Pharmacokinetic and Pharmacodynamic Modeling of Recombinant Human Erythropoietin after Intravenous and Subcutaneous Dose Administration in Cynomolgus Monkeys

ROHINI RAMAKRISHNAN, WING K. CHEUNG, FRANCIS FARRELL, LINDA JOFFEE, and WILLIAM J. JUSKO

Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, State University of New York at Buffalo, Buffalo, New York (R.R., W.J.J.); and R.W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey (W.K.C., F.F., L.J.)

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ABSTRACT
The pharmacokinetics (PK) and pharmacodynamics (PD) of recombinant human erythropoietin (rHuEpo) were investigated in monkeys. A two-compartment model with dual input and nonlinear disposition could adequately characterize the PK of rHuEpo upon three intravenous and six s.c. administrations. The kinetic model suggests rapid zero-order absorption of part of the s.c. dose (35%) followed by a slow first-order entry through the lymphatics. The s.c. treatments caused a delayed dose-dependent rise in reticulocyte numbers peaking between 100 and 200 h and returning to baseline by 300 to 400 h. This was followed by steady rises in red blood cell (RBC) and hemoglobin counts. A physiological catenary model based on a life span concept with rHuEpo stimulating the production of two cell populations (progenitor cells and erythroblasts) was applied. The model could adequately describe the reticulocyte responses upon the various s.c. treatments, giving estimates of maturation times for cells in the various stages of differentiation including the early progenitor cells (70.4 h), erythroblasts (15.0 h), and reticulocytes (141.6 h) that are close to the literature reported values. An $S_{max}$ of 3.13 was estimated indicating a moderate maximum stimulation of erythropoiesis, whereas the $SC_{50}$ was 842 IU/l. The model was used to effectively predict the increases in RBC and hemoglobin counts as well. In conclusion, the physiological PK/PD model developed could adequately describe the time course of rHuEpo effects, yielding realistic estimates of cell life span parameters.

Erythropoiesis involves a sequence of cellular differentiations that are controlled by specific hematopoietic growth factors. Erythropoietin (Epo) is a key lineage-specific humoral regulator of mammalian erythropoiesis. The recombinant form of human erythropoietin (rHuEpo) is structurally very similar to endogenous Epo (Egrie, 1990). It exerts its biological effects by binding to specific receptors in the bone marrow cells, which causes them to undergo a 5- to 9-day process of cellular proliferation, differentiation, and maturation leading to an increase in reticulocyte counts followed by rises in hematocrit and hemoglobin levels in the blood (Flaharty, 1990).

rHuEpo has been shown to be an effective alternative to blood transfusion, ameliorating anemia associated with a variety of indications and producing consequent improvements in quality of life in many renal (Lundin et al., 1990) and nonrenal applications (Markham and Bryson, 1995) in humans. Several investigators have reported pharmacokinetic and dynamic studies of rHuEpo in humans and many animal species, including mouse, rat, dog, rabbit, sheep, and horse (Fu et al., 1988; Jaussaud et al., 1994; Bleuel et al., 1996; Souillard et al., 1996; Widness et al., 1996; Yoon et al., 1997; Cheung et al., 1998; Chapel et al., 2000; Kato et al., 2001). A clear mathematical quantification of the kinetics and dynamics of rHuEpo effects would greatly facilitate rational design of optimal dosage regimens and aid therapy. The Epo receptor structure is reasonably conserved among mammalian species, allowing for cross-species biological activity. The cynomolgus monkey is one species that has shown response to rHuEpo and has been used in previous studies with related compounds and hence was chosen as a suitable preclinical model.

Our main objective was to develop a mechanistic PK/PD model to characterize the pharmacokinetics (PK) and pharmacodynamics (PD) of rHuEpo in terms of increased reticulocyte, red blood cell, and hemoglobin counts in blood after intravenous administration of three single doses and subcutaneous administration of six single doses of rHuEpo (Eprex;
J&J, Raritan, NJ) in male cynomolgus monkeys. The pharmacokinetics of rHuEpo has been shown to be strikingly different depending on the route of administration (Markham and Bryson, 1995). Regular intravenous administration in humans is inconvenient, needs very frequent dosing, and is impractical for certain conditions such as continuous ambulatory peritoneal dialysis and nondialysis patients with restricted vascular access (Lui et al., 1990). The prolonged stimulation of the erythroid cells by persisting low levels of rHuEpo upon s.c. dosing have been implicated in the better efficiency of this route of administration in maintaining a target hemoglobin level.

