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ORIGINAL ARTICLE

Treatment with Ombitasvir/Paritaprevir/Ritonavir and Dasabuvir in Chronic Hepatitis C Genotype 2k/1b

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ABSTRACT

AIM: Hepatitis C virus (HCV) infection is one of the most prevalent etiologies for liver cirrhosis and hepatocellular carcinoma. As a result of being highly heterogeneous, HCV has seven confirmed major genotypes and 67 confirmed subtypes. The importance of correctly determining each genotype and its subtype is dependent on choosing the relevant combination and duration of anti-viral treatment. The recombinant HCV genotype 2k/1b was first detailed in St. Petersburg in 2002 among intravenous drug users. Treatment of HCV 2k/1b patients with sofosbuvir and ribavirin resulted in a lowered sustained virologicresponse rate (SVR). The high efficacy of the 3D regimen (ombitasvir/paritaprevir/ritonavir and dasabuvir) has been proven for genotype1b population; however it has not been evaluated for patients with 2k/1b HCV genotype. The aim of this study was to evaluate the efficacy and safety of a twelve week treatment for patients infected with HCV 2k/1b utilizing the 3D regimin - ombitasvir/paritaprevir/ritonavir and dasabuvir (3D).

MATERIALS AND METHODS: This is an open-label, single arm study. Seven patients received ombitasvir/paritaprevir/ritonavir 25/150/100 mg once daily as well as dasabuvir 250 mg twice daily for twelve weeks.

RESULTS: Six patients achieved a SVR without significant adverse events. One patient discontinued the treatment at week four due to headaches and vomiting.

CONCLUSIONS: This study demonstrates the high efficacy and safety of a twelve week treatment of ombitasvir/ paritaprevir/ritonavir and dasabuvir (3D) for adult patients with HCV 2k/1b.

Key words: Direct acting antiviral treatment; Genotype 2k/1b; Hepatitis C; Sustained virologic response

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Abbreviations list
HCV: Hepatitis C virus; SVR: sustained virologicresponse rate; PegIFN-alfa: Pegylated interferon; DAA: Direct Acting Antiviral; NS3: nonstructural protein 3; RNA: ribonucleic acid; RBV: ribavirin; RT-PCR: Reverse Transcription Polymerase Chain Reaction; 5'-UTR: 5'-untranslated region

INTRODUCTION

Hepatitis C virus (HCV) infection is one of the most prevalent etiologies of liver cirrhosis, hepatocellular carcinoma, liver failure

and liver-related mortality [1].

With seven confirmed major genotypes and 67 confirmed subtypes[2,3], HCV is highly heterogeneous with up to 30% divergence in the nucleotide sequences[4]. Each genotype corresponds to a different geographic distribution[2]. Accurate identification and choosing of the type of treatment as well the duration is essential since different HCV genotypes respond differently to the available antiviral therapies. The recombinant HCV genotype 2k/1b was first recorded in 2002, in conjunction with intravenous drug users in St. Petersburg[5].

Compromised identification of the recombinant HCV can occur, specifically when routine testing for the genotype, since most assays are based on sequencing a single genome region[6,7]. Regarding commercial genotyping testing, in order to ensure precise genotype identification, either special sequencing strategies or utilization of testing methods that amplify both the 3' and the 5' regions of the Hepatitis C virus genome are required. In contrast to using the second generation VERSANT HCV Genotype 2.0 Assay for routine HCV genotyping, clinical laboratories misclassify the 2k/1b strains as HCV genotype 2a/2c[8, 9]. Taking this in to consideration, the number of infected patients with HCV 2k/1b may be underestimated.

Studies dealing with identifying and quantifying the prevalence of recombinant HCV genotypes are sparse. In 2015 Georgia reported that 76% of patients with defined genotype 2 also harbored the 2k/1b chimeric variant[8]. Subsequently, as a result of migration in Germany and Israel[7], a relatively high prevalence (17-25%) of patients infected with HCV genotype 2 also harbored the 2k/1b chimeric variant.

The geographic origin of 50 patients infected with 2k/1b was reveled in a recent extensive study aimed at detailing the geographical distribution of genotype 2k/1b.

The results were as follows: 88% (n = 44/50) originated from the former Soviet Union; eighteen patients were of Russian origin; eleven from Georgia; four from Ukraine; three from Azerbaijani; three from Chechenia; two from Armenia; two from Kazakhstan; one from Tadzhikistan. The remaining six patients were from Western Europe (Germany n = 2; Greece n = 1, Romania n=1 and Israel=2)[7].

In Israel, a 24 week treatment of Pegylated interferon (PegIFN -alfa) and ribavirin of the chimera virus was conducted in four out of eleven patients. Two patients achieved a SVR and two failed to respond.

In Germany[10], the use of sofosbuvir and ribavirin in treating viral chimera led to virologic relapse 25 of 27 patients (93%). This in contrast to ten other patients with the 2k/1b genotype, where the treatment consisted of a genotype 1-targeted Direct Acting Antiviral (DAA) regimens.

