Mutations in non-structural 5A and rapid viral response to pegylated interferon-α-2b plus ribavirin therapy are associated with therapeutic efficacy in patients with genotype 1b chronic hepatitis C

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Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver disease, with an estimated 170 million people infected worldwide. In Japan, the carrier rate is estimated to be approximately 1% of the general population. This rate increases depending on age and reaches approximately 5% in individuals over 70 years of age. The main goal of treatment for chronic hepatitis C is prevention of cirrhosis and hepatocellular carcinoma by eradication of the virus. Interferon (IFN)-based therapy was initiated in 1992, and efficacy of treatment regimens has...
improved year by year. Although the HCV viral eradication rate is approximately 5% following 24 weeks of treatment with conventional IFN therapy, the therapeutic result of combined pegylated IFN and ribavirin is ~55%. However, approximately half of patients treated with pegylated-IFN do not achieve a sustained viral response (1-3).

Due to the numerous side effects and the high cost of treatment, it is important to understand the individual mechanisms involved in non-response to treatment and to predict therapeutic efficacy prior to treatment. It has been reported that various viral and host factors are associated with the therapeutic response.

The role of amino acid (aa) mutations within the functional regions of non-structural 5A (NS5A) in relation to therapeutic response has been reported by several researchers. In 1996, it was reported that a high number of mutations in the IFN-sensitivity-determining region (ISDR) (aa 2209-2248) was strongly related to the sustained viral response (SVR) to IFN monotherapy in genotype 1b Japanese patients (4,5). In 2008, high mutations in the IFN-ribavirin resistance-determining region (IRRDR) (aa 2334-2379) were also related to the SVR to combined pegylated-IFN and ribavirin therapy (6). The significance of these mutations was also confirmed by studies carried out in different populations in different countries (7).

Based on previous studies, factor analysis and determination of NS5A viral mutations in relation to SVR of patients treated with pegylated-IFN and ribavirin combination therapy for HCV genotype 1b and a high viral load was carried out in a collaborative study in Kobe, Japan.

Materials and methods

Sample collection. Serum samples were collected from chronic hepatitis C patients with genotype 1b and a high viral load. A total of 96 patients (age 57.7±8.3 years; 45 males, 51 females) who were treated by subcutaneous injections of pegylated-IFN-α-2b once every week (1.5 μg/kg) (Peginteron; Schering-Plough, Innishannon, Country Cork, Ireland) in combination with oral ribavirin (400-800 mg) daily for 48 weeks between September, 2006 and June, 2008 were enrolled. HCV-RNA in serum samples was examined at 4 weeks, at the end of treatment and 6 months after the end of treatment. Serum samples were collected and stored at -80°C until virological examination. The rapid virological response (RVR) was defined as undetectable HCV-RNA at 4 weeks. Patients who had persistent undetectable serum HCV-RNA and normal serum alanine aminotransferase (ALT) levels 6 months after the end of treatment were considered to have an SVR.

The standard dosage of PEG-IFN (1.5 μg/kg) and ribavirin (12 mg/kg) was determined depending on the weight-based dose. Patients treated with >80% of the standard dosage were considered as high drug adherence and patients treated with at least one drug at <80% of the standard dosage were categorized as a low drug adherence group.

This study was conducted by Kobe University Hospital and 25 affiliated hospitals in Hyogo prefecture. The study protocol was approved by the Ethics Committee of Kobe University Hospital, and written informed consent was obtained from each patient before treatment.

| Table I. Comparison of the base characteristics of the SVR and the non-SVR groups. |
|---------------------------------|----------------|----------------|----------------|
| Factor                          | SVR            | Non-SVR        | P-value        |
| No. of patients (%)             | 42 (44%)       | 54 (56%)       | 0.005          |
| Age, years                      | 55.1±8.6       | 59.7±7.5       | 0.057          |
| Males:Females                   | 22:20          | 23:31          | 0.901          |
| BMI (kg/m²)                     | 24.0±3.4       | 23.2±3.4       | 0.85           |
| ALT (IU/l)                      | 72.3±69.4      | 75.8±61.8      | 0.66           |
| PLT (x10^12/mm³)                | 17.7±4.9       | 17.0±5.3       | 0.68           |
| RVR                             | 15/38          | 3/49           | <0.001         |
| PPB/ITT                         | 30/41 (73%)    | 25/54 (46%)    | 0.031          |

SVR, sustained viral response; BMI, body mass index; PLT, platelets; ALT, alanine aminotransferase; RVR, rapid viral response; PPB, per-protocol-based analysis; ITT, intention-to-treat analysis.

