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Pharmacokinetic differentiation of drug candidates using system analysis and physiological-based modelling. Comparison of C.E.R.A. and erythropoietin

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Abstract

Evaluation of the pharmacokinetics (PKs) in a proper physiological context is paramount to elucidate the factors that may improve a drug's PK properties. Using modern system analysis-based physiological modelling principles, this work applies a novel kinetic analysis framework to a PK comparison of two erythropoietically active drugs, C.E.R.A. (continuous erythropoietin receptor activator) and recombinant human erythropoietin (Epo), aimed at elucidating the main factors responsible for the substantial PK differences seen. The evaluation according to the new model is compared with a compartmental model analysis. Sheep (n = 7 for Epo; n = 8 for C.E.R.A.) received intravenous bolus injections of Epo and C.E.R.A. Baseline and 20–30 blood samples per injection were assayed by radioimmunoassay. Fundamental physiologically based PK building block principles were introduced, proceeding to the construction of a general PK model and several sub-models from which a final PK model was selected based on information theoretical principles. The compartmental comparison analysis use a two-compartment model with central Michaelis–Menten elimination. Several lines of evidence support the hypothesis that the desirable slow elimination of C.E.R.A. relative to Epo is mainly caused by a smaller recirculation extraction fraction, which appears more influential on the elimination kinetics than the mean circulation transit time. The compartmental analysis demonstrates large differences in several PK parameters that contribute to C.E.R.A.'s slower elimination, consistent with the recirculation model analysis. It is hypothesized that C.E.R.A.'s smaller recirculatory extraction fraction is due to a reduced receptor-mediated elimination, consistent with in-vitro measurements where C.E.R.A. shows Epo-receptor binding with a lower association constant and a larger dissociation constant.

Introduction

Differences in the pharmacokinetics (PKs) of a competing drug with similar pharmacology can make a significant therapeutic difference and can result in simpler, more practical drug dosing. Thus, the PK properties are of central importance in drug design. It is important to evaluate such PK differences in a reliable and physiologically meaningful manner in order to gain better insight into the important factors useful for differentiating the kinetics and improving the PK properties.

Traditionally, PK analysis in this context has largely been done using classical compartmental principles and simple non-compartmental analysis. The compartmental approach to a large extent uses a mathematical abstraction that can be difficult to justify in a physiological context and also makes interpretation of structure-dependent parameters troublesome. The non-compartmental approach to a large extent avoids such abstractions, but suffers from being short on structure and thus limits the depth to which the kinetics can be differentiated.

Physiologically based PK modelling was recognized early on as an important modelling paradigm (Bischoff 1980, 1986). Application of such models to endogenous drug substances is complicated by confounding factors such as saturable enzyme biotransformation, receptor-mediated elimination, endogenous synthesis, feedback processes and presence of baseline concentration (Marzo & Rescigno 1993). Early attempts in this area were quite ambitious in

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trying to consider the exceedingly many physiological factors (e.g. blood flow, organ size, tissue partition coefficients). This largely resulted in models that, unfortunately, were of more theoretical than practical interest. Other attempts recognized this limitation of the highly structured physiological models and introduced simplifying system analysis principles (e.g. convolution, deconvolution, transit time density functions) to reduce the complexity of the analysis, while still providing a rational connection to the physiology (Cutler 1979; Verotta et al 1989; Weiss et al 2007).

The present work builds on these modern principles, motivated by the need to analyse and compare the PKs of two biotechnology-produced drugs, namely recombinant human erythropoietin (rHuEpo) and a new drug candidate C.E.R.A. (continuous erythropoietin receptor activator), which is produced by chemical synthesis and differs from Epo by the formation of a chemical bond between an amino group present in erythropoietin and methoxy polyethylene glycol butanoic acid (Brandt et al 2006). The approach presented demonstrates the use of simple system analysis tools for structuring a general physiological, system analysis based recirculation type PK model. This model is the source for the formation of sub-models that are then compared. The final model used in the PK comparison of the two drugs is selected from information theoretical considerations and strikes a compromise between complexity and analysis practicality.

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' (NIH publication #85-23, revised in 1985). Seven healthy young adult sheep, 2–4 months old, 25.0 kg (14%) (mean, coefficient of variation), were selected for the Epo experiments, and eight sheep, 2–4 months old, 29.7 kg (8.70%), were used for the C.E.R.A. experiments. The animals were housed in an indoor, light- and temperature-controlled environment, with free access to feed and water. Before the start of the study, jugular venous catheters were aseptically placed under pentobarbital anaesthesia. Intravenous ampicillin (1 g) was administered daily for 3 days following catheter placement.

