Successful Treatment of Living Donor Liver Transplantation Patients with Recurrent Hepatitis C Virus by Interferon-Free Therapy with Direct-Acting Antivirals

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Abstract

Re-infection of Hepatitis C Virus (HCV) to the transplanted liver happens in virtually all cases and is refractory to treatment. Until now, Interferon (IFN)-based therapy has been the standard treatment for post-transplantation hepatitis with HCV. IFN-based therapy had a poor outcome and an issue with respect to rejection of the transplanted liver. Recent progress in drug development of direct-acting antivirals has improved the therapeutic outcome for many HCV-infected patients. This study aimed to evaluate the efficacy and safety of Daclatasvir (DCV) and Asunaprevir (ASV) therapy for Living Donor Liver Transplantation (LDLT) recipients with recurrent HCV genotype 1b (GT1b). In this study, 6 LDLT recipients with recurrent HCV GT1b were treated with DCV and ASV. Five cases without prior resistance-associated variants, both in nonstructural 3 and 5A regions, achieved sustained virological response (SVR). Severe adverse effects were not observed. In conclusion, DCV and ASV therapy for recurrent HCV in LDLT patients resulted in a high SVR rate (83%, 5/6) and was safe and well tolerated.

Keywords: HCV; Living donor liver transplantation; Direct-acting antiviral agents

Abbreviations


Introduction

Hepatitis C Virus (HCV) infection is still the main cause of end-stage liver disease and the leading indication for Liver Transplantation (LT) worldwide [1]. HCV infection invariably affects the graft in nearly all LT patients with detectable HCV viremia, leading to rapid progression of liver fibrosis, resulting in graft loss without re-transplantation. Up to 30% of post LT patients with HCV develop liver cirrhosis within 5 years and about half of those cirrhotic patients reach liver failure within 1 year of follow-up: which is an immensely high rate compared to the natural course of HCV patients [2,3].

The management of HCV in LT patients was challenging in the era of interferon (IFN)-based therapy because of poor treatment effectiveness and treatment-related side effects, including an increased risk of rejection reaction against graft liver [4-6]. Recent progress in Drug Development of...
Direct-Acting Antivirals (DAAs) has improved therapeutic outcomes for many HCV-infected patients. However, the evidence is limited in the light of treatment of patients with post LT HCV recurrence. An IFN-free DAA combination therapy with a nonstructural (NS) 5A replication complex inhibitor (daclatasvir, DCV) and an NS3-4A serine protease inhibitor (PI) (asunaprevir, ASV) was approved for HCV genotype 1 in 2014 in Japan [7-10]. DCV has an acceptable safety profile with a lack of drug-drug interactions (DDIs) with cyclosporine and tacrolimus [11]. ASV has a profile with a DDI with cyclosporine [12], so living donor liver transplantation (LDLT) recipients treated only with tacrolimus were recruited. This study aimed to evaluate the efficacy and safety of DCV and ASV combination therapy for LDLT recipients with recurrent HCV (GT1b).

**Methods**

**Patients and study design**

In this prospective, observational study at Hokkaido University Hospital, 6 LDLT recipients with HCV re-infection to the transplanted liver received DCV and ASV combination therapy between September 2014 and April 2015. Patients were excluded if they had de-compensated liver cirrhosis, poorly controlled cardiac disease: administration of prohibited concomitant medications for DCV or ASV or malignancy. The presence of liver cirrhosis was diagnosed by liver biopsy.

The study protocol was approved by the ethics committee of Hokkaido University Hospital as IRB0140092. Written, informed consent to participate in this study was obtained from each patient. This study was registered at the UMIN Clinical Trials Registry as UMIN000016355.

**Therapeutic protocol and follow-up**

Patients were treated with DCV (60 mg once daily) and ASV (100 mg twice daily) for 24 weeks. All patients were admitted for at least 2 weeks after the beginning of DAA treatment. Patients were assessed as outpatients every 2 or 4 weeks for the duration of treatment. Patients were followed every month for an additional 24 weeks after the end of treatment. Physical examinations and blood tests were performed at each time point.

