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Pharmacodynamic Analysis of Recombinant Human Erythropoietin Effect on Reticulocyte Production Rate and Age Distribution in Healthy Subjects

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Abstract

Objective—To evaluate the effect of recombinant human erythropoietin (rHuEPO) on the reticulocyte production rate and age distribution in healthy subjects.

Methods—Extensive pharmacokinetic and pharmacodynamic data collected from 88 subjects who received a single subcutaneous dose of rHuEPO (dose range 20–160 kIU) were analysed. Four nonlinear mixed-effects models were evaluated to describe the time course of the percentage of reticulocytes and their age distribution in relation to rHuEPO pharmacokinetics. Model A accounted for stimulation of the production of progenitor cells in bone marrow, and model B implemented shortening of differentiation and maturation times of early progenitors in bone marrow. Model C was the combination of models A and B, and model D was the combination of model A with an increase in the maturation times of the circulating reticulocytes. Model evaluation was performed using goodness-of-fit plots, a nonparametric bootstrap and a posterior predictive check.

Results—Model D was selected as the best model, and evidenced accurate and precise estimation of model parameters and prediction of the time course of the percentage of reticulocytes. At baseline, the estimated circulating reticulocyte maturation time was 2.6 days, whereas the lifespan of the precursors in the bone marrow was about 5 days. The rHuEPO potency for the stimulatory effect (7.61 IU/L) was higher than that for the increase in reticulocyte maturation times (56.3 IU/L). There was a significant 1- to 2-day lag time in the reticulocyte response. The effect of rHuEPO on the reticulocyte age distribution consisted of a transient increase in the reticulocyte maturation time from baseline up to 6–7 days, occurring 1 day after administration. The dose-dependent amplitude of the changes in the age distribution lasted for 12–14 days. The model-predicted peak increase in the reticulocyte release rate ranged from 140% to 160% of the baseline value and was maximal on days 7–8 following rHuEPO administration.

Conclusions—A semiphysiological model quantifying the effect of rHuEPO on the reticulocyte production rate and age distribution was developed. The validated model predicts that rHuEPO

increases the reticulocyte production rate and modifies the reticulocyte age distribution in a dose-dependent manner.

Background

Erythropoietin (EPO) is a 30.4-kD glycoprotein that is the major growth factor regulating the production of circulating red blood cells (RBCs), a process known as erythropoiesis.[1] EPO is released from the liver and kidney in response to hypoxia, and acts by binding and dimerizing EPO receptors on the surface of the erythroid progenitor cells in the bone marrow and other haematopoietic tissues, leading to an increase in the survival, proliferation and differentiation of erythroid progenitor cells, which ultimately results in increased numbers of reticulocytes, RBCs and haemoglobin levels.[2,3] Recombinant human EPO (rHuEPO) has been approved for treatment of anaemia associated with renal failure, cancer chemotherapy and treatment of AIDS, among other indications.

Reticulocytes are formed from orthochromatic erythroblasts by the process of nuclear extrusion. The reticulocytes then degrade internal organelles and assume a uniform shape. The moment when no residual RNA can be detected in the cytosol defines the reticulocyte maturation into a young RBC. Flow cytometry assesses the maturation of reticulocytes by quantifying the fractions of reticulocytes in low-fluorescence, middle-fluorescence and high-fluorescence intensity regions.[4] Reticulocyte fluorescence intensity is directly proportional to the quantity of intracellular RNA, and thus expresses a function of cellular maturity.[5] Consequently, the reticulocyte population can be subdivided by maturity according to high, medium and low RNA content, corresponding to young, middle-aged and old reticulocytes, respectively.[6]

The total maturation time of erythroblasts and reticulocytes is in the order of 4 days, of which 3 days are spent in the bone marrow and 1 day in the peripheral circulation.[7] A single intravenous bolus administration of rHuEPO in healthy subjects results in an immediate release of immature reticulocytes into the circulation, followed by a 1.5-day delayed release of reticulocytes arising from stimulation of erythropoiesis by rHuEPO.[8] This indicates that in addition to an increase in the number of reticulocyte precursors, rHuEPO shortens the transit time of early erythroblasts to reticulocytes in the bone marrow. Another consequence of rHuEPO is a transient change in the age distribution of circulating reticulocytes. The maturation time of about 1 day for homeostatic reticulocytes in the circulation extends to about 3 days of maturation time in the circulation for stress reticulocytes.[8] This phenomenon has been confirmed by other investigators for phlebotomy-induced stress erythropoiesis.[9] Recently, it has been demonstrated that the maturation time of stress reticulocytes in the circulation depends on the rHuEPO dose in healthy subjects.[10]

The objective of this article was to evaluate pharmacokinetic and pharmacodynamic models that account for a major mechanism of the rHuEPO action on the reticulocyte age distribution and production rate from data obtained in phase I studies, where healthy subjects received single doses of rHuEPO subcutaneously. A previously established pharmacodynamic model was applied to quantify dynamic changes in reticulocyte counts and, indirectly, their ages in relation to time-dependent rHuEPO serum concentrations.[11–13] Several models accounting for one or more possible mechanisms of rHuEPO action were developed, tested and evaluated.

Methods

Study Design and Subject Eligibility Criteria

Data from three open-label, randomized, placebo-controlled, parallel-group phase I studies performed by Johnson & Johnson Pharmaceutical Research & Development, LLC (formerly the R.W. Johnson Pharmaceutical Research Institute) were used in the current analysis. In these studies, the pharmacokinetics and pharmacodynamics of a single subcutaneous dose of rHuEPO were investigated in 88 adult male healthy volunteers. Subjects aged 18–45 years with a body weight of 63.6–100 kg and confirmed as being healthy by a physical examination, ECG and standard laboratory tests were eligible to participate in the studies. In addition, subjects were required to have baseline serum erythropoietin levels of <30 IU/L; haemoglobin and haematocrit values of 13.8–16.4 g/dL and 41–49%, respectively; baseline percentage of reticulocytes $\leq$ 3%; and normal serum folate, vitamin B₁₂ (cyanocobalamin) and iron parameters. A detailed description of the exclusion criteria has been published elsewhere.[14]

Study A was conducted in 1996 and randomized 20 subjects to receive rHuEPO subcutaneously at doses of 300, 600, 1200 or 2400 IU/kg.[14] Study B was conducted in 1996 and randomized 20 subjects to receive rHuEPO subcutaneously at doses of 450, 900, 1350 or 1800 IU/kg.[14] The doses per kg of body weight were converted to absolute doses for the pharmacokinetic and pharmacodynamic analyses. Studies A and B were conducted at the South Florida Bioavailability Clinic (Miami, FL, USA). Study C was conducted at Chiltern International (Buckinghamshire, UK) in 2002 and randomized 48 subjects to receive rHuEPO subcutaneously at doses of 20, 40, 60, 90, 120 and 160 kIU; however, one subject receiving 60 kIU discontinued the study and was not included in the pharmacodynamic analysis.[15] All subjects received daily oral iron supplementation (equivalent to 210 mg of elemental iron) through day 29 of the study. In the three studies, the randomization schedule was balanced by using randomly permuted blocks. The studies were conducted in accordance with the principles for human experimentation as defined in the International Conference on Harmonisation and Good Clinical Practice and the principles of the Declaration of Helsinki. The studies were approved by the Human Investigational Review Board of each study centre. Informed consent was obtained from each subject after explanation of the potential risks and benefits, as well as the investigational nature of the study.

