Published in final edited form as:

J Pharmacokinet Pharmacodyn. 2008 June ; 35(3): 349–377. doi:10.1007/s10928-008-9092-6.

Basic pharmacodynamic models for agents that alter the lifespan distribution of natural cells

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Abstract

A new class of basic indirect pharmacodynamic models for agents that alter the loss of natural cells based on a lifespan concept are presented. The lifespan indirect response (LIDR) models assume that cells (R) are produced at a constant rate (k_{in}), survive during a certain duration T_R , and finally are lost. The rate of cell loss is equal to the production rate but is delayed by T_R . A therapeutic agent can increase or decrease the baseline cell lifespan to a new cell lifespan, T_D , by temporally changing the proportion of cells belonging to the two modes of the lifespan distribution. Therefore, the change of lifespan at time t is described according to the Hill function, $H(C(t))$, with capacity (E_{max}) and sensitivity (EC_{50}), and the pharmacokinetic function $C(t)$. A one-compartment cell model was examined through simulations to describe the role of pharmacokinetics, pharmacodynamics and cell properties for the cases where the drug increases ($T_D > T_R$) or decreases ($T_D < T_R$) the cell lifespan. The area under the effect curve ($AUCE$) and explicit solutions of LIDR models for large doses were derived. The applicability of the model was further illustrated using the effects of recombinant human erythropoietin (rHuEPO) on reticulocytes. The cases of both stimulation of the proliferation of bone marrow progenitor cells and the increase of reticulocyte lifespans were used to describe mean data from healthy subjects who received single subcutaneous doses of rHuEPO ranging from 20 to 160 kIU. rHuEPO is about 4.5-fold less potent in increasing reticulocyte survival than in stimulating the precursor production. A maximum increase of 4.1 days in the mean reticulocyte lifespan was estimated and the effect duration on the lifespan distribution was dose dependent. LIDR models share similar properties with basic indirect response models describing drug stimulation or inhibition of the response loss rate with the exception of the presence of a lag time and a dose independent peak time. The current concept can be applied to describe the pharmacodynamic effects of agents affecting survival of hematopoietic cell populations yielding realistic physiological parameters.

Keywords

Lifespan indirect response models; Erythropoietin; Reticulocytes; Delay differential equations

Introduction

Many pharmacological agents exhibit an indirect mechanism of action by inhibiting or stimulating production or loss of a biological signal or a biomarker that ultimately controls a therapeutically relevant response. Four basic pharmacodynamic (PD) models have been introduced that combined the turnover of the response variable with an indirect drug effect [1]. Since then, these types of models have been extensively studied and extended to account for complexities of biological systems such as multiple compartments, tolerance effects, feedback mechanisms and others [2]. Lifespan-based indirect response (LIDR) models have been proposed to describe drug effects on such systems like cell populations where the loss rate is more likely to be controlled by the natural cell senescence or its conversion to another cell type rather than by the size of the cell population [3]. However, the drug effects implemented in the LIDR models are limited to the inhibition or stimulation of the production rate of the response [3], which makes them similar to the basic indirect response Models I and III [1]. A description of drug effects on the loss rate of the response, equivalent to the basic indirect response Models II and IV, requires the implementation of a mechanism that affects the distribution of lifespans in a drug concentration-dependent manner. Contrary to the basic indirect response models, such a process introduces a great deal of mathematical complexities to the pharmacodynamic models. However, models dealing with drug effects that alter the lifespan distribution of cell populations are of great interest to analyze the changes in cell lifespan due to the entry of younger cells in the circulation, the inhibition or stimulation of the apoptosis, and the modification of mature cell survival.

Among others, the hematopoietic cell populations are natural examples of biological systems governed by lifespan-based processes of cell proliferation, differentiation, maturation, and senescence [3]. Endogenous hematopoietic growth factors like erythropoietin (EPO), thrombopoietin, and granulocyte colony stimulating factor (G-CSF) and their therapeutic protein analogs fall into the category of agents that regulate not only the production of cells in the bone marrow and in blood, but also the apoptosis and cell survival. Consequently, their pharmacological effects could be adequately described by the LIDR models [4–6]. The major mechanism of action for erythropoietin is the receptor-mediated activation of the Janus kinase 2 signaling pathway that results in stimulation of proliferation of progenitors cells in bone marrow and inhibition of apoptosis [7]. Erythropoietin has been reported also to act as a survival factor [8]. The hypothesised role of EPO in neocytolysis also suggests that this cytokine exhibits a mechanism of action that affects the lifespan distribution of reticulocytes and red blood cells (RBC) [9]. Pharmacodynamic models of recombinant human erythropoietin (rHuEPO) effects on reticulocyte age markers have been developed recently [10]. The relationship between reticulocyte and RBC counts in healthy subjects receiving a single dose of rHuEPO indicated a dose dependent effect on the mean reticulocyte lifespan [11]. Therefore, we use rHuEPO and reticulocytes as an example of a system where a therapeutic agent affects the lifespan distribution of a clinically relevant biomarker. Other hematopoietic growth factors have also been reported to affect lifespan distributions of blood cells. The effect of pegylated recombinant megakaryocyte growth and development factor (PEG-rHuMGDF) on the mean platelet lifespan has been investigated [12]. Administration of PEG-rHuMGDF to normal subjects prolonged the mean platelet lifespan from the baseline 8.5 days to 9.4 days on day 8 of the study. This effect was attributed to an increased number of young circulating platelets due to a prior stimulation of the production of bone marrow precursor cells. Similarly, Roskos et al. evidenced that pegfilgrastim stimulates early release of younger neutrophils from bone marrow in peripheral blood [13].

The objective of this work is to introduce and systematically evaluate the basic indirect pharmacodynamic models for agents that alter the loss of natural cells based on the modification of cell lifespan distribution. The general theory is presented first and subsequently limited to

a very simplistic distribution of cell lifespans. The one-compartment cell model is examined through a simulation exercise to describe the role of pharmacokinetics, pharmacodynamics and cell properties for the cases where the drug increases or decreases the cell lifespan. The differential equations, the explicit solutions of LIDR models for large doses and the area under the effect curve (AUC_E) are derived. A pharmacokinetic and pharmacodynamic model of rHuEPO effect on reticulocyte accounting for both stimulation of the proliferation of bone marrow progenitor cells and increase of reticulocyte lifespans was applied to describe mean data from healthy subjects who received a single subcutaneous doses of rHuEPO ranging from 20 to 160 kIU.

Theoretical

The number of cells R in a population is controlled by two processes: cell production and cell loss. The net change in the cell number is determined by the difference between the production rate $k_{in}(t)$ and the elimination rate $k_{out}(t)$:

$$\frac{dR}{dt} = k_{in}(t) - k_{out}(t) \quad (1)$$

The fundamental assumption is that every cell in the population at any moment of time t is assigned to have a specific lifespan. The loss process is assumed to be a consequence of natural senescence or conversion to another cell type. Each cell lives for the same period of time and then disappears, therefore, the cells of the same age will leave the population at the same time. In general, the lifespans of cells can be time dependent. Then, the distribution of the cell lifespan is described by the following probability density function (p.d.f.) $\ell(t, \tau)$:

$$\ell(t, \tau) \Delta\tau = \# \text{ cells with a specific lifespan at time } t \text{ between } \tau \text{ and } \tau + \Delta\tau \quad (2)$$

The loss of cells from the population is totally determined by their lifespan distribution, then the elimination rate must satisfy the following relationship:

$$k_{out}(t) = \int_0^{\infty} k_{in}(t - \tau) \ell(t - \tau, \tau) d\tau \quad (3)$$

The derivation of Eq. 3 has been presented previously elsewhere [14]. In other words, the elimination rate for a cell population with a known lifespan distribution is equal to the convolution integral of the cell production rate and the p.d.f. for the lifespan distribution as shown in Eq. 4:

$$k_{out}(t) = k_{in} * \ell(t) \quad (4)$$

where the convolution operator '*' is defined by Eq. 3. In the simplest case of the point lifespan distribution $\delta(\tau - T_R)$, where T_R is the lifespan common for all cells:

$$k_{out}(t) = k_{in}(t - T_R) \quad (5)$$

Previously, it has been shown that the baseline cell count corresponding to the time-invariant lifespan distribution $\ell_0(\tau)$ is equal to the product of the baseline production rate k_{in0} and the mean lifespan T_R [11]:

$$R_0 = k_{in0} \cdot T_R = k_{in0} \int_0^{\infty} \tau \ell_0(\tau) d\tau \quad (6)$$

