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Combination Therapy with Ombitasvir/Paritaprevir/Ritonavir for Dialysis Patients Infected with Hepatitis C Virus: A Prospective Multi-Institutional Study

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Hepatitis C virus (HCV) infection is common in dialysis patients worldwide and nosocomial HCV spread within dialysis facilities continues to develop. Combination therapy with daclatasvir and asunaprevir (DCV/ASV) that has proven efficacy for dialysis patients infected with genotype 1b HCV (HCV/1b) has several concerns in Japan. The recently available combination therapy with ombitasvir, paritaprevir, and ritonavir (OBV/PTV/r) is not contraindicated in patients with chronic renal failure and has more safety profile and shorter treatment period than that with DCV/ASV. We evaluated the effects of combination therapy with OBV/PTV/r in four dialysis patients infected with HCV/1b, who were eligible for our study. On-treatment assessments included standard laboratory testing, serum HCV RNA and symptom-directed physical examinations. Three patients had a sustained virological response at 12 weeks after treatment, but one remaining patient had viral breakthrough. Notably, the patient with viral breakthrough had been coinfected with HCV/1b and HCV/2b; namely, HCV/2b with resistance-associated variations was not eradicated by the combination therapy. Among the three patients responsive to the combination therapy, one patient complained of appetite loss and itching, while in another patient the therapy was discontinued due to itching, exacerbation of wamble, and a falling tendency probably due to interaction with valsartan. These AEs were ameliorated or disappeared after the completion of the therapy. The significance of our study is persuasive virological evaluation associated to the combination therapy and reasonable interpretation of AEs. In conclusion, combination therapy with OBV/PTV/r may have promise as an efficacious therapy, but caution regarding AEs should be practiced.

Keywords: dialysis; hepatitis C; ombitasvir; paritaprevir; ritonavir
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Introduction

Hepatitis C virus (HCV) infection is common in dialysis patients worldwide. The Dialysis Outcomes and Practice Patterns Study showed that 4,735 patients (9.5%) had HCV infection of 49,762 dialysis patients in 12 nations enrolled between 1996 and 2011 (Goodkin et al. 2013). Based on epidemiologic evidence and viral sequencing, nosocomial HCV spread within dialysis facilities continues to develop (Health Advisory CDC: http://emergency.cdc.gov/han/han00386.asp). The eradication for HCV infection in dialysis patients is an unmet need to address.

The most recent version (ver. 5.1) of the Japan Society of Hepatology (JSH) guideline for the management of HCV infection recommends combination therapy with daclatasvir and asunaprevir (DCV/ASV) as interferon-free direct-acting antiviral agents (DAAs) for dialysis patients with chronic infection of HCV genotype 1b (HCV/1b) in Japan. While the antiviral efficacy of DCV/ASV combination therapy for dialysis patients has been reported in several studies (Miyazaki and Miyagi 2016; Kawakami et al. 2016; Sato et al. 2016; Toyoda et al. 2016; Suda et al. 2016), this therapy

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has several flaws compared to more updated regimens with DAAs such as sofosbuvir and ledipasvir (SOF/LDV) or ombitasvir, paritaprevir, and ritonavir (OBV/PTV/r), as will be discussed later.

Combination therapy with OBV/PTV/r has recently become available, and each of these drugs is metabolized largely in the liver, with minimal renal clearance; as such, this therapy is not contraindicated in patients with chronic renal failure (Khatri et al. 2016; Polepally et al. 2016). Combination of OBV/PTV/r, administered with dasabuvir (DSV), was recently reported to show a sustained virological response at 12 weeks after treatment (SVR12) in 90% of patients with HCV/1b infection and stage 4 or 5 chronic kidney disease (CKD) (Pockros et al. 2016). We therefore examined the efficacy and safety of OBV/PTV/r combination therapy in four dialysis patients infected with HCV/1b.

This case series of dialysis patients is part of an ongoing, prospective, multi-institutional study evaluating the efficacy and safety of DAA-based therapy for patients infected with genotype 1 HCV at our university hospital and its affiliated hospitals. This study was approved by the Institutional Review Board at each institute. We obtained written informed consent from each subject. In addition, this ongoing prospective study complies with the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan. The registration number with Japan's UMIN-CTR is UMIN000019212.

