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Receptor-Based Dosing Optimization of Erythropoietin in Juvenile Sheep after Phlebotomy

Matthew Rosebraugh, John A. Widness, and Peter Veng-Pedersen

Colleges of Pharmacy (M.R., P.V-P.) and Medicine (J.A.W.) and Department of Pediatrics, the University of Iowa, Iowa City, Iowa

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ABSTRACT:

The primary objective of this work was to determine the optimal time for administration of an erythropoietin (Epo) dose to maximize the erythropoietic effect using a simulation study based on a young sheep pharmacodynamic model. The dosing optimization was accomplished by extending a Hb production pharmacodynamic model, which evaluates the complex dynamic changes in the Epo receptor (EpoR) pool from the changes in Epo clearance. Fourteen healthy 2-month-old sheep were phlebotomized to Hb levels of 3 to 4 g/dl. Epo clearance was evaluated longitudinally in each animal by administering tracer doses of ^{125}I-recombinant human Epo multiple times during the experiment. Kinetic parameters were estimated by simultaneously fitting to Hb data and Epo clearance data. The phlebotomy caused a rapid temporary increase in the endogenous Epo plasma level. The Hb began to increase after the increased in the Epo level with a lag time of 1.13 ± 0.79 days. The average correlation coefficients for the fit of the model to the Hb and clearance data were 0.953 ± 0.018 and 0.876 ± 0.077, respectively. A simulation study was done in each sheep with fixed individual estimated model parameters to determine the optimal time to administer a 100 U/kg intravenous bolus Epo dose. The optimal dose administration time was 11.4 ± 6.2 days after phlebotomy. This study suggests that the Hb produced from Epo administration can be optimized by considering the dynamic changes in the EpoR pool.

Introduction

Erythropoietin (Epo) is a glycoprotein hormone responsible for regulating erythrocyte production. Epo exerts its mechanism of action by binding to erythropoietin receptors (EpoRs) located on erythroid progenitor burst-forming units and colony-forming units found primarily in the bone marrow(Casadevall, 1995).

Information regarding the in vivo clearance of Epo remains incomplete. Some studies have shown that Epo is cleared by the liver (Fukuda et al., 1989; Spivak and Hogans, 1989) and the kidney (Jensen et al., 1994); however, their effects do not contribute significantly to the total clearance (Jelkmann, 2002). It has been hypothesized that in vivo desialidation is the rate-limiting step for Epo metabolism by the liver (Nielsen et al., 1990). Receptor-mediated endocytosis in the bone marrow by erythroid progenitors followed by lysosomal degradation is the primary mechanism of Epo elimination from the body (Sawyer et al., 1987). Additional evidence for this elimination mechanism has been shown by studies investigating different degrees of bone marrow activity (Beguin et al., 1993; Cazzola et al., 1998). Several preclinical and clinical studies have reported an increase in Epo clearance within 1 month after Epo treatment (Kinoshita et al., 1992; Ohls et al., 1996; Widness et al., 1996; Sans et al., 2000), whereas others found no statistical difference (Salmonson et al., 1990; Kampf et al., 1992). An additional study modeled the change in the EpoR level over time after the induction of anemia (Chapel et al., 2001). In a recent study by our group, we reported up-regulation of EpoR mRNA levels of 4.97 ± 3.92 times baseline at 9 days after the induction of anemia (Nalbant et al., 2010). Although EpoR mRNA levels do not necessarily correlate directly with the number of receptors, an increase in the mRNA level is probably associated with an increase in the quantity of EpoRs. An important aspect of Epo dosing is that receptor-mediated Epo clearance (CL_R) leads to Hb production, whereas the nonreceptor-mediated clearance (CL_L) does not produce Hb. Thus, the greatest efficacy in Epo dosing is achieved when the biggest fraction of the dose is eliminated via the erythropoietic elimination pathway. One study was able to characterize and quantify both types of clearances through a chemical bone marrow ablation method (Veng-Pedersen et al., 2004). With two different types of clearances, optimal Epo dosing involves using the receptor-mediated clearance pathway as much as possible and minimizing the fraction of Epo eliminated via the nonerythropoietic elimination pathway. Thus, the primary objective of this study was to determine the optimal Epo dosing time in neonatal sheep with phlebotomy-induced anemia, considering the complex, receptor-mediated elimination mechanism of Epo. The dosing optimization was based on a mechanistic, pharmacodynamic model for predicting Hb production, which considers the dynamic Epo-dependent changes in the EpoR pool that determines erythropoietic efficacy.