Results of the treatment were: two patients responded positively to daclatasvir/sofosbuvir; two responded positively to paritaprevir/ombitasvir/dasabuvir (3D) therapy; four out five of the patients achieved a SVR with treatment consisting of ledispasvir/sofosbuvir ± ribavirin therapy; and one patient was lost to follow-up[10].

Of the 25 patients who relapsed, 14 received salvage therapy with an NS5A-Inhibitor plus sofosbuvir or 3D, with or without ribavirin (ledispasvir/sofosbuvir n = 2; ledispasvir/sofosbuvir/ribavirin n = 3; daclatasvir/sofosbuvir/ribavirin n = 1; paritaprevir /ombitasvir / dasabuvir n = 5; velpatasvir/sofosbuvir n = 3), with 13 of them achieving SVR12, while in one patient treatment was still ongoing[10]. The 3D regimen includes 3 DAAs with distinct, non-overlapping mechanisms of action. Ombitasvir is a potent NS5A inhibitor that is co-formulated with paritaprevir, a potent NS3 (nonstructural protein 3)/4A protease inhibitor co-dosed with ritonavir to boost drug exposure. Dasabuvir is a non-nucleoside NS5B ribonucleic acid (RNA) polymerase inhibitor[11,12]. The high efficacy of the 3D regimen has been proven in registration clinical trials for 1b population[13]. The aim of this study was to prospectively assess the efficacy and safety of the 3D regimen as a treatment for the recombinant HCV genotype 2k/1b.

METHODS

Study Design and Patients

The study was conducted according to Good Clinical Practice guidelines, the Declaration of Helsinki and all applicable regulations, with independent ethics committee and institutional review board approval. Access to the study data was open to all authors, all of whom reviewed and approved the final text.

This was an open-label, single arm study which included 1 group of HCV genotype 2k/1b- infected subjects who were untreated or failed to respond to previous therapy with pegIFN-alfa and ribavirin (RBV). A 12 week 3D regimen was used to treat the patients.

All patients received ombitasvir/paritaprevir/ritonavir (25/150/100 mg) once a day and dasabuvir (250 mg) twice daily. Subjects were assessed for virologic response, clinical outcome and adverse events. A follow up for up to 24 weeks was also included, commencing from the conclusion of the treatment (Figure 1).

The eligible age for accepting patients was 18 years and above and all patients provided written informed consent. The accepted patients all had HCV RNA > 15 IU/mL and a laboratory result at screening, indicating infection with HCV genotype 2k/1b subtype. Patients who tested positive for hepatitis B surface antigen, anti-HIV antibody or displayed evidence of HCV genotype or subtype other than genotype 2k/1b at the time of screening were all excluded from the study.

Other reasons for exclusion from the study included: Previous study drug administration, a history of organ transplant, hepatocellular carcinoma, severe renal impairment, clinically significant disorders or co-morbidities other than HCV infection.

HCV genotyping

HCV genotyping was performed by real-time based assays (Abbott RealTime HCV Genotype II, Abbott Molecular, Illinois, USA) according to manufacturer instructions. In short, the Abbott m2000sp instrument was used to extract RNA from serum samples, after which Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed, using both the kit amplification reagent packs and the Abbott m2000rt instrument. The Abbott RealTime HCV Genotype II kit (Abbott-RT-HCV) is a commonly used FDA-approved platform for HCV genotyping. It relies on consensus primer amplification

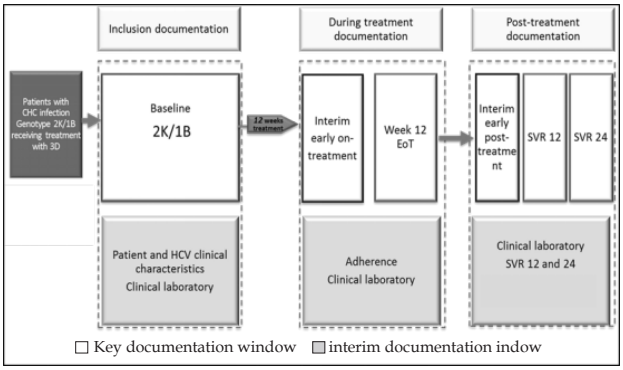

Figure 1 Study flowchart. EOT-end of treatment; SVR-sustained virologic response.

Table 1 Summary of patients' demographic and clinical parameters.