Materials and Methods

Patients

Patients were enrolled from October 2015, and recruitment is currently underway in Japan. Patients were required to be chronically infected with HCV/1b and with viremia before enrollment. The patients with chronic hepatitis or compensated cirrhosis were included. All subjects intended to undergo 12 weeks of DAA treatments including 25 mg OBV/150 mg PTV/100 mg r, administered orally once daily. On-treatment assessments included standard laboratory testing, serum HCV RNA, and symptom-directed physical examinations. Adverse events (AEs) were evaluated at study visits. Clinical laboratory testing was performed at visits during the treatment period and after the end of the treatment period. In this paper, the subjects were confined to dialysis patients.

Quantitation of HCV RNA

The quantification of HCV RNA was measured by a TaqMan HCV Test, version 2.0, real-time polymerase chain reaction (PCR) assay (F. Hoffmann, La Roche Ltd., Basel, Switzerland), with a lower limit of qualification of 15 IU/mL and a range of quantitation of 1.2-8.0 log IU/mL.

HCV serogrouping and genotyping

The serogroups and genotypes of HCV were determined in commercial laboratories (Showa Medical Science, Tokyo, Japan; and BML, Inc., Tokyo, Japan, respectively). For confirmation of the HCV genotype in Patient 3, a core region-based multiplex PCR (Okamoto et al. 1993) was performed. Briefly, part of the HCV core gene spanning nt 480-751 (272 base pairs [bp]) was amplified on HCV cDNA with universal primers. A portion of the product was then amplified by PCR with universal primers (sense) and a mixture of five primers (antisense) derived from the sequences of the HCV core gene, each of which was specific to genotype 1a, 1b, 2a, 2b, or 3a. The genotypes were distinguished from each other by the size of the products: 49 bp for 1a; 144 bp for 1b; 174 bp for 2a; 123 bp for 2b; and 88 bp for 3a.

Analysis of resistance-associated variations

The presence of resistance-associated variations (RAVs) at amino acids (aa) V36, T54, Q80, R155, A156, D168, and V170 in the HCV/1b-NS3 and L31, Q54, and Y93 in the HCV/1b-NS5A was determined in commercial laboratories (SRL, Inc., Tokyo, Japan; and LSI Medience Corporation, Tokyo, Japan).

For a detailed analysis of the genome sequences in the NS3 and NS5A regions of HCV isolates from all four patients, the serum samples obtained before treatment and at viral breakthrough were subjected to PCR amplification, and the nucleotide sequences were determined by direct sequencing. The primers used for cDNA synthesis and amplifications are shown in Table 1. Thirty-five cycles of first amplification and 25 cycles of second amplification were performed with TaKaRa *Ex Taq* DNA polymerase (TaKaRa Bio Inc., Shiga, Japan) as follows: denaturation for 30 s at 94°C, annealing of primers for 30 s at 54-57°C, extension for 60 s at 72°C, and final extension for 7 min at 72°C. The amplification products were purified using a FastGene Gel/PCR Extraction Kit (NIPPON Genetics Co. Ltd., Tokyo, Japan), and both strands were then sequenced directly using an Applied Biosystems 3130xl Genetic Analyzer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with a BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Inc.). The sequence analysis was performed using the Genetyx software program (version 13.0.3; Genetyx Corporation, Tokyo, Japan).

Results

Baseline patient demographics and characteristics

All four patients were male Japanese with HCV/1b or serogroup 1 HCV before combination therapy of OBV/PTV/r. The severity of liver disease was judged as chronic hepatitis in 3 patients and liver cirrhosis in 1 patient based on the laboratory data and imaging. After starting the combination therapy, we principally checked the laboratory data and AEs at least once a fortnight. The laboratory findings, treatments and outcomes of all patients are shown in Table 2.

Patient 1

A 61-year-old male was receiving dialysis due to chronic renal failure arising from diabetic nephropathy in 2008. The transmission source of HCV could not be identified. He had not received interferon-based therapy. He had HCV with a mutation in the non-structural region 5A (NS5A) (Q54H), which is known as a RAV against BMS-790052 (DCV); however, without Y93H or L31V-Y93H, this mutation could not confer reduced susceptibility to DCV (Fridell et al. 2011). This mutation is unlikely to be associated with resistance to OBV (Krishnan et al. 2015; Interview form of VIEKIRAX® ver. 4 [http://www.abbvie.co.jp/content/dam/abbviecorp/japan/docs/if_Viekirax_j.

