Pharmacokinetic Analysis of Continuous Erythropoietin Receptor Activator Disposition in Adult Sheep Using a Target-Mediated, Physiologic Recirculation Model and a Tracer Interaction Methodology

Mohammed H. El-Komy, John A. Widness, and Peter Veng-Pedersen

Division of Pharmaceutics, College of Pharmacy (M.H.E., P.V-P.) and Department of Pediatrics, College of Medicine (J.A.W.), University of Iowa, Iowa City, Iowa

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ABSTRACT:
The pharmacokinetics (PK) of continuous erythropoietin receptor activator (CERA), a PEGylated erythropoietin (EPO) derivative, was studied in sheep after bone marrow (BM) busulfan ablation by using a receptor-based recirculation model and tracer interaction method (TIM) experiments. The nontracer CERA component of the TIM was analyzed using a noncompartmental approach. In contrast to EPO elimination that is linear after the BM ablation, CERA elimination remains nonlinear. After busulfan treatment, initial EPO receptors (EPOR) normalized production rate constant, EPOR degradation rate constant, and CERA-EPOR complex internalization rate constant decreased \( (p < 0.01) \), whereas no change in CERA/EPOR equilibrium dissociation constant was detected \( (p > 0.05) \). After BM ablation, noncompartmental analysis showed that CERA-PK parameters underwent 1) a decrease in plasma clearance \( (p < 0.01) \); 2) a concomitant increase in elimination half-life and mean residence time; and 3) no significant change in volume of distribution, distribution half-life, or distributional clearance \( (p > 0.05) \). These results suggest that CERA elimination is mediated through saturable hematopoietic and nonhematopoietic EPOR pathways, with possible contribution of another EPOR-independent pathway(s). Compared with the nonhematopoietic EPOR population, the hematopoietic receptors have similar affinity to CERA but are significantly more involved in CERA’s in vivo elimination. The saturable nature of the nonerythropoietic, non-BM pathway(s) for CERA in contrast to EPO predicts two fundamental differences: 1) an increasing fraction of CERA is used for erythropoiesis for increasing concentrations; and 2) the clearance of CERA becomes more limited for increasing concentrations. Taken together, these differences favor a more efficacious and prolonged action for CERA.

Introduction

Continuous erythropoietin receptor activator (CERA) is a novel erythropoiesis-stimulating agent (ESA) that differs from epoetin \( \beta \) by the integration of amide bonds between amino groups and methoxy polyethylene glycol-succinimidyl butanoic acid, resulting in a molecular mass of approximately 60 kDa (Brandt et al., 2006). CERA has unique binding characteristics to erythropoietin (EPO) receptors (EPOR) that involve slow association but slightly faster dissociation than EPO, resulting in reduced internalization and degradation properties of CERA, together with its receptor-binding properties, are responsible for improved pharmacodynamics reflected in an increased reticulocyte count and a stable hemoglobin level in vivo (Haselbeck et al., 2002; Sulowicz et al., 2007).

In contrast to Michaelis-Menten kinetics, which is the frequently used empirical model to address saturation kinetics, the target-mediated drug disposition (TMDD) model can adequately describe several molecular processes such as drug-receptor binding, drug-receptor complex internalization, receptor production, and degradation (Mager and Jusko, 2001). The TMDD model can be simplified to its quasi-

