approximately 3% to 5%. This event does not simply represent end-stage acute cellular rejection (ACR), although the two may be temporally related. The pathogenesis of CR is not completely understood, although its association with donor-specific human leukocyte antigen antibodies was recently reported (*16*). Additional immunosuppressive therapy is unlikely to be beneficial for CR patients, particularly those with late disease in which bile duct loss affects more than 50% of the portal tracts, and retransplantation is required (*15*).

Several studies have suggested an association of CR with IFN-based antiviral therapy (*17–20*). Two recent reports found that CR was associated with antiviral therapy for recurrent hepatitis C after LT (*11, 12*). Stanca et al. (*12*) reported that 12 of 70 LT recipients with HCV infection treated with pegylated IFN (peg-IFN) and ribavirin developed CR. Their study indicated that ACR and CR are not strongly associated and that CR progresses rapidly, terminating in graft failure. Fernandez et al. (*11*) reported that 7 of 79 (9%) patients developed CR during antiviral therapy. They found that the use of cyclosporine in immunosuppression therapy, achievement of an SVR, and ribavirin discontinuation were factors associated with CR development.

Although the details of patients with antiviral therapy-associated CR after deceased-donor liver transplantation (DDLT) have been reported (*11, 12*), no study of antiviral therapy-associated CR in patients receiving living-donor liver transplantation (LDLT) has been published thus far. The features specific to LDLT, including blood-relative donors, posttransplantation liver regeneration, and ABO-incompatible LT, might result in characteristic differences between LDLT and DDLT patients.

We aimed to clarify the details of antiviral therapy-associated CR after LDLT and to identify the factors associated with CR.

RESULTS

Patient Characteristics and Treatment Outcomes

The study included 125 HCV-infected LT patients treated with standard IFN and/or peg-IFN in combination with ribavirin for recurrent hepatitis C after LDLT. Of these, 69 (55%) were men (median [range] age at the beginning of therapy, 57 [32–70] years). Most patients were infected with HCV genotype 1b (n=101 [81%]). The HCV genotype for the remaining patients was 2a (n=14), 2b (n=6), 3a+3b (n=1), and indeterminate (n=2). Genotype was not examined in one patient. The median (range) serum HCV RNA load at the beginning of antiviral therapy after LDLT was 3980 (31 to <69,000) kIU/mL. The median (range) donor age was 42 (19–65) years. Seventy-three (58%) donors were men, and 84 (67%) were blood relatives of the recipients. The graft type was the right lobe for 108 (86%) patients and the left lobe for 17 (14%) patients. The blood type combination was incompatible for 27 (22%) patients. Thirty-six (29%) patients had histologically diagnosed ACR before antiviral therapy, 16 of whom had moderate or severe ACR. No patient had shown ACR findings in the liver biopsy examination immediately before antiviral therapy. The median (range) time to treatment initiation after LDLT was 8.9 (1.1–72.4) months. Before treatment, necroinflammatory activity of levels A1, A2, and A3 based on the METAVIR score was found in 82 (66%), 40 (32%), and 3 (2%) patients, respectively. Fibrosis scores of F0, F1, F2, and F3 were found in 19 (15%), 82 (66%), 19 (15%), and 5 (4%) patients, respectively. Tacrolimus-based immunosuppression was administered to 117 (94%) patients and cyclosporine was administered to 7 (6%) patients. Mycophenolate mofetil (MMF) without calcineurin inhibitor (CNI) was administered to one patient because of renal failure at the beginning of antiviral therapy. In the patients who received tacrolimus, the mean (range) serum trough level at therapy initiation was 6.2 (2.0–12.7) ng/mL. In addition to CNIs, MMF and prednisolone were administered at the start of the antiviral treatment to 39 (31%) and 21 (17%) patients, respectively.

Of the 123 patients in whom the final treatment outcomes could be evaluated, 54 (44%) patients achieved SVR, 12 (10%) relapsed, 30 (24%) were nonresponders, and 27 (22%) withdrew from treatment. The remaining two patients were still undergoing treatment during the analysis.

Characteristics of Patients with Antiviral Therapy-Associated CR

Seven of 125 (6%) patients developed CR during or within 6 months after the end of antiviral therapy. The characteristics and clinical courses of these seven patients are shown in Table 1. Although four patients had a history of ACR before antiviral therapy was initiated (three of whom had moderate or severe ACR), three had no previous ACR episodes. The METAVIR score-based fibrosis level before antiviral therapy was F0 in three of the seven patients, F1 in three patients, and F2 in one patient, indicating that the antiviral therapy had been initiated at an early stage of fibrosis. The median (range) time from transplantation to initiation of antiviral therapy in these seven recipients was 9 (2–72) months. Tacrolimus was administered to five patients and cyclosporine was administered to one patient when the antiviral therapy was initiated. One patient did not receive a CNI because of renal failure (patient 7). Four patients received MMF, and one patient received prednisolone in combination with tacrolimus and MMF. The trough levels of tacrolimus and cyclosporine were within the therapeutic range. Standard amounts of immunosuppressant were therefore used for all patients, except for patient 7 who received MMF only. Immunosuppressant doses were reduced during therapy in five of seven patients. The tacrolimus dose was reduced for two patients (patients 2 and 3), as a result of which the blood trough level of tacrolimus decreased by approximately 2 ng/mL. In patient 3, MMF (500 mg/day) was also stopped during treatment. In patient 4, the MMF dose was reduced from 1000 to 250 mg per day, and prednisolone treatment (2.5 mg/day) was also terminated during treatment. In patient 5, MMF (1000 mg/day) was stopped immediately after initiation of antiviral therapy. Patient 6 received no CNI, and MMF dose was reduced from 500 to 250 mg per day during treatment. Three patients received standard IFN, and four received peg-IFN. Ribavirin was not administered to three patients immediately before the diagnosis of CR because of anemia.