If $C(t)$ denotes the drug plasma concentration that affects the cell lifespan distribution, then in the simplest case of the point distribution $\delta(\tau - T_R)$, the most natural way of describing this drug effect would be via temporal changes of the value of T_R :

$$\delta(\tau - H(t)T_R) \quad (7)$$

where $H(t)$ is a function that relates $C(t)$ to the effect (e.g. the Hill function). This approach leads to difficult mathematical problems in calculation of the convolution integral Eq. 3 that have been solved by Freise et al. [15]. An alternative approach is presented here for lifespan distributions that are combinations of a finite number of the Dirac delta functions:

$$\ell(t, \tau) = \alpha_1(t) \delta(\tau - T_1) + \dots + \alpha_M(t) \delta(\tau - T_M) \quad (8)$$

where

$$\alpha_1(t) + \dots + \alpha_M(t) = 1 \quad \text{and} \quad \alpha_i(t) \geq 0, \quad i=1, \dots, M. \quad (9)$$

The time dependent coefficients can be considered as weights determining the contribution of each point distribution to the overall distribution. Our basic postulate is that the drug does not affect the location of points $T_1, \dots, T_M$ on the lifespan axis, but rather impacts the weights $\alpha_1(t), \dots, \alpha_M(t)$, and consequently temporally changes the lifespan distribution. Following the formalism of Eq. 7, the drug effect on the lifespan distribution can be described as

$$\alpha_1(H(t)) \delta(\tau - T_1) + \dots + \alpha_M(H(t)) \delta(\tau - T_M) \quad (10)$$

Contrary to Eq. 7, the convolution integral Eq. 3 can be easily calculated for the lifespan distribution described by Eq. 10 yielding

$$k_{out}(t) = k_{in}(t - T_1) \alpha_1(H(t - T_1)) + \dots + k_{in}(t - T_M) \alpha_M(H(t - T_M)) \quad (11)$$

Notice that if $M = 1$, then according to Eq. 9, $\alpha_1(t) \equiv 1$ is a constant function and no drug effect can be exercised. Further, we consider the simplest non-trivial lifespan distribution that, in the absence of drug, is centered about T_R . The drug effect is manifested by changing the weights between two point distributions $\delta(\tau - T_R)$ and $\delta(\tau - T_D)$, where T_D is the lifespan of a cell exposed to the drug that can be longer or shorter than the lifespan of a cell in the absence of drug. The Hill function is used to describe the effect as shown in Eq. 12:

$$\ell(t, \tau) = \left(1 - \frac{E_{max}C(t)}{EC_{50} + C(t)}\right) \delta(\tau - T_R) + \frac{E_{max}C(t)}{EC_{50} + C(t)} \delta(\tau - T_D) \quad (12)$$

In the absence of drug ($C(t) = 0$), the p.d.f. in Eq. 12 reduces to $\delta(\tau - T_R)$. According to Eqs. 1 and 11, the equations describing cell counts in the population with a constant production rate $k_{in}(t) \equiv k_{in}$ is as follows

$$\frac{dR}{dt} = k_{in} - k_{in} \left(1 - \frac{E_{max} C(t - T_R)}{EC_{50} + C(t - T_R)} \right) - k_{in} \frac{E_{max} C(t - T_D)}{EC_{50} + C(t - T_D)} \quad (13)$$

Equation 13 can be further simplified to

$$\begin{aligned} \frac{dR}{dt} &= \frac{k_{in} E_{max} C(t - T_R)}{EC_{50} + C(t - T_R)} - \frac{k_{in} E_{max} C(t - T_D)}{EC_{50} + C(t - T_D)} \\ &= k_{in} \cdot H(C(t - T_R)) - k_{in} \cdot H(C(t - T_D)) \end{aligned} \quad (14)$$

with the baseline condition

$$R_0 = k_{in} \cdot T_R \quad (15)$$

A schematic diagram for the LIDR model described by Eq. 14 is shown in Fig. 1. Equation 12 allows one to determine the effect of drug on the mean lifespan according to Eq. 16.

$$ML_R(t) = \int_0^\infty \tau \ell(t, \tau) d\tau = T_R + \frac{E_{max} C(t)}{EC_{50} + C(t)} (T_D - T_R), \quad \text{with } E_{max} \leq 1 \quad (16)$$

Since the drug effect increases the contribution of $\delta(\tau \mu T_D)$ into the cell lifespan distribution, if $T_D > T_R$, then the drug effect transiently increases the cell mean lifespan, whereas, if $T_D < T_R$, the mean lifespan transiently decreases and returns to T_R after $C(t)$ becomes 0.

The increase in the mean lifespan results in accumulation of cells in the population followed by a return to the baseline number of cells and hence to the baseline mean lifespan. Such a time course is characteristic for the indirect response Model II where drug inhibits response removal [1]. Similarly, the transient decrease in the mean lifespan accelerates depletion of cells and a decrease in the cell count can be observed followed by its return to the baseline. This is a characteristic pattern for the indirect response Model IV where drug stimulated the response removal [1]. In that respect, the case $T_D > T_R$ is analogous to IDR Model II, and the case $T_D < T_R$ to IDR Model IV. These and other important properties of the R vs. t curve can be deduced from the following relationship derived in Appendix A:

$$R(t) = R_0 + k_{in} E_{max} \int_{t-T_D}^{t-T_R} \frac{C(z) dz}{EC_{50} + C(z)} \quad (17)$$

Methods

Model exploration

The one-compartment cell model (Fig. 1, Eq. 14) was examined through a simulation exercise to describe the role of pharmacokinetics, pharmacodynamics and cell properties. Computer simulations of the basic LIDR were conducted using the ADAPT II software [16]. The effect of increasing doses was assessed through simulations of two distinct cases. In the first case, the drug effect increases the lifespan of exposed cells from T_R to T_D ($T_D > T_R$). In the second case, the drug effect decreased the lifespan of exposed cells from T_R to T_D ($T_D < T_R$). Escalating doses ranging from 100 to 100,000 units were modelled as IV bolus inputs into the central compartment. The monoexponential kinetics was assumed to follow Eq. 18:

$$C(t) = \frac{D}{V} e^{-k_{el}t} \quad (18)$$

where $V = 31$, $k_{el} = 0.1 \text{ h}^{-1}$. The basic pharmacodynamic parameter values used for simulations were $E_{max} = 1$, $EC_{50} = 100 \text{ ng/ml}$, $T_R = 48 \text{ h}$, $T_D = 24$ and 72 h , and $R_0 = 100$ units. The responses were simulated over a 200 h range and the role of each model parameter was examined by exploring the effect of a range of model parameter values on the pharmacodynamic profile, while other model parameters were kept fixed at the basic values. Therefore, simulations included E_{max} values ranging from 0.2 to 1, EC_{50} values ranging from 1 to 10,000 ng/ml, and R_0 values ranging from 80 to 120 units. The sensitivity of the basic models to T_R was explored for two additional scenarios: one where k_{in} was expressed by Eq. 12 and another where k_{in} was set to 100/48 units/h and R_0 was given by Eq. 15. The sensitivity of the basic models to T_R was performed for values ranging from 60 to 120 h when $T_D > T_R$, and for 12–36 h if $T_D < T_R$. For each scenario, the production rate constant was calculated from the baseline Eq. 15.

Calculus (limits, derivatives, and integration) was employed to explore the behavior of the pharmacodynamic response R as a function of time and dose. The characteristic points of the R vs. t curve such as the response peak (R_{peak}), time to response peak (T_{peak}), lag time (T_{lag}), and the area between the baseline and the response curve ($AUCE$) were calculated from Eq. 17 as integrals of the drug effect Hill function. Additionally, the limiting values of $R(t)$ for large doses ($D/V/EC_{50} \rightarrow \infty$) were obtained.

