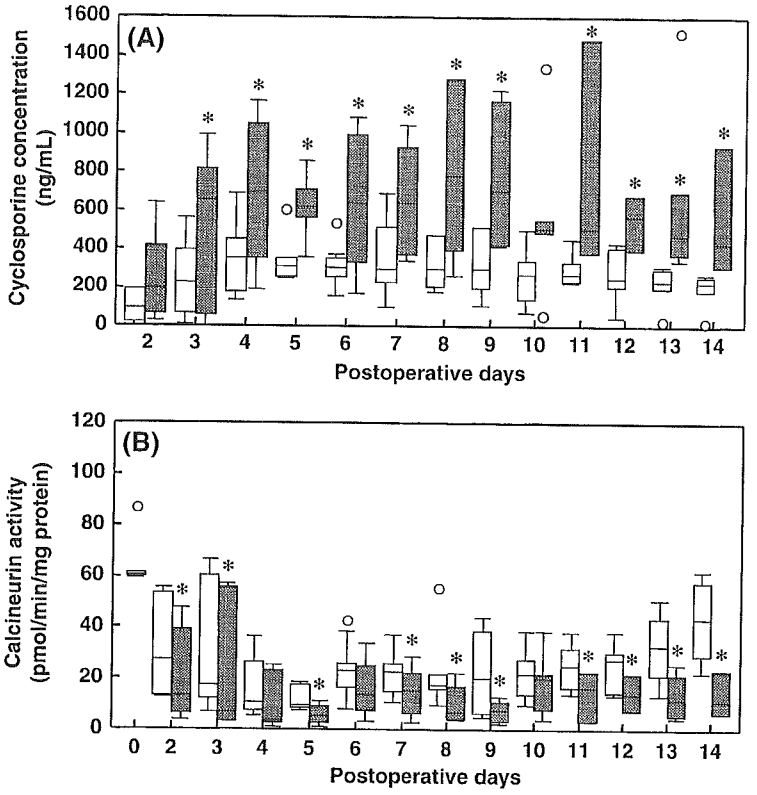

Fig 2. Blood cyclosporine concentration (**A**) and calcineurin phosphatase activity in PBMCs (**B**) during first 14 days after living-donor liver transplantation (N = 10). *Open* and *shaded boxes* represent data measured at the trough time point and at 2 hours after dosing of cyclosporine, respectively. Each *box plot* includes the median (*horizontal line*), length of the interquartile range r (*box*), whisker at greatest value lower than or equal to upper limit of interquartile range + 1.5r, whisker at lowest value greater than or equal to lower limit of interquartile range − 1.5r, and all outliers beyond the whiskers (*open circles*). *Asterisk*, $P < .05$, significantly different from data measured at the trough time point on the same postoperative day (Wilcoxon test).

mL), the inhibition of calcineurin activity by cyclosporine was almost complete, although the enzyme activity was only partially inhibited by tacrolimus (Fig 1).

Blood drug concentrations and calcineurin phosphatase activity after transplantation

Cyclosporine. Blood concentrations of cyclosporine gradually increased and showed substantial interindividual variability after liver transplantation (Fig 2, *A*). During the first 14 postoperative days, 42% of cyclosporine C_2 measurements were within the target range, although 36% of the C_0 measurements were greater than 300 ng/mL. By postoperative day 3, only 50% of patients had attained a C_0 level greater than 200 ng/mL, and the target C_2 level was achieved in 60% of patients. The median calcineurin activity at the trough time point abruptly decreased to less than half of the median activity at baseline on postoperative days 2 and 3 (Fig 2, *B*). Furthermore, the enzyme activity at 2 hours after dosing of cyclosporine was significantly lower than that at the trough time point on the same postoperative day. Thereafter the C_0 level was maintained between 200 and 400 ng/mL, and the median C_2 level was within the range of 600 to 800 ng/mL and decreased to around 500 ng/mL by postoperative day 14. The median calcineurin activity at the trough time point remained low relative to the median baseline activity. About half of the measurements of calcineurin activity at the trough time point were lower than 20 pmol · min^{-1} · mg $protein^{-1}$ within the first 2 weeks of transplantation. Furthermore, the enzyme activity at the trough time point was significantly suppressed at 2 hours after the administration on each postoperative day, except