CR was diagnosed after a median (range) of 9 (1–16) months of antiviral therapy. Two patients were diagnosed

TABLE 1. Characteristics of patients with CR associated with antiviral therapy

Patient	1	2	3	4	5	6	7
Age (years)	62	41	45	67	50	59	49
Gender	Female	Male	Female	Female	Female	Male	Male
ABO mismatch with donor	Match	Match	Match	Mismatch	Match	Mismatch	Match
Relation to donor	Related	Related	Nonrelated	Related	Nonrelated	Nonrelated	Nonrelated
Graft type (lobe)	Right	Right	Right	Right	Left	Right	Right
Splenectomy	No	No	No	No	Yes	Yes	No
Previous ACR	Yes	Yes	Yes	No	Yes	No	No
Previous moderate/severe ACR	Yes	No	Yes	No	Yes	No	No
Previous steroid pulse	Yes	No	No	No	Yes	No	No
HCV genotype	1b	1b	1b	2a	1b	1b	1b
HCV RNA (kIU/mL) before IFN	>850	3620	1790	>5000	>5000	>5000	16,000
METAVIR score before IFN	A2 F2	A2 F0	A1 F0	A1 F1	A2 F1	A1 F0	A1 F1
Months from LT to IFN	13	2	5	13	7	9	72
Months from initiation of IFN to diagnosis of CR	9	1	16	10	15	8	7
Immunosuppressant at initiation of IFN	Tacrolimus	Tacrolimus,	Tacrolimus, MMF	Tacrolimus, MMF, PSL	Cyclosporine, MMF	Tacrolimus, MMF	MMF
Trough level of CNI	7.8	7.9	7.9	6.8	152	5.9	—
Reduction of immunosuppressant during IFN (reduced drugs)	No	Yes (tacrolimus)	Yes (tacrolimus, MMF)	Yes (MMF, PSL)	Yes (MMF)	No	Yes (MMF)
Type of IFN	Standard	Standard	Standard	Pegylated	Pegylated	Pegylated	Pegylated
Ribavirin discontinuation	No	No	Yes	Yes	No	No	Yes
IFN at diagnosis of CR	On treatment	On treatment	1 month after end of IFN	5 months after end of IFN	On treatment	On treatment	On treatment
At diagnosis of CR							
Liver biopsy	Foam cell arteriopathy, bile duct atrophy	Bile duct atrophy	Bile duct atrophy	Bile duct atrophy, bile duct loss	Bile duct atrophy, bile duct loss	Bile duct atrophy, bile duct loss	Foam cell arteriopathy, bile duct atrophy
AST (IU/L)	121	90	53	73	331	124	36
ALT (IU/L)	67	37	43	63	288	52	32
ALP (IU/L)	2034	906	494	1751	2143	528	1164
γ-GTP (IU/L)	561	768	155	209	515	27	1489
Bilirubin (mg/dL)	18.6	18.8	31.5	38.1	11.8	16.4	22.6
HCV RNA (kIU/mL)	Undetectable	460	Undetectable	Undetectable	16,000	Undetectable	0.40
Treatment for CR	Tacrolimus, MMF	Tacrolimus	Tacrolimus, steroid pulse, MMF	Tacrolimus, MMF, PSL	Tacrolimus, MMF, rapamycin, steroid pulse	Tacrolimus, steroid pulse, MMF	Tacrolimus, MMF, rapamycin, steroid pulse
Outcome	Died	Alive	Died	Died	Died	Died	Died
Months from diagnosis of CR to death	64	—	1	1	1	3	1

ALT, alanine aminotransferase; AST, aspartate aminotransferase; PSL, prednisolone.

with CR after antiviral therapy was terminated. Antiviral therapy was discontinued in the remaining five patients. Of note, six patients were treated with IFN for more than 7 months, suggesting that long-term administration of IFN is associated with CR. Liver biopsy was performed for diagnosis of CR because of abnormal liver function tests in all cases. All patients with documented CR had high levels of alkaline phosphatase (ALP). Total bilirubin levels were extremely high (11.8–38.1 mg/dL) at diagnosis, suggesting a delayed diagnosis of CR. All liver biopsies showed atrophy affecting most bile ducts as well as hepatocanalicular cholestasis. Two patients (patients 1 and 7) showed foam cell obliterative arteriopathy. Bile duct loss was shown in 100%, 67%, and 20% of the portal tracts in patients 4, 5, and 6, respectively. In none of the seven patients was evidence of ACR found in the biopsy specimens. Hepatic artery or biliary tract obstruction or structuring was excluded by imaging in all patients.

Serum HCV RNA was undetectable in four patients at CR diagnosis and remained undetectable in all four patients during the follow-up period. Two of the four patients were considered to have SVR. Final outcomes could not be determined in the remaining two patients who died within 24 weeks after termination of treatment.

Various intensive treatment protocols were used for these seven patients after CR diagnosis, including increase of tacrolimus dose, addition or increase in MMF and/or prednisolone dose, administration of steroid pulse therapy, and inclusion of rapamycin in the therapy. CR progressed rapidly to liver failure in five patients (patients 3–7). These five patients died within 3 months after diagnosis of CR due to liver failure and infection. The liver damage in patient 1 gradually progressed to liver failure, and the patient died at 64 months after CR was diagnosed. Only one patient (patient 2) recovered from CR and survived, although a follow-up liver biopsy showed chronic hepatitis C.

Risk Factors of CR Associated with Antiviral Therapy

Factors associated with the development of CR during and after antiviral therapy were analyzed by comparing the features of 7 CR patients with those of 76 patients who did not develop CR despite receiving antiviral therapy for more than 1 year (Table 2). A reduction of the immunosuppressant dose during antiviral therapy (P=0.034) and a low fibrosis stage before antiviral therapy (P=0.045) were significantly associated with antiviral therapy-related CR. No significant associations were found with other variables, including donor factors, ribavirin discontinuation, and undetectable HCV RNA. The rate of previous ACR (P=0.065), rate of previous moderate or severe ACR (P=0.059), ALP level (P=0.121), and γ-glutamyl transpeptidase (γ-GTP) level (P=0.051) before antiviral therapy was higher in the patients who developed CR, but the differences from patients without CR were not significant.