Although all PD model parameters are identifiable, concerns can be raised about precision of parameter estimates if data is limited and exposed to noise due to assay or intra-individual variability. Estimability of the PD model parameters were studied by generating responses for the basic LIDR model with $T_D > T_R$ at specified times 0, 24, 48, 60, 72, 96, 120, and 144 h for 100 subjects with the following residual error model

$$R_{ind}(t_i) = R(t_i) e^{\varepsilon_i} \quad (19)$$

where t_i denote the simulated times, and ε_i are random variables $\varepsilon_i \sim N(0, 0.1)$. The basic PK/PD parameter values were used with $Dose = 100\text{mg}$ and $E_{max} 0.8$. The individual responses were simulated by NONMEM® V level 1.1 software package (GloboMax, Hanover, MD, USA). Subsequently, all individual responses were fitted by the same model and individual parameter estimates recorded and presented in the form of histograms. The estimates obtained after successful minimization were used for further analysis. The quartiles of the parameter estimate distributions were used as measures of the bias and precision of estimation.

Model application

Previously published data were used to illustrate the implementation and the application of the basic LIDR model with $T_D > T_R$ into a more complex pharmacokinetic and pharmacodynamic (PK/PD) model of rHuEPO effect on reticulocytes accounting for both stimulation of the proliferation of bone marrow progenitor cells and increase of reticulocyte lifespans [11]. In this study, 48 healthy subjects were randomized to receive rHuEPO subcutaneously at doses of 20, 40, 60, 90, 120 and 160 kIU. All subjects received daily oral iron supplementation through day 29 of the study. Blood samples were collected 30, 20, and 10 min 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 h after drug administration. Reticulocyte counts were measured before drug administration, daily up to 9 days, and then every other day up to 25 days and further at 28, 34

and 43 days after drug administration. The mean PK and PD data were used to quantify the effect of rHuEPO on reticulocyte survival. An average time a reticulocyte spends in the circulation prior to maturation to an erythrocyte is about one day [17]. Administration of a single dose of rHuEPO to healthy volunteers has been reported to temporally increase reticulocyte count [18]. Additionally, a transient increase in the reticulocyte residence time has been reported [10]. The rHuEPO effect has been attributed to release of immature reticulocytes from the bone marrow that need to spend more time in the circulation before they convert to mature RBC [19]. This phenomenon can be interpreted as a transient increase in the mean lifespan of the circulating reticulocytes [11].

A previously published PK model has been applied to describe the time course of rHuEPO after subcutaneous administration [4]. The model structure used to characterize the rHuEPO disposition is displayed in Fig. 2. Briefly, it is an open one-compartment model with non-linear elimination. Subcutaneous absorption of rHuEPO was described by a dual absorption model with a faster absorption process characterized by a zero-order input, and a parallel slower absorption process that started after the zero-order input ended, and was characterized by a first-order input into the central compartment. The model after subcutaneous administration can be described by the following system of differential equations:

$$\frac{dA_{SC}}{dt} = -k_a A_{SC} \quad (20)$$

$$\begin{aligned} \frac{dA_E}{dt} = & k_{EPO} + (1 - Fr) \theta(t_{lag} - t) \frac{F \cdot Dose}{t_{lag}} \\ & + Fr \theta(t - t_{lag}) \cdot k_a \cdot A_{SC} - \frac{V_{max} A_E}{K_m + V_d + A_E} \end{aligned} \quad (21)$$

with the initial conditions

$$A_{SC}(0) = F \cdot Dose \quad \text{and} \quad A_E(0) = C_0 \cdot V_d \quad (22)$$

where A_{SC} and A_E is the amount of erythropoietin in the subcutaneous and central compartments, k_a is the absorption rate constant, F is the bioavailability, Fr denotes the fraction of a dose that is absorbed via a first-order process, whereas $1 - Fr$ is absorbed at the zero-order rate during the time t_{lag} . The endogenous EPO production rate constant k_{EPO} forms another input to the central compartment. EPO is eliminated via a saturable process described by the Michaelis—Menten equation with parameters V_{max} and K_m , and V_d denotes the volume of distribution. The step function $\theta(z) = 1$ for $z > 0$ and $\theta(z) = 0$ for $z \leq 0$, and C_0 is the endogenous EPO concentration. rHuEPO serum concentration was expressed by the ratio:

$$C = \frac{A_E}{V_d} \quad (23)$$

Since C_0 was selected as a model parameter, the endogenous EPO production rate was calculated from the steady-state equation for the compartment A_E :

$$k_{EPO} = \frac{V_{max} C_0}{K_m + C_0} \quad (24)$$

A previously published PD model was modified to describe the reticulocyte responses to rHuEPO treatment [5]. It is assumed that the progenitor cells BFU-E (*BFU*) are generated at a constant rate of k_{in} and the differentiation of BFU-E cells into CFU-E (*CFU*) is controlled by a first-order process, k_p , and stimulated by rHuEPO according to the Hill function

$$H(C) = \frac{S_{max}C}{SC_{50} + C} \quad (25)$$

where C is serum rHuEPO concentration given by Eq. 23, S_{max} is the maximum stimulation of BFU-E to differentiate to CFU-E, and SC_{50} is the EPO concentration eliciting 50% of S_{max} . Consequently,

$$\frac{dBFU}{dt} = k_{in} - k_p H(C(t)) BFU(t) \quad (26)$$

The CFU-E cells proliferate on average M_{CFU} times and transform to normoblasts (*NOR*) after their mean lifespan T_{CFU} . Hence, the differential equation representing the change of CFU-E vs. time can be described as follows:

$$\frac{dCFU}{dt} = 2^{M_{CFU}} k_p H(C(t)) BFU(t) - 2^{M_{CFU}} k_p H(C(t - T_{CFU})) BFU(t - T_{CFU}) \quad (27)$$

It is further assumed that normoblasts (*NOR*) proliferate M_{NOR} times and are transformed to reticulocytes (*RET*) after their maturation time T_{NOR} . Therefore, the differential equation representing the change of *NOR* vs. time can be described as follows

$$\frac{dNOR}{dt} = 2^{M_{CFU} + M_{NOR}} k_p H(C(t - T_{CFU})) BFU(t - T_{CFU}) - 2^{M_{CFU} + M_{NOR}} k_p \times H(C(t - T_{CFU} - T_{NOR})) BFU(t - T_{CFU} - T_{NOR}) \quad (28)$$

The production rate for *RET* is equal to the elimination rate for *NOR*. However, an additional effect of rHuEPO on the distribution of the *RET* lifespan was postulated. We assumed that rHuEPO transiently increases the baseline reticulocyte residence time in the circulation T_{RET} to T_D according to Eq. 12 where the E_{max} function was corrected for the presence of the endogenous EPO. Consequently, employing Eq. 11 to calculate the elimination rate for *RET* results in the following equation that is analogous to Eq. 13:

$$\begin{aligned} \frac{dRET}{dt} = & 2^{M_{CFU} + M_{NOR}} k_p H(C(t - T_{CFU} - T_{NOR})) BFU(t - T_{CFU} - T_{NOR}) \\ & - 2^{M_{CFU} + M_{NOR}} k_p H(C(t - T_{CFU} - T_{NOR} - T_{RET})) BFU(t - T_{CFU} - T_{NOR} - T_{RET}) (1 - E(C(t - T_{RET}))) \\ & - 2^{M_{CFU} + M_{NOR}} k_p H(C(t - T_{CFU} - T_{NOR} - T_D)) BFU(t - T_{CFU} - T_{NOR} - T_D) E(C(t - T_D)) \end{aligned} \quad (29)$$

where,

$$E(C) = \frac{C - C_0}{EC_{50} + C - C_0} \quad (30)$$

and the E_{max} value was fixed at 1, to reduce the number of model parameters. The initial conditions were obtained from the baseline (steady-state) equations as follows:

$$RET(0) = RET_0 \quad (31)$$

$$NOR(0) = \frac{T_{NOR}}{T_{RET}} RET_0 \quad (32)$$

$$CFU(0) = \frac{T_{CFU}}{T_{RET}} RET_0 \quad (33)$$

$$BFU(0) = BFU_0 = \frac{SC_{50} + C_0}{2^{M_{CFU} + M_{NOR}} k_p S_{max} T_{RET} C_0} RET_0 \quad (34)$$

The BFU-E production rate constant was calculated as a secondary parameter according to Eq. 35.

$$k_{in} = \frac{RET_0}{2^{M_{CFU} + M_{NOR}} T_{RET}} \quad (35)$$

The number of mitoses M_{CFU} and M_{NOR} were fixed at 4. The parameters k_p and S_{max} are not independently identifiable, therefore, the product $k_p \cdot S_{max}$ was directly estimated from the data. Also, since only the BFU variable is present in the equation describing RET , Eqs. 27 and 28 are not necessary to determine RET . Equation 16 describing the mean reticulocyte lifespan becomes Eq. 36.

$$ML_{RET}(t) = T_R + \frac{C(t) - C_0}{EC_{50} + C(t) - C_0} (T_D - T_{RET}) \quad (36)$$

During the estimation process, the measured rHuEPO serum concentrations and model predictions were transformed into logarithms. The magnitude of residual variability in the transformed serum concentrations was modelled using an additive error model. The residual variability in the reticulocyte counts was described by a combination of the additive and constant coefficient of variation models according to the equation:

$$R_{obs} = RET(1 + \varepsilon_1) + \varepsilon_2 \quad (37)$$

where R_{obs} was the observed reticulocyte count and ε_1 and ε_2 were independent, normally distributed random variables with zero means and variances σ_1^2 and σ_2^2 . Nonlinear regression fittings were performed using the NONMEM® V level 1.1 software package (GloboMax, Hanover, MD, USA) including NM-TRAN (version III level 1.0) and PREDPP (version IV level 1.0). NONMEM® was run under the Microsoft Windows operating system and compiled with Intel Fortran 9.0 for Windows. The implementation of the cell lifespan concept in NONMEM was done as previously described [20].

