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Induction of inosine triphosphatase activity during ribavirin treatment for chronic hepatitis C

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ABSTRACT

Background: Ribavirin (RBV) is an antiviral agent and the primary component for chronic hepatitis C (CHC) therapy. Hemolytic anemia is limitation for RBV treatment. Inosine triphosphatase (ITPA) activity has been associated with severity of RBV-induced anemia. However, changes in ITPA activity during CHC therapy are unknown. The aim of this study was to measure the time-dependent change in ITPA activity over the RBV treatment.

Methods: Forty-two patients with CHC were evaluated for ITPA activity over the course of RBV treatment.

Results: The median value of ITPA activity at start of RBV treatment was 134.2 μmol/h/g hemoglobin (Hb; range, 26.3–251.0 μmol/h/g Hb). The ITPA activity values at 4, 8, and 12 weeks during RBV treatment were 143.2, 202.2, and 225.7 μmol/h/g Hb, respectively, and these ITPA values were significantly elevated compared with the start of treatment ($p < 0.001$). In patients with ITPA variants, patients with anemia (Hb < 10 g/dL) had greater elevated ITPA activities compared with patients without anemia at 12 weeks.

Conclusion: Our findings indicate that ITPA activities are elevated with RBV therapy, and this elevation may be a risk of anemia in late therapeutic phase in patients that began with low ITPA activity.

1. Introduction

Ribavirin (RBV) is an anti-viral agents, and still main component for chronic hepatitis C (CHC) therapy [1,2]. RBV is phosphorylated to RBV mono-, di-, and triphosphate, which is responsible for its antiviral efficacy. However, RBV-induced severe hemolysis is a limitation of treatment due to the accumulation of RBV and RBV phosphate in erythrocytes during therapy. *ITPA* genetic variation has been reported as one of the factors underlying RBV-induced anemia [3].

ITPA encodes inosine triphosphatase (ITPA), which dephosphorylates nucleotides, including the deoxy and ribose forms of inosine, xanthine, and other nucleotides. The *ITPA* variants c.94C > A and IVS2 + 21A > C are known as ITPA depletion variants. In the c.94C > A variant, the ITPA activity for c.94C > A heterozygous and homozygous has been found to be 22.5% and 0% for controls, while the IVS2 + 21A > C heterozygous variant had 60% activity for controls [4]. Fellay et al. predicted ITPA enzyme activity using a combination of *ITPA* c.94C > A and IVS2 + 21A > C, and reported that the frequency of a hemoglobin (Hb) decline > 3 g/dL was associated with ITPA enzyme activity and no patients had severe anemia that were ITPA deficient [5].

In vitro, RBV-induced anemia is believed to result from a depletion of endogenous purine. The mechanism of RBV-induced anemia has been reported to involve the reduction of ATP synthesis and the depletion of cellular guanosine triphosphate (GTP) levels by RBV treatment. GTP is an energy source in the synthesis of adenylsuccinate catalyzed by adenylosuccinate synthase, and GTP depletion reduces ATP synthesis. Hitomi et al. reported that inosine triphosphate (ITP) replaced GTP in ATP synthesis. ITP is dephosphorylated to inosine monophosphate (IMP) by ITPA [6].

Although ITPA deficiency and predicted genetic variants are protective factors for the severity of RBV-induced anemia, some patients have decreased Hb levels similar to patients with normal ITPA activity by 12 weeks of treatment. To date, these patients' characteristics were unknown. The aim of this study was to measure the time-dependent change in ITPA enzyme activity at the start of therapy and during and after RBV therapy in patients with CHC. Moreover, we evaluated the association between the dynamics of ITPA activity and RBV-induced Hb depletion.

2. Materials and methods

Patients receiving therapy with RBV, including treatments involving

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RBV/Peg-interferon/simeprevir (PR + SMV), RBV/Peg-interferon/teraprevir (PR + TVR), RBV/sofosbuvir (R + SOF), were recruited into this study. The standard treatments for PR + SMV and PR + TVR were PR and a protease inhibitor for the first 12 weeks and then PR for 12 weeks. The standard treatment for R + SOF was a combination of these drugs for 12 weeks. In this study, severe anemia was defined as Hb levels < 10 g/dL after the start of therapy. A total of 42 patients with CHC were enrolled at Kitasato University Medical Center in Japan. The protocol was approved by the Ethics Committee of Kitasato University Medical Center (approval no. 29–8). The study was conducted after approval had been obtained from the institutional ethics committees.

Blood samples were obtained in heparinized tubes; erythrocytes were then separated from plasma and leucocytes by centrifugation and washed three times with saline. Erythrocytes were stored at −20 °C until measurement of ITPA activity. ITPA activity was measured in erythrocyte lysates using a high-performance liquid chromatography procedure based on the conversion of ITP to IMP, and the ITPA activity was normalized by hemoglobin levels of isolated erythrocytes as previously described [7]. In brief, ITPA activity values were defined as the IMP level converted from the ITP by ITPA enzyme reaction. The enzymatic reaction was stopped using perchloric acid and saturated dipotassium hydrogen phosphate. IMP was quantified by measuring the absorbance at 262 nm, after separation on a C18 column using 20 mmol/L phosphate buffer (pH 2.5) as the mobile phase. Within-day and between-day imprecision values of < 3% and < 7%, respectively, were allowed, and an inaccuracy value of < 1.2% was verified using pooled erythrocytes.