DISCUSSION

Of the 125 patients, 7 (6%) who received antiviral therapy for hepatitis C after LDLT developed CR. CR progressed rapidly, resulting in death within 3 months after diagnosis, in 5 of these 7 patients.

The risk of rejection have been suggested to increase with IFN administration because of the drug's theoretical immunomodulatory actions, such as up-regulation of human leukocyte antigen class II antigens and induction of proinflammatory cytokines (*21*). Previous studies have reported that the frequency of CR in patients who received IFN was substantially higher compared with patients who did not receive antiviral therapy (*11, 12, 17*). In the present study, the rate of antiviral therapy-associated CR was 6%. This rate is high, because no CR occurred in the entire study period other than during or within 6 months after termination of antiviral therapy in the 230 HCV-positive recipients analyzed. Some cases showed sudden onset of CR after a long transplantation period in the absence of preexisting ACR, supporting the association of antiviral therapy with CR.

In our analysis, the two significant risk factors for CR were reduction of the immunosuppressant dose during antiviral therapy and low fibrosis score at antiviral therapy initiation. Additional characteristics associated with CR were elevated cholestatic enzyme levels at the time of diagnosis, onset of CR more than 7 months after treatment initiation (excluding one patient) and poor prognosis after the diagnosis. The MMF dose was reduced or stopped during antiviral therapy in four of five patients who had received MMF at the start of the treatment. We had initially tried to reduce the MMF dose during antiviral therapy, because MMF is known to suppress the bone marrow and could therefore augment the cytopenic effects of IFN and ribavirin. We had reduced immunosuppressant according to our reduction protocol even during antiviral therapy. Based on the data, we subsequently changed our strategy to maintaining the MMF dose and increasing the trough level of CNIs during antiviral therapy. The reason for the association between the low fibrosis score and CR is currently unclear. Although some institutions recommend early introduction of antiviral therapy (*8, 9*), our data suggest that antiviral therapy should not be administered to patients with no or mild fibrosis. On the contrary, it is reported that tolerance to therapy decreases significantly in patients with a fibrosis stage ≥3 on baseline liver biopsy (*22*). Therefore, the antiviral therapy should be initiated in patients with a fibrosis stage 2, as the recent review articles recommended (*23, 24*).

All our patients underwent LDLT, but no characteristics specific to LDLT, including blood-relative donors, graft size, and ABO incompatibility, were identified as risk factors for CR in our study. This appears to indicate that LDLT and DDLT patients do not differ with respect to antiviral therapy-associated CR.

Early diagnosis of CR, as well as prevention, is important for ensuring improved outcomes in LT recipients. CR was diagnosed in our patients after liver damage had already progressed. Histologic diagnosis of CR was difficult in all these cases, despite repeated liver biopsy examination. However, all the patients had elevated ALP and γ-GTP levels before jaundice was observed. CR should therefore be suspected when a cholestatic liver enzyme pattern develops during antiviral therapy for hepatitis C. When imaging has excluded large bile duct and/or hepatic artery changes as the

TABLE 2. Risk factors for CR

	CR (n=7)	No CR (n=76)	*P*
Age at LT (years)	50 (41−67)	56 (36−69)	0.506[a]
Gender, male/female	3/4	44/32	0.352[b]
HCV genotype, 1/non-1	6/1	71/5	0.421[b]
Donor age at LT (years)	46 (28−60)	42 (21−65)	0.857[a]
Donor gender, male/female	4/3	40/36	0.568[b]
Sex mismatch, match/mismatch	0/7	26/50	0.064[b]
ABO mismatch, match/mismatch	5/2	59/17	0.507[b]
Relation to donor, related/nonrelated	3/4	48/28	0.254[b]
HLA-A matched number, 0/1/2/unknown	0/5/2/0	13/44/16/3	0.332[a]
HLA-B matched number, 0/1/2/unknown	2/4/1/0	21/47/5/3	0.778[a]
HLA-DR matched number, 0/1/2/unknown	3/3/1/0	18/47/8/3	0.487[a]
Graft type, left lobe/right lobe	1/6	9/67	0.608[b]
Splenectomy, yes/no	2/5	38/38	0.247[b]
Previous ACR, yes/no	4/3	17/59	0.065[b]
Previous moderate/severe ACR, yes/no	3/4	9/67	0.059[b]
Previous steroid pulse therapy, yes/no	2/5	8/68	0.198[b]
Months from LT to therapy	9.0 (1.8−72.4)	9.1 (2.2−68.8)	0.694[a]
Valuables at initiation of IFN			
Age (years)	55 (41−68)	57 (37−70)	0.599[a]
CNI tacrolimus/cyclosporine	5/1	71/5	0.376[b]
Trough level for tacrolimus (ng/mL)	7.3 (0−7.9)	6.2 (2.6−10.9)	0.641[a]
AST (IU/L)	68 (24−464)	76 (21−331)	0.908[a]
ALT (IU/L)	88 (25−354)	79 (20−392)	0.842[a]
ALP (IU/L)	878 (283−2977)	462 (168−2818)	0.121[a]
γ-GTP (IU/L)	317 (48−1623)	112 (15−1704)	0.051[a]
Bilirubin (mg/dL)	0.8 (0.3−10.4)	0.9 (0.3−4.6)	0.861[a]
Activity grade, A1/A2/A3	4/3/0	50/24/2	0.693[a]
Fibrosis stage, F0/F1/F2/F3	3/3/1/0	4/56/13/3	0.045[a]
Reduction of immunosuppressant during IFN, yes/no	5/2	22/54	0.034[b]
Ribavirin discontinuation during IFN, yes/no	3/4	26/50	0.468[b]
Undetectable HCV RNA during IFN, yes/no	4/3	51/25	0.439[b]

[a] Wilcoxon rank-sum test.
[b] Chi-square test.
Comparison was made between 7 patients with CR and 76 patients without CR despite receiving antiviral therapy for more than 1 year (No CR). Qualitative variables expressed as number. Quantitative variables expressed as median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HLA, human leukocyte antigen.

potential etiology of abnormal liver function, we believe that cessation of antiviral therapy and initiation of intensive immunosuppressive therapy should be considered, even without histologic confirmation of CR.