Table 1. Primers used for amplification of the NS3 and NS5A regions of the genotype 1b and 2b HCV genomes.

Primer name	Sequence (5' to 3')[a]	Nucleotide position[b]	Notes
HCV/1b-NS3 region			
HC626	TCTCYGACATGGAGACCAAG	3268-3287	1st sense
HC630	YCCTTGTACCCYTGGGCTGC	4074-4093	RT and 1st antisense
HC628	GGAGACCAAGRTCATYACCTG	3278-3298	2nd sense
HC631	TTAGTGCTCTTRCCGCTGCC	4038-4057	2nd antisense
HCV/1b-NS5A region			
HC632	CCTCTCYRGCCTYACCATCAC	6182-6202	1st sense
HC635	RGAGGGRTCGGTRAGCATGG	6859-6878	RT and 1st antisense
HC634	CAGTGGATYAATGARGACTGC	6225-6245	2nd sense
HC636	AYTGGTTGAGCCCGACCTGG	6782-6801	2nd antisense
HCV/2b-NS3 region			
HC690	CYTRGCRRTCGCCGTGGAGC	3251-3270	1st sense
HC702	CATGTASGCCCCRAARCCAAG	4137-4157	RT and 1st antisense
HC692	CTGTGRTGTTYAGYCCRATGGAG	3271-3293	2nd sense
HC703	AGYGTRGCCGCKACAGAGGG	4119-4138	2nd antisense
HCV/2b-NS5A region			
HC698	GRCTACAYRCCTGGATCACTG	6229-6249	1st sense
HC699	GGGTCYGTCARCATGGAGGC	6867-6886	RT and 1st antisense
HC700	CCRGTYCCRTGYTCGGGGTC	6258-6277	2nd sense
HC701	AGGCYARYACCTCGGTGTCC	6851-6870	2nd antisense

[a]R=A/G, S=G/C, K=T/G and Y=T/C.
[b]The nucleotide positions are numbered in accordance with the HC-J4/91 strain (D10750) with the HCV/1b reference, whereas the nucleotide positions are numbered in accordance with the HC-J8 strain (D01221) with the HCV/2b reference.

pdf]). V170I is not known to be associated with resistance to NS3/4A protease inhibitors (Romano et al. 2010; Halfon and Locarnini 2011). In addition, there were no mutations at aa 155, 156, or 168 in the NS3 region or at aa 28, 31, or 93 in the NS5A region, which are known to be associated with resistance to PTV and OBV, respectively (Krishnan et al. 2015; Pilot-Matias et al. 2015; Interview form of VIEKIRAX® ver. 4 [http://www.abbvie.co.jp/content/dam/abbviecorp/japan/docs/if_Viekirax_j.pdf]) (Table 3). He had a history of cerebral infarction, duodenal ulcer, bilateral vitreous hemorrhaging, and bilateral retinal detachment.

He experienced a rush and vomiting after injection of iodinated contrast material. He had several comorbidities, including hypertension, right central facial paralysis, and gallbladder polyp. He was taking clopidogrel sulfate, aliskiren fumarate, calcium carbonate, and sennoside A • B calcium, as well as injections of insulin. After starting combination therapy of OBV/PTV/r, he complained of appetite loss, lumbago, and itching during the therapy. The lumbago had been reported before the combination therapy, and it was not clearly improved after the completion of therapy. Thus, the treatment-emergent adverse events (TEAEs) were considered to be appetite loss and itching, as these disappeared after the completion of therapy. However, the patient's adherence to OBV/PTV/r was 100%. He achieved rapid virological response (RVR) and SVR12.

Patient 2

A 71-year-old male was receiving dialysis due to chronic renal failure arising from diabetic nephropathy in 2005. The transmission source of HCV could not be identified. He had not received interferon-based therapy. He had no RAVs known to be associated with resistance to NS3/4A protease inhibitors, including linear ketoamids and macrocyclic compounds (Romano et al. 2010; Halfon and Locarnini 2011), or NS5A inhibitors, including DCV (Fridell et al. 2011) and OBV (Krishnan et al. 2015) (Table 3). He had several comorbidities, such as atrial septal defect, atrial fibrillation, hypertension, upper gastrointestinal disease, and liver cysts with calcification. He was also

Table 2. Laboratory findings at baseline, treatments and outcomes of HCV-infected dialysis patients.