ABBREVIATIONS: CERA, continuous erythropoietin receptor activator; BM, bone marrow; CL, CERA total clearance; CL\(_{\text{d}}\), distribution clearance; EPO, erythropoietin; EPOR, erythropoietin receptor; ESA, erythropoiesis-stimulating agent; CV, coefficient of variation; HLS, heart-lung segment; MRT, mean residence time; MSE\%, mean percent standard error; NESP, novel erythropoiesis-stimulating protein; NTM, nontarget mediated; PEG, polyethylene glycol; PK, pharmacokinetic; PBPK, physiologically based PK; QE, quasi-equilibrium; RIA, radioimmunoassay; \( t_{\text{lag}}(a) \), distribution half-life; \( t_{\text{lag}}(\beta) \), CERA elimination half-life; \( t_{\text{inj}} \), time of injection of unlabeled CERA; TIM, tracer interaction method; TM, target-mediated; TMDD, target-mediated drug disposition; \( V_{\text{in}} \), initial volume of distribution; \( V_{\text{ss}} \), steady-state volume of distribution; AUC, area under the curve; \( k_{\text{syn}}/R_{\text{D}} \), EPOR-normalized production rate constant; \( k_{\text{deg}} \), EPOR degradation rate constant; \( k_{\text{syn}} \), CERA-EPOR complex internalization rate constant; \( K_{\text{D}} \), dissociation constant; \( k_{\text{syn}} \), EPOR synthesis rate constant; \( R_{\text{D}} \), initial total EPOR concentration.
equilibrium (QE) form, if drug-receptor binding is assumed to be fast (Mager and Krzyzanski, 2005), or to its quasisteady-state form, if both binding of drug to its receptor and internalization of drug-receptor complex are assumed to be rapid (Gibiansky et al., 2008).

Since its publication, the TMDD model synthesized in the context of two- or three-compartment model for drug disposition has been extensively used to study the PK of several drugs exhibiting receptor-mediated endocytosis, including interferon-β (Mager et al., 2003), thrombopoietin (Jin and Krzyzanski, 2004), and recombinant human EPO (Woo et al., 2007). However, physiologically based PK (PBPK) models have become a powerful alternative to classical compartmental models because they provide a mechanistic approach that 1) relates drug disposition to various physiological, anatomical, and physicochemical factors (Thygesen et al., 2009) and 2) offers a strong basis for interspecies, tissue, route, and drug extrapolations (Nestorov, 2003).

A major obstacle to the widespread implementation of the physiologic approach is high dimensionality of PBPK models that oftentimes makes them far too complex to be fitted to plasma concentration-time data. Accordingly, lumping dynamically similar tissues to form dimensionally smaller but still PBPK models and focusing on target tissue(s) has been considered a successful strategy (Nestorov et al., 1998).

The classic PBPK models are based on organ/tissue models that assume perfusion or permeability rate-limited structure (described using systems of differential equations) (Nestorov, 2003). An improvement that keeps any restrictive assumptions to the minimum is characterization of organ or tissue regions by model-independent transit time distributions in the framework of linear systems principles (i.e., input-output convolution relationship). This approach has been termed “stochastic modeling” (Weiss and Roberts, 1996; Veng-Pedersen et al., 2008).

The tracer interaction method (TIM) is a tracer/nontracer-based methodology for analyzing nonlinear, saturation kinetics under dynamic conditions by monitoring perturbations in the level of a tracer form of the drug after the introduction of the parent drug (Veng-Pedersen et al., 1997). The TIM approach provides not only a sensitive and an accurate method to assess drug-receptor in vivo-binding characteristics but also a way to differentiate between receptor populations in distinct tissues (Veng-Pedersen et al., 2003, 2004).

It has been hypothesized that elimination of CERA in sheep is capacity limited, and greater nonlinearity was observed for CERA than for EPO (Veng-Pedersen et al., 2008). The aim of this work is to get a greater insight into CERA’s complex, nonlinear disposition and to get a better understanding of the interaction between CERA and EPOR in bone marrow (BM) and non-BM tissue. This strategy is done by a minimal stochastic receptor-based, recirculation model used for analyzing data from TIM experiments in adult sheep before and after chemical ablation of EPOR in the BM. The use of a sheep model allows the comparison of CERA TIM behavior with what was reported for EPO (Veng-Pedersen et al., 2004).