Some limitations of this study are its retrospective nature and relatively small sample size. Because the frequency of CR was low, the sample size was not adequate for multivariate analysis.

In conclusion, CR developed in association with antiviral therapy for recurrent hepatitis C after LDLT. Reduction of the immunosuppressant dose during antiviral therapy should be avoided and antiviral therapy should not be administered to patients with no or mild fibrosis to prevent antiviral therapy-associated CR. Early CR diagnosis should be suspected when a cholestatic liver enzyme pattern develops during antiviral therapy. In these cases, discontinuation of antiviral therapy and increase in the immunosuppressant dose are recommended when other causes of liver dysfunction are excluded.

MATERIALS AND METHODS

Patients

A total of 232 patients with HCV-related end-stage liver disease underwent LDLT at Kyoto University Hospital between March 1999 and September 2012. Two patients who received a liver graft from an identical twin were excluded from this study, because they did not require immunosuppression because of genetic identity. Of the remaining 230 patients, 157 patients were followed up for more than 6 months after LDLT in our hospital. Antiviral therapy was administered to 125 of the 157 patients with recurrent hepatitis C between January 2001 and September 2012. They were diagnosed with recurrent hepatitis C after LDLT via serum HCV RNA analysis and histologic evidence. The remaining 32 patients did not receive antiviral therapy for various reasons: serum HCV RNA negative after LDLT (n=4), no histologic hepatitis C recurrence in the follow-up period (n=13),

no fibrosis seen by liver histology (n=8), and ongoing treatment for the other complications (n=7). CR was defined histologically according to the updated International Banff Schema for Liver Allograft Rejection with the following criteria: (a) the presence of bile duct atrophy/pyknosis affecting most of the bile ducts with or without bile duct loss, (b) convincing foam cell obliterative arteriopathy, or (c) bile duct loss affecting more than 50% of the portal tracts (*13*). Patients who were diagnosed with CR based on these diagnostic criteria during or within 6 months after terminating antiviral therapy were examined for antiviral therapy-associated CR. The clinical features of these 7 patients with CR were compared with those of 76 patients who did not have CR despite receiving antiviral therapy for more than 1 year to determine the risk factors for CR.

The study protocol was approved by the ethics committee at Kyoto University and performed in compliance with the Helsinki Declaration.

Treatment Protocol and Definition of Responses to Treatment

Between January 2001 and April 2004, 40 patients with recurrent hepatitis C after LDLT received treatment with IFN-α-2b plus ribavirin (*25*). From May 2004 to June 2011, patients received combination therapy with peg-IFN-α-2b plus ribavirin (*26*). Patients who acquired a negative serum HCV RNA status within 12 months after treatment initiation continued to receive the treatment for an additional 12 months. Patients who tested negative for serum HCV RNA for more than 6 months after completing IFN therapy were defined as having achieved SVR. For those who tested positive for serum HCV RNA after 12 months of treatment, therapy was discontinued or switched to maintenance therapy with low-dose peg-IFN (*27*), and patients were classified as having shown no response.

Histologic Assessment

Liver biopsy examination was performed when patients showed abnormal liver function tests, or at yearly intervals, with informed consent. Biopsy specimens were evaluated by two pathologists (H.H. and A.M.-H.) with extensive experience in the pathology of LT. Necroinflammatory activity (A0–A3) and fibrosis stage (F0–F4) were assessed using METAVIR scores (*28*).

Immunosuppression

Tacrolimus with low-dose steroid or MMF was administered to most patients for immunosuppression (*25*). The target whole blood lower level for tacrolimus was 10 to 15 ng/mL during the first 2 weeks, 10 ng/mL thereafter, and 5 to 8 ng/mL starting from the second month. Steroid therapy was initiated at a dose of 10 mg/kg methylprednisolone before graft reperfusion then tapered down from 1 mg/kg per day on days 1 to 3, to 0.5 mg/kg per day on days 4 to 6, and to 0.3 mg/kg per day on day 7. Subsequently, oral prednisolone was continued at 0.3 mg/kg per day until the end of the first month, and this was followed by 0.1 mg/kg per day until the end of the third month. After that, steroid administration was terminated. MMF was initiated at a starting dose of 10 to 15 mg/kg on day 1, which was gradually increased to a target dose of 30 mg/kg, and this was continued for 6 months. Thereafter, MMF administration was terminated. Four patients received cyclosporine microemulsions instead of tacrolimus. MMF and/or prednisolone was administered again to patients who experienced refractory rejection or required reduction of the tacrolimus or cyclosporine dose because of adverse events and then tapered down gradually. Twenty-seven patients who received ABO-incompatible transplants were treated with rituximab, plasma exchange, and hepatic artery or portal vein infusion with prostaglandin E1 and methylprednisolone (*29*).

Virologic Assays

HCV genotype was determined using a genotyping system based on polymerase chain reaction (PCR) to amplify the core region using genotype-specific primers (*30*). The serum HCV RNA load was evaluated before LDLT, before IFN treatment, once a month during treatment, and 24 weeks after treatment using PCR and an Amplicor HCV assay (Cobas Amplicor HCV Monitor; Roche Molecular Systems, Pleasanton, CA) until April 2008. A real-time PCR-based quantitation method for HCV (COBAS AmpliPrep/COBAS TaqMan HCV Test; Roche Molecular Systems) was used alternatively from May 2008.

Statistical Analysis

To evaluate the association between patient characteristics and CR, the characteristics were defined and compared between patients with and without CR. Medians and ranges were determined for continuous variables, and data were analyzed using the Wilcoxon rank-sum test. Categorical variables were expressed as counts, and data were analyzed using the chi-square test. A significance level of $P<0.05$ was considered significant. Statistical analyses were performed using PASW Statistics version 18.0.0 (SPSS, an IBM company).

