Interspecies Comparisons of Pharmacokinetics and Pharmacodynamics of Recombinant Human Erythropoietin

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ABSTRACT:

Erythropoietin (EPO) has a highly conserved structure among mammals, and thus recombinant human EPO (rHuEPO) has biological activity in various species. This study explores the interspecies relationships of the pharmacokinetics (PK) and pharmacodynamics (PD) of rHuEPO. The PK parameters such as clearance (CL) and volume of distribution (V\textsubscript{ss}) after i.v. doses of rHuEPO were obtained in several species via noncompartmental analysis and were assessed using the traditional allometric approach. Also, PK/PD modeling of rHuEPO concentrations and responses [reticulocytes, red blood cells (RBCs), and hemoglobin] was performed following a range of i.v. and s.c. doses in rats, monkeys, and humans. Nonlinear disposition (V\textsubscript{max}, K\textsubscript{m}) and s.c. absorption rate and bioavailability parameters of rHuEPO were examined. A cascade, indirect, lifespan PD model was applied to recover efficacy (S\textsubscript{max}) and potency (SC\textsubscript{50}) of rHuEPO on erythropoiesis and erythroid cell lifespan parameters. Despite nonlinear rHuEPO disposition, CL and V\textsubscript{ss} were highly correlated with body weight (R\textsuperscript{2} > 0.92) with allometric scaling exponents of 0.708 for CL and 0.853 for V\textsubscript{ss}. The s.c. bioavailability increased with dose in monkeys and humans but appeared to be dose-independent in rats. A correlation between S\textsubscript{max} or SC\textsubscript{50} and body weight was not obvious. However, RBC lifespans obeyed allometric principles. Size dependence was found for PK and lifespan parameters, whereas pharmacologic parameters were independent of body weight.

Recombinant human erythropoietin (rHuEPO) has been widely used clinically for treatment of anemia associated with chronic renal failure and chemotherapy. Erythropoietin (EPO) is the primary hormone of erythropoiesis and mainly synthesized in the kidney in response to hypoxia. On binding to its receptor on progenitor cells in the bone marrow, EPO stimulates proliferation and differentiation of erythroid cells, leading to an increase in reticulocytes followed by increases in red blood cells (RBCs) and hemoglobin (Hb) in the blood.

EPO is a glycosylated protein with molecular mass of 30.4 kDa. Amino acid sequences in the coding region of mature EPO protein show a high degree of homology among mammals. The human EPO sequence is 91% homologous to monkey EPO, 85% to cat and dog EPO, and 80 to 82% to sheep, pig, mouse, and rat EPO (Wen et al., 1993). This explains the biological activity of rHuEPO that has been observed across species.

Interspecies similarities in structural, physiological, and biochemical properties result in allometric equations that characterize the dependence of biological variables on body weight (Dedrick, 1973; Mordenti, 1986). Allometric scaling has been widely used to predict pharmacokinetic (PK) parameters of small molecules but has been applied to a limited extent to macromolecules. It is well accepted that interspecies scaling works best for drugs that are eliminated primarily by physical processes (i.e., biliary or renal excretion) compared with metabolism or drugs with nonlinear disposition. Because of their large molecular mass (1–400 kDa), clearance (CL) mechanisms for proteins may be significantly different from small molecules, i.e., proteolysis, renal filtration and catabolism, and hepatic uptake via sugar-recognizing receptors (Braeckman, 1999). In addition, many macromolecules are endogenously present in the body, which may influence the disposition of exogenously given molecules. Effects of species specificity and immune-mediated CL on scaling preclinical data to humans have been reported (Richter et al., 1999). However, despite these factors that may complicate interspecies scaling of proteins, studies have shown that CL of proteins may be predicted with a reasonable accuracy from preclinical data (Mordenti et al., 1991; Mahmood, 2004; Tang and Mayersohn, 2006).