Patient number	Gender	Age	† COB	genotype	Fibrosis Stage (fibroscan)	Cut-off value (KPa)	Previous treatment	Baseline HCV RNA level (IU/ml)	SVR12 HCV RNA level (IU/ml)
1	Male	47	Russia	2K/1B	F4	25	untreated	$1.7x10^6$	§ND
2	Male	61	Israel	2K/1B	F3-F4	8.8	untreated	$1.5X10^6$	ND
3	Female	22	Ukraine	2K/1B	F0-F1	6.5	untreated	$3.2X10^6$	
4	Male	52	Israel	2K/1B	F0-F1	4.6	untreated	$2.4X10^6$	ND
5	Male	41	Russia	2K/1B	F0-F1	6.6	¶pegIFN/RBV	$1X10^6$	ND
6	Female	40	Georgia	2K/1B	F0-F1	3.9	untreated	$1.7X10^6$	ND
7	Male	46	Azerbaijan	2K/1B	F0-F1	7	pegIFN/RBV	$2.9X10^6$	Early discontinuation

† COB-country of birth; ‡ SVR- sustained virologic response; § ND-not detected; ¶ PegIFN/RBV-Pegylated interferon-alfa and ribavirin

of the HCV 5'-untranslated region (5'-UTR) and specific primers for HCV genotype 1a and 1b NS5b, located at the 3'-untranslated region (3'-UTR) sequences. Amplicons are then detected with the use of genotype specific fluorescent labelled probes against 5'-UTR of genotypes 1-6 and NS5B of subtypes 1a and 1b. The advantage of utilizing two pairs of primers (from the 5-UTR and the NS5b regions) is such that one can concurrently detect recombinant HCV genotype 2/1 strains.

HCV RNA viral load detection

HCV RNA viral load was determined using the Abbott RealTime HCV assay (Abbott Molecular, Inc. (Des Plaines, IL) according to the manufacturer's specifications. Abbott RealTime HCV assay combines RT-PCR technology and homogeneous real time fluorescent detection for the quantitation of HCV RNA. The test is standardized against the Second WHO International Standard for Hepatitis C Virus RNA (NIBSCCode 96/798). Results are reported in International Units/mL (IU/mL). HCV RNA < lower limit of quantification (LLOQ) is 12 IU/mL.

Assessments

The SVR at 12 weeks post-treatment was used to assess treatment efficacy.

SVR is defined as a sustained virologic response when found less than the LLOQ.

At the end of the four week treatment, 12 week post-treatment as well as 24 week post-treatment, serum samples were collected and examined for HCV RNA viral load at baseline.

Adverse event monitoring, vital signs measurements, physical examination, and laboratory tests were performed throughout the study to assess safety and tolerability.

RESULTS

HCV genotype 2k/1b- infected subjects who were untreated (5/7) or failed to respond to pegIFN/RBV (2/7) were treated with ombitasvir/paritaprevir/ritonavir (25/150/100mg once daily) and dasabuvir (250 mg twice daily) (3DAA) for 12 weeks. HCV RNA level was determined at baseline and 12 weeks post treatment. Six patients completed 3DAA treatment without any adverse events. In all 6 patients HCV RNA was not detected at 12 weeks post treatment (SVR12) (Table 1). One patient discontinued treatment early at week 4 due to headaches and vomiting.

CONCLUSIONS

Recombinant HCV genotype 2/1 strains represent a challenge for direct-acting antiviral (DAA) therapy. Over the past years various direct-acting antiviral drugs (DAAs) targeting the HCV non-structural proteins NS3, NS5A, or NS5B have become available, leading to notable improvements in sustained virologic response rate[14].

One of the main requirements when allocating the optimal treatment for each patient is the precise determination of the HCV genotype and its subtype. This accurate determination of both the genotype and its subtype allows the optimal combination of treatment and duration. Patients infected with un-diagnosed chimeric viruses may be treated inappropriately[15].

Since its first detailing in 2002[15], the inter-genotypic recombinant HCV strain 2k/1b has been detected rarely, but consistently, in HCV infected patients[8,15] . As of yet the HCV strain 2k/1b HCV has been detected in 50 patients from eleven different countries[10]. Eighty nine percent of these patients originate from countries of the former Soviet Union. Treatment of viral chimera 2k/1b with the DAA's sofosbuvir/ribavirin or daclatasvir/sofosbuvir led to a relatively low SVR rate[10]. However, treatment with other DAAs such as ledispasvir/sofosbuvir, ledispasvir/sofosbuvir/ribavirin, daclatasvir/sofosbuvir/ribavirin, paritaprevir/ombitasvir/dasabuvir, velpatasvir/sofosbuvir, has demonstrated a high SVR rate[10]. The aim of this study was to evaluate for the first time the efficacy and safety of the 3D regimen for the treatment of the the2k/1b chimera virus. Seven new patients infected with 2k/1b HCV were identified. As expected[10], majority of the patients originated from the Soviet Union area, two patients originated from Russia, one from Ukraine, one from Georgia, and one from Azerbaijan. Two patients were born in Israel. Five patients were naive to treatment; others were non responders to pegIFN/RBV treatment. All patients were treated with 3DAA treatment for 12 weeks. All six patients who completed 3DAA treatment without any adverse events displayed a high SVR rate. One patient discontinued treatment after four weeks due to headaches and vomiting. In conclusion, the 3D regimen was found to be highly efficient and safe for treating patients with recombinant HCV genotype 2k/1b, both for naïve patients as well as for patients who failed treatment with pegIFN/RBV.

Data availability: All available data used to support the findings of this study are included within the article.

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