Variant ITPA Phenotypes are Associated with Increased Ribavirin Triphosphate Levels

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Abstract

Background: Single nucleotide polymorphisms (SNPs) in the rs1127354 and/or rs7270101 genes encoding the inosine triphosphatase (ITPA) enzyme provide protection against ribavirin (RBV)-induced hemolytic anemia. The effect of these SNPs on the active triphosphate (TP) form of RBV has not been assessed. We sought to determine if there was an association between ITPA genetics and RBV-TP concentrations in red blood cells (RBC).

Methods: RBC were collected from individuals receiving RBV-based HCV treatment. RBV-TP concentrations were determined in RBC or dried blood spots using a validated LC-MS/MS assay linear in the range of 0.5-200 pmol/sample, then normalized to cell count (pmol/10^6 cells). Genotyping of ITPA SNPs was performed using the ABI TaqMan allelic discrimination kit and the BIO-RAD CFX Connect Real-Time PCR Detection System using standard TaqMan Universal PCR conditions. ITPA phenotypes were defined as 100%, 60%, 30%, and 5-10% activity. ANOVA was used for overall comparison between ITPA phenotypes with individual t-tests for differences relative to 100% activity.

Results: RBC were obtained from 187 individuals [60% male, 63% white/29% African American, median (range) age and weight of 53 (21, 78) years and 85 (45, 153) kg] receiving RBV for a median (range) of 84 (19, 336) days. RBV-TP was significantly different between ITPA phenotypes (figure, p<0.0001), with RBV-TP in the 30% and 5-10% activity phenotypes being approximately 2-fold higher than wild-type (100%).

Conclusion: ITPA variant phenotypes have higher RBV-TP concentrations in RBC despite having less RBV-induced hemolytic anemia.

Methods

- 187 patients consented with IRB approved protocols. One sample per subject analyzed.
- RBC, dried blood spots (DBS), or lysed whole blood were used for analysis.
- RBV-TP were extracted by first separating MP, DP and TP with strong anion exchange followed by de-salting and concentration prior to analysis.
- Quantification performed using validated LC-MS/MS method in the range of 0.5-200 pmol/sample[1].
- DNA isolated from either whole blood or DBS for genotyping.
- Genotyping of ITPA rs1127354 and rs7270101 was performed on all patients using TaqMan assays and real time PCR.
- Graphpad Prism software was used to analyze RBV-TP and ITPA activity by One-way ANOVA.
- SAS software was used to perform linear regression on demographic and clinical data with RBV-TP.
- P<0.05 was considered significant.

Results

Table 1: Demographics of the study participants.

Demographics	N=187
% Male	59.4%
Race	63.1% white, 29.4% black, 5.3% Hispanic
Age in yrs: mean (SD)	53.4 (10.0)
Fibrosis score ≥ 3.0	40.6%
Weight in Kg: mean (SD)	84.6 (20.0)
Δ Hgb ≥ 3.0 g/dL (baseline to blood collection)	38.5%
Duration of treatment in days: Median (range)	84 (19, 336)

Table 2: Percent of subjects receiving each treatment combination: Number, percent. PEG is pegylated interferon.

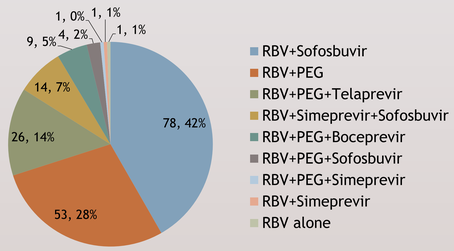


Table 3: Number (percent) of subjects in each ITPA activity phenotype[3]. Variant allele frequencies were 8.79% and 11.0% for rs1127354 and rs7270101, respectively.

rs1127345 C>A	rs7270101 A>C	ITPA Activity	Number
Wild type (C/C)	Wild type (A/A)	100%	119 (63.6%)
Wild type (C/C)	Heterozygous (A/C)	60%	32 (17.1%)
Heterozygous (C/A)	Wild type (A/A)	30%	23 (12.3%)
Wild type (C/C)	Homozygous variant (C/C)	30%	2 (1.1%)
Heterozygous (C/A)	Heterozygous (A/C)	10%	6 (3.2%)
Homozygous variant (A/A)	Wild type (A/A)	<5%	1 (0.5%)
--	--	Unknown	5 (2.7%)

Figure 1: RBV-TP was significantly increased (~2 fold) in subjects with ITPA activity ≤ 30% compared to 100%. P<0.0001 from One-way ANOVA analysis. Multiplicity adjusted P values reported in figure (Dunnett test).