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ABBREVIATIONS: Epo, erythropoietin; EpoR, erythropoietin receptor; PK/PD, pharmacokinetic/pharmacodynamic; rhEpo, recombinant human erythropoietin; RBC, red blood cell.

Pharmacodynamic analysis studies have modeled different variables in response to the erythropoietic stimulatory effect of Epo. Prior studies have examined the effect of Epo on reticulocytes (Chapel et al., 2000) and Hb (Al-Huniti et al., 2004) individually, and reticulocytes and Hb together (Veng-Pedersen et al., 2002). An additional mechanistic PK/PD study took into consideration the Epo, reticulocyte, and EpoR levels to determine the Hb response to Epo (Woo et al., 2007). Although it is commonly stated that the binding of Epo to EpoR accounts for erythrocyte production and target-mediated disposition, the quantity of EpoRs has not previously been used to predict the pharmacodynamic response of Epo. Nonetheless, target-mediated drug disposition pharmacokinetics has been considered in different contexts previously (Shepherd, 1989; Levy, 1994; Mager and Jusko, 2001). The modeling-based methodology used in this work is not limited to Epo but has been applied to other therapeutic agents with target-mediated disposition such as recombinant tumor necrosis factor (Blick et al., 1987) and selected angiotensin-converting enzyme inhibitors (Till et al., 1984). The fundamental modeling objective was to determine the kinetic mechanism governing the complex relationship between the Epo concentration and the EpoR pool size, both of which are determinants of Hb production. The analysis we have chosen proposes a feedback regulation mechanism for EpoRs and is based on the assumption that the receptor-mediated Epo elimination pathway results in an Epo clearance that is linearly related to the quantity of EpoRs.

Epo therapy has been well established in patients with renal failure (Salmonson et al., 1990) and patients undergoing chemotherapy treatment (Rizzo et al., 2010). However, the benefit of Epo therapy in anemic preterm infants is still under investigation. Clinical trials involving Epo administration in preterm infants have shown some significant results, although the clinical significance is inconsistent (Ohlsson and Aher, 2006). Young sheep have previously been used to model human anemia (Veng-Pedersen et al., 1999) and may provide guidelines for how to optimally administer Epo in preterm infants.

Materials and Methods

All surgical and experimental procedures were approved before the study by the local animal care review committee. Fourteen healthy sheep ages 21 to 60 days were studied. All animals were housed in an indoor light- and temperature-controlled environment. Jugular venous catheters were inserted into the sheep for undisturbed plasma sampling. To prevent infection, intravenous ampicillin (1 g) was administered for the first 3 days after insertion of the venous catheters. Anemia was induced by an exchange transfusion using 0.9% NaCl. The procedure for the exchange transfusion included removing blood from the venous catheter until Hb levels had fallen to 3 to 4 g/dl. While blood was being removed from the animal, an equal volume of 0.9% NaCl was infused back into the animal to keep a constant total volume of blood in the animal. Hb concentrations were determined using a veterinary flow cytometer (XT-2000; Sysmex, Kobe, Japan).

To determine the Epo clearance, tracer doses of biologically active ^{125}I-rhEpo equivalent to 0.1 U/kg Epo were given intravenously over less than 30 s. Ten to 15 plasma samples were drawn over the next 7- to 8-h period after Epo dosing. The concentration of ^{125}I-rhEpo was determined by immunoprecipitation as described previously (Widness et al., 1992). To evaluate dynamic changes in the Epo clearance, ^{125}I-rhEpo clearance studies were completed before and after the phlebotomy. In addition to the tracer doses of Epo, endogenous plasma Epo concentrations were measured at multiple time points during the study by using a radioimmunoassay as described previously (Widness et al., 1992).

Pharmacokinetic Analysis. The Epo plasma concentration data from a single intravenous ^{125}I-rhEpo tracer bolus dose was fitted using a biexponential function to determine the Epo clearance based on the dose administered and the resulting area under the curve. Curve fitting and parameter estimation were performed using WINFUNFIT, a general nonlinear regression program evolved from the FUNFIT program (Veng-Pedersen, 1977). The dosing optimization algorithms used with WINFUNFIT were programmed using FORTRAN.

