

Polymorphism of the inosine triphosphate pyrophosphatase gene predicts ribavirin-induced anemia in chronic hepatitis C patients

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Received August 15, 2011; Accepted October 25, 2011

DOI: 10.3892/mmr.2011.659

Abstract. Ribavirin (RBV)-induced anemia is a serious side effect of pegylated interferon (PEG-IFN) plus RBV therapy which is the standard care most effective for hepatitis C virus (HCV) infection. In the present study, we investigated the association of inosine triphosphate pyrophosphatase (ITPA) genotypes with RBV-induced hemoglobin (Hb) reduction in HCV patients treated with PEG-IFN/RBV therapy. The genotypes of the *ITPA* rs1127354 single nucleotide polymorphism were determined in 179 patients with HCV infection. Among them, 52 patients were treated with PEG-IFN/RBV. The frequency of the *ITPA* major allele (CC) was 76.3% and that of the minor allele (CA and AA) was 23.7%. A rapid decrease in Hb levels during the initial 4 weeks was observed in patients with the *ITPA* major allele (CC), but not in patients with the *ITPA* minor allele (C/A and AA). Hb levels at 4 weeks were significantly lower in patients with the *ITPA* major allele than the levels in patients with the minor allele. Out of the 41 patients, 6 (14.6%) with *ITPA* major allele had Hb levels <10 g/dl and 11 patients (26.8%) had a decline in Hb of >3 g/dl. None of the patients with the *ITPA* minor allele had such data. There were no significant differences in virological responses of HCV-RNA between patients with the *ITPA* major allele and those with the minor allele. In conclusion, the *ITPA* genotypes may be a useful marker for prediction of RBV-induced anemia.

Introduction

Hepatitis C virus (HCV) is the leading cause of chronic liver disease. It accounts for 70% of all chronic hepatitis cases, 40% of all cases of liver cirrhosis and 60% of hepatocellular carcinoma (HCC) (1,2). Since the successful eradication of HCV is associated with a reduced risk of liver cirrhosis and HCC (3,4), antiviral therapy plays a crucial role in the management of HCV-infected patients. Currently, pegylated interferon (PEG-IFN) plus ribavirin (RBV) is considered to be the standard of care most effective for chronic hepatitis C (5-7), but the rate of sustained virological response (SVR; HCV RNA negative for 24 weeks after the cessation of therapy) is approximately 50% in patients with HCV genotype 1, the most common genotype found in populations in many countries (5,6). Furthermore, 20-30% of HCV genotype 1 patients are non-responders to PEG-IFN/RBV therapy (3).

PEG-IFN therapy sometimes causes bone marrow suppression (8,9), and dose reduction may be required in such cases. However, dose reduction is associated with a potentially compromised treatment outcome. Rates of viral clearance are significantly reduced in patients who cannot be maintained on at least 80% of their PEG-IFN/RBV dosage for the duration of treatment (10). Prediction of risk for such complications would be clinically useful for selecting patients for therapy, as well as planning the monitoring frequency. Since bone marrow suppression remains prevalent in patients with early-stage fibrosis, a genetic biomarker for predicting risk of treatment for bone marrow suppression would be particularly useful at the start of therapy.

RBV-induced anemia is a serious side effect of therapy which leads to dose reduction of RBV, but the mechanism underlying RBV-induced anemia remains to be determined. De Franceschi *et al* previously reported that membrane oxidative damage as well as impairment of erythrocyte Na/K pump activity are responsible for RBV-induced hemolytic anemia (11). Studies show that single nucleotide polymorphisms (SNPs) in the inosine triphosphate pyrophosphatase (*ITPA*) locus are associated with anemia in patients treated with combination therapy (12-14). Two SNPs that are associated with *ITPA* enzyme activity were identified in the Caucasian population (12), but the Japanese population lacks one of these SNPs (14,15). A limited number of reports have demonstrated the association of *ITPA* genotypes with RBV-induced anemia, but further studies concerning the role of *ITPA* genotypes on RBV-induced anemia are required. Furthermore, the effect of the SNPs on the therapy outcome should be investigated.

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Key words: hepatitis C virus, ribavirin, hemolytic anemia

In the present study, we investigated the association of ITPA genotypes with hemoglobin reduction and therapeutic outcome in HCV patients treated with PEG-IFN/RBV therapy.