The assessment of pharmacodynamics (PD) among species is seldom carried out with mechanistic PK/PD models. These offer the opportunity to assess both pharmacologic (capacity, sensitivity) factors and systemic variables (biochemical, physiological). The former are likely to exhibit genetic differences, whereas the latter are more apt to obey allometric principles (Lepist and Jusko, 2004).

In this study we evaluated the allometric relationships of PK and PD properties of rHuEPO in various animals and humans. The kinetic characteristics of rHuEPO include nonlinear PK, prolonged absorption, and variable incomplete bioavailability on s.c. administration, which has often been observed for other therapeutic protein drugs (Radwanski et al., 1998; Mager and Jusko, 2002) and can be a challenge in assessment of the allometry. In the presence of nonlinear kinetics, direct comparisons of PK parameters of interest such as CL,
volume of distribution, and bioavailability are difficult because these values change with dose when calculated by traditional methods. Our laboratory has previously reported the PK/PD analysis of rHuEPO following i.v. and s.c. doses in rats, monkeys, and humans using comprehensive PK/PD models that share general common structures, thereby allowing interspecies comparisons of PK/PD parameters. To extend our findings, additional information from the literature was included.

Materials and Methods

CL and Steady-State Volume of Distribution by Noncompartmental Analysis. The PK data following i.v. administration of rHuEPO were obtained from the literature for various species, including rats (Kato et al., 2001; Woo et al., 2006), rabbits (Yoon et al., 1997), monkeys (Ramakrishnan et al., 2003), dogs (Fu et al., 1988), sheep (Widness et al., 1996; McLennan et al., 2005), and humans (Ramakrishnan et al., 2004). The values of CL and steady-state volume of distribution \( V_{ss} \) were calculated based on a noncompartmental approach (Gibaldi and Perrier, 1982). When the reference did not report the values but included time profiles of rHuEPO concentrations, the data were digitized, and WinNonlin (Pharsight, Mountain View, CA) was used to calculate the parameters with the noncompartmental analysis option. Because of the nonlinearity, the values of CL and \( V_{ss} \) were calculated at each dosage.

PK and PD Parameters by PK/PD Modeling Approach. The PK and PD parameters of rHuEPO were obtained from rats (Woo et al., 2006), monkeys (Ramakrishnan et al., 2003), and humans (Ramakrishnan et al., 2004). Each study reported the parameter estimates by computational fittings using the PK and PD data from i.v. and s.c. administration of a wide dosage range of rHuEPO. A general structure of the PK/PD model of rHuEPO applied to the three species is shown in Fig. 1. The PK model depicts nonlinear elimination \( (V_{\text{max}}, K_m) \) and s.c. absorption kinetics where the bioavailable fraction \( (F) \) of rHuEPO gets absorbed via a zero-order rate \( (k_a) \) followed by a first-order rate \( (k_e) \) from the injection site. The PD model mimics the process of erythropoiesis from bone marrow erythroid cells \( (P1 \text{ and } P2) \) to peripheral blood cells \( (RET \text{ and } RBC_{ss}) \). The PD model is based on cell lifespan concepts where the cell conversion from a predecessor to a successor is controlled by its own lifespan (Krzyszanski et al., 1999). The mean lifespans of successive cell populations were represented by \( T_{P1}, T_{P2}, T_{RET}, \) and \( T_{RBC} \). The RBCs and Hb increase as rHuEPO stimulates the proliferation and differentiation of progenitor cells \( (S_{\text{max}}, SC_{\text{IC0}}) \), but a feedback regulatory mechanism prevents their excessive increase by inhibiting the production of progenitors \( (T_{\text{max}}, IC_{\text{IC0}}) \). Details about the PK/PD model equations were described in the original publications. Representative time profiles of the observed and predicted rHuEPO concentrations and reticulocytes in rats and humans are shown in Fig. 2.