Pharmacodynamic Model for Hb Response. A pharmacodynamic model was developed to predict the Hb response to induced anemia based on current understanding of the mechanism of action of Epo. In particular, the model assumes that Hb is produced only after the binding of Epo to its receptor, EpoR. It was also assumed that the receptor-based clearance of Epo (CL_R) is proportional to the EpoR pool size (Chapel et al., 2001). Finally, it was assumed that before the start of the study the system was at steady state; i.e., the Hb production was initially equal to the Hb removal resulting from red blood cell (RBC) senescence. RBC senescence was accounted for in the model by assuming that no senescence occurred among newly produced RBCs after the phlebotomy within the time span of the experiment. This assumption is justified on the basis of the assumption of a 120-day lifespan of RBCs reported for sheep (Mock et al., 1997), which is longer than the approximately 30-day duration of the kinetic experiment. The model incorporated a lag time (t_{lag}) between the Epo-EpoR binding and the production of Hb that corresponded to the time from the binding of Epo to EpoRs in the bone marrow and the appearance of newly formed RBCs, i.e., reticulocytes in the peripheral bloodstream.

Modeling for the CL_R is similar to our group's previous work (Chapel et al., 2001) in that the feedback mechanism for the CL_R is modeled through the use of an intermediate variable denoted M. The intermediate variable in this model provides a mathematical method to account for the clearance rebounding above the baseline value. A representative fitted Hb profile with the Epo concentrations is illustrated in Fig. 1. The following numerically integrated delay-type differential model equations (eq. 1) were simultaneously fit in all animals to the Hb and clearance versus time data:

$$\begin{aligned} \frac{d(\mathrm{Hb})}{dt} &= E(t) - E(t-\tau) \\ \frac{d\mathrm{CL_R}}{dt} &= k_3 \cdot M(t) - k_1 \cdot \mathrm{Epo}(t) \cdot \mathrm{CL_R}(t) \\ \frac{dM}{dt} &= k_0 + k_2 \cdot (\mathrm{CL_{R,SS}} - \mathrm{CL_R})_+ - k_3 \cdot \mathrm{M} \end{aligned} \tag{1}$$

where Hb is the Hb amount, CL_R is the receptor-based clearance of Epo assumed to be proportional to the quantity of EpoR, $CL_{R,\,SS}$ is steady-state value of CL_R, M is the intermediate variable used to account for the feedback, Epo is the Epo plasma concentration represented with a linear spline, τ is the lifespan of RBCs fixed to 120 days (Mock et al., 1997), k_3 is a first-order elimination rate constant for M, k_1 is the rate constant for Epo-EpoR binding; k_0 is the zero-order rate constant for M formation, and k_2 is a first-order feedback rate constant. The + subscript denotes the truncation function (eq. 2); i.e.,

$$(\mathrm{CL_{R,SS}} - \mathrm{CL_R})_+ = \begin{cases} \mathrm{CL_{R,SS}} - \mathrm{CL_R} & \mathrm{CL_{R,SS}} > \mathrm{CL_R} \\ 0 & \text{otherwise} \end{cases} \tag{2}$$

$E(t)$ is the Hb production given by a modified Hill equation model (eq. 3):

$$\begin{aligned} E(t) &= \frac{E_{\max}(t) \cdot \mathrm{Epo}(t - t_{\mathrm{lag}})}{\mathrm{EC_{50}} + \mathrm{Epo}(t - t_{\mathrm{lag}})} \\ E_{\max}(t) &= E_{\max,\mathrm{SS}} \frac{\mathrm{Cl_R}(t - t_{\mathrm{lag}})}{\mathrm{Cl_{R,SS}}} \end{aligned} \tag{3}$$

where t_{lag} is the lag time between Epo-EpoR binding and production of Hb, EC_{50} is the concentration of Epo resulting in half of the maximum Hb production rate, and $E_{max}(t)$ is the maximum Hb production possible at the current time. $E_{max,\,SS}$ is the initial SS value of E_{max}; i.e., $E_{max,\,SS} = E_{max}(0)$.

In the differential equations presented, Hb is the amount of Hb, but the experimental data represent the Hb concentration. Thus, the output variable of the differential equation must be divided be $V(t)$ (eq. 4):