Pharmacokinetic-Pharmacodynamic Sampling and Bioanalytical Methods

Serial blood samples were drawn by direct venipuncture before and after drug administration, collected in heparinized tubes and centrifuged. Separated serum was stored at -20°C until the analysis was conducted. For studies A and B, blood samples were drawn for determination of rHuEPO serum concentrations at 30, 20 and 10 minutes before drug administration and at 0.5, 3, 6, 9, 12, 15, 18, 24, 27, 30, 36, 48, 72, 96, 144, 168, 216, 264, 336, 408, 504, 600 and 672 hours after drug administration. For study C, blood samples were collected 30, 20 and 10 minutes before drug administration and at 0.5, 1, 2, 5, 8, 12, 18, 24, 30, 36, 48, 60, 72, 96, 120, 144, 168, 192, 216, 264, 312 and 360 hours after drug administration. A modified radioimmunoassay kit procedure (Diagnostic Systems Laboratories [DSL], Webster, TX, USA) was used for determination of serum erythropoietin concentrations, as described by Cheung et al.[14] The lower limit of quantification was 7.8 IU/L and the mean overall coefficient of variation was <15% across the validated range of concentrations, which included up to 5000 IU/L.

The percentage of reticulocytes was measured before drug administration in the three studies analysed. In studies A and B, serial blood samples were drawn daily up to 15 days and then

weekly up to 29 days after drug administration. In study C, blood samples were drawn daily up to 9 days and then every other day up to 25 days and at 28, 34 and 43 days after drug administration. A flow cytometric procedure with the H-1 system (Technicon Instruments Corp., Tarrytown, NY, USA) was used for determination of the percentage of reticulocytes, as described elsewhere.[16]

Software

Nonlinear mixed-effects modelling was employed to characterize the time course of the rHuEPO serum concentration and the percentage of reticulocytes, using NONMEM® V level 1.1 software (GloboMax, Hanover, MD, USA) including NM-TRAN (version III level 1.0) and PREDPP (version IV level 1.0). NONMEM® analyses were performed on the Johnson & Johnson Computational Grid, which is based on the United Devices' Metaprocessor product running with an Oracle 9i repository and a mixture of Intel-based processing nodes running Microsoft Windows and Red Hat Linux operating systems. The NONMEM® on the Johnson & Johnson Computational Grid executes models compiled with Intel Fortran 9.0 for Windows software. A maximum *a posteriori* (MAP) Bayesian estimation of individual pharmacokinetic parameters was performed using the POSTHOC option in NONMEM®. The pharmacodynamic analysis was conducted using the first-order conditional estimation method (FOCE). Nonparametric bootstrap analysis was performed using Wings for NONMEM® software.[17] Graphical data visualization, evaluation of NONMEM® outputs, construction of goodness-of-fit plots, and graphical model comparisons were performed using S-Plus version 7.0 software for Windows (Data Analysis Products Division, Insightful Corporation, Seattle, WA, USA).

Pharmacokinetic Analysis

A MAP Bayesian estimation of individual pharmacokinetic parameters of rHuEPO was implemented in NONMEM® software, using the POSTHOC option. The results of a previous population pharmacokinetic analysis of rHuEPO using data from 16 clinical studies were used to describe the time course of rHuEPO after intravenous and subcutaneous administration.[18] Briefly, the model structure used to characterize the rHuEPO disposition is displayed in figure 1. It was an open two-compartment model with linear distribution and linear and nonlinear elimination. Subcutaneous absorption of rHuEPO was described by a dual absorption model with a faster absorption process characterized by a sequential zero-order input into the depot compartment, followed by first-order absorption from the depot compartment into the central compartment and a parallel slower absorption process starting after a lag time, and characterized by a zero-order process directly into the central compartment. The model after subcutaneous administration can be described by the following system of differential equations (equations 1, 2 and 3):

$$\frac{dA_1}{dt} = \begin{cases} \frac{\text{Dose} \cdot \text{fr} \cdot F}{D_1} - k_a A_1 & t \leq D_1 \\ -k_a A_1 & t > D_1 \end{cases} \quad (\text{Eq. 1})$$

$$\frac{dA_2}{dt} = \begin{cases} \frac{\text{Dose} \cdot (1 - \text{fr}) \cdot F}{D_2} + k_a A_1 + k_{32} A_3 - k_{23} A_2 - k_{20} A_2 \\ -\frac{V_{\max} A_2 / V_2}{K_m + A_2 / V_2} + k_{\text{EPO}}, & t_{\text{lag}2} \leq t \leq D_2 \\ k_a A_1 + k_{32} A_3 - k_{23} A_2 \\ -\frac{V_{\max} A_2 / V_1}{K_m + A_2 / V_1} + k_{\text{EPO}}, & t < t_{\text{lag}2} \text{ or } t > D_2 \end{cases} \quad (\text{Eq. 2})$$

$$\frac{dA_3}{dt} = k_{23}A_2 - k_{32}A_3 \quad (\text{Eq. 3})$$

where Dose is the absolute amount of rHuEPO dose administered; A_1 , A_2 and A_3 are the amounts of erythropoietin in the depot, central and peripheral compartments, respectively; V_1 is the volume of the central compartment; K_m (the Michaelis-Menten constant) is the concentration at which the saturable elimination pathway operates at half the maximal rate; $V_{\max}$ is the maximum elimination rate of the saturable pathway; f_r , which describes the relative bioavailability through the two absorption pathways, is the fraction of the absolute bioavailability, F ; k_{20} is the linear elimination rate constant, equivalent to CL_I/V_2 ; k_{23} and k_{32} are the intercompartmental rate constants, equivalent to Q/V_2 and Q/V_3 , respectively, where Q is the intercompartmental flow; D_1 and D_2 are the duration of zero-order processes for the faster and slower absorption pathways, respectively; k_a is the first-order absorption rate constant; and t_{lag2} is the lag time. In the absence of exogenous rHuEPO, the system is at steady state and, therefore, the production rate of endogenous EPO, k_{EPO} , can be expressed as a function of the baseline endogenous EPO concentration (BSL) as follows (equation 4):

$$k_{EPO} = CL_I \cdot BSL + \frac{V_{\max} \cdot BSL}{K_m + BSL} \quad (\text{Eq. 4})$$

where CL_I is the linear clearance.

The bioavailability through the faster pathway was $F \cdot f_r$, while the bioavailability through the slower pathway was $F \cdot (1 - f_r)$. The dose-dependent absolute bioavailability, F , of subcutaneous administration was described by a hyperbolic function (equation 5):

$$F = F_0 + \frac{E_{\max(F)} \cdot \text{Dose}}{ED_{50(F)} + \text{Dose}} \quad (\text{Eq. 5})$$

where F_0 is the minimum absolute bioavailability. $E_{\max(F)}$ is the maximum increase in bioavailability with Dose, and $ED_{50(F)}$ is the dose giving 50% of the increase in F . Finally, the endogenous EPO serum concentration was added to the concentrations generated by rHuEPO administration. Figure 1 displays the schematics of the pharmacokinetic model for rHuEPO. For some model parameters, interindividual variability (IIV) could not be estimated and, therefore, they were fixed to the typical value reported by Olsson-Gisleskog et al.[18] Additional details about this model have been published elsewhere.[18]

Pharmacodynamic Analysis

The development of the pharmacodynamic model was performed using a sequential process. [19] Individual model parameters obtained from the pharmacokinetic analysis were used to predict the individual time course of rHuEPO serum concentrations, which was used as an input function into the pharmacodynamic model.

