A double-blind, placebo-controlled study of the pharmacokinetics and safety of multiple doses of recombinant human erythropoietin (rHuEPO) has been shown in clinical trials to be safe and effective in treating anemia of chronic renal failure. 

Materials and Methods

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). The dosages needed to achieve the desired rate of hematocrit increase in these other conditions may be higher than the dosage required in chronic renal failure, however (eg, 50 U/kg three times a week).

Studies of rHuEPO at higher dosages in normal subjects have demonstrated the safety of a single intravenous (IV) dose of 100 U/kg and of multiple IV doses up to 300 U/kg for 14 days (Ortho Pharmaceutical, Raritan, NJ; unpublished observations on file). To obtain information of potential value for dosing, we investigated the pharmacokinetics and effects of rHuEPO in normal volunteers. Because subcutaneous (SC) rather than IV administration of the hormone would simplify outpatient treatment, serum concentrations of EPO were compared at various times after SC and IV administration to establish the kinetics and systemic availability of rHuEPO administered by these two routes.

Because hypertension and the potential for increased cardiovascular morbidity are concerns attending rHuEPO administration to dialysis patients, we also examined the effects of multiple doses of rHuEPO on hemodynamics and associated clinical parameters in male volunteers with uncompromised cardiovascular systems.

Recombinant Human Erythropoietin (rHuEPO) has been shown in clinical trials to be safe and effective in treating anemia of chronic renal failure. Clinical observations as well as theoretic considerations suggest that it may also be effective in treating anemia in other conditions associated with insufficient endogenous EPO production and possibly with reduced bone marrow (BM) response to normal EPO levels (eg, anemias accompanying chronic disease, cancer and its treatment, and acquired immunodeficiency syndrome [AIDS]). The dosages needed to achieve the desired rate of hematocrit increase in these other conditions may be higher than the dosage required in chronic renal failure, however (eg, 50 U/kg three times a week).

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Resistance (TPR), and mean arterial pressure (MAP) were determined. MAP was derived from the formula: \( \text{MAP (mm Hg)} = \text{SBP (mm Hg)} - \text{DBP (mm Hg)} / 3 + \text{DBP (mm Hg)} \). 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 monoeponential 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 (Cl_s), serum EPO concentration 24 hours after injection (C_{24h}), the elimination rate constant (\( k_{el} \)), and the resultant harmonic mean half-life (Hmt%). 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\(^9,10\) 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\(^11\) and dogs.\(^12\) A distributional \( t/2 \) might also be predicted in
The observation suggests that the decrease in is not a dose may not reflect the steady-state clearance of the administration of rHuEPO at doses of 100, 500, and parameter occurred between the first and second dose. This in this study indicated that the greatest change in this from a multiple-dose study in cynomolgus monkeys. After IV 

hours postdose concentration over the five doses administered have not yet been determined. Monitoring the serum EPO 24

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 $t_{1/2}$ 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 $t_{1/2}$ 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 ($C_{\text{max}}$) achieved, even though this did not occur ($T_{\text{peak}}$) 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 $t_{1/2}$.

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 response for each treatment is 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 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>
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<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.

Hemodynamic effects. Statistical analysis of differences in echocardiographic test results from baseline (before injection on day 1) to 2 hours after injection on day 10 for the six patients treated with 300 U/kg rHuEPO IV showed some changes that were statistically significant (Table 4). These changes are not believed to be clinically relevant, however, particularly because the predose day 1 MAP (94 mm Hg) for the rHuEPO 300-U/kg group appeared to be atypically high as compared with the prestudy value and subsequent mean daily values for this group (Fig 3).

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,14 these events were far more common in previously hypertensive patients treated with rHuEPO than in normotensive patients1; 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
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.

Fig 3. MAP over treatment time, daily average of pre- and postdose readings, in normal subjects receiving rHuEPO IV or SC. Episode of epistaxis on day 5; this resolved spontaneously after 15 minutes. Another patient receiving 300 U/kg rHuEPO IV had mild polycythemia. His hematocrit value (54.0) continued to be elevated 26 days after study. Neither of these adverse experiences was considered clinically significant.

Serum chemistry and urinalysis. Aside from the events already described, no abnormalities in serum chemistry or any clinically significant changes in urinalysis parameters were noted.

Physical examination and vital signs. No clinically significant changes between findings before and after the study were observed in the physical examination findings for any of the subjects. MAP in the various groups is shown in Fig 3. No clinically significant changes in MAP occurred during the study, and no hypertensive events were reported.

ECGs. One subject who received 150 U/kg rHuEPO IV had a borderline abnormality (sinus tachycardia) on the 12-lead ECG taken on day 13. The sinus rate had returned to normal on a repeat ECG taken during the follow-up visit.

REFERENCES
Pharmacokinetics and effects of recombinant human erythropoietin after intravenous and subcutaneous injections in healthy volunteers

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