| Table II. Drug adherence of patients to pegylated-interferon and ribavirin therapy. |
|---------------------------------|----------------|----------------|
| No. of patients (%)             | High drug adherence | Low drug adherence | P-value |
| Age, years                      | 57.4±8.2       | 59.3±7.2       | 0.25          |
| Males:Females                   | 33:32          | 13:18          | 0.072         |
| BMI (kg/m²)                     | 23.6±2.8       | 23.5±4.3       | NS            |
| ALT (IU/l)                      | 78.2±54.5      | 72.7±68.5      | 0.74          |
| PLT (x10^12/mm³)                | 16.3±5.6       | 16.7±4.6       | 0.83          |
| SVR                             | 30/65 (46%)    | 11/31 (35%)    | NS            |
| ISDR ≥1                         | 26/50 (52%)    | 12/26 (46%)    | NS            |
| IRRDR ≥6                        | 18/50 (36%)    | 11/26 (42%)    | NS            |

BMI, body mass index; ALT, alanine aminotransferase; PLT, platelets; SVR, sustained viral response; ISDR, IFN sensitivity-determining region; IRRDR, IFN resistance-determining region.

NS5A sequence analysis. HCV-RNA was extracted from 140 µl serum using a commercial kit according to the manufacturer's protocol (QIAamp Viral RNA kit; Qiagen, Tokyo, Japan). The NS5A region of the HCV genome was amplified and sequenced by nested RT-PCR using primer sets (6). The aa sequences were deduced and aligned using GENETYX Win software version 7.0 (Genetyx Corp., Tokyo, Japan).

Statistical analysis. Differences in parameters, including all available patient demographic, biochemical, hematological, and virological data, as well as ISDR and IRRDR sequence variations factors, were determined between the different patient groups by the Student's t-test for numerical variables, and Fisher's exact probability test for categorical variables.

Subsequently, univariate and multivariate logistic analyses were performed to identify variables that independently predict SVR. The odds ratios (OR) and 95% confidence intervals

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(CIs) were also calculated. Positive and negative predictive values of SVR were computed, and their significance levels were evaluated using the sign test. All statistical analyses were performed using the SPSS version 16 software (SPSS Inc., Chicago, IL). Unless otherwise stated, a P-value of <0.05 was considered to indicate a statistically significant result.

Results

Baseline characteristics and on-treatment response in association with SVR. Baseline characteristics and on-treatment response are summarized in Table I. Overall, 42 cases out of 96 (44%) achieved an SVR. SVR patients were significantly younger in age and had a higher rate of RVR than the non-SVR patients. The prevalence of high drug adherence in SVR patients (73%) was significantly higher than that in non-SVR patients (46%) (P=0.03).

Drug adherence to pegylated interferon and ribavirin therapy. Due to various side effects, 31 patients were not treated with a sufficiently high dosage. Table II summarizes the patient groups with low and high drug adherence. Sixty-five (68%)
patients had high drug adherence to the therapy. Older age and women tended to require dose reductions. The SVR rate (35%) in patients with low drug adherence was significantly lower than those (46%) with high drug adherence.

Mutations in the NS5A region and predictive indicators for SVR. Factor analysis in association with the SVR was performed by per-protocol-based (PPB) analysis. The average number of mutations in IRRDR was significantly higher in the SVR group (6.1±2.7) than that in the non-SVR group (3.7±1.9) (P=0.0003). The average number of mutations in ISDR was also higher in the SVR group (1.9±2.2) than that in the non-SVR group (0.9±1.7), but this difference did not achieve statistical significance (Fig. 1). The SVR group and the non-SVR group were compared based on the number of mutations in the NS5A region. The prevalence of patients with ≥6 aa mutations within IRRDR in the SVR group (60%) was significantly higher than that in the non-SVR group (18%) (P=0.02). Similarly, the prevalence of patients with ≥1 aa mutation within ISDR in the SVR group (73%) was higher than that in the non-SVR group (41%), but this difference was not statistically significant (P=0.06). All patients with ≥6 aa mutations in IRRDR and ≥1 aa mutation in ISDR achieved an SVR (Table III). The positive predictive values of SVR in patients with ≥6 aa mutations in IRRDR was 78%. The sensitivity and specificity were 64 and 86%, respectively.

Factor analysis in association with the SVR. Univariate and multivariate analyses are summarized in Table IV. Univariate analysis showed that ≥6 aa mutations in IRRDR and ≥1 aa mutation in ISDR were strongly associated with an SVR. In addition, RVR and LVR were also significant between the two groups. Multivariate analysis revealed that ≥6 aa mutations in IRRDR was significantly related to the SVR.