To investigate possible mechanisms by which rHuEPO increases the production of reticulocytes and affects their age distribution, the concept of the maturation-structured cytokinetic model, introduced previously by Harker et al.[11] and exploited further by Roskos et al.,[12] was applied. The backbone structure of such a model is a series of compartments linked in a catenary fashion by first-order cell transfer rates (figure 2). Each compartment represents a pool of cells of the increased mean age by $1/k$, where k denotes the first-order rate constant between the age compartments. A cascade of $N_p = 10$ age

compartments with transfer rate constants equal to N_p/T_p were selected to account for the development and maturation of reticulocyte precursor cells in bone marrow, where T_p is the mean lifespan of the precursor cell. Similarly, a cascade of $N_R = 10$ age compartments represents the circulating reticulocytes with the mean lifespan T_R . The number of 10 compartments was arbitrarily selected in order to have a large enough number of compartments that would result in a smooth distribution of the cell lifespan by reducing the variability in the cellular transit time. The precursor cells in the first age compartment P_1 were assumed to be produced at the zero-order rate k_{in} . Four possible mechanisms of rHuEPO action on reticulocyte production were considered (figure 2): (a) stimulation of production of progenitor cells in bone marrow; (b) shortening of the differentiation and maturation times of early progenitors in bone marrow; (c) the combination of the two previous mechanisms of action; and (d) stimulation of the production of progenitor cells in bone marrow and an increase in the maturation times of circulating reticulocytes.

Model A

Model A encompassed mechanism (a), where the stimulatory effect of rHuEPO is on the production rate of precursor cells, as follows (equation 6):

$$\frac{dP_1}{dt} = \frac{k_{in} \cdot C}{SC_{50} + C} - \frac{N_p}{T_p} \cdot P_1 \quad (\text{Eq. 6})$$

where C denotes the serum concentration of endogenous EPO and exogenous rHuEPO and is equivalent to $BSL + A_2/V_1$, k_{in} represents the maximal endogenous production rate of progenitor cells, SC_{50} denotes the EPO serum concentration necessary to maintain the progenitor cell production rate at 50% of its maximum, and P_1 represents the number of precursor cells in the first age compartment. The remaining $N_p - 1$ precursor cell age compartments maintain the catenary structure of the signal transduction model (equation 7). [20]

$$\frac{dP_i}{dt} = \frac{N_p}{T_p} \cdot (P_{i-1} - P_i), \quad i=2, \dots, N_p \quad (\text{Eq. 7})$$

The precursor cells in bone marrow are released to the blood as the youngest circulating reticulocytes, according to equation 8:

$$\frac{dR_1}{dt} = \frac{N_p}{T_p} \cdot P_{N_p} - \frac{N_R}{T_R} \cdot R_1 \quad (\text{Eq. 8})$$

where R_1 represents the number of reticulocytes in the first age compartment. The remaining $N_R - 1$ reticulocyte age compartments maintain the catenary structure of the model in order to mimic the maturation process to RBCs (equation 9):

$$\frac{dR_j}{dt} = \frac{N_R}{T_R} \cdot (R_{j-1} - R_j), \quad j=2, \dots, N_R \quad (\text{Eq. 9})$$

As T_p and T_R represent the lifespan of precursor cells and reticulocytes, respectively, the transition between age compartments is set to N_p/T_p and N_R/T_R . Therefore, the circulating reticulocytes are the sum of the reticulocytes (RET) at all ages (equation 10):

$$RET = R_1 + \dots + R_{N_R} \quad (\text{Eq. 10})$$

The initial conditions for P_i and R_j were determined from the baseline reticulocyte count (RET_0), as follows (equations 11 and 12):

$$P_{i0} = \frac{T_p}{N_p \cdot T_R} \cdot RET_0, i=1, \dots, N_p \quad (\text{Eq. 11})$$

$$R_{j0} = \frac{RET_0}{N_R}, j=1, \dots, N_R \quad (\text{Eq. 12})$$

The endogenous progenitor production rate constant at baseline was calculated from equation 6 at steady state (equation 13):

$$k_{in} = \left(1 + \frac{SC_{50}}{BSL}\right) \cdot \frac{RET_0}{T_R} \quad (\text{Eq. 13})$$

Model B

The acceleration of the differentiation and maturation of progenitor cells due to exposure to rHuEPO was described by model B, which is consistent with mechanism of action (b). This process was modelled as the stimulation of the transfer rates between the progenitor cell age compartments. Since only the early progenitor cells express EPO receptors, the first half of the precursor pools were stimulated, whereas the second half was not affected by rHuEPO (figure 2. equation 14):

$$\frac{dP_1}{dt} = k_{in} - \frac{S_{max} \cdot C}{SC_{50P} + C} \cdot \frac{N_p}{T_p} \cdot P_1 \quad (\text{Eq. 14})$$

where S_{max} is the maximum stimulation factor of the precursor cell maturation process, and SC_{50P} denotes the EPO serum concentration eliciting 50% of the maximum stimulation of the precursor cell maturation process. Consequently, the catenary model structure for precursor cells is defined as follows (equations 15, 16 and 17):

$$\frac{dP_i}{dt} = \frac{S_{max} \cdot C}{SC_{50P} + C} \cdot \frac{N_p}{T_p} \cdot (P_{i-1} - P_i), i=2, \dots, N_p/2 \quad (\text{Eq. 15})$$

$$\frac{dP_{(N_p/2)+1}}{dt} = \frac{S_{max} \cdot C}{SC_{50P} + C} \cdot \frac{N_p}{T_p} \cdot P_{(N_p/2)} - \frac{N_p}{T_p} \cdot P_{(N_p/2)+1} \quad (\text{Eq. 16})$$

$$\frac{dP_i}{dt} = \frac{N_p}{T_p} \cdot (P_{i-1} - P_i), i=N_p/2+2, \dots, N_p \quad (\text{Eq. 17})$$

The differential equations describing the circulating reticulocytes are the same as for model A (equations 8, 9 and 10), including the same initial conditions (equation 11). However, the production rate constant of the endogenous progenitor cells was calculated according to equation 18:

$$k_{in} = \frac{S_{max} \cdot BSL}{SC_{50p} + BSL} \cdot \frac{RET_0}{T_R} \quad (\text{Eq. 18})$$

Model C

Model C combined the mechanisms described by models A and B, reflecting the fact that both the stimulatory effect on the production of reticulocyte progenitors cells and the acceleration of maturation and differentiation of their precursors cells in bone marrow due to exposure to rHuEPO occur simultaneously. Therefore, the equation describing the P_1 compartment was as follows (equation 19):

$$\frac{dP_1}{dt} = \frac{k_{in} \cdot C}{SC_{50} + C} - \frac{S_{max} \cdot C}{SC_{50p} + C} \cdot \frac{N_p}{N_t} \cdot P_1 \quad (\text{Eq. 19})$$

The remaining equations for $P_2, \dots, P_{N_p}$ and $R_1, \dots, R_{N_R}$ were the same as for model B with the initial conditions stated in equation 11. In this particular model, the endogenous progenitor production rate constant was calculated as follows (equation 20):

$$k_{in} = S_{max} \cdot \frac{SC_{50} + BSL}{SC_{50p} + BSL} \cdot \frac{RET_0}{T_R} \quad (\text{Eq. 20})$$