Materials and Methods

Animals. All animal care and experimental procedures were approved by the University of Iowa Institutional Animal Care and Use Committee and adhere to the Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised in 1985). Sixteen healthy young adult sheep (mean b.w.t. ≥ S.D. = 27 ± 2.6 kg) were selected for the study. Four sheep were chosen for comparing the PK of labeled and unlabeled CERA. Two sheep were used in TIM control studies. Ten sheep were used for the TIM experiments conducted under normal BM conditions and after BM ablation (five animals for each case). The animals were housed in an indoor, light- and temperature-controlled environment, with ad libitum access to feed and water. Before study initiation, jugular venous catheters were aseptically placed under pentobarbital anesthesia. Intravenous ampicillin (1 g) was administered daily for 3 days after catheter placement. The long-term infusion of CERA required in the TIM experiments were done with a portable infusion pump (Pegasus infusion pump; Instech Laboratories, Plymouth Meeting, PA) mounted on a specially designed sheep jacket.

Ablation Protocol. Busulfan was administered orally twice a day at a dose of 11 mg/kg per day for 3 consecutive days. Ampicillin (1 g b.i.d.) was administered daily for the first 3 days before the busulfan treatment and again after the start of the treatment. Animals were clinically monitored for adverse effects of the chemotherapy such as weight loss, hair loss, blood in urine or stools, fever, unusual bleeding or bruising, and loss of appetite.

Study Protocol. 125I-labeled versus unlabeled CERA PK study. Identical or near-identical disposition of the tracer and the nontracer forms of a drug is a prerequisite for TIM methodology. Accordingly, a 200 mU/kg i.v. bolus dose of 125I-CERA and a 109 U/kg i.v. bolus dose of unlabeled CERA were simultaneously administered to the same subjects, and plasma concentrations of both forms were followed for approximately 86 h.

TIM experiments. A detailed description of the theory and principles of the TIM was published previously (Veng-Pedersen et al., 1997). In the current study, each animal underwent a single TIM PK experiment. A 50 mU/kg i.v. bolus dose of 125I-CERA was initially administered, then immediately followed by an intravenous infusion at 1.7 mU/h · kg of the tracer to the end of the experiment. An intravenous bolus injection (113 U/kg) of unlabeled CERA was administered when the plasma 125I-CERA level approached steady state (i.e., at approximately 24 h). An average of 53 blood samples (approximately 0.5 ml/sample) were collected for plasma 125I-CERA from 0 to 130 h, whereas approximately 50 samples for plasma unlabeled CERA were collected from 24 to 360 h. To minimize hemoglobin and red cell loss as a result of frequent blood sampling, blood was centrifuged, the plasma was removed, and the red cells were reinforced.

TIM control experiments. The TIM experiment assumes that the drug disposition kinetics is time invariant. To investigate the validity of this assumption in CERA kinetics, a “half” TIM experiment was conducted. The plasma level of 125I-CERA was followed over a period of 300 h after a single intravenous bolus (66 mU/kg) and a constant rate infusion [2 mU/(h · kg)] were administered to a normal BM and BM-ablated subjects. No unlabeled drug was given.

Assay. Radiolabeled CERA. 125I-CERA was prepared by iodination of 1 mg of CERA with 1 ml of 125I-Na (PerkinElmer Life and Analytical Sciences, Waltham, MA) using 8 µg of chloramine-T. The 125I-CERA plasma concentrations and infusion solutions were determined by nonspecific protein precipitation followed by separation by centrifugation. In particular, 1-ml duplicate aliquots of plasma sample or infusion solution were added to 12 × 75 glass test tubes, 1 ml of trichloroacetic acid (10% w/v) was added, and then the precipitated proteins were pelleted by a 30-min centrifugation; the supernatant was decanted, and the pellet was counted for radioactivity.

Unlabeled CERA. CERA was provided as 5.9 mg protein/ml solution (Lot R782388600; F. Hoffman-La Roche, Basel, Switzerland) and was stored at −70°C. This stock was used to prepare working stocks [in 50 mM sodium phosphate with 0.02% sodium azide and 5% bovine serum albumin (pH 7.4)] at a concentration of 0.14 mg protein/ml. Preparation and analysis of the unknown CERA plasma, standard curve, nonspecific binding, and zero standard samples were done using a double-antibody radioimmunoassay (RIA) procedure (lower limit of quantitation, 1 mU EPO/ml) (Widness et al., 1992). All plasma samples from the same animal were measured in the same assay to reduce variability.

