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

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Figures: 6

References: 36

Abstract: 230

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Recommended section assignment: Hematology

Abbreviations

Aₚ - amount of drug in the plasma compartment; Aᵦ - amount of drug in the peripheral compartment; k₁₂/ k₁₂ – first-order rates of transfer between the central and peripheral compartments; Vₘₐₓ - Michaelis-Menten constant representing capacity of the process; Kₘₐₚ - Michaelis-Menten constant representing Epo concentration at one-half of Vₘₐₓ; Fr - fraction of the dose associated with the first-order pathway of absorption k₁; τ - time at which the zero-order input (kₒ) stops and first-order input (k₁) starts; F - bioavailability of rHuEpo upon subcutaneous administration; k₀ - zero-order rate of production of cells; Cₑₚₒ - concentration of Epo in plasma at time t; Sₘₐₓ - maximum possible stimulation factor for cell production; SC₅₀ - Epo concentrations at which one-
half $S_{\text{max}}$ is reached; $R$ - reticulocyte count at time $t$; $\text{RBC}_t$ - red blood cell count at time $t$; $T_P$ - amount of time each cell spends in the precursor compartment $P_1$; $T_{P2}$ - amount of time each cell spends in the precursor compartment $P_2$; $T_R$ - amount of time each cell spends in the reticulocyte compartment $R$; $T_{\text{RBC}}$ - amount of time each cell spends in the red blood cell compartment $\text{RBC}$; $\text{Hb}$ - hemoglobin levels contributed by cells in compartments $R$ and $\text{RBC}$. 
ABSTRACT

The pharmacokinetics (PK) and pharmacodynamics (PD) of recombinant human erythropoietin (rHuEpo) was investigated in monkeys. A two-compartment model with dual input and nonlinear disposition could adequately characterize the PK of rHuEpo upon 3 intravenous and 6 subcutaneous (SC) administrations. The kinetic model suggests rapid zero-order absorption of part of the SC dose (35%) followed by a slow first-order entry through the lymphatics. The SC treatments caused a delayed dose-dependant rise in reticulocyte numbers peaking between 100 and 200 hr and returning to baseline by 300-400 hr. This was followed by steady rises in red blood cell (RBC) and hemoglobin counts. A physiological catenary model based on a lifespan 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 SC treatments giving estimates of maturation times for cells in the various stages of differentiation including the early progenitor cells (70.4 hr), erythroblasts (15.0 hr) and reticulocytes (141.6 hr) which are close to the literature reported values. An $S_{\text{max}}$ of 3.13 was estimated indicating a moderate maximum stimulation of erythropoiesis while the $SC_{50}$ was 842 IU/L. The model was utilized 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 lifespan 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 biologic 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).

Recombinant humanized Epo (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 non-renal applications (Markham and Bryson, 1995) in man. Several investigators have reported pharmacokinetic and dynamic studies of rHuEpo in man and many animal species including mouse, rat, dog, rabbit, sheep and horse (Bleuel et al., 1996; Chapel et al., 2000; Cheung et al., 1998; Fu et al., 1988; Jaussaud et al., 1994; Kato et al., 2001; Souillard et al., 1996; Widness et al., 1996; Yoon et al., 1997). 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 in order 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) 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 non-dialysis patients with restricted vascular access (Lui et al., 1990). The prolonged stimulation of the erythroid cells by persisting low levels of rHuEpo upon SC 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 man and other species as well.
Methods

Data were obtained from two studies performed by RW Johnson Pharmaceutical Research Institute. The first study was a parallel group study performed in 12 male cynomolgus monkeys. Monkeys were divided into 4 groups, one group being the control while the other three being injected intravenously with 500, 2000 and 4000 IU/kg of Eprex. Blood samples were drawn predose and up to 48 hours for measuring rHuEpo concentrations. The second study was a parallel group study done in 21 male cynomolgus monkeys, which were divided into 7 groups with 3 monkeys per group. The control group received subcutaneously sterile saline while the remaining six groups were administered 400, 1000, 2400, 5000, 20000 and 40000 IU/kg of 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 previously 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 Figure 1.

Pharmacokinetics: A two-compartment model was chosen to account for the polyexponential decline in the kinetic profiles upon intravenous administration. Non-compartmental analysis indicated non-linearity 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 in order 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:

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

\[
\frac{dA_t}{dt} = k_{12} \cdot A_p - k_{21} \cdot A_t \quad \text{where} \quad A_t(0) = 0 \quad (2)
\]

The subcutaneous data were modeled with the following equations:

\[
\frac{dA_p}{dt} = k_{a(0-\tau)} + k_{a(\tau-t)} - \frac{V_{\text{max}} \cdot A_p}{(K_m \cdot V_d + A_p)} - k_{12} \cdot A_p + k_{21} \cdot A_t \quad (3)
\]

\[
\frac{dA_t}{dt} = k_{12} \cdot A_p - k_{21} \cdot A_t \quad (4)
\]

where \( A_p(0) \) and \( A_t(0) \) are 0.