Study protocol

Blood samples (~0.5 mL per sample) for plasma Epo and plasma C.E.R.A. were collected before intravenous bolus dosing to determine baseline values. Blood samples were collected subsequent to a single or dual intravenous bolus dosing. Some 22 samples were collected over an 8-h period for Epo. Some 30 samples were collected over a 30-h period for C.E.R.A. following a single intravenous bolus administration. A longer sampling period for C.E.R.A. was required due to the slower elimination of C.E.R.A. from the plasma. In the C.E.R.A. low-dose/high-dose double intravenous dosings, about 30 samples were collected over the first 30 h, and about 36 samples were collected over ~40 h following the second dosing, which was given 190 h following the first dose. To minimize haemoglobin and red blood cell loss due to frequent sampling, blood was centrifuged, the plasma removed, and the red blood cells re-infused. The doses given are summarized in Table 1.

Sample analysis

Plasma Epo concentrations were measured in triplicate using a double antibody radioimmunoassay procedure as previously described by Widness et al (1992) (lower limit of quantitation 1 mU mL^{-1}). All samples from the same animal were measured in the same assay to reduce variability.

C.E.R.A. was provided as 5.9 mg of protein mL^{-1} solution (lot no. R78238600; Hoffmann-LaRoche Inc. Nutley, New Jersey, USA) and 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^{-1}. Preparation and analysis of the C.E.R.A. plasma, standard curve, non-specific binding, and zero standard samples was identical to the unlabelled Epo determination. The C.E.R.A. plasma concentrations were determined using the rHuEpo standard curve between the rHuEpo EC80 and EC20 as mU Epo equivalents mL^{-1}. The rHuEpo standard curve was used instead of the C.E.R.A. standard curve for convenience and our extensive experience with the Epo radioimmunoassay.

The use of the rHuEpo standard curve to measure C.E.R.A. was validated by performing 1:2 dilutions of the C.E.R.A. stock solution until the response was between the EC80 and EC20 on the rHuEpo standard curve. The dilution corrected responses were determined and had a coefficient of variation of 8.3% across the linear range of the rHuEpo standard curve, demonstrating a 1-to-1 relationship between the determined mU Epo equivalents mL^{-1} and the ng mL^{-1} of C.E.R.A. within this range. These validations resulted in a C.E.R.A. conversion constant of 71 300 mU (μg of protein)$^{-1}$ (n = 13). All C.E.R.A. samples were measured in duplicate or triplicate and diluted between the EC80 and EC20 on the rHuEpo standard curve, which corresponds to a linear C.E.R.A. range of 65.5–647 pg of protein mL^{-1}. The unit conversions used were: Epo and C.E.R.A. molecular weight (protein only): 18 236 Da; Epo and C.E.R.A.: 54.5 pmol (μg of protein)$^{-1}$; Epo: 1 mU mL^{-1} = 0.305 pmol; C.E.R.A.: 1 mU mL^{-1} = 0.769 pmol.

Computer analysis

All kinetic analysis and modelling was conducted using WINFUNFIT, an interactive Microsoft Windows program evolved from the general nonlinear regression program FUNFIT (Veng-Pedersen 1977).

Statistical analysis

The Epo versus C.E.R.A. comparisons of population mean values for the PK parameters (Table 1) were done using a one-tailed, equal or non-equal variance Student's *t*-test, with variance testing done by an *F*-test using Microsoft Excel

Table 1 Pharmacokinetic parameters determined for C.E.R.A. and erythropoeitin according to the recirculation model (Eqns 1–7)

		Dose 1	Dose 2	Disposition mean residence time (b)	Terminal half-life	Apparent steady state volume	Residence volume	Linear clearance[b]	Linear elimination rate parameter	Nonlinear elimination parameters		Nonlinearity index	Peak recirculation time	Body-weight	Correlation coefficient
		D_1 (pmol kg^{-1})	D_2 (pmol kg^{-1})	MRT (h)	t½ (h)	V_{ss} (mL kg^{-1})	V_{res} (mL kg^{-1})	CL (mL h kg^{-1})	K_L (1/h)	E_0	K_N (pmol h^{-1})		t_{peak} (s)	BW (kg)	r
C.E.R.A.[a] (n = 8)	Mean	52.9	91.5	771	27.1	71.3	67.3	1.87	0.0264	0.0464	844	1.38	4.26	29.7	0.989
	CV (%)	73.0	8.82	32.5	16.9	19.4	21.1	21.8	18.2	14.2	24.7	72.0	40.7	8.70	0.611
Erythropoeitin (n = 7)	Mean	28.6	56.3	4.91	2.55	239	132	73.0	0.310	0.688	98.4	0.0755	4.98	25.0	0.997
	CV (%)	14.0	10.1	32.1	38.7	38.7	26.8	47.9	28.0	38.3	48.9	51.9	29.1	14.0	0.147
	t-test			$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS	NS	$P < 0.01$
	Ratio[c]			157	10.6	3.35	1.96	39.0	11.7	14.8	8.58	18.3	1.17		