The unknown CERA concentrations were determined using the EPO standard curve between the EPO EC40 and EC20 as mU EPO equivalents per milliliter. The EPO standard curve was used instead of CERA standard curve for convenience and because of our extensive experience with the EPO RIA (Widness et al., 1992). The use of the EPO standard curve to measure CERA was validated by performing 1/2 dilutions of the CERA stock solution until the response was between the EC40 and EC20 on the EPO standard curve. The dilution-corrected responses were determined and had a coefficient of variation (CV) of 8.3% across the linear range of the EPO standard curve, demonstrating a one-to-one relationship between the determined mU EPO equivalents per milliliter and the nanograms per milliliter of CERA within this range. These
validations resulted in a CERA conversion constant of 71,300 mU/μg protein (n = 13). All unknown CERA samples were measured in duplicate or triplicate and diluted between the EC80 and EC20 on the EPO standard curve, which corresponds to a linear CERA range of 65.5 to 647 pg protein/ml.

The RIA cannot distinguish between endogenously produced EPO and exogenously administered CERA in the same plasma sample. To minimize the contribution of endogenous EPO in the measured CERA concentration, only CERA plasma samples with concentrations greater than 300 mU EPO equivalents/ml were reported. Endogenous EPO contribution was calculated in sheep typically range from 10 to 30 mU/ml (Chapel et al., 2000; Al-Hunind et al., 2005).

No compartmental Analysis. To assess the effect of BM amination on CERA total plasma clearance (CL), initial volume of distribution (V0), steady-state volume of distribution (Vss), distribution clearance (CLd), distribution half-life [1/(α)], elimination half-life [1/(β)], and mean residence time (MRT), a noncompartmental analysis was performed by fitting a biexponential equation [C(0)(t) = C1 · exp(−P1 · t) + C2 · exp(−P2 · t), P1 > P2 > 0] to unlabeled CERA plasma concentrations.

PK Model. A PBPK model was used to analyze CERA TIM tracer data. The proposed model consists of three tissues, heart-lung segment (HLS), nontarget-mediated disposition (NTM), and target-mediated (TM) disposition, interconnected by arteries and veins (see Fig. 1A). The cardiac output is given by Q, and Pi is the fraction of blood flow to ith tissue for i = TM or NTM (P1TM + P1NTM = 1). Drug disposition in the HLS and NTM tissues was assumed to be linear. Therefore, the output rates from both tissues (fTM(out)(t), for i = HLS or NTM) are described by the following convolution equations (* denotes convolution) that relate the input and output rates for a given tissue to a unit impulse response function (UIR(t)):

\[ f_{\text{HLS}}(\text{out})(t) = [D_in \cdot \delta(t) + h(t)] + \beta_{\text{TM}}(\text{out})(t) + \beta_{\text{NTM}}(\text{out})(t) \cdot UIR(t) \]  

\[ f_{\text{TM}}(\text{out})(t) = P_{\text{TM}} \cdot f_{\text{HLS}}(\text{out})(t) \cdot UIR(t) \]

where D_in and h(t) are intravenous bolus dose and constant rate infusion of the tracer, respectively, and δ(t) denotes the Dirac delta function.

With the assumption that both HLS and NTM tissues are nonextracting, the UIR(t) function reduces to a transit time density function (g(t)) that was empirically described as a biexponential function:

\[ UIR(t) = g(t) = \lambda \alpha \exp(-\lambda t) + (1 - \lambda)\beta \exp(-\beta t), \quad 0 \leq \lambda \leq 1 \]

where α and β are rate parameters and λ is a mixing parameter.

