Letters to the Editor

and comparable to that of hemoglobin whereas the increase in CHr and the fast decrease of %HYPOr could be observed already after 5 to 7 days. This suggests that CHr and particularly %HYPOr rapidly reflect increased iron availability for erythropoiesis and could be useful for monitoring the effectiveness of oral iron medication.

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Key words: anemia, hypochromic RBC, reticulocytes, iron medication

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References


Combined therapy with desferrioxamine and deferiprone in thalassemic patients: effect on urinary iron excretion

Desferrioxamine B mesylate (DFO) and deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one) (DFP) have been used for the treatment of hemosiderosis in patients with thalassemia major.2 Preliminary studies have suggested that chelation is enhanced by the simultaneous administration of DFO and DFP.2,4 In this study we evaluated the urinary iron excretion (UIE) of patients during treatment with DFO or DFP or the two drugs simultaneously.

Sixty patients (32 females) with transfusion-dependent β-thalassemia major, aged 16 - 37.7 years (mean: 24±4.5), were included in the study. Their ferritin levels ranged between 512 and 9359 µg/L (mean: 3118±1861). Nine patients were splenectomized. Eleven had anti-hepatitis C antibodies. All patients had been on regular chelation therapy with either DFO and comparable to that of hemoglobin whereas the increase in CHr and the fast decrease of %HYPOr could be observed already after 5 to 7 days. This suggests that CHr and particularly %HYPOr rapidly reflect increased iron availability for erythropoiesis and could be useful for monitoring the effectiveness of oral iron medication.

Figure 1. The responses of %HYPOm (top), %HYPOr (middle), and CHr (below) to oral iron medication in each of the iron deficient subjects (n=8). The samples were analysed on days 2, 7, 14, 21 and 28 after the start of iron medication.

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References


The UIE of DFP was related to ferritin ($r_s = -0.29$, $p = 0.036$). These findings reflect the substantially high UIE with DFP observed in patients with significant hemosiderosis. No relationship was documented between UIE and gender, age, spleen or hepatitis C status.

Mean daily iron accumulation from transfusions was estimated at 0.48±0.08 mg/kg/day. Iron balance in the various phases is shown in Table 1.

Table 1. Treatment protocol and results.

<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFO</td>
<td>30-55 mg/kg/day</td>
<td>30-55 mg/kg/day</td>
<td>30-55 mg/kg/day</td>
<td></td>
</tr>
<tr>
<td>DFP</td>
<td>25 mg/kg every 8 hours</td>
<td>2 doses of 25 mg/kg*</td>
<td>3 doses of 25 mg/kg*</td>
<td></td>
</tr>
<tr>
<td>UIE (mg/kg/day)</td>
<td>median 0.28</td>
<td>0.30</td>
<td>0.54</td>
<td>0.65</td>
</tr>
<tr>
<td>25th-75th percentile</td>
<td>0.21-0.45</td>
<td>0.18-0.43</td>
<td>0.40-0.79</td>
<td>0.48-0.72</td>
</tr>
<tr>
<td>Iron balance° (mg/kg/day)</td>
<td>median 0.18</td>
<td>0.19</td>
<td>-0.06</td>
<td>-0.12</td>
</tr>
<tr>
<td>25th-75th percentile</td>
<td>to 0.27</td>
<td>to 0.32</td>
<td>to 0</td>
<td>to -0.12</td>
</tr>
<tr>
<td>Positive iron (%)</td>
<td>78.6</td>
<td>69.6</td>
<td>23.6</td>
<td>15.6</td>
</tr>
</tbody>
</table>

The first dose of DFP was taken at the start, the second dose 2 hours before the end of the DFO infusion and the third dose 6-8 hours (during phase 4 only) after the end of the DFO infusion. *Iron balance is defined as: [daily iron accumulation from transfusions] – UIE. Possible fecal excretion, which has previously been estimated at 30-200% of UIE for DFO and 10%-20% of UIE for DFP, was not taken into account.

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Figure 1. Dot plots of the ratios of UIE of combined therapy of DFO with 2 doses of DFP to UIE during treatment with either DFO (phase 3/phase 1, △) or DFP (phase 3/phase 2, □) and of the ratios of the observed UIEs to the expected UIEs during treatment with combined therapy of DFO with 2 doses of DFP (phase 3, ▲).
With the current chelation regimen, the balance between iron accumulation and excretion is fine. In contrast to the iron balance achieved by monotherapy with either DFO or DFO, iron balance achieved with combined therapy was negative in the majority of patients.

In conclusion, combined therapy with DFO and DFP showed an additive and occasionally synergistic effect on UIE, which could reach levels higher than iron accumulation from transfusions, leading to a negative iron balance. Long-term studies are required to validate the efficacy and safety of combined therapy.

Key words: thalassemia, chelation, hemosiderosis.

References


Lack of Bcr-Abl point mutations in chronic myeloid leukemia patients in chronic phase before imatinib treatment is not predictive of response

Despite the positive results of treatment with imatinib mesylate (IM) in patients with chronic myelogenic leukemia (CML), a number of patients develop clinical resistance to this drug, resulting in progression of the disease at 18 months in 11% of interferon resistant/intolerant patients. Most patients in blast crisis will eventually suffer disease progression despite continuous treatment with imatinib. Among the different mechanisms of in vivo resistance to IM, the most frequently detected in patients with advanced phase (accelerated or blast crisis) CML is point mutations in the kinase domain of Abl. We studied the presence of Bcr-Abl mutations in a homogeneous group of CML patients in chronic phase with primary cytogenetic resistance to IM in order to determine the incidence of point mutations and whether the presence of these substitutions before treatment could predict resistance to IM therapy.

We studied a group of 89 patients with CML enrolled in an extended access trial of IM (chronic phase CML patients resistant to or intolerant of interferon-α). All patients had 100% Philadelphia positive metaphases. Patients with no cytogenetic response after at least 6 months of therapy were defined as having primary resistance to IM and analyzed for the presence of Abl mutations. Bone marrow mononuclear cells were obtained before initiating treatment with IM and every 3 months thereafter.

Total RNA was extracted using RNeasy®Mini Kit (Qiagen, Hilden, Germany) from frozen cells. Total RNA (1 µg) was used for cDNA synthesis using SuperScript™ II RNase H-RT (Invitrogen Life Technologies, Paisley, UK) with random hexamers. A BCR-ABL transcript of 1.3 kb was amplified by PCR using 4 µL of cDNA and CM 10 (5'-GAAGCTTCTCCCTGACATCCGT-3') and 3ABL2 (5'-GGGACTGATGCCAGAATGTTG-3') primers under the following conditions: 94°C for 10 min, 30 cycles at 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 90 seconds, and a final elongation cycle at 72°C for 10 min. The Abl kinase domain was amplified in a second PCR using 1 µL of the first PCR product and 5ABLKD (5'-GGCAAGCAACCAAGCCACTGCATCGGT-3') and 3ABLKD (5'-GGGACTGATGCCAGAATGTTG-3') primers under the following conditions: 94°C for 10 min, 30 cycles at 94°C for 30 seconds, 70°C for 30 seconds and 72°C for 90 seconds, followed by an elongation cycle at 72°C for 10 min. All PCR reactions were carried out in a total volume of 25 µL with 2.5 µL of native PFU polymerase (Stratagene, Amsterdam, The Netherlands), 0.4 mM dNTPs and 20 pmol of each primer. The second PCR product (597 bp) was subcloned into pCR®-4. Letters to the Editor