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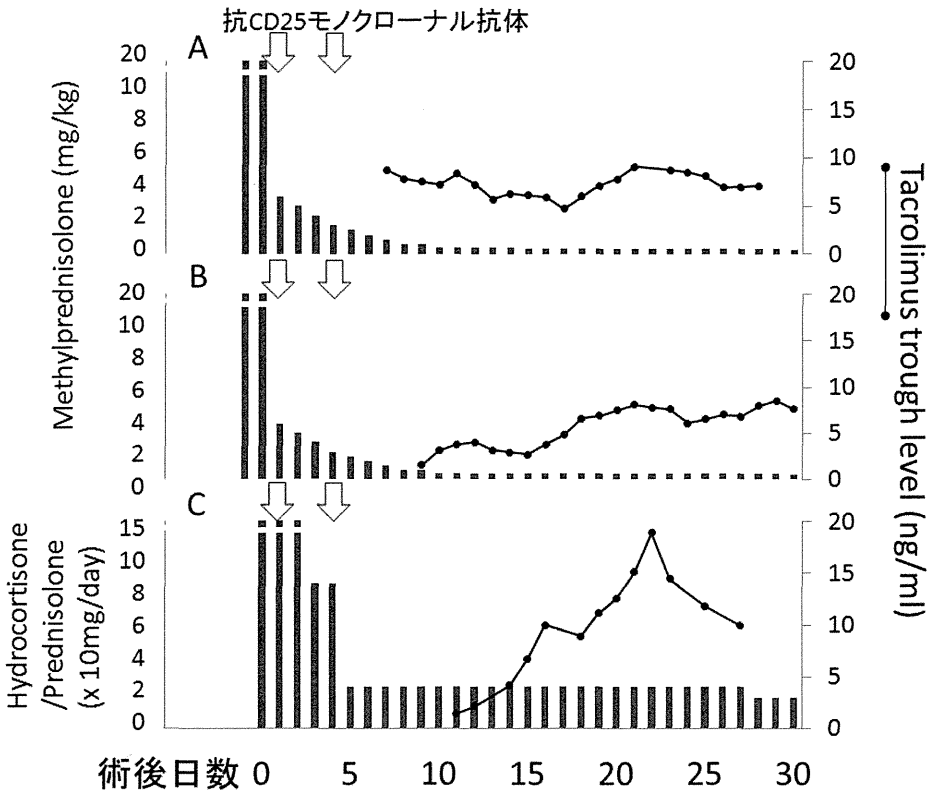
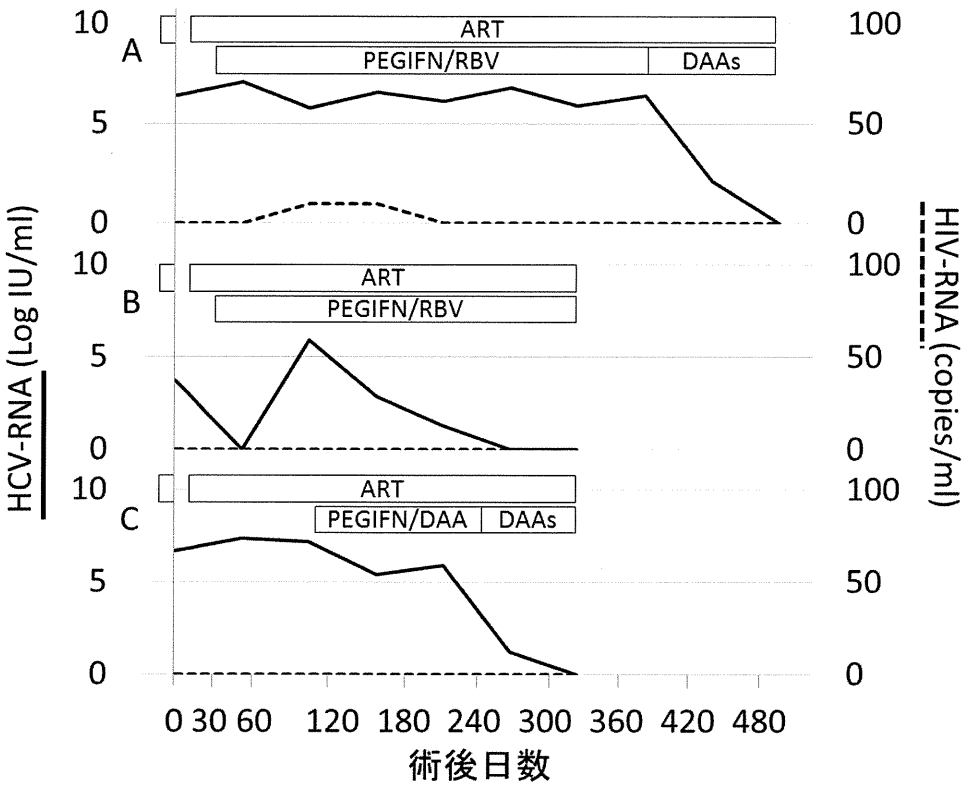
図

図 1.　抗 CD25 モノクローナル抗体を用いた免疫抑制療法



折れ線は Tacrolimus 開始後の血液中トラフ濃度の推移を示す。A から C は症例 1 から 3 を示す。

図 2.　HIV/HCV に対する抗レトロウイルス療法(ART)と HCV に対する治療



点線は HIV-RNA のウイルス量(右軸)を、実線は HCV-RNA のウイルス量(左軸)の推移を示す。A から C は症例 1 から 3 を示す。略語、ART、抗レトロウイルス療法; PEGINF、ペグインターフェロン; RBV、リバビリン; DAA、直接作用型抗ウイルス薬(HCV)