Model D

The dilating effect of rHuEPO on the age distribution of circulating reticulocytes was included in model D along with stimulation of the production of progenitor cells in bone marrow, which is consistent with mechanism of action (d). The stimulation of k_{in} was described as in model A as well as equations for $P_1, \dots, P_{N_p}$. To account for an observed transient increase in the mean lifespan of circulating reticulocytes, inhibition of the transfer rates between the age compartments was applied.[10] An additional feature introduced by model D was a signal transduction between the rHuEPO serum concentration and a hypothetical effect site. This was necessary since rHuEPO returned to baseline values for the highest doses after about 7 days, whereas the peak reticulocyte count occurred at 9 days. The signal transduction was described by the model introduced previously[21] with $M = 5$ transit compartments according to equations 21 and 22:

$$\frac{dS_1}{dt} = \frac{1}{\tau} \left(\frac{C}{EC_{50} + C} - S_1 \right) \quad (\text{Eq. 21})$$

$$\frac{dS_k}{dt} = \frac{1}{\tau} (S_{k-1} - S_k), \quad k=2, \dots, M \quad (\text{Eq. 22})$$

where τ is the signal transduction transit time, EC_{50} is the concentration of rHuEPO eliciting 50% of the maximum signal, which was assumed to be 1.0, and S_k represents the signal transduction at the k th compartment. In the presence of the ratio S_0/S_M in equations

describing reticulocyte compartments, a separate maximum effect ($E_{\max}$) parameter could not be identified. At baseline conditions, S_k was represented by equation 23:

$$S_k = S_0 = \frac{BSL}{EC_{50} + BSL}, \quad k=1, \dots, M \quad (\text{Eq. 23})$$

The signal S_M was used to drive the inhibitory effect on reticulocyte aging rates (equations 24 and 25):

$$\frac{dR_1}{dt} = \frac{N_p}{T_p} P_{N_p} - \frac{N_R}{T_R} \left(\frac{S_0}{S_M} \right) R_1 \quad (\text{Eq. 24})$$

$$\frac{dR_j}{dt} = \frac{N_R}{T_R} \left(\frac{S_0}{S_M} \right) (R_{j-1} - R_j), \quad j=2, \dots, N_R \quad (\text{Eq. 25})$$

The initial conditions for the P_i and R_j compartments were given by equation 11.

Statistical Model

The IIVs in the pharmacokinetic and pharmacodynamic model parameters were assumed to follow the lognormal distribution according to equation 26:

$$P_j = P \bullet \exp^{\eta_{pj}} \quad (\text{Eq. 26})$$

where P_j was an individual model parameter for the j th individual, P^* was the typical value of the model parameter, and η_{pj} was an independent and normally distributed random variable with a zero mean and variance ω_p . [21] In order to keep the individual values of P_j between 0 and 1, IIV in this parameter was modelled using a normal distribution in the logit domain, as previously described. [18]

For the residual error, the measured rHuEPO serum concentrations and model predictions were transformed into logarithms (transform-both-sides approach). The magnitude of the residual variability in the transformed serum concentrations was modelled using an additive error model. However, the residual variability in the percentage of reticulocytes was assumed to follow the normal distribution according to equation 27:

$$R_{\text{obs}} = R_{\text{pred}} + \varepsilon \quad (\text{Eq. 27})$$

where R_{obs} was the percentage of reticulocytes observed, R_{pred} was the corresponding model-predicted percentage of reticulocytes, and ε was an independent, normally distributed random variable with a zero mean and variance σ . [2]

Model Selection

The improvement in the fit obtained for models B, C and D as compared with the reference model (model A) was assessed in several ways. First, the resulting minimum objective function value (MOFV) after fitting the models evaluated was used to test the statistical improvement of the fit by the likelihood ratio test (LRT). This test is based on the change in the MOFV (ΔMOFV), which is equal (up to a constant) to minus twice the log-likelihood of the data and is asymptotically distributed like χ^2 , with the degrees of freedom (df) equal to

the number of parameters added to the model. For hierarchical models, a ΔMOFV of ≥ 6.63 is required to reach statistical significance ($p = 0.01$) for the addition of one fixed effect. In addition, the improvement in the fit was assessed by examination of diagnostic plots as the scatter plots of the observed versus the predicted percentages of reticulocytes, and scatter plots of weighted residuals versus the predicted percentage of reticulocytes and the time since the last dose. The magnitude of the IIV and residual variability and the precision of the parameter estimate were also evaluated to select the optimal structural model.

Pharmacodynamic Model Validation

A nonparametric bootstrap analysis was performed as an internal model evaluation technique for the four pharmacodynamic models. A new replication of the original dataset (a bootstrap sample) was obtained by N random draws of individual data (with replacement) from the original dataset. The final population pharmacodynamic model was refitted to each new dataset, and this process was repeated 1000 times with different random draws. The stability of the final model was evaluated by visual inspection of the distribution of the model parameter estimates from the new datasets and compared with that obtained from the fit of the original dataset.[22] Bootstrap runs with unsuccessful minimization were excluded from further analysis. The final model parameter estimates were compared with the mean and 95% confidence intervals of the nonparametric bootstrap replicates of the final model.

In addition, a posterior predictive check was performed to evaluate the pharmacokinetic model and the selected pharmacodynamic model.[23] The parameter estimates obtained by fitting the final model were used to simulate the time course of the rHuEPO serum concentrations and the percentage of reticulocytes after subcutaneous administration of rHuEPO at doses of 20,40,60,90, 120 and 160kIU. A nonparametric 95% prediction interval around the median rHuEPO serum concentrations and the percentage of reticulocytes was constructed to quantify the variability and uncertainty in the model predictions, and for visual comparison with the observations obtained.

Reticulocyte Residence Time Distribution

The j th reticulocyte compartment R_j can be considered as a subpopulation of reticulocytes with the mean residence time (MRT) in the circulation a_j , calculated as the sum of the mean MRTs for compartments $R_1, \dots, R_j$ (equation 28):

$$a_j(t) = j \bullet \text{MRT}(t) \quad (\text{Eq. 28})$$

where both a_j and MRT ($k = 1, \dots, j$) are time dependent for model D, since the first-order transfer rates between the reticulocyte pools were dependent on the transduction signal. For this model, and for time $t = t_0$, $\text{MRT}(t_0)$ values were calculated as follows (equation 29) [see the Appendix]:

$$\text{MRT}(t_0) = \int_{t_0}^{\infty} A(t, t_0) dt \quad (\text{Eq. 29})$$

where $A(t, t_0)$ was a solution to the following differential equation (equation 30):

$$\frac{dX}{dt} = - \frac{N_R}{T_R} \left(\frac{S_0}{S_M} \right) X, t > t_0 \quad (\text{Eq. 30})$$

with the initial condition (equation 31):

$$X(t_0)=1 \quad (\text{Eq. 31})$$

Since the actual reticulocyte residence time distribution is difficult to determine, it was assumed that all of the reticulocytes in the R_j compartment have the same residence time a_j . Then, at a time $t = t_0$, the probability density function (pdf) of the reticulocyte age distribution is determined as follows (equation 32):

$$\text{pdf}(a, t_0) = \frac{R_1(t_0)}{\text{RET}(t_0)} \delta(a - a_1) + \dots + \frac{R_{NR}(t_0)}{\text{RET}(t_0)} \delta(a - a_{NR}) \quad (\text{Eq. 32})$$

where the Dirac delta function $\delta(a - a_j)$ represents the point distribution of the residence times of the reticulocytes in the R_j compartment. Consequently, the mean reticulocyte residence time at time $t = t_0$ can be calculated as follows (equation 33):

$$\text{MRT}_{\text{RET}}(t_0) = \frac{\text{MRT}(t_0)}{\text{RET}(t_0)} \sum_{j=1}^{N_R} j \cdot R_j(t_0) \quad (\text{Eq. 33})$$

An analogous equation can be derived for the MRT of the reticulocyte precursors (MRT_{PREC}) in the bone marrow (equation 34):

$$\text{MRT}_{\text{PREC}}(t_0) = \frac{T_P/N_P}{P(t_0)} \sum_{i=1}^{N_P} i \cdot P_i(t_0), \text{ where} \\ P(t_0) = P_1(t_0) + \dots + P_{NP}(t_0) \quad (\text{Eq. 34})$$

The MRT for each P_i compartment is time independent and equal to T_P/N_P .