Parameters	Patient 1	Patient 2	Patient 3	Patient 4
Age (yr)	61	71	68	64
Sex	Male	Male	Male	Male
BMI (kg/m^2)	20.9	20.8	20.8	23.8
HCV genotype	1b	1b	1*	1b
Cause of dialysis	DM	DM	DM	Unknown
IFN-based therapy: Outcome	Naive: NA	Naive: NA	Naive: NA	Naive: NA
At the start of therapy				
HCV RNA (log IU/mL)	4.4	3	6.4	5.7
AST (IU/L)	9	25	15	16
ALT (IU/L)	14	24	11	25
WBC (cells/μL)	4,600	7,420	5,480	3,060
Hemoglobin (g/dL)	14	9.3	9.9	9.5
Platelets (cells/μL)	127,000	171,000	214,000	95,000
RAVs at baseline†	None	None	L28F and L31M‡	None
Severity of liver disease	Chronic hepatitis	Chronic hepatitis	Chronic hepatitis	Liver cirrhosis
Treatments and outcomes				
Ombitasvir/paritaprevir/ritonavir dosage (mg)	25/150/100	25/150/100	25/150/100	25/150/100
Achievement of rapid virological response	Yes	Yes	Yes	Yes
Adherence to ombitasvir/paritaprevir/ritonavir	100%	100%§	100%	100%
Weeks of therapy	12	6	12	12
Response	SVR12	SVR12	Breakthrough	SVR12
Concomitant drugs	Clopidogrel sulfate, aliskiren fumarate, calcium carbonate, sennoside A·B calcium, insulin	Warfarin, valsartan, voglibose, epalrestat, calcium carbonate, lanthanum carbonate, mecobalamin, vildagliptin, rabeprazole, polaprezinc	Azilsartan medoxomil, rabeprazole, ezetimibe, limaprost alfadex, adenosine triphosphate disodium hydrate, L-carbocisteine, aspirin, doxazosin mesilate, epinastine hydrochloride, tramadol hydrochloride/acetaminophen, epalrestat, miglitol, falecalcitriol, sennoside A·B calcium	Calcium carbonate, febuxostat, sodium polystyrene sulfonate, brotizolam, cinacalcet hydrochloride, epoetin beta pegol, calcitriol
Treatment-emergent adverse events	Appetite loss, itching	Itching, exacerbation of wamble and falling tendency	None	None

BMI, body mass index; CGN, chronic glomerulonephritis; DM, diabetes mellitus; NA, not applicable; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; WBC, white blood cells; RAVs, resistance-associated variations; SVR, sustained virological response. *Serogroup. Genotype turned out to be 1b and 2b as we examined after viral breakthrough. †RAVs against ombitasvir or paritaprevir. ‡HCV/2b-NS5A RAVs. No HCV/1b RAVs were detected. §The patient's adherence to ombitasvir/paritaprevir/ritonavir was 100% until the date of the discontinuation of the combination therapy.

Table 3. Resistance profile of four dialysis patients treated with ombitasvir/paritaprevir/ritonavir.

Patient	Genotype	NS3							NS5A			
		V36	T54	Q80	R155	A156	D168	V170	L28	L31	Q54	Y93
Patient 1	1b[a]	WT	WT	WT	WT	WT	WT	I[b]	WT	WT	H[c]	WT
Patient 2	1b[a]	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
Patient 3	1b[a]	WT	WT	K[b]	WT	WT	WT	I[b]	WT	WT	WT	WT
Patient 4	1b[a]	WT	WT	L[b]	WT	WT	WT	I[b]	WT	WT	WT	WT

Patient	Genotype	NS3						NS5A		
		E79	F154	A160	D168	E173	A178	L28	L31	C92
Patient 3	2b[a,d]	WT	WT	WT	WT	WT	WT	**F**	**M**	WT
	2b[e]	WT	WT	**T/A**	**D/V**	WT	WT	**F**	**M**	WT

WT, wild-type.
[a]Before treatment.
[b]Not resistant to paritaprevir.
[c]Not resistant to ombitasvir.
[d]Genotype of co-infected HCV.
[e]At breakthrough.

taking nifedipine, warfarin, valsartan, voglibose, epalrestat, calcium carbonate, lanthanum carbonate, mecobalamin, vildagliptin, rabeprazole, and polaprezinc. Calcium antagonist combined with combination therapy of OBV/PTV/r has been reported to cause edema (Kumada et al. 2015), so we discontinued nifedipine before starting combination therapy. Because his blood pressure remained stable after discontinuing nifedipine, we pressed ahead with the combination therapy.

