Effectiveness of Sirolimus in Combination with Cyclosporine against Chronic Rejection in a Pediatric Liver Transplant Patient

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The patient is a 3-year-old boy who received living-donor liver transplantation (LDLT) for hepatoblastoma, with his mother as the donor. Oral tacrolimus was started at a dose of 0.3 mg every 12 h from day 1, with the dosage adjusted on the basis of trough concentrations. The levels of aspartate aminotransferase (AST), alanine transferase (ALT), and total bilirubin (T-bil) were 110 U/L, 182 U/L, and 12.6 mg/dL, respectively, when chronic rejection (CR) was pathologically diagnosed. Then, sirolimus at a dose of 1.0 mg/d was added to the tacrolimus-based regimen. The T-bil level rapidly decreased to 5.4 mg/dL, without changes in AST and ALT. Because the intracellular receptor of sirolimus and tacrolimus is FK506-binding protein 12, we switched tacrolimus to cyclosporine at a dose of 60 mg/d to avoid competitive inhibition between these 2 drugs. The target trough concentration of sirolimus and cyclosporine was set to around 15 ng/mL and 180 ng/mL, respectively. The concentration/dose ratio of sirolimus was significantly correlated with the blood cyclosporine level ($r=0.5293$, $p<0.05$), suggesting the pharmacokinetic interaction between these 2 drugs. Thereafter, the levels of AST and ALT as well as the T-bil were successfully decreased to 73 U/L, 83 U/L, and 3.0 mg/dL, respectively. These results suggest that sirolimus therapy in combination with cyclosporine may be an effective treatment against CR after liver transplantation.

Key words liver transplantation; humoral rejection; immunosuppressant; tacrolimus; hepatoblastoma; chemotherapy

Living-donor liver transplantation (LDLT) with subsequent immunosuppressive therapy with tacrolimus is used to treat pediatric patients with end-stage liver disease. However, intensive therapeutic drug monitoring (TDM) is required to control the concentration of tacrolimus in the blood between the narrow therapeutic ranges, because of the large inter- and intra-individual variation of tacrolimus pharmacokinetics. In addition, several adverse effects of tacrolimus, such as neurotoxicity, kidney injury, and malignancy, have been raised as serious problems that need to be considered in pediatric patients.

Hepatoblastoma develops in children, until the age of 4 year, with highly elevated alpha-fetoprotein (AFP) levels of >100 ng/mL.[1,2] Several chemotherapeutic drugs such as anthracyclines, cisplatin, taxanes, or irinotecan have been evaluated as treatments against hepatoblastoma for patients who are contraindicated for surgical treatment.[3,4] Liver transplantation has been considered an attractive optional treatment for patients with hepatoblastoma.[5,6]

Although acute cellular rejection (ACR) after LDLT is curable with high-dose steroid pulse therapy, there is no effective treatment against chronic rejection (CR) mediated by humoral immunity. Tacrolimus and cyclosporine, common immunosuppressive agents, inhibit calcium-dependent T-cell activation.[7] On the other hand, a mammalian target of rapamycin (mTOR) inhibitor acts on B-cells independently of its effects on helper T-cells, causing an inhibition of antigen- and cytokine-driven B-cell proliferation.[8] Maintenance immunosuppression with mTOR inhibitors is reported to be associated with a significantly reduced risk of developing any posttransplant malignancy.[9] mTOR is acknowledged as a major player in cell proliferation. Recently, the mTOR inhibitors sirolimus (rapamycin) and everolimus have been used for cancer treatment.[10,11] Elsharkawi *et al.*[12] reported that sirolimus administration diminished the lung metastasis of hepatocellular carcinoma after liver transplantation. On the basis of these findings, mTOR inhibitors such as sirolimus and everolimus are considered to act as both anticancer agents and immunosuppressants.

In the present study, we examined the effectiveness of sirolimus against CR and the relapse of hepatoblastoma in a boy who received LDLT. CR was successfully controlled, without a relapse of hepatoblastoma, using sirolimus therapy in combination with cyclosporine rather than with tacrolimus.