Results

Pharmacokinetic Analysis

Pharmacokinetic analysis of rHuEPO was performed on 2018 rHuEPO serum concentrations obtained from 88 healthy subjects. A descriptive summary of the individual Bayesian pharmacokinetic parameters of rHuEPO is shown in table I. The pharmacokinetic model used in this analysis was suitable to describe the observed rHuEPO serum concentrations following subcutaneous administration of a single dose (figures 3a and 3b). The observed rHuEPO serum concentrations following subcutaneous administration of 20, 40, 60, 90, 120 and 160 kIU are displayed in figure 4a together with the model-predicted time course of rHuEPO serum concentrations. Taken together, these results show the similarity between the rHuEPO model parameters and those reported previously, and also confirm the validity of the model to describe the rHuEPO pharmacokinetic profile in healthy subjects.[18]

Pharmacodynamic Analysis

Pharmacodynamic analysis of rHuEPO was performed on 1628 percentage of reticulocytes counts obtained from 88 subjects. In subjects treated with subcutaneous rHuEPO, a dose-dependent increase in the percentage of reticulocytes was evident (figure 4b). The percentage of reticulocytes after an initial lag time of 1–2 days increased rapidly to attain the peak value on day 8–9 and declined slowly thereafter. This signature in the reticulocyte profiles exhibited a delay in the response onset and a prolonged duration of the effect. The presence of the lag time in the reticulocyte response was statistically tested. As shown in

figure 5, there were no differences between the reticulocyte counts at 0 and 24 hours, whereas such a difference was observed between 0 and 48 hours. The tag time was interpreted as a delay necessary for maturation of reticulocyte precursor cells in the bone marrow and implemented in the pharmacodynamic models by means of a series of aging precursor compartments.

The estimated population parameters for the four pharmacodynamic models displayed in figure 2 are presented in table II. together with the results of the nonparametric bootstrap analysis. Diagnostic plots confirmed that all of the pharmacodynamic models used in this analysis were suitable to describe the observed percentage of reticulocytes following subcutaneous administration of a single dose. Improvement of the fit was achieved with model C with respect to models A ($\chi^2 = 81.9$, $df = 2$, $p < 0.0001$) and B ($\chi^2 = 29.9$, $df = 1$, $p < 0.0001$). Model D provided a substantially better fit of the data with respect to model C, as evidenced by a decrease of 154 units in the MOFV, given the same number of parameters for both models. In addition, model D provided 18%, 22% and 25% reductions in the magnitude of the residual variability with respect to models C, B and A, respectively.

Diagnostic plots for pharmacodynamic model D showed random normal scatter around the identity line, indicating the absence of bias and confirming the adequacy of the model to describe the data (figures 3c and 3d). The population mean predictions determined by pharmacodynamic model D for the percentage of reticulocytes counts are shown in figure 4b, together with the 95% prediction intervals, for the doses of 20, 40, 60, 90, 120 and 160 kIU. The visual analysis confirms the adequacy of the pharmacodynamic model to reproduce the time course of the percentage of reticulocytes and its variability for each dose evaluated in the current analysis.

The model D estimates of the system-related parameters RET_0 and T_p were very similar and consistent with previous values reported using different models.[24,25] However, the estimated typical value of T_R (2.6 days) is shorter than that reported previously and closer to the time of 1 day that a normal reticulocyte spends in the circulation.[7] From a therapeutic standpoint, the drug-related parameters SC_{50} and EC_{50} are the most important model parameters. These values should be interpreted as a descriptor of drug potency for the stimulatory effect on the proliferation of precursor cells and the prolongation of reticulocyte survival, respectively. The estimated value of the transduction time τ multiplied by the number of transduction compartments provides an assessment of the delay of 1 day between the exposure to rHuEPO and its effect on reticulocyte maturation.

Bootstrap Analysis

From the 1000 bootstrap replicates of the model, 7.3%, 23.1%, 37.8% and 29.8% failed to minimize successfully in models A, B, C and D. respectively, and were excluded from further analysis. Except for model A, these rates are similar to what has been published previously for the lifespan models.[26] The parameter estimates of the pharmacodynamic models evaluated were very similar to the median of the nonparametric bootstrap replicates, and all were contained within the 95% confidence intervals obtained from the bootstrap analyses. The precision of the NONMEM[®] parameter estimates was excellent, since the relative standard error from the bootstrap analysis for the fixed and random effects was <25%, except for the variability of the lifespan of reticulocytes in models B and C and the variability of the lifespan of precursor cells in model B. Also, the precision of the drug effect parameters of model D was >25% but <60%. Overall, these results suggest the absence of bias and a reasonable precision in the NONMEM[®] parameter estimates for the four pharmacodynamic models evaluated.

Reticulocyte Residence Time Distribution

Since model D was selected as the best model describing the reticulocyte data, equation 32 was used to determine the residence time distribution. Figure 6 shows several pdf values calculated at various times for a typical patient receiving a rHuEPO dose of 20 kIU and using the population estimates of the pharmacokinetic and pharmacodynamic parameters obtained from tables I and II, respectively. According to equation 32, the drug can affect the residence time distribution by changing the $MRT(t)$ and $R_j(t)/RET(t)$ values. The exposure to rHuEPO resulted in a rapid increase in the $MRT(t)$ for times that peaked at $t = 2.5$ days followed by a slower return to the baseline values of 0.26 days. Consequently, the pdf transiently shifted to the right and increased in range from the baseline 0.26–2.6 days to the highest 1.2–12 days on day 2.5. The changes in the $MRT(t)$ values were regulated by inhibition of the reticulocyte transfer between the age compartments. Starting at $t = 1$ day, a peak can be observed at the pdf bars at residence time $MRT(t)$ that increases up to day $t = 5$ and moves towards larger MRTs for later times. These changes are described by $R_j(t)/RET(t)$ and can be attributed to a delayed transient increase in the reticulocyte release from the bone marrow precursor compartments. A similar pattern of changes in the reticulocyte residence time distributions was observed in typical patients receiving higher rHuEPO doses.

The mean reticulocyte residence times $MRT_{RET(t)}$ were plotted against time t for rHuEPO doses of 20, 60 and 160 kIU (figure 7). All curves start at about 2 days, increase rapidly to peak on $t = 1.3$ days and return slowly to the baseline values of 1.4 days. The peak MRT_{RET} increased with the dose and ranged from 6.0 to 6.8 days for the analysed doses. The offset part of the $MRT_{RET(t)}$ curve consists of two phases. The one with the steeper slope is determined by the loss of the rHuEPO inhibitory effect on the reticulocyte transfer between age compartments. The beginning of the terminal phase coincides with the time of the peak reticulocyte release rate from the bone marrow to the circulation. The presence of the age compartments for reticulocyte precursors allowed us to calculate the $MRT_{PREC(t)}$ by means of equation 34. The plots of $MRT_{PREC(t)}$ versus time corresponding to $MRT_{RET(t)}$ are shown in figure 7. The typical patient $MRT_{PREC(t)}$ started at the baseline of 2.7 days, decreased to the minimum of 2.4 days on day 2, increased and passed the baseline to reach the rebound peak of 2.8 days, and subsequently returned to baseline. The rebound peak seemed to be independent of the dose, whereas the peak times increased with the dose and ranged from 8 to 11 days for analysed doses. Consequently, given that the difference between the peak and the minimum was only 15% of the baseline MRT_{PREC} , it can be concluded that the rHuEPO effect on the distribution of the precursor residence time was limited as expected, as no drug effect was present in model D. This phenomenon is consistent with the physiological understanding, as EPO is believed to play a major role in determining the fate of colony-forming unit erythroids, proerythroblasts and basophilic erythroblasts, increasing the number of erythroid precursors but without altering the length of the cell cycle or the number of mitotic divisions involved in the differentiation process.[27] In addition, the time course of MRT_{PREC} was determined by the peak times of precursors P_i that occurred later for larger indices i .