We present here a comprehensive PK/PD model describing the time course of rHuEpo effects, which provides direct mechanistic linkages between drug kinetics and the sequential responses. Such a model would help in designing future studies in monkeys and physiological parameters in the model could be scaled to perform simulations to predict responses in human and other species as well.

**Materials and Methods**

Data were obtained from two studies performed by R. W. Johnson Pharmaceutical Research Institute. The first study was a parallel group study performed in 12 male cynomolgus monkeys. Monkeys were divided into four groups, one group being the control and the other three being injected intravenously with 500, 2,000, or 4,000 IU/kg Eprex. Blood samples were drawn predose and up to 48 h for measuring rHuEpo concentrations. The second study was a parallel group study done in 21 male cynomolgus monkeys, which were divided into seven groups with three monkeys per group. The control group received subcutaneously sterile saline, whereas the remaining six groups were administered 400, 1,000, 2,400, 5,000, 20,000, and 40,000 IU/kg Eprex subcutaneously. Blood samples were drawn predose and at various times after administration up to day 30 for rHuEpo concentrations as well as reticulocyte, erythrocyte, and hemoglobin counts. Animals were assigned so as to have a uniform body weight distribution across groups in all the studies.

The serum rHuEpo concentrations were measured using an established and validated radioimmunoassay with a limit of quantification of 7.8 mIU/ml as described by Cheung et al. (1998). The assay probably measures endogenous monkey Epo, but baseline values were below the limit of quantification. Hematocrit and hemoglobin were measured by conventional clinical methods. Reticulocyte counts were measured using flow cytometry. The mean data were used for all analyses.

**Model.** A schematic representation of the PK/PD model is depicted in Fig. 1.

**Pharmacokinetics.** A two-compartment model was chosen to account for the polyexponential decline in the kinetic profiles upon intravenous administration. Noncompartmental analysis indicated nonlinearity in the kinetics, which was modeled using the Michaelis-Menten disposition function. A dual absorption kinetic model with a rapid zero-order input of a fraction of the dose followed by a slow first-order input of the remainder was used to characterize the absorption of rHuEpo upon subcutaneous administration. The six single subcutaneous doses as well as the three intravenous doses in male monkeys were fitted simultaneously to this model to obtain a common set of parameters to characterize all the data.

The differential equations used for modeling the intravenous kinetics were as follows:

\[
\frac{dA_p}{dt} = - \frac{V_{max} \cdot A_p}{(K_m + V_d + A_p)} - k_{12} \cdot A_p + k_{21} \cdot A_t \quad \text{where } A_{p(0)} = \text{Dose} \quad (1)
\]

\[
\frac{dA_t}{dt} = k_{12} \cdot A_p - k_{21} \cdot A_t \quad \text{where } A_{t(0)} = 0 \quad (2)
\]
and recently adapted for Epo and reticulocyte responses by Chapel et al. (2000).

As depicted in Fig. 2, erythropoiesis involves a cascade of events. The first precursor compartment in the model is representative of all the earliest progenitor cells in the bone marrow involved in this process, which are eventually converted to erythroblasts. The time $T_{P1}$ therefore essentially serves as an average length of time taken for the earliest precursor cell stimulated by rHuEpo to undergo the cascade of differentiation processes to finally get converted to an erythroblast. The time $T_{P2}$ represents the average time taken for an erythroblast to be converted to a reticulocyte. As a result, $T_{P2}$ accounts for the initial time delay seen for reticulocytosis to be initiated by rHuEpo. Once a reticulocyte is formed, it slowly matures over a time equal to $T_R$ at which point it is converted into a RBC. It is assumed that the primary way by which a reticulocyte could be lost is by conversion to an erythrocyte. The model does not account for random destruction of cells such as bleeding. Hence, the production and elimination rate of all these cells can be represented by a single zero-order rate constant $k_0$. Once a RBC is produced, it in turn survives for a period of $T_{RBC}$ days after which it disappears from blood.

The baseline rHuEpo concentrations were assumed to be zero and hence the baseline reticulocyte level was given by $k_0 \cdot T_R$. The differential equation used for estimation purposes were as follows:

$$\frac{dR}{dt} = k_0 \cdot \left( (1 + S(t - T_{P2}))/(-1 + S(t - T_{P2} - T_{R})) \right) \cdot \left( (1 + S(t - T_{P1} - T_{P2}))/(-1 + S(t - T_{P1} - T_{P2} - T_{R})) \right)$$

with the stimulation function given by the simple nonlinear equation:

$$S(t) = \frac{S_{max} \cdot C_{EPO}}{SC_{50} + C_{EPO}}$$

The reticulocyte numbers after administration of the six dose levels of Epres were fitted to eq. 5 to get a single set of dynamic parameters characterizing the data across all doses. The parameters for the kinetic model were fixed and used as the forcing function for the dynamics. The predose reticulocyte counts were fixed to be the baseline values. Area under the effect curve values were calculated using the trapezoidal rule.