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Successful Telaprevir Treatment in Combination of Cyclosporine against Recurrence of Hepatitis C in the Japanese Liver Transplant Patients

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Telaprevir (TVR) is a protease inhibitor used in combination with pegylated interferon alfa-2b and ribavirin for hepatitis C, and TVR strongly inhibits CYP3A4 and CYP3A5. We reported successful TVR treatment of liver transplant patients with recurrence of hepatitis C during receiving immunosuppressive therapy. Before initiation of triple therapy, all patients switched from tacrolimus to cyclosporine, which has a lower inhibitory effect on CYP3A4 and CYP3A5 than tacrolimus. To avoid graft failure, we measured the cyclosporine blood concentrations at 0, 2, and 6 h after administration to maintain the target level (150–200 ng/mL) within 1 week after initiation of TVR and adjusted the dose of cyclosporine. The dose of cyclosporine was decreased 0.24–0.40 fold in all patients after initiation of TVR treatment. In 3 patients, the dose of TVR was decreased two-thirds of starting dose because of adverse effects, including anorexia and skin rash. However, the HCV RNA level rapidly decreased to undetectable levels within 1 month. Furthermore, all patients completed the TVR therapy in 12 weeks and did not experience liver graft rejection. In addition, we found the rapid elimination of inhibitory effect of TVR on the disposition of cyclospirne in the all four cases and therefore, rapid increase in the dosage of cyclosporine would be required immediately after the end of TVR administration. These results suggest that frequent measurement of cyclosporine levels was important for successful TVR triple therapy and prevention of rejection.

Key words telaprevir; cyclosporine; drug interaction; hepatitis C; liver transplantation

Telaprevir (TVR), a protease inhibitor, is a new drug to treat hepatitis C.[1–3] Triple therapy with pegylated interferon alpha 2b (PEG-IFN α-2b), ribavirin, and TVR for 12 weeks and double therapy with PEG-IFN α-2b and ribavirin for 12 weeks strongly affects the hepatitis C virus (HCV), and 73.0% of patients achieve sustained viral responses (SVRs).[4] TVR is metabolized by CYP 3A4 and CYP3A5 and strongly inhibits CYP3A4 and CYP3A5.[5,6] Therefore, TVR has strong drug interactions with immunosuppressants like tacrolimus and cyclosporine.[7]

Some hepatitis C patients develop liver cirrhosis or hepatocellular carcinoma, which require a liver transplant. After liver transplantation, they have to take immunosuppressive agents to prevent graft loss, and the blood concentrations of these drugs have to be carefully monitored. However, patients can show recurrence of hepatitis C even after liver transplantation.[8,9] Therefore, it is difficult to control the blood concentration of immunosuppressive agents with TVR triple therapy in patients with recurrence of HCV infection. To prevent graft rejection and treat hepatitis C, we carefully adjusted the dose of immunosuppressive agents to maintain the target blood concentrations.

This case report describes successful treatment of transplant patients with recurrence of hepatitis C with TVR, PEG-IFN α-2b, and ribavirin triple therapy to prevent liver graft rejection. We carefully controlled the blood concentration of immunosuppressive agents to that of the target level by frequent measurement.

MATERIALS AND METHODS

Treatment Protocol The day of initial administration of TVR was set as Day 1. The primary immunosuppressive agent was changed from tacrolimus to cyclosporine around one week before the initiation of TVR administration. The target trough and C_2 level of cyclosporine was set between 150 ng/mL and 200 ng/mL and between 600 ng/mL and 800 ng/mL, respectively, beginning around 2 weeks of TVR administration. TVR 750 mg was orally administered twice daily. Around day 7, PEG-IFN α-2b and ribavirin were added to start the triple therapy with TVR. The administration of TVR was terminated after 12 weeks, and then combination treatment with PEG-IFN α-2b and ribavirin was continued for 24 weeks. All periods of HCV treatment were 24 weeks.

Blood Samples Blood samples for measuring the levels of immunosuppressive agents were collected immediately before the morning dosage and at 2 h and 6 h after the administration between days 1 and 7. After day 8, the blood samples were collected for measuring the morning trough level of immunosuppressants.

The authors declare no conflict of interest.

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Measurements of Blood Concentration of Tacrolimus and Cyclosporine Whole blood concentrations of tacrolimus and cyclosporine were determined using chemiluminescence immunoassay (CLIA) using an ARCHITECT® i2000SR analyzer (Abbott Laboratories, Chicago, IL, U.S.A.) and affinity column-mediated immunoassay (ACMIA) using Dimension® (Siemens Healthcare Diagnostics Inc., Newark, DE, U.S.A.), respectively, according to the manufacturer's instructions.

Ethics This study was conducted in accordance with the Declaration of Helsinki and its amendments, and the study protocol was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee. Written informed consent was obtained from each patient.

CASE REPORTS

Although TVR strongly inhibits the dispositions of tacrolimus and cyclosporine, the interaction between TVR and cyclosporine is relatively mild compared to that between TVR and tacrolimus.[7] In addition, the clinical efficiency of cyclosporine was found to be similar with tacrolimus in the 39 living donor liver transplant patients.[10] Therefore, we carefully switched the primary immunosuppressant from tacrolimus to cyclosporine about a week before the initiation of TVR administration to avoid toxicity from calcineurin inhibitors. In addition, the dosage of TVR was decreased to 1500 mg/bid, because previous studies in Japan indicated that the standard dose of TVR 2250 mg/tid was toxic to Japanese patients.[11,12]

The clinical characteristics of the patients in this study are summarized in Table 1. All patients showed a recurrence of HCV genotype 1b after liver transplantation. The median (range) of duration between liver transplantation and initiation of TVR treatment was 21 (1–75) months.