The PK parameters for rats and humans based on single-dose studies were directly taken from the original articles. The PD data from monkey and two multiple-dose studies in humans were reanalyzed with the PK model used for rats (Woo et al., 2006). All the computer fittings were performed by ADAPT II (Biomedical Simulation Resources, Los Angeles, CA).

Allometric Analysis. An allometric equation was used to relate PK or PD parameters of rHuEPO with body weight: \( Y = a \cdot BW^b \), where \( Y \) is the parameter of interest, \( BW \) is body weight in kilograms, \( a \) is the allometric coefficient, and \( b \) is the allometric exponent. To include a wide range of body weights, the RBC lifespans for other animals (Allison, 1960) were included.

Results

Comparisons of CL and \( V_{ss} \) of rHuEPO. Table 1 lists the body weights, ranges of dosages, CL, and \( V_{ss} \) for i.v. administration of rHuEPO from six species. Based on the noncompartmental analysis, sets of CL and \( V_{ss} \) exist for as many as the number of dosages evaluated; thus, these values are presented as a range. Most studies have reported the nonlinear PK of rHuEPO, mainly characterized by a decrease in CL with increasing dose. With respect to \( V_{ss} \), across species, it appeared that the values were greater than plasma volume (i.e., 4.5% of body weight or 45 ml/kg) but less than about half of the extracellular water space volume (i.e., 27% of body weight or 270 ml/kg). Although there did not appear dose dependence in \( V_{ss} \), especially in small animals, the values in some species such as monkey, sheep, and humans showed changes for a wide range of dosages. They were not necessarily dose-related but tended to be slightly higher at low and high doses compared with those in medium doses.

The interspecies relationships of CL and \( V_{ss} \) taken from all the dosages of rHuEPO were described by allometric equations. As shown in Fig. 3, a good correlation \( (R^2 > 0.92) \) between body weight and CL and \( V_{ss} \) was observed for rHuEPO. The parameters from humans were not included in the allometric analysis but predicted for a body weight of 70 kg from five animal species. The predicted values for CL (4.58 ml/h/kg) and \( V_{ss} \) (48.21 ml/kg) in humans were located within the range of observed values, close to ones obtained at the dosage of 150 IU/kg in humans. The exponent of CL (0.78) for rHuEPO was close to 0.75, whereas the exponent of \( V_{ss} \) (0.853) was slightly less than 1. The values of CL and \( V_{ss} \) for rHuEPO-B in mice, rats, dogs (Bleuel et al., 1996), and humans (Halstenson et al., 1991), although they were not included in the analysis, are also shown in Fig. 3. In general, CL and \( V_{ss} \) for rHuEPO-B fell closely near the regression line for those of rHuEPO except in mice, whose values were higher than expected from the allometric equations.

Comparisons of PK Parameters from PK Modeling. The PK parameters from the fittings were retrospectively compared for rats, monkeys, and humans. The PK data were described by a two-compartment model for rats and monkeys and a one-compartment model for humans. Besides the Michaelis-Menten process, rats had a non-saturable elimination pathway \( (k_e) \). Figure 4 illustrates comparisons of nonlinearity parameters \( (V_{\text{max}}, K_m) \), central volume of distribution...
(Vc), and first-order absorption rate constant (ka) among three species. The Vmax was scaled with body weight (R^2 > 0.98) with the exponent of 0.504, whereas there was no correlation between Km and body weight. The value of Km was similar between monkeys and humans but much smaller in rats, probably because of having keI in addition to saturable CL in the PK model for rats. The Vc was smaller than Vss for rats and monkeys, which explains the need of the peripheral compartment in their PK model. The correlation between Vc and body weight was nearly unity (R^2 > 0.999), and Vc was almost directly proportional to body weight. The first-order absorption rate constants (ka) associated with s.c. doses were negatively related to body weight. There was no obvious trend in the duration of the zero-order rate (13.5, 10, and 44–60 h) or the fraction absorbed via the zero-order process (68, 35, and 88%) among rats, monkeys, and humans.