CV, coefficient of variation. NS, non significant ($P > 0.05$). [a]Three animals given C.E.R.A. were given two intravenous bolus doses: D1 = 8.36 (3.97) pmol kg^{-1} and D2 = 91.5 (8.82) pmol kg^{-1} (mean, CV%) and the fitting was simultaneously done to the data from two administrations. [b]Value when kinetics is operating in the linear range. [c]Relative magnitude of the mean C.E.R.A. and erythropoeitin parameter values arranged as max value/min value.

2002 SP3 (Microsoft Corporation, Redmond, WA, USA). Statistically significant levels were at the 0.05 and 0.01 level for the type 1 null hypotheses error.

Pharmacokinetic analysis

Recirculation model analysis

The pharmacokinetic analysis was done using a recirculation model based on systems analysis (Figure 1E). The model employs heterogeneous, non-compartmental flow-through systems (FTS) as building blocks. The FTS encapsulate stochastic drug transport through the FTS via blood flow and other mass transport (e.g. diffusion) in a linear convolution context. Nonlinear disposition is considered by Front-end/Back-end (FEBE) nonlinear elimination components (modules N; Figure 1E) that bracket the linear FTS to account for a nonlinear extraction from the heterogeneous FTS. The synthesis of the proposed recirculation model and the derivations of model equations are presented in Appendix A.

The recirculation model (Figure 1E) is described by the following key differential equations, which were used in the model fittings (Figures 2 and 3) and parameter estimation (Table 1):

$$dy_1/dt = N(E_0, K_N, (f_{in}(t) + N(E_0, K_N, f_{out1}) + N(0, f_{out2}))/2) - \alpha y_1 \quad (1)$$

$$df_{out1}/dt = \alpha\beta y_1 - \beta f_{out1} \quad (2)$$

$$dy_2/dt = N(0, (f_{in}(t) + N(E_0, K_N, f_{out1}) + N(0, f_{out2}))/2) - \alpha y_2 \quad (3)$$