**Analysis and detection of resistance-associated variants**

Before treatment, the resistance-associated variants (RAVs) in NS5A (at the amino acid positions of L31 and Y93) and NS3 (at the amino acid positions of Q80 and D168) were determined by direct sequencing methods and PCR-invader assay, as described previously [13].

**Efficacy of treatment and safety monitoring**

The primary endpoint of this study was sustained virological response (SVR), which was defined as undetectable serum HCV RNA at 24 weeks after the end of treatment (SVR24). Changes in blood parameters and fibrosis markers at pre-treatment, during-treatment and post-treatment points were also analyzed.

Safety evaluations were conducted every 2 or 4 weeks at a minimum for the duration of treatment by clinical laboratory tests and physical examinations. Alanine aminotransferase (ALT) elevation was defined as an ALT level greater than the upper limit of normal.
Results

Patients’ characteristics

Six patients with post LDLT HCV recurrence were enrolled and treated at Hokkaido University Hospital in Japan between September 2014 and April 2015. The baseline characteristics of these 6 patients are shown in (Table 1). LT occurred 7 months to greater than 10 years prior to enrollment. Four patients had the F1 fibrosis stage of METAVIR and 2 patients had the F2 fibrosis stage of METAVIR by liver biopsy, but none had cholestatic hepatitis and any hepatic diseases other than chronic hepatitis C. Five patients had previously received HCV therapy, including pegylated interferon (Peg-IFN) with ribavirin (RBV) (4 of 5) and Peg-IFN/RBV with simeprevir (SMV) (1 of 5).

Virological response

In the overall study population, 5 of 6 patients (83.3%) achieved SVR24. HCV RNA levels decreased rapidly after initiation of treatment in all patients. At two weeks, HCV RNA was below the quantitation limit (1.2 log10 IU/ml) in 5 of 6 patients (Figure 1). HCV RNA remained detectable at Week 2 in 1 patient who did not achieve SVR. Thereafter, viral breakthrough (VBT) occurred and HCV RNA increased at Week 4 in this patient.

Changes in liver disease parameters

Therapy was associated with a modest tendency toward improvement in clinical and biochemical indicators of liver disease between baseline and the end of treatment (EOT) through post-treatment at Week 24 in the patients who achieved SVR24. ALT, albumin and AFP levels in patients who achieved SVR24 all showed incremental improvement (Figure 2). Interestingly, fibrosis markers including the FIB-4 score, Type IV collagen 7S, hyaluronic acid and Mac-2 binding protein glycosylation isomer (M2BPGi) also improved with time (Figure 3).

Virologic failure and resistance analyses

One patient who did not achieve SVR experienced virologic failure. This patient previously ceased Peg-IFN/RBV treatment at week 16 because of VBT. NS3 RAVs, Q80R and D168E, were detected at the time of SMV failure (Table 2). At the baseline of DCV and ASV therapy (5 months after cessation of SMV therapy), only the NS3-D168E RAV was detected. At the time points of VBT against DCV and ASV therapy, deletion of P32 at the NS5A region was observed with the NS3-D168E RAV. Twelve weeks after DCV and ASV therapy, the NS3-Q80R RAV appeared instead of the NS3-D168E RAV with the existence of NS5-Delta P32.