Nonlinear drug disposition through TM tissues was considered by a receptor-based model (see Fig. 1B) that follows the generalized TMDD model (Mager and Jusko, 2001) and its QE approximation (Mager and Krzyzsniak, 2005). Accordingly, the interaction between CERA tracer input concentration (C\text{TM}(t)) and free EPO in the extracellular space (R) occurs with a second-order rate constant (k\text{tot}), to form a CERA-EPO complex (R*), which dissociates at a first-order rate constant (k\text{off}), is internalized into the intracellular space with a first-order rate constant (k\text{in}), and is subsequently degraded by lysosomes. The EPOR is produced at a zero-order rate constant (k\text{syn}) and degraded at a first-order rate constant (k\text{deg}). The changes in R and R* were described by the following:

\[ \frac{dR}{dt} = k_{\text{syn}} + k_{\text{deg}} R^* - k_{\text{off}} C_{\text{TM}}(t) - k_{\text{deg}} R \]  

\[ \frac{dR^*}{dt} = - (k_{\text{deg}} + k_{\text{in}}) R^* + k_{\text{off}} C_{\text{TM}}(t) \]

At QE conditions, binding and debinding of the drug with receptors are much faster than the other processes of the system resulting in

\[ R \frac{C_{\text{TM}}(t)}{R^*} = \frac{(R_in - R^*)C_{\text{TM}}(t)}{R^*} = K_0 \]

where R\text{in} is total EPOR (R\text{in} = R + R*) and K0 is CERA/EPOR equilibrium dissociation constant (K0 = k\text{deg}/k\text{off}).

Rearranging eq. 6 for R* and normalizing the resulting equation by initial total EPOR (R0) gives the following result:

Equations 4 and 5 were added to obtain the change in R\text{out}, and the resulting equation was normalized by R0:

\[ \frac{dR_{\text{out}}}{dt} \bigg|_{R_0} = k_{\text{deg}} R_{\text{in}} - k_{\text{off}} R_{\text{out}}(t) + (k_{\text{deg}} - k_{\text{off}}) R^* R_0, \quad R_{\text{out}}(0)/R_0 = 1 \]

The normalization in eqs. 7 and 8 is necessary because R0 cannot be estimated from drug plasma concentrations.

To establish a link between the receptor-based model in TM tissues and the rest of the circulations model, a receptor-dependent extraction function, E_{TM}(t), was defined as follows:

\[ E_{TM}(t) = \frac{R^*/R_0}{C_{\text{in}}(t)} \]

where C\text{TM}(t) is given by:

\[ C_{\text{TM}}(t) = P_{\text{TM}} f_{\text{HLS}}(\text{out})(t) / Q \]

Assuming that distribution equilibrium is achieved rapidly between the tissues and the emerging venous blood, the output rate from TM tissues is given by:

\[ f_{\text{TM}}(t) = P_{\text{TM}}(1 - E_{TM}(t)) f_{\text{HLS}}(\text{out})(t) \]

Finally, the total plasma tracer concentration that is measured in the venous blood (C_out(t)) is given by:

\[ C_{\text{out}}(t) = (f_{\text{TM}}(\text{out})(t) + f_{\text{HLS}}(\text{out})(t))/Q \]

Equations 1 to 3 were conveniently converted to the following equivalent equations (eqs. 13–18). The latter six equations are simpler to deal with numerically and computationally, because they include first-order differential equations and avoid the convolution operator.

\[ d y_1/dt = - \alpha y_1 + \lambda \alpha (\text{Inf}(t) + f_{\text{TM}}(t) + f_{\text{HLS}}(t)) - y_1(0) = D_0 \lambda \alpha \]

\[ d y_2/dt = - \beta y_2 + (1 - \lambda)\beta \text{Inf}(t) + f_{\text{TM}}(t) + f_{\text{HLS}}(t), \quad y_2(0) = D_0(1 - \lambda)\beta \]

\[ f_{\text{HLS}}(t) = y_1 + y_2 \]

\[ d y_3/dt = - \beta y_3 + P_{\text{TM}}(1 - \lambda)\beta y_1(t), \quad y_3(0) = 0 \]

\[ f_{\text{TM}}(t) = y_1 + y_4 \]

