Promotion of platelet production by hTPO cDNA injection

Sirs,

Thrombopoietin (TPO) is a regulator of megakaryocyte differentiation that stimulates platelet production and possesses great value in treatment of primary and secondary marrow failure. Previously, we observed that mice which received human TPO (hTPO) cDNA injection were resistant to the toxicity of cyclophosphamide. Here we report the activity of TPO cDNA injection in elevating platelet count.

hTPO cDNA was isolated from the total RNA of fetal liver by RT-PCR and subcloned in pCDNA3. It was packed with lipotecin (10 µg/µL, Gibco BRL) for injection. One-hundred and twenty Babl/c mice (20 g body weight, female) were injected with 60 µg of the reconstructed plasmid; another 40 non injected mice were used as controls. Platelets and leukocytes were counted directly. Paraffin sections of bone marrow were prepared to estimate megakaryocyte densities. Rabbit anti-hTPO serum was collected from rabbits immunized with truncated hTPO expressed in E. coli.

The average platelet number of the hTPO cDNA-injected mice began to rise on the third day. A significant increase lasted for about 1 week. The average increase was about 184% that of age-matched controls, and the highest number was over 3 times greater than the control values. Furthermore, platelet counts fluctuated but for over 2 weeks they were still higher than controls (Figure 1). Leukocyte counts were not significantly affected except for the first 2 days. Megakaryocyte densities began to rise on the second day following gene injection; the density change occurred 1 day earlier than the rise in platelet number. They remained elevated for over three weeks, but the highest levels were observed on the 12th day.

Slot hybridization detected hTPO mRNA in total RNA isolated from local muscular tissue within 24 hr of injection. Positive mRNA transcription lasted for three weeks, with the strongest signal appearing on the 3rd day after injection. The earliest detection of hTPO in the blood of mice was at the 3rd day, but it was still increased even after 3 weeks.

It has been reported that recombinant hTPO can elevate the average platelet counts of mice. The gene injection here increased platelet counts by about 84% and avoided complication treatment. Our results suggested that hTPO cDNA injection may have potential clinical value.

Despite the increase in platelet numbers, their function was not significantly changed. We believe that is due to the balance behavior of the coagulation system. We suppose that the merits of TPO cDNA injection might be sufficiently estimated in the thrombocytopenia bleeding model.

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Figure 1. Dynamic change in the platelets count of hTPO-injected mice. A significant increase was observed in days 3 to 9.

Pregnancy in patients with immune thrombocytopenic purpura

Sirs,

Management of patients with immune thrombocytopenic purpura (ITP) during pregnancy should be based on the incidence of thrombocytopenia and bleeding in mothers as well as in neonates. Earlier studies suggest a high incidence of hemorrhagic complications, which have not been confirmed by more recent reports. The authors present their experience concerning 14 pregnancies in 11 women with ITP. Median age at diagnosis was 21. ITP was diagnosed during pregnancy in 4 patients, who had persistent thrombocytopenia for at least 6 months thereafter. The other ten pregnancies occurred on average 6.2 years after the diagnosis. Data concerning mothers and neonates are presented in Table 1. Four patients presented antinuclear antibodies (patients D, E, H and I), but only 2 developed systemic lupus erythematosus (patients H and I).

Three patients had pre-eclampsia and there were 6 preterm deliveries. There were 5 Cesarian sections, performed for obstetrical complications (severe pre-eclampsia, abruptio placentae, acute fetal distress, and uterine dysfunction), and 9 vaginal deliveries. Eight mothers had platelet count less than 30 x109 platelets/L (57%), 5 of them had mild cutaneous and mucous membrane purpura. Significant bleeding during labor, requiring blood transfusion, was seen in 3 patients: one patient (G9) had vaginal bleeding requiring reoperation, another (I12) presented vaginal hemorrhage and wound hematoma after a Cesarian section, and the third patient (G9) had vaginal bleeding not related to severe thrombocytopenia. None of the 5 patients who received prednisone during pregnancy bled.

Table 1. Data from women with ITP and their neonates (NA = not available).

<table>
<thead>
<tr>
<th>Case</th>
<th>Weeks gestation</th>
<th>Way of delivery</th>
<th>Maternal bleeding</th>
<th>Maternal platelet count x109/L</th>
<th>Neonatal platelet count x109/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/1</td>
<td>39</td>
<td>vaginal</td>
<td>No</td>
<td>70</td>
<td>110</td>
</tr>
<tr>
<td>B/2</td>
<td>36</td>
<td>vaginal</td>
<td>No</td>
<td>99</td>
<td>119</td>
</tr>
<tr>
<td>C/3</td>
<td>36</td>
<td>vaginal</td>
<td>No</td>
<td>56</td>
<td>224</td>
</tr>
<tr>
<td>D/4</td>
<td>40</td>
<td>vaginal</td>
<td>Purpura</td>
<td>5</td>
<td>200</td>
</tr>
<tr>
<td>E/5</td>
<td>40</td>
<td>vaginal</td>
<td>No</td>
<td>35</td>
<td>38</td>
</tr>
<tr>
<td>F/6</td>
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<td>vaginal</td>
<td>No</td>
<td>18</td>
<td>36.5</td>
</tr>
<tr>
<td>G/7</td>
<td>40</td>
<td>vaginal and vaginal</td>
<td>Purpura</td>
<td>10</td>
<td>NA</td>
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<tr>
<td>G/8</td>
<td>36</td>
<td>vaginal</td>
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<td>120</td>
<td>300</td>
</tr>
<tr>
<td>G/9</td>
<td>40</td>
<td>Cesarean</td>
<td>Purpura</td>
<td>10</td>
<td>NA</td>
</tr>
<tr>
<td>H/10</td>
<td>36</td>
<td>Cesarean</td>
<td>No</td>
<td>25</td>
<td>200</td>
</tr>
<tr>
<td>H/11</td>
<td>39</td>
<td>Cesarean</td>
<td>No</td>
<td>197</td>
<td>250</td>
</tr>
<tr>
<td>H/12</td>
<td>22</td>
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<td>Purpura</td>
<td>26</td>
<td>Dead</td>
</tr>
<tr>
<td>K/13</td>
<td>28</td>
<td>Cesarean</td>
<td>No</td>
<td>162</td>
<td>200</td>
</tr>
<tr>
<td>J/14</td>
<td>38</td>
<td>Cesarean and wound hematoma</td>
<td>No</td>
<td>5</td>
<td>NA</td>
</tr>
</tbody>
</table>