$$V(t) = V_{\mathrm{Kg}} W(t) \tag{4}$$

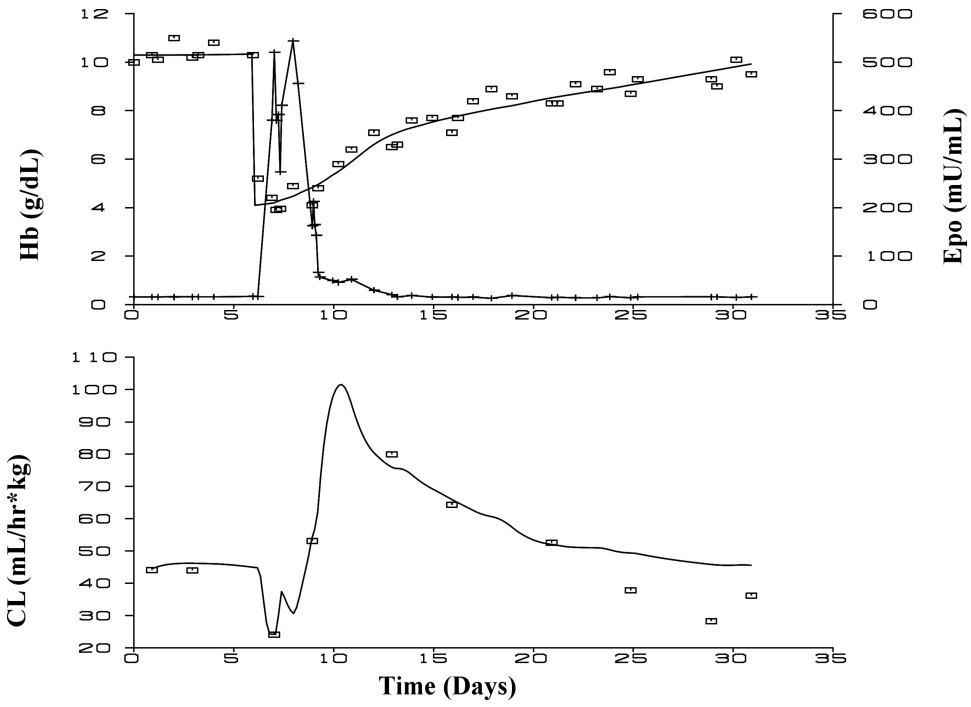

FIG. 1. Representative fit of pharmacodynamic model to Hb (top) and CL data (bottom). Erythropoietin data are represented with a linear spline function (top).

where V_{kg} is the volume of distribution per kilogram, which is considered a constant and $W(t)$ is the weight of the animal at time t. The weight function is represented by a linear spline fit to the weight data of each animal.

Previous literature has shown that the red cell lifespan (τ) for sheep is approximately 120 days (Mock et al., 1997). For the 14 sheep used in this study, the average study time was 34.6 ± 5.7 days. Therefore, the lifespan (τ) of RBCs is much longer than the study length. Thus, the above differential equations become (eq. 5):

$$\begin{aligned} \frac{d(\mathrm{Hb})}{dt} &= E(t) - \frac{\mathrm{Hb_{SS}}}{\tau} \\ \frac{d\mathrm{CL_R}}{dt} &= k_3 \cdot M(t) - k_1 \cdot \mathrm{Epo}(t) \cdot \mathrm{CL_R}(t) \\ \frac{dM}{dt} &= k_0 + k_2 \cdot (\mathrm{CL_{R,SS}} - \mathrm{CL_R})_+ - k_3 \cdot M \end{aligned} \tag{5}$$

subject to the SS initial conditions in eq. 6:

$$\begin{aligned} E(0) &= \mathrm{Hb_{SS}}/\tau \\ \mathrm{Cl_{R,SS}} &= k_0/(k_1 \cdot \mathrm{Epo_{SS}}) \\ M_{SS} &= k_0/k_3 \end{aligned} \tag{6}$$

The Epo concentration was used as a forcing function for the differential equations by representing the Epo response by a linear spline fit to the plasma Epo data. The tracer kinetic data enable the total clearance (CL) of Epo to be obtained. Previous studies have shown that the total clearance includes a second linear clearance ($\mathrm{CL_L}$) that is assumed to be unaffected by anemia and independent of the Epo concentration (Veng-Pedersen et al., 2004). Therefore, the total Epo clearance may be expressed as eq. 7:

$$\mathrm{CL}(t) = \mathrm{CL_L} + \mathrm{CL_R}(t) \tag{7}$$

where $\mathrm{CL_L}$ is an estimated constant and $\mathrm{Cl_R}(t)$ is the receptor-based clearance that changes according to the dynamic changes in the receptor pool. The above total clearance equation and the numerically integrated differential equations define the model that was fit to the clearance and Hb data.

Dosing Optimization. The objective for the dosing optimization was to find the optimal time to administer a single intravenous 100 U/kg bolus Epo dose to anemic neonatal sheep based on a simulation study. To accomplish this, an additional differential equation (eq. 8) was added to the model:

$$\begin{aligned} \frac{d(\mathrm{Hb})}{dt} &= E(t) - \frac{\mathrm{Hb_{SS}}}{\tau} \\ \frac{d\mathrm{CL_R}}{dt} &= k_3 \cdot M(t) - k_1 \cdot \mathrm{Epo}(t) \cdot \mathrm{CL_R}(t) \\ \frac{dM}{dt} &= k_0 + k_2 \cdot (\mathrm{CL_{R,SS}} - \mathrm{CL_R})_+ - k_3 \cdot \mathrm{M} \\ \frac{d\mathrm{Epo_x}}{dt} &= \frac{-(\mathrm{Cl_L} + \mathrm{Cl_R})}{\mathrm{V(t)}} \cdot \mathrm{Epo_x} \end{aligned} \tag{8}$$