表

表 1. 患者背景

		症例 1	症例 2	症例 3
術前	年齢	47	50	41
	性別	男性	男性	男性
	BMI (kg/m2)	21.7	18.8	24.6
	血友病	A	A	A
	MELD/Child-Pugh スコア	22 / 12	13 / 10	18 / 12
	総ビリルビン値 (mg/dL)	4.3	3.4	4.6
	HIV-RNA 量 (copies/mL)	検出感度以下	検出感度以下	検出感度以下
	抗レトロウイルス療法	raltegravir, lamivudine, abacavir, etravirine	raltegravir, tenofovir, emtrivitabine	raltegravir, tenofovir, emtrivitabine
	CD4 陽性細胞数(個/μl)	247	351	258
	HCV Genotype	1b	1a	1b
	HCV-RNA 量 (LogIU/mL)	6.5	3.7	6.2
	肝細胞癌の合併	なし	なし	なし
手術	ドナー	妻	妻	脳死ドナー
	ドナー年齢	43	48	40 代
	グラフト肝の種類	右肝	左肝	全肝
	グラフト肝重量 (g)	474	361	1590
	標準肝容量に対する割合 (%)	39	35	123
	手術時間	11 時間 8 分	12 時間 2 分	11 時間 45 分
術後	出血量(ml)	6,690	1,900	16,500
	拒絶	なし	なし	あり
	サイトメガロウイルス感染症	なし	なし	なし
	合併症	術後出血	細菌感染症	術後出血
	生存期間 (月)	23	15	14

略語; BMI、body mass index; MELD、model for end-stage liver disease

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CASE REPORT

Interleukin-2 receptor antagonist immunosuppression and consecutive viral management in living-donor liver transplantation for human immunodeficiency virus/hepatitis C-co-infected patients: a report of 2 cases

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Abstract Management of immunosuppression for human immunodeficiency virus/hepatitis C (HIV/HCV) in living-donor liver transplantation (LDLT) has not been established. We performed LDLT for two patients with HIV/HCV-co-infected end-stage liver disease. The immunosuppression protocol consisted of early calcineurin inhibitor-free and interleukin-2 receptor antagonist (IL2Ra) induction and methylprednisolone. Maintenance low-dose tacrolimus was started and anti-retroviral therapy for HIV was re-started 1 week after LDLT. Consecutively, pegylated interferon and ribavirin therapy were successfully added as pre-emptive therapy for HCV. HIV-RNA and HCV-RNA were undetectable on anti-retroviral therapy and HCV treatment at 17 and 8 months after LDLT, respectively, with normal liver function. This study is the first report of early calcineurin inhibitor-free and IL2Ra induction with methylprednisolone immunosuppression in LDLT for HIV/HCV-co-infected patients with a favorable outcome. Consecutive HIV/HCV treatment was well tolerated.

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Keywords Interleukin-2 receptor antagonist · Living-donor liver transplantation · Human immunodeficiency virus · Hepatitis C · Co-infection

Introduction

Hepatitis C virus (HCV) co-infections are identified in 19 % of human immunodeficiency virus (HIV)-infected people in Japan, because most of them contracted both viruses from administration of unheated concentrated coagulation factors for hemophilia [1]. Since the recent development of anti-retroviral therapy (ART) has drastically reduced HIV infection-related mortality, HCV-related end-stage liver diseases are now a leading cause of death in HCV/HIV-co-infected patients [2]. Although liver transplantation is a therapeutic option for end-stage liver disease, living-donor liver transplantation (LDLT) for HIV/HCV-co-infected patients remains challenging [3] because immunosuppression and postoperative HIV/HCV management for LDLT have not been established. The incidence of acute rejection is paradoxically high in HIV/HCV-co-infected liver transplant recipients and management of acute rejection and HIV/HCV has an impact on the outcome [2]. Meanwhile, an increased serum creatinine level and high calcineurin inhibitor trough levels during the early period after liver transplantation has a significant impact on the risk of developing chronic renal disease [4]. The postoperative immunosuppression regimen for HIV/HCV-co-infected patients should avoid acute rejection while maintaining satisfactory liver and renal function for further consecutive HIV/HCV management by carefully monitoring drug-to-drug interactions. The aim of the present study was to investigate the feasibility of early calcineurin inhibitor-free and interleukin-2 receptor

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antagonist (IL2Ra) induction with concomitant methylprednisolone immunosuppression, ART and consecutive interferon and ribavirin pre-emptive therapy for co-infected HIV/HCV patients undergoing LDLT.

Case report

Patient 1

Patient 1 was a 47-year-old male with hemophilia A and HIV/HCV co-infection detected at the age of 22 years (Table 1). He achieved undetectable HIV-RNA on ART (raltegravir 800 mg/day, lamivudine 300 mg/day, abacavir 600 mg/day, and etravirine 400 mg/day); however, his serum HCV-RNA level was 6.5 log IU/ml, and genotype was 1b (null responder to interferon treatment). The model for end-stage liver disease score was 22 at the time of LDLT. Serum total bilirubin, serum albumin and prothrombin time international normalized ratio were 4.3, 2.8 and 1.78 mg/dl, respectively. Creatinine level and estimated glomerular filtration rate were 1.53 mg/dl and 40.4 ml/min/1.73 m^2, respectively [5] and CD4-positive T-cell count was 247 cells/mm^3. Body mass index was 21.7 kg/m^2. He had mild ascites and no hepatic encephalopathy or history of AIDS-defining events or previous opportunistic infections. In 2013, informed consent was obtained and LDLT was performed with administration of recombinant coagulation factor VIII. The donor was the patient's 43-year-old spouse. Splenectomy was also performed for further interferon therapy. Operation time was 11 h 8 min and estimated blood loss was 6,690 ml. The weight of the right liver graft was calculated to be 39 % of the patient's standard liver volume. An emergent re-laparotomy was performed for intra-abdominal hemorrhage on day 0. IL2Ra, basiliximab (20 mg), was administered on days 1 and 4. Methylprednisolone was administered as described previously [6]. Administration of recombinant coagulation factor VIII was not necessary due to an adequate factor VIII level. The same ART regimen was resumed on day 6. Low-dose tacrolimus was started on day 8 with a target trough level of 8–10 ng/ml. Pegylated interferon (alpha-2b, 75 μg/week) and ribavirin (400 mg/day) were added on day 28 (Fig. 1a). The postoperative course after day 1 was uneventful and he was discharged on day 43. His serum creatinine level and estimated glomerular filtration rate at 30 days after transplantation were 0.86 mg/dl and 75.8 ml/min/1.73 m^2, respectively. One year after LDLT, pegylated interferon and ribavirin were changed to nonstructural protein 5A replication complex inhibitor, daclatasvir, and nonstructural protein 3/4A protease inhibitor, asunaprevir. At the same time, etravirine (ART) was changed to tenofovir to

