CONCISE REPORT

Cooperative Enhancement of F-Cell Formation in Baboons Treated With Erythropoietin and Hydroxyurea

By Adil Al-Khatti, Thalia Papayannopoulou, Glenn Knitter, Edward F. Fritsch, and George Stamatoyannopoulos

Chronically anemic baboons on a continuous hydroxyurea regimen were treated with pulsed doses of recombinant human erythropoietin (rHuEpo) to test whether the combination of these two compounds, which individually induce F-cell production, can enhance further F-cell output. A low-F-cell-responding animal under chronic hydroxyurea treatment was given three separate pulses of Epo and responded with F-reticulocyte increments that were similar to the sum increments caused by either hydroxyurea alone or rHuEpo alone. The same results were obtained in a high-F-responding animal similarly treated. These findings suggest that rHuEpo and hydroxyurea can increase F-cell numbers in an additive fashion. It is speculated that both compounds act through perturbation of erythroid differentiation kinetics.

PREVIOUS STUDIES have shown that several cytotoxic drugs can induce F-cell formation in primate models and humans.1, 11 The mechanism of this phenomenon remains speculative; however, several pieces of evidence indicate that the response is partly due to changes in regenerative erythroid kinetics triggered by drug treatment.14 Hydroxyurea is among the various cytotoxic drugs that have been found to effectively induce F-cell formation in primates and humans, and this agent is currently in use in clinical trials to raise fetal hemoglobin (HbF) in patients with sickle cell anemia.13 In addition to cell cycle-specific drugs, administration of pulses of high doses of recombinant human erythropoietin (rHuEpo) has been found to stimulate HbF synthesis in normal and anemic baboons.16 The purpose of the experiments described herein was to test whether administration of hydroxyurea and erythropoietin can stimulate F-cell production beyond the level attained by each compound alone.

MATERIALS AND METHODS

Two 5-year-old baboons were kept chronically anemic (steady-state hematocrit, 23% to 27%) by daily phlebotomy and received regular supplements of iron and multivitamins. rHuEpo was given as a bolus intravenous (IV) injection over five minutes every 12 hours. Hydroxyurea was administered IV over 15 minutes three times a week. Hematologic studies including determinations of reticulocytes, HbF determinations, F cells, F reticulocytes, and globin chain biosynthesis were done as previously described.16

RESULTS

Table 1 shows results obtained with animal A. In the nonanemic state, this animal had 1.2% (±0.3%) F reticulocytes and a γ/γ + β ratio that was below the sensitivity of the globin biosynthesis method. Chronic anemia (Hct, 23% to 27%) increased the proportion of F reticulocytes to 6.0% ± 1.5% and the γ/γ + β ratio to 0.02. Administration of rHuEpo (3,000 IU/kg every 12 hours for one day) to this anemic animal resulted in further increment of F reticulocyte percentages, which reached a peak value of 13%. Subsequently, the animal was treated with hydroxyurea (25 mg/kg three times a week) for 118 days. The F reticulocyte proportion gradually increased to a level of 16.5% ± 3.1%, while the γ/γ + β ratio increased to 0.08. To test whether the administration of rhEpo to this hydroxyurea-treated animal would further increase F-reticulocyte formation, two separate pulses of 3,000 IU/kg two times a day were given; the first resulted in an F-reticulocyte peak of 24%, the second in a peak of 28% (Fig 1). Thus, in this animal rHuEpo increased F reticulocyte numbers by 116% above the chronic anemia level and hydroxyurea alone by 175%, while the combination of the two compounds resulted in an increment of 333% (Table 1). An increment of 291% would have been obtained if the effects of rhEpo and hydroxyurea were strictly additive.

Animal A was also treated with a higher dose of rHuEpo (3,000 IU/kg two times a day for three days), which resulted in a peak of F reticulocytes of 23%. Administration of this dose of rHuEpo when the animal was under hydroxyurea treatment increased the F reticulocyte proportion to 30%. This corresponds to an increment (over the chronic anemia level) of 430%. If the effects of rHuEpo and hydroxyurea were strictly additive, the expected increment would be 458% above the level of F reticulocytosis in the chronically anemic state.