Case I: A 62-year-old man who underwent cadaveric donor liver transplantation because of hepatocellular carcinoma after HCV-related liver cirrhosis. HCV RNA was detected in his serum after transplantation; therefore, he was administered anti-HCV therapy with PEG-IFN α-2b and ribavirin at the 2nd post-transplant month. Because of fatigue and nausea, the double therapy (consisting of PEG-IFN α-2b and ribavirin) was interrupted twice; once between months 9 and 10 and once between months 45 and 67. Then, the double therapy for recurrence HCV was withdrawn 75 months after liver transplantation. Before initiation of triple therapy (consisting of TVR, PEG-IFN α-2b, and ribavirin), the calcineurin inhibitor was switched from tacrolimus to cyclosporine about a week before the administration of TVR. The dosage and trough concentration of cyclosporine at the day before the administration of TVR were 150 mg/bid and 212 ng/mL, respectively (Figs. 1A, B). To avoid an excessive increase in the blood concentration of cyclosporine, the dosage of cyclosporine was reduced to one-third of the original on the day of TVR administration (1500 mg/bid). Although the blood concentration of cyclosporine varied, dosage adjustment was carefully performed on the basis of the blood concentration of cyclosporine in the morning during the two weeks after initiation of TVR therapy. On day 7 of TVR therapy, PEG-IFN α-2b (100 μg/week) and ribavirin (400 mg/bid) were also added to constitute the triple therapy. The levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) decreased to normal levels during the first 7 d with TVR, without PEG-IFN α-2b and ribavirin. The levels of uric acid, serum creatinine, and blood urea nitrogen (BUN) moderately increased; an increase in the dosage of allopurinol from 100 mg/d to 200 mg/d was effective against TVR-related kidney injury. The patient successfully completed the 12-week regimen of the triple therapy; subsequently, the administration of TVR was discontinued. Immediately after the termination of TVR administration, the dosage of cyclosporine was increased to 100 mg/bid to maintain a sufficient trough level to prevent rejection. Over the course of 100 d, the patient did not experience any severe adverse reactions related to TVR and cyclosporine and such as graft rejection.

Case II: A 50-year-old man who underwent living-donor liver transplantation from his offspring because of HCV-related liver cirrhosis. Because the serum HCV RNA level rapidly increased after the transplantation, he received liver-supporting therapy with monoammonium glycyrrhizinate for 1 month. The calcineurin inhibitor was carefully switched from tacrolimus to cyclosporine about 10 d before the admin-

Table 1. Pretreatment Profile and Clinical Characteristics

Case number	I	II	III	IV
Male/Female	Male	Male	Female	Female
Age (years)	62	50	67	68
Body weight (kg)	68.6	120.2	53.4	43.0
Primary disease for transplantation	HCC	LC	LC, HCC	LC, HCC
Milan criteria for HCC treatment	Within		Within	Within
ABO blood type (donor/recipient)	O/O	A/A	B/B	O/O
Donor	Cadaveric	Offspring	Spouse	Offspring
HCV genotype	1b	1b	1b	1b
Months after liver transplantation	77	22	74	37
Months after transplantation recurrence of HCV	75	1	69	33
Post-transplant anti-HCV treatment	PEG-IFN, RBV	Not treated	PEG-IFN, RBV	PEG-IFN, RBV
Duration of post-transplant anti-HCV treatment (months)	2–8, 11–44, 68–71		8–22	5–15
Outcome	Withdraw		SVR*	Withdraw
Positive conversion of HCV after SVR (month after liver transplantation)			66	

HCC, hepatocellular carcinoma; LC, liver cirrhosis; HCV, hepatitis C virus; LT, liver transplantation; PEG-IFN, pegylated interferon alfa-2b; RBV, ribavirin; SVR, sustained viral response.

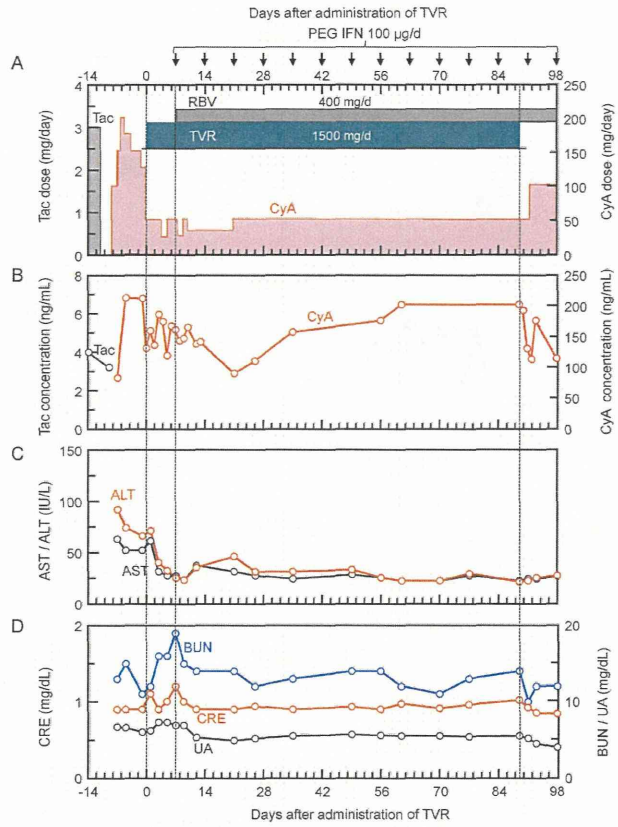

Fig. 1. Dosage of Immunosuppressants and TVR (A); Blood Concentrations of Immunosuppressants (B); Monitoring of Levels of Transaminases (C); Creatinine, Blood Urea Nitrogen, and Uric Acid Levels (D) in Case I

(A) The daily doses of tacrolimus (black) and cyclosporine (red) were documented. (B) The blood concentration of tacrolimus (black) and cyclosporine (red) were quantified by chemiluminescence immunoassay (CLIA) and affinity column-mediated immunoassay (ACMIA), respectively. (C) Aspartate aminotransferase (black) and alanine aminotransferase (red) values were determined. (D) Creatinine (red), blood urea nitrogen (blue), and uric acid (black) values were determined. TVR, telaprevir; Tac, tacrolimus; CyA, cyclosporine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRE, creatinine; BUN, blood urea nitrogen; UA, uric acid.