The precursor cells eliminated from the last compartment P_{N_p} become the circulating reticulocytes. Therefore, the elimination rate $N_p \cdot P_{N_p}/T_p$ is the rate at which the reticulocytes are released from the bone marrow into the blood. Since model D postulated two mechanisms by which rHuEPO could affect the reticulocyte residence time distribution, analysis of the time course of the reticulocyte release is important in assessing the contribution of the newly produced reticulocyte to the distribution. As shown in figure 8, the time course of the reticulocyte production rate for a typical subject exhibited a lag time of 2 days, followed by an increase to a peak value and a return to the baseline value of 0.48% per day. Both the peak and the peak time were slightly increased with the dose and ranged from

0.71% to 0.78% per day and from 7 to 8 days, respectively, for the different analysed doses. The lag times and delayed peaks precluded contribution of the bone marrow reticulocytes to the rapid increase in $MRT_{RET(t)}$, which peaked at $t = 2.5$ days. The delayed times of the peak reticulocyte release rates coincided with the beginning of the slower phases of the $MRT_{RET(t)}$ time profiles (see figure 7). Consequently, the effect of rHuEPO on circulating reticulocytes dominates the effect on bone marrow reticulocytes with respect to the distribution of residence times.

Discussion

Several major mechanisms of rHuEPO action on reticulocyte production, including stimulation of the production of early progenitor cells in bone marrow, shortening of the differentiation and maturation times of progenitors in bone marrow, increase in the maturation times of the circulating reticulocytes, or the combination of these mechanisms, have been postulated in the literature. In this article, four nonlinear mixed-effects models were evaluated to describe the time course of the percentage of reticulocytes and to evaluate the effect of rHuEPO on the reticulocyte production rate and age distribution in healthy subjects according to the proposed major mechanism of rHuEPO action. The underlying assumption for further analysis was that the best performing model would indicate the dominant mechanisms controlling reticulocyte responses to rHuEPO in healthy volunteers and their relative contribution. The results presented should not be directly extrapolated to patients with a disease or treatment-related anaemia, where endogenous erythropoietin production and/or exogenous and endogenous erythropoietin disposition may be affected. In this situation, the mechanisms of rHuEPO action and/or their relative contribution may be altered as a consequence of the disease, and similar studies would be needed in different patient populations to fully explore the relative contribution of each mechanism to the overall effect of rHuEPO on reticulocytes.

One of the previously reported effects of rHuEPO is stimulation of the release of immature reticulocytes from the bone marrow into the circulation, which could be explained by shortening of the differentiation and maturation times of progenitors in bone marrow.[8] However, a model-independent assessment showed that for doses of 20–160 kIU, there was a significant lag time of 24–48 hours that was not consistent with the immediate increase in circulating reticulocyte counts due to systemic exposure to rHuEPO, which is also in agreement with the time course of young reticulocytes in mice, according to recently published data.[28] Such a lag time cannot be explained by a kinetic delay for subcutaneously administered rHuEPO, since the serum concentrations at 24 hours ranged from 400 to 5500 IU/L (see figure 5), and the time to reach the peak concentration values were about 8–24 hours for all doses. As a consequence, a delay between the stimulatory effect of rHuEPO on bone marrow precursors and their release into the blood was incorporated into all analysed models.

Our analysis confirmed that inclusion of dose-dependent shortening of the differentiation and maturation times of progenitors in bone marrow in the pharmacodynamic model improved the fit as compared with the model that only considered the stimulatory effect of rHuEPO on the proliferation of early progenitor cells. However, substantial further improvement of the model fit was achieved when the effect of rHuEPO on the stimulation of the release of immature reticulocytes from the bone marrow into the circulation was replaced by the time-dependent increase in the maturation times of the circulating reticulocytes. The stimulatory effect of rHuEPO on the proliferation and survival of erythroid progenitor cells is attributed to receptor-mediated inhibition of apoptosis via the STAT5 pathway and activation of the mitogen-activated protein kinase pathway.[1,2] A release of immature reticulocytes from bone marrow has been postulated as a consequence

of a direct action of rHuEPO on the sinusoid endothelial cells.[8] rHuEPO-mediated alterations of the interaction between splenic endothelial cells and macrophages has been reported as a factor determining the survival of young RBCs.[29] Consequently, the effect of rHuEPO on the reticulocyte age distribution can also be attributed to its effect on endothelial cells. Our results confirm the importance of the stimulatory effect of rHuEPO on the production of marrow progenitor cells in the increase in reticulocyte counts. However, this effect will have a very moderate effect on changes in the reticulocyte maturation time in the blood. We also conclude that the release of stress reticulocytes alone would not explain the time course of the reticulocyte response as accurately as a mechanism involving rHuEPO action that was not mediated by the bone marrow precursor cells. Inclusion of both processes into a pharmacodynamic model was not possible because of the problems with identifiability of the model parameters, and therefore it is difficult to determine which mechanism dominates. Consequently, a part of the rHuEPO effect on the distribution of reticulocyte maturation times has to be attributed to the release of stress reticulocytes. On the other hand, the observed rHuEPO-induced increase in the reticulocyte mean age cannot be explained solely by changes in interactions with the endothelial cells, since the reticulocyte age is determined by the levels of the residual RNA.

The assumption of a fixed lifespan for reticulocytes was made in previously published pharmacokinetic/pharmacodynamic models of the effect of rHuEPO on the production of RBCs, and led to estimates of 3–5 days.[24,25] These values are comparable to the reticulocyte lifespan that combines their maturation in the bone marrow and in the circulation. However, the reticulocyte lifespan calculated from the baseline reticulocyte count of 1–2% and a typical RBC lifespan of 120 days is about 1–2 days, which is consistent with the reported reticulocyte transit time in the circulation.[7] This discrepancy is caused by the fact that the estimates of the reticulocyte lifespan obtained by the lifespan-based pharmacokinetic/pharmacodynamic models are determined by the difference between the peak and lag times for the percentage of reticulocytes. For the data presented here, we have previously reported that this difference is about 6 days.[10] The estimates of T_R obtained by models A, B and C were 4–5 days, whereas model D yielded an estimate of 2.6 days. This indicates that incorporation of an rHuEPO effect on the reticulocyte lifespan into pharmacokinetic/pharmacodynamic models might improve their performance and remove the discrepancy between the estimated and baseline values of the reticulocyte lifespan.