Patients and methods

Study populations. A total of 176 patients with chronic HCV infection (HCV serotype 1, $n=126$ and serotype 2, $n=50$), who were treated at the Hospital of the Shiga University of Medical Science, the Notogawa Hospital and the Social Insurance Shiga Hospital were included in the study. Among them, 52 HCV1 patients were treated with PEG-IFN/RBV therapy. Table I lists the demographic features of the patients receiving PEG-IFN/RBV therapy. The patients received weekly injections of PEG-IFN α 2b at 1.5 μ g/kg body weight or PEG-IFN α 2a at 180 or 90 μ g, and the oral administration of RBV for 24-72 weeks. The amount of RBV was adjusted based on the subject's body weight (600 mg for <60 kg, 800 mg for 60-80 kg, 1,000 mg for >80 kg). Only patients with >75% compliance with the prescribed doses of PEG-IFN/RBV were included in this study. Informed consent was obtained from all patients. The ethics committee of each participating medical center approved this study.

Serotyping. Serotyping was performed using an enzyme immunoassay-based Murex assay (Murex Diagnostics Inc., Norcross, GA, USA).

Genotyping. The samples were genotyped for *ITPase* C94A (rs1127354) using the TaqMan[®] SNP Assays (Applied Biosystems Inc., Foster City, CA, USA) as previously described (16). Homozygosity (AA) or heterozygosity (CA) of the minor sequence was defined as having the ITPA minor allele, whereas homozygosity for the major sequence (CC) was defined as having the ITPA major allele. The HCV RNA levels were analyzed using the TaqMan RT-PCR test. The measurement ranges of these assays were 1.2-7.8 log IU/ml.

Statistical analysis. Hardy-Weinberg equilibrium (HWE) analysis was performed in these subjects by comparing the detected distribution of allele frequencies to the theoretical distribution estimated from the SNP allelic frequencies. $P>0.05$ (Chi-square test) was considered to indicate equilibrium. The categorical variables were presented as frequencies and percentages when required. The continuous variables were reported as the means \pm SD (range). Multivariate logistic regression analysis with stepwise forward selection was performed with a criterion of $P<0.05$ for the inclusion or removal of variables. All statistical analysis used the Ekuseru-toukei 2008 (Social Survey Research Information Co., Ltd., Tokyo, Japan) software. A P -value <0.05 was considered to denote statistical significance.

Results

The genotype frequencies of the ITPA polymorphisms in patients with HCV ($n=176$) are shown in Table II. The frequency of the ITPA major allele, defined as homozygosity for the major sequence (CC), in HCV serotype 1 patients (76.2%) was similar to that of serotype 2 (76.5%). In total, the

Table I. Patient baseline characteristics.

No.	52
Gender (male/female)	32/20
Age (years)	62.0 \pm 10.5 (38-76)
Weight (kg)	62.0 \pm 11.1 (40-90.7)
Body mass index (kg/m ²)	22.3 \pm 2.8 (17.0-30.1)
Baseline hemoglobin level (g/dl)	13.7 \pm 1.4 (10.8-17.9)
Baseline platelet level (10 ⁶ / μ l)	15.5 \pm 5.2 (3.9-29.9)
Initial ribavirin dose (mg/day), n (%)	
200	1 (1.9%)
400	4 (7.7%)
600	23 (44.2%)
800	22 (42.3%)
1,000	2 (3.8%)
Hemoglobin level at week 4 (g/dl)	12.1 \pm 1.4 (8.3-14.1)
Hemoglobin decline at week 4 (g/dl)	2.2 \pm 1.3 (0.7-5.2)
Data unless otherwise specified are presented as the means \pm SD (range).	

Table II. Genotype distribution of ITPA SNP rs1127354.

	rs1127354				
	CC (%)	CA (%)	AA (%)	C (%)	A (%)
HCV serotype 1 (n)	96 (76.2)	27 (21.4)	3 (2.4)	- (86.9)	- (13.1)
HCV serotype 2 (n)	38 (76.5)	10 (19.6)	2 (3.9)	- (86.3)	- (13.7)
Total (n)	134 (76.3)	37 (20.9)	5 (2.8)	- (86.7)	- (13.3)

frequency of the rs1127354 major allele (CC) was 76.3% and the frequency of the minor allele (CA and AA) was 23.7%. These data are compatible with a recent report of the Japanese population (5).