$$df_{out2}/dt = \alpha\beta y_2 - \beta f_{out2} \quad (4)$$

$$c_{out}(t) = K_{out}(f_{out1} + f_{out2}) \quad (5)$$

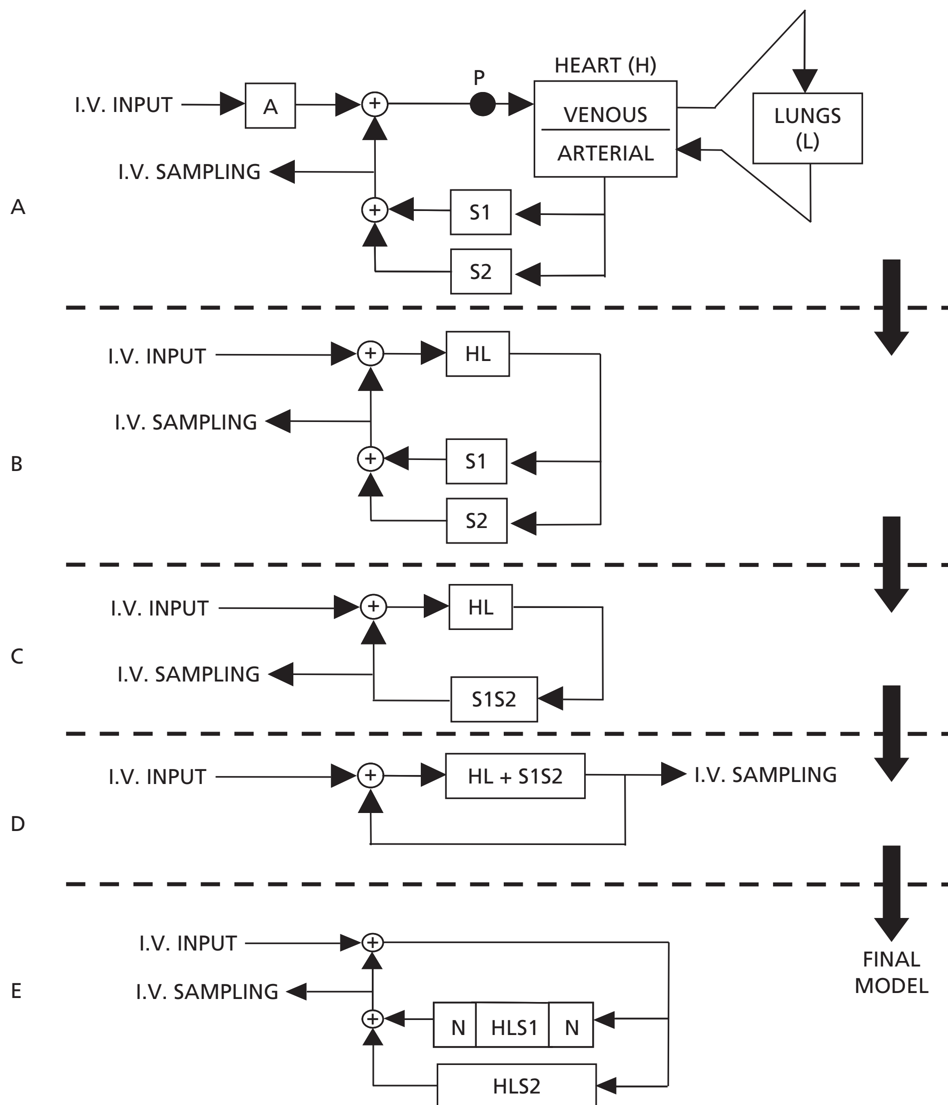

Figure 1 Schematics of synthesis of the proposed recirculation model.

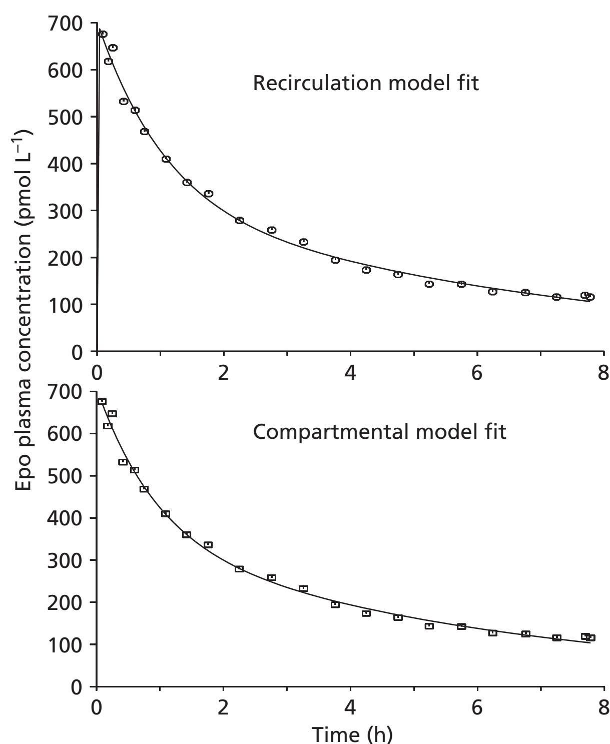

Figure 2 Representative plots of the pharmacokinetic recirculation model and compartmental model fits to plasma concentration data from an intravenous bolus dose of erythropoeitin (Epo).

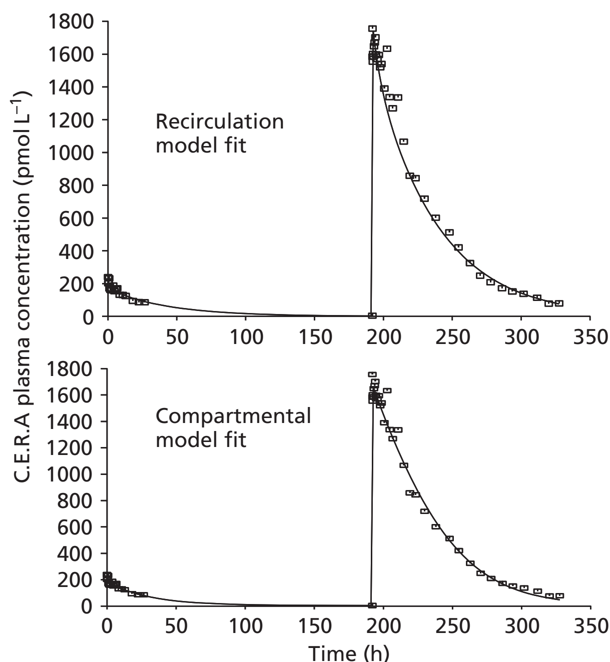

Figure 3 Representative plots of pharmacokinetic recirculation model and compartmental model fits to plasma concentration data from a low and high intravenous bolus dose of C.E.R.A.