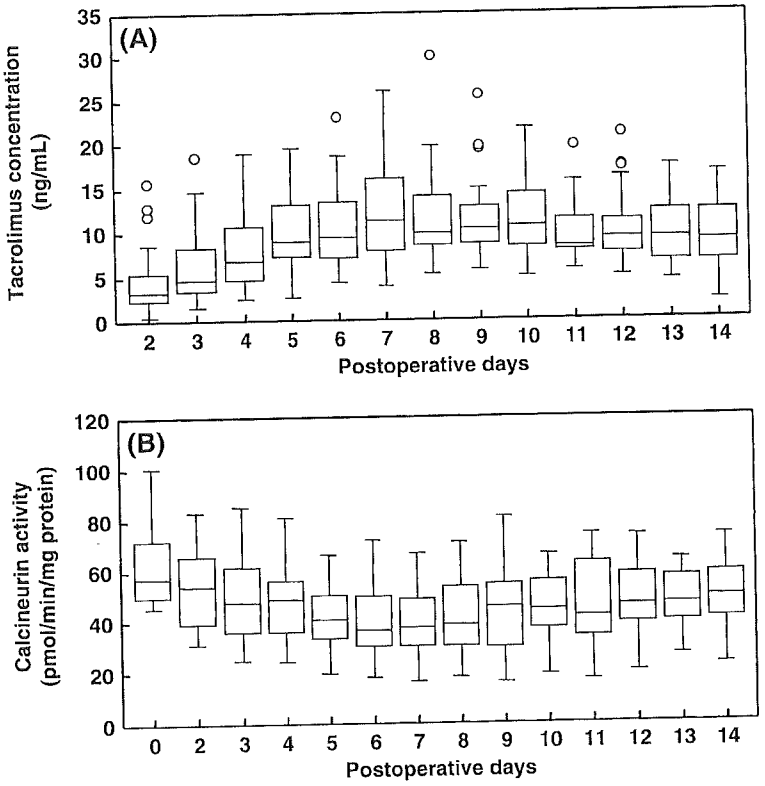

Fig 3. Blood tacrolimus concentration (**A**) and calcineurin phosphatase activity in PBMCs (**B**) during first 14 days after living-donor liver transplantation (N = 30). *Open boxes* represent data measured at the trough time point after dosing of tacrolimus. Each *box plot* includes the median (*horizontal line*), length of the interquartile range r (*box*), whisker at greatest value lower than or equal to upper limit of interquartile range + 1.5r, whisker at lowest value greater than or equal to lower limit of interquartile range − 1.5r, and all outliers beyond the whiskers (*open circles*).

for days 4, 6, and 10 of transplantation. Approximately 80% of the measurements of calcineurin activity at 2 hours after dosing fell below 20 pmol · min^{-1} · mg $protein^{-1}$ during the first 14 days after liver transplantation. Moreover, the calcineurin activity at 2 hours after treatment was less variable than the corresponding blood cyclosporine concentrations.

Tacrolimus. During the first 14 postoperative days, 72% of tacrolimus C_0 measurements were within the target range and only 10 measurements from 6 patients exceeded 20 ng/mL (Fig 3, *A*). The calcineurin activity gradually decreased according to the increase in trough blood concentrations immediately after liver transplantation (Fig 3, *B*). By postoperative day 7, 90% of patients had reached the target C_0 level. The calcineurin activity was lowest around 1 week after transplantation (median, 37.5 pmol · min^{-1} · mg $protein^{-1}$ on day 7), although the enzyme activity between day 7 and the other days (days 5, 6, and 8-12) was not statistically different. During postoperative days 8 to 14, most of the trough concentrations were controlled at between 5 and 15 ng/mL, and the median calcineurin activity was within the range of 40 to 50 pmol · min^{-1} · mg $protein^{-1}$. Moreover, only 9 measurements of the enzyme activity from 5 patients fell below 20 pmol · min^{-1} · mg $protein^{-1}$ within the first 2 weeks of transplantation. Extensive interindividual variability was observed in the calcineurin activity, as well as in the blood drug concentrations, as demonstrated by the large differences between the minimal and maximal measurements on each postoperative day.