A total of 52 patients with HCV1 infection were treated with PEG-IFN/RBV therapy. These patients were stratified according to their ITPA allele type, and the decrease in hemoglobin (Hb) levels was analyzed. As shown in Fig. 1, a rapid decrease in Hb levels during the initial 4 weeks was observed in patients with the ITPA major allele (CC), but such a phenomenon was not observed in patients with the ITPA minor allele (C/A and AA). Hb levels at 4 weeks were significantly lower in patients with the ITPA major allele than levels in patients with the minor allele. The rate of clinically significant anemia, defined as either a decline in Hb levels of >3 g/dl or Hb <10 g/dl, was analyzed. This criteria is the threshold at which RBV dose reduction is recommended. As shown in Fig. 2, none of the patients with the ITPA minor allele had Hb levels <10 g/dl and a decline in Hb of >3 g/dl at 4 weeks. On the other hand,

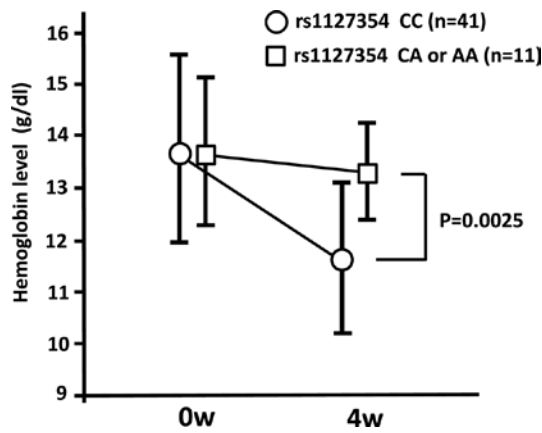


Figure 1. Association between ITPA genotypes and changes in Hb levels. A total of 52 patients with HCV1 infection were treated with PEG-IFN/RBV therapy. These patients were stratified according to their ITPA genotypes, and decrease in Hb levels was analyzed at 4 weeks after the start of therapy. Data are expressed as the means \pm SD.

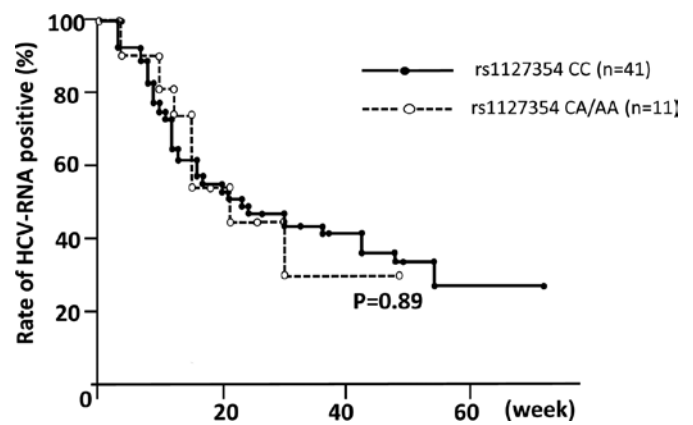


Figure 3. Comparison of the effects of ITPA genotypes on HCV positivity. Data were analyzed using the Kaplan-Meier method. There were no significant differences between the effects of the ITPA genotypes.

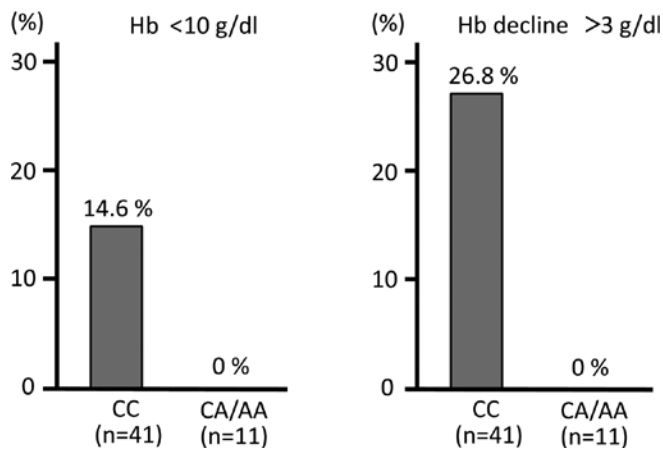


Figure 2. Association between ITPA genotypes and the rate of clinically significant anemia at 4 weeks (a decline of >3 g/dl or <10 g/dl in Hb levels).