Animal B had higher baseline numbers of F reticulocytes and F cells and also responded to all manipulations with a higher induction of HbF synthesis. Chronic anemia (Hct, 23% to 27%) increased F reticulocyte numbers to a steady-state level of 20.8% (±2.7%). The administration of rHuEpo (3,000 IU/kg twice for one day) to the chronically anemic animal further stimulated F-reticulocyte production to a peak value of 34%. Administration of hydroxyurea to the anemic animal increased F reticulocyte proportions from 20.8% ± 1.5% to a level of 40.3% ± 2.8% (Fig 2). Administration to this hydroxyurea-treated animal of rHuEpo (3,000 IU/kg twice for one day) produced a further increase in F...
Table 1. Effects of rHuEpo and Hydroxyurea on Anemic Baboons

<table>
<thead>
<tr>
<th>Animal</th>
<th>Manipulation</th>
<th>Mean ± SD</th>
<th>Increment Over Chronic Anemia (%)</th>
<th>( \gamma/\gamma + \beta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None</td>
<td>1.2 ± 0.3</td>
<td>—</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Chronic Anemia</td>
<td>6.0 ± 1.5</td>
<td>0</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>rHuEpo (3,000 IU/kg twice for 1 d)*</td>
<td>13.0 ± 2.2</td>
<td>0</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>HU (25 mg/kg 3 times per week)</td>
<td>16.5 ± 3.1</td>
<td>175</td>
<td>0.08-0.09</td>
</tr>
<tr>
<td></td>
<td>HU + rHuEpo (3,000 IU/kg twice for 1 d)*</td>
<td>26 (24-28)</td>
<td>333</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>rHuEpo (3,000 IU/kg twice a day for 3 d)*</td>
<td>23.0 ± 1.5</td>
<td>283</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>HU + rHuEpo (3,000 IU/kg twice a day for 3 d)*</td>
<td>30.0 ± 1.5</td>
<td>383</td>
<td>0.16</td>
</tr>
<tr>
<td>B</td>
<td>None</td>
<td>2.4 ± 0.6</td>
<td>—</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Chronic Anemia</td>
<td>20.8 ± 2.7</td>
<td>0</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>rHuEpo (3,000 IU/kg twice for 1 d)*</td>
<td>34.1 ± 2.5</td>
<td>64</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>HU (25 mg/kg 3 times per wk)</td>
<td>40.3 ± 2.5</td>
<td>93</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>HU + Epo*</td>
<td>52 ± 1.0</td>
<td>150</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Abbreviation: HU, hydroxyurea.

*Peak value.

reticulocyte numbers to a peak of 52% by day 5 (Fig 2). Thus, in this animal Epo increased F reticulocyte proportions by 64% above the chronic anemia level and hydroxyurea did by 93%, while the combination of hydroxyurea and Epo increased it by 150% (Table 1).

DISCUSSION

Our findings show that rHuEpo enhances the induction of F cells in animals chronically treated with hydroxyurea. The increase in F reticulocytes is very similar to the one expected if the effects of the two compounds, rHuEpo and hydroxyurea, were additive.

Although synergistic effects might be indicative of different modes of action, an additive effect does not necessarily point to a particular mode. As far as hydroxyurea is concerned, available evidence favors the alteration in marrow cell kinetics as a major mechanism of its action\(^4\), other proposed mechanisms, ie, direct reprogramming of progenitors,\(^5\) cannot be excluded. Similarly, alterations in the kinetics of erythroid differentiation and proliferation were thought to underlie the induction of F-cell production by erythropoietin.\(^6\) Thus, it is possible that both hydroxyurea and erythropoietin act through a common pathway, ie, induction of rapid erythroid regeneration kinetics, in bringing about F-cell production. The additive increase in F-cell output by the combination treatment would indicate that the combination of the two compounds accelerates erythroid regeneration beyond the level achieved by each compound.

It has been suggested that sudden shortening of erythroid progenitor cell cycling could explain the activation of \( \gamma \) genes during acute erythroid regeneration.\(^14\) A similar model has been previously proposed to explain the induction of HbC by erythropoietin in genetically AA sheep.\(^18\) Erythropoietin or hydroxyurea could affect in vivo progenitor cell cycling either indirectly (because of the rapid erythroid regeneration kinetics induced by the drug treatment) or through a direct action on erythroid progenitors.

The results presented here are relevant to the question of
pharmacological induction of HbF in patients with sickle cell anemia. If rHuEpo is shown to produce significant induction of F-cell formation in such patients, undesirable elevations of Hcts may appear in response to the erythropoietic effect of the hormone. Combined administrations of rHuEpo and hydroxyurea may decrease the chance of elevation of the Hct.

REFERENCES

3. Charache S, Dover G, Smith K, Talbot CC, Jr, Moyer M, Boyer S: Treatment of sickle cell anemia with 5-azacytidine results in increased fetal hemoglobin production and is associated with nonrandom hypomethylation of DNA around the \( \gamma-\delta-\beta \)-globin gene complex. Proc Natl Acad Sci USA 80:4842, 1983
Cooperative enhancement of F-cell formation in baboons treated with erythropoietin and hydroxyurea

A Al-Khatti, T Papayannopoulou, G Knitter, EF Fritsch and G Stamatoyannopoulos