The patient achieved RVR; however, he developed itching of the head and body as well as exacerbation of wamble and a falling tendency during the therapy. Besides, he fell and hurt his leg and developed a femoral ulcer, whereupon he was admitted to an orthopedic ward. He developed delirium, probably due to the stress of hospitalization. After being discharged from the hospital, his delirium completely disappeared. However, the therapy was discontinued after six weeks of therapy due to his and his family's wishes. The TEAEs were considered to be itching, exacerbation of wamble, and a falling tendency because these disappeared or grew less severe after the completion of therapy. Fortunately, he achieved SVR12 despite completing only half of the standard treatment duration of 12 weeks.

Patient 3

A 68-year-old male was receiving dialysis due to chronic renal failure arising from diabetic nephropathy in 2003. The transmission source of HCV could not be identified, although he had received a blood transfusion about 40 years ago and did not manifest posttransfusion hepatitis at that time. He had not received interferon-based therapy. He was infected with HCV of serogroup 1 (most likely genotype 1b). There were no mutations in the NS3 or NS5A regions of the infected HCV/1b, which are known to be associated with resistance to PTV and OBV, respectively (Table 3). Although Q80K, which is known to be an RAV against the NS3/4A protease inhibitor TMC435 (simeprevir) (Romano et al. 2010; Halfon and Locarnini 2011), was found, this mutation is unlikely to be associated with resistance to PTV (Pilot-Matias et al. 2015; Interview form of VIEKIRAX® ver. 4 [http://www.abbvie.co.jp/content/dam/abbviecorp/japan/docs/if_Viekirax_j.pdf]). V170I is not known to be associated with resistance to NS3/4A protease inhibitors (Romano et al. 2010; Halfon and Locarnini 2011). His comorbidities were diabetes mellitus and hypertension. He was taking azilsartan medoxomil, rabeprazole, ezetimibe, limaprost alfadex, adenosine triphosphate disodium hydrate, L-carbocisteine, aspirin, doxazosin mesilate, epinastine hydrochloride, tramadol hydrochloride/acetaminophen, epalrestat, miglitol, falecalcitriol, and sennoside A • B calcium. He was not taking any calcium antagonist.

Although he achieved RVR, HCV RNA reappeared at week 8 of the therapy at 2.1 log IU/ml and reached 5.4 log IU/ml at week 12, which corresponded to the end of therapy. We therefore diagnosed this event as viral breakthrough. However, there were no AEs during the therapy, and the combination therapy was well-tolerated. At 12 weeks after completing combination therapy, we checked the RAVs. Surprisingly, the genotype of the reappeared HCV was 2b. We evaluated the HCV genotype in the stored serum sample obtained just before the start of the combination therapy and found that the patient was coinfected with HCV/1b and HCV/2b (Fig. 1). As indicated in Table 3, HCV/2b in this patient had RAVs of L28F and

L31M in the NS5A; RAVs of A160T and D168V in the NS3 emerged along with the viral breakthrough. The patient's adherence to OBV/PTV/r was 100%. The time course after the start of the combination therapy is shown in Fig. 2.

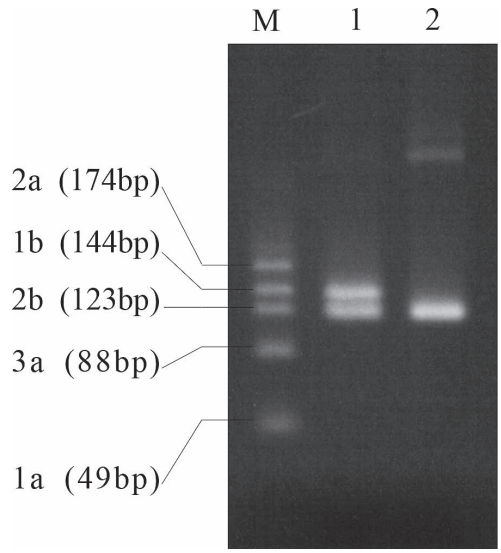

Fig. 1. Detection of HCV genotypes.
Agarose gel electrophoresis of the PCR products in Patient 3. Lane M, size markers of genotypes 1a, 1b, 2a, 2b, and 3a; lane 1, before treatment (typed as 1b + 2b); lane 2, at viral breakthrough (typed as 2b).