Results

Model exploration

The purpose of the sensitivity analysis was to determine which part of the response vs. time curve is most sensitive to the perturbation of a model parameter and to interpret the dynamics of the system described by qualitatively analyzing the simulated profiles. In addition, this analysis helped to understand how and to what extent certain parameters control the behavior of the response described by the model.

The effect of dose on the response-time course is presented in Fig. 3 using the basic PK/PD parameters. If $T_D > T_R$, the response remains for all doses at the baseline level up to T_R and starts to increase to reach the peak value at time T_D , followed by a gradual return to the baseline. Both the peak response and the time needed for the response to return to baseline increases with increasing doses (Fig. 3, middle panel). Similarly, if $T_D < T_R$, the response remains at the baseline level up to T_D for all doses and starts to decrease to reach the peak value at time T_R , followed by a gradual return to the baseline. The peak decreases and the time for the response to return to the baseline increases with increased doses. In each case, the time to reach the peak response was dose independent and equivalent to $T_D + T_R$. Similarly, both the peak and the initial slope approached limiting values with large doses. The sharpness of the peaks was highest for the doses in the range 10^3 – 10^4 units and decreased for smaller and larger doses.

The changes in the response vs. time profiles-corresponding to 0.2 increments in the E_{max} values are shown in Fig. 4 (upper panels). Relative to the baseline, the response increases ($T_D > T_R$) or decreases ($T_D < T_R$) proportionally to E_{max} values. Consequently, E_{max} acts as a scaling factor for the difference $R_{max} - R_0$. As expected for basic LIDR, the time to peak response did not depend on E_{max} . The lower panels of Fig. 4 show the effect of EC_{50} on the response vs. time profiles. They are identical to the plots presented in Fig. 3, except that they appear in inverse order with respect to the EC_{50} values. This is expected for monoexponential kinetics where both *Dose* and EC_{50} appear in the Hill function only as the combination $Dose/V/EC_{50}$. Therefore, an increase in the EC_{50} value causes the same effect on the response curve as a proportional decrease in *Dose* and vice versa.

The effects of T_D on the R vs. t curve are presented in Fig. 5 (upper panel). Since for $T_D > T_R$, T_D is equal to the time to peak response, an increase in T_D value results in a shift of the time to peak response to the right with a corresponding increase in the peak response value. The sharpness of the peak decreases with increased T_D . Since for $T_D < T_R$, T_D is equal to the lag time, a decrease in T_D value results in a decrease of the lag time with a corresponding decrease in the peak value of the response variable. Since the time to peak response remains at T_R , the sharpness of the peak increases with decreased T_D . The lower panels of Fig. 5 show the effect of a change in the baseline R_0 on the R vs. t curves. For both models, R_0 acts as a scaling factor where an increase in the R_0 value causes a proportional increase in the R values.

Figure 6 represents the effects of T_R on the R vs. t curve. Since T_R occurs in the baseline Eq. 15, two sets of simulations were performed. The first assumed that k_{in} was calculated according to Eq. 15, which resulted in a baseline that is independent of T_R . The second was free of this constraint (Fig. 6, lower panel). Since for $T_D > T_R$, T_R is equal to the lag time, a decrease in T_D value results in a decrease of the lag time in the response curve with a corresponding increase in the peak value of the response variable for simulations under the constraint and a decrease for simulations without the constraint. The time to peak remains at T_D and the sharpness of the peak increases with decreased T_R . Since for $T_D < T_R$, T_R is equal to the time to reach peak, an increase in T_R value results in a shift of the maximum response value to the right with a corresponding decrease in the peak response for simulations with the constraint and an increase

for simulations without the constraint. The sharpness of the peak increases with increased T_R .

The models describe an effect of drug on the lifespan distribution of cells. This effect is concentration-dependent and consequently a temporal change in the lifespan distribution can be observed. Figure 7 shows the mean lifespan $ML_R(t)$ as a function of time for both models simulated according to Eq. 16. For monoexponential kinetics and the scenario where $T_D > T_R$, the model predicts an immediate increase in the mean lifespan followed by a gradual decrease to the baseline mean lifespan of T_R . The maximal value of the mean lifespan is $T_R + E_{max}(T_D - T_R)$. Similarly, if $T_D < T_R$, the model predicts immediate decrease in the mean lifespan followed by a gradual increase to the baseline mean lifespan of T_R . The minimal value of the mean lifespan is $T_R - E_{max}(T_R - T_D)$. Equation 16 implies that if ML_R is plotted against the drug concentration C , then the EC_{50} will correspond to the concentration eliciting 50% of the difference between the maximal (minimal) and baseline lifespans.

Equation 17 provides a geometrical interpretation of the difference between $R(t)$ and R_0 as the area below the Hill function that extends from $t - T_D$ to $t - T_R$ with an appropriate correction for the sign. This implies that for strictly decreasing concentrations $C(t)$, the response has a unique peak at time

$$t_{peak} = \begin{cases} T_D, & \text{if } T_D > T_R \\ T_R, & \text{if } T_D < T_R \end{cases} \quad (38)$$

Consequently, the peak responses can be obtained from Eq. 17 by evaluating $R(t_{peak}) = R_{peak}$

$$R_{peak} = \begin{cases} R_0 + k_{in} E_{max} \int_0^{T_D - T_R} \frac{C(z) dz}{EC_{50} + C(z)}, & \text{if } T_D > T_R \\ R_0 - k_{in} E_{max} \int_0^{T_R - T_D} \frac{C(z) dz}{EC_{50} + C(z)}, & \text{if } T_D < T_R \end{cases} \quad (39)$$

Equation 16 also implies the existence of a lag time for the response curve

$$T_{lag} = \begin{cases} T_R, & \text{if } T_D > T_R \\ T_D, & \text{if } T_D < T_R \end{cases} \quad (40)$$

Integration of both sides of Eq. 17 allows one to calculate the area between the baseline and the response curve, $AUCE$, as follows,

$$AUCE = \int_0^\infty |R(t) - R_0| dt = R_0 E_{max} \left| \frac{T_D}{T_R} - 1 \right| \int_0^\infty \frac{C(z) dz}{EC_{50} + C(z)} \quad (41)$$

Equation 41 holds for any drug concentration for which the first moment is finite

$$\int_0^\infty t C(t) dt < \infty \quad \text{and} \quad t^2 C(t) \rightarrow 0 \quad \text{as} \quad t \rightarrow \infty \quad (42)$$

The absolute value notation has been used to avoid differentiation between T_D and T_R . Derivations of the equations described above are shown in detail in Appendix A. In particular, for monoexponential drug concentrations, the integral in Eq. 41 can be calculated explicitly as follows:

$$AUC_E = \frac{R_0 E_{\max}}{k_{el}} \left| \frac{T_D}{T_R} - 1 \right| \ln \left(1 + \frac{D/V}{EC_{50}} \right) \quad (43)$$

