SLC29A1 polymorphism and prediction of anaemia severity in patients with chronic hepatitis C receiving triple therapy with telaprevir

Laura Milazzo1*, Anna Maria Peri1, Cristina Mazzali2, Carlo Magni3, Elisa Calvi1, Amedeo De Nicolò4, Emilio Clementi5, Stefania Cheli5, Antonio D’Avolio4, Spinello Antinori1 and Felicia Stefania Falvella5

1Department of Biomedical and Clinical Sciences L. Sacco, University of Milan, Milan, Italy; 2Department of Clinical Sciences, Section of Biostatistics, University of Milan, Milan, Italy; 3I Division of Infectious Diseases, Luigi Sacco Hospital, Milan, Italy; 4Unit of Infectious Diseases, University of Turin, Amedeo di Savoia Hospital, Turin, Italy; 5Unit of Clinical Pharmacology, Luigi Sacco University Hospital, Milan, Italy

*Corresponding author. Tel: +390239043350; Fax: +390250319758; E-mail: laura.milazzo@unimi.it

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Objectives: The equilibrative nucleoside transporter 1 (ENT1) is the main protein involved in ribavirin cellular uptake. Polymorphisms at the SLC29A1 gene, encoding ENT1, may influence ribavirin-associated anaemia, which is observed at a higher incidence with telaprevir in combination with pegylated-IFNα and ribavirin than with pegylated-IFNα and ribavirin alone. In this study, we investigated the role of the rs760370 SLC29A1 variant in ribavirin-induced anaemia in chronic hepatitis C patients treated with telaprevir-based triple therapy.

Methods: Forty patients infected with hepatitis C virus (HCV) genotype 1 and starting anti-HCV therapy with telaprevir in combination with pegylated-IFNα/ribavirin were prospectively evaluated for SNPs at the SLC29A1 gene and inosine triphosphatase (ITPA) genes using a real-time PCR system.

Results: 40% of patients developed severe anaemia with a haemoglobin (Hb) decline ≥5 g/dL from the pretreatment value. The SLC29A1 rs760370 GG genotype was associated with the severity of Hb decrease as expressed by the median (IQR) Hb nadir change from baseline [−5.4 (−5.6; −5.0) g/dL in GG versus −4.2 (−5.1; −3.4) in AA/AG genotype; P = 0.05] and by the Hb decrease ≥5 g/dL by week 12 (77.8% of GG carriers versus 24% of AA/AG; P < 0.01). In multivariate analysis, older age (P = 0.03), lower baseline Hb concentration (P = 0.02) and SLC29A1 rs760370 GG (P = 0.02) were associated with the development of severe anaemia during treatment, whereas no association was found with ITPA SNPs in our population receiving telaprevir-based therapy.

Conclusions: In patients with chronic hepatitis C receiving telaprevir-based therapy, SNP rs760370A>G at the SLC29A1 gene influences the severity of ribavirin-induced anaemia, possibly mirroring the erythrocyte uptake of ribavirin.

Keywords: HCV, ribavirin, nucleoside transporters, single nucleotide polymorphisms

Introduction

Anaemia is one of the major adverse events in patients treated with pegylated-IFNα+ribavirin and it is mainly due to the haemolytic effect of ribavirin. The addition of a PI, boceprevir or telaprevir, has been shown to increase the frequency and/or severity of anaemia.1–3 Indeed, in patients treated with pegylated-IFNα+ribavirin plus telaprevir, haemoglobin (Hb) concentrations <10 and 8 g/dL were achieved in 34% and 8%, respectively, compared with 14% and 2%, respectively, of those treated with dual therapy.1–5