where $\mathrm{Epo_x}$ is the exogenous Epo concentration resulting from an intravenous bolus injection and $V(t)$ is the weight-adjusted volume of distribution at time t. The objective function for the optimization is based on the total amount of postphlebotomy Hb produced to the end of the study when a fixed 100 U/kg intravenous bolus dose of Epo is administered at a certain time (eq. 9):

$$\text{Hb Produced} = \text{Hb End} - \text{Hb Start} + \text{Hb Senescence} \tag{9}$$

where Hb End is the amount of Hb in the animal at the end of the study, Hb Start is the amount of Hb in the animal immediately after the phlebotomy, and Hb Senescence is the Hb amount removed from the circulation because of natural erythrocyte senescence. It is assumed that $\mathrm{CL_L}$ is constant, that $\mathrm{CL_R}$ is proportional to the EpoR pool, and that the Epo dosing does not significantly change the endogenous Epo production.

Simulation Study. The simulation study involved a dose simulation for each of the 14 sheep with fixed individual PK/PD parameters that were determined individually from the model based on eq. 5. The individual E_{max}, EC_{50}, k_1, k_2, k_3, τ, and lag-time parameters were fixed for each individual dose simulation. The software developed for the analysis determined the postphlebotomy Hb response for a sheep when a single 100 U/kg intravenous bolus dose is given at a particular time. For each sheep and each simulated Epo dosing, the software determined the postphlebotomy Hb produced on the basis of eq. 9. The postphlebotomy dosing time that produced the most Hb was considered to be the optimal dosing time. This optimal dosing time was determined by numerical optimization using the derivative-free optimization algorithm developed by Nelder and Mead (1965), commonly referred to as the simplex method. Likewise, the simplex optimization algorithm was also set up to determine the lowest postphlebotomy Hb produced according to eq. 9 for a single 100 U/kg intravenous Epo bolus dose. The dosing time with the lowest Hb produced was considered to be the worst possible dosing time.

TABLE 1

Parameter value summary for pharmacodynamic model

Parameter	Mean Value	S.D.
E_{max} (g/day)	0.333	0.412
EC_{50} (mU/ml)	578	1160
Lag time (days)	1.13	0.794
k_3 (l/day)	0.807	0.359
k_1 (ml/mU per day)	9.75×10^{-3}	4.63×10^{-3}
k_2 (1/day^2)	16.3	10.9
Hb correlation r	0.953	0.0180
Clearance correlation r	0.876	0.0770

Results

Study animals began with a mean prephlebotomy Hb of 10.5 ± 2.22 g/dl and a mean starting weight of 14.4 ± 6.26 kg; mean ending weight was 21.8 ± 7.07 kg. All animals were phlebotomized to a similar Hb value of 3.87 ± 0.450 g/dl. Figure 1 shows a representative fit of the proposed model to the clearance and the Hb data. As expected, in all cases there was a marked increase in the plasma Epo concentration after the phlebotomy followed by an increase in Hb. Furthermore, every animal showed a decrease in Epo clearance immediately after the phlebotomy followed by an increase in clearance above each animal's baseline clearance once plasma Epo levels had declined to nearly normal levels. The sheep reached an average peak clearance of 110.3 ± 25.90 ml/h · kg at 5.57 ± 2.12 days after the phlebotomy.

The simultaneous curve fitting was able to capture the behavior of the data for all animals. Although there was an age difference of up to 39 days for some animals, there was no statistical difference ($p > 0.05$) in baseline clearance values or parameter values for the different age groups. Table 1 summarizes the parameters and goodness-of-fit values. The curve fitting showed a trend toward a rapid increase in Hb level of production followed by a more gradual increase in Hb concentration. In all cases, the Hb levels continued to increase when plasma Epo concentrations were at baseline as a result of the EpoR pool size being above the baseline value. The average lag time for the study was 1.13 ± 0.794 days. The average Epo steady-state clearance value for all of the animals was 62.7 ± 17.5 ml · h^{-1} · kg^{-1}. The average maximum and minimum clearance values for all of the animals were 94.3 ± 28.8 and 24.7 ± 9.8 ml · h^{-1} · kg^{-1}, respectively.