The "template" function N() above is introduced for convenience to simplify the mathematical notation:

$$N(x_1, x_2, x_3) = (1 - x_1 x_2 / (x_2 + x_3)) x_3 \tag{6}$$

$$N(x_1, x_2) = (1 - x_1) x_2 \tag{7}$$

The model has only five parameters, namely E_0, K_N, α, β and K_{out}. The E_0 and K_N parameters deal with the elimination kinetics according to the FEBE elimination model, while the α and β parameters are parameters for the FTS. The K_{out} parameter converts from rate to measured drug concentration c_{out} (Eqn 5). The specific details of the above model equations are given in Appendix A.

Recirculation model development

The full model (Figure 1B) (Eqns 27A–36A) in addition to several simplifying sub-models were analysed for their agreement with the observed data by fitting the numerical solution of the associated ordinary differential equations (ODEs) to the data. The fairly comprehensive structure of the full model allowed combinatorially for a large number of sub-models to be considered. In the preliminary analysis, the selection for testing of these sub-models were not totally comprehensive but largely driven by intuition, trial and error, and time constraints. First, a sub-model was excluded if it showed an obvious lack of fit to the data. Second, if the fit was acceptable, the exclusion was done on the basis of information theoretical considerations, that is by comparing the Akaike information criteria (AIC) of the competing sub-models. In this preliminary analysis, it became evident that there was no need for both pathways to be nonlinear (as is the case in the full model in Figure 1B) to account for the known disposition nonlinearity that we identified in previous studies. Thus, the nonlinearity was assigned to a single pathway (Figure 1E). Comparison of a Front end, Back end and both Front end and Back end (FEBE) showed that the FEBE structure was to be favoured (AIC) if FE and BE shared the same two parameters. Sharing the parameters reduced the total number of parameters by two, with little change in the goodness-of-fit. Another somewhat surprising finding in the search for the optimal model was that sharing the parameters for the transit time functions for the two pathways (which resulted in two additional parameters being eliminated) resulted in a more favourable model (AIC). These considerations lead to the final model described by Equations 1–7 and diagrammatically illustrated in Figure 1E. The parameters shown in Table 1 are obtained from this model.

Compartmental model analysis

For comparison, and to better discuss the main differences between the current model and the more common compartmental models, the data were also analysed according to a conventional two-compartmental model with a central Michaelis–Menten nonlinear elimination. This model also has five parameters, namely K_M, K_{10} ($K_{10} = V_M/K_M$), K_{12}, K_{21}, and V, simplifying the comparison.

Both models were formulated to account for endogenous drug and assumed a steady state before the exogenous drug administration.

Table 2 Pharmacokinetic parameters determined for C.E.R.A. and erythropoeitin according to the compartmental model

Drug		Disposition mean residence time[b]	Terminal half-life	Steady state volume	Volume of distribution	Linear clearance[b]	Elimination parameters		Correlation coefficient
		MRT (h)	t½ (h)	V_{ss} (mL kg^{-1})	V (mL kg^{-1})	CL (mL h kg^{-1})	K_M (pmol L^{-1})	K_{10} (1/h)	*r*
C.E.R.A.[a] (n = 8)	Mean	28.2	19.5	79.5	65.5	3.60	1330	0.0537	0.971
	CV (%)	44.5	46.2	25.4	24.1	58.9	99.7	53.7	2.3
Erythropoeitin (n = 7)	Mean	2.02	3.01	217	42.2	117	1470	2.90	0.989
	CV (%)	27.6	19.9	25.8	16.5	37.3	20.2	43.2	1.5
	t-test	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS	$P < 0.01$	NS	$P < 0.01$	$P < 0.01$
	Ratio[c]	14.0	6.48	2.73	1.55	32.5	1.10	54.0	

CV, coefficient of variation. NS, non significant ($P > 0.05$). [a]Three animals given C.E.R.A. were given two intravenous bolus doses: D1 = 8.36 (3.97) pmol kg^{-1} and D2 = 91.5 (8.82) pmol kg^{-1} (mean, CV%) and the fitting was simultaneously done to the data from two administrations. [b]Value when kinetics is operating in the linear range. [c]Relative magnitude of the mean C.E.R.A. and erythropoeitin parameter values arranged as max value/min value.

Since the two models contain a number of different structure parameters that cannot be directly compared, the comparison was done in terms of parameters (Tables 1 and 2) that have similar or identical definitions/meanings (Appendix A).