Among individual factors associated with anaemia, including age, BMI, renal function and advanced fibrosis, genetic variants have been widely studied. SNPs mapping in the inosine triphosphatase gene (ITPA) have been significantly associated with ribavirin-induced anaemia;6,7 in particular, a missense variant in exon 2 (rs1127354 C>A, P32T) and an intron splicing-altering polymorphism (rs7270101 A>C) in the ITPA gene were shown to reduce the inosine triphosphate pyrophosphorylase (ITPase) activity.8 As the accumulation of ITP in erythrocytes enhances the biosynthesis of ATP, by substituting for erythrocyte GTP, which is depleted by ribavirin, individuals with ITPase deficiency are partly protected from ribavirin-induced haemolytic anaemia.9–11 Recent studies confirmed that ITPA variants influence the Hb concentrations during triple therapy.12–14 Indeed, individuals carrying the WT rs1127354 anaemia-susceptible genotype for ITPA (CC) required ribavirin dose reductions significantly earlier than patients with other genotypes.13
In addition, ribavirin accumulation in erythrocytes was associated with Hb reduction in IFN and ribavirin combination therapy, as a result of active unidirectional transmembrane transport. The main protein involved in ribavirin cellular uptake is the equilibrative nucleoside transporter 1 (ENT1), encoded by the solute carrier family 29, member 1 (SLC29A1), which has been associated with ribavirin uptake into erythrocytes and haematological toxicity. Polymorphisms of the SLC29A1 gene are associated with SLC29A1 mRNA concentrations; in particular, AA genotype (WT) at rs6932345 polymorphism, highly linked with rs760370, is associated with a 1.7-fold increase in SLC29A1 mRNA concentrations. Furthermore, the GG variant at rs760370, compared with WT genotype (AA), has been shown to be positively associated with rapid virological response (RVR) to pegylated-IFNα + ribavirin therapy (50% versus 17%, in GG carriers and AA/AG carriers, respectively), as a result of the increased ribavirin uptake into hepatocytes.

In this study, we investigated the role of rs760370 SLC29A1 variant in ribavirin-induced anaemia, in chronic hepatitis C patients treated with telaprevir-based triple therapy.

Methods

Patients

All patients infected with hepatitis C virus (HCV) genotype 1 and starting anti-HCV therapy with telaprevir in combination with pegylated-IFN/ribavirin at the I and III Division of Infectious Diseases at Luigi Sacco Hospital, Milan, between January 2012 and December 2013, were enrolled in this prospective study. Patients with other forms of liver disease, decompensation of liver disease, decompensated liver disease, hepatocellular carcinoma or serum creatinine >1.15 mg/dL were excluded from the study.

Treatment consisted of pegylated-IFNα-2a at standard dose (180 μg/week) plus weight-adjusted ribavirin (1000 mg/day for patients weighing <75 kg and 1200 mg/day for patients weighing ≥75 kg). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Institutional Ethic Committee. Informed consent was obtained from all patients before enrolment. Hb levels at baseline and weeks 4, 8 and 12 were registered for a decrease in Hb concentration to 600 mg or Hb stabilization) for a decrease in Hb concentration to 5 g/dL from the pretreatment value.

Genotyping

Genomic DNA was extracted from whole blood samples using Maxwell 16 (Promega). Genotyping was carried out with the LightSNp typing assay (TIB-MolBiol, Berlin, Germany) by analysing the melting curves with the LightCyler® 480 Real-Time PCR System (Roche Applied Science, Mannheim, Germany) according to the manufacturer’s instructions. We evaluated the functional variants rs7270101A>C and rs1127354A>G mapping in the ITPA gene and rs760370A>G mapping in the SLC29A1 gene.

Ribavirin plasma concentration

Ribavirin plasma concentrations were measured in a subgroup of patients at the end of the dosing interval (Cmax) at week 2 of therapy, using a previously described chromatographic method.

Statistical analysis

Continuous variables are described as median (IQR), whereas categorical variables are described as frequency and percentage. Continuous variables were compared using the non-parametric Mann–Whitney–Wilcoxon test and categorical data were compared using a χ² test or a Fisher exact test. The association between individual SLC29A1 SNPs and the incidence of significant Hb decline was tested by a basic allelic test and calculated using the Fisher exact test. Comparison of anaemia-free survival times between groups defined by SLC29A1 SNPs was performed by Kaplan–Meier curves and with a two-sided log-rank test. Values <0.05 were considered significant. Univariate and multivariate logistic regression analysis was performed to identify significant predictors for significant Hb decline. All variables with a significance level <0.1 at univariate analysis were included in the final model; the Firth penalized estimation was performed to reduce bias in the parameter estimates. Logistic regression analysis was also applied to identify predictors of RVR.