The dynamic parameters obtained from the reticulocyte fittings were used to simulate the RBC numbers and hemoglobin levels for all the doses. The RBC in monkeys are known to have a mean potential life span of 70 to 90 days (Landaw, 1988), and therefore it was assumed that the RBC produced simply accumulate over the 1-mo period of the study. The 0- and 48-h RBC counts were used as the baseline, whereas the hemoglobin content per cell was fixed for each group from the ratio of the predose hemoglobin count to the predose total number of cells (RBC + reticulocytes) for that group.

$$Hb = Hb_{pred} \cdot (RBC_i + R_i)$$

The differential equations used for simulation purposes were as follows:

$$\frac{dRBC}{dt} = k_0 \cdot \frac{(1 + S(t - T_{P2} - T_{R})) \cdot (1 + S(t - T_{P1} - T_{P2} - T_{R})) -}{(1 + S(t - T_{P2} - T_{R} - T_{RBC})) \cdot (1 + S(t - T_{P1} - T_{P2} - T_{R} - T_{RBC}))}$$

All the fittings and simulations were performed using the ADAPT II program (D’Argenio and Schumitzky, 1997) by the maximum likelihood method. It was assumed that the observed and predicted values for any specific time point are normally distributed. The extended least-squares variance model was applied as follows: $V_i = \sigma_i^2 \cdot M_i$ where $V_i$ is the variance of the $i$th data point, $\sigma_i$ is the vector of structural parameters, $\sigma_1$ and $\sigma_2$ are the vectors of variance parameters, $t_i$ is the $i$th time, and $M$ is the $i$th predicted value.

**Results**

Figure 3 shows the fittings for the rHuEpo concentration-time profiles after administration of three single intravenous doses and six single subcutaneous doses of Epres in male cynomolgus monkeys. The parameters obtained are listed in Table 1. The program was unable to estimate coefficient of variation percent. The two-compartment kinetic model with nonlinear disposition could adequately capture the multiphasic intravenous kinetic profiles, although the terminal phase for the lowest dose is slightly overestimated. A high $K_m$ value was estimated, which indicates that the nonlinearity in disposition is mild and would be prominent only at high doses. The central volume of distribution $V_d$ was estimated to 57 ml/kg, which is close to the plasma volume. For the subcutaneous administrations, the bioavailability increased with dose with the lowest dose showing a bioavailability of 26.8% and the next higher dose 73%. The lowest dose has a slightly different $k_d$ value compared with the rest of the doses. It can be inferred from the parameter estimates that a major fraction of the bioavailable dose follows the slow first-order pathway. The zero-order route of entry (where input = Fr Dose/r) seems to be fast and accounts for a smaller fraction (35.5%) of the bioavailable dose. We attempted to fit a Michaelis-Menten function to account for drug loss at the s.c. site, but the fittings were worse than presently shown. The present model assumes that the unasorbed drug never participates in the input process. A model two-step model was applied by Radwanski et al. (1998) after s.c. interleukin-10, a compound that also exhibited incomplete availability (42%). Better understanding of the absorption and loss processes associated
with s.c. doses of proteins and lymphatic transfer is needed (Porter and Charman, 2000).

All the s.c. doses resulted in dose-dependent increases in reticulocyte counts that peaked between 100 and 200 h and returned to baseline by 300 to 400 h. All the treatments with the exception of the lowest caused steady increases in RBC numbers that were followed by a corresponding rise in the hemoglobin concentrations. The reticulocyte fittings in male monkeys are shown in Fig. 4, and Table 2 lists the pharmacodynamic parameters estimated. The lag time that is accounted for by the second precursor compartment $T_{p2}$ was small (~15 h). The estimated reticulocyte life span was close to 6 days. The $S_{max}$, which signifies the maximum possible increase in production rate, was 3.13, whereas a high SC$_{50}$ value of 842 IU/l was estimated. Figures 5 and 6 show simulations for the RBC counts and hemoglobin response over the month period. There was a good agreement between the observed rise in hemoglobin levels and the model predictions. The reticulocyte counts could well predict the rise in RBC counts at times later than 9 days, including the plateau. However, the model consistently underpredicted the early two RBC time points of 6 and 9 days. The predicted reticulocyte life span being 141.6 h, the high RBC numbers seen at this time frame of 144 and 216 h should be reflective of the reticulocyte levels at 2.4 to 74.4 h. One explanation for the discrepancy between the observed and expected RBC counts at the early time points would be a higher than expected blood reticulocyte count at these times. Unfortunately, there was only one data point in this time range. Another possibility is that there might be a nonuniform effect on late-stage mitotic precursors that is not represented in the model structure. There is evidence that Epo accelerates the release of reticulocytes from the marrow (Jelkmann, 1992). The early release of immature reticulocytes from the marrow upon dosing may have resulted in a higher than predicted RBC count on days 6 and 9.