For large doses, the responses will approach a limiting curve that can be characterized by explicit equations for dose-proportional concentrations and times t such that $C(t) > 0$. If $T_D > T_R$

$$R(t) \rightarrow \begin{cases} R_0, & 0 < t \leq T_R \\ R_0 + k_{in} E_{\max} (t - T_R), & T_R \leq t \leq T_D \\ R_0 + k_{in} E_{\max} (T_D - T_R), & t \geq T_D \end{cases} \quad \text{as } D/V/EC_{50} \rightarrow \infty \quad (44)$$

and if $T_D < T_R$, then

$$R(t) \rightarrow \begin{cases} R_0, & 0 < t \leq T_D \\ R_0 - k_{in} E_{\max} (t - T_D), & T_D \leq t \leq T_R \\ R_0 + k_{in} E_{\max} (T_R - T_D), & t \geq T_R \end{cases} \quad \text{as } D/V/EC_{50} \rightarrow \infty \quad (45)$$

Derivations of Eqs. 44 and 45 are presented in detail in Appendix B. Consequently, for large doses the slope of the onset part of the response curve is $k_{in} E_{\max} (T_D > T_R)$, $-k_{in} E_{\max} (T_D < T_R)$, and the maximal response is defined as follows:

$$R_{\max} = R_0 + k_{in} E_{\max} (T_D - T_R) \quad (46)$$

To address estimability of the PD parameters 100 individual responses were simulated for model $T_D > T_R$ with 10% constant coefficient of variation residual error. Next, the same model was fitted to such data and parameter estimates for successful minimizations were recorded. The histograms of their frequency distributions are shown in Fig. 8. The number of successful minimizations was 68 out of 100. The ranges for parameter estimate distributions as well as the first, second, and third quartiles are presented in Table 1. The true EC_{50} value was 100 ng/ml. The ranges for $E_{\max}$ estimates were 0.011–1 with the first (Q1), second (Q2), and third (Q3) quartiles 0.762, 1, and 1, respectively. If the quotient $(\text{True} - Q2)/\text{True} \times 100\%$ used as a relative measure the bias the parameter estimate, then biases for estimates of EC_{50} , T_D , T_R , and R_0 were 1.6%, -25%, 6.7%, 1.7%, and 2.1%, respectively. Similarly, the ratio $(Q3 - Q1)/\text{True} \times 100\%$ can be used as a measure of the precision of the parameter estimate. Then, the precisions for EC_{50} , $E_{\max}$, T_D , T_R , and R_0 were 440%, 29.6%, 19.1%, 10.9%, and 7.9%, respectively. To determine if fixing $E_{\max}$ improves bias and precision of the remaining parameters, the simulated data were re-fitted with $E_{\max} = 0.8$ (results not shown). The number of successful minimizations increased to 79. The biases for estimates of EC_{50} , T_D , T_R , and R_0 were 8.5%, 7.7%, 2.9%, and 2.5%, respectively. The precisions were 295%, 15.1%, 12.9%, and 7.4%, respectively.

Model application

The estimates of parameters for the rHuEPO PK/PD model are shown in Table 2. The Michaelis—Menten saturable elimination process was sufficient to describe the disposition of rHuEPO

as shown in Fig. 9. Since our goal was to develop a relatively simple and minimalistic PK model that would adequately describe the rHuEPO serum concentrations the parameter estimates differ from their analogs from previously published models [4,21]. The PK parameters were fixed and used to generate the rHuEPO serum concentration C that was the driving force for two pharmacodynamic effects: stimulation of the production of the progenitor cells (proliferation) and perturbation of the reticulocyte lifespan distribution. The mean reticulocyte data fitted by the PD model are shown in Fig. 10. The estimate of the precursor cell lifespan in the bone marrow $T_{CFU} + T_{NOR}$ was 5 days. This value is similar to the reticulocyte precursor lifespan reported previously [3]. The parameters k_p and S_{max} were not identifiable, and the estimate of the product $k_p \cdot S_{max}$ yielded the mean resident time for the BFU-E cells under the maximal erythropoietic stimulation of 16.9 days that is unphysiologically long, but consistent with analogous estimates published previously [5]. The value of the estimate for the rHuEPO potency parameter for the stimulatory effect on the proliferation was $SC_{50} = 16.3$ IU/l, which is slightly higher than the baseline EPO levels and comparable with the values reported previously [4].

The objective of this case study was to assess the effect of rHuEPO on the reticulocyte lifespan distribution in the circulation. The estimate of the baseline reticulocyte lifespan T_R was 1.7 days, which is the average time a homeostatic reticulocyte spends in the circulation. However, this value is in contrast with T_{RET} values obtained from the LIDR models previously used [4, 5]. Our study indicates that this discrepancy can be attributed to the assumption that rHuEPO in those models was not allowed to change the reticulocyte lifespan. The PK/PD model presented here predicts that rHuEPO might increase the RET lifespan in a dose-dependent manner by as much as 4.1 days. As presented in Fig. 11, the mean reticulocyte lifespan ML_{RET} increases within $\Delta T_D = 4.1$ days to the maximal value of $T_R + \Delta T_D = 5.8$ days. Then, it gradually returns to the baseline value, $T_R = 1.7$ days, + within 6 days for doses of 20 kIU and 9 days for doses of 160 kIU. The parameter EC_{50} for this effect is estimated to be 73.2 IU/l, which implies that rHuEPO is about 4.5-fold less potent as an agent changing the reticulocyte lifespan distribution than as stimulator of the precursor production.

Discussion

The concept of the mean lifespan of a cell population as a delay time in the system variable has been used in various mathematical models of cell kinetics [22]. The idea of using a cell lifespan as a determinant of the removal rate was applied in the PK/PD area by Uherlinger et al. [23]. Its implementation in the context of indirect response models followed [3]. Subsequent applications of LIDR have been extended to modeling of toxic effects of anticancer agents, or effects of rHuEPO on erythropoiesis in humans and animals [4,24–26]. These models were also applied to analyze data from patient populations [27,28]. Another applications based on the convolution integral has been presented by Veng-Pedersen and collaborators [15,29,30]. Since the complex lifespan based PK/PD models require delay differential equations that are not supported by major PK/PD software packages, implementation of these concepts is faced with numerical challenges [20]. The transit or aging compartment PD models have been successfully used as an alternative approach to model delays in cell kinetics without numerical complexities associated with lifespan implementation [12,13,27,31]. The drug effect on the mean lifespan of the cell population has been also adequately described by these type of models [12,32].

Sensitivity analysis has revealed similarities between the LIDR model with $T_D > T_R$ and IDR Model II as well as between the LIDR model with $T_D < T_R$ and IDR Model IV [33]. All these models describe drug action on the response removal rate. A transient increase in the mean lifespan qualitatively results in a similar behavior of the response as a transient inhibition of the loss rate. Analogously, a transient decrease in the mean lifespan yields a response time

course that is similar to the response to a stimulation of the loss rate. However, there are two distinct differences between these LIDR and IDR models. The presence of a lag time, and the independence of the time to peak response to dose for LIDR models are the main difference with the corresponding IDR models [33]. The latter property is also common to the basic LIDR models that describe stimulatory and inhibitory drug effects on the response production rate [3]. However, these two distinct features are consequences of the assumed simplistic lifespan distribution, which was centered at two points, T_R and T_D . In the case of continuous lifespan distributions that extend from 0 to infinity, the lag time in the response will be absent. Also, as shown previously for the basic LIDR models with continuous lifespan distributions, the time to peak response becomes dose dependent [14].

Another conclusion that can be drawn from the exploratory analysis of the LIDR models is the high sensitivity of the response vs. time curve to all PD parameters, both system-related like T_R , T_D and R_0 , and drug-related like E_{max} , and EC_{50} . The parameters R_0 , T_R , and T_D can be clearly identified from the response at baseline, the lag time, and the time to maximum response. The drug-related parameters E_{max} and EC_{50} might require a relatively large dose to be resolved from single response data [34]. Also, since these parameters control the R vs. t curve in a similar manner, a higher correlation between their estimates might be expected, as observed for basic IDR models [34]. The simulations performed for model $T_D > T_R$ with a dense sampling and 10% coefficient of variation of the residual error indicate that for a single dose response the estimates of EC_{50} , T_D , T_R , and R_0 are not biased. The only biased parameter is E_{max} with a distribution skewed to the right owing to an imposed upper limit of 1 on the parameter estimates. An acceptable precision of estimates was observed for parameters E_{max} , T_D , T_R , and R_0 . The precision of EC_{50} estimates was very low implicating that this parameter cannot be precisely estimated from single dose data. The second least precise parameter was E_{max} . Fixing E_{max} to its true value did not improve the bias of parameter estimates and increased the precision, but the precision of EC_{50} estimate remained very low. These findings are similar to estimability of the I_{max}/S_{max} and IC_{50}/SC_{50} parameters from single response data for the basic IDR models reported previously [34]. Further studies are necessary to address the question of estimability of these parameters from single dose data, optimal dosing designs, as well as optimal sampling time designs.