Table 1 Patient characteristics at LDLT and outcome

Patient no.	Age/sex	Type of hemophilia	HCV genotype	HCV-RNA at LDLT (logIU/ml)	HIV-RNA at LDLT (copy/ml)	MELD score	BMI (kg/m^2)	Graft	Graft size (%SLV)	eGFR at LDLT	eGFR 30 days after LDLT	ACR	CMV	Survival (months)
1	47/male	A	1b	6.5	ND	22	21.4	Right	38.8	40.4	75.8	0	0	Alive (17)
2	50/male	A	1a	3.7	ND	13	19.6	Left	34.6	73.5	106.5	0	0	Alive (8)

HCV hepatitis C virus, *LDLT* living-donor liver transplantation, *HIV* human immunodeficiency virus, *MELD* model for end-stage liver disease, *eGFR* estimated glomerular filtration rate (ml/min/1.73m^2), *BMI* body mass index, *SLV* standard liver volume, *ACR* acute cellular rejection, *CMV* cytomegalovirus, *ND* not detected

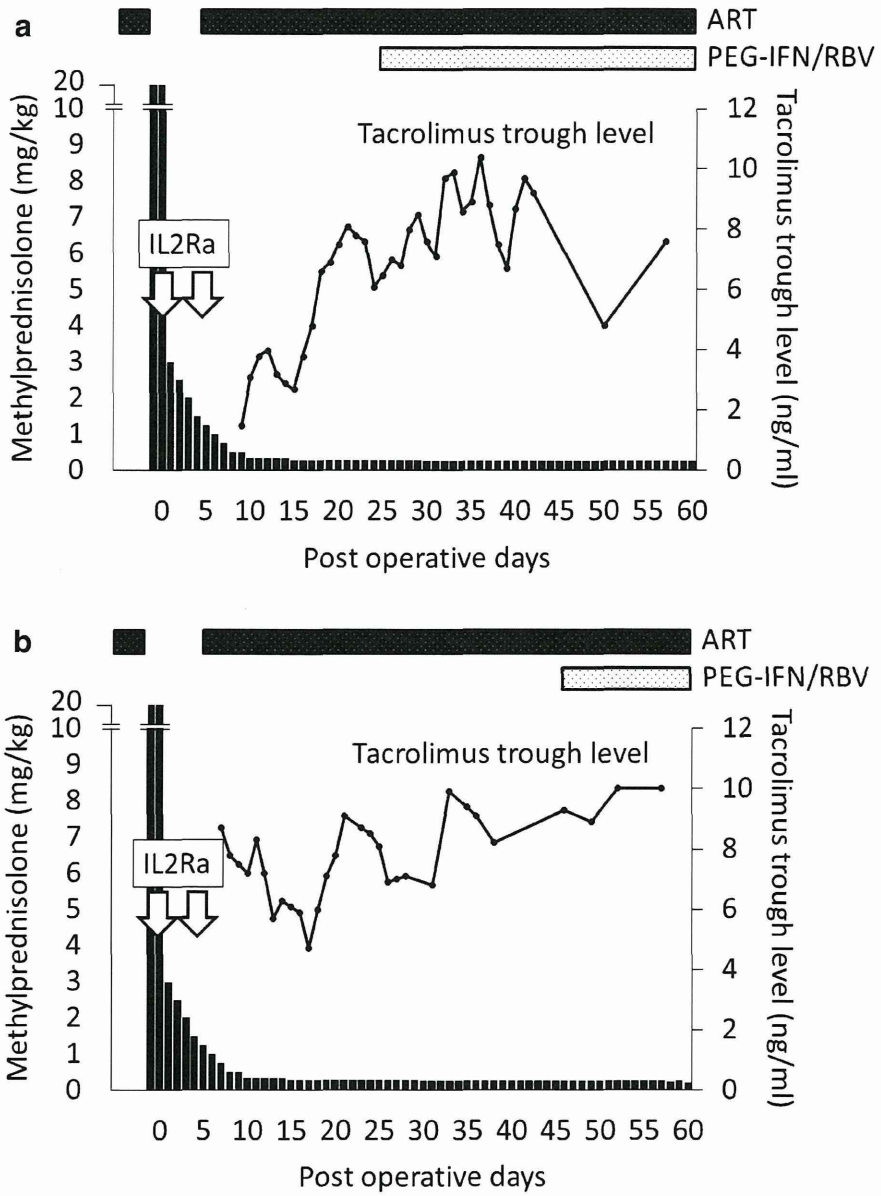

Fig. 1 Immunosuppression protocol and consecutive viral management of HIV/HCV. **a** Patient 1, **b** patient 2. *IL2Ra* interleukin-2 receptor antagonist, *ART* anti-retroviral therapy, *PEG-IFN/RBV* pegylated interferon and ribavirin

avoid drug-to-drug interactions. HIV-RNA and HCV-RNA were undetectable at 17 months after LDLT and the patient remains in stable condition (Fig. 2a).