Equation 7 was modified to account for the TIM procedure:

\[ R^*/R_0 = \frac{R_{\text{out}}(t) C_{\text{TM}}(t)}{K_0 + C_{\text{TM}}(t)} \quad \text{for} \quad 0 < t \leq t_{\text{cold}} \]

\[ R^*/R_0 = \frac{R_{\text{out}}(t) C_{\text{TM}}(t)}{K_0 + C_{\text{cold}}(t)} \quad \text{for} \quad t > t_{\text{cold}} \]

where C_{\text{cold}}(t) is plasma nontracer concentrations represented as a cross-validation cubic spline (Hutchinson and de Hoog, 1985) when fitting the model to tracer data, and t_{\text{cold}} is time of injection of the nontracer.

To improve the estimation of the parameters, the sheep physiological parameters, Q and P_{\text{TM}}, were fixed to values drawn from literature (Upton, 2008), with \( Q = 7122.7 \text{ml/(h } \times \text{kg)} \), and P_{\text{TM}} was assumed to be equal to the fraction of blood flow to the BM (0.0597).

The selection of the proposed model (eqs. 8–19) was based on comparing the Akaike information criterion of several competing nested models. In a
was calculated for each parameter as follows: The mean percent standard error (MSE%) of the estimate points.

The numerical solution for $C_{\text{TM}}(t)$ (eq. 12) was fitted to tracer data for the pre- ($t < t_{\text{1/2 TM}}$) and post- ($t > t_{\text{1/2 TM}}$) unlabeled drug administration phases using WINFUNFIT, an interactive Microsoft Windows program evolved from the general nonlinear regression program FUNFIT (Veng-Pedersen, 1977). The best fit was accepted only if simulated $E_{\text{TM}}(t)$ (eq. 9) values were ≤ 1 at all time points.

To summarize the uncertainty in the individual subject receptor-related parameter estimates, the mean percent standard error (MSE%) of the estimate was calculated for each parameter as follows:

$$\text{MSE}\% = \frac{1}{n} \sum_{i=1}^{n} \frac{SE_i}{P_i} 100/n$$

where $SE_i$ and $P_i$ are the S.E. of the parameter and the estimate of the parameter for the $i^{th}$ subject, respectively, and $n$ is the number of subjects.

Statistical comparisons of mean values for the receptor-related (Table 1) and noncompartmental PK parameters (Table 2) between normal and ablated BM sheep populations were done using a one- or two-tailed Student’s $t$ test with correction for nonequal variance, if needed. $p$ values of 0.05 and 0.01 were taken as the levels of significance for the type I null hypotheses error.

### Results

#### Checking TIM Assumptions

TIM experiment is based on the assumption that the tracer behaves in the same kinetic manner as the parent drug (Veng-Pedersen et al., 1997). Figure 2 indicates that the proposed PK model is well suited for capturing the behavior of the observed data. From Table 1, the receptor-related parameters were well estimated with MSE% of less than 30% for all parameters, with most of them less than 15%. The relatively high MSE% for CERA-EPOR complex internalization rate constant, $K_{\text{int}}$, is due to two subjects having a high relative S.E. of 69 and 85%, respectively. Calculation of the MSE% for $K_{\text{int}}$ without those subjects results in a MSE% of less than 17%. A relatively small amount of subject-to-subject variability was observed in all parameters with maximal CV% less than 60%, and a larger variability was found between ablated subjects compared with normal subjects (Table 1).

#### Effect of Busulfan Ablation on Elimination Kinetics

TIM data before ablation (Fig. 4, top panel) show a significant perturbation in the $^{125}$I-CERA plasma level (□) caused by the bolus injection of unlabeled CERA (●) at approximately 24 h. The pronounced perturbation, which is a characteristic of nonlinear elimination pathways (Veng-Pedersen et al., 1997), is persistent after busulfan treatment (Fig. 4, bottom panel). This pronounced phenomenon was consistently observed in all of the animals with ablated BM. This behavior of CERA differs from what has been reported for EPO whose elimination is completely linearized by BM ablation (Veng-Pedersen et al., 2004).