Results and Discussion

C.E.R.A. versus Epo comparison based on the recirculation model

C.E.R.A. showed dramatically different PK parameter values compared with those of Epo (Table 1). The most marked difference was seen for the disposition mean residence time (MRT) parameter where the mean value for C.E.R.A. was more than 100-fold different compared with Epo. The linear clearance was also about 40-fold smaller for C.E.R.A., while the terminal half-life was about 10-fold longer for C.E.R.A. A similar 10-fold difference was seen in the linear elimination rate parameter. The recirculation extraction fraction parameter (E_0) was similarly about 10-fold smaller for C.E.R.A., and the nonlinear elimination parameter (K_N) was also about 10-fold larger for C.E.R.A. The nonlinearity indices for Epo and C.E.R.A. showed an operational nonlinearity that was about 20-fold larger for C.E.R.A. No significant difference was found in the peak recirculation times. The smallest differences were found in the apparent steady state volumes and residence volume terms. The above large-magnitude differences seen between C.E.R.A. and Epo resulted in a high degree of significance ($P < 0.01$) for all parameters except the peak recirculation time. They were all consistent with the overall much slower plasma elimination of C.E.R.A. generally seen in all the C.E.R.A. experiments.

The largest relative difference between C.E.R.A. and Epo was seen in the MRT parameter. This parameter may therefore contain a clue to what factors are the greatest contributors to the substantial differences seen in the PKs between these two compounds. The expression for MRT (Eqn 39A) is made up of two terms, namely the single pass extraction fraction, E, and the mean transit time (MTT). It was observed that E appears in a squared form in the denominator. Thus, changes in E are expected to have a greater effect on the average time that the drug molecules stay in the circulation than changes in MTT. The MRT and the linear elimination rate parameter, K_L, together provide some additional support to the hypothesis that the extraction fraction E is the most important source for the large PK difference. The expression for K_L (Eqn 41A) is simply the ratio between E and MTT and is thus to a smaller extent influenced by a change in E. In comparing the relative difference between the MRT and the K_L parameters for C.E.R.A. and Epo (Table 1), it is seen that the MRT difference is in fact much larger than the K_L difference. This indicates that the PK difference is due more to a difference in the extraction fraction E, than to a difference in MTT, although both parameters appear to be important contributing factors. A third clue that E is the main contributing factor is given by the about 10-fold smaller E_0 value for C.E.R.A. Although this parameter is a part of the nonlinear elimination function (Eqn 21A), the E_0 parameter becomes the extraction fraction, E, for the system when it operates in the linear range.

The second largest difference between C.E.R.A. and Epo was seen in the clearance parameter, CL, the clearance of C. E.R.A. was about 40-fold less than that for Epo (Table 1). The expression for CL (Eqn 40A) shows that this parameter, as expected, depends on the extraction fraction E. Accordingly, the observation of the large difference seen in the clearance values is consistent with the hypothesis that E is the major factor for the PK difference between C.E.R.A. and Epo. The clearance also depends on the K_{out} parameter. Thus, the possibility also exists that the large difference seen in the clearance may be due to the K_{out} parameter. However, this does not seem very likely because of the residence volume parameter V_{res} (Eqn 43A), which differs only little between C.E.R.A. and Epo. In fact, the smallest relative difference (not accounting for the peak circulation time parameter) was seen for the residence volume parameter. A similar only modest difference was seen in the steady state volume of distribution V_{ss}. The expression for V_{ss} (Eqn 42A) shows this parameter to be the ratio between the clearance, CL, and the linear elimination rate parameter, K_L. The large difference in the clearance does not have much of an effect on the steady state volume because the large clearance

difference is to a large extent counteracted by a similar large change in the linear elimination rate parameter (Table 1).

The basic properties associated with a saturable elimination process is another factor that can slow down (in the nonlinear range) the elimination process. This effect is difficult to quantify and compare in simple terms. However, the nonlinearity index (Table 1) provides some crude guidance for assessing this effect. The about 20-fold larger value for this nonlinearity metrics shows that C.E.R.A. exhibits a higher degree of nonlinearity, which may contribute to a slower elimination due to a higher degree of saturation of the elimination mechanism.

No significant difference was seen between C.E.R.A. and Epo in the peak recirculation time parameter t_{peak}. This peak parameter is not very dependent on the drug's MTT, since it is primarily determined by blood flow physiology and little influenced by the drug disposition. The MTT parameter depends to a large extent on the tail end of the transit time distribution and less on the location of the peak of the highly skewed transit time distribution. The high degree of distribution skewness is well recognized in cardiovascular research in dye dilution curves used in blood flow measurements (Fonseca-Costa & Zin 1979; von Spiegeland & Hoeft 1998; Picker et al 2001).

It would have been valuable to individually determine MTT to test the above hypothesis that E contributes more than MTT to the large MRT difference seen. However, such an estimation is complicated by the fact the there exists a confounding interaction between MTT and E in their effect on the drug elimination. This is logically understood since a lengthening of the MTT for a constant E will slow down the elimination process due to the extraction being repeated at a 'slower frequency'. A similar decrease in the elimination can also be achieved by a smaller E. Thus, it is difficult to resolve these two parameters.