Relationship between blood drug concentrations and calcineurin phosphatase activity

Baseline characteristics did not differ between the tacrolimus and cyclosporine treatment groups except

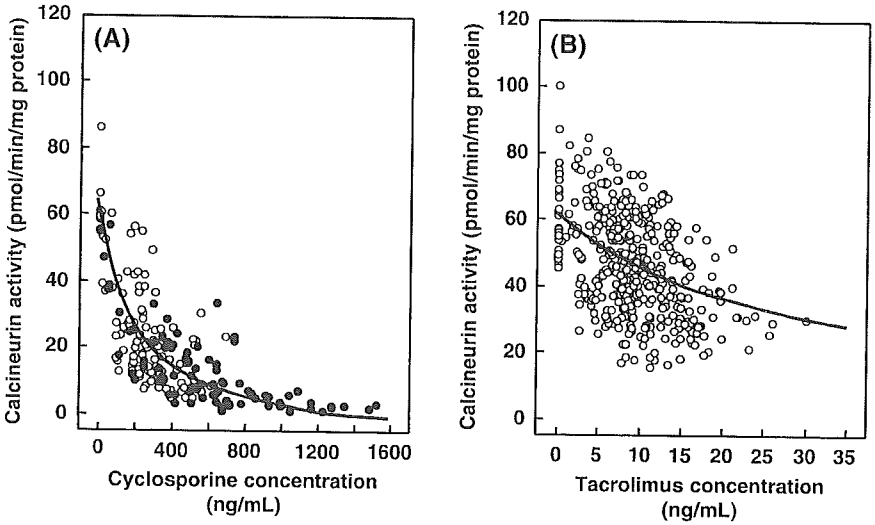

Fig 4. Relationship between calcineurin phosphatase activity in PBMCs and blood drug concentration of cyclosporine (**A**) (N = 10) or tacrolimus (**B**) (N = 30) in living-donor liver transplant patients. *Open* and *closed circles* represent data measured at the trough time point and at 2 hours after administration, respectively. The *bold lines* show the predicted calcineurin phosphatase activity versus blood drug concentration profile by use of the pharmacodynamic parameter mean estimates (shown in Table II) and the mean value of baseline calcineurin activity in each treatment arm.

Table II. Pharmacodynamic parameters of cyclosporine and tacrolimus in living-donor liver transplant patients

Parameter	*Cyclosporine (N = 10)*	*Tacrolimus (N = 30)*
Mean parameter and 95% CI		
E_{max} (pmol · min^{-1} · mg protein^{-1})	74.2 (63.6-84.8)	59.8 (49.5-70.1)
EC_{50} (ng/mL)	200 (127-274)	26.4 (15.7-37.1)
Interindividual and residual variability and 95% CI		
ω_{EC50} (%)	84.0 (21.3-117)	81.4 (49.8-104)
σ (pmol · min^{-1} · mg protein^{-1})	8.5 (5.9-10.4)	8.6 (7.7-9.5)

CI, Confidence interval; E_{max}, maximum effect; EC_{50}, blood concentration that yields half-maximal effect; ω_{EC50}, interindividual variability in EC_{50}; σ, residual variability.

for grafted liver weight (Table I). We used a simple E_{max} model to describe the concentration and response relationship of tacrolimus and cyclosporine. The final estimates of the pharmacodynamic parameters are shown in Table II. Because the interindividual variability for the E_{max} of the 2 drugs was not significantly different from 0, it was not incorporated into the final model. The calcineurin activity in patients receiving cyclosporine showed a steep decline according to the increase in blood drug concentrations and reached a plateau above a specific blood concentration, approximately 700 ng/mL (Fig 4, *A*). The population mean estimate of the EC_{50} for cyclosporine was 200 ng/mL (95% confidence interval [CI], 127-274 ng/mL), and large interindividual variability in the EC_{50} value was found (mean % CV, 84.0%). Although a marked variability in the calcineurin activity was observed around the EC_{50} value, the enzyme activity converged to a minimal level at high blood cyclosporine concentrations. On the other hand, tacrolimus showed less dynamic change in its effect on calcineurin activity with the increase of the blood drug concentration than did cyclosporine (Fig 4, *B*). The population mean estimate of the EC_{50} for tacrolimus was 26.4 ng/mL (95% CI, 15.7-37.1 ng/mL), and extensive interindividual variability in the EC_{50} value was again identified (mean % CV, 81.4%). In addition, remarkable variability in the level of calcineurin activity was evident within the

Table III. Safety and efficacy of cyclosporine and tacrolimus in living-donor liver transplant patients