Results

The baseline characteristics of the 40 patients studied are summarized in Table 1. Most of the patients carried HCV subtype 1a (52.5%) and had advanced fibrosis defined by transient elastography (F3–F4 Metavir score, 90%). Sixty per cent were coinfected with HIV, and showed a median CD4+ T cell count of 724 cells/μL; HIV-RNA was <37 copies/mL in all the coinfected patients. Antiretroviral therapy included etravirine/rilpivirine in 6/24 (25%), ritonavir-boosted lopinavir/protease inhibitor in 6/24 (25%), darunavir/ritonavir in 12/24 (50%). Moreover, 13/24 (54%) were on treatment with tenofovir. Overall, 13/40 (32.5%) were naive to anti-HCV therapy, 6/40 (15%) were relapsers and 21/40 (52.5%) were non-responders to a previous treatment. HIV-positive subjects were younger (P = 0.01), whereas male sex (20 versus 11; P = 0.28), baseline concentrations of Hb (median g/dL: 14.9 versus 14.85; P = 0.86) and creatinine (0.81 versus 0.8; P = 0.8) were similar between HIV-infected and uninfected patients. A significant Hb reduction (≥3 g/dL) by week 12 of treatment was observed in 87.5% of patients; in 17 patients (42.5%) Hb concentrations dropped below 10 g/dL and 40% showed an Hb decline ≥5 g/dL from the pretreatment value.

There were no significant differences in sex (male 89% and 74%, P = 0.35), age (median, 51 and 52 years, P = 0.86), baseline Hb concentration (median, 14.7 and 15 g/dL; P = 1.0), creatinine concentration (median, 0.82 and 0.8 mg/dL; P = 0.9) and eGFR (median, 101 and 115 mL/min; P = 0.56) between SLC29A1 rs760370 genotypes (GG versus AA/AG). Also the percentage of HIV-infected subjects was similar in the two genotype groups (55.5% versus 61.3% in GG and AA/AG, respectively; P = 0.75).

Thirty-eight patients completed the 12 weeks of pegylated-IFNα + ribavirin + telaprevir treatment, one patient discontinued treatment at week 4 having reached the virological stopping rule and in one case treatment was interrupted at week 8 because of the patient’s own decision. In 29.4% of the patients who showed an Hb decline below 10 g/dL, this was reached at week 4 of treatment, in 41.1% it was at week 8 and in 29.4% it was at week 12. The onset of severe anaemia (defined by Hb decline ≥5 g/dL and/or erythropoietin use or blood transfusion) was most frequently seen from weeks 8 to 12. When median decline in Hb values was stratified by ITPA deficiency (CC/AA versus CA for rs1127354 and rs7270101), a trend...
for a higher decline was seen only at week 4 in the ITPA CC/AA genotypes (week 4 Hb decline: 3.0 g/dL versus 2.2 g/dL; P = 0.06), whereas no statistical significant difference emerged by the comparison at weeks 8 and 12 (P = 0.087 and 0.3, respectively), also considering the Hb decrease ratio from baseline to week 12 and the Hb nadir change from baseline (data not shown).

Hb crude values from baseline to week 12 were not significantly different in patients with SLC29A1 rs760370 GG as compared with AA/AG genotypes (from a median g/dL (IQR) of 14.7 (14.3–15.7) at baseline to 9.3 (8.9–10.6) at week 12 and from 15 (14.2–15.3) at baseline to 11 (9.8–11.8) at week 12, in GG and AA/AG, respectively; P = 0.08); moreover, although the Hb decrease ratio showed a higher decrease in Hb at week 12 in the SLC29A1 rs760370 GG versus 7/29, 24.1% of AA/AG; P = 0.01; Figure 2a).

A comparison of severe anaemia-free survival among SLC29A1 rs760370 genotypes is reported in Figure 2b. Patients with GG SNP developed severe anaemia more frequently and at an earlier timepoint (log-rank, P = 0.01).