Figure 5 displays the values of s.c. bioavailability versus doses of rHuEPO obtained from simultaneous fittings of both i.v. and s.c. data in each species. The bioavailability observed in monkeys and humans clearly showed a dose-dependent increase with increasing doses and, more interestingly, resulted in 100% absorption at dosages >2400 IU/kg rHuEPO in both species. Unlike these two species, the s.c. bioavailability in rats was constant (58%) and fell in the middle of the range.

**PD Parameters of rHuEPO.** The mechanism-based PD model (Fig. 1) was used to quantitatively describe the effects of rHuEPO, including reticulocytes, RBCs, and Hb. The typical response profiles of reticulocytes to rHuEPO administration are presented in Fig. 2. The only difference in PD models for the three species was the component used to model the feedback inhibition (IC50): reticulocytes for humans (Ramakrishnan et al., 2004), Hb for rats (Woo et al., 2006), and none for monkeys (Ramakrishnan et al., 2003). For comparison purposes, the PD data from the multiple-dosing studies in humans were modeled with Hb being the regulator of the feedback. For monkeys, however,
the IC50 could not be estimated owing to very modest rebound phases observed in reticulocyte profiles.

The values of Smax, SC50, and IC50 are provided in Table 2. Although the Smax in humans based on the single-dose studies was higher than others, it ranged 1.5 to 2.0 across species. The values of SC50 varied from species to species: 26 to 45 mIU/ml for humans, 65 mIU/ml for rats, and 153 mIU/ml for monkeys, suggesting size independence. The values of IC50 with the same units were similar for rats (1.79 g/dl) and humans (2.88 g/dl).

Except for RBC lifespans that were usually fixed to the values from the literature, lifespan parameters for other RBC precursor cells were obtained through the computational fittings. As can be seen in Fig. 6, the mean lifespans of P1 and RBCs were well correlated to body weight ($R^2 > 0.8$), whereas the scaling of $T_{REP}$ with weight was less obvious ($R^2 = 0.432$). Adding additional values from a wide range of species clearly showed that the RBC lifespans were dependent on their body size. Interestingly, the exponents of lifespan parameters were very similar, especially for $T_p$, $T_R$, and $T_{RBC}$, ranging from 0.124 to 0.148.

Discussion

PK. Interspecies scaling has been frequently used to predict human PK parameters based on animal data and is a useful tool for drug development. Application of allometric scaling for protein drugs is particularly interesting because of their different characteristics from small molecules. Mordenti et al. (1991) showed excellent allometric relationships of CL and V for five proteins. Subsequent studies by Mahmood (2004) using 15 proteins and by Tang and Mayersohn (2006) using 10 proteins further indicated that CL of proteins may be scaled to body weight with reasonable consistency. The exponents for CL ranged widely from 0.577 to 1.287. On average, CL has an exponent of 0.75 and volume of 1.0 (Mordenti, 1986). Whether this is also applicable for CL of proteins is not known. Although V was not included in those studies, distribution of many biologic agents is often concentrated in the vascular space because of their large molecular size; thus, it can be expected that V, like blood volume, may be directly proportional to body weight.

For rHuEPO the exponent of $V_e$ (0.85) was less than 1, whereas the exponent of $V_c$ (0.983) was almost 1. The difference between $V_e$ and $V_c$ appeared to be larger in small animals, suggesting more distribution of rHuEPO outside the central compartments in small animals. Mahmood (2004) evaluated the interspecies scaling for CL of rHuEPO using rat (Kato et al., 2001), rabbit (Yoon et al., 1997), and dog (Fu et al., 1988), as well as rHuEPO-β from mouse, rat, and dog (Bleuel et al., 1996), and reported the exponents of 0.748 and 0.775. This slight difference from our study (0.708) is related to which value of CL was chosen from the original studies. Kato et al. (1997) obtained the allometric exponent of 0.70 for nonsaturable CL of rHuEPO from rats, dogs, and humans.