	Cyclosporine (n = 8)	*Tacrolimus (n = 21)*	*P value**
Nephrotoxicity			
Incidence	4 (50%)	8 (38%)	NS
Time to event (d)	8 ± 2 (6-9)	8 ± 2 (6-11)	
Acute rejection			
Incidence	2 (25%)	5 (24%)	NS
Time to event (d)	14† (11-16)	9 ± 3 (6-13)	

Data are expressed as number of patients and percent or mean ± SD and range.
NS, Not significant.
*Statistical significance was examined with the Fisher exact probability test.
†The mean value alone is indicated.

therapeutic range (5-15 ng/mL). Even at trough blood concentrations higher than 20 ng/mL, the enzyme activity was not completely inhibited by tacrolimus. Residual variability in both treatment arms was relatively large compared with the assay variability.

To evaluate the significance of the E_{max} model for tacrolimus and cyclosporine, the EC_{50} value was set to nearly 0 (10^{-4}) or an infinite value (10^{4}). The value of −2 LLD increased by more than 7.88 in each hypothesis for both drugs. Therefore we confirmed that the estimated EC_{50} values were significantly different from 0 and the E_{max} model for tacrolimus and cyclosporine was significantly better in terms of goodness of fit compared with a linear model.

Clinical outcome and its relationship with pharmacokinetics and pharmacodynamics

Cyclosporine. After 11 patients were ruled out on the basis of the exclusion criteria, baseline characteristics were again similar in the tacrolimus and cyclosporine treatment groups, with the exception of the greater grafted liver weight in patients receiving tacrolimus. The incidence of nephrotoxicity or acute rejection was not significantly different between the 2 groups (Table III). The mean C_0 level, but not C_2 level, of cyclosporine in patients with nephrotoxicity was significantly higher than in those without this adverse event (Fig 5, *A*). The mean values for calcineurin activity at the trough time point and at 2 hours after dosing were comparable between the patients with and without nephrotoxicity (Fig 5, *A*). Only 2 patients had an acute rejection episode during cyclosporine therapy. In one patient the administration of cyclosporine was interrupted because of an infectious event on postoperative day 8. In the other patient difficulty in achieving adequate blood cyclosporine concentrations occurred as a result of poor absorption, and no remarkable calcineurin inhibition was observed in comparison with the baseline activity for the initial few days after transplantation. The C_0 and C_2 levels from the 2 patients were both subtherapeutic, although most patients without acute rejection had high C_2 levels and C_0 levels within the therapeutic range (Fig 5, *B*). In addition, the mean calcineurin activity in patients with no rejection episode was lower than in the 2 patients who had acute rejection and remained below 20 pmol · min^{-1} · mg $protein^{-1}$ at both the trough time point and 2 hours after dosing (Fig 5, *B*).

Tacrolimus. The mean C_0 level of tacrolimus and the corresponding calcineurin activity in patients with nephrotoxicity were significantly different from those in patients without this adverse event (Fig 6, *A*). Five patients had an acute rejection episode during tacrolimus therapy. In 3 of these patients the individual C_0 levels were still within the therapeutic range (5-15 ng/mL). The mean C_0 level of tacrolimus in patients with acute rejection was significantly lower than in those without a rejection episode (Fig 6, *B*). Moreover, the mean calcineurin activity in patients who had acute rejection was significantly higher than in those with no rejection episode, whereas large interindividual variability was evident (Fig 6, *B*).

DISCUSSION

This is the first study to compare the inhibitory effects of tacrolimus and cyclosporine on calcineurin phosphatase activity in PBMCs in the setting of living-donor liver transplantation. Furthermore, we have investigated the clinical relevance of pharmacokinetics and pharmacodynamics of the 2 drugs in terms of nephrotoxicity and acute rejection during the first 2 weeks after liver transplantation.