\[
k_o = 0 \quad \text{when} \quad t > \tau
\]

\[
k_o = \frac{F \cdot (1 - Fr) \cdot \text{Dose}}{\tau} \quad \text{when} \quad 0 < t \leq \tau
\]

\[
k_1 = 0 \quad \text{when} \quad t \leq \tau
\]

and

\[
k_1 = k_a \cdot F \cdot Fr \cdot \text{Dose} \cdot e^{-(k_a \cdot (t - \tau))} \quad \text{when} \quad t > \tau
\]
The time period ($\tau_1$) for the zero-order input ($k_o$) was fixed to 10 hours based on the data and initial runs. A single first-order rate of absorption $k_a$ could describe all the doses except the lowest dose (400 IU/kg) for which a separate $k_a$ was estimated. The bioavailability $F$ appeared to change with dose and was estimated for the lowest two doses and fixed to 100% for the remaining doses.

**Pharmacodynamics:** A catenary pharmacodynamic model (Figure 1) with two precursor cell compartments having different lifespans was applied for the pharmacodynamics of rHuEpo. Stimulation of production was assumed to occur for both precursor pools.

According to this model, all cells involved in the process of erythropoiesis are assumed to be produced in a zero-order fashion ($k_0$), they exist for a fixed time period at the end of which they die or are converted to other cells. As a result, the cells are lost at a rate that is exactly equal to the rate at which they were born, except that their elimination is delayed by a time period, which is equal to the lifespan of the cell. It is assumed that the lifespan of any single set of cells is constant with respect to time and is the same for each cell of that type. A review of basic PK/PD models based on lifespan concepts has been published (Krzyzanski *et al.*, 1999) and recently adapted for Epo and reticulocyte responses by Chapel *et al* (2000).

As depicted in Figure 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 period 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 to 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:

\[
\frac{dR}{dt} = k_0 \left( (1 + S(t - T_{P2})) \cdot (1 + S(t - T_{P1} - T_{P2})) \right) \\
\left( - (1 + S(t - T_{P2} - T_R)) \cdot (1 + S(t - T_{P1} - T_{P2} - T_R)) \right)
\]

with the stimulation function given by the simple nonlinear equation:

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

The reticulocyte numbers after administration of the six dose levels of Eprex were fitted to equation 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 curves (AUEC) 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 lifespan of 70 to 90 days (Landaw, 1988) and therefore, it was assumed that the RBC produced simply accumulate over the one-month period of the study. The 0 and 48-hr RBC counts were used as the baseline while 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.

\[
Hb_t = Hb_{cell} \cdot (RBC_t + R_t)
\]

The differential equations used for simulation purposes were as follows:

\[
\frac{dRBC}{dt} = k0 \cdot \left( \frac{(1 + S(t - T_{p1} - T_R)) \cdot (1 + S(t - T_{p2} - T_R))}{(1 + S(t - T_{p2} - T_R - T_{RBC})) \cdot (1 + S(t - T_{p1} - T_{p2} - T_R - T_{RBC}))} \right)
\] (6)

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:

\[
V_i = V(\theta, \sigma, t_i) = \sigma_1^2 \cdot M(\theta, t_i)^\sigma; \text{ where } V_i \text{ is the variance of the } i\text{th data point, } \theta \text{ is the vector of structural parameters, } \sigma_1 \text{ and } \sigma_2 \text{ are the vectors of variance parameters, } t_i \text{ is the } i\text{th time, and } M(\theta, t_i) \text{ is the } i\text{th predicted value.}
\]
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 Eprex in male cynomolgus monkeys. The parameters obtained are listed in Table 1. The program was unable to estimate CV%. The two-compartment kinetic model with non-linear 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 non-linearity 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 $k_a$ value compared to 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 = $F_r \times \text{Dose}/\tau$) 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 SC site, but the fittings were worse than presently shown. The present model assumes that the unabsorbed drug never participates in the input process. A similar model two-step model was applied by Radwanski et al (1998) following SC interleukin-10, a compound that also exhibited incomplete availability (42%). Better understanding of the absorption and loss processes associated with SC doses of proteins and lymphatic transfer is needed (Porter and Charman, 2000)
All the SC doses resulted in dose-dependant increases in reticulocyte counts that peaked between 100 and 200 hr and returned to baseline by 300-400 hr. 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 Figure 4, and Table 2 lists the pharmacodynamic parameters estimated. The lag time which is accounted for by the second precursor compartment T_{P2} was small (~15 hr). The estimated reticulocyte lifespan 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 Hb 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 lifespan being 141.6 hr, the high RBC numbers seen at this time frame of 144 and 216 hr should be reflective of the reticulocyte levels at 2.4 hr to 74.4 hr. 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 non-uniform 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 man (Veng-Pedersen et al, 1995) have demonstrated that a simple monoexponential 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 non-linear disposition. Although the elimination half-life has been shown to vary between species, the Vd 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 non-specific binding of rHuEpo. The terminal phase for the lowest dose was overestimated which could be due to a different kinetic behavior are 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 man which could also be occurring in these monkeys and explain the low-dose IV and SC 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, RES, or proteolytic disposition of Epo. An improved model would include both nonlinear components, however.