The simulation of exogenous Epo administration caused a variable increase in postphlebotomy Hb production, which was dependent on the time the Epo dose was administered (Fig. 3). A representative plot of this increased Hb production is shown in Fig. 2, top. These Hb profiles are based on the Epo profiles shown in Fig. 2, bottom. The two Hb profiles in Fig. 2 are superimposable until the Epo dose is given. In all cases, there were two local solutions for the dosing optimization algorithm, which are summarized in Tables 2 and 3. Figure 3 shows the objective function values (Hb produced) as a function of the intravenous bolus dosing time. The plot demonstrates two local solutions found for a particular animal. For 11 of the 14 animals, the later dosing time was more optimal than the first. The average for the optimal dose administration time was 11.4 ± 6.2 days postphlebotomy, which corresponded to an average increase in Hb production of 21.1 ± 9.7% over the worst possible dosing time. The average for the second local solution for the optimal dosing time was 4.6 ± 4.4 days postphlebotomy, which corresponded to an average increase in Hb production of 10.2 ± 5.4% over the most unfavorable possible dosing time. The averages for the two optimal dosing times could be estimated in a different manner as dealt with under *Discussion*. The average worst possible dosing time was 6.6 ± 3.5 days

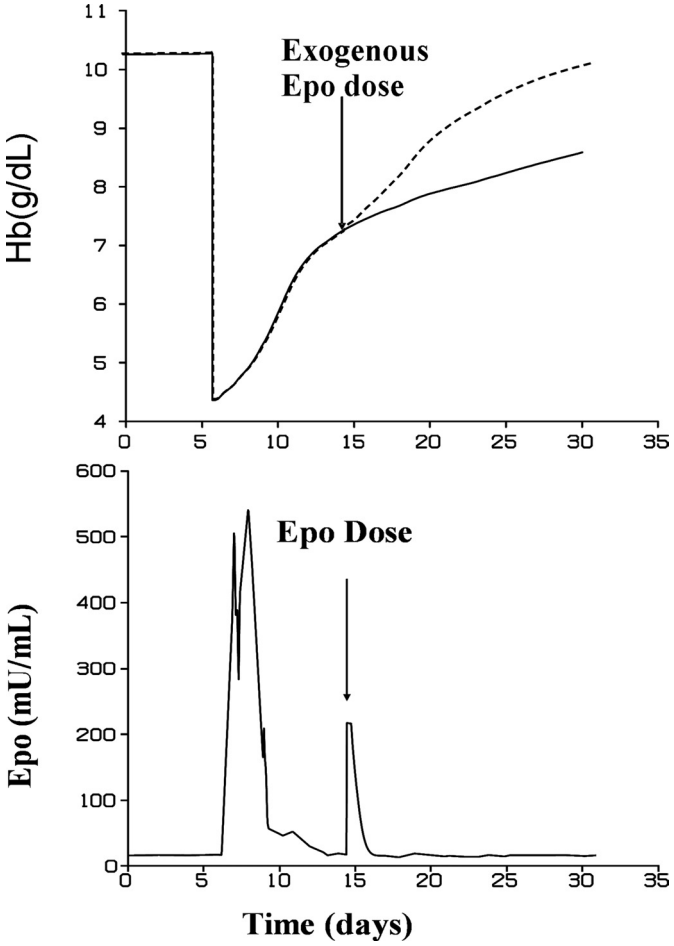

FIG. 2. Representative estimated Hb profile (top) for a model with a simulated intravenous bolus Epo dosing (broken curve) and without any Epo dosing (continuous curve). Representative Epo profiles (bottom) with an intravenous bolus Epo dosing simulation.

postphlebotomy. The average Hb produced was 186.2 ± 50.5 g for the optimal dose time, which was significantly larger ($p < 0.05$) than for the worst possible dose time (152.1 ± 33.6 g).

Discussion

Judged from the goodness-of-fit parameters, the proposed kinetic model was able to accurately predict the Hb production in phleboto-

TABLE 2

Summary of dosing optimization for best possible solution

Subject No.	Optimal Postphlebotomy Dose Time 1	Increase in Objective Function
	days	*%*
1	1.40	23.0
2	13.2	20.9
3	2.20	24.3
4	13.7	33.5
5	14.1	30.0
6	7.90	26.5
7	13.0	31.3
8	16.8	12.3
9	18.1	26.0
10	18.0	7.80
11	19.1	6.50
12	1.90	6.10
13	12.9	15.6
14	7.40	31.2
Mean	11.4	21.1
S.D.	6.20	9.70