Using blood samples from a healthy volunteer, we first examined the in vitro concentration-dependent inhibition of calcineurin activity by tacrolimus and cyclosporine in PBMCs (Fig 1). The mean IC_{50} value of cyclosporine was comparable to that in 5 healthy volunteers reported by Caruso et al.[19] The mean IC_{50} value

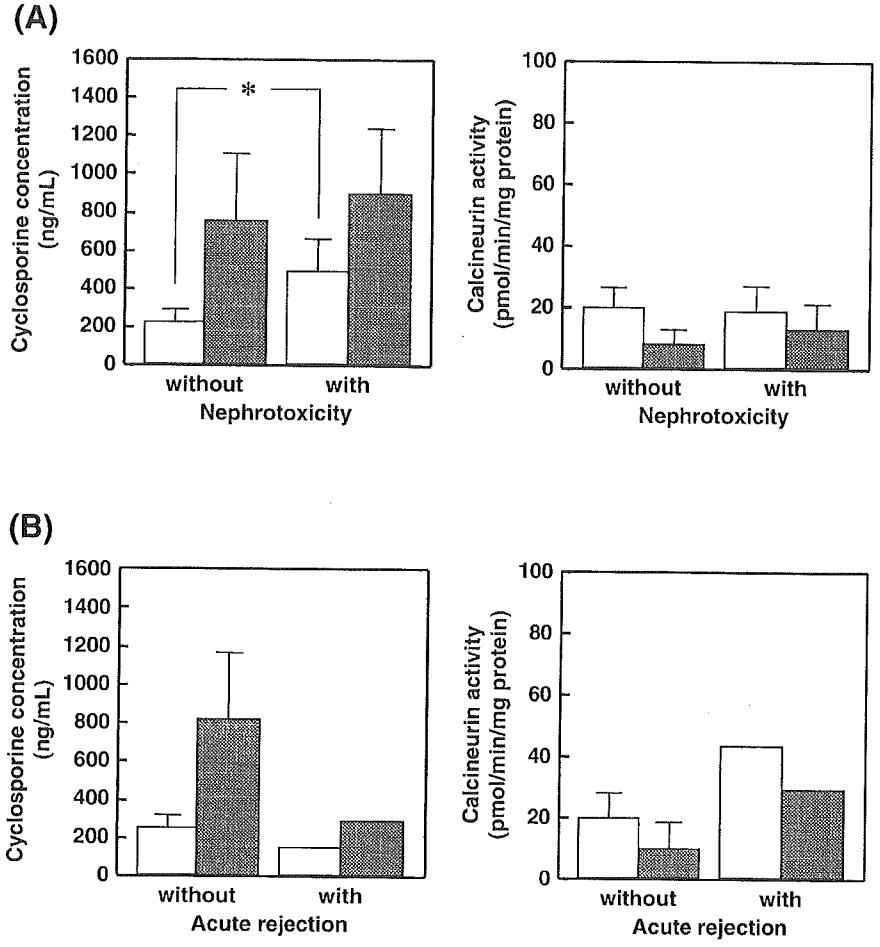

Fig 5. Blood cyclosporine concentration and calcineurin phosphatase activity in PBMCs in living-donor liver transplant patients (n = 8) with and without nephrotoxicity (**A**) or acute rejection (**B**). *Open* and *shaded columns* represent data measured at the trough time point and at 2 hours after dosing, respectively. Each column indicates the mean ± SD. For data from 2 patients with acute rejection, the mean value alone is indicated by each column. *Asterisk*, $P < .05$, significantly different from the mean value in patients without nephrotoxicity (unpaired *t* test).

of tacrolimus was approximately 10 times smaller than that of cyclosporine, indicating that tacrolimus is more potent than cyclosporine in calcineurin inhibition. However, at higher blood drug concentrations (≥1000 ng/mL), the inhibition by tacrolimus was incomplete whereas that by cyclosporine was almost complete. It has been reported that calcineurin inhibition by tacrolimus can be increased by the addition of exogenous FK506-binding protein.[20] A possible explanation for the incomplete inhibition by tacrolimus is that FK506-binding protein in PBMCs may be limiting.

In patients treated with tacrolimus, the calcineurin activity gradually decreased according to the increase in the trough blood concentration after liver transplantation (Fig 3). On the other hand, the enzyme activity abruptly decreased in the cyclosporine treatment group immediately after transplantation and tended overall to be lower than that in the tacrolimus treatment group (Fig 2). These findings may be supported by the observations from in vitro experiments that cyclosporine could reduce the enzyme activity more effectively than tacrolimus within the respective therapeutic ranges (Fig 1). The population mean estimates of EC_{50} for tacrolimus and cyclosporine in liver transplant patients agreed well with the respective IC_{50} values obtained in a healthy volunteer (Table II). Furthermore, the mean