MATERIALS AND METHODS

Case Report The patient is a 3-year-old boy admitted to Kyoto University Hospital (Kyoto, Japan) with poorly differentiated hepatoblastoma and placed on the waiting list for liver transplantation. He was affected with chronic heart failure, chronic pulmonary disorder, bronchial asthma, chronic colon pseudo-obstruction and inguinal hernia. Before the transplantation, he was admitted in another hospital. Because of heart failure, the first-line therapy for hepatoblastoma, anthracycline antitumor drugs, could not be given to this patient. Therefore, he was treated with cisplatin (80 mg/sqm) biweekly. The tumor marker, alpha-fetoprotein (AFP), was effectively decreased from 602000 to 14900 ng/mL. However, because cisplatin-induced nephropathy occurred, the regimen was changed to weekly carboplatin (120 mg/sqm) 4 times. AFP was decreased further to 3000 ng/mL; however, the portal vein tumor throm-

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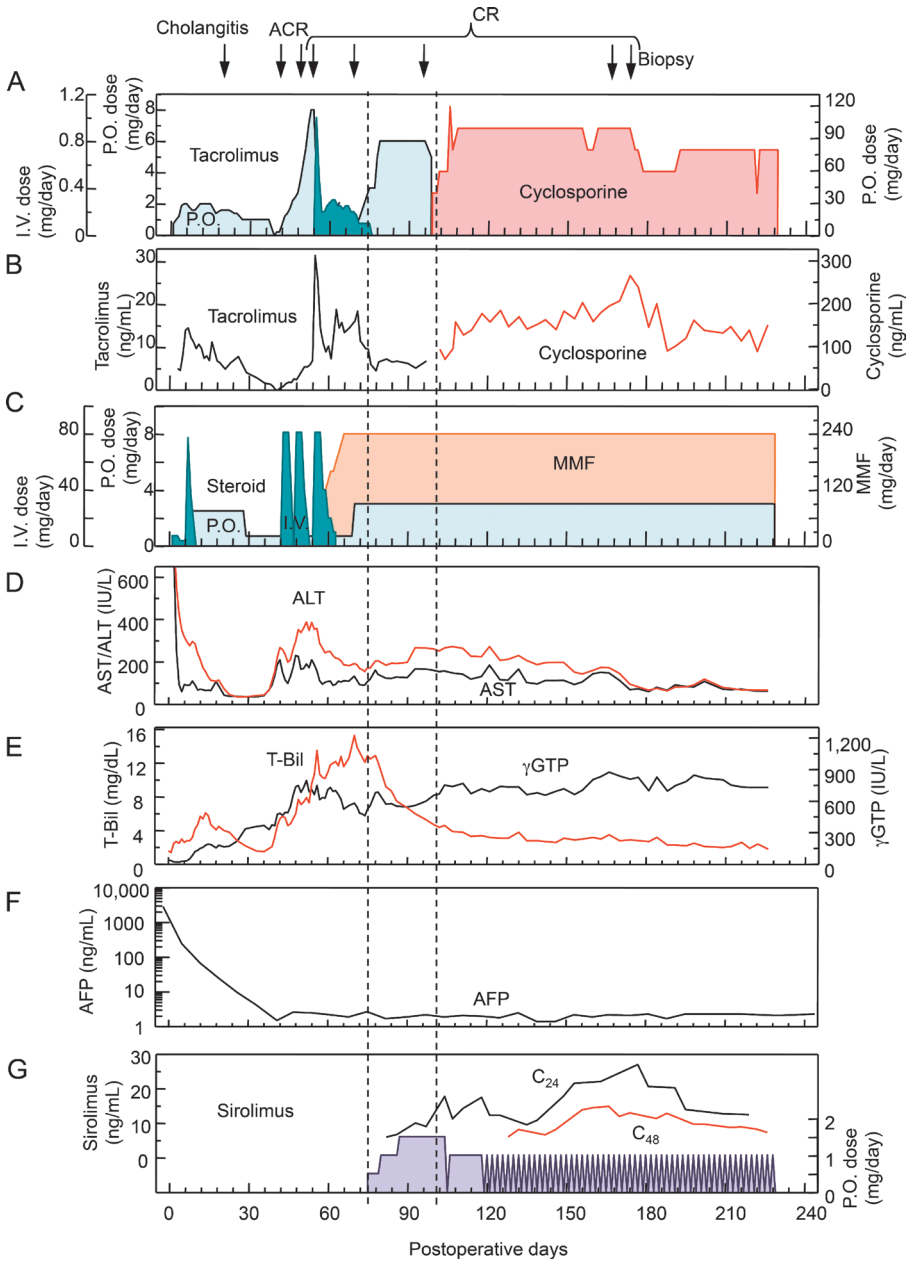