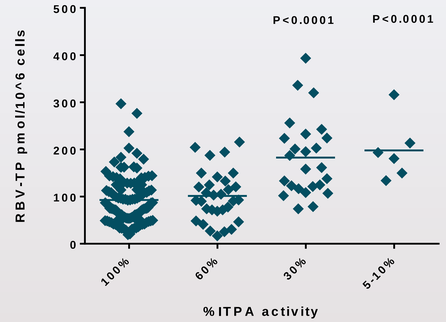


Table 4: Mean (SD) RBV-TP values for each ITPA activity phenotype.

ITPA Activity	Mean (SD) RBV-TP (pmol/10^6 cells)	Multiplicity adjusted P values
100%	92.8 (51.6)	NA
60%	101.3 (53.5)	0.9934
30%	184.8 (84.5)	<0.0001
5-10%	197.9 (64.6)	<0.0001

Figure 2: The change in hemoglobin from beginning of treatment to the time of sample collection was not significantly different in those with lower ITPA activity. Listed for each group is the median and range of treatment duration at the time of sample collection in days.

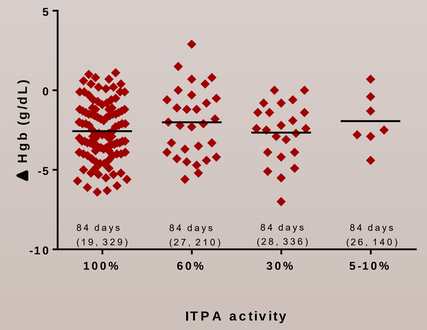


Table 5: Linear regression analysis of demographic and clinical factors on RBV-TP levels. ITPA phenotype remained the most significant predictor of increased RBV-TP.

Variable	% change RBV-TP	%Error	Univariate P value	Multivariate P value
ITPA	98.4	12.3	<.0001	<.0001
Telaprevir	52.5	27.7	0.0004	0.0015
Race	17.4	46.7	0.0336	0.427
Fibrosis	-5.1	-65.0	0.1263	0.236
ΔHgb (g/dL)	-2.1	-120.4	0.4073	0.749
Sofosbuvir	-7.1	-124.1	0.4215	0.550
Simeprevir	-12.7	-124.6	0.4232	0.514
Sex	-6.4	-140.2	0.4767	0.576
Boceprevir	14.5	144.7	0.4903	0.178
PEG	5.3	178.9	0.5768	0.316
Weight	0.0	-501.8	0.8423	0.617
Age	0.1	776.5	0.898	0.833

Conclusions

- ITPA variant phenotypes have higher RBV-TP concentrations in RBC despite having less RBV-induced hemolytic anemia as shown in previous studies[4].
- Change in hemoglobin was not significantly different for ITPA variants compared to wild type.
- ITPA was significantly associated with increased RBV-TP levels after controlling for sex, age, race, weight, HCV drug regimen, change in hemoglobin and fibrosis stage in linear regression analysis.
- **Potential Mechanisms for increased RBV-TP levels:**
  - ITPA degrades RBV-TP in RBC and thus phosphorylated RBV levels are higher in subjects with ITPA variant phenotypes.
  - ITPA variants may have more ATP in RBC[5]. ATP increases activity of the RBV phosphorylation enzyme cytosolic 5"-Nucleotidase II by 17-fold[6]. Thus, excess ATP may be a cause for increased RBV-TP levels.
- Prospective studies are needed to confirm and extend these findings, to establish the mechanism, and to evaluate additional cell types other than RBC.

References

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Acknowledgments

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