Patient 4

A 64-year-old male was receiving dialysis in 2009; however, the cause of dialysis was unknown. The transmission source of HCV could not be identified. He had not received interferon-based therapy. There were no mutations in the NS3 or NS5A regions of the infected HCV/1b, which are known to be associated with resistance to PTV and OBV, respectively (Table 3). Although Q80L, which is known to be an RAV against simeprevir (Romano et al. 2010), was found, this mutation is unlikely to be associated with resistance to PTV (Pilot-Matias et al. 2015; Interview form of VIEKIRAX® ver. 4 [http://www.abbvie.co.jp/content/dam/abbviecorp/japan/docs/if_Viekirax_j.pdf]). V170I is not known to be associated with resistance to NS3/4A protease inhibitors (Romano et al. 2010; Halfon and Locarnini 2011). His comorbidities were hyperuricemia, insomnia, secondary hyperparathyroidism, gallbladder polyp, enlarged prostate, ureter stone, and intraductal papillary mucinous neoplasms of the pancreas. He was taking calcium carbonate, febuxostat, sodium polystyrene sulfonate, brotizolam, cinacalcet hydrochloride, and injections of epoetin beta pegol and calcitriol. The patient encountered no AEs during the therapy, and the combination therapy was well-tolerated. The patient's adherence to OBV/PTV/r was 100%. He achieved RVR and SVR12.