The partially integrated solutions of the presented LIDR models, the behavior of the responses for large doses, and the explicit equations for $AUCE$ are similar to those presented previously for LIDR models, which described drug effects on the response production rate [3]. These relationships can be used to obtain initial values for the PD parameter estimates assuming large dose responses. In particular, the E_{max} value can be obtained either from the maximal response or the initial slope. Resolving the EC_{50} value from single dose response data is not straightforward and might involve a technique that uses $AUCE$ that has been developed for the basic IDR models [35]. If additional experimental data regarding the mean lifespan dependence on time or concentration are available, one might apply Eq. 16 to determine EC_{50} . The equation that relates $AUCE$ and the area under the Hill function (Eq. 41) holds true not only for the LIDR models presented here, but also for the other type of LIDR models, as well as for basic IDR Models I and III. These universal characteristics serve as an additional argument for using $AUCE$ as a measure of the net pharmacological effect for broadly understood indirect responses [36].

The presented case study of the rHuEPO effect on reticulocyte lifespan in healthy subjects is meant to illustrate the implementation and the applicability of the LIDR models for drugs affecting cell lifespan distributions. The mean data were used for analysis. A limitation of the naïve pooled data analysis has been recognized previously [37]. However, for balanced study designs as in the example presented here, the naïve pooled data approach has been shown to provide unbiased estimates of model parameters [38]. Although the rHuEPO prolongation of

the reticulocyte lifespan is well documented [14], up to now this drug effect has been included only in few models used to describe the rHuEPO effects on reticulocytes [15]. Including such a process in the PK/PD model dramatically improves the estimate of the mean reticulocyte lifespan $T_{RET} = 1.7$ days that has been overestimated by LIDR models that only account for the stimulation of the production of the progenitor cells, $T_{RET} = 3.1\text{--}6.7$ days [3,14]. A similar improvement can be expected for the estimates of the mean lifespan of RBC if such data are modeled by an analogous model. Such an effect of rHuEPO on the RBC lifespan distribution has been indicated by our previous study [14].

A relatively high value of the estimate of $K_m = 3860$ IU/l is consistent with the estimates of this parameter published previously and reflects moderate nonlinearity in rHuEPO disposition [4,5]. A rHuEPO mechanism of action on survival of RBC is not currently known and might be difficult to determine in view of still not fully understood major processes that control RBC senescence and is further hampered by the duration of clinical studies [39]. Since the class of therapeutic proteins with the back bone structure identical to erythropoietin increases, it might be expected to observe similar effects of this class on the survival of RBC. Consequently, the model presented here could be applicable to analyze results of studies involving those agents. These types of models may be useful to explore the role of erythropoietin stimulating agents' half-life in the stimulation of the production rate of erythropoietic precursor cells and the prolongation of the reticulocytes' lifespan, which would be helpful to differentiate compounds that are already in the market (rHuEPO, darbepoietin, PEG-rHuEPO). Also, it can be used in dealing with preclinical information and as the basis for the interspecies PK/PD extrapolation and the optimization of first in human studies as recently has been demonstrated using a similar mechanistic PK/PD model for rHuEPO [40,41]. The examples of PEG-rHuMGDF and pegfilgrastim provide other potential applications of our concept to biologics affecting the thrombopoietic cell populations and neutrophils, respectively [12,13]. Therefore, the current model is able to deal with drug effects that alter the lifespan distribution of cell populations and, as a consequence, it is useful to analyze the increase in lifespan of cells due to the entry of younger cells in the circulation, the inhibition of the apoptosis, and/or the prolongation of mature cell survival.

In summary, new LIDR models describing the pharmacological effects of agents that alter the lifespan distribution of cell populations have been developed. The properties of these models have been analyzed by computer simulations and methods of mathematical calculus. The new LIDR models exhibit similarities to the basic IDR Models II and IV both in the shape of the response curves and the way model parameters control the responses. However, the LIDR preserve distinct features like the presence of lag times and dose-independent time of peak response. Inclusion of the effect of rHuEPO on the reticulocyte lifespan distribution into a previously developed PD model resulted in an improved estimate of the mean reticulocyte lifespan. The concept presented here can be applied to other therapeutic agents that alter the lifespan distribution of cell populations. More in particular, they can be used to understand the changes in cell lifespan due to the entry of younger cells in the circulation, the inhibition or stimulation of apoptosis, and/or the modification of mature cell survival.

Acknowledgements

This study was supported by Johnson & Johnson Pharmaceutical Research & Development, A Division of Janssen Pharmaceutica, NV, Beerse, Belgium, and in part by the National Institute of General Medical Sciences, National Institutes of Health Grant GM 57980.

Appendix A

Derivation of Eq. 17

Integration of both sides of Eq. 1 from 0 to t yields

$$R(t) = R_0 + k_{in} E_{max} \int_0^t \frac{C(\tau - T_R) d\tau}{EC_{50} + C(\tau - T_R)} - k_{in} E_{max} \int_0^t \frac{C(\tau - T_D) d\tau}{EC_{50} + C(\tau - T_D)} \quad (A1)$$

Changing the variables $z = \tau - T_R$ in the first integral and $z = \tau - T_D$ in the second results in

$$R(t) = R_0 + k_{in} E_{max} \int_0^{t-T_R} \frac{C(z) dz}{EC_{50} + C(z)} - k_{in} E_{max} \int_0^{t-T_D} \frac{C(z) dz}{EC_{50} + C(z)} \quad (A2)$$

Combining the two integrals in Eq. A2 into one gives Eq. 17.

Derivation of Eq. 41

Consider the case $T_R < T_D$. Subtraction of R_0 from both sides of Eq. A2 and integration from 0 to t yields

$$\int_0^t (R(s) - R_0) ds + k_{in} E_{max} \int_0^t \int_0^{s-T_R} \frac{C(z) dz}{EC_{50} + C(z)} ds - k_{in} E_{max} \int_0^t \int_0^{s-T_D} \frac{C(z) dz}{EC_{50} + C(z)} ds \quad (A3)$$

Integration by parts results in

$$\begin{aligned} \int_0^t \int_0^{s-T_R} \frac{C(z) dz}{EC_{50} + C(z)} ds &= s \int_0^{s-T_R} \frac{C(z) dz}{EC_{50} + C(z)} \Big|_0^t - \int_0^t \frac{sC(s-T_R) ds}{EC_{50} + C(s-T_R)} \\ &= t \int_0^{t-T_R} \frac{C(z) dz}{EC_{50} + C(z)} - \int_0^{t-T_R} \frac{(\tau - T_R) C(\tau) d\tau}{EC_{50} + C(\tau)} \end{aligned} \quad (A4)$$

The l'Hospital rule and assumption imply that

$$t \int_0^{t-T_R} \frac{C(z) dz}{EC_{50} + C(z)} \rightarrow 0 \quad \text{as } t \rightarrow \infty \quad (A5)$$

The limit of the second integral in Eq. A5 as $t \rightarrow \infty$ is

$$\int_0^{t-T_R} \frac{(\tau - T_R) C(\tau) d\tau}{EC_{50} + C(\tau)} \rightarrow \int_0^\infty \frac{\tau C(\tau) d\tau}{EC_{50} + C(\tau)} - T_R \int_0^\infty \frac{C(\tau) d\tau}{EC_{50} + C(\tau)} \quad (A6)$$

Analogously,

$$\int_0^t \int_0^{s-T_D} \frac{C(z) dz}{EC_{50}+C(z)} ds \rightarrow \int_0^\infty \frac{\tau C(z) dz}{EC_{50}+C(z)} - T_D \int_0^\infty \frac{C(\tau) d\tau}{EC_{50}+C(\tau)}, \quad \text{as } t \rightarrow \infty \quad (\text{A7})$$

Combining Eqs. A3–A7, one can conclude that

$$\begin{aligned} \int_0^\infty (R(z) - R_0) dz = & T_D k_{in} E_{max} \int_0^\infty \frac{C(z) dz}{EC_{50}+C(z)} \\ & - T_R k_{in} E_{max} \int_0^\infty \frac{C(\tau) d\tau}{EC_{50}+C(\tau)} \end{aligned} \quad (\text{A8})$$

The baseline Eq. 15 implies that $T_D k_{in} = R_0 T_D / T_R$ and $T_R k_{in} = R_0$, and Eq. 41 follows. The derivation of Eq. 41 in the case $T_D < T_R$ is analogous to the above.

