Ribavirin PBMC accumulation and ribavirin plasma concentration as determinants of anemia after one month of anti-HCV therapy

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Background

Epidemiology of HCV infection

The World Health Organization estimates around 2-3% prevalence of infection worldwide, with approximately 170 million HCV-positive individuals.

- In Africa and Eastern Mediterranean are present the highest rates of seroprevalence (> 10%)
- In Western Europe, Canada and Australia the lowest rates (<1%)
- In Italy we have an average prevalence rate of 2%, with an incidence of 0.5 / 100,000
Background

Treatment of chronic hepatitis C

At the time 6 viral genotypes were identified, and more than 50 subtypes. The therapy currently used is based on pegylated-interferon (PEG-IFN) and ribavirin (RBV), administered according to HCV genotypes:

- **Genotypes 1 and 4**
  PEG-IFN α (α2a or α2b) + ribavirin at higher doses (1000-1200 mg / day) for 48 weeks.
  Numerous new anti-HCV drugs acting as true antivirals (DAAs) are being developed. Two protease inhibitors (telaprevir and boceprevir) have been approved for clinical use as third component of PEG-INF/ribavirin regimens.

- **Genotypes 2 and 3**
  PEG-IFN α (α2a or α2b) + ribavirin in lower doses (800 mg / day) for 24 weeks.

Background

RIBAVIRIN TOXICITY

Therapy combines PEG-IFN alpha and Ribavirin (RBV) [and PIs], and is associated with a range of adverse effects.

RBV-induced haemolytic anaemia, takes place in most patients (about 30%, without PIs and up to 50% with new PIs) and might require dose modification.
Background

PREDICTORS OF RBV TOXICITY

ITPA polymorphisms, RBV plasma trough concentration, sex, age, weight-adjusted RBV dose, pre-treatment platelet count and Hb level were found to be associated with anaemia.
Background

RIBAVIRIN AND TOXICITY

Plasma exposure of RBV and ITPA SNPs as determinants of anaemia

**FIGURE 1.** The possession of at least 1 variant allele in the functional ITPA SNPs (n = 49) was associated with a smaller decrement of Hb (grams per deciliter) at week 4 (P = 4.09 × 10⁻⁸). Median values (horizontal line), IQR (bars), patient values (black square), highest and lowest values (whiskers) are shown.

**FIGURE 2.** The predicted probability of developing anemia considering the possession of variant alleles and RBV concentrations (cut-off of 2.3 µg/mL) in multivariate logistic regression. The predicted probability of anemia is 13.8% for Yes-Carrier and Yes-RBV <2.3, 27.5% for Yes-Carrier and No-RBV <2.3, 35.5% for No-Carrier and Yes-RBV <2.3, and 42% for No-Carrier and No-RBV <2.3.

D’Avolio et al

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Background

RIBAVIRIN TOXICITY

The intracellular (Red Blood Cells) concentration of ribavirin is associated with anaemia.

No data are available on intracellular (PBMC) concentrations of ribavirin.
Objectives

- To measure peripheral blood mononuclear cells (PBMCs)-associated concentration of RBV and RBV-P in RBV intakers

- To evaluate the relationship with the development of anaemia
Methods

- 38 HCV patients were recruited in Turin, Italy

- **Inclusion criteria** were:
  - Older than 18 years
  - No concomitant interacting drugs
  - No hepatic or renal function impairment
  - Self-reported adherence more than 95%

- Blood sampling at the end of dosing interval (C\text{trough}) was performed at **week 4** on therapy.

- Plasma samples were analyzed by **HPLC-UV** method [D’Avolio A. et al. 2006] and intracellular samples by a validated **UPLC-MS/MS** method [D’Avolio A. et al. submitted 2012] (with and without **phosphatase digestion**).
- Quantification of mean cellular volume (MVC) and cell count were assessed using **Coulter Counter**.
Methods

**Significant anaemia** was defined as a decline in Hb of 3 g/dL or Hb levels <10 g/dL, which is the threshold at which RBV dose reduction is recommended.
## Results

### Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>IQR Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (Tot. 38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males (%)</td>
<td>29 (76.3)</td>
<td>-</td>
</tr>
<tr>
<td>Age, years</td>
<td>44</td>
<td>35 – 52</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.0</td>
<td>22.2 – 27.4</td>
</tr>
<tr>
<td>HCV Log BL (IU/mL)</td>
<td>6.4</td>
<td>2.9 – 7.8</td>
</tr>
<tr>
<td>ALT BL (IU/mL)</td>
<td>112</td>
<td>14 – 664</td>
</tr>
<tr>
<td>Hb BL (g/dL)</td>
<td>12.8</td>
<td>11.3 – 14.3</td>
</tr>
<tr>
<td>RBV dose (mg)</td>
<td>1000</td>
<td>800 – 1050</td>
</tr>
<tr>
<td>HCV 1/4 (%)</td>
<td>25 (65.8)</td>
<td>-</td>
</tr>
</tbody>
</table>
## Results