Safety

Table 3 shows the frequency of adverse events. There were no
were treated with DCV and ASV for 24 weeks. In the present study, in which 6 living LDLT recipients with recurrent HCV GT1b were treated with DCV and ASV [20-23]. The present study was conducted as a pilot study, in which 6 living LDLT recipients with recurrent HCV GT1b were treated with DCV and ASV. Several case reports described one or three LDLT patients treated with dual therapy has been approved for HCV GT1 since 2014. Recently, DAA regimens have enhanced therapeutic efficacy, with an SVR rate of around 85%-100%. Compared to IFN-based regimens, IFN-free DAA regimens have enhanced therapeutic efficacy, with an SVR rate of around 85%-100%. Compared to IFN-based regimens, IFN-free DAA regimens have enhanced therapeutic efficacy, with an SVR rate of around 85%-100%

**Table 2:** Analysis of RAVs in the patient who did not achieve SVR.

<table>
<thead>
<tr>
<th>Region of RAVs</th>
<th>At the initiation of Peg/RBV/SMV</th>
<th>At the VBT with Peg/RBV/SMV</th>
<th>At the initiation of DCV/ASV</th>
<th>At the VBT of DCV/ASV</th>
<th>At 12 weeks after DCV/ASV</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS3</td>
<td>-</td>
<td>Q80R</td>
<td>-</td>
<td>-</td>
<td>Q80R</td>
</tr>
<tr>
<td>NS5A</td>
<td>-</td>
<td>D168E</td>
<td>D168E</td>
<td>D168E</td>
<td>-</td>
</tr>
</tbody>
</table>

RAV: Resistance-Associated Variant; SVR: Sustained Virologic Response; Peg: Pegylated Interferon; RBV: Ribavirin; SMV: Simeprevir; VBT: Viral Breakthrough; DCV: Daclatasvir; ASV: Asunaprevir.

**Table 3:** Adverse events in the patients.

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>LDLT (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngitis</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>Fever</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>Itching or rash</td>
<td>4 (66.7%)</td>
</tr>
</tbody>
</table>

Transaminase elevation

<table>
<thead>
<tr>
<th>Transaminase elevation</th>
<th>LDLT (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0</td>
</tr>
<tr>
<td>Grade 3</td>
<td>0</td>
</tr>
</tbody>
</table>

LDLT: Living Donor Liver Transplantation.

Transaminase Elevation Means Elevation of Alanine Aminotransferase. Grade 1: Mean >Upper Limit of Normal (ULN)–3.0xULN; Grade 2: Mean >3.0-5.0xULN; and Grade 3: Mean >5.0-20.0xULN.

Treatment-related serious adverse events or deaths during treatment. Although 66.7% (4 of 6) of patients developed adverse events: none stopped treatment due to an adverse event.

**Discussion**

HCV treatment for patients with post LT recurrence remains an important issue due to the potential increase in serious life-threatening outcomes [14]. Graft loss and deterioration of survival are significantly worse in HCV-positive recipients than other recipients [15]. This difference is mainly the result of re-infection of the liver graft with HCV. HCV recurrence is unavoidable for post LT recipients and its natural course is accelerated in LT recipients. Within 10 years, more than 40% of recipients developed cirrhosis and half of them developed liver failure within a year [14]. Before the era of IFN-free DAA regimens, LT patients with HCV were treated by IFN-based therapy, resulting in unsatisfactory SVR rates of 50%-60% with severe adverse events [5,16-18]. Therefore, IFN-free DAA treatment for LT patients is urgently needed.

Since 2011, several DAs targeting NS3-4A, NS5A and NS5B of HCV nonstructural proteins have been approved for the treatment of HCV worldwide. With the DAA revolution, IFN-free all-oral DAA regimens have enhanced therapeutic efficacy, with an SVR rate of around 85%-100%. Compared to IFN-based regimens, IFN-free regimens have profiles with less adverse events, shorter treatment duration and better efficacy. DCV and ASV combination therapy was assessed in all-oral therapy for HCV [9,19]. These studies also included patients with cirrhosis and proved its safety and efficacy in a difficult-to-cure patient population. In Japan, DCV and ASV dual therapy has been approved for HCV GT1 since 2014. Recently, several case reports described one or three LDLT patients treated with DCV and ASV [20-23]. The present study was conducted as a pilot study, in which 6 living LDLT recipients with recurrent HCV GT1b were treated with DCV and ASV for 24 weeks.