### Effect of Busulfan Ablation on PK Parameters

The estimated receptor-related microparameters obtained from the physiological modeling of the tracer component of the TIM are displayed in Table 1. The nonlinear behavior of the observed data. From Table 1, the receptor-related parameters were well estimated with MSE% of less than 30% for all parameters, with most of them less than 15%. The relatively high MSE% for CERA-EPOR complex internalization rate constant, $K_{\text{int}}$, is due to two subjects having a high relative S.E. of 69 and 85%, respectively. Calculation of the MSE% for $K_{\text{int}}$ without those subjects results in a MSE% of less than 17%. A relatively small amount of subject-to-subject variability was observed in all parameters with maximal CV% less than 60%, and a larger variability was found between ablated subjects compared with normal subjects (Table 1).

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#### Checking TIM Assumptions

TIM experiment is based on the assumption that the tracer behaves in the same kinetic manner as the parent drug (Veng-Pedersen et al., 1997). Figure 2 indicates that $^{125}$I-CERA can be considered a proper tracer by having very similar distribution curve to unlabeled CERA, when both forms are mixed together. This finding was confirmed by a nonsignificant difference ($p > 0.05$) in AUC_{[0-last]} and concentrations at various time percentages.

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### Effect of Busulfan Ablation on PK Parameters

The estimated receptor-related microparameters obtained from the physiological modeling of the tracer component of the TIM are displayed in Table 1. The
macroparameters estimated from the noncompartmental analysis performed on the nontracer component of the TIM are depicted in Table 2. The ablation resulted in a highly significant reduction \((p < 0.01)\) in the EPOR-normalized production rate constant \((k_{\text{syn}}/R_0)\), EPOR degradation rate constant \((k_{\text{deg}})\), CERA-EPOR complex internalization rate constant \((k_{\text{int}})\), and CERA total clearance (CL) to approximately 44, 28, 0.4, and 60% of the normal subjects mean values, respectively. A pronounced increase \((p < 0.01)\) in CERA elimination half-life \([t_{1/2}]\), and MRT, by approximately 2.4-fold was detected. No significant change \((p > 0.05)\) was observed in CERA/EPOR equilibrium dissociation constant \((K_D)\), CERA initial volume of distribution \((V_d)\), steady-state volume of distribution \((V_{ss})\), distribution half-life \([t_{1/2}(\alpha)]\), or distribution clearance \((CL_d)\).

Discussion

The main assumption of our model is that CERA is degraded only by cells expressing EPOR through binding to those receptors, followed by internalization via endocytosis and subsequent degradation in the lysosomes. Although no specific sites and mechanisms of CERA metabolism have been identified yet, several lines of evidence support our key assumption. In vitro cellular trafficking of EPO and novel erythropoiesis-stimulating protein (NESP), a hyperglycosylated EPO analog, showed that only cells expressing surface EPOR were able to degrade both compounds (Gross and Lodish, 2006). Under the assumption that hyperglycosylation and PEGylation exert similar effects on EPO molecule, the role of EPOR in CERA elimination must be considered.

CERA-PK parameters were similar in patients with severe hepatic impairment and healthy subjects after a single intravenous bolus dose (Kupcova et al., 2008). The same observation can be made when comparing the bioavailability normalized clearance \([0.66 \text{ versus } 0.90 \text{ ml/(h \cdot kg)}]\) and terminal half-life \((160 \text{ versus } 139 \text{ h})\) of CERA in healthy volunteers and patients with chronic kidney diseases after subcutaneous administration of a single dose (Macdougall et al., 2006; Fishbane et al., 2007). In accordance with EPO whose clearance in sheep is not affected by hepatectomy and nephrectomy (Widness et al., 1996), CERA in vivo elimination seems to be independent of liver and kidneys. CERA PEG chains stabilize the core glycoprotein against the action of proteases, reducing the probability of biotransformation taking place in blood.