TABLE 3

Summary of second dosing optimization solution

Subject No.	Optimal Postphlebotomy Dose Time 2	Percentage Increase in Objective Function
	days	*%*
1	13.0	15.6
2	4.10	13.7
3	12.0	4.50
4	2.00	14.8
5	2.40	11.5
6	1.50	12.2
7	1.20	12.9
8	2.10	3.30
9	4.10	6.70
10	0.900	6.80
11	3.40	4.30
12	12.8	4.30
13	3.00	10.4
14	2.50	21.7
Mean	4.60	10.2
S.D.	4.40	5.40

mized sheep on the basis of the Epo concentration and Epo clearance values. Furthermore, the model was extended to determine the optimal time to administer an intravenous bolus Epo dose to anemic sheep. Because the Epo CL_R is assumed to be proportional to the change in receptors and because the linear clearance is assumed to be constant (Veng-Pedersen et al., 2004), any change in the total clearance, CL, is due to a change in the quantity of EpoRs. In addition, because the EpoRs are located primarily in the bone marrow, phlebotomy does not significantly change the EpoR pool. Our group recently showed that EpoR mRNA is up-regulated after the induction of anemia in sheep, which is consistent with the model used in this study (Nalbant et al., 2010). Because this study used the *GAPDH* gene as a control, it can be suggested that the number of EpoRs per cell increased rather than the total number of cells. Another study released recently shows that the Epo receptors decrease after large plasma Epo concentrations, demonstrating consistency with our model (Becker et al., 2010). However, this study does not show EpoRs increasing above the baseline value, which is most likely due to the short time scale (5 h) of the study. The present simulation study provides additional evidence that the increased total clearance of Epo is probably due to an increase in the number of EpoRs present in the bone marrow. The agreement of the present model provides additional indirect evidence for a receptor-mediated clearance mechanism for Epo. In particular, the decrease in the total Epo clearance immediately after the endogenous Epo surge suggests that the EpoR is consumed in a receptor-mediated endocytic process.

There are several limitations of this study that may be improved in future experimental work. One limitation is that an indirect method was used to determine the EpoR pool. In addition, the lifespan (τ) of red blood cells is fixed to 120 days to account for senescence. In reality, the red blood cell lifespan could be variable throughout the course of the animal's life. Although the simulation study suggested a potential Hb production benefit based on consideration of the dynamic EpoR state, experimental work needs to be done to verify the simulations. The study is also based on the assumption of an initial EpoR steady state, which may not be true.

Work by others has shown the potential for scaling preclinical animal Epo studies to humans (Mager et al., 2009). Thus, with proper allometric pharmacodynamic scaling, it should be possible to make use of the sheep model for pharmacodynamic dosing predictions useful in planning mechanism-based human efficacy studies.

Dosing Optimization Solutions. The dosing optimization algorithm obtained two solutions for the optimal time to administer an intravenous bolus Epo dose. The average dose administration time was reported as the average for the best solution and the second best solution. To better analyze the optimal dosing times, it is more informative to average the solutions by the early solution and the late solution. If the averages are computed in this manner, then the average early postphlebotomy Epo administration solution is 2.3 $\pm$ 1.0 days and the average late solution is 13.7 $\pm$ 3.5 days postphlebotomy. The later dosing time was expected and is probably due to the EpoR state being up-regulated around the optimal dosing time. The earlier solution for the dosing optimization was not expected at first. However, this solution may be explained by an earlier Epo dosing, allowing the endogenous Epo to make use of the up-regulated EpoRs, resulting in a greater efficacy of the endogenous Epo.

The optimization algorithm showed a 21.1 $\pm$ 9.7% increase in the amount of Hb produced for the best dosing time above that of the worst dosing time. This percentage corresponds to an average difference in Hb of 34.0 g for the best dosing time compared with the worst dosing time. The estimate for sheep blood volume for our study is 98.1 ml/kg. With this value we can estimate, on the basis of the average weight of the sheep in this study, that a Hb difference of 34.0 g would correspond to an improvement in Hb concentration of 1.57 g/dl.

Linear Clearance Mechanism. Understanding of the linear nonerythropoietic clearance component of Epo remains incomplete. Our previous bone marrow ablation study in nonanemic lambs showed that the linear clearance of Epo accounts for only 7.38 $\pm$ 2.7% of the total Epo clearance and that this clearance does not change under anemic conditions (Veng-Pedersen et al., 2004). Even though the total linear Epo clearance does not change under anemic conditions, the proportional contribution of the linear clearance to the total clearance does change. The linear clearance in the present study was shown to contribute an average of 26.8% when the EpoRs were the most down-regulated.