Genotyping was performed before therapy. DNA was extracted from buffy coat samples using the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Total genomic DNA was quantified using a Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). Real-time TaqMan allelic discrimination polymerase chain reaction (StepOne; Applied Biosystems, Foster city, CA, USA) was used for *ITPA*c.94C > A (rs1127354) and IVS2 + 21A > C (rs7270101) genotyping. Primers and probes were obtained from Applied Biosystems (Foster city, CA, USA).

The normal probability distribution was tested using the Shapiro–Wilk test. Statistical differences among patient characteristics were evaluated using Fisher's exact test and the Mann–Whitney *U* test. The ITPA activity change was evaluated using the Wilcoxon signed-rank test. ITPA activity during RBV treatment was evaluated using two-way analysis of variance (ANOVA). Statistical correlation between ITPA activity and declining Hb levels was evaluated using Pearson's correlation coefficient. A two-sided p value of < 0.05 (two side) was considered statistically significant. Statistical analysis was conducted using the SPSS software version 19 (IBM, Armonk, NY, USA) and R statistical software (version 3.4.1; http://www.r-project.org/).

3. Results

3.1. ITPA activity at the start of RBV treatment

In this study population, the number of patients treated by PR + SMV, PR + TVR, and R + SOF was 14, 13, and 15, respectively. These patients' characteristics are shown in Table 1. The median ITPA activity value at the start of RBV treatment was 134.2 μmol/h/g Hb (range, 26.3–251.0 μmol/h/g Hb). The association between enzyme activity and ITPA genotype is shown in Table 1. There were 10 patients that were c.94C > A heterozygous and no one with IVS2 + 21A > C. In patients at the start of RBV treatment, the median ITPA activity in the variants was significantly lower than wild types (148 vs. 43 μmol/h/g Hb, $p < 0.001$).

3.2. ITPA activity change depends on RBV treatment

In this study population, the elevated value of ITPA activity was observed in 41 of 42 patients (97.6%) at 12 weeks from the start of therapy. The ITPA activity value for 24 of 42 patients (57%) was up to 1.50-fold greater compared with the start of RBV treatment. The values of ITPA activity at 4, 8, and 12 weeks during RBV treatment are shown in Fig. 1. At 4, 8, and 12 weeks after the start of therapy, ITPA activity was significantly elevated compared with the values at the start of RBC treatment ($p < 0.001$). The change ratios in the ITPA activity value at 4, 8, and 12 weeks compared with the start of treatment were 1.21-fold, 1.50-fold, and 1.69-fold, respectively. For patients treated with PR + SMV and PR + TVR, they were treated with RBV and interferon from 13 to 24 weeks of treatment. The ITPA activity for these patients after 20 weeks was 2.12-fold higher compared with the start of RBV treatment. The value of ITPA activity at 120 days after the end of RBV treatment was back to a comparable degree to the start of RBV treatment.

3.3. Influence of ITPA genotype and combination drugs on ITPA activity

The value of ITPA activity from 4- to 12-week was significantly elevated compared with the start of therapy in patients with *ITPA* wild type ($p < 0.005$), and the value from 8 to 12 weeks was significantly elevated in patients with variants ($p < 0.05$). The values of ITPA activity for the variants were significantly lower than wild type during whole RBV treatment (Fig. 1, $p < 0.001$), but the change ratio of ITPA activity during RBV treatment (4 week, 1.14-fold; 8 week, 1.51-fold; 12 week, 1.66-fold) compared with the start of RBV treatment did not differ between wild type and the variants (4 week, 1.33-fold; 8 week, 1.18-fold; 12 week, 1.72-fold, $p < 0.05$). The ITPA activity values at 12 weeks for 5 of 10 patients with the *ITPA* c.94C > A variant exceeded the 10 percentiles of ITPA activity values at the start of therapy for *ITPA* wild type.

The influence of combination direct-acting agents (DAAs) for ITPA activity values and change ratios compared with the start of RBV treatment did not differ during treatment.

3.4. The association between ITPA activity and the severity of Hb decline

The decreased Hb levels in SOF were less than triple therapy. The patients treated with R + SOF and RBV did not demonstrate anemia (Hb < 10 g/dL). At 4 weeks, the values of ITPA activity during RBV treatment were correlated with Hb decline level (Fig. 2, $r = 0.41$, $p = 0.027$). However, at 8 and 12 weeks, the values of ITPA activity were not associated with Hb decline level. In PR + SMV and PR + TVR, patients with Hb levels < 10 g/dL had significantly increasing ITPA activity ratios compared with patients with Hb levels > 10 g/dL.

In patients with the *ITPA* c.94C > A variant, 3 of the 10 patients suffered from anemia. The ITPA activity values and change ratios at 12 weeks tended to increase compared with patients without anemia by 12 weeks (Table 2, 123.7 vs. 79.5 μmol/h/g Hb and 3.14-fold vs. 1.68-fold, respectively) although the ITPA activity value was not different at the start of therapy between groups.

4. Discussion

ITPA activity in patients with CHC was monitored from the start of RBV treatment and after the completion of therapy. We first found that ITPA activity was significantly elevated during RBV treatment, and ITPA activity induced by RBV therapy returned to values before RBV therapy. In patients with the *ITPA* c.94C > A heterozygous variant with anemia, the ITPA activity value was elevated to a high level after treatment compared with patients without anemia. However, we did not find a correlation between the values of ITPA activity and Hb levels at 4, 8, and 12 weeks from the start of therapy.