Patient 2

Patient 2 was a 50-year-old male with hemophilia A and HIV/HCV co-infection detected at the age of 41 years (Table 1). He achieved undetectable HIV-RNA on ART (raltegravir 800 mg/day, tenofovir 300 mg/day, and emtricitabine 200 mg/day). His HCV-RNA level was 3.7 log IU/ml, and genotype was 1a without any treatment for HCV. The model for end-stage liver disease score and serum total bilirubin were 13 and 3.4 mg/dl, respectively, and serum albumin and prothrombin time international normalized ratio were 2.7 and 1.23 mg/dl, respectively. Creatinine level and estimated glomerular filtration rate were 0.87 mg/dl and 73.5 ml/min/1.73 m^2, respectively. The CD4-positive T-cell count was 351 cells/mm^3. Body mass index was 19.6 kg/m^2. He had mild ascites and no history of hepatic encephalopathy or AIDS-defining events or previous opportunistic infections. In 2014, informed consent was obtained and LDLT was performed. The donor was the patient's 48-year-old spouse. Splenectomy was also performed for further interferon therapy. Operation time was 12 h 2 min and estimated blood loss was

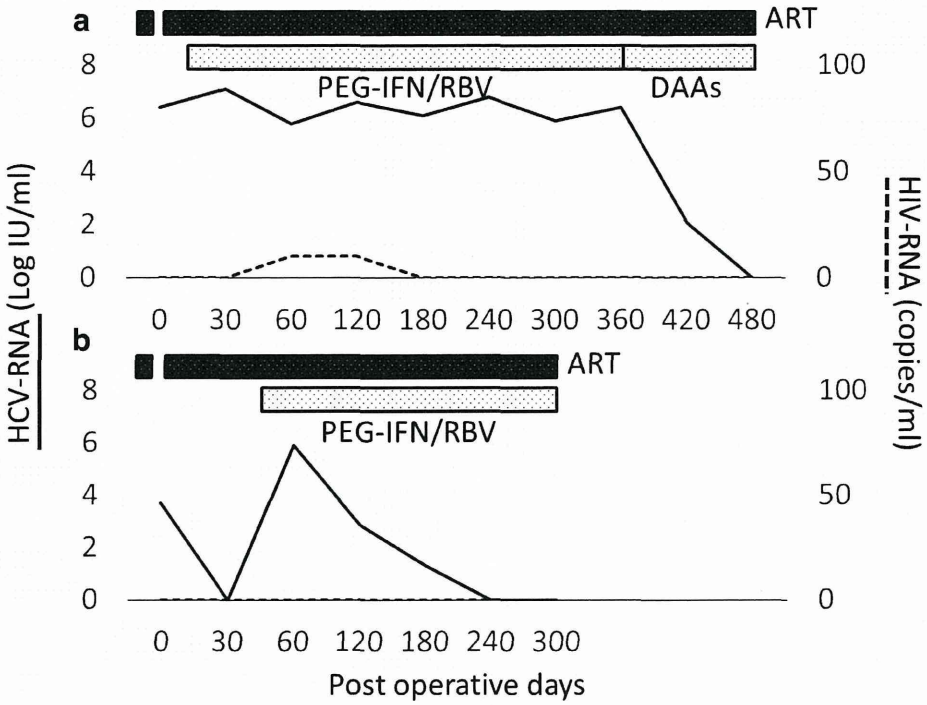

Fig. 2 Trend of viral roads of HIV/HCV on consecutive viral management. **a** Patient 1, **b** patient 2. *DAA* direct-acting antiviral, *HCV* hepatitis C virus, *RNA* ribonucleic acid, *HIV* human immunodeficiency virus

1,900 ml. The left liver graft weight was calculated to be 35 % of the patient's standard liver volume. Use of recombinant coagulation factor VIII and immunosuppression protocol was the same as patient 1. Tacrolimus was started on day 6. The same ART regimen was resumed on day 7. On day 38, he was discharged after a catheter-related bloodstream infection and intra-abdominal abscess cured by antibiotic treatment. Creatinine level and estimated glomerular filtration rate were 0.62 mg/dl and 106.5 ml/min/1.73 m^2, respectively. Pegylated interferon (alpha-2b, 60 μg/week) and ribavirin (400 mg/day) were started on day 46 (Fig. 1b). HIV-RNA and HCV-RNA were undetectable at 8 months after LDLT, and the patient remains in stable condition (Fig. 2b).

Discussion

Liver transplantation is an established therapeutic option for end-stage liver disease with a 5-year survival rate of ~70 % in deceased-donor liver transplantation (DDLT) based on data from the United Network for Organ Sharing [7]. However, outcome of liver transplantation for HIV/HCV-co-infected recipients has not been favorable. A study in the United States of liver transplantation for 27 HIV/HCV-co-infected recipients reported a 5-year survival rate of 33 % [8]. A French study reported a 5-year patient survival rate of 51 % for 35 HIV/HCV-co-infected recipients [9]. There has been extremely limited experience in LDLT for HIV/HCV-co-infected recipients. Our previous report on 6 HIV/HCV-co-infected patients found 3- and 5-year survival rates of 66 % and 50 %, respectively, and appears to be the only literature to date [3].

Unfavorable results after liver transplantation for HIV/HCV-co-infected patients may be largely attributed to the difficulty of immunosuppression in this setting. In fact, we experienced a high incidence (67 %) of acute rejection in our initial series [3]. Similarly, in DDLT, >50 % of moderate to severe rejection episodes occurred within 21 days of transplantation [10]. Treatment of acute rejection requires extensive immunosuppression therapy and delaying initiation of ART; HCV-related cholestatic hepatitis and other infections may develop such as cytomegalovirus infection, which are difficult to manage following LDLT. One of the problems might be impaired renal function due to the conventional use of calcineurin inhibitors. For instance, an increased serum creatinine level during the first week post-transplant and tacrolimus trough levels >15 ng/ml at day 15 significantly impact on the risk of developing chronic renal disease [4]. Furthermore, renal impairment causes limitation of use of HIV/HCV drugs. For instance, tenofovir for HIV is associated with nephrotoxicity and sofosbuvir is not recommended for HCV in patients with severe renal impairment or end-stage renal disease.