Although this study does not attempt to identify a mechanism for the linear Epo clearance, its significant contribution to the total clearance when the EpoRs are down-regulated merits further examination. From previous research, it has been well established that EpoRs are found in all tissues of the body and not just in the bone marrow (Yamaji et al., 1996; Juul et al., 1998, 2001; Carlini et al., 1999). It is possible that these receptors have different Epo binding characteristics in mediating a linear (nonsaturable) clearance of Epo (Veng-Pedersen et al., 2004). However, it is not known whether these additional receptors are responsible for the linear Epo clearance.

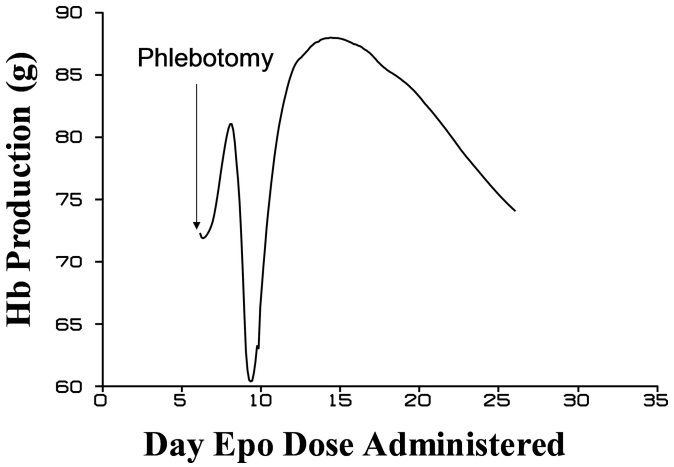

FIG. 3. Representative plot of amount of Hb produced as a function of simulated Epo dosing time for one animal.

Lag Time in the Present Study. In the present study, the lag time between receptor stimulation and the corresponding newly formed reticulocytes appearing in the circulation was calculated to be 1.13 ± 0.794 days. Other studies have looked at the lag time between Epo presence and reticulocyte production. One study found this lag time to be 1.7 days in adult humans (Krzyzanski and Perez-Ruixo, 2007), whereas another study calculated the lag time as 0.873 days in young sheep (Veng-Pedersen et al., 2002). These results seem to be similar to the results found in this study, although the variability in the lag time in this study was high. One publication has shown that under anemic conditions reticulocytes may be prematurely released into the bloodstream (Freise et al., 2007). Early release of reticulocytes will lead to earlier detection of new Hb in the bloodstream, which could contribute to study-to-study variability in lag time.

Potential Clinical Application. There have been a number of clinical trials in which Epo was administered to preterm infants with the goal of reducing or eliminating the need for blood transfusions. A review study done on 27 different Epo clinical trials with preterm infants concluded that although Epo administration reduces the use of RBC transfusions and the quantity of RBCs transfused, these reductions were claimed to be of limited clinical importance (Ohlsson and Aher, 2006). However, because these published clinical trials used a "trial and error" methodology that does not consider the complex PK/PD of Epo, it is not clear how well they can be used as a guide to the real benefit of Epo when Epo is optimally administered to anemic infants.

The results from the current study show that the Epo receptor state plays an important role in Hb production. Furthermore, in the sheep studied, the endogenous Epo drops sharply at a certain point (Fig. 1, day 9). In a similar way, preterm infants' endogenous Epo concentrations drop sharply in the first few weeks of life (Freise et al., 2010). This similarity in the drop in Epo indicates a similarity in the Epo clearance/receptor pool dynamics in sheep and humans. Thus, knowledge of the efficacy of Epo relative to the Epo receptor pool dynamics gained from sheep experiments may provide a guide for how to optimally administer Epo in anemic infants. The hypothesis based on this similar kinetic mechanism is that a larger Epo receptor pool provides a greater potential for Hb production. Thus, Epo dosing should optimally be "in sync" with the variable status of the Epo receptor pool so that the peak Epo concentration coincides with the maximally unregulated quantity of Epo receptors.

In conclusion, a substantial increase in the efficacy of Epo is possible by a dosing optimization that considers the dynamic receptor regulation mechanism. This study was conducted under anemia induced by a major phlebotomy, but the basic approach may be extended to other anemic conditions for which similar large improvements in erythropoietic efficacy may be possible by considering the EpoR dynamics.

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Authorship Contributions

Participated in research design: Rosebraugh, Widness, and Veng-Pedersen.

Performed data analysis: Rosebraugh and Veng-Pedersen.

Wrote or contributed to the writing of the manuscript: Rosebraugh, Widness, and Veng-Pedersen.

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Address correspondence to: Dr. Peter Veng-Pedersen, University of Iowa, College of Pharmacy, 115 S. Grand Ave., Iowa City, IA 52242. E-mail: veng@uiowa.edu