Pharmacokinetics and Effects of Recombinant Human Erythropoietin After Intravenous and Subcutaneous Injections in Healthy Volunteers

By F. Gilbert McMahon, Ramon Vargas, Michael Ryan, Adesh K. Jain, Robert I. Abels, Barbara Perry, and Ian L. Smith

A double-blind, placebo-controlled study of the pharmacokinetics and safety of multiple doses of recombinant human erythropoietin (rHuEPO) 150 or 300 U/kg either by intravenous (IV) bolus or subcutaneously (SC) in normal male subjects demonstrated that rHuEPO had a dose-related effect on the hematocrit independent of the route of administration and that multiple doses of rHuEPO had no direct pressor effects. When rHuEPO was injected IV, a monoeponential decrease in serum EPO level was evident for 18 to 24 hours postdose. Absorption of SC injected rHuEPO occurred more slowly, with relatively low serum EPO levels being maintained for 48 hours. All rHuEPO antibody titer determinations were negative. With the exception of significant increases in hemoglobin and hematocrit, no clinically significant changes occurred. No hypertensive, convulsive, or thrombotic events were observed.

Of the adverse experiences observed in 10 subjects, none was considered clinically significant, and none of the subjects dropped out because of adverse experiences.

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MATERIALS AND METHODS

Study medication. rHuEPO (EPREX®) and placebo preparations for this study were supplied by Amgen, Inc (Thousand Oaks, CA). The carrier was human serum albumin (HSA 2.5 ± 0.5 mg/mL) buffered in citrated saline. Placebo solution was identical to the study medication but did not contain rHuEPO. These solutions were refrigerated at 2° to 8°C and warmed to room temperature before administration.

Study population. The subjects enrolled in this study were in good health, as evidenced by medical history, complete physical examination, and 12-lead electrocardiogram (ECG). They had no clinically significant abnormalities in hemolytic parameters, measured within 2 weeks before study entry. Before enrollment, each subject signed an informed consent form after full explanation of the nature of the study. The study was approved by the local institutional review board.

This study was a double-blind, placebo-controlled, randomized, parallel-group, phase I study. The 32 normal men, ranging in age from 19 to 45 years, consisted of 20 whites, 10 blacks, and 2 American Indians. None had a history of hypertension [diastolic blood pressure (DBP) >90 mm Hg].

Protocol. The 32 subjects were divided into four groups of eight subjects each. Within each group, six subjects were randomly assigned to receive either 150 or 300 U/kg rHuEPO, by IV bolus or SC injection, and two subjects were randomly assigned to receive matching placebo.

rHuEPO was administered on days 1, 4, 6, 8, and 10 of the study. Subjects were confined from at least 12 hours before injection of the first dose until not less than 72 hours after the last injection. Injections were performed 2 hours before breakfast, and no food was ingested for at least 8 hours before each injection.

Pharmacokinetic study. Serum samples were obtained before study and on days 1 and 10 in a 48-hour period after IV or SC injection of rHuEPO. Serum samples were also obtained before injection on days 4, 6, and 8 and also on days 5, 7, and 9 (24 hours after injection). Serum EPO concentrations were determined using a modification of a competitive-binding radioimmunoassay (RIA). The assay has a usable range from 2.5 to 200 mU/mL and does not discriminate between endogenous and recombinant-derived hormone. All assays were performed at R.W. Johnson Pharmaceutical Research Institute. Kinetic parameters were estimated using standard pharmacokinetic equations, assuming a single first-order elimination of rHuEPO from the circulation.

Hemodynamic parameters. Two-dimensional echocardiograms were obtained on the eight patients in the 300 U/kg IV group (six treated with rHuEPO and two controls) before injection of medication on day 1, 2 hours after injection on day 1, and 2 hours after injection on day 10. In addition, systolic BP (SBP), DBP, pulse rates, and ECGs were obtained along with the echocardiograms. On the basis of these data, cardiac output (CO), total peripheral vascular
rhUePO PHARMACOKINETICS IN NORMALS

resistance (TPR), and mean arterial pressure (MAP) were determined. MAP was derived from the formula: MAP (mm Hg) = SBP (mm Hg) - DBP (mm Hg)/3 + DBP (mm Hg). A two-sided paired $t$ test was used to test the significance of changes from baseline in MAP, TPR, and CO among the six subjects who received the study medication. Each of these parameters was tested at an individual level of significance of $P = .05$.

