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CASE REPORT

Successful direct-acting antiviral treatment of three patients with genotype 2/1 recombinant hepatitis C virus

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Abstract
There have been a few reports on the treatment of patients infected with recombinant hepatitis C virus (HCV) genotype 2/1 strains with direct-acting antivirals (DAAs). We experienced three patients, with genotype 2/1 recombinant HCV, treated with DAAs successfully. The first, a 39-year-old man, was infected with recombinant HCV genotype 2a/1b, a rare variant. The sequence of the relapsed virus showed chimeric HCV 2a/1b with the recombinant breakpoint found at nucleotide +49 from the start of the NS3 region. Sofosbuvir plus ribavirin, a regimen recommended for HCV genotype 2, did not lead to a sustained viral response (SVR). Retreatment with grazoprevir plus elbasvir resulted in an SVR. The second case, a 70-year-old woman, was infected with recombinant HCV genotype 2b/1b. DAA therapy with sofosbuvir plus ledipasvir resulted in an SVR. The third case, a 48-year-old woman, was also infected with recombinant HCV genotype 2b/1b. DAA therapy with daclatasvir plus asunaprevir resulted in an SVR. The baseline sequences of the viruses from both the second and third cases showed chimeric HCV 2b/1b with the recombinant breakpoint found at nucleotide +10 from the NS3 start. We report three cases with 2/1 chimeras and discuss the prevalence and response to therapy.

Keywords Chimeric HCV · Direct-acting antivirals · HCV genotype 2/1

Introduction

Hepatitis C virus (HCV) infection is a global problem [1]. With the introduction of direct-acting antivirals (DAAs), approximately 95% of naïve patients with HCV can achieve a sustained virological response (SVR) [2]. Before the development of pangenotypic DAAs, the selection of an HCV genotype-guided DAA regimen was recommended. However, roughly 14–25% of patients in Germany and Israel with HCV genotype 2 have a recombinant (chimeric) HCV strain, which consists of two types of HCV [3]. There are few reports on the efficacy of DAA therapy for such patients [4]. In Japan, HCV typing is performed using one or two of the three available methods. The virus is serotyped as type 1 or 2 using an enzyme-linked immunosorbent assay with type-specific antibodies against the non-structural (NS) 4 region [5]. Genotyping is determined by PCR with genotype-specific primers for the core region [6], or direct sequencing of the NS5B region [7]. The discrepancy in typing using the three methods suggests the possibility of recombinant HCV infection. It was reported that 1–2% of the patients with genotype 2 in Japan were infected with recombinant HCV [8].

We identified the nucleotide breakpoint of the recombinant HCV in three patients who achieved an SVR to interferon-free DAA regimens.

Case presentation

Case 1

A 39-year-old man had failed interferon-based therapy for chronic hepatitis C (Table 1). He had a hetero-type of *interleukin 28B* single-nucleotide polymorphism. He had no history of surgery, blood transfusion, illegal drug use, or

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Table 1 Clinical characteristics of the three patients

	Case 1	Case 2	Case 3
Age (years)	39	70	48
Gender	Male	Female	Female
Cirrhosis/non-cirrhosis	Non-cirrhosis	Non-cirrhosis	Cirrhosis
Liver stiffness (kPa)	4.7	4.1	20.2
Past IFN-based therapy	No responder	–	–
WBC (/μL)	4300	3900	2800
Hb (g/dL)	16.8	12.2	9.8
Plt (×104/μL)	17.7	15.2	9.7
T-Bil (mg/dL)	1.4	0.7	0.8
ALT (IU/L)	20	19	50
FIB-4 index	0.94	3.11	4.89
AFP (ng/mL)	3.2	2.3	375.7
HCV RNA viral load ($\log_{10}$ IU/mL)	7	6.2	6.5
Serotype	1	1	1
Genotype; core/NS5B	2a/1b	2b/1b	2b/1b
Interleukin 28B rs8099917 SNP	TG	TT	TT
DAA regimen	SOF + RBV	SOF + LDV	DCV + ASV
Outcome of DAA therapy	Relapse	SVR	SVR
Outcome retreatment	SVR by GZR + EBR	–	–