Fig. 1. Dosage (A) and Blood Concentration (B) of Calcineurin Inhibitors; Dosage of Steroid or Mycophenolate Mofetil (MMF) (C); Monitoring of Transaminases (D), γ-GTP or Total Billirubin (E), and α-Fetoprotein (F); and Dosage and Blood Concentration of Sirolimus (G)

The trough levels of calcineurin inhibitors (B) were determined approximately 12 h after the evening dose every day. The blood concentrations of tacrolimus and cyclosporine were measured with the CLIA and ACMIA methods, respectively. The blood concentration of sirolimus was measured by CLIA method. The dotted line shows the time points of the start and end of sirolimus therapy in combination with tacrolimus. ALT. alanine aminotransferase; AST, asparate aminotransferase; T-bil, total bilirubin; γ-GTP, gammma-glutamyl transpeptidase; CR, chronic rejection; ACR, acute cellular rejection; P.O., per oral administraion; I.V., intravenous administration; AFP, alpha-fetoprotein.

bus was not diminished. His physicians changed the regimen again to irinotecan (100 mg/sqm) and etoposide (100 mg/sqm) for 3 d. Although the patient developed adverse effects of chemotherapy, such as myelosuppression, diarrhea, and sepsis, he was treated with a reduced dose of irinotecan (3.3 mg/kg) and etoposide (3.3 mg/kg) and/or carboplatin (2.6 mg/kg) for 3 d. Then, he was referred to our hospital in August 2009. His AFP level was 5110 ng/mL; however, portal vein tumor thrombus persisted and metastasis to the lymph nodes was suspected. Therefore, he was not approved for liver transplantation. He was then treated with irinotecan for 6 months from August 2009 to February 2010 in the other hospital, and his AFP decreased to 700 ng/mL. He was eventually admitted to our hospital for LDLT. In June 2010, he underwent successful LDLT, with his 41-year-old mother (ABO blood type identical) as donor. He weighed 7665 g and had a height of 74.5 cm. The graft size was 190 g, and the graft-to-recipient weight ratio (GRWR) was 2.53%. Oral administration of immunosuppressive agent tacrolimus (FK506) was started at a dose of 0.3 mg every 12 h with the target window around 10 ng/mL, and its dose was adjusted on the basis of trough concentrations measured 12 h after the evening dose (Figs. 1A, B). However, at postoperative day (POD) 43, ACR was observed on biopsy (Fig. 1A). He received steroid pulse therapy to control ACR, 3 times at POD 43, 48, and 53 (Fig. 1C). Intravenous injection of tacrolimus was also started at the time. The high