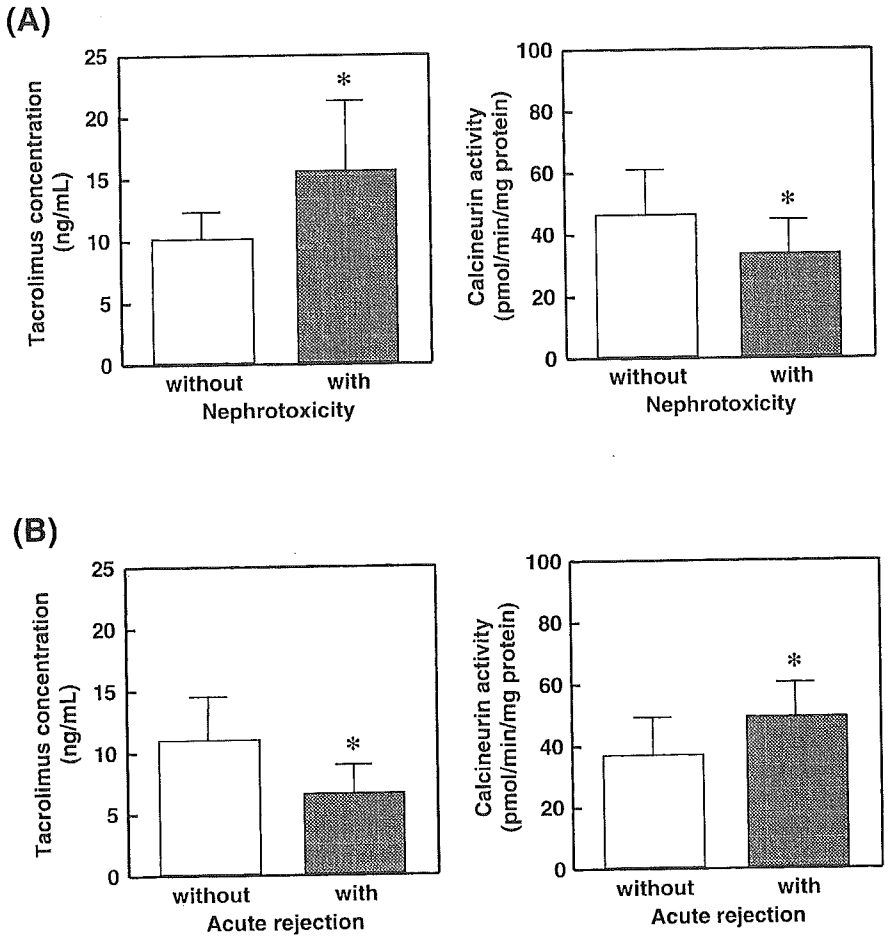

Fig 6. Blood tacrolimus concentration and calcineurin phosphatase activity in PBMCs in living-donor liver transplant patients (n = 21) with and without nephrotoxicity (**A**) or acute rejection (**B**). Each column indicates the mean ± SD of data measured at the trough time point. *Asterisk*, $P < .05$, significantly different from the mean value in patients without the given event (unpaired t test).

EC_{50} value for cyclosporine was reasonably consistent with the IC_{50} values reported in kidney transplant patients.[14,19] Large interindividual variability in the EC_{50} value was demonstrated in patients receiving tacrolimus and cyclosporine (Table II). The variability could be explained by the difference in drug concentrations in PBMCs or the difference in function or content of proteins relating to the pharmacodynamics, such as immunophilins and calcineurin. We have reported that intestinal P-glycoprotein plays an important role in limiting oral absorption of tacrolimus and cyclosporine from the gut lumen after living-donor liver transplantation.[21,22] Because P-glycoprotein is also expressed in PBMCs, it may contribute to the difference in drug distribution into the cells.[23]

Clearly, the concentration and response relationship differed between tacrolimus and cyclosporine in liver transplant patients, suggesting that their pharmacodynamic properties for calcineurin inhibition are not identical in vivo (Fig 4). Cyclosporine produced a steep decline in calcineurin activity and exerted no additional effect at blood concentrations above a certain threshold (approximately 700 ng/mL) (Fig 4, *A*). These results of the effects of cyclosporine were similar to those in a study that measured the inhibition of stimulated interleukin 2 (IL-2) production in whole blood by cyclosporine.[24] Although tacrolimus was demonstrated to completely suppress lymphocyte proliferation and IL-2 production in human mixed lymphocyte reactions,[25] the calcineurin activity was only partially inhibited by