Modelling of cell dynamics by means of the sequence of transit compartments is a technique that was introduced previously to assess pharmacologically induced changes in the lifespan of circulating platelets, neutrophils, red blood cells and tumour cells.[11–13,30] However, interpretation of a transit time as the reciprocal of the first-order rate constant becomes problematic if this rate becomes dependent on drug pharmacokinetics, as in model D. The standard calculation of the MRT for the one-compartment model based on the statistical moments was applied to the situation where the elimination rate varies with time. Another limitation is the approximation of the distribution of the reticulocyte residence times in the circulation by a combination of point distributions centred at the model mean transit times. Our goals were not only to determine the time course of the mean maturation time but also to visualize which fraction of the reticulocytes is affected by rHuEPO. This allowed us to differentiate between the effects of the shift of the distribution and temporal changes in the contributions of each reticulocyte age group to the overall distribution. A more adequate model would have included the reticulocyte age or residence time distribution as a dependent variable. Although plausible, such an approach would require a mathematical and numerical complexity that extends beyond the scope of this work.[31]

The analysed pharmacodynamic models described the major mechanisms controlling the production of reticulocytes and the pharmacological effects of rHuEPO on this process. We

purposely ignored other known processes that contribute to erythropoiesis. All progenitor cells in bone marrow – along with proliferation, differentiation, and maturation – undergo natural death (apoptosis), which makes the estimates of the current parameter apparent.[1] Similarly, young circulating RBCs are subject to neocytolysis, a preferential loss of the newly produced RBCs.[29] All circulating cells can also be eliminated due to random destruction processes. The production of RBCs following rHuEPO administration is downregulated by a process that could be linked to an increase in haemoglobin levels or depletion of the marrow progenitors.[24,25,32] This causes a rebound in the reticulocyte response that is observable in the data presented here. Inclusion of one or more of these processes in the structure of the pharmacodynamic models would require additional parameters. This would increase the degrees of freedom of nonlinear regression analysis and subsequently impact on the identifiability of model parameters and the precision of the estimates of the typical and IIV parameters. The pharmacodynamic models were structured to avoid problems with overparameterization, and only the major erythropoietic processes were considered.

Conclusion

The semiphysiological model for the reticulocyte responses obtained from phase I clinical studies in healthy volunteers implicated that rHuEPO both stimulates the production of marrow progenitor cells and transiently increases the mean transition time of the circulating reticulocytes. The validated model predicts that both effects contribute to the increase in reticulocyte counts in a dose-dependent manner. However, the change in the reticulocyte maturation time cannot be explained solely by the release into the circulation of immature stress reticulocytes. The presence of another mechanism by which rHuEPO affects the reticulocyte age distribution is apparent.

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Appendix

Mean Residence Time for One-Compartment Model with Time-Dependent First-Order Elimination Rate

Consider a one-compartment model where drug amount A is eliminated at the first-order rate $k(t)$, which can depend on time (equation 35):

$$\frac{dA}{dt} = -k(t) \cdot A \quad (\text{Eq. 35})$$

Let t_0 be an arbitrary time and the amount of drug at this time is $A(t_0)$. For an incrementally small Δt , the number of drug particles comprising $A(t_0)$ that exit the compartment between times t and $t + \Delta t$ is $k(t) \cdot (A(t)/m) \cdot \Delta t$, where m is the mass of a single drug particle. In addition, let t_{out} denote the time at which a particle exits the compartment. Then t_{out} can be considered as a random variable, and the probability that a particle will leave the compartment between times t and $t + \Delta t$ can be calculated as equation 36:

$$P(t < t_{\text{out}} < t + \Delta t) = \frac{k(t) \cdot [A(t)/m] \cdot \Delta t}{A(t_0)/m} \quad (\text{Eq. 36})$$

Consequently, the probability density function for the t_{out} distribution is represented by equation 37:

$$\text{pdf}(t_{\text{out}}) = \frac{k(t_{\text{out}}) \cdot A(t_{\text{out}})}{A(t_0)} \quad (\text{Eq. 37})$$

and the expected t_{out} $[E(t_{\text{out}})]$ can be expressed as follows (equation 38):

$$E(t_{\text{out}}) = \int_{t_0}^{\infty} t_{\text{out}} \cdot \text{pdf}(t_{\text{out}}) \cdot dt_{\text{out}} = \frac{\int_{t_0}^{\infty} t \cdot k(t) \cdot A(t) \cdot dt}{A(t_0)} \quad (\text{Eq. 38})$$

The time during which a particle of $A(t_0)$ resides in the compartment is the time elapsing between t_0 and t_{out} . Hence the MRT is (equation 39):

$$\text{MRT}(t_0) = E(t_0 - t_{\text{out}}) = t_0 - E(t_{\text{out}}) \quad (\text{Eq. 39})$$

To simplify the integral in equation 38, multiply both sides of equation 35 by t and integrate from t_0 to infinity (equation 40):

$$\int_{t_0}^{\infty} t \frac{dA}{dt} dt = - \int_{t_0}^{\infty} t \bullet k(t) \bullet A(t) dt \quad (\text{Eq. 40})$$

Integration by parts implies that (equation 41):

$$\int_{t_0}^{\infty} t \frac{dA}{dt} dt = - t_0 \bullet A(t_0) - \int_{t_0}^{\infty} A(t) \bullet dt \quad (\text{Eq. 41})$$

Combining equations 38 and 41 results in equation 42:

$$\text{MRT}(t_0) = \frac{\int_{t_0}^{\infty} A(t) \bullet dt}{A(t_0)} \quad (\text{Eq. 42})$$

Note that introducing a new variable $X(t, t_0) = A(t)/A(t_0)$ [equation 43]:

$$\text{MRT}(t_0) = \int_{t_0}^{\infty} X(t, t_0) \bullet dt \quad (\text{Eq. 43})$$

where $X(t, t_0)$ is a solution to equation 44:

$$\frac{dX}{dt} = - k(t) \bullet X, \text{ for } t > t_0 \quad (\text{Eq. 44})$$

with the initial condition (equation 45):

$$X(t_0) = 1 \quad (\text{Eq. 45})$$

Fig. 1.

Pharmacokinetic model describing the absorption and disposition of recombinant human erythropoietin (rHuEPO). A_1 = amount of rHuEPO in the depot compartment; A_2 = amount of rHuEPO in the central compartment; A_3 = amount of rHuEPO in the peripheral compartment; C = concentration in the central compartment (A_2/V_2); CL_I = linear clearance; D_1 = estimated duration of faster absorption phase; D_2 = estimated duration of slower absorption phase; F = bioavailability; fr = bioavailability fraction; k_a = first-order absorption rate constant; K_m = Michaelis-Menten constant; Q = intercompartmental clearance; t_{lag2} = lag time for the slower absorption phase; V_2 = volume of distribution of the central compartment; V_3 = volume of distribution of the peripheral compartment; V_{max} = maximum elimination rate of the saturable pathway.

Fig. 2.

Schematic diagrams of the four pharmacodynamic models. The dashed arrows indicate links to the recombinant human erythropoietin (rHuEPO) central compartment of the pharmacokinetic model. The model operation and symbols are explained in the Methods section. τ = signal transduction transit time; EC_{50} = concentration of rHuEPO eliciting 50% of maximum signal; k_{in} = maximal zero-order endogenous production rate constant of progenitor cells; N = number of compartments; P = precursor cell age compartment; R = reticulocyte compartment; SC_{50} = EPO serum concentration necessary to maintain the progenitor cell production rate at 50% of its maximum; SC_{50p} = EPO serum concentration eliciting 50% of maximum stimulation of precursor cell maturation, S_{max} = maximum stimulation factor of precursor cell maturation; T_p = mean lifespan of precursor cells; T_R = mean lifespan of circulating reticulocytes.