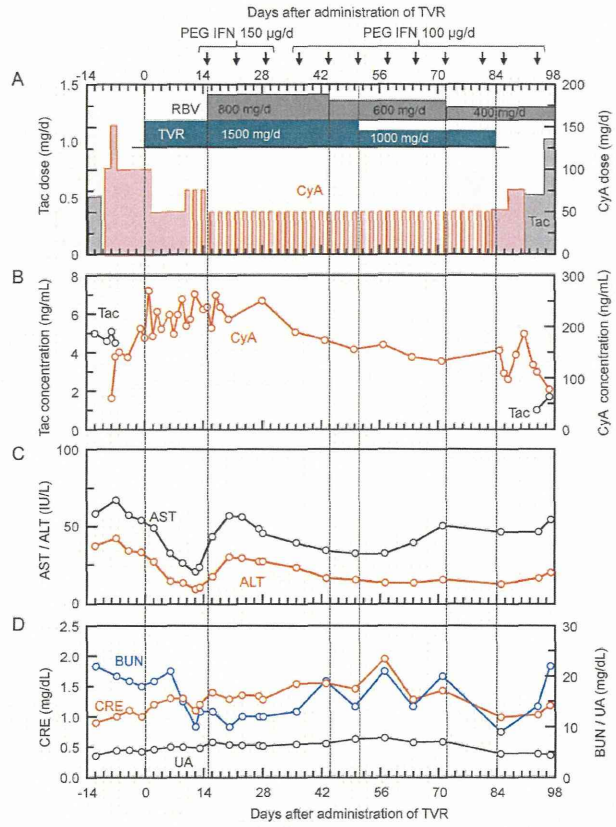

Fig. 2. Dosage of Immunosuppressants and TVR (A); Blood Concentrations of Immunosuppressants (B); Monitoring of Levels of Transaminases (C); Creatinine, Blood Urea Nitrogen, and Uric Acid Levels (D) in Case II

(A) The daily doses of tacrolimus (black) and cyclosporine (red) were documented. (B) The blood concentration of tacrolimus (black) and cyclosporine (red) were quantified by CLIA and ACMIA, respectively. (C) Aspartate aminotransferase (black) and alanine aminotransferase (red) values were determined. (D) Creatinine (red), blood urea nitrogen (blue), and uric acid (black) values were determined. TVR, telaprevir; Tac, tacrolimus; CyA, cyclosporine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRE, creatinine; BUN, blood urea nitrogen; UA, uric acid.

istration of TVR. Before TVR administration (1500 mg/bid), the dosage and trough concentration of cyclosporine were 100 mg/bid and 197 ng/mL, respectively (Figs. 2A, B). Because of interactions between cyclosporine and TVR, the dosage of cyclosporine was reduced to half on day 1 of TVR administration. We carefully adjusted the dosage of cyclosporine on the basis of the measurement of morning blood concentration during 3 weeks after administration of TVR, because of the unstable pharmacokinetics of cyclosporine. Finally, the dosage of cyclosporine was 50 mg/qod and the trough level of cyclosporine was 215 ng/mL at day 21. Hepatorenal syndrome and diabetic nephropathy deteriorated his renal function at surgery; therefore, the initiation of PEG-IFN α-2b (150 μg/week) and ribavirin (800 mg/bid) was carefully set back to day 14 to avoid further kidney dysfunction. Fatigue and anorexia worsened from 1 month after initiation of TVR treatment, and the patient developed intense pruritus on his back from day 51 after TVR treatment. Because of these complications, the dose of TVR was decreased to 1000 mg/bid; subsequently, these adverse reactions subsided. Although the blood concentration of cyclosporine decreased to 141 ng/mL at the 20th day after reducing the TVR dosage, the dosage of cyclosporine of 50 mg on alternate days (qod) was unchanged without any rejection episode. On the 19th day, the uric acid level was 7.0 mg/dL; therefore, the patient was administered febuxostat (10 mg/d). Moreover, the serum creatinine level moderately increased, but it decreased after reduction of TVR dose. The patient completed the 12-week TVR therapy. After the end of TVR administration, the dosage of cyclosporine was gradually increased from 50 mg/qod to 50 mg/qd, and finally to 75 mg/qd to maintain the target trough level. At that point, the blood concentration of cyclosporine was stable. The calcineurin inhibitor was switched again from cyclosporine to tacrolimus. Over the course of 100 d, the patient showed no rejection episode.

Case III: A 67-year-old woman who underwent living-donor liver transplantation from her spouse because of hepatocellular carcinoma after HCV-related liver cirrhosis, which was within the Milan criteria. The patient experienced a recurrence of hepatitis C at 6 months after transplantation; therefore, she was treated with PEG-IFN α-2b and ribavirin for 14 months and achieved SVR. At the 66th post-transplant month after achieving SVR, she experienced relapse of HCV infection and was followed-up without PEG-IFN α-2b treat-

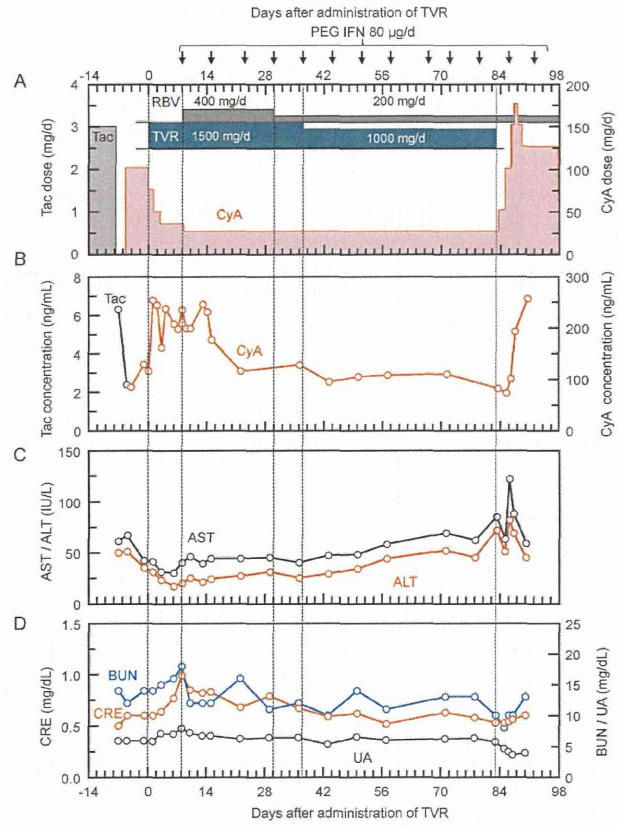

Fig. 3. Dosage of Immunosuppressants and TVR (A); Blood Concentrations of Immunosuppressants (B); Monitoring of Levels of Transaminases (C); Creatinine, Blood Urea Nitrogen, and Uric Acid Levels (D) in Case III

