Limitations of daclatasvir/asunaprevir plus beclabuvir treatment in cases of NS5A inhibitor treatment failure

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Abstract
Combined daclatasvir (DCV)/asunaprevir (ASV) plus beclabuvir (BCV) treatment shows a high virological response for genotype 1b chronic hepatitis C patients. However, its efficacy for patients for whom previous direct-acting antiviral (DAA) therapy failed is not known. We analysed the efficacy of DCV/ASV/BCV treatment for HCV-infected mice and chronic hepatitis patients. Human hepatocyte chimaeric mice were injected with serum samples obtained from either a DAA-naïve patient or a DCV/ASV treatment failure and were then treated with DCV/ASV alone or in combination with BCV for 4 weeks. DCV/ASV treatment successfully eliminated the virus in DAA-naïve-patient HCV-infected mice. DCV/ASV treatment failure HCV-infected mice developed viral breakthrough during DCV/ASV treatment, with the emergence of NS5A-L31V/Y93H HCV resistance-associated variants (RAVs) being observed by direct sequencing. DCV/ASV/BCV treatment inhibited viral breakthrough in NS5A-L31V/Y93H-mutated HCV-infected mice, but HCV relapsed with the emergence of NS5B-P495S variants after the cessation of the treatment. The efficacy of the triple therapy was also analysed in HCV-infected patients; one DAA-naïve patient and four prior DAA treatment failures were treated with 12 weeks of DCV/ASV/BCV therapy. Sustained virological response was achieved in a DAA-naïve patient and one of the DCV/ASV treatment failures through DCV/ASV/BCV therapy; however, HCV relapse occurred in the other patients with prior DCV/ASV and/or sofosbuvir/ledipasvir treatment failures. DCV/ASV/BCV therapy seems to have limited efficacy for patients with NS5A RAVs for whom prior DAA treatment has failed.

INTRODUCTION
Hepatitis C virus (HCV) infection currently affects more than 180 million people and is a serious health problem worldwide [1]. Infected individuals develop acute or chronic hepatitis, liver cirrhosis and potentially die due to liver failure or hepatocellular carcinoma [2, 3].

Direct-acting antivirals (DAAs) have dramatically improved the outlook for the treatment of HCV-infected patients. The combination of oral asunaprevir (ASV) and daclatasvir (DCV) was the first interferon (IFN)-free drug combination approved in Japan for the treatment of genotype 1 HCV-infected patients. This drug combination showed high rates of sustained virological response (SVR) and better tolerability compared to earlier IFN-based treatments [4, 5]. In recent years, many new DAA combination therapies, including sofosbuvir (SOF)/ledipasvir (LDV), elbasvir/grazoprevir and ombitasvir/paritaprevir plus ritonavir, have become available for clinical use and have achieved high rates of SVR in Japan [6–8]. Despite the high rates of SVR achieved with DAA treatments, the treatment response is lower among patients who have drug resistance-associated variants (RAVs) [9]. Substitutions in the non-structural protein 3 (NS3) and non-structural protein 5A (NS5A) greatly reduce the effectiveness of the drugs. In particular, NS3-D168, NS5A-L31 and NS5A-Y93 RAVs are detected in most patients who fail to respond to treatment using NS3/4A protease inhibitors (PIs) and NS5A inhibitors,

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Abbreviations: ASV, asunaprevir; BCV, beclabuvir; DAA, direct-acting antiviral; DCV, daclatasvir; HCV, hepatitis C virus; LDV, ledipasvir; RAV, resistance-associated variant; SOF, sofosbuvir; SVR, sustained virological response; PCR, polymerase chain reaction; SCID, severe combined immunodeficiency; uPA, urokinase-type plasminogen activator.
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respectively. The frequency of NS3 RAVs has been decreasing for several years, but they are thought to remain at low frequency even after the threshold at which RAVs become undetectable by deep sequencing [10]. On the other hand, NS5A RAVs persist at high frequencies long after the cessation of DAA therapy [11]. The presence or emergence of RAVs is one of the most important factors associated with treatment failure or DAA retreatment.

Beclabuvir (BCV), a non-nucleoside NS5B polymerase inhibitor, was used in combination with DCV/ASV for HCV treatment. This therapy was well tolerated and achieved high SVR rates in DAA-naïve patients with genotype 1 and 4 HCV infection [12–16]. In Japan, this triple-combination therapy was approved for the treatment of genotype 1 HCV-infected patients in 2016. A 12-week phase III clinical trial of this triple-combination therapy for DAA-naive patients revealed SVR12 rates of 95% [17]. However, the efficacy of triple DAA therapy for patients whose previous DAA treatment had failed in real-world studies is not so clear.