IL2Ra, a chimeric monoclonal antibody that binds to CD25, inhibits IL-2-mediated proliferation on the surface of activated T lymphocytes. A recent meta-analysis of IL2Ra induction in liver transplant recipients reported that IL2Ra immunosuppression therapy reduces the incidence of acute rejection and impaired renal function [11]. IL2Ra induction is also used to minimize the use of calcineurin inhibitors. Detailed meta-analysis found that acute rejection at 12 months or later favored the use of IL-2Ra (relative risk 0.83) and steroid-resistant rejection was also less frequent in patients receiving IL-2Ra (relative risk 0.66). Patients who received IL-2Ra in addition to reduced or delayed calcineurin inhibitors had better renal function and a lower incidence of renal dysfunction (relative risk 0.46) [11]. On the basis of these advantages of IL2Ra, we employed the use of IL2Ra for HIV/HCV-co-infected patients. In our study, the preliminary results of early calcineurin inhibitor-free and IL2Ra induction with concomitant methylprednisolone immunosuppression in LDLT were favorable for HIV/HCV-co-infected patients as well as showing improved renal function at 30 days after LDLT. Healthy renal function in immunosuppressed patients on HIV/HCV treatment ensures drug dosing errors related to renal impairment are avoided. In DDLT, although meta-analysis of IL2Ra was reported, only a limited number of cases of IL2Ra induction for HIV/HCV-co-infected patients were reported successfully [12]. To the best of our knowledge, this study is the first report of the use of IL2Ra induction in HIV/HCV-co-infected end-stage liver disease patients in the LDLT setting.

Early treatment of HCV following LDLT is important for preventing the progression of liver fibrosis. Pre-emptive therapy, initiation of pegylated interferon and ribavirin for HCV-infected patients early after liver transplantation, was reported in LDLT. An end-of-treatment response was achieved in 45 % of patients, and a sustained viral response was achieved in 34 % with a 5-year survival rate of 79 % for HCV-mono-infected patients, which did not significantly differ from non-HCV patients [13]. Pre-emptive therapy may be more important in HIV/HCV-co-infected patients, because HIV infection accelerates the progression of liver fibrosis due to HCV. HCV recurrence appears earlier in HIV/HCV-co-infected patients than in HCV-mono-infected patients, and results in graft loss [8]. In one controlled study, the proportion of patients with bridging fibrosis or cirrhosis at 2 years post-transplantation was 28 % in HIV/HCV-co-infected patients and 10 % in HCV-mono-infected patients [9]. Although interferon and ribavirin therapy is still an important pre-emptive therapy following LDLT for HCV, recently introduced direct-acting antiviral agents for HCV were used in patient 1 with careful monitoring of drug-to-drug interactions because he had not achieved a sustained viral response on a conventional interferon and ribavirin therapy. Interferon-free direct-acting antiviral agents have the potential to achieve high rates of sustained virological response with infrequent serious adverse events [14]. Considering the advantage of interferon-free direct-acting antiviral agents, further study as a first-line treatment is needed in HIV/HCV-co-infected liver transplant patients.

Although protease inhibitors are key drugs for suppressing HIV, they are potentially hepatotoxic, especially in HCV-co-infected patients [15]. Protease inhibitor-related hepatitis also occurs in ~9 % of patients and has a more aggressive course in HCV-co-infected patients [16]. A previous report of LDLT for HIV/HCV-co-infected patients found that protease inhibitors for HIV could not be started within 1 month after LDLT because of strong drug-to-drug interactions with tacrolimus [3]. Raltegravir, an HIV-1 integrase inhibitor, is not a substrate of CYP450 enzymes and has fewer drug-to-drug interactions with calcineurin inhibitors. The combination of two nucleoside reverse-transcriptase inhibitors (nucleoside reverse-transcriptase inhibitors, tenofovir and emtricitabine or abacavir and lamivudine) and raltegravir is currently the antiretroviral regimen of choice for HIV-infected liver transplant recipients to avoid pharmacokinetic interactions with immunosuppression [2]. A shorter ART cessation period is the key to maintaining control of HIV-RNA during LDLT. In the present protocol, ART was resumed at days 6 and 7 after LDLT, respectively, including nucleoside reverse-transcriptase inhibitors and raltegravir.

In conclusion, we have reported two successful cases of early calcineurin inhibitor-free and IL2Ra induction with methylprednisolone immunosuppression in LDLT for HIV/HCV-co-infected patients. Consecutive HIV/HCV treatment was well tolerated after LDLT. This is a promising strategy for LDLT in this setting and warrants further evaluation.

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Compliance with ethical standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Human Rights: All procedures followed have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed Consent: Informed consent was obtained from all patients for being included in the study.

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Hepatology Research

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Case Report

Daclatasvir and asunaprevir for recurrent hepatitis C following living donor liver transplantation with HIV co-infection

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Antiviral treatment in liver transplant recipients co-infected with hepatitis C virus (HCV) and HIV remains a challenge. We herein report a case of HCV recurrence that was successfully treated using interferon-free anti-HCV therapy with daclatasvir and asunaprevir. A 48-year-old man underwent antiviral therapy with a 24-week course of daclatasvir and asunaprevir for biopsy-proven recurrent HCV 15 months after living donor liver transplantation, following non-response to pre-emptive antiviral treatment with pegylated interferon plus ribavirin. Anti-HIV and immunosuppressive regimens were modified safely. Renal function was feasibly preserved. The anti-HCV effect was remarkable with an undetectable viral load confirmed within 2 weeks, and this patient achieved a sustained virological response after 12 weeks of post-transplantation treatment. No serious adverse events were observed. This case indicates that daclatasvir and asunaprevir for recurrent HCV in a HIV co-infected recipient after liver transplantation is safe and effective.