Clinical laboratory evaluations. The total RBC, WBC (including differential), and platelet counts, prothrombin time, and partial thromboplastin time were determined under fasting conditions before the initial dose on day 1 and at study termination on day 13. Samples for rhUePO antibody titers, determined by RIA (Smith Kline Beecham, Van Nuys, CA), were obtained before treatment on day 1, at study termination, and 1 month after administration of the initial dose.

Assessment of hematologic response. Hemoglobin and hematocrit responses were defined as the difference between the baseline value and the mean of values obtained on days 9, 11, and 13 of the study. The mean was calculated to reduce random variations in these values. The reticulocyte count, defined as the percentage of reticulocytes multiplied by the RBC and divided by 100, was measured on day 1 before injection and on day 13 at termination of the study. The effect of treatment on hematocrit, hemoglobin, and reticulocyte count was analyzed by a least-squares approach.

RESULTS

Pharmacokinetics. After administration of 150 or 300 U/kg rhUePO IV on days 1 and 10, a dose-proportional increase in serum EPO concentrations was observed at 0.5 hours after injection. Because no samples were drawn earlier than this time, the EPO concentrations at 0.5 hours were taken as the maximum concentrations attained when EPO was injected by IV bolus. A monoexponential decrease ensued for 18 to 24 hours after injection on both days 1 and 10 for both the 150 U/kg and 300 U/kg doses; the data for the latter group are shown in Fig 1.

After multiple IV doses, rhUePO appeared to be eliminated more rapidly than after a single dose. Although the changes from day 1 to day 10 were not always statistically significant, this trend was reflected in the area under the curve (AUC), systemic clearance ($C_{IS}$), serum EPO concentration 24 hours after injection ($C_{24}$), the elimination rate constant ($k_{el}$), and the resultant harmonic mean half-life ($H_{mt%}$). The apparent volume of distribution ($V_d$) appeared to remain constant over the course of the study and was approximately equal to the plasma volume that would normally be associated with healthy volunteers. These results are summarized in Table 1.

There also appeared to be a trend toward slower elimination of rhUePO as the dose was increased from 150 to 300 U/kg. These trends appear to indicate that dosing regimen and, to a lesser extent, dose level, influence the disposition of rhUePO.

Early studies in rats and dogs\textsuperscript{9,10} suggested that the clearance of EPO from plasma may not be a simple monophasic process. A rapid initial decrease in EPO levels was noted in these investigations. More recent work with iodinated rhUePO has confirmed these observations in rats\textsuperscript{11} and dogs.\textsuperscript{12} A distributional $t/2$ might also be predicted in
humans, but could not be estimated in this study because the earliest blood sample was drawn 0.5 hours postdose.

The reasons for the shortening of the serum t½ over time have not yet been determined. Monitoring the serum EPO 24 hours postdose concentration over the five doses administered in this study indicated that the greatest change in this parameter occurred between the first and second dose. This observation suggests that the decrease in t½ is not a time-dependent process. The kinetic profile after a single dose may not reflect the steady-state clearance of the hormone. This concept is given further support by findings from a multiple-dose study in cynomolgus monkeys. After IV administration of rHuEPO at doses of 100, 500, and 1,000 U/kg, the elimination kinetics remained unchanged from day 5 to day 89 at all three dose levels (Ortho Pharmaceutical, Raritan, NJ; unpublished observations on file).

On both days 1 and 10, SC administered hormone was absorbed slowly, yielding serum EPO concentrations which were low as compared with initial concentrations after IV administration but which were maintained for several hours. On day 1, there appeared to be a reasonable dose proportionality in the maximum serum EPO concentration (Cmax) achieved, even though this did not occur (Tpeak) for several hours postdose (Table 2). This relationship did not hold at day 10, when serum EPO concentrations were very similar to levels achieved with both the 150- and 300-U/kg doses. Figure 2 shows mean serum EPO concentrations after SC administration of 150 and 300 U/kg rHuEPO on days 1 and 10. Serum EPO concentrations were not monitored for a sufficiently long period after SC dosing to estimate a terminal t½.