**Discussion**

Previous studies in rats (Steinberg et al., 1986), dogs (Fu et al., 1988), sheep (Chapel et al., 2000), and humans (Veng-Pedersen et al., 1995) have demonstrated that a simple monoeponential process may not account for the disposition of Epo from plasma. We observed that the kinetics of rHuEpo followed a biexponential decline upon intravenous administration in monkeys, and this was captured using a two-compartment model with nonlinear disposition. Although the elimination half-life has been shown to vary between species, the $V_d$ is generally consistent with distribution in plasma among species similar to what we observed in monkeys. The primary site of action of rHuEpo is the bone marrow, which is a highly perfused tissue, and so the peripheral compartment in the model may only represent some nonspecific binding of rHuEpo. The terminal phase for the lowest dose was underestimated, which could be due to a different kinetic behavior at very low Epo concentrations. Indeed, Veng-Pedersen et al. (1995) have found a low-capacity, high-affinity process for removal of tracer doses of Epo in humans that could also be occurring in these monkeys and explain the low-dose i.v. and s.c. results. However, this pathway would be easily saturated and contribute very little to the disposition at high Epo concentrations. The kinetics of intravenous Epo were nearly linear and well characterized for most of the dose range. The modest nonlinearity may be due to saturation in renal or proteolytic disposition of Epo. An improved model, however, would include both nonlinear components.

A population PK analysis of rHuEpo in healthy adult male volunteers has been performed previously (Hayashi et al., 1998) using a model with first-order absorption and linear
elimination. There were only two low s.c. doses administered in that study and the rHuEpo concentrations had almost returned to baseline in 3 days. We observed in our study in monkeys that after s.c. dosing, the peak concentrations of rHuEpo were attained within 1 day and rHuEpo remained in circulation for a much longer time. Hence, it was necessary for us to apply empirically a dual absorption model with a fast zero-order absorption component governing the early rise in concentrations and the slow first-order absorption governing the terminal phase. Occurrence of the flip-flop phenomenon and incomplete availability has been observed to be fairly common with subcutaneous absorption of macro-molecules (Radwanski et al., 1998; Mager and Jusko, 2002). The initial concentrations for the highest subcutaneous dose were slightly overestimated. However, this should be acceptable considering the fact that a single set of parameters was used to describe all the dose levels. Our kinetic model can be used to explain the different pathways of absorption of the drug from the subcutaneous site. The rapid zero-order input of a part of the dose might suggest a direct entry via blood vessels in the subcutaneous site to the blood. On the other hand, a major fraction of the dose can be assumed to enter the lymphatics and undergo a slow process of first-order absorption from the lymph to the blood. It has been reported (Porter and Charman, 2000) that compounds with molecular mass above 4 kDa are absorbed increasing in proportion to molecular mass via the lymph. This phenomenon might explain the 10-h time lag we saw for start of the first-order absorption. The bioavailability increased with dose and was 100% for doses of 2400 IU/kg and higher.

Complex mathematical models describing regulation of erythropoiesis in mice and rats have been developed previously (Loeffler et al., 1989; Wichmann et al., 1989; Wulff et al., 1989). These physiological models elaborately include most components of the erythropoietic system using closed interacting feedback loops. Although these models can give a comprehensive mathematical means to describe the process of hematopoiesis, their applicability in characterizing typical pharmacodynamic data obtained from preclinical and clinical studies is limited due to the complexity involved and lack of feasibility of obtaining data on various cell populations in the bone marrow. A linear pharmacodynamic model using the Bateman function has been developed previously to describe the pharmacodynamics of rHuEpo in hemodialysis patients (Brockmoller et al., 1992). However, the model was highly empirical and loses utility in extrapolating to doses beyond that used in the study. Uehlinger et al. (1992) have proposed

### TABLE 2
Pharmacodynamic parameters for rHuEpo in male cynomolgus monkeys

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{p1}$ (h)</td>
<td>70.38</td>
<td>32.19</td>
</tr>
<tr>
<td>$T_{p2}$ (h)</td>
<td>14.95</td>
<td>73.25</td>
</tr>
<tr>
<td>$T_{R}$ (h)</td>
<td>141.6</td>
<td>19.07</td>
</tr>
<tr>
<td>$S_{max}$</td>
<td>3.133</td>
<td>8.07</td>
</tr>
<tr>
<td>$SC_{50}$ (IU/l)</td>
<td>842.5</td>
<td>28.18</td>
</tr>
</tbody>
</table>

CV%, coefficient of variation percentage.