At univariate analysis older age (P = 0.06), lower baseline Hb concentration (P = 0.07) and SLC29A1 rs760370 GG (P = 0.01) were associated with the development of severe anaemia during treatment, while no association was found for HIV infection and eGFR with severe anaemia. In multivariable logistic regression analysis these three variables remained independently associated with the occurrence of severe anaemia: age (P = 0.03), baseline Hb value (P = 0.02) and GG genotype (P = 0.02) (Table 2). No association was found with ITPA SNPs in our population receiving telaprevir-based therapy. In a subgroup of 15 patients we evaluated plasma ribavirin concentration at week 2 of treatment. Although not statistically significant, patients carrying GG SNP (n = 4) showed a trend towards higher plasma ribavirin concentrations than AA/AG genotypes (n = 10; median ng/mL, 2117 versus 1597, respectively; P = 0.059).

Finally, when logistic regression analysis was performed to identify main predictors of the RVR to pegylated-IFNα + ribavirin + telaprevir therapy (obtained in 22/40 patients), the only variable found significantly associated with RVR was the baseline ALT value (OR 1.014, 95% CI 1.0 – 1.026; P = 0.05), whereas no association was seen between age, gender, HCV-RNA concentrations, HCV subtype (1a versus 1b) and the rs760370A>G polymorphism.

**Discussion**

One of the most common adverse effects associated with telaprevir is haematological alterations (anaemia, thrombocytopenia and leucopenia), and moderate to severe anaemia has been shown to occur more frequently in telaprevir-based triple therapy than in pegylated-IFNα + ribavirin alone. As previously reported, in our cohort severe anaemia was most frequently seen from weeks 8 to 12, possibly reflecting the increasing ribavirin plasma concentrations observed after 4 weeks of treatment with telaprevir. SNPs in the ITPA gene were identified as predictors of ribavirin treatment-associated anaemia.

Among them the most widely studied are ITPA rs1127354 C>A, ITPA rs7270101 A>C and, more recently, rs6051702 A>C, which play a protective role against severe anaemia in pegylated-IFNα + ribavirin alone. Few studies have evaluated the predictive role of ITPA polymorphisms in the development of anaemia in pegylated-IFNα + ribavirin + telaprevir-treated patients. In agreement with Aghemo et al., we did not find ITPA deficiency to play a protective role against pegylated-IFNα + ribavirin + telaprevir severe anaemia, apart from a close to significance impact at week 4, although the small number of patients studied does not allow a conclusive statement to be drawn. Moreover, since in most studies the association between ITPA genotype and ribavirin-related anaemia was evaluated only at week 4, we cannot exclude the ribavirin dose reduction adopted in a large proportion of patients after week 4 having possibly reduced the association of ITPA genotype with the occurrence of anaemia.

More recently, a rs760370 polymorphism at the SLC29A1 gene, encoding ENT1, the primary transporter involved in ribavirin
uptake into hepatocytes, has been associated with the rate of virological response to pegylated-IFNα + ribavirin therapy; the GG genotype (compared with AA/AG) was significantly and independently associated with higher RVR to pegylated-IFNα + ribavirin in HIV/HCV-coinfected patients.19 The authors of the study hypothesized that differences in the expression of this transporter might influence the intrahepatocyte concentration of ribavirin and hence its anti-HCV activity. In addition, a correlation between ribavirin plasma and erythrocyte concentrations and the achievement of both RVR and sustained virological response was previously reported,29,30 highlighting the essential role of ribavirin exposure in the clearance of HCV with dual therapy. The lack of an association between the rs760370 GG genotype and RVR in our patients might be explained by the higher antiviral potency of triple therapy with telaprevir.

The SLC29A1 genotypes at rs760370 are in Hardy–Weinberg equilibrium and their frequency is similar to that reported by Morello et al.19 (35% AA; 42.5% AG and 22.5% GG). We observed an association between polymorphisms at the SLC29A1 gene and the risk of ribavirin-associated severe anaemia. Older age, 

![Figure 1](https://academic.oup.com/jac/article-abstract/70/4/1155/806603)

Figure 1. Mean Hb decrease ratio and Hb nadir change from baseline stratified by SLC29A1 SNPs. (a) A higher Hb decrease ratio from baseline to week 12 was seen in patients with rs760370 GG than in those with rs760370 AA/AG. (b) The change in Hb concentration from baseline to nadir was higher in those with rs760370 GG than in AA/AG carriers. W12, week 12.