Key words: asunaprevir, co-infection, daclatasvir, HIV, living donor liver transplantation

INTRODUCTION

RECENT UPDATES[1,2] INDICATE that liver transplantation is a feasible treatment option for patients with end-stage liver disease and co-infection with hepatitis C virus (HCV) and HIV, primarily due to the high liver-related mortality and comparatively improved post-transplant survival due to the evolution of antiretroviral treatment (ART) in this population. Patient and graft survival rates in HCV/HIV co-infected liver transplant recipients, however, remain poorer than those in HCV mono-infected recipients. Recurrent HCV is a primary reason for the poor prognosis following liver transplantation, mainly because co-infected recipients have a more aggressive recurrence, namely, severe necroinflammation and rapid progression of fibrosis in the graft, or the occurrence of fibrosing cholestatic hepatitis.

Until recently, combined treatment with pegylated interferon (PEG IFN) and ribavirin (RBV) was the only option for recurrent HCV. The virological response to this regimen in HIV/HCV co-infected recipients, however, has been poor (10–20%).[2,3] There are several reasons for the poor virological response of HIV/HCV co-infected recipients to PEG IFN/RBV, including higher rates of premature discontinuation due to intolerability, higher severity of liver disease at initiation of treatment and/or host factors related to HIV co-infection.[1]

Here, we describe a case of established recurrent HCV after living donor liver transplantation (LDLT) with HIV co-infection and renal dysfunction that previously exhibited a null-response to conventional treatment with PEG IFN/RBV and was safely and successfully treated using IFN-free therapy with daclatasvir and asunaprevir.

CASE REPORT

A 48-YEAR-OLD MAN received antiviral therapy with daclatasvir and asunaprevir for recurrent HCV post-LDLT. He underwent right liver LDLT at the age of 47 years, with his spouse as the living donor, for HCV-related cirrhosis; this patient was known to be co-infected with HIV. The HCV genotype was 1b. The interleukin-28B (IL28B) genotype rs8099917, examined using the Invader assay, was recipient TT (major homo) and donor TG (hetero). The

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source of infection of both HCV and HIV was thought to be contamination of blood-derived clotting factor products (factor VIII) for hemophilia. Since the initial diagnosis of HIV/HCV co-infection 25 years prior to LDLT, the patient had undergone two courses of IFN monotherapy and one course of PEG IFN/RBV, and was a non-responder to both treatments. He had also received ART with feasible control, achieving undetectable HIV RNA and CD4 counts of approximately 200–300/μL until LDLT. The regimen at the time of the LDLT consisted of etravirine (ETR), raltegravir and abacavir sulfate/lamivudine. In addition, he had been exposed to azidothymidine, lamivudine, emtricitabine, stavudine, tenofovir disoproxil fumarate (TDF) and efavirenz.

The immunosuppression regimen was based on tacrolimus and methylprednisolone. The immediate postoperative course was uneventful except for reoperation for intra-abdominal bleeding on postoperative day (POD) 1. ART with the combination of ETR, raltegravir and abacavir sulfate/lamivudine was restarted on POD 6 during the first admission, and the patient was discharged on POD 45 without infectious complications or acute cellular rejection.

On POD 69, according to our protocol treatment for HCV positive recipients,[4] pre-emptive anti-HCV treatment was initiated (pretreatment HCV-RNA load was 6.4 log IU/mL) with low-dose PEG IFN-α-2b (0.5 μg/kg) and RBV 200 mg/day, which was gradually increased to 1.0 μg/kg PEG IFN and 400 mg RBV. Although the treatment was tolerated, the virological response was null after 48 weeks of PEG IFN/RBV, leading us to convert to treatment with the IFN-free direct-acting antivirals (DAA) daclatasvir and asunaprevir. At that time, the resistance-associated substitutions at L31 and Y93 in the NS5A region, and at Q80, R155, A156, D168 and V170 in the NS3 region of the HCV genome were tested using a direct sequencing method, which confirmed only the resistance-associated variant of V170I. Renal function was impaired with a serum creatinine level of 1.45 mg/dL and estimated glomerular filtration rate (eGFR) of 43.2 mL/min,[5] and low-grade proteinuria, indicative of chronic kidney disease stage G3b/A2 as per the Kidney Disease: Improving Global Outcomes Work Group.[6] The daily form of tacrolimus was administrated with a trough level of 3–7 ng/mL. Liver biopsy indicated liver injury compatible with recurrent HCV activity grade 1 and fibrosis stage 1, as per METAVIR. The ETR was switched to TDF, based on the strong drug–drug interaction of asunaprevir with ETR[7] as well the absence of viral mutations to TDF despite the patient's previous use of TDF as part of his anti-HIV regimen. After this switch, 4 weeks after cessation of the PEG IFN/RBV, we started the patient on daclatasvir (60 mg once a day) and asunaprevir (100 mg twice a day). The clinical course after introduction of this therapeutic regimen is shown in Figure 1. The HIV RNA levels increased somewhat (260 copies/mL) in week 16 of