The major route of elimination for compounds greatly influences the predictability of human CL via allometry. As seen for small molecules, successful applications for renally eliminated macromolecules have been shown, including digoxin-specific Fab (Grene-Lerouge et al., 1996), interferon-α (Lave et al., 1995), Apo2L/tumor necrosis factor-related apoptosis-inducing ligand (Kelley et al., 2001), and recombinant CD4 (Mordenti et al., 1991). The disposition of rHuEPO has been assessed in context of ablation of kidneys (Emmanouel et al., 1984; Fu et al., 1988; Yoon et al., 1997), liver (Szykowski, 1980; Widness et al., 1996), and bone marrow (Pirso et al., 1991; Chapel et al., 2001) in various animals. The results have consistently indicated that the kidneys contribute in a very minor fashion to rHuEPO elimination, i.e., only 4 to 7% of its overall elimination rate in animals and at most 10% in humans. The alteration of bone marrow function results in a significant change in rHuEPO PK, indicating that bone marrow may be a major route of rHuEPO elimination for low doses and may contribute to its nonlinear PK via receptor-mediated endocytosis. Although Michaelis-Menten kinetics does not directly reflect receptor-mediated drug elimination in bone marrow, the $K_m$ value is similar to SC50 in rats (Woo et al., 2006) and sheep (Veng-Pedersen et al., 1999), suggesting that the saturable elimination and receptor binding may be closely related. The $V_m$ max correlated to body weight with an exponent of 0.504, suggesting a relationship of enzyme and/or receptor capacity.

The rate and extent of s.c. absorption of rHuEPO were compared based on results from three species. The characteristics of s.c. absorption of rHuEPO include a fast rising and flip-flop kinetics (Fig. 2). In PK modeling, these features were captured by a zero-order followed by a first-order ($k_p$) input from the injection site. The $k_p$ was found to be inversely correlated to body weight. This may be related to tissue size and blood flow determinants of drug loss from the site of absorption. The zero-order process probably reflects rHuEPO absorption via the lymphatic pathway. This was based on studies that have shown involvement of lymphatics in the absorption of s.c. administered proteins and >50% of an s.c. administered dose being absorbed by the regional lymphatics for molecules with molecular mass >16...
kDa (Supersaxo et al., 1990; Porter and Charman, 2000). The PK model suggested >68% of fraction absorbed via the zero-order input for rats and humans but to a lesser extent in monkeys (35%).

The contribution of the lymphatics to absorption of s.c. rHuEPO has been measured in sheep as 75% of the administered dose (McLennan et al., 2005). The rate and bioavailability of s.c. absorption are influenced by the site of injection (Jensen et al., 1994) because regional lymphatic flow is highly dependent on the density of lymphatic vessels and frequency of muscle movement. This may explain why the duration of zero-order input and fraction of absorbed dose via the zero order were not correlated to body size but are rather specific to species. A similar reason may indicate why bioavailability was independent of body weight. Bioavailability has been cited as a difficult parameter to predict from preclinical data (Mahmood, 2000; Tang and Mayersohn, 2006). The bioavailability for monkeys and humans varied with dosages <2400 IU/kg, but above that complete absorption was observed. The cause of incomplete s.c. bioavailability of rHuEPO is not known. The s.c. administration of rHuEPO encapsulated in liposomes produces almost 3 times higher lymph concentrations and bioavailability of rHuEPO in rats (Moriya et al., 1997), suggesting that liposomes may protect rHuEPO from enzymatic degradation during absorption or transport into the circulation through the lymphatic pathway.