![Figure 4](image-url)

**Fig. 4.** Time course of blood reticulocyte counts for the different s.c. dosage regimens. Solid circles are the mean data and error bars are the standard deviations. Solid lines are the simultaneous fittings for data from all the doses using eq. 5.
a model based on the cell life span concept that describes changes in hematocrit during rHuEpo therapy. Although the approach they suggested seems to have potential value for clinical use, they had to approximate the pharmacokinetics of the drug as a constant rate infusion. Thus, the model loses relevance in assessing the importance of drug kinetics in modifying the responses or comparing different drug formulations. We have presented here a simple mechanistic approach to modeling the changes in cell response variables based on a multiple-pool life span concept with temporal changes in drug concentrations controlling the rate and extent of changes in responses. This catenary life span approach was recently adapted by Chapel et al. (2000) to describe reticulocyte responses to Epo in phlebolomized sheep using a linear function to capture the effects of Epo. There are imperfections in modeling both the kinetics and dynamics in our studies that yield insights into added complexities and that may stimulate further investigation and development of improved models.

The exact mode of action of erythropoietin is still not fully understood. The primary action of rHuEpo was thought to be stimulation of the proliferation of early progenitor cells. However, there is evidence from studies on experimental animals that erythropoietin acts on the differentiated erythroblasts as well (Krantz and Jacobson, 1970). This has led to the proposal that rHuEpo acts on the mature erythroblasts to give rise to an early 24-h reticulocyte response followed by a macrocytosis owing to an additional effect on normoblasts. Based on this theory, we developed the mechanistic catenary aging model with rHuEpo stimulation occurring at two precursor cell populations, which might represent the erythroblasts and the earlier progenitor cells.

Erythroblasts are known to undergo two to five cell divisions with a mean maturation or turnover time of 11 to 48 h, depending on the species (Osgood, 1954; Alpen and Cranmore, 1959; Fliedner et al., 1959; Krantz and Jacobson, 1970). Our model predicts that there is a 15- to 18-h lag time before the newly produced reticulocytes are actually released into circulation, and this reflects the erythroblast maturation time. The estimated reticulocyte life span was 6 days. In humans, the normal life span of cells in the reticulocyte stage is around 2 days in the marrow and 1 to 2 days in the blood (Hillman and Finch, 1967). However, in animal models of severe anemia, it has been demonstrated that the marrow reticulocyte pool is shifted to the circulation (Bessis and Weed, 1973; Hillman and Finch, 1967). These displaced marrow reticulocytes take up to 3 days longer than normal reticulocytes to produce erythrocytes. Hence, we could expect that the average life span of reticulocytes estimated by our model reflects the sum of the maturation times in the marrow and blood. It has been reported that in humans, it takes an average of 5 days for an erythroid precursor to form a reticulocyte in the marrow (Krantz and Jacobson, 1970). This time actually reflects the sum of the times a cell spends in the P1 and P2 compartments, which was estimated to be 85 to 119 h. Thus, our physiological PK/PD model seems to well approximate the kinetics and rHuEpo effects, yielding realistic estimates of cell aging parameters.
The present study was a parallel group design, and the data from each dosing and treatment group were averaged for analysis. Preliminary examination showed that this did not introduce any bias into the absorption and disposition profiles and allowed us to formulate and resolve the proposed PK/PD model. Assessment of nonlinear kinetic and dynamic processes requires data across a range of doses that can be simultaneously fitted. The large number of doses and routes, the complexity of the overall model, and the necessity of operating delay differential equations created difficulties in performing the nonlinear regression analysis even with mean data. It would be desirable to expand the modeling to a population assessment to jointly fit the kinetics and responses and recover information about interindividual variability. It would be a formidable computational and modeling challenge at present.

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Address correspondence to: Dr. William J. Jusko, Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, 565 Hochstetter Hall, State University of New York at Buffalo, Buffalo NY 14260. E.mail: wjjusko@buffalo.edu