Appendix B

Derivation of Eqs. 44 and 45

We will provide a proof of Eq. 44. An analogous derivation holds for Eq. 45. Let $T_R < T_D$ and the time t be such that $C(t) > 0$. The assumption of dose-proportionality of $C(t)$ implies, that

$$C(t) = \frac{D}{V} c(t) \quad (\text{B1})$$

and the variable $C(t)$ does not depend on dose. We will exploit Eq. 17 transformed to the following form

$$R(t) = R_0 + k_{in} E_{max} \int_{t-T_D}^{t-T_R} \frac{c(z) dz}{EC_{50}/D/V + c(z)} \quad (\text{B2})$$

Consider the case $0 < t < T_R$. Since for negative times the drug concentration is 0, the integral in Eq. B2 becomes 0, and the response $R(t) = R_0$. If $T_R \leq t \leq T_D$, then the integral lower limit can be set to 0 and

$$R(t) = R_0 + k_{in} E_{max} \int_0^{t-T_R} \frac{c(z) dz}{EC_{50}/D/V + c(z)} \quad (\text{B3})$$

Since for $0 < z < t - T_R$, then

$$\frac{c(z)}{EC_{50}/D/V + c(z)} \rightarrow 1 \quad \text{as } D/V/EC_{50} \rightarrow \infty \quad (\text{B4})$$

and consequently

$$R(t) \rightarrow R_0 + k_{in} E_{max} \int_0^{t-T_R} dz = R_0 + k_{in} E_{max} (t - T_R) \quad (B5)$$

Similarly, if $t \geq T_D$, then

$$R(t) \rightarrow R_0 + k_{in} E_{max} \int_{t-T_D}^{t-T_R} dz = R_0 + k_{in} E_{max} (T_D - T_R) \quad \text{as} \quad D/V/EC_{50} \rightarrow \infty \quad (B6)$$

This completes the derivation of Eq. 44.

References

1. Dayneka NL, Garg V, Jusko WJ. Comparison of four basic models of indirect pharmacodynamic responses. *J Pharmacokinet Biopharm* 1993;21:457–478. [PubMed: 8133465]
2. Mager DE, Wyska E, Jusko WJ. Diversity of mechanism-based pharmacodynamic models. *Drug Metabol Disp* 2003;31:510–519.
3. Krzyzanski W, Ramakrishnan R, Jusko WJ. Basic pharmacodynamic models for agents that alter production of natural cells. *J Pharmacokinet Biopharm* 1993;21:457–478. [PubMed: 8133465]
4. Ramakrishnan R, Cheung WK, Wacholtz MC, Minton N, Jusko WJ. Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after single and multiple doses in healthy volunteers. *J Clin Pharmacol* 2004;44:991–1002. [PubMed: 15317827]
5. Krzyzanski W, Jusko WJ, Wacholtz MC, Minton N, Cheung WK. Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after multiple subcutaneous doses in healthy subjects. *Eur J Pharm Sci* 2005;26:295–306. [PubMed: 16102948]
6. Samtani, MN.; Perez-Ruixo, JJ.; Brown, K.; Cerneus, D.; Molloy, C. Pharmacokinetic and pharmacodynamic model of pegylated thrombopoietin mimetic peptide in healthy subjects: comparison of verification procedures for assessing model predictability. Abstracts of the XVI annual meeting of the population approach group in Europe; 2007. Abstr. 1220 [www.page-meeting.org/?abstract=1220]
7. Lacombe C, Mayeux P. Biology of erythropoietin. *Haematologica* 1998;83:724–732. [PubMed: 9793257]
8. Polenakovic M, Sikole A. Is erythropoietin a survival factor for red blood cells? *J Am Soc Nephrol* 1996;7:1178–1182. [PubMed: 8866410]
9. Trial J, Rice L, Alfrey CP. Erythropoietin withdrawal alters interactions between young red blood cells, splenic endothelial cells, and macrophages: an in vitro model of neocytolysis. *J Invest Med* 2001;49:335–345.
10. Al-Huniti NH, Widness JA, Schmidt RL, Veng-Pedersen P. Pharmacodynamic analysis of changes in reticulocyte subtype distribution in phlebotomy-induced stress erythropoiesis. *J Pharmacokinet Pharmacodyn* 2005;32:359–376. [PubMed: 16284920]
11. Krzyzanski W, Perez-Ruixo JJ. An assessment of recombinant human erythropoietin effect on reticulocyte production rate and lifespan distribution in healthy subjects. *Pharm Res* 2007;24:758–771. [PubMed: 17318417]
12. Harker LA, Roskos LK, Marzec UM, Carter RA, Cherry JK, Sundell B, Cheung EN, Terry D, Sheridan W. Effects of megakaryocyte growth and development factor on platelet production, platelet life span, and platelet function in healthy human volunteers. *Blood* 2000;95:2514–2522. [PubMed: 10753829]
13. Roskos LK, Lum P, Lockbaum P, Schwab G, Yang B-B. Pharmacokinetic/pharmacodynamic modeling of pegfilgrastim in healthy subjects. *J Clin Pharmacol* 2006;46:747–757. [PubMed: 16809800]

14. Krzyzanski W, Woo S, Jusko WJ. Pharmacodynamic models for agents that alter production of natural cells with various distributions of lifespans. *J Pharmacokinet Pharmacodyn* 2006;33:125–166.
15. Freise KJ, Widness JA, Schmidt RL, Veng-Pedersen P. Pharmacodynamic analysis of time-variant cellular disposition: reticulocyte disposition changes in phlebotomized sheep. *J Pharmacokinet Pharmacodyn* 2007;34:519–547.
16. D'Argenio, DZ.; Schumitzky, A. Biomedical Simulations Resource. Los Angeles: 1997. ADAPT II user's guide.
17. McKenzie, SB. Textbook of hematology. Williams & Wilkins; Baltimore: 1996. p. 101-102.
18. Cheung WK, Goon BL, Guilfoyle MC, Wacholtz MC. Pharmacokinetics and pharmacodynamics of recombinant human erythropoietin after single and multiple subcutaneous doses to healthy subjects. *Clin Pharmacol Ther* 1998;64:412–423. [PubMed: 9797798]
19. Major A, Bauer C, Breymanann C, Huch A, Huch R. rh-Erythropoietin stimulates immature reticulocyte release in man. *Br J Haematol* 1994;87:605–608. [PubMed: 7993803]
20. Perez-Ruixo JJ, Kimko HC, Chow AT, Piotrovsky V, Krzyzanski W, Jusko WJ. Population cell life span models for effects of drugs following indirect mechanisms of action. *J Pharmacokinet Pharmacodyn* 2005;32:767–793. [PubMed: 16328102]
21. Olsson-Gisleskog P, Jacqmin P, Perez-Ruixo JJ. Population pharmacokinetics meta-analysis of recombinant human erythropoietin in healthy subjects. *Clin Pharmacokinet* 2007;46:159–173. [PubMed: 17253886]
22. Belair J, Mackey MC, Mahaffy JM. Age-structured and two-delay models for erythropoiesis. *Math Biosci* 1995;128:317–346. [PubMed: 7606142]
23. Uehlinger DE, Gotch FA, Sheiner LB. A pharmacodynamic model of erythropoietin therapy for uremic anemia. *Clin Pharmacol Ther* 1992;51:76–89. [PubMed: 1732079]
24. Krzyzanski W, Jusko WJ. Multiple-pool cell lifespan model of hematologic effects of anticancer agents. *J Pharmacokinet Pharmacodyn* 2002;29:311–337. [PubMed: 12518707]
25. Ramakrishnan R, Cheung WK, Farrell F, Kelley M, Jolliffe L, Jusko WJ. Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after intravenous and subcutaneous single dose administrations in cynomolgus monkeys. *J Pharmacol Exp Ther* 2003;306:324–331. [PubMed: 12676891]
26. Woo S, Krzyzanski W, Jusko WJ. Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after intravenous and subcutaneous administration in rats. *J Pharmacol Exp Ther* 2006;319:1297–1306. [PubMed: 16973883]
27. Holford, NHG. PK/PD models for red blood cell responses to erythropoietic stimulation with and without chemotherapy and iron supplements. Symposium annual meeting; Orlando, FL, USA. American Society for Clinical Pharmacology and Therapeutics; 2005.
28. Gieschke, P.; Chanu, P.; Charoin, J-E.; Pannier, A.; Reigner, B. Modelling of haematological responses to erythropoiesis-stimulating agents in healthy volunteers and patients. Fifth international symposium on measurement and kinetics of in vivo drug effects; The Netherlands: Noordwijkerhout; 2006.
29. Chapel SH, Veng-Pedersen P, Schmidt RL, Widness JA. A pharmacodynamic analysis of erythropoietin-stimulated reticulocyte response in phlebotomized sheep. *J Pharmacol Exp Ther* 2000;295:346–351. [PubMed: 10992000]
30. Veng-Pedersen P, Chapel SH, Schmidt RL, Widness JA. An integrated pharmacodynamic analysis of erythropoietin, reticulocyte, and hemoglobin responses in acute anemia. *Pharm Res* 2002;19:1630–1635. [PubMed: 12458668]
31. Friberg LE, Freijs A, Sandstrom M, Karlsson MO. Semiphysiological model for the time course of leukocytes after varying schedules of 5-fluorouracil in rats. *J Pharmacol Exp Ther* 2000;295:734–740.
32. Perez-Ruixo JJ, Krzyzanski W, Hing J. Pharmacodynamic analysis of recombinant human erythropoietin effect on reticulocyte production rate and age distribution in healthy subjects. *Clin Pharmacokinet* 2008;46:399–415. [PubMed: 18479174]
33. Sharma A, Jusko WJ. Characterization of four basic models of indirect pharmacodynamic responses. *J Pharmacokinet Biopharm* 1996;24:611–634. [PubMed: 9300353]