Discussion

The major finding from this case series is that combi-

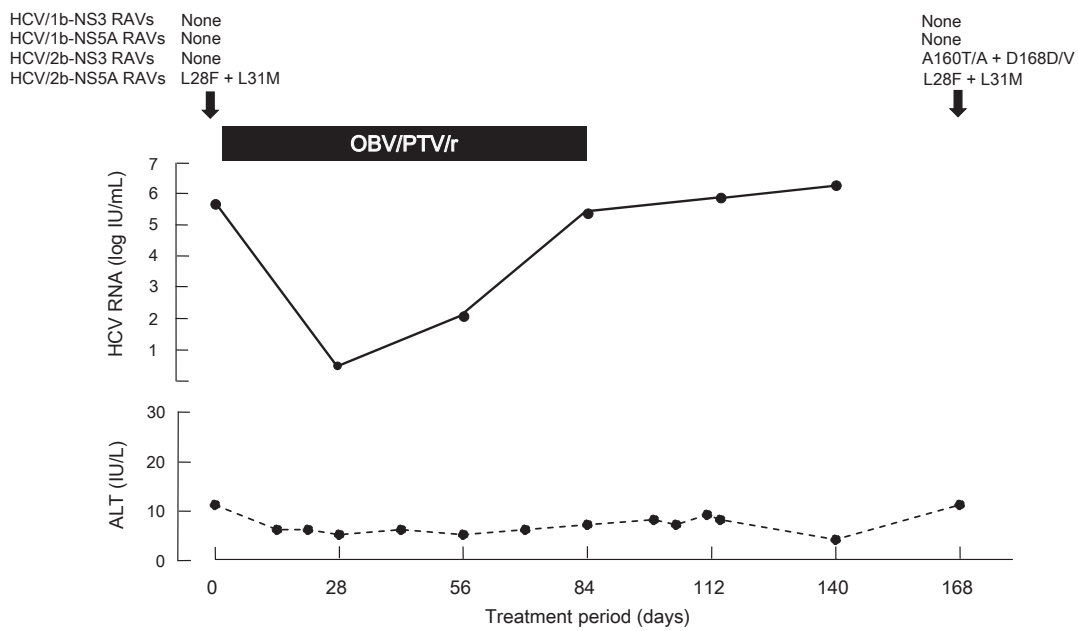

Fig. 2. Clinical course of Patient 3 with viral breakthrough during combination therapy with ombitasvir, paritaprevir, and ritonavir (OBV/PTV/r).
Serum HCV RNA level become undetected once, but it reappeared and reached the pretreatment level at the end of therapy by the combination therapy of OBV/PTV/r. Later, serum HCV RNA level gradually increased. Regarding RAVs, L28F and L31M as HCV/2b-NS5A RAVs were detected in the pretreatment serum. However, A160T/A (mixture of Thr and Ala at aa 160) and D168D/V (mixture of Asp and Val at aa 168) as HCV/2b-NS3 RAVs were detected at post-treatment week 12 of the combination therapy in addition to the pre-existent L28F and L31M. RAVs: resistance-associated variations; HCV: hepatitis C virus; ALT: alanine aminotransferase; OBV: ombitasvir; PTV: paritaprevir; r: ritonavir.

nation therapy of OBV/PTV/r may have promise as an efficacious therapy in HCV-infected dialysis patients, but caution regarding AEs should be practiced, especially in patients taking concomitant drugs that may interact with the combination therapy medicines, such as Patient 2.

At present, combination therapy of DCV/ASV is the first-line choice for dialysis patients infected with HCV/1b based on the most recent version (ver. 5.1) of the JSH guideline. However, this combination therapy takes 24 weeks to complete, which is twice as long as standard DAA combination therapy for patients with HCV/1b (SOF/LDV or OBV/PTV/r). In addition, combination therapy of DCV/ASV causes liver dysfunction, headache, and pyrexia (Kumada et al. 2014) at rates more frequent than combination therapy of SOF/LDV (Mizokami et al. 2015) or OBV/PTV/r (Kumada et al. 2015). Furthermore, combination therapy of DCV/ASV was not normally used for patients with RAVs associated with resistance to NS5A inhibitors, such as Y93 or L31 mutations; combination therapy of SOF/LDV or OBV/PTV/r was found to be more efficient for these patients than DCV/ASV. However, combination therapy of SOF/LDV is contraindicated in patients with severe CKD (eGFR < 30 mL/min/1.73 m^2) and those on dialysis. Given that OBV, PTV, and r are all mainly metabolized in the liver with minimal renal clearance (Khatri et al. 2016; Polepally et al. 2016), combination therapy of OBV/PTV/r may be safely used in dialysis patients, at least in theory; we therefore examined the efficacy of this combination therapy in a series of dialysis patients.

Despite its promise, however, OBV/PTV/r contains a major flaw: drug-drug interactions (DDIs) can happen especially with this regimen; for example, r, which is used as a pharmacologic booster for PTV, induces edema when administered in patients concomitantly taking calcium antagonists. We therefore discontinued a calcium antagonist before administering combination therapy of OBV/PTV/r in Patient 2. Another flaw is that the antiviral efficacy of OBV/PTV/r for patients infected with HCV/1b is slightly but not decisively inferior in patients with the Y93 mutation compared to those without it (Kumada et al. 2015). We therefore examined the RAVs to determine the indications for combination therapy of OBV/PTV/r and started this therapy only after confirming the absence of the Y93 mutation.

The pharmacokinetics study of OBV, PTV, DSV, and r performed using patients who tested negative for hepatitis A, B, C, or human immunodeficiency virus with renal impairment (mild: creatinine clearance [Ccr] 60-89 mL/min, moderate: Ccr 30-59 mL/min, severe: Ccr 15-29 mL/min) showed that the renal function did not affect the fraction of drugs unbound to plasma proteins, nor did it change the urinary fraction of drugs excreted unchanged (Khatri et al. 2016). Another recent study (Pockros et al. 2016) showed that the mean plasma trough concentration of OBV, PTV, DSV, and r in patients with stages 4 and 5 CKD were generally similar to those infected with HCV/1b without end-stage renal disease (ESRD) in phase 3 studies of these drugs. The arterial and venous concentrations of all DAAs in stage 5 CKD were similar to those before the start of dialysis, 1 h after beginning dialysis, and at the end of dialysis (Pockros et al. 2016). However, in post-marketing surveillance, according to the most recent version of package insert, combination therapy of OBV/PTV/r causes rapid deterioration of the renal function in patients with an already reduced renal function or in those receiving the therapy in conjunction with calcium antagonist. We should therefore pay close attention to the development of renal dysfunction or AEs during the combination therapy of OBV/PTV/r in ESRD patients.