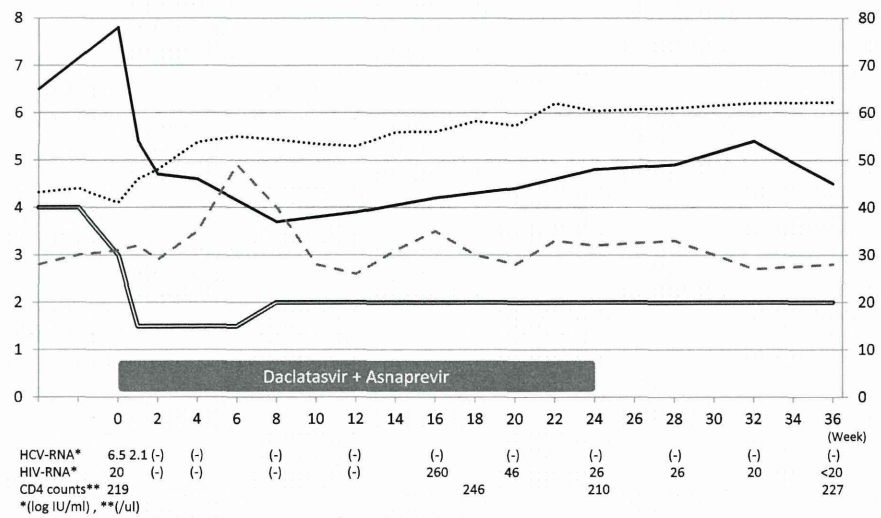

Figure 1 Clinical course of a patient who received daclatasvir and asunaprevir for recurrent hepatitis C virus (HCV) post-living donor liver transplantation (LDLT) with HIV co-infection. The blood concentrations of tacrolimus at the trough level (FK trough, ng/mL), p.o. dose of tacrolimus (FK dose, mg/day), estimated glomerular filtration rate (eGFR, mL/min), and alanine aminotransferase (ALT, IU/L) are indicated by the solid, doubled, dotted and dashed lines, respectively. The box below the chart indicates the duration of daclatasvir and asunaprevir treatment. The serum HCV RNA and HIV RNA levels (log IU/mL) are shown as their values or (−), indicating below lower limit of quantification. CD4 counts (/μL) are also shown.

the treatment, and then spontaneously decreased to approximately the lower limit of quantification. Alanine aminotransferase levels slightly increased in week 6, then also spontaneously returned to normal. TDF was again switched back to ETR at the time of discontinuation of daclatasvir and asunaprevir in view of the transient elevation in HIV RNA, and no relapse was observed during the observation period. No other remarkable adverse events were observed. The dose of tacrolimus administration was successfully modified throughout the observation period with a stable trough level. The patient showed a rapid virological response with daclatasvir and asunaprevir treatment, and HCV RNA became undetectable during week 2 of treatment. A sustained virological response 12 weeks after completion of 24 weeks of treatment with daclatasvir and asunaprevir was achieved.

DISCUSSION

TO OVERCOME THE poor prognosis for HCV/HIV co-infected patients undergoing liver transplantation due to not only aggressive HCV recurrence, but also the inferior response rate to anti-HCV treatment, especially conventional PEG IFN/RBV,[1] there have been several noteworthy attempts to treat recurrent HCV in HIV co-infected recipients using protease inhibitors such as telaprevir or boceprevir in combination with PEG IFN/RBV,[8,9] although the side-effects and actual antivirological potency have remained unclear. More recently, sofosbuvir/simeprevir/RBV was reported to be effective and much safer for treating recurrent HCV in HIV co-infected recipients.[10]

The present case experienced established recurrent HCV after LDLT despite the pre-emptive use of PEG IFN/RBV and absence of meaningful viral mutations in the NS3 and NS5A area, and he had renal dysfunction (eGFR 43.2 mL/min, chronic kidney disease G3b/A2). We selected daclatasvir and asunaprevir not only because this regimen is especially effective for genotype 1b without NA3 and/or NS5A mutation,[11] but because it is estimated to be safe even for patients with impaired renal function,[12,13] although results of clinical trials of these drugs for patients with renal dysfunction have not been published to date. Importantly, a more potent antiviral regimen including an NS5B inhibitor such as sofosbuvir is not recommended for patients with a low GFR.[13] We understand, however, the regimen of sofosbuvir and ledipasvir was approved in Japan recently (September 2015)[14] and was already widely used in Western countries for post-transplant recipients,[15,16] and is contraindicated only for those with GFR of less than 30 mL/min. Our intention in choosing daclatasvir and asunaprevir, not awaiting sofosbuvir and ledipasvir, was to treat this HIV/HCV co-infected patient as safely, effectively and soon as possible. It should be noted that the patient developed established recurrent hepatitis C despite the aggressive pre-emptive PEG IFN/RBV, which suggests the aggressive progression of the HCV disease and also the possibility of worsening renal function while awaiting sofosbuvir and ledipasvir.

We also successfully modified the dose of TDF and calcineurin inhibitors with close monitoring. The fall of the trough level of tacrolimus soon after the introduction of the therapy may be related to a drug–drug interaction with asunaprevir and recovered liver function, which accelerates drug metabolism. We considered it acceptable, however, in the absence of the risk for IFN-induced/immune-mediated graft dysfunction.[17,18] In addition, presumably due to the cessation of PEG IFN/RBV and decreased HCV RNA by daclatasvir and asunaprevir, or their combination, renal function in the current case was reasonably preserved or even better throughout the observation period. As for the HIV treatment in this setting, careful selection and management of ART considering drug–drug interactions with the HCV DAA and HIV ART are key. CYP450 enzyme induction/inhibition accounts for many of the pharmacokinetic interactions between HCV DAA and HIV ART, although other transporter-mediated interactions are now also recognized.[19] Through those mechanisms, it is reported that protease inhibitors for both HIV and HCV, such as asunaprevir, non-nucleoside HCV and HIV polymerase inhibitors, interact. In contrast, comparatively newer HCV DAA and HIV ART, such as HIV and HCV nucleoside/nucleotide polymerase inhibitors, and most HCV NS5A inhibitors such as daclatasvir, and HIV integrase inhibitors rarely affect CYP450, suggesting that such drugs are free from significant drug interactions.[20] In transplant recipients, further consideration of HIV ART, HCV DAA and calcineurin inhibitors must be taken into account.[1] In the present case, conversion from ETR to TDF might have led to a transient elevation of HIV RNA in week 16, which spontaneously recovered to normal levels within the next 4 weeks and did not recur. HIV RNA has remained at approximately the lower limit of quantification even after switching back to TDF from ETR after the cessation of daclatasvir and asunaprevir. The CD4 count has been preserved at more than 200/μL during the observation period.

We herein report that IFN-free therapy with daclatasvir and asunaprevir for recurrent HCV in a HIV co-infected recipient was safe and effective. Selection of the appropriate regimen for each individual based on consideration of the

background conditions, such as renal dysfunction, is desirable in the coming era of access to multiple DAA for HCV.