Even with the difficulty of determining the AUC accurately after SC administration, any estimate will be considerably less than that obtained from the corresponding IV dose. Comparison of AUC on day 1 at both 150 and 300 U/kg indicates that the AUC from SC dosing would be only slightly greater than 15% of the value obtained after IV administration.

Because of the prolonged elevations in serum EPO concentrations with SC injection, injection every 48 hours SC resulted in higher preinjection serum EPO concentrations than with IV injection of the same dose. Despite the apparently low systemic availability resulting from SC injection, rHuEPO exhibited a dose-related effect on hematocrit, hemoglobin, and reticulocyte count that was independent of the route.

**Antibody titers.** Results of RIAs for EPO antibody titers on sera drawn before injection on day 1, at study termination, and 1 month after the initial dose were always negative.

**Hematologic response.** The mean hematocrit responses for each treatment are summarized in Table 3. There was no statistically significant effect of route of administration, nor any statistically significant interaction between dose and route on hematocrit response. The hematocrit response was, however, significantly related to the dose of rHuEPO (P = .0038). The mean increases in hematocrit for 300 U/kg rHuEPO, 150 U/kg rHuEPO, and placebo, IV and SC combined, were 4.22%, 2.57%, and 0.79%, respectively.

Statistical analysis of the hemoglobin response showed no significant effect of route and no significant interaction between dose and route. A significant dose effect (P = .0121) was observed for the increase in hemoglobin. The mean hemoglobin response was greater in groups treated with 300 and 150 U/kg rHuEPO than in the controls. There was no significant difference in the response induced by 300 U/kg and that induced by 150 U/kg rHuEPO (Table 3). In addition, a trend toward higher mean reticulocyte counts with higher doses of rHuEPO was observed, but statistical significance was not achieved. This was largely owing to the variability of reticulocyte response.

No clinically significant or unexpected changes occurred in the means for the total erythrocyte, total leukocyte, or platelet counts from baseline values on day 1 to study termination on day 13. No effect was observed on the prothrombin time or partial thromboplastin time.

### Table 1. Pharmacokinetic Parameters of IV-Administered rHuEPO in 12 Normal Male Subjects

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>rHuEPO 150 U/kg IV Day 1</th>
<th>Day 10</th>
<th>rHuEPO 300 U/kg IV Day 1</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (mU/mL)</td>
<td>3,544 ± 515</td>
<td>3,692 ± 439</td>
<td>7,332 ± 1,314*</td>
<td>6,209 ± 1,000*†</td>
</tr>
<tr>
<td>Cmax (mU/mL)</td>
<td>154 ± 67</td>
<td>67 ± 30†</td>
<td>440 ± 95</td>
<td>181 ± 57†</td>
</tr>
<tr>
<td>AUC (U · h/mL)</td>
<td>25.73 ± 4,71</td>
<td>20.92 ± 5.10†</td>
<td>55.00 ± 5.63*</td>
<td>43.31 ± 1.68*†</td>
</tr>
<tr>
<td>Cl (mL/h/kg)</td>
<td>6.0 ± 1.0</td>
<td>7.5 ± 1.7†</td>
<td>5.6 ± 0.6</td>
<td>6.9 ± 0.2†</td>
</tr>
<tr>
<td>K1 (h⁻¹)</td>
<td>0.131 ± 0.020</td>
<td>0.180 ± 0.024†</td>
<td>0.114 ± 0.007</td>
<td>0.149 ± 0.017††</td>
</tr>
<tr>
<td>Hmat* (h)</td>
<td>5.3</td>
<td>3.9</td>
<td>6.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Vd (mL/kg)</td>
<td>46.7 ± 4.5</td>
<td>41.4 ± 5.3</td>
<td>48.6 ± 7.3</td>
<td>47.1 ± 6.8</td>
</tr>
</tbody>
</table>

*Significantly different (P < .05) from the 150 U/kg dose on the same day.
†Significantly different (P < .05) from day 1 at the same dose.
‡Statistical comparison inappropriate.