Fig. 3. Diagnostic plots for the pharmacokinetic model **(a)** and **(b)** and pharmacodynamic model D **(c)** and **(d)** of subcutaneous administration of a single dose of recombinant human erythropoietin (rHuEPO).

Fig. 4.

Time course of recombinant human erythropoietin (rHuEPO) serum concentrations (**a**) and percentage of reticulocytes counts (**b**) following subcutaneous administration of rHuEPO at different doses. The lines and the shaded area represent the median and 90% prediction interval from the posterior predictive check, respectively.

Fig. 5.

Mean (+SD) recombinant human erythropoietin (rHuEPO) serum concentrations (**a**) and reticulocyte counts (**b**) at predose, 24 h and 48 h following subcutaneous administration in selected dosing groups. None of the mean reticulocyte counts at time $t = 24$ h were significantly different from the corresponding one at time $t = 0$ h. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs the mean reticulocyte count at time 0; paired two-tailed t-test.

Fig. 6.

Age distributions of circulating reticulocytes at various timepoints for a typical subject who received a subcutaneous recombinant human erythropoietin dose of 20 kIU. The j th bar represents the R_j reticulocyte compartment where reticulocytes are assumed to have the same age $j \cdot \text{MRT}(t)$ at time t . **MRT** = mean residence time.

Fig. 7.

Mean age of reticulocyte precursors in bone marrow **(a)** and mean residence time of circulating reticulocytes **(b)** as functions of time for a typical subject who received subcutaneous recombinant human erythropoietin doses of 20 kIU, 60 kIU and 160 kIU. The curves in **(a)** and **(b)** were calculated according to equations 34 and 33, respectively.

Fig. 8. Rate of reticulocyte release from the bone marrow as a function of time for a typical subject who received subcutaneous recombinant human erythropoietin doses of 20 kIU, 60 kIU and 160 kIU.

Table I

Descriptive statistics of the recombinant human erythropoietin individual maximum *a posteriori* Bayesian estimates of pharmacokinetic parameters from the population model^a

Parameter	Prior mean (% CV)	Posterior mean (% CV)	Range
Baseline serum erythropoietin (IU/L) ^b	13.9 (30)	11.4 (34)	6.2–26.6
CL _l (L/h)	0.358 (33)	0.325 (15)	0.226–0.468
V _{max} (IU/h)	211 (29)	239 (44)	83–634
V ₂ (L)	3.89 (31)	4.17 (25)	1.81–7.10
F ₀ (%)	62 (35)	47 (42)	15–100
fr (%)	60 (45)	78 (46)	51–93
k _a (/h)	0.034 (36)	0.039 (47)	0.016–0.155
D ₁ (h)	0.725 (125)	1.678 (102)	0.128–12.718
t _{lag2} (h)	2.72 (53)	2.75 (31)	1.00–5.47

^a K_m was fixed at 394 IU/L, Q was fixed at 0.044 L/h, V₃ was fixed at 1.64 L, D₂ was fixed at 37.8 h, E_{max}(F) was fixed at 64.9% and ED₅₀(F) was fixed at 63.2 kIU.

^b Circadian rhythm was fixed to values previously reported by Olsson-Gisleskog et al.[18]

CL_l = linear clearance; CV = coefficient of variation; D₁ = estimated duration of faster absorption phase; D₂ = estimated duration of slower absorption phase; ED₅₀(F) = dose giving 50% of increase in absolute bioavailability; E_{max}(F) = maximum increase in bioavailability with dose; F₀ = minimum absolute bioavailability; fr = bioavailability fraction; k_a = first-order absorption rate constant; K_m = Michaelis-Menten constant; Q = intercompartmental clearance; t_{lag2} = lag time for the slower absorption phase; V₂ = volume of distribution of the central compartment; V₃ = volume of distribution of the peripheral compartment; V_{max} = maximum elimination rate of the saturable pathway.

Table II

Estimates of the population parameters of pharmacodynamic models

Model and value parameter	Structural model parameters				Interindividual variability (ω) [%]				Residual variability (σ) [%]			
	RE T_0 (%)	T R (h)	T p (h)	SC $_{50}$ (IU/L)	S $_{max}$	EC $_{50}$ (IU/L)	τ (h)	RE T_0		T R	T p	SC $_{50}$
Model A (n = 927)												
Original dataset	1.29	127	59.7	42.8				19.0	17.9	29.5	37.0	70.5
BS median (RSE %)	1.29 (2.5)	127 (4.8)	59.7 (6.2)	42.9 (5.7)				18.7 (11.5)	17.7 (23.2)	28.9 (12.4)	37.0 (12.7)	70.5 (5.0)
BS 95% CI	1.23, 1.36	115, 139	52.4, 67.1	38.2, 47.8				14.0, 22.6	6.3, 22.8	21.7, 36.0	26.9, 45.8	64.1, 78.0
Model B (n = 769)												
Original dataset	1.58	96.2	165	31.8 ^a	0.411			18.4	25.2	15.9	41.8 ^a	69.6
BS median (RSE %)	1.57 (3.4)	96.5 (16.9)	166 (11.4)	32.3 (6.1) ^a	0.416 (18.9)			18.1 (13.8)	25.5 (50.1)	15.2 (32.7)	41.4 (11.4)	69.4 (5.2)
BS 95% CI	1.46, 1.67	55.3, 116	137, 213	28.8, 36.6	0.273, 0.588			13.2, 22.7	0.01, 55.8	5.0, 23.6	32.4, 50.5 ^a	63.1, 77.1
Model C (n = 702)												
Original dataset	1.51	121	158	15.4/42.2 ^a	0.806			19.9	14.5	21.3	32.2 ^a	68.6
BS median (RSE %)	1.51 (2.6)	119 (7.0)	160 (6.5)	15.2 (16.8)/42.4 (10.5) ^a	0.819 (22.0)			19.7 (10.1)	10.8 (96.6)	21.3 (12.1)	32.1 (13.9) ^a	68.6 (5.4)
BS 95% CI	1.44, 1.59	106, 137	139, 179	9.5, 20.0/35.6, 52.2 ^a	0.51, 1.19			15.5, 13.2	<0.01, 21.9	16.2, 26.2	22.7, 40.1 ^a	62.3, 76.5
Model D (n = 622)												
Original dataset	1.24	62.2	118	7.61 ^a		56.3	4.89	19.8	36.2	27.4	107 ^a	63.1
BS median (RSE %)	1.23 (3.23)	62.2 (8.39)	117 (13.9)	7.91 (35.9)		71.1 (49.5)	4.83 (60.7)	19.7 (10.5)	35.8 (11.5)	25.1 (23.8)	108 (18.9) ^a	62.9 (4.62)
BS 95% CI	1.15, 1.30	44.1, 78.6	86, 144	2.81, 14.25 ^a		34.4, 166.5	0.24, 10.8	15.6, 23.8	27.9, 44.2	14.2, 36.5	74.1, 154 ^a	57.5, 68.3

^aSC₅₀P.

τ = signal transduction transit time; **BS** = nonparametric bootstrap; **EC₅₀** = concentration of recombinant human erythropoietin eliciting 50% of maximum signal; n = number of replicates; **RET₀** = baseline reticulocyte count; **RSE** = relative standard error; **SC₅₀** = erythropoietin serum concentration necessary to maintain progenitor cell production at 50% of maximum production; **SC₅₀P** = erythropoietin serum concentration eliciting 50% of maximum stimulation; **S_{max}** = maximum stimulation factor; **T_P** = mean lifespan of precursor cells; **T_R** = mean lifespan of circulating reticulocytes.