**PD.** Erythropoiesis involves a series of cell proliferation and differentiation steps from hematopoietic stem cells through the mature RBCs. Because erythroid cells in the bone marrow were not individually measured, these cells were lumped into two subgroups in the PD modeling based on their similar characteristics, mainly dependence on EPO. Direct experimental measurements on survival half-lives of these RBC precursors are not available in the literature; thus, their lifespan values relied on the estimates from the PD modeling. Biological time periods (e.g., circulation time, maximum lifespan potential) tend to have an exponent of 0.25 (Mordenti, 1986). Based on the results from rats, monkeys, and humans, the cell lifespans appeared to be dependent of body size. The exponents (0.124–0.148) were less

<table>
<thead>
<tr>
<th>Species</th>
<th>(S_{\text{max}})</th>
<th>(SC_{50})</th>
<th>(IC_{50})</th>
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<tbody>
<tr>
<td>Rat</td>
<td>1.87</td>
<td>65.37</td>
<td>1.79 g/dl</td>
</tr>
<tr>
<td>Monkey</td>
<td>1.92</td>
<td>153</td>
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<tr>
<td>Human</td>
<td>4.25</td>
<td>26.53</td>
<td>38.71 (\times 10^{10}) cells/l</td>
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<tr>
<td>Human</td>
<td>1.53</td>
<td>45</td>
<td>2.88 g/dl</td>
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* Taken from the original study.
* Represented as unit of hemoglobin.
* Remodeled PD data from Ramakrishnan et al. (2003).
* Represented as reticulocyte units.
* Remodeled PD data from multiple dosing studies (Ramakrishnan et al., 2004).
than 0.25, but their slopes were very similar to each other. Interspecies scaling of RBC lifespan based on a rich data set from diverse sources supported this finding.

Little is known about allometric relationships in PD. Few studies have reported allometric scaling of PD-related parameters via modeling approaches (Gronert et al., 1995; Lepist and Jusko, 2004). As expected, the pharmacologic parameters of rHuEPO \( (S_{\text{max}} \text{ and } SC_{50}) \) did not follow allometric principles but may be more related to other factors such as receptor density and/or structural homology of EPO or erythropoietin receptor between species. However, the sequence homology of EPO alone was not enough to explain these observations.

Interspecies comparisons of the PK/PD of rHuEPO may provide insights into the selection of animal models to study rHuEPO or its analogs. Rats appear to be a suitable preclinical small animal model for rHuEPO kinetics and dynamics. The PK/PD properties observed in rats were qualitatively similar to those observed from humans except for dose-dependent bioavailability. The RBC lifespan of rats (60 days) is half of that in humans (120 days). Other cell lifespan were also estimated to be about half of those for humans. However, changes in hematological baselines in rats with age add complexity in model development and interpretation of results (Woo et al., 2006). Despite the high EPO homology (91%), monkeys well reflect the PK of rHuEPO for humans, especially in s.c. bioavailability, compared with the PD. The tolerance/rebound phenomenon observed in monkeys (Ramakrishnan et al., 2003) was not as prominent as in rats or humans, resulting in no need of the IC\(_{50}\). Sheep are often used as a developmental animal model for erythropoiesis, probably because sheep have almost the same lifespan of reticulocytes and RBCs and resemble developmental erythropoiesis in humans (Moritz et al., 1997).

We examined interspecies comparisons of PK and PD of rHuEPO via noncompartamental and mechanism-based PK/PD modeling approaches. Based on our findings, despite nonlinear PK behavior of rHuEPO, CL and \( V_{\text{ss}} \) were highly correlated with body weight. Bioavailability of rHuEPO following s.c. administration was dependent on dosage rather than body size. The pharmacologic parameters \( S_{\text{max}} \) and \( SC_{50} \) appeared to be species-specific, but system parameters for RBCs and precursor lifespans generally obeyed principles of allometry. The assessment of PD among species with mechanistic PK/PD models provided the opportunity to separate pharmacologic factors from systemic variables that are not dependent on drugs. This accumulating experience with PK/PD of rHuEPO will certainly help to understand PK/PD characteristics of EPO analogs and mimetics and to facilitate their drug development processes.

**References**


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