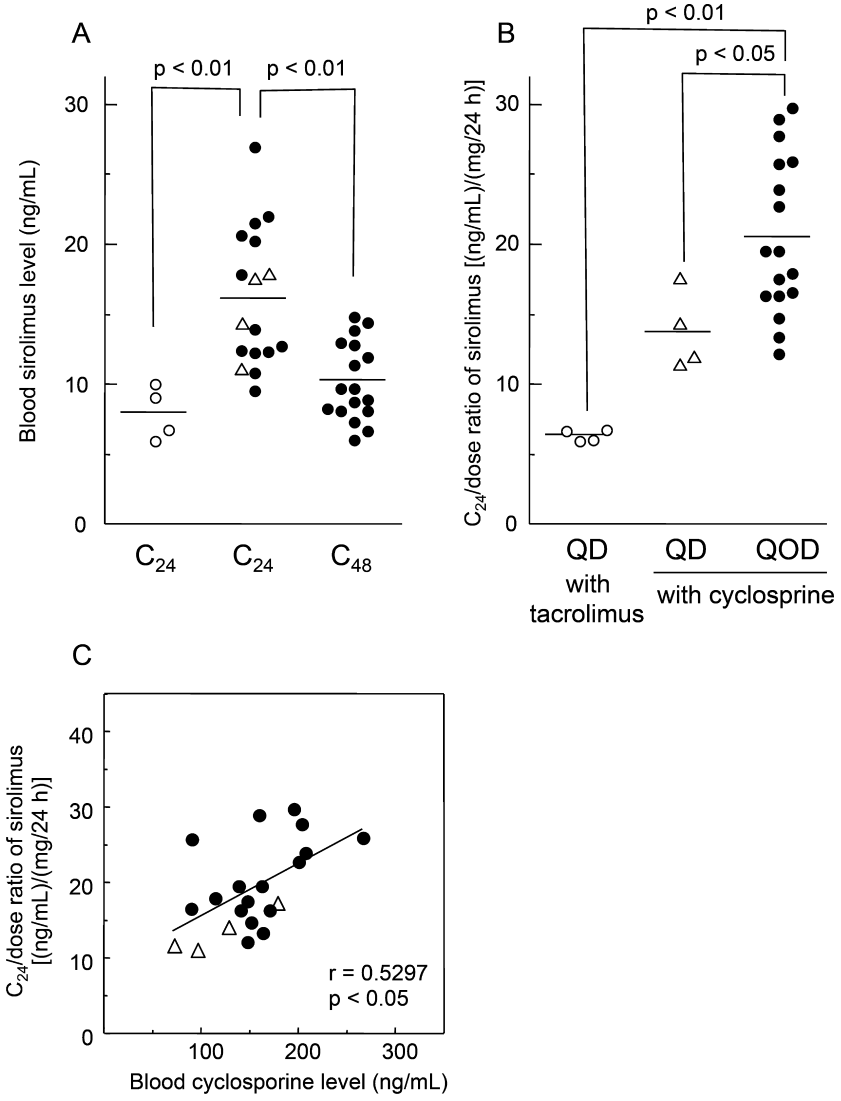

Fig. 2. The Blood Concentration of Sirolimus Was Elevated When Given in Combination with Cyclosporine

The dosage regimen of sirolimus was changed from QD to QOD because the calcineurin inhibitor was switched from tacrolimus to cyclosporine. The blood concentration of sirolimus (A) and the 24 h concentration of sirolimus (ng/mL) per dosage (mg/d) ratio (B) were significantly elevated when given in combination with cyclosporine. (C) The blood cyclosporine level significantly correlated with the C_{24}/dose ratio of sirolimus. Data are derived from Figs. 1B and 2B. White circle, QD sirolimus with tacrolimus; black circle, QOD sirolimus with cyclosporine; white triangle, QD sirolimus with cyclosporine.

levels of ALT and AST were decreased, while T-bil level was increased (Figs. 1D, E). Although the pathological findings related to ACR were diminished, CR was observed at POD 52. The levels of AST, ALT and T-bil were 110 U/L, 182 U/L, and 12.6 mg/dL, respectively. At POD 72, the findings related to CR were still positive. We started to administer the mTOR inhibitor sirolimus (rapamycin) orally with intravenous tacrolimus from postoperative day 75. The T-bil level rapidly decreased to 5.4 mg/dL without changes in AST and ALT. Therefore, treatment with sirolimus seems to be effective against CR. However, the gamma-glutamyl transpeptidase (γ-GTP) was slightly increased. At POD 96, he underwent biopsy and late-phase CR was diagnosed. Because the intracellular receptor of sirolimus and tacrolimus is FK506-binding protein 12 (FKBP1A), we switched tacrolimus to cyclosporine at a dose of 60 mg/day at postoperative day 100 to avoid competitive inhibition between sirolimus and tacrolimus.[13] We thought cyclosporine was more effective against CR than tacrolimus in combination with sirolimus. The target trough concentration of sirolimus and cyclosporine was set to around 15 ng/mL and 180 ng/mL, respectively. The blood concentration of sirolimus was elevated in combination with cyclosporine, but not with tacrolimus. At POD 177, when the final biopsy was performed, the levels of AST and ALT as well as T-bil were found to be successfully decreased to 73 U/L, 83 U/L, and 3.0 mg/dL, respectively. Thereafter, the liver function did not improve but remained stable. Finally, the patient was discharged without deterioration of liver function and relapse of the hapatoblastoma at POD 247.

Measurement of the Blood Concentration of Immunosuppressant Drugs The dose of tacrolimus and sirolimus was adjusted on the basis of trough concentrations measured 12 h after the evening dose by means of the chemiluminescent enzyme immunoassay (CLIA) method (ARCHITECT™; Abbott Japan, Tokyo, Japan). The lower limit of this system is 0.5 ng/mL for tacrolimus or 1.0 ng/mL for sirolimus with a whole blood sample. The blood concentration of cyclosporine was measured by the antibody-conjugated magnetic immuno-

assay (ACMIA) method (Dimension™; Siemens Japan, Tokyo, Japan). The lower limit for cyclosporine is 25 ng/mL.