### Table 2. Pharmacokinetic Parameters of SC-Administered rHuEPO in 12 Normal Male Subjects

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>rHuEPO 150 U/kg SC Day 1</th>
<th>Day 10</th>
<th>rHuEPO 300 U/kg SC Day 1</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (mU/mL)</td>
<td>144 ± 10</td>
<td>228 ± 68</td>
<td>288 ± 77</td>
<td>285 ± 53</td>
</tr>
<tr>
<td>Tpeak (h)</td>
<td>8-24</td>
<td>5-12</td>
<td>12-24</td>
<td>5-24</td>
</tr>
<tr>
<td>Cmax (mU/mL)</td>
<td>121 ± 24</td>
<td>140 ± 37</td>
<td>241 ± 50</td>
<td>228 ± 56</td>
</tr>
<tr>
<td>AUC0-48 (U · h/mL)</td>
<td>4.06 ± 0.43</td>
<td>6.30 ± 1.42</td>
<td>8.52 ± 2.14</td>
<td>8.28 ± 2.21</td>
</tr>
</tbody>
</table>
Table 3. Mean Hematocrit and Hemoglobin Increase (average of Days 9, 11, and 13 minus baseline) in 32 Normal Male Subjects

<table>
<thead>
<tr>
<th>Dose/Route</th>
<th>N</th>
<th>Mean Hematocrit Increase (%)</th>
<th>Mean Hemoglobin Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 U/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>3.73</td>
<td>1.07</td>
</tr>
<tr>
<td>SC</td>
<td>6</td>
<td>4.71</td>
<td>1.39</td>
</tr>
<tr>
<td>IV + SC*</td>
<td>12</td>
<td>4.22†</td>
<td>1.23†</td>
</tr>
<tr>
<td>150 U/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>2.40</td>
<td>1.56</td>
</tr>
<tr>
<td>SC</td>
<td>6</td>
<td>2.73</td>
<td>0.84</td>
</tr>
<tr>
<td>IV + SC*</td>
<td>12</td>
<td>2.57†</td>
<td>1.20†</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>1.67</td>
<td>0.64</td>
</tr>
<tr>
<td>SC</td>
<td>4</td>
<td>0.008</td>
<td>0.08</td>
</tr>
<tr>
<td>IV + SC*</td>
<td>8</td>
<td>0.79</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*Statistically significantly greater response than placebo IV and SC combined (P < .01).
†Data from IV and SC routes of administration combined.

Figure 3 shows the daily MAP for IV and SC administration routes, respectively, for the duration of the study. There was no change in the daily BP readings within or between treatment groups for either route of administration. In addition, there were no ECG changes from prestudy to poststudy within or between treatment groups for either route of administration.

Adverse experiences. Fifteen adverse experiences were reported in 10 subjects (eight experiences in six treated subjects and seven in four placebo subjects). Three subjects, all in the placebo group, developed liver function test abnormalities. At follow-up, one of these subjects admitted IV drug abuse. Two patients receiving 300 U/kg rHuEPO, one by the SC and the other by the IV route, had increases in serum phosphorus levels. No other adverse experiences were reported by more than one patient.

We observed no hypertensive, convulsive, or thrombotic events. Although such events have been reported in patients with chronic renal failure treated with rHuEPO, these events were far more common in previously hypertensive patients treated with rHuEPO than in normotensive patients; hypertensive patients were excluded from the present study. The absence of these occurrences in healthy volunteers suggests that events of this type observed during treatment of anemia with rHuEPO in chronic renal failure may be owing to the underlying disease or the effect of increasing hematocrit rather than to a direct pressor effect of rHuEPO itself.

One patient (300 U/kg rHuEPO SC) experienced a brief
DISCUSSION

The present study confirmed the safety of rHuEPO administration at 150 and 300 U/kg in normal men. No clinically significant changes in hemodynamic parameters were observed. Even with the apparently low systemic availability of rHuEPO from SC dosing, the observed increases in hemoglobin and hematocrit suggest that SC administration could be as effective as IV administration of rHuEPO for treating anemia in suitably selected patients. Results from clinical trials must be awaited to determine whether rHuEPO administration will benefit patients (and if so, at what dosages) with anemias accompanying a variety of medical conditions.

ACKNOWLEDGMENT

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REFERENCES


![Fig 3. MAP over treatment time, daily average of pre- and postdose readings, in normal subjects receiving rHuEPO IV or SC.](image-url)
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