To avoid such parameter estimation problems, Table 1 only reports 'robust' parameters. This robustness is consistent with the lack of very excessive coefficient of variation values in the parameter estimation (Table 1) and with the lack of excessive standard errors for the parameters in the fit to individual animal data. Although some coefficient of variation values appear somewhat large in some cases this is to be expected due to subject-to-subject variability often seen in PK analysis.

Extrapolations used in determining disposition function parameters such as volume of distribution and clearance has always been a problem in PK analysis. Specific to compartmental analysis, the extrapolation to $t = 0$ to estimate the initial volume of distribution poses a significant problem. This problem is particularly severe when dealing with drugs with a fast/short initial distribution phase and with the often poor initial sampling. Very significant errors may be introduced in such cases not only in the estimation of the initial volume of distribution but also in the estimation of the total area under the curve used in determining drug clearance.

The physiological approach, taken in this work, does not have this problem because a recirculation system does not have an initial volume of distribution. Also, while the compartmental approach extrapolates to an unknown and highly non-physiological $t = 0$ point, the physiological recirculation approach extrapolates to a known and physiologically exact point ($c = 0$ at $t = 0$ i.v. bolus case). (Resorting to a constant i.v. infusion in the compartmental approach does not solve this problem since the determination of the initial slope required by this approach is very inaccurate). As discussed above, the recirculation approach does also have volume of distribution terms, but these are defined from the total curve and are not based on error prone, one-point estimations and extrapolations.

The known $t = 0$ extrapolation constraint in recirculation-based physiological modelling generally creates a better estimation of the disposition function. This constrain facilitates a more consistent parameter estimation. This may explain the quite small variability in the parameters seen in this study.

C.E.R.A. versus Epo comparison based on compartmental model

Similar to the recirculation analysis, C.E.R.A. had a substantially larger MRT than Epo, although not as pronounced as for the recirculation model (Tables 1 and 2). The large difference in the MRT value for C.E.R.A. evaluated by the two models is discussed (recirculation versus compartmental model evaluation).

As before, C.E.R.A. had a larger terminal half-life (t½). The relative magnitude for C.E.R.A./Epo t½ (6.48) was similar to that obtained by the recirculation model (10.6).

Consistent with the recirculation analysis, the apparent steady state volume, V_{ss}, for C.E.R.A. was smaller than for Epo and the relative sizes were similar between the two model evaluations (2.73 and 3.35 for compartmental modes vs recirculation model, respectively).

The volume of distribution, V, was not significantly different between C.E.R.A. and Epo and was similar to the plasma volume.

As for the recirculation model, the linear clearance, CL, for C.E.R.A. was substantially less than that of Epo with a comparable relative magnitude difference (32.5 and 39.0 for the compartmental vs recirculation model evaluations, respectively).

While the elimination parameter K_M did not appear to differ significantly between C.E.R.A. and Epo, the K_{10} parameter was much smaller (Table 1) resulting in a significantly slower elimination of C.E.R.A.

Overall, the analysis by the compartmental model was consistent with the analysis using the recirculation model, with C.E.R.A. versus Epo parameter differences of the same order of magnitude (Tables 1 and 2).

Recirculation versus compartmental model evaluation

Judged on the basis of the AIC, the recirculation model appears as the preferred model. The recirculation model produces a more favourable AIC in the majority of cases (13 out of 15). In addition to AIC, it is valuable to consider the interpretation and physiological relevance of the model. The recirculation model is a physiologically more relevant model. It avoids the mathematical abstraction of homogenous compartments and the need for considering first-order transfer

between such compartments. Instead, the recirculation model approaches the kinetics in a more general way with a foundation (linear disposition case) based on generalized stochastic independent molecular disposition principles, which include diffusion, flow and biotransformation that lead to superposition and simple convolution/linear operator relationships. Furthermore, the recirculation model is formulated in a physiological sound structural context based on cardiovascular recirculation principles.

Parameter comparisons

Direct comparison of parameters from the two modelling paradigms is complicated by a fundamentally different structure in the two models. This difficulty is mainly due to the fact that the recirculation model is not employing homogenous compartments. Accordingly, the (initial) volume of distribution, V, is specific to the compartmental model. Back-extrapolation to t = 0 from an intravenous bolus injection in the recirculation model gives a zero concentration, not allowing an initial volume of distribution to be defined. Although the recirculation model does not have an (initial) volume of distribution, V, it appears that the residence volume, V_{res}, defined by Equation 43A comes close to the compartmental values for V (Tables 1 and 2). In fact, for C.E.R.A. V_{res} is not significantly different from V and the two volume terms are quite close for Epo (Tables 1 and 2). More importantly, the parameter ratios for the two drugs (1.96 vs 1.55 recirculation vs compartmental) are quite close. Accordingly, we propose that V_{res} may be used as a substitute for the volume of distribution when comparing the distribution of drugs using a recirculation model.