14.6% (6 of 41) of patients with the ITPA major allele had Hb levels <10 g/dl and 26.8% (11 of 41) had a decline in Hb of >3 g/dl.

The association of the ITPA genotypes with virological responses was analyzed by the Kaplan-Meier method. As shown in Fig. 3, there were no significant differences in the negative rate of HCV-RNA between patients with the ITPA major allele and those with ITPA minor allele.

Discussion

PEG-IFN/RBV is the standard of care therapy for chronic HCV infection (5-7), but this therapy is associated with a range of treatment-limiting adverse effects. One of the most serious adverse effects is RBV-induced hemolytic anemia (12,14), which affects most patients and is severe enough to require dose modification in approximately 15% of patients. Recently, a genome-wide association study of determinants of treatment-related anemia in individuals with chronic HCV genotype 1 infection identified that genetic variants leading to ITPA deficiency protect against hemolytic anemia in HCV-infected patients receiving RBV (12). In the Caucasian population,

there are two SNPs (rs7270101 and rs1127354) that are associated with ITPA enzyme activity (12,13), but one of these SNPs (rs7270101) is absent in Japanese patients (14). In this study, we confirmed the absence of rs7270101; the frequency of the rs1127354 major allele (CC) was 76.3% and that of the minor allele (CA and AA) was 23.7%. These results are compatible with recent reports on the Japanese population (5,14).

We investigated an association between ITPA genotypes and incidence of RBV-induced anemia. As reported by recent studies (5,12-14), Hb decrease is faster and more severe during the first 4 weeks of treatment in patients with the ITPA major allele (CC). The rapid decrease of Hb observed in major allele CC patients was associated with early reduction of RBV dosage, resulting in a lower total RBV administration. Hb decrease was slightly milder in this study as compared to the results of Thompson *et al* (13), and this may be due to the early reduction of the RBV dose in Japanese patients resulting from lower pre-treatment Hb levels. Initial Hb levels indeed had a strong influence on reduction of the RBV dose. Since reduction of RBV to $<80\%$ results in a decreased rate of SVR (10), patients with pre-treatment Hb levels below 13.5 g/dl or between 13.5 and 15 g/dl who have the ITPA major allele should receive treatment with drugs such as erythropoietin to prevent reduction of RBV. Thus, we confirmed the clinical relevance of this genetic discovery. Patients who have the ITPA major allele (CC type) have a greater likelihood of rapid progress of RBV-induced hemolytic anemia and should be monitored extensively. By contrast, patients with the minor allele (CA or AA type) are unlikely to present a rapid decrease in Hb levels. However, since the gradual and continuous decrease of Hb has been reported in ITPA minor allele patients (5), a reduction in RBV dosage, but not as drastically as in patients with the major allele, should be considered.

We did not observe any significant association between the ITPA genotypes and early or late anti-HCV treatment outcomes. Similar observations have been reported by several authors (5,12,14). This may reflect decreased treatment efficacy due to dose reduction of RBV in patients showing severe anemia.

The mechanism of RBV-induced hemolytic anemia remains to be elucidated (17), but a role of RBV accumulation in red blood cells is suspected. Accumulation of RBV induces membrane oxidative damage as well as impairment of

erythrocyte Na/K pump activity and leads to hemolysis (11). It has been reported that ITPA deficiency leads to an accumulation of inosine triphosphate in red blood cells (18). Fellay *et al* (12) hypothesized that ITPA competes with RBV and thereby protects cells from the lytic effects of RBV. An alternative explanation is that ITPA activity and/or ITPA levels directly or indirectly influence RBV pharmacokinetics.

In conclusion, the present study may provide a rationale for developing a therapeutic approach for targeting ITPA genotypes in PEG-IFN/RBV therapy of chronic hepatitis C patients. The ITPA genotypes would be a useful marker for prediction of RBV-induced anemia. Moreover, genetic testing of ITPA genotypes may be applied to establish personalized dosages in PEG-IFN/RBV therapy.

Acknowledgements

This study was partly supported by the MSD Co., Ltd., Tokyo, Japan.

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