DISCUSSION

Sirolimus treatment rapidly decreased the high level of T-bil. However, the levels of AST and ALT tended to increase when sirolimus was added to the tacrolimus-based regimen. Recently, Nielsen *et al.*[14] reported that 4 and 6 of 12 pediatric patients with chronic graft dysfunction after liver transplantation developed completely normal liver function and showed partial response, respectively. However, there was no precise analysis in drug interaction between tacrolimus and everolimus. In the present study, both AST and ALT became well controlled after switching from tacrolimus to cyclosporine. Therefore, part of the patients with partial response of the past report[14] might develop completely normal liver function when tacrolimus was switched to cyclosporine. These results suggested that sirolimus in combination with cyclosporine might be an effective treatment against CR after liver transplantation. That is to say that sirolimus is efficacious against CR, and cyclosporine is better calcineurin inhibitor compared to tacrolimus to obtain pharmacological effects of both mTOR inhibitor and calcineurin inhibitor for concomitant administration.

Several groups have shown that the mTOR pathway is required for the development and maturation of B-cells.[15,16] It is also reported that mTOR inhibitors block B-cell development and antibody production.[17] On the basis of these findings and the present experience, sirolimus treatment might be effective against CR mediated by humoral immunity.

The target sirolimus trough level of between 10 and 15 ng/mL was recommended for pediatric renal transplantation.[18] However, the frequency of leukopenia was increased with the elevation of the sirolimus trough concentration to as high as 15 ng/mL.[19] Nevertheless, we have reported the initial target trough concentration of sirolimus at 15 ng/mL in patients receiving islet transplantation.[20,21] Based on these reports, we have set the initial target trough concentration of sirolimus at 15 ng/mL in this case.

The blood concentration of the immunosuppressive drugs used is shown in Fig. 2. The mean blood concentration of sirolimus, at 24 h after the treatment in combination with tacrolimus, was 8.0 ng/mL (range, 6–10.1 ng/mL). However, the blood concentration of sirolimus when given in combination with cyclosporine was significantly increased to 16.2 ng/mL (range, 9.6–27.0 ng/mL; $p<0.01$). The 24 h concentration of sirolimus (ng/mL) per dosage (mg/d) ratio was also significantly elevated in combination with cyclosporine ($p<0.01$). It is well acknowledged that sirolimus is mainly metabolized in the intestine and liver by cytochrome P450 3A4 (CYP3A4).[22] In addition, the apparent IC_{50} values of tacrolimus and cyclosporine on for CYP3A4 inhibition were determined to be 53 μM and 90 μM, respectively.[23] However, the target trough concentration of tacrolimus was approximately 10 μg/mL and that of cyclosporine was approximately 180 μg/mL in this case. Therefore, the inhibitory effect of cyclosporine on CYP3A4-mediated sirolimus metabolism would be stronger than that of tacrolimus in clinical use. Taking together these findings, the blood concentration of sirolimus could be expected to be elevated in combination with cyclosporine, but not with tacrolimus. In addition, as Fig. 2C clearly indicates, the C_{24}/dose ratio of sirolimus correlates with blood cyclosporine concentration. These results clearly demonstrate the pharmacokinetic interaction between cyclosporine and sirolimus for the first time in a clinical liver transplant case.

Previously, it has been reported that sirolimus effectively inhibits hepatoblastoma growth both *in vitro* and *in vivo*.[24] Therefore, in the present case, it is indicated that sirolimus treatment may not only be effective against CR but also contributes to the avoidance of hepatoblastoma relapse. In fact, since the treatment with sirolimus was started, the AFP levels remained markedly decreased to around 2.0 ng/mL (Fig. 1F), showing that the hepatoblastoma did not spread.

In summary, sirolimus therapy in combination with cyclosporine may be an effective treatment against CR after liver transplantation. Because of the competitive inhibition between tacrolimus and sirolimus, cyclosporine would be better than tacrolimus from use in combination with sirolimus. However, further investigation with more cases is required to confirm whether sirolimus treatment in combination with cyclosporine is effective against humoral immunity and hepatoblastoma.

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