(A) The daily doses of tacrolimus (black) and cyclosporine (red) were documented. (B) The blood concentration of tacrolimus (black) and cyclosporine (red) were quantified by CLIA and ACMIA, respectively. (C) Aspartate aminotransferase (black) and alanine aminotransferase (red) values were determined. (D) Creatinine (red), blood urea nitrogen (blue) and uric acid (black) values were determined. TVR, telaprevir; Tac, tacrolimus; CyA, cyclosporine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRE, creatinine; BUN, blood urea nitrogen; UA, uric acid.

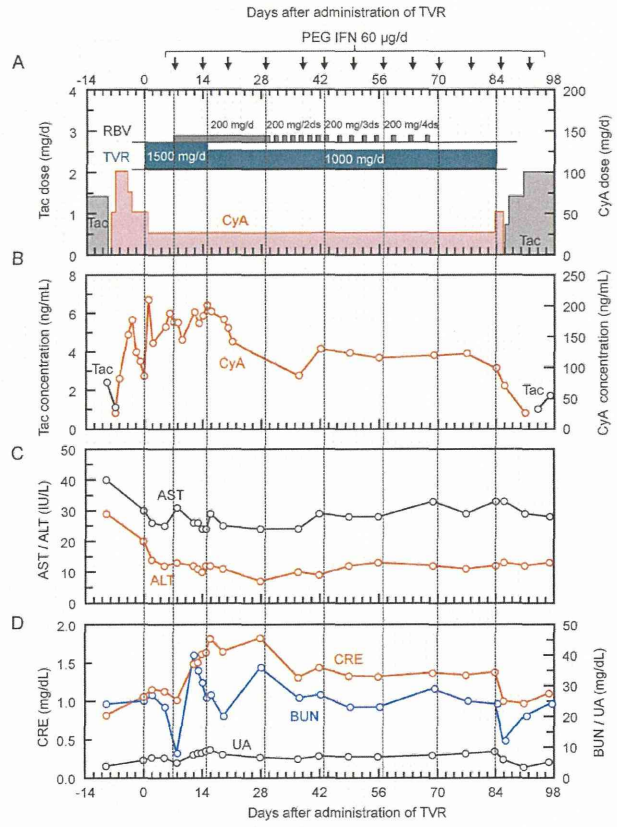

Fig. 4. Dosage of Immunosuppressants and TVR (A); Blood Concentrations of Immunosuppressants (B); Monitoring of Levels of Transaminases (C); Creatinine, Blood Urea Nitrogen, and Uric Acid Levels (D) in Case IV

(A) The daily doses of tacrolimus (black) and cyclosporine (red) were documented. (B) The blood concentration of tacrolimus (black) and cyclosporine (red) were quantified by CLIA and ACMIA, respectively. (C) Aspartate aminotransferase (black) and alanine aminotransferase (red) values were determined. (D) Creatinine (red), blood urea nitrogen (blue) and uric acid (black) values were determined. TVR, telaprevir; Tac, tacrolimus; CyA, cyclosporine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRE, creatinine; BUN, blood urea nitrogen; UA, uric acid.

ment. Before treatment with TVR, PEG-IFN α-2b, and ribavirin, the calcineurin inhibitor was switched from tacrolimus to cyclosporine about a week before TVR administration. The dosage and trough concentration of cyclosporine at the day before the administration of TVR were 100 mg/bid and 129 ng/mL, respectively (Figs. 3A, B). To achieve the target trough concentration of cyclosporine (150–200 ng/mL), the dosage of cyclosporine was gradually decreased from 100 mg/bid to 35 mg/qd within 4 d after initiation of TVR administration (1500 mg/bid). The dosage of cyclosporine decreased to 25 mg/qd when the trough concentration of cyclosporine was greater than 200 ng/mL. On day 8 of TVR therapy, PEG-IFN α-2b (80 μg/week) and ribavirin (400 mg/bid) were also added to constitute the triple therapy. The levels of AST and ALT decreased during the first 7 d with TVR without PEG-IFN α-2b or ribavirin. Administration of allopurinol (50 mg/d) was effective against TVR-related kidney injury such as temporary increases in the levels of uric acid, serum creatinine, and BUN (Fig. 3D). Because of anemia and anorexia, the doses of TVR and ribavirin were decreased to 1000 mg/bid on day 37 and 200 mg/day on day 30, respectively. Subsequently, the severity of these adverse decreased. The patient completed the 12-week TVR therapy. After the final day of TVR administration, the dose of cyclosporine was gradually increased from 25 mg/qd to 50 mg/bid, and finally to 100 mg/bid to maintain the target trough level. At that point, the blood concentration of cyclosporine was stable at the dosage of 125 mg/bid. Over the course of 100 d, the patient showed no rejection episode.

Case IV: A 68-year-old woman who underwent living-donor liver transplantation from her offspring because of hepatocellular carcinoma and liver cirrhosis. The patient experienced hepatitis C recurrence at 5 months after transplantation, and was immediately treated with PEG-IFN α-2b and ribavirin. However, she was withdrawn from this therapy because of nausea at 15 months after liver transplantation. The calcineurin inhibitor was switched from tacrolimus to cyclosporine about a week before the administration of TVR. The dosage and trough concentration of cyclosporine at the day before TVR administration were 50 mg/bid and 109 ng/mL, respectively (Figs. 4A, B). To achieve the target trough concentration of cyclosporine (150–200 ng/mL), the dosage of cyclosporine was decreased from 50 mg/bid to 25 mg/qd at the first day of TVR administration (1500 mg/bid). At Day 7 of TVR therapy, PEG-IFN α-2b (60 μg/wk) and ribavirin (200 mg/d) were also