It is proposed, but is still a controversial issue (Jelkmann et al., 2008), that EPOR is expressed not only on erythroid progenitor cells found mainly in the BM but also on nonhematopoietic cells present outside the BM \([\text{e.g., capillary endothelial cells, myocytes, liver parenchyma cells, kidney interstitial cells, retinal cells, myoblasts, neural cells, glia, and astrocytes (Anagnostou et al., 1994; Juul et al., 1998; Ogilvie et al., 2000; Nagai et al., 2001)}]\). Busulfan ablation is considerably more selective in ablating BM cells than most other tissue’s cells in the body, including tissues where mRNA for EPOR has been detected (Carlin et al., 1999). Thus, it does not seem very likely that nonhematopoietic EPOR-expressing cells outside of the BM will be much affected by busulfan treatment. From our previous work with chemical ablation by busulfan (Chapel et al., 2001; Veng-Pedersen et al., 2003, 2004), no erythroid colony-forming units, and only extremely few burst-forming unit-erythroid colonies, were found after 6 days of incubation of BM aspirates drawn at days 8 and 13 after busulfan treatment of sheep. Thus,
The internalization and lysosomal degradation processes in our model were presented by $k_{int}$. Study of the fate of $^{125}$I-EPO and EPOR in human EPOR-expressing UT-7 cells demonstrated that EPO was degraded completely after endocytosis, whereas few EPORs were recycled back to the surface of the cells (Sawyer et al., 1987; Walrafen et al., 2005). In a different study in cultured Ba/F3 and UT-7/EPO cells, 60% of internalized EPO or NESP was resorbed intact. In spite of this process, however, the absence of differences in the proportions of EPO and NESP subject to degradation or recycling, as well as the identical internalization rate of both compounds, ruled out the importance of the fate of the ESAs after binding with surface EPO in influencing their half-lives (Gross and Lodish, 2006). In the current model, fractions of recycled CERA or EPOR were not considered due to our desire to keep the model simple. The interesting finding that CERA-EPOR complex internalization rate constant, but not the dissociation constant, was significantly reduced by busulfan treatment (Table 1) suggests that although erythropoietic and nonerythropoietic populations have the same affinity to CERA molecules, the former contributes more effectively to CERA in vivo elimination.

Our finding that some of CERA-PK parameters determined by noncompartmental analysis were significantly affected by busulfan-induced BM ablation (Table 2) provides a strong evidence for the importance of BM in CERA disposition. Substantial changes in CL, $t_{1/2}(\beta)$, and MRT, but not in $V_{ss}$, $t_{1/2}(\alpha)$, or CL indicate that busulfan treatment affects the elimination phase ($\beta$ phase) of CERA and is consistent with the finding of the physiological model that BM plays a major role in CERA elimination. Similar changes in EPO CL, $t_{1/2}(\beta)$, MRT, $V_{ss}$, and $t_{1/2}(\alpha)$ were previously reported from PK studies performed in pre- and post–BM-ablated sheep (Chapel et al., 2001), suggesting that ESAs have a common elimination pathway that depends on hematopoietic EPOR.

The substantial reduction in $k_{int}$ after busulfan treatment can explain the significant decrease in CL, as well as the significant increase in $t_{1/2}(\beta)$ and MRT. Although CERA CL was significantly reduced after BM ablation, CERA elimination, which accounted for approximately 60% of the total elimination in sheep with normal BM, did occur. Such elimination could be mediated by EPOR located outside the BM, but the disproportionality between changes in CL and $k_{int}$ (percent decrease is 40 versus 100) suggests that other EPOR-independent elimination pathway(s) are involved. However, questions regarding location and nature of such pathway(s) require additional investigations.

The saturable nature of the nonerythropoietic, non-BM pathway(s) for CERA in contrast to EPO predicts two fundamental differences: 1) an increasing fraction of CERA is used for erythropoiesis for increasing concentrations; and 2) the clearance of CERA becomes more limited for increasing concentrations. Taken together, these differences favor a more efficacious and prolonged action for CERA.

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Authorship Contributions

Participated in research design: Widness and Veng-Pedersen.

Conducted experiments: Widness.

Performed data analysis: El-Komy and Veng-Pedersen.

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


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