34. Krzyzanski W, Dmochowski, Matsushima JN, Jusko WJ. Assessment of dosing impact on intra-individual variability in estimation of parameters for basic indirect response models. *J Pharmacokinet Pharmacodyn* 2006;33:635–655.
35. Wyska E, Mager DE, Krzyzanski W. Methods of estimation IC_{50} and SC_{50} parameters for indirect response models from single dose data. *J Pharm Sci* 2003;92:1438–1454. [PubMed: 12820148]
36. Krzyzanski W, Jusko WJ. Application of moment analysis to the sigmoid effect model for drug administered intravenously. *Pharm Res* 1997;14:949–952. [PubMed: 9244156]
37. Mandema, JW. Population pharmacokinetics and pharmacodynamics. In: Welling, PG.; Tse, FLS., editors. *Pharmacokinetics: regulatory, industrial academic perspectives*. Marcel Dekker; New York: 1995. p. 411-450.
38. Egan TD, Lemmens HJ, Fiset P, Hermann DJ, Muir KT, Stanski DR, Shafer SL. The pharmacokinetics of the new short-acting opioid remifentanyl (GI87084B) in healthy adult male volunteers. *Anesthesiology* 1993;79:881–892. [PubMed: 7902032]
39. Glader, B.; Greer, JP.; Rodgers, GM.; Forster, J. Destruction of erythrocytes. In: Paraskevans, F.; Lukens, JN.; Glader, B., editors. *Wintrobe's clinical hematology*. 11. Lippincot Williams and Wilkins; Philadelphia: 2004.
40. Ruixo, JJ Perez. Optimizing the design of phase I studies of erythropoietin receptor agonist through mechanism-based PK/PD modeling and simulation. Abstracts of the annual meeting of the population approach group in Europe; 2006. p. 15Abstr. 1024 [www.page-meeting.org/?abstract=1024]
41. Perez-Ruixo, JJ.; De Ridder, F.; Kimko, H.; Samtani, M.; Cox, E.; Mohanty, S.; Vermeulen, A. Simulation in clinical drug development. In: Bertau, M.; Mosekilde, E.; Westerhoff, H., editors. *Biosimulation in drug development*. Wiley-VCH; Weinheim: 2007. p. 1-24.

Fig. 1.

Schematic diagram of basic LIDR models that alter the lifespan distribution. The response R is produced at a zero-order rate constant k_{in} . The removal rate is determined by the baseline lifespan T_R and the maximum or minimum lifespan T_D . The drug changes the distribution of cell lifespans between T_R and T_D according to the Hill function characterized by E_{max} and EC_{50} (*open box*). $C(t)$ denotes time-dependent drug concentrations at the effect site

Fig. 2.

Schematic diagram of the PK/PD model of rHuEPO effect on reticulocyte counts in healthy subjects. Processes, variables, and parameters presented in this diagram are explained under Methods

Fig. 3. Simulated profiles of drug plasma concentrations (*upper panel*) and corresponding responses for the LIDR model $T_D > T_R$ (*middle panel*) and model $T_D < T_R$ (*lower panel*) for various doses

Fig. 4. Sensitivity of LIDR models that alter the lifespan distribution to E_{max} (upper panels) and to EC_{50} (lower panels). The response curves were simulated for $Dose = 10^4$

Fig. 5. Sensitivity of LIDR models that alter the lifespan distribution to T_D (upper panels) and to R_0 (lower panels). The response curves were simulated for Dose = 10^4

Fig. 6. Sensitivity of LIDR models that alter the lifespan distribution to T_R . For the responses shown in the lower panels k_{in} was set to 2.083 unit/h and R_0 was calculated according to Eq. 15

Fig. 7. Time courses of the mean lifespans, $MLR(t)$ for LIDR models that alter the lifespan distribution: model $T_D > T_R$ (upper panel) and model $T_D < T_R$ (lower panel)

Fig. 8.

Frequency distributions of estimates of the PD parameters for model $T_D > T_R$. One hundred individual responses were simulated with the residual error model described by Eq. 19. The estimates of EC_{50} , E_{max} , T_D , and T_R obtained from successful minimizations are shown as histograms. The histogram for R_0 is omitted. The value of parameters used for simulations were $Dose = 10^4 \mu g$, $EC_{50} = 100 \text{ ng/ml}$, $E_{max} = 0.8$, $T_D = 72 \text{ h}$, $T_R = 48 \text{ h}$, and $R_0 = 100 \text{ units}$

Fig. 9. Observed (*open symbols*) and model predicted (*solid lines*) mean rHuEPO serum concentrations after subcutaneous administration in healthy subjects

Fig. 10.
Observed (*open symbols*) and model predicted (*solid lines*) mean reticulocyte counts for healthy subjects who received subcutaneous doses of rHuEPO

Fig. 11. Time courses of the model-predicted mean reticulocyte lifespan $ML_{RET}(t)$ in healthy subjects after subcutaneous administration of rHuEPO at indicated doses (kIU)

Table 1
Characteristics of the frequency distributions of PD parameter estimates obtained by fitting model $T_D > T_R$ to 100 individual responses simulated with the 10% coefficient of variation residual error

Parameter	True	Min	Q1	Q2	Q3	Max
EC_{50} (ng/ml)	100	0.62	17.91	98.39	457.91	1000.00
E_{max}	0.8	0.01	0.76	1.00	1.00	1.00
T_D (h)	72	55.27	67.37	72.81	82.23	107.68
T_R (h)	40	1.06	44.33	47.19	49.58	57.71
R_0 (unit)	100	79.81	94.23	97.90	102.13	112.46

The number of successful minimization was 68. Q1, Q2, and Q3 denote first, second, and third quartiles, respectively

Table 2

Estimated values of the PK/PD model parameters of the rHuEPO effect on reticulocyte count

Parameter	Estimate	SE
V/F (L)	20.3	1.71
$V_{max} F$ (IU/h)	3900	963
K_m (IU/L)	3860	1380
k_a (h^{-1})	0.183	0.0224
t_{lag} (h)	1.31	0.216
Fr	0.928	0.0164
C_0 (IU/l)	10.5	0.494
$T_{CFU} + T_{NOR}$ (h)	147	2.71
T_{RET} (h)	41.5	2.87
RET_0 (10^9 cells/l)	76.0	4.92
$k_p S_{max}$ (h^{-1})	0.00247	0.00118
SC_{50} (IU/l)	16.3	1.25
ΔT_D (h)	98.6	5.74
EC_{50} (IU/l)	73.2	23.0