IFN interferon, *WBC* white blood cell, *Hb* hemoglobin, *Plt* platelet, *T-Bil* total bilirubin, *ALT* alanine aminotransferase, *FIB-4 index* Fibrosis-4 index, *AFP* α-fetoprotein, *HCV* hepatitis C virus, *NS5B* non-structural 5B, *DAA* direct-acting antiviral, *SOF* sofosbuvir, *RBV* ribavirin, *GZR* grazoprevir, *EBR* elbasvir, *LDV* ledipasvir, *DCV* daclatasvir, *ASV* asunaprevir, *SVR* sustained viral response

tattooing. The HCV genotype was determined as 2a using PCR of the core region. Combination therapy with sofosbuvir 400 mg/day and ribavirin 800 mg/day was initiated in 2015 at previous hospital. The serum HCV RNA fell rapidly from 6.4 $\log_{10}$ IU/mL at baseline to below the detection limit of the COBAS TaqMan assay (1.2 $\log_{10}$ IU/mL) at 4 weeks. Although the viral load was below the detection level during treatment, HCV relapsed 4 weeks after the end of treatment.

He moved because of his job and was referred to our hospital in 2016. Our screening test for HCV-infected patients showed that his HCV serotype was 1. Consequently, we examined the core, and NS5B regions to determine the HCV genotype, which was determined to be 2a by PCR of the core region, but as 1b by sequencing NS5B. Next, the NS5A region was successfully sequenced using HCV genotype 1b-specific primers to examine the resistant-associated substitutions (RAS) [2]. After confirming that there was no RAS in the NS5A region, we started retreatment with grazoprevir 100 mg/day and elbasvir 50 mg/day. The viral load fell rapidly from 7.0 $\log_{10}$ IU/mL at baseline to <1.2 $\log_{10}$ IU/mL at 4 weeks and an SVR at 12 weeks (SVR_{12}) was achieved.

HCV 2a/1b recombinant breakpoint in Case 1

We assumed that the patient was infected with a recombinant HCV strain, which consisted of genotype 2a in the first half of the viral genome and genotype 1b in the second half. Because the recombinant sites were often identified around the NS2/NS3 junction in previous studies [3, 9, 10], we designed primer pairs to amplify a 438-bp fragment covering the NS2/NS3 junction. The HCV sequences amplified before and after sofosbuvir treatment both aligned with the HCV2a_HC-J6 and HCV1b_Con1 strains. The recombinant breakpoint was found at nucleotide position +49 relative to the start of the NS3 region (Fig. 1).

Case 2

The patient was a 70-year-old woman with chronic hepatitis C (Table 1). She had undergone an appendectomy at the age of 48. She had no history of blood transfusion or anti-HCV therapy. The HCV serotype was determined as 1 and the genotype as 2b by PCR of the core region. Therefore, we checked the genotype by sequencing NS5B. This showed that her HCV genotype was 2b by PCR of the core region, but as 1b by sequencing NS5B. The NS5A region was amplified and sequenced using genotype 1b-specific primers. No RAS was detected in the NS5A region. Combination therapy with sofosbuvir 400 mg/day and ledipasvir 90 mg/day was initiated in 2016. The viral load fell rapidly from 6.2 $\log_{10}$ IU/mL at baseline to <1.2 $\log_{10}$ IU/mL at 4 weeks and SVR_{12} was achieved.