### After One month of Therapy

<table>
<thead>
<tr>
<th>Number of patients (Tot. 38)</th>
<th>Median</th>
<th>IQR Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma RBV concentrations (ng/mL)</td>
<td>1584</td>
<td>1095 – 2072</td>
</tr>
<tr>
<td>PBMC RBV concentrations (ng/mL)</td>
<td>3781</td>
<td>2391 – 5138</td>
</tr>
<tr>
<td>PBMC RBV-P concentrations (ng/mL)</td>
<td>42630</td>
<td>27384 – 56156</td>
</tr>
<tr>
<td>PBMC RBV/Plasma RBV (Ratio)</td>
<td>2.68</td>
<td>1.71 – 3.28</td>
</tr>
<tr>
<td>PBMC RBV-P/Plasma RBV (Ratio)</td>
<td>27.74</td>
<td>20.71 – 35.19</td>
</tr>
<tr>
<td>Delta Hb (g/dL)</td>
<td>- 2.00</td>
<td>- 1.20 – - 3.17</td>
</tr>
<tr>
<td>Delta Hb (%)</td>
<td>- 13.07</td>
<td>- 8.31 – - 21.86</td>
</tr>
</tbody>
</table>
Results

Plasma ribavirin concentrations were correlated with both intracellular unphosphorylated and phosphorylated ribavirin concentrations.

\[ \text{rho} = 0.343 \quad p = 0.035 \]

[Graph showing correlation between plasma and intracellular concentrations]
Results

After 1 month of therapy 12/26 (31.6%) patients presented anaemia.

Plasma ribavirin concentrations were significantly higher in patients presenting anaemia.
Results

After 1 month of therapy 12/26 (31.6%) patients presented anaemia.

PBMC phosphorylated-ribavirin (RBV-P) and PBMC ribavirin (unphosphorylated) concentrations were significantly higher in patients presenting anaemia.
Results

Loss of hemoglobin correlated with ribavirin plasma and unphosphorylated intracellular concentrations.

\[
\rho = -0.390 \quad p = 0.015
\]

\[
\rho = -0.562 \quad p < 0.001
\]
Results

Predictors of anaemia in univariate and multivariate logistic regression analysis.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P value</td>
<td>P value</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.011</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.171</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hb BL</td>
<td>0.141</td>
<td>n.s.</td>
</tr>
<tr>
<td>RBV dose (mg/kg)</td>
<td>0.006</td>
<td>0.008</td>
</tr>
<tr>
<td>RBV Plasma concentrations &gt; 2300 ng/mL</td>
<td>0.034</td>
<td>0.048</td>
</tr>
<tr>
<td>RBV PBMC concentrations &gt; 6000 ng/mL</td>
<td>0.061</td>
<td>n.s.</td>
</tr>
<tr>
<td>RBV-P PBMC concentrations &gt; 50000 ng/mL</td>
<td>0.039</td>
<td>n.s.</td>
</tr>
<tr>
<td>Carrier of variant allele (ITPA SNP 354 and 101)</td>
<td>0.267</td>
<td></td>
</tr>
</tbody>
</table>
Results

Preliminary results on the virological outcome

<table>
<thead>
<tr>
<th>OUTCOME</th>
<th>HCV Genotype</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCV 2/3</td>
<td>HCV 1/4</td>
</tr>
<tr>
<td>RVR NO (Rapid Virological Response) YES</td>
<td>0 (100%)</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>25</td>
</tr>
</tbody>
</table>

Factors and RVR (HCV 1/4) N=25

<table>
<thead>
<tr>
<th></th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma RBV Concentrations (1 month)</td>
<td>0.509</td>
</tr>
<tr>
<td>PBMC RBV Conc. Concentrations (1 month)</td>
<td>0.039</td>
</tr>
<tr>
<td>PBMC RBV-P Conc. Concentrations (1 month)</td>
<td>0.024</td>
</tr>
<tr>
<td>IL-28b 860 CC</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-28b 917 TT</td>
<td>0.027</td>
</tr>
</tbody>
</table>
Summary

- This is, to our knowledge, the first study reporting RBV concentrations in PBMCs.

- High concentrations of both phosphorylated and unphosphorylated RBV were measured in PBMC.

- Plasma RBV concentrations and PBMC-RBV/RBV-P concentrations were associated with the development of anaemia in RBV recipients.
Conclusion

- These are preliminary findings just based on 1st month analysis of 38 patients.

- Measurement of PBMC concentrations of RBV is a time-consuming procedure and, concerning anaemia, does not provide more predictive indication than more easily achievable measures of plasma exposure.
Discussion

- However, looking at the virological efficacy side, the study of intracellular PBMC RBV concentrations might help to verify whether PBMC pharmacokinetic can be considered as a surrogate of exposure at the target site (hepatocytes).

*Could PBMCs be considered as a surrogate for hepatocytes?*
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