DDIs between DAs and immunosuppressive agents were a concern, especially with first-generation PIs, such as telaprevir and boceprevir. Tacrolimus is also metabolized by cytochrome P450 3A4, but DDIs between DCV and ASV combination therapy with tacrolimus were not well understood. In the present study, all patients were admitted for at least 2 weeks at the initiation of DAA treatment for careful monitoring and dose adjustment of tacrolimus (Figure 4). Drug monitoring is necessary during anti-HCV therapy: but small dosage adjustments might be adequate with DCV and ASV treatment.

Experience with PI use is a concern for retreatment without an IFN regimen [24,25]. In the present study, one patient who was treated with Peg-IFN/RBV with SMV had an NS3-D168E RAV at the initiation of DCV and ASV treatment. After the cessation of Peg-IFN/RBV/SMV treatment, ALT levels increased rapidly. After full consideration including waiting for the permission of other regimens, we decided to put priority on protecting graft liver from rapid progression of liver damage. This patient also developed the NS5A-Delta P32 RAV at the VBT of DCV and ASV. Then, 12 weeks after DCV and ASV therapy, the NS3-Q80R RAV appeared instead of the NS3-D168E RAV with the existence of NS5-Delta P32. Except for one patient, other patients obtained SVR24. Safe and effective DAA regimens against HCV have been dramatically improved during recent years. The best available treatment varies from one country to the next in accordance with the period background. Clinical judgment for therapeutic objectives is needed with consideration of HCV GT, RAVs and past treatment, with particular attention to PI therapy. The one failed patient was eventually obtained SVR24 with ledipasvir and sofosbuvir (SOF) plus RBV for 12weeks.

The rate of serious adverse effects was low in LDLT patients and varied among patients, with no consistent adverse events. The frequency of adverse events was also low compared with a previous cohort of LT patients receiving boceprevir or telaprevir with Peg-IFN/RBV that showed high rates of anemia (84%-100%) and rehospitalization (32%-33%) for an acute flare-up of chronic kidney disease [5].
One of the key drug in recent regimens is a nucleotide analog inhibitor of NSSB polymerase (sofosbuvir: SOF). SOF is eliminated by renal excretion, so the treatment of HCV infected patients with severe (CrCl< 30 mL/min) or end stage renal disease requiring hemodialysis by SOF is prohibited. Impaired renal dysfunction is a critical complication in LT patients [26,27]. Our group and another group reported the efficacy and safety of DCV and ASV combination therapy in hemodialysis patients with HCV [13,28]. Both DCV and ASV are eliminated primarily by hepatic metabolism: so that these drugs do not have much effect on renal function. Therefore, we think DCV and ASV combination therapy is still an important option for HCV GT1b infected LDLT patients with renal impairment without RAVs.

Conclusion

The combination of DCV and ASV achieved high SVR rates with LDLT recurrence. The DCV and ASV combination regimen was effective, safe and well-tolerated for LDLT recipients. Further studies including large patient numbers and long-term follow up are needed to prove the efficacy of DAAs therapy in LDLT patient with HCV recurrence. We intend to conduct future studies involving large samples especially in HCV re-infected LDLT patients with renal impairment.

Authors' Contributions

Conceived and designed the work, KO KM GS KY TS AK NS. Performed the work, KO KM KS AN MO NK TI MU JI MN TS GS. Wrote the paper, KO KM TS.

Conflict of Interests

Professor, Naoya Sakamoto received lecture fees from Bristol Myers Squibb and Pharmaceutical K.K, grants and endowments from MSD K. K and Chugai Pharmaceutical Co., Ltd; and research grant from Gilead Sciences. Inc. Dr. Kenichi Morikawa received research grants from Gilead Sciences. Inc. Dr. Goki Suda received research grants from Bristol Myers Squibb.

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