Fig. 1 HCV 2a/1b recombination breakpoint in Case 1. Alignment of the nucleotide sequences from the recombinant strains before and after sofosbuvir treatment with reference strains HCV2a_HC-J6 and HCV1b_Con1. The breakpoint is at +49, referring to the nucleotide position relative to the NS3 start

Case 3

The patient was a 48-year-old woman with HCV-related cirrhosis (Table 1). She had no history of surgery, blood transfusion, illegal drug use, tattooing, or anti-HCV therapy. HCV serotype was determined to be 1 and genotype as 2b by PCR of the core region. Therefore, we checked the genotype by sequencing NS5B. This determined that her HCV genotype was 2b by PCR of the core region, but as 1b by sequencing NS5B. The NS5A region was amplified and sequenced using genotype 1b-specific primers. No RAS was detected in the NS5A region. Combination therapy with daclatasvir 60 mg/day and asunaprevir 200 mg/day was initiated in 2014, after confirming that there was no substitution associated with resistance in the NS5A region. The viral load fell from 6.5 $\log_{10}$ IU/mL at baseline to < 1.2 $\log_{10}$ IU/mL at 12 weeks and SVR_{12} was achieved. Of interest, her daughter was also infected with HCV and the serotype was determined to be 1 and the genotype as 2b by PCR of the core region.

HCV 2b/1b recombinant breakpoint in Cases 2 and 3

We assumed that the patients were infected with recombinant HCV strains in which the first half was genotype 2b and the second half was genotype 1b. Both HCV strains were aligned with the HCV2b_HC-J8 and HCV1b_Con1 strains. In both cases, the recombinant breakpoint was found to be at nucleotide position +10 relative to the NS3 start (Fig. 2).

Discussion

We treated three patients with genotypes 2 and 1 recombinant HCV strains using interferon-free DAAs. One patient (Case 1), who was initially treated with the DAA regimen recommended for genotype 2, did not achieve an SVR, but the DAA regimen recommended for genotype 1 led to an SVR in all three patients, as the initial DAA treatment in Cases 2 and 3 and retreatment in Case 1.

In our hospital, the HCV type is determined using NS4 serotyping and PCR with genotype-specific primers for the core region. NS4 serotyping could not be determined in 65 patients treated with DAA therapy between September 2014 and July 2017. Of the remaining 819 patients treated with DAA therapy, 3 (0.4%) had typing results that differed with the two methods.

Further examination by direct sequencing of NS5B showed that the serotyping results matched the genotypes. To our knowledge, the HCV recombinant viruses in previous studies consisted of the structural region of genotype 2 and the NS4–NS5 region of genotype 1 [3, 10]. We identified the recombinant breakpoint of the HCV genotype 2/1b in all cases. The breakpoint in Case 1 was located +49 nucleotides from the NS3 start, which was the same position reported by Susser et al. (Fig. 1) [3]. The breakpoint in Cases 2 and 3 was at the same position at +10 nucleotides (Fig. 2). In previous reports, the breakpoint of 2/1 chimera HCV was located within 80 acids of the NS2/NS3 junction [10]. The prevalence and characteristics of chimeric HCV differ regionally. The first report was on an HCV genotype 2 k/1b chimera derived from a Russian patient in 2001 [11].