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Title: Living-donor liver transplantation for hemophilia with special reference to the management of perioperative clotting factor replacement

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Short Title: Living-donor liver transplantation for hemophilia

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Footnote page

Abbreviations: APTT, activated partial thromboplastin time; ART, antiretroviral therapy; AUC, area under the plasma concentration versus time curve; CL, clearance; DDLT, deceased donor liver transplantation; ESLD, end stage liver disease; FVIII, factor VIII; FIX, factor IX; HCV, hepatitis C virus; HIV, human immunosuppressive virus; IVR, in vivo recovery; LDLT, living-donor liver transplantation; MELD, model for end-stage liver disease; MRT, mean residence time; PEG-IFN, pegylated-interferon alpha-2b; Vdss, steady-state volume of distribution

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Introduction

Patients with hemophilia contract hepatitis C virus (HCV) through clotting factor concentrates. Due to contamination of blood-derived clotting factor products, nearly all patients treated with large-pool donor products before the early 1990s were infected with HCV, and up to 25% of these patients developed cirrhosis. Indeed, among patients with hemophilia, end-stage liver disease (ESLD) due to HCV is the leading cause of death. This population is also estimated to have an approximately 70% incidence of coinfection with human immunodeficiency virus (HIV) through the same route of transmission . It is well recognized that HIV coinfection accelerates HCV-related liver disease; thus, a large number of hemophilia patients develop ESLD, which accounts for 10% of liver transplantation for HCV/HIV-coinfected patients (1).

With the advances in antiretroviral therapy (ART) in the mid-1990s and the improved outcome of HIV-infected patients, the indication for liver transplantation in ESLD patients with HCV/HIV coinfection is almost the same as that for HCV-monoinfected patients in all centers worldwide. Liver transplantation for hemophilia patients with ESLD due to HCV cures not only the liver failure but also coagulation abnormalities, making life-long clotting factor replacement unnecessary. There are several reports of a relatively a small number of cases of deceased-donor liver transplantation (DDLT) for hemophilia patients with an acceptable outcome (2, 3), but liver transplantation in these patients remains difficult with regard to the perioperative clotting factor replacement strategy and postoperative antiviral treatment for both HCV and HIV.

Living-donor liver transplantation (LDLT) is a mainstay for patients with ESLD in Japan, as well as for hemophilia patients. A partial liver graft in LDLT may be disadvantageous, however, in terms of the production of clotting factor. We performed 10 consecutive LDLTs for hemophilia patients with HCV-related ESLD, including 8 with HIV-coinfection, which is the largest series of LDLT for hemophilia reported to date. Here we present our case series, with special reference to the clotting factor replacement strategy in LDLT for hemophilia.

Materials and Methods

Patients

Among 562 liver transplants performed at the University of Tokyo Hospital from 1996 to the end of 2014, including 540 LDLTs and 22 DDLTs, 10 patients with hemophilia were indicated for liver transplantation, and they were the subjects of the present study. Six patients had hemophilia A and four patients had hemophilia B. The preoperative clotting factor activity of 11.6 (0.5 – 48.1) %. All patients were adult males with a mean age of 32 years (range 28-50), and infected with HCV. Among them, eight patients were coinfected with HIV. The indication for liver transplantation was ESLD caused by HCV in all patients with a mean model for end-stage disease score of 20 (range, 13-48), and elective LDLT was performed in all cases. To prevent early vascular thrombosis, anticoagulation therapy was started just after transplantation and continued for two weeks in all recipients, utilizing dalteparin (25 IU/kg/d) regardless of the presence of hemophilia. Immunosuppression protocol and anti-HCV treatment after LDLT were exactly same with those in HCV-monoinfected recipients, consisted of tacrolimus/ methylprednisolone and pegylated interferon alpha-2b/ribavirin, respectively. ART was transiently terminated during the perioperative period. The timing of reintroduction was individualized according to the CD4 count, HIV viral load, general status such as surgical complications, and the result of liver function tests. Choice of the anti-HIV drug was individualized according to each patient's treatment history and accumulated resistance mutations.

Perioperative optimization of prophylaxis dosing

Hematologists were involved in the perioperative management. All recipients underwent an individual pharmacokinetic study to determine the preoperative dosing of clotting factors to establish the amount of bolus and continuous injection of factor VIII (FVIII) or factor IX (FIX) during liver transplantation.

Briefly, the pharmacokinetic studies were performed after a single bolus infusion of 50 IU/kg, and included area under the plasma concentration versus time curve (AUC), clearance (CL) = dose/AUC, mean residence time (MRT) = area under the first moment curve/AUC, volume of distribution (Vdss) = MRT x CL, terminal half-life, and in vivo recovery (IVR) = maximum rise in concentration x body weight/bolus dose. The details of the pharmacokinetic studies are

described elsewhere (4).

Protocol for perioperative clotting factor replacement

The targeted trough level of the clotting factor was set at 120% from the start of transplantation until reperfusion of the graft. The initial bolus dose and continuous maintenance dose at the time of liver transplantation were calculated as follows:

Bolus dose (U) = body weight (kg) x 120 (targeted trough) /IVR

Continuous infusion dose (U/kg/h) = CL x 120 (targeted trough)

During the operation, the clotting factor activity and the serum activated partial thromboplastin time were measured every 2 h, and when the trough was under 100%, an additional bolus injection was given according to the following calculation:

Additional bolus injection (U)= (100-trough value) x 100 (U)

In the case of an unexpected massive blood loss, sufficient fresh frozen plasma was the first choice for the replacement, but a bolus injection of 1000 U of clotting factor was considered in cases with insufficient hemostasis.

Upon reperfusion of the graft, the continuous infusion dose was decreased with a targeted trough of 60% till the end of the operation. Then, after admission to the intensive care unit, continuous infusion was gradually tapered off within 72 h after liver transplantation following the reduction of the continuous does every 6 hours. Afterward, no additional infusion of the clotting factor was planned as a protocol in principle, but the clotting factor activity was monitored daily until postoperative day 7, and an additional bolus was given to those with low clotting factor activity or presenting with clinical bleeding tendencies. The perioperative clotting factor replacement strategy is summarized in Figure 1.

Statistical analysis