Discussion

Pegylated-IFN and ribavirin combination therapy has been a standard treatment for patients with chronic hepatitis C. However, HCV genotype 1 is more resistant to IFN treatment than genotypes 2 or 3. In Japan, genotype 1b is the most prevalent and it is important to predict the therapeutic response for these patients prior to therapy (7-9). In general, approximately 50% of patients with genotype 1b do not achieve SVR even when using a combination of pegylated-IFN plus ribavirin treatment (10). In the present study, the overall SVR rate was 44% and this value was slightly lower than that in a previous study (8). The reason for this is possibly related to the patient age and drug adherence. The present study showed that age, drug adherence and RVR in the SVR group were significantly different than these values in the non-SVR group. The SVR rate in patients younger than 65 years was 52% and was significantly higher than that in patients over 65. In addition, the SVR rate (46%) in patients with high drug adherence was higher than that (35%) in patients with low drug adherence. There is no doubt that older patients have difficulties continuing therapy and are forced to reduce the dosage or terminate treatment because of side effects. In the present study, the percentage of patients having low drug adherence was 32%, and the majority of patients in this group were aged women. Physically and mentally, it is frequently difficult to continue therapy for older patients. The average age of patients in Japan is older than that in most other European countries and this is one of the important reasons for the therapeutic difference among Japanese studies and those carried out in other countries.

On-treatment response is an important factor for predicting SVR; RVR 4 weeks following the initiation of treatment has been reported to be a good predictor of SVR (11-13). In this study, RVR was an important factor for predicting SVR by multivariate analysis. The positive predictive value was 82% and RVR was confirmed to be a good predictor in this study. However, even when patients are predicted as good responders for IFN/RBV therapy, they do not always achieve SVR as side effects result in dose reduction or termination of the planned IFN/RBV treatment. It was also reported that drug adherence is related to SVR (14). In this study, 3 patients relapsed after achieving RVR. The first case was over 65 years of age, the second case had low drug adherence, and the third was an older patient over 65 years with low drug adherence. Incomplete treatment is an important factor contributing to the failure of achieving SVR. This result suggests the necessity for prolonged therapy or therapeutic modification in patients with RVR receiving a dosage reduction.

Mutations in several amino acids in the NS5A protein have been described and are thought to play an important role in response to IFN treatment. It has been reported that a high number of mutations in ISDR and IRRDR are significantly associated with SVR (6). In the present study, patients with ≥1 aa mutation in ISDR and ≥6 aa mutations in IRRDR tended to achieve SVR, which was supported by previous data (6). For ISDR, the mutation results are similar to previous studies (4,5). Compared with ISDR, IRRDR was more strongly associated with SVR in this study. Based on the multivariate analysis, only IRRDR was associated with an SVR. Patients with more than 6 IRRDR mutations had a higher SVR rate and it was the same as previous studies (6). The positive predictive value and sensitivity was >80%, suggesting it to be a good predictive marker. All patients with ≥6 aa mutations and ≥1 aa mutation in ISDR achieved SVR following pegylated-IFN and ribavirin combination therapy. The importance of the NS5A mutation is still controversial. It has been reported that a mutation in NS5A is not related to the IFN response in European and American HCV strains (15-18). However, the importance of NS5A was reported in Asian HCV strains including Taiwan and Chinese strains (19,20). To date, this inconsistency is unclear but is partly related to the fact that HCV strains are different depending on geographic distribution (21). Meta-analysis revealed that the prevalence of a mutation in ISDR was 44.1% in Japanese and 24.8% in European patients, respectively (21). Mutational studies are sometimes inconsistent even among Japanese studies, suggesting that mutations in the NS5A region vary based on different geographical regions even in Japan.

The NS5A protein has a transcriptional activation function and represses IFN-induced gene expression (22). In addition, the NS5A protein interacts with antiviral protein PKR resulting in suppressed PKR activity (23). It is possible that mutations in the NS5A protein may affect the structural and/or biological functions of NS5A and inhibit IFN activity (23,24).
Mutations in E2-PePHD (aa 659-670), PKRBD (aa 2209-2274) and NS5A-V3 (aa 2356-2379) are also reported to be associated with IFN sensitivity (24,25).

Recent studies have shown that SNPs in the IL28B region are strongly associated with response to IFN therapy (26). In this study, genomic factors in the host were not analyzed due to the pre-treatment study design and informed consent. Therapeutic prediction can be more accurate upon examination of host factors as well as viral factors. In the near future, new drug therapies such as protease and polymerase inhibitors called new direct-acting antivirals (DAAs) will become available (27). Standard therapy for hepatitis C virus will include combination therapies using DAAs and pegylated-IFN plus ribavirin. However, the SVR rate by telaprevir-based pegylated-IFN plus ribavirin combination therapy (REALIZE study; phase III, randomized, double blind, placebo-controlled study) was found to be as high as 31% in patients who were non-responders to prior treatment (28). The viral response to pegylated-IFN and ribavirin combination therapy is important for the development of future combination therapies.

In conclusion, mutations in the NS5A region, particularly in patients with more than 6 aa mutations in the IRRDR region are strongly associated with the therapeutic response to pegylated-IFN and ribavirin combination therapy.

References