The human hepatocyte chimaeric mouse model, prepared from cDNA-urokinase-type plasminogen activator (uPA) transgenic severe combined immunodeficiency (SCID) mice transplanted with human hepatocytes, serves as a mouse model for HCV infection and is useful for evaluating antiviral drugs [18, 19]. Using this animal model, we previously reported the successful elimination of HCV genotype 1b by treatment with a combination of DCV/ASV [20].

In this study, we investigated the efficacy of DCV/ASV/BCV therapy for NS3/4A PI and NS5A inhibitor treatment failures using the humanized mouse model. We also assessed the real-world efficacy of the triple-DAA combination therapy for chronic hepatitis C patients, including patients who had experienced previous DAA treatment failures.

RESULTS

Effect of DCV/ASV in genotype 1b HCV-infected mice

Six mice were inoculated with serum samples obtained from either a DAA-naïve patient with wild-type NS3-D168 and NS5A-L31/Y93 or a patient who experienced treatment failure with DCV/ASV therapy and then administered with DCV/ASV for 4 weeks. NS5A-L31M and Y93H RAVs and wild-type NS3-D168 were detected in these DCV/ASV treatment-failure serum samples. In mice inoculated with serum samples obtained from a DAA treatment-naïve patient, the serum HCV RNA levels decreased below the detectable limit at 1 week and remained negative throughout the treatment (Fig. 1a). In these three mice, the serum HCV RNA levels remained undetectable after the cessation of the treatment, and elimination of the virus was assumed since HCV RNA was undetectable by nested polymerase

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Fig. 1. Daclatasvir plus asunaprevir treatment in mice. Six mice were inoculated with serum samples obtained from either a DAA-naïve patient (a and b) or a patient who had experienced the failure of daclatasvir plus asunaprevir treatment (c) and then administered with daclatasvir and asunaprevir (DCV/ASV) for 4 weeks. (a) Changes in serum HCV RNA levels (upper panel). The grey bar indicates the lower limit of detection (3.5 log copies ml\(^{-1}\)). The dagger symbol (†) indicates that the mouse was sacrificed at that time point and its liver was subjected to nested PCR. (b) Nested PCR of HCV RNA and human β-actin (ACTB) in mouse liver samples before treatment and after cessation of treatment (weeks 7, 8 and 9). A liver sample without HCV infection was also analysed. (c) Changes in serum HCV RNA levels at the indicated points are shown. Sequence analysis was performed at baseline and end of treatment.
chain reaction (PCR) in livers at 7, 8 and 9 weeks (3, 4 and 5 weeks after the cessation of the treatment), respectively (Fig. 1b). Direct sequence analysis showed the development of viraemia containing NS5A-L31M and Y93H RAVs in mice inoculated with serum samples obtained from a DCV/ASV treatment failure. In contrast to wild-type HCV-infected mice, the serum HCV RNA levels were positive at 1 week, and rebounded during the treatment in these three mice (Fig. 1c).

**Effect of beclabuvir in mice inoculated with HCV from a patient with DCV/ASV treatment failure**

We next analysed the efficacy of BCV in combination with DCV/ASV for cases of prior DCV/ASV failure. Since DCV/ASV treatment-failure HCV-infected mice showed resistance to DCV/ASV treatment (Fig. 1b), it is possible that BCV monotherapy shows a similar effect to DCV/ASV plus beclabuvir triple therapy for cases of prior DCV/ASV failure, i.e. DCV/ASV/BCV triple therapy in the presence of DCV/ASV-associated RAVs might effectively result in BCV monotherapy. To compare the effect of these treatments, we first investigated the efficacy of BCV monotherapy for cases of prior DCV/ASV treatment failure. Three mice were inoculated with serum samples obtained from a patient who experienced failure of DCV/ASV treatment and were then administered with BCV alone for 4 weeks (Fig. 2a). The serum HCV RNA levels decreased slightly at 1 week but rebounded at 2 weeks. Direct sequence analysis showed the emergence of NS5B-P495L, a known RAV against BCV, at 4