In Patient 2, TEAEs including itching, exacerbation of wamble, and a falling tendency resulted in the termination of the combination therapy. Regarding DDIs, valsartan and rabeprazole are reported to be subject to interactions with combination therapy of OBV/PTV/r on the "HEP Drug Interactions" site of the University of Liverpool (http://www.hep-druginteractions.org/). Valsartan is a substrate of OATP1B1; therefore, valsartan exposure may increase when administered with combination therapy of OBV/PTV/r. Because the AEs of valsartan include dizziness, wamble, and hypotension, this DDI may have caused the TEAEs observed in Patient 2. Indeed, the blood pressure in Patient 2 remained stable during the combination therapy of OBV/PTV/r despite the discontinuation of nifedipine before starting combination therapy, which supports this understanding of the DDI. Rabeprazole is a substrate of CYP2C19 and CYP3A4; therefore, rabeprazole exposure may decrease when administered with combination therapy of OBV/PTV/r, suggesting that rabeprazole will likely not influence TEAEs via DDIs.

In Patient 3, the viral breakthrough was likely attributed to coinfection with HCV/1b and HCV/2b. Unfortunately, only the HCV serogroup (and not the HCV genotype) before the combination therapy was measured in this patient. However, the RAVs in NS3 and NS5A were able to be measured, and the initial hepatologist thought that the patient was infected with genotype 1 HCV because the measurement of RAVs in the NS3 and NS5A regions was not commercially available in genotype 2 HCV in Japan. The antiviral efficiency of combination therapy of OBV/PTV/r was found to vary depending on the infected subgenotype in patients infected with genotype 2 HCV, although the number of subjects tested was small (Chayama et al. 2015). The SVR24 rate of 25 mg OBV/150 mg PTV/100 mg r was 100% in patients with HCV/2a but only 37.5% in those with HCV/2b (Chayama et al. 2015). In our detailed RAV analysis, L28F and L31M concerning HCV/2b-NS5A RAVs were detected before the combination therapy, although no RAVs were found in the NS3 or NS5A regions of the co-infected HCV/1b. These combination mutations confer 247-fold-reduced susceptibility to OBV in HCV/2b (Krishnan et al. 2015). In addition to these mutations, A160T/A and D168D/V mixed mutations

concerning HCV/2b-NS3 RAVs developed after viral breakthrough. These combination variations confer 15-fold-reduced susceptibility to PTV in HCV/2b (Interview form of VIEKIRAX®, ver. 4 [http://www.abbvie.co.jp/content/dam/abbviecorp/japan/docs/if_Viekirax_j.pdf]). Thus, only HCV/1b was eradicated, but HCV/2b with increased resistance against the combination therapy increased, eventually developing as viral breakthrough. These mutations support the theoretical basis of viral breakthrough in Patient 3.

A recent brief paper (Abei et al. 2016) reported on the effects of OBV/PTV/r in dialysis patients infected with genotype 1 HCV. However, the study did not mention HCV genotyping, the RAVs, or the mechanisms underlying the observed AEs.

Several limitations associated with the present case series warrant mention. The sample size of our case series was very small, and the serum concentrations of OBV, PTV, and r during the therapy were not measured.

In summary, combination therapy with OBV/PTV/r may have promise as an efficacious therapy, but caution regarding AEs should be practiced, especially in patients taking concomitant drugs that may interact with the combination therapy medications. As measurement of only the HCV serogroup may misjudge the exact HCV genotype, the HCV genotype should be measured before starting combination therapy. Larger prospective studies of combination therapy with OBV/PTV/r for dialysis patients are needed in the near future to confirm the findings in our case series.

Author Contributions

K.S. and K.H. recruited the patients. K.S., Y.Y., T.K., S.T., N.H., and S.K. analyzed and interpreted the data. K.S. drafted the article. M.K. and M.Y. gave a critical advice. H.Oh. and H.Ok. performed virological analysis. H.Ok. edited the article and approved the final version to be published. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest. However, K.S. received research funding from AbbVie, Inc, MSD K.K., and BMS K.K. and lecture fees from AbbVie, Inc, MSD K.K., BMS K.K., and Gilead Sciences, Inc., outside the submitted work. S.K. received research funding and lecture fees from AbbVie, Inc, MSD K.K., BMS K.K., and Gilead Sciences, Inc., outside the submitted work. H.Ok. received research funding from BMS K.K., outside the submitted work.

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