Neonate platelet count was available in 11 out of 14 infants: 2 of them had less than 30 x109 platelets/L (18%), but none bled. There was an early neonatal death associated to severe pre-eclampsia at 22 weeks, but not related to hemorrhage (I12). There was a significant correlation between maternal and newborn platelet count suggesting that thrombocytopenic women are at greater risk of having thrombocytopenic neonates. The incidence of severe maternal thrombocytopenia was 57% but significant bleeding due to thrombocytopenia was
uncommon, regardless the mode of delivery, as shown by Kelton et al., in a review of 94 cases.1 Data from this study are in agreement with those from literature showing that children born to mothers with ITP have low risk of having severe thrombocytopenia and associated hemorrhage.2 Thrombocytopenic mothers should be treated in order to avoid maternal bleeding during labor and invasive procedures, such as cordocentesis and preventive Cesarean section are not justified.1

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References

Sir,

Even though severe hypokalemia has been associated with acute leukemia, other acid-base and/or electrolyte disturbances have also been reported infrequently in such patients. We describe an interesting case of severe, multiple and interrelated electrolyte abnormalities in a patient with acute leukemia. A 76-year-old man was admitted to the hospital with a 4-week history of malaise, fatigue, muscle weakness, 5 kg weight loss, fever up to 39°C, cough and rusty sputum. The patient was not on any drugs that affect electrolyte levels, such as diuretics. Upon admission he had a temperature of 38.6°C, a pulse rate 109, and respiratory rate of 22 breaths per minute. The examination revealed a diminution of breath sounds at the base of the left lung associated with dullness to percussion was found. Enlargement of the left tonsil and of bilateral cervical lymph nodes was also observed. The remainder of the physical examination was normal. An electrocardiogram revealed inverted T-waves, prolonged Q-T intervals and the presence of U waves. An X-ray of the chest showed right pleural effusion.

The results of laboratory tests on patient admission are shown in Table 1. A urine dipstick was positive for leukocytes and trace positive for blood, but negative for protein; the urine sediment contained 3-4 erythrocytes, 4-5 white blood cells and many granular casts per high-power field. Intravenous and oral potassium and magnesium supplements were given, and ceftazidime 2 g/8 h IV was administered. Four days later serum electrolytes were within normal limits. Bone marrow aspirate and biopsy analysis confirmed the diagnosis of acute myelomonocytic leukemia and the patient was treated with etoposide and cytosine arabinoside. The patient died two months later of septicemia due to Pseudomonas aeruginosa. Of the various electrolyte abnormalities our patient developed, severe hypokalemia with inappropriate kaliuresis (FEK+ > 6.4%, TTKG > 2) was prominent and could be ascribed: 1) to lysozymuria, which may induce renal tubular dysfunction with kaliuresis;2) to the concomitant severe hypomagnesemia, since it was well known that hypomagnesemia of any cause can lead to potassium depletion through both urinary and fecal losses, and 3) to potassium entry into metabolically active leukemic cells.3 Severe hypomagnesemia with inappropriate magnesiuria (FEMg++ > 2.5%) was also found, which could be the result of a leukemia-induced or even lysozyme-induced tubular dysfunction,4 or of the coexisting phosphate depletion.5 Hypophosphatemia with appropriate renal phosphate conservation (FEPO4 < 20%, TmPO4 /GFR: renal threshold phosphate concentration, calculated using the

Table 1. Laboratory values found in the patient.

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (%)</td>
<td>9.3</td>
</tr>
<tr>
<td>White-cell count (L)</td>
<td>9.1</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>62.8</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>37.2</td>
</tr>
<tr>
<td>Serum glucose (mmol/L)</td>
<td>5.5</td>
</tr>
<tr>
<td>Serum total proteins (g/L)</td>
<td>60</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>45</td>
</tr>
<tr>
<td>Serum K+ (mmol/L)</td>
<td>3.3</td>
</tr>
<tr>
<td>Serum Ca++ (mmol/L)</td>
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</tr>
<tr>
<td>Serum Mg++ (mmol/L)</td>
<td>0.46</td>
</tr>
<tr>
<td>Serum TmPO4 /GFR</td>
<td>2.85</td>
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<tr>
<td>Serum urine sodium (mmol/L)</td>
<td>135</td>
</tr>
<tr>
<td>Serum creatinine (µKat/L)</td>
<td>115</td>
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<tr>
<td>Serum uric acid (µmol/L)</td>
<td>375</td>
</tr>
<tr>
<td>Serum total proteins (g/L)</td>
<td>60</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>45</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.4</td>
</tr>
<tr>
<td>Serum phosphorus (mmol/L)</td>
<td>0.77</td>
</tr>
<tr>
<td>Serum sodium (mmol/L)</td>
<td>135</td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
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</tr>
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<td>Serum magnesium (mmol/L)</td>
<td>0.46</td>
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