A population PK analysis of rHuEpo in healthy adult male volunteers has been performed earlier (Hayashi et al., 1998) using a model with first-order absorption and
linear elimination. There were only two low SC 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 SC dosing, the peak concentrations of rHuEpo were attained within one 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 macromolecules (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 weight above 4 KDa are absorbed increasing in proportion to molecular weight via the lymph. This phenomenon might explain the 10-hour 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 earlier (Loeffler et al., 1989; Wichmann et al., 1989; Wulff
et al., 1989). These physiologic 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 haematopoiesis, 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 earlier 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 a model based on the cell lifespan 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. Due to this reason, 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 lifespan concept with temporal changes in drug concentrations controlling the rate and extent of changes in responses. This catenary lifespan 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-hour 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 2 to 5 cell divisions with a mean maturation or turnover time of 11 to 48 hours depending on the species (Alpen and Cranmore, 1959; Fliedner et al., 1959; Krantz and Jacobson, 1970; Osgood, 1954). Our model predicts that there is a 15-18 hr lag time before the newly produced reticulocytes are actually released into circulation, and this reflects the erythroblast maturation time. The estimated reticulocyte lifespan was 6 days. In humans, the normal lifespan 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 lifespan 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 85-119 hours. 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 inter-individual variability, but this would be a formidable computational and modeling challenge at present.
REFERENCES

Alpen EL and Cranmore D (1959) Cellular kinetics and iron utilization in the bone marrow as observed by Fe^{59} radioautography. *Ann NY Acad Sci* 77:753-759.


Jelkmann W (1986) Erythropoietin research, 80 years after the initial studies by Carnot and Deflandre. *Respir Physiol* 63:257-266


Footnotes:

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**Figure Legends**

**Fig. 1.** PK/PD model for rHuEpo effects on blood reticulocyte, RBC and Hb counts of cynomolgus monkeys. Symbols are defined in Abbreviations.

**Fig. 2.** Process of erythropoiesis in relation to model components. Erythropoietin stimulates the proliferation and differentiation of the erythrocyte progenitors (BFUe, burst-forming-unit-erythroid; CFUe, colony-forming-unit-erythroid) represented by the P1 compartment as well as the erythroblasts represented by the P2 compartment. The stimulation is indicated by the nonlinear function S(t). Figure partially adapted from Jelkmann (1986).

**Fig. 3.** Bottom panel shows serum rHuEpo concentration versus time profiles after IV administration of 500 (closed circles), 2000 (open circles) and 4000 IU/kg (closed triangles) Eprex. Top panel shows the kinetics of rHuEpo after SC administration of 400 (open squares), 1000 (closed squares), 2400 (open triangles), 5000 (closed triangles), 20000 (open circles) and 40000 (closed circles) IU/kg Eprex. Symbols are the mean data and error bars are the standard deviations while the solid line is obtained from a simultaneous fitting of all the doses to the model using equations 1 and 2 for the IV data and equations 3 and 4 for the SC data.

**Fig. 4.** Time course of blood reticulocyte counts for the different SC 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 equation 5.
**Fig. 5.** Time course of blood RBC counts for the different SC dosage regimens. Solid circles are the mean data, error bars are the standard deviations and the solid lines are simulations using equation 6 and the estimated parameters from the reticulocyte fittings.

**Fig. 6.** Time course of blood hemoglobin counts for the different SC dosage regimens. Solid circles are the mean data, error bars are the standard deviations and the solid lines are simulations using the estimated parameters from the reticulocyte fittings.
### TABLE 1
Pharmacokinetic parameters for rHuEpo in male cynomolgus monkeys

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
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<tbody>
<tr>
<td>$V_{\text{max}}$ (IU/kg/hr)</td>
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<tr>
<td>$K_m$ (IU/L)</td>
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</tr>
<tr>
<td>$V_d$ (L/kg)</td>
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<td>$k_{21}$ ($\text{hr}^{-1}$)</td>
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<tr>
<td>$k_a$ ($\text{hr}^{-1}$)</td>
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<td>$k_a$ ($\text{hr}^{-1}$)-lowest dose</td>
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<tr>
<td>Fr</td>
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<tr>
<td>$F$ (400 IU/kg dose)</td>
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<td>$F$ (1000 IU/kg dose)</td>
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<td>$F$ (higher doses)</td>
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TABLE 2
Pharmacodynamic parameters for rHuEpo in male cynomolgus monkeys

<table>
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<th>Parameter</th>
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</thead>
<tbody>
<tr>
<td>$T_{P1}$ (hr)</td>
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<td>$T_{P2}$ (hr)</td>
<td>14.95</td>
<td>73.25</td>
</tr>
<tr>
<td>$T_{R}$ (hr)</td>
<td>141.6</td>
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<tr>
<td>$S_{max}$</td>
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<tr>
<td>$S_{C_{50}}$ (IU/L)</td>
<td>842.5</td>
<td>28.18</td>
</tr>
</tbody>
</table>
Fig. 3
Number of Reticulocytes (x10^10 cells/L)

Time (hr)

Fig. 4
Fig. 5
Fig. 6