Determination of the volume of distribution at steady state, V_{ss}, is similarly complicated. This is due to the fact that the elimination in the recirculation model is based on probability of elimination (E) and not simple first-order principles. In the compartmental case it is possible to describe the rate of elimination at any given time and thus by simple mass balance principles determine the amount of drug in the body, including at steady state, which when related to the steady state concentration enables evaluation of V_{ss}. In contrast, the elimination model in the recirculation model, due to its greater generality, does not consider the instantaneous rate of elimination, only the probability of elimination from a FTS (linear case); therefore, the exact rate of elimination is unknown and does not enable an exact mass balance and V_{ss} evaluation. Nevertheless, the recirculation model appears to provide some approximation to V_{ss} by the Equation 42A. Thus, heuristically, we propose to use the notation ‘apparent’ steady state volume (Table 1) as an approximation to the unknown true V_{ss} when the evaluation is done according to Equation 42A. The compartmental V_{ss} and the apparent V_{ss} values (Tables 1 and 2) are quite similar with no statistically significant difference ($P > 0.05$).

Comparisons of the disposition mean residence time (MRT) between the two models is similarly complicated by the fundamentally different structure and drug elimination assumptions in the compartmental and recirculation models. The compartmental model calculation of MRT according to the well known moment principle AUMC/AUC (Veng-Pedersen 1989a, b) assumes that all elimination is from the central sampling compartment and is first order. In contrast, the recirculation MRT calculation is based on the MTT though the system and the mathematical expectation (mean) of the number of times a drug molecule goes through the system before being eliminated (Eqns 3A, 39A). Accordingly, it is not surprising to find differences between the compartmental- and recirculation-based estimates of the MRT parameter (Tables 1 and 2). The question of which MRT estimate is closest to the true in-vivo value is difficult to answer. However, in recognizing that the compartmental model is more of an abstraction of the physiological system employing more specific (less general) and stronger assumptions than the recirculation model, we propose that the recirculation estimates are better estimates. The difference in the MRT evaluations results in a substantial difference in the C.E.R.A./Epo MRT ratios between the two model evaluations. The ratio is about 10-times larger when evaluated by the recirculation model (Tables 1 and 2). The different MRT evaluation by the recirculation model may also explain the large MRT mean value of 771 h for C.E.R.A., which appears large relative to the mean value for the half-life of 27.1 h.

As expected, comparison of the non-compartmental, ‘model independent’ parameters t½ and CL posed no problem. There were no significant differences between the two models in these parameters ($P < 0.05$) as well as the parameter ratio for the two drugs when evaluated by the two models (Tables 1 and 2).

Extension of the model to consider other input

Although the current analysis was based on an intravenous bolus dosing, the same analysis can readily be extended to consider any parametric or nonparametric absorption model by simply adding the absorption rate, $f_{abs}(t)$ to $f_{in}(t)$ in Equation 1.

Conclusion

Based on the analysis using the recirculation model, several lines of evidence support the hypothesis that the desirable slow elimination of C.E.R.A. relative to Epo is mainly caused by a smaller recirculation extraction fraction, E. The mathematical analysis indicates that E is more influential in its effect on the elimination kinetics than the MTT.

Our previous work with Epo and current preliminary work with C.E.R.A. indicate that both are to a large extent eliminated via a receptor-based mechanism. The reason for C.E.R.A.’s apparent smaller extraction fraction is not clear. However, based on our previous work with Epo we hypothesize that the smaller extraction fraction, E, is due to reduced receptor-mediated elimination, consistent with the work of Jarch et al (2008) who determined a smaller Epo receptor binding association constant and a slightly larger dissociation constant for C.E.R.A. relative to Epo.

The compartmental comparison of C.E.R.A. and Epo, although being less specific about the mechanism for the large differences in the elimination kinetics between C.E.R.A. and Epo, is consistent with the recirculation model-based analysis in clearly showing similar large differences between the two drugs in the disposition mean residence time, terminal half-life and clearance parameters.

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Appendix A

Physiological, system analysis-based PK modelling framework

Figure 1A illustrates the basic structure of the system analysis-based model considered in this work. This structure differs in several ways from the very common compartmental structure seen in PK modelling. The basic