![Figure 2](https://academic.oup.com/jac/article-abstract/70/4/1155/806603)

Figure 2. (a) Percentage of patients experiencing severe anaemia (Hb decrease ≥5 g/dL) by week 12 of treatment according to the SLC29A1 SNPs. (b) Severe anaemia-free survival of patients with rs760370 GG versus AA/AG. Patients with the GG SNP developed severe anaemia more frequently and at an earlier timepoint (log-rank, P = 0.01).
baseline Hb concentrations and SLC29A1 rs760370 GG genotype were independently associated with severe anaemia in our population, and GG SNP carriers were shown to develop severe anaemia earlier and at a higher rate. This result differs from that recently described by Tsubota et al.,\(^3\) who did not find any significant impact of SLC29A1 SNPs on pegylated-IFN\(\alpha\)+ribavirin treatment-induced anaemia. A possible explanation might arise from the different definition of severe anaemia adopted and the different timepoints considered. Hence, the higher rate of anaemia in our cohort was observed from week 8 to week 12 of treatment. In agreement with Tsubota et al.,\(^3\) no association emerged when Hb decrease was analysed at week 4, suggesting an accumulation effect of ribavirin on the development of haematological toxicity. To obtain a higher homogeneity of the studied population, we chose to define severe anaemia as a decrease in Hb \(\geq 5\) g/dL and/or the use of erythropoietin or blood transfusion rather than considering a decrease below a defined threshold, the baseline concentrations of Hb varying among patients. Since SLC29A1 encodes ENT1, the primary transporter of ribavirin into erythrocytes, it is likely that an increase in the ENT1 transporter on erythrocytes may lead to an enhanced accumulation of ribavirin. Indeed, Endres et al.,\(^3\) have reported that the uptake of ribavirin into erythrocytes is a saturable form of transport, showing that the ribavirin uptake into erythrocytes from Ent1(+/+) mice was significantly higher than that observed from Ent1(+/−) and Ent1(−/−) mice.

This study has several limitations, including the limited size of the study population, which might have lowered the power of the statistical analyses. In addition, although the anti-HCV regimen was homogeneous among the enrolled patients, we cannot exclude the heterogeneity of antiretroviral therapies in the HIV-infected population having influenced the expression and/or activity of ENT1 through different drug/nucleoside transporter interactions.\(^4\) Another limitation is represented by the small number of plasma ribavirin determinations obtained on a single timepoint measurement and the lack of intra-erythrocytic concentration dosing. Finally, we did not analyse the recently described ITPA rs6051702 polymorphism\(^2\) and this might have caused the impact of ITPA deficiency to be underestimated in our cohort.

To assess the role of SLC29A1 polymorphism in ribavirin uptake into erythrocytes, the concentrations of ribavirin in individual carriers of different genotypes are needed. Plasma ribavirin concentration at week 2 of treatment in a subgroup of 15 patients showed a trend of higher ribavirin plasma concentrations in GG carriers, possibly mirroring the influence of GG genotype in the cellular uptake of ribavirin by the intestinal and renal epithelia, where ENT1 is also expressed.\(^5\) Indeed, besides hepatocytes and erythrocytes, ENT1 is also expressed in enterocytes\(^3\) and renal epithelial cells,\(^6\) where it works as an influx and efflux transporter, possibly influencing ribavirin plasma and cellular concentration.

In conclusion, SNP rs760370 at the major ribavirin transporter ENT1 gene SLC29A1 was for the first time associated with ribavirin-induced anaemia in telaprevir-based triple therapy of HCV chronic hepatitis. Further studies are required to investigate the relationship of SLC29A1 polymorphism with intracellular ribavirin concentrations at different timepoints of therapy and to identify the cellular mechanisms responsible for haemolysis. If our observations are confirmed, the identification of SLC29A1 risk genotypes might represent a helpful tool for physicians to predict the need for ribavirin dose reduction early during anti-HCV IFN/ribavirin-based triple therapy.

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Transparency declarations
None to declare.
Ribavirin dose reduction.