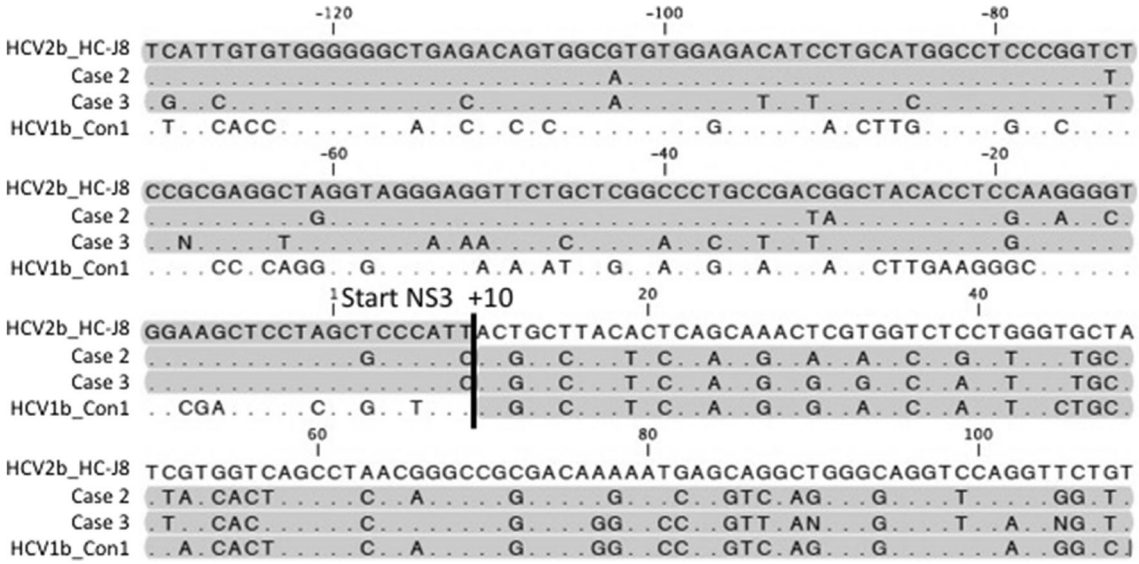

Fig. 2 HCV 2b/1b recombination breakpoint in Cases 2 and 3. Alignment of the nucleotide sequences from the recombinant strains in Case 2 (daclatasvir + asunaprevir) and Case 3 (ledipasvir + sofosbuvir) with the reference strains HCV2b_HC-J8 and HCV1b_Con1. The breakpoint is at +10, referring to the nucleotide position relative to the NS3 start

The original 2 k/1b chimera is prevalent (14–25%) in Russia, Georgia, Germany, and Israel [3, 9, 10]. In comparison, the original 2b/1a chimeric HCV was predominant in Western Europe and North America [10, 12], while the original 2b/1b chimera was identified in Japan and the Philippines [13, 14]. The first 2a/1b chimeric HCV variant originated from Russia [3], and our case was the second report of 2a/1b recombinant HCV.

There are several issues that need to be solved regarding chimeric HCV, including the mechanism of recombination, the cause of the limited G2/G1 recombination pattern, and how HCV is spread. Our cases did not share clinical characteristics, including the infection route. However, mother-to-child transmission of chimeric HCV was suspected in Case 3.

Previously, it was reported that the RF1-2 k/1b recombinant HCV was responsive to PEG–interferon therapy in a chimeric mouse model [15]. However, Hoshino *et al*. showed that patients with recombinant HCV 2b/1b did not achieve an SVR with PEG-interferon and ribavirin combination therapy [8]. Excluding recent pangenotypic DAA regimens, the guidelines recommend an HCV genotype-specific DAA regimen. It was not clear which DAA regimen was effective for patients with HCV genotype 2/1 chimera. In our patient with HCV 2a/1b, sofosbuvir plus ribavirin, which is recommended for genotype 2, did not result in an SVR, while three DAA regimens recommended for genotype 1 (daclatasvir + asunaprevir, sofosbuvir + ledipasvir, and elbasvir + grazoprevir) all led to an SVR. We speculate that the HCV genotype 2/1b chimera has NS3/4, NS5A, and NS5B derived from genotype 1b. These HCV regions are targets for DAAs. It is expected that patients with chimeric HCV would achieve SVR after receiving a recent pangenotypic DAA regimen.

In conclusion, we experienced three patients with chimeric HCV that consisted of genotypes 2 and 1b who achieved an SVR to DAA regimens recommended for genotype 1. Our data suggest that the exact determination of HCV genotype is important for choosing the optimal treatment for patients with chronic hepatitis C.

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Compliance with ethical standards

Conflict of interest Dr. H Kumada has received lecture fees from AbbVie Inc., Bristol-Myers Squibb, Gilead Sciences, and MSD K.K. Dr. N Kawada has received lecture fees from Gilead Sciences, and MSD K.K. Dr. A Tamori has received a lecture fee from Gilead Sciences. Other authors declare that they have no conflict of interest.

Human/animal 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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