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**Fig. 2.** Effect of beclabuvir in mice. Mice were inoculated with serum samples obtained from a patient who had experienced the failure of daclatasvir plus asunaprevir treatment, and were then administered with beclabuvir (BCV) alone (a) or in combination with daclatasvir and asunaprevir (DCV/ASV/BCV) (b) for 4 weeks. Changes in serum HCV RNA levels at the indicated time points are shown. Sequence analysis was performed at baseline and end of treatment (a) and at baseline and after HCV relapse (2–6 weeks after cessation of treatment) (b), respectively.
weeks in all three mice (Table 1). Because treatment with BCV alone resulted in the development of viral rebound in mice inoculated with HCV from a DCV/ASV treatment failure, we next analysed the effect of BCV in combination with DCV/ASV for mice infected with NS5A-L31M/Y93H-mutated HCV. Six mice were administered with BCV plus DCV/ASV for 4 weeks (Fig. 2b). In contrast to the results for BCV treatment alone, the serum HCV RNA levels decreased to a lower level of detection and remained negative throughout the treatment in all mice. However, relapse of viraemia was observed in all mice after the cessation of the treatment. NS5A-L31M and Y93H RAVs persisted after the relapse of viraemia in these mice. In addition, the NS3A-D168G/A mutation emerged in one mouse and the NS5B-P495A/L mutation emerged in another after relapse (Table 1). These results indicate that DCV/ASV/BCV triple-combination therapy seems to be able to inhibit viral breakthrough during treatment, but is likely to result in HCV relapse after the cessation of treatment in patients whose DCV/ASV treatment has failed and who have high frequencies of NS5A-L31M and Y93H RAVs.

**Effect of DCV/ASV/BCV treatment in genotype 1b HCV-infected patients**

Five genotype 1b HCV-infected patients received 12 weeks of DCV/ASV/BCV treatment. The characteristics of these five patients at baseline are shown in Table 2. Four out of five patients had cirrhosis. One patient was DAA-naïve (case 1) and the others were DAA treatment failures. The prior DAA treatment regimens for each patient were: DCV/ASV (cases 2 and 3); SOF/LDV (case 4); and DCV/ASV and SOF/LDV (case 5). The serum HCV RNA levels decreased below the detectable limit and remained negative in all five patients during treatment. SVR was achieved in the DAA-naïve patient (case 1) and a DCV/ASV failure (case 2); however, serum HCV RNA relapsed in the other prior DAA treatment failures (cases 3, 4 and 5) at 4 and 12 weeks after the cessation of the treatment, respectively (Fig. 3a). The prior DCV/ASV treatment failure (case 3) had NS5A-L31M/V RAVs and the prior SOF/LDV treatment failure (case 4) had NS5A-L31V and Y93H RAVs before treatment. These RAVs persisted during HCV relapse and additionally acquired an NS3-D168E substitution at the time of HCV relapse. The prior DCV/ASV and SOF/LDV treatment failure (case 5) had NS5A-L31I/L and Y93H RAVs before treatment, and additionally acquired NS3-D168E and NS5B-P495S substitutions at the time of HCV relapse (Fig. 3b).

**DISCUSSION**

Patients who have failed to respond to DAA treatment often acquire RAVs. Although the frequency of treatment-emergent NS3/4A RAVs gradually decreases, high proportions of NS5A RAVs have been reported to persist for a long time [11]. Therefore, DCV/ASV treatment failures are likely to fail when treated with SOF/LDV therapy. The SVR rate of SOF/LDV treatment for patients whose prior DCV/ASV treatment had failed was approximately 70% [21]. Thus, it is important to choose an appropriate DAA retreatment strategy that takes the presence of RAVs into account.

Although DCV/ASV/BCV therapy showed an SVR12 rate of 92% for genotype 1 chronic hepatitis C patients who had NS5A-L31 and/or Y93 HCV viraemia, similar to the case of DCV/ASV failure, and, as expected, these mice developed viral breakthrough following DCV/ASV treatment failure. The DCV/ASV failure HCV-infected mice developed NS5A-L31M/Y93H HCV viraemia, similar to the case of DCV/ASV failure, and, as expected, these mice developed viral breakthrough following DCV/ASV treatment, with the emergence of additional NS3-D168T RAVs (Fig. 1c). BCV mono-therapy also resulted in viral breakthrough in NS5A-L31M/Y93H HCV-infected mice, with the emergence of NS5B-P495L RAVs (Fig. 2a), which have 53-fold higher resistance to BCV.
relative to the wild-type, based on a genotype 1b HCV replication system [22]. DCV/ASV/BCV treatment inhibited viral breakthrough, but HCV relapsed in NS5A-L31M/Y93H HCV-infected mice (Fig. 2b). Despite there being no NS3-D168 RAVs at baseline, triple therapy with DCV/ASV/BCV failed to eliminate HCV. Using the same mouse model, we previously reported that the virological response to NS3/4A PIs was low in patients with NS3/4A PI treatment failure, even if NS3 RAVs were undetectable by deep sequencing [10]. According to these results, as well as previous findings [10], it seems that DCV/ASV treatment failure strains not only have resistance to NS5A inhibitors, but also to NS3/4A PIs, even if NS3-D168 RAVs are not detected. Due to the presence of NS5A-L31M/Y93H RAVs at high frequency and with high resistance to NS3/4A PIs, triple therapy with DCV/ASV/BCV seemed to fail to eliminate the virus in mice inoculated with HCV from a DCV/ASV treatment failure. It is unclear whether the efficacy of DCV/ASV/BCV varies with natural resistance and RAVs due to DCV/ASV treatment failure or not. It would be interesting to compare the efficacy of DCV/ASV/BCV for mice infected with HCV obtained from DCV/ASV treatment failures and DAA-naive patients who have the same natural resistance profiles for NS3/4A and/or NS5A, and this analysis might be able to provide helpful findings to consider the treatment strategy for HCV-infected patients with natural resistance.

In the present study, five HCV-infected patients received a 12-week course of DCV/ASV/BCV triple therapy. Three out of four patients with prior DAA treatment failure developed HCV relapse after the cessation of the treatment (Fig. 3a), which is consistent with the results from the mouse experiment (Fig. 2b). Based on the results from the mouse and the chronic hepatitis C patients, the effect of DCV/ASV/BCV therapy seems to be limited for patients who have experienced prior DAA treatment failure and maintain high frequencies of NS5A RAVs. In the patient with a previous DCV/ASV and SOF/LDV failure (case 5), NS5B-P495S emerged following DCV/ASV/BCV treatment. In the clinical trial of DCV/ASV/BCV treatment in Japan, no NS5B-P495S RAVs emerged in any of the patients with treatment failure [17]. Therefore, it seems that DAA treatment failures have a low genetic barrier to resistance to DAA with similar resistance profiles. Three out of four patients in the present study who had experienced previous DAA treatment failure had liver cirrhosis, and only one patient without cirrhosis (case 2) succeeded in achieving SVR12 by DCV/ASV/BCV therapy. Advanced liver fibrosis and high frequencies of RAVs were risk factors for non-response to DAA therapy [5, 23]. It is possible that patients with advanced liver fibrosis and high frequencies of RAVs are also resistant to DCV/ASV therapy. Further large-scale clinical studies are necessary to investigate the efficacy of DCV/ASV/BCV therapy for cases involving prior DAA treatment failure and non-cirrhotic patients, and the relationship between the frequencies of NS3 and NS5A RAVs, liver fibrosis and treatment efficacy.

Although HCV RNA relapsed after the cessation of the four weeks of DCV/ASV/BCV treatment, the triple therapy inhibited viral breakthrough during the treatment period in mice inoculated with HCV from a DCV/ASV treatment failure (Fig. 2b). Consistent with the results from mouse models, the serum HCV RNA remained below the detectable

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<th>Table 2. Clinical characteristics of patients treated with daclatasvir/asunaprevir plus beclabuvir</th>
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DCV/ASV, daclatasvir plus asunaprevir; eGFR, estimated glomerular filtration rate; SOF/LDV, sofosbuvir plus ledipasvir.
As an alternative to DCV/ASV/BCV combination therapy, glecaprevir plus pibrentasvir combination therapy has been shown to be effective for patients with prior DAA treatment failure and has been approved for pan-genotypic HCV-infected patients in Japan [24, 25]. Sofosbuvir/velpatasvir plus ribavirin or sofosbuvir/velpatasvir/voxilaprevir with or without ribavirin therapy also showed SVR rates of 96–100% for genotype 1 HCV-infected patients who had experienced prior DAA treatment failure [26, 27]. Moreover, the prolongation of the treatment period or the addition of ribavirin could improve the virological response of second-line treatments [28]. These therapies are expected to overcome the problems of DAA retreatment in the near future.

In conclusion, DCV/ASV/BCV therapy seems to have limited efficacy for patients who have experienced prior DAA treatment failure and for whom NS5A RAVs are present. Drug resistance is an important consideration during DAA

![Fig. 3. Effect of daclatasvir/asunaprevir plus beclabuvir treatment for genotype 1b HCV-infected patients. One DAA-naive patient and four prior DAA treatment failures received 12 weeks of treatment with daclatasvir/asunaprevir plus beclabuvir (DCV/ASV/BCV). (a) Changes in serum HCV RNA levels. (b) Nucleotide and amino acid sequences before and at HCV relapse in three patients.]
therapy because it drastically reduces the efficacy of DAAs, increasing the risk of treatment failure. It is important to choose an appropriate strategy that considers RAVs when planning DAA retreatment.

**METHODS**

**Generation of HCV-infected mice**

The generation of cDNA- uPA+/+/SCID+/+ (uPA/SCID) mice and the transplantation of human hepatocytes were performed as described previously [18]. Twelve weeks after hepatocyte transplantation, mice were injected intravenously with HCV-infected serum. After serum inoculation, mouse blood samples were obtained serially, and serum HCV RNA levels were measured.

We used two types of genotype 1b HCV-infected serum in order to establish HCV infection; one was obtained from a treatment-naïve patient and the other was obtained from a patient who had experienced treatment failure during DCV/ASV therapy. The DAA-naïve patient was infected with wild-type NS3-D168, NS51-L31/Y93 HCV. The serum obtained from the DCV/ASV failure patient contained high frequencies of NS5A-L31M/Y93H RAVs. At the time of viral breakthrough in this patient, NS3-D168T/A/G, NS5A-L31M/L and NS5A-Y93H RAVs had emerged. The frequency of NS3-D168 RAVs gradually decreased and became undetectable after the cessation of treatment [10]. Human serum samples were obtained from patients who had provided written informed consent for participation in the study. All serum samples were divided into small aliquots and stored in liquid nitrogen until use. All animal protocols described in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) and the local committee for animal experiments. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the Graduate School of Biomedical Sciences, Hiroshima University (A14-195).

**Direct-acting antiviral treatment for HCV-infected mice**

After the establishment of stable viraemia, HCV-infected mice were treated with a combination of DAAs for 4 weeks. The mice received orally 75 mg/kg/day of ASV (Bristol-Myers Squibb, New York, USA), 20 mg/kg/day of DCV (Bristol-Myers Squibb), and 50 mg/kg/day of BCV (Bristol-Myers Squibb). The drug dose for the mice was equivalent to the dose for humans.

**RNA extraction and amplification**

RNA was extracted from mouse serum samples and liver samples using Sepa Gene RV-R (EIDIA Co., Ltd, Tokyo, Japan) and NucleoSpin RNA II (TaKaRa Bio, Shiga, Japan), respectively. The extracted RNA was reverse transcribed using a random primer (Takara Bio Inc., Shiga, Japan) and M-MLV reverse transcriptase (ReverTra Ace; Toyobo Co., Ltd, Osaka, Japan) according to the instructions provided by the manufacturer. Complete suppression of HCV in humanized liver was confirmed by nested PCR. The primers and amplification conditions for nested PCR were described previously [29].

**Clinical assessment**

Five patients with chronic genotype 1b HCV infection were treated with a combination of DCV, ASV and BCV therapy between March and November 2017 at Hiroshima University Hospital. All patients were administered 200 mg of ASV, 30 mg of DCV and 75 mg of BCV (Ximency, Bristol-Myers Squibb) twice daily for 12 weeks. SVR12 was defined as undetectable HCV RNA both at the end of treatment and 12 weeks after the end of treatment. All subjects provided written informed consent for participation in the study according to the process approved by the ethical committee of the hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

**Quantification of serum markers**

The human albumin concentration in chimaeric mouse blood was measured as described previously [18]. The HCV RNA levels in the mice and patients were measured using the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). The lower detection limits for the assay were 3.5 log copies ml⁻¹ and 1.2 log IU ml⁻¹ in the mice and patients, respectively. The HCV genotype was determined by sequence determination in the 5' nonstructural region of the HCV genome, followed by phylogenetic analysis.

**Detection of DAA-resistant variants**

The nucleotide and amino acid sequences of NS3-D168, NS5A-L31, NS5A-Y93 and NS5B-P495 were determined by direct sequence analysis. The amplification conditions and primers used for the amplification of the NS3 and NS5A regions were described previously [10]. The NS5B region was amplified by nested PCR. The outer primers were 5'-TTYRCRGARGCYATGACVAGGTACTC-3' (forward) and 5'-ATGCCTACCCCTACAGAAAGTAG-3' (reverse) (nt: 8613–9351), and the inner primers were 5'-RGARGC YATGACVAGGTACTCNGCYC-3' (forward), 5'-CTACTACAGAAATGAGTAGGCAC-3' (reverse) (nt: 8618–9342). The amplified DNA fragments were purified after separation by 2% agarose gel electrophoresis. The nucleotide sequences were determined using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Inc., CA, USA) according to the manufacturer's instructions. Furthermore, in some of the clinical cases, the sequences and populations of NS3-D168, NS5A-L31 and NS5A-Y93 were determined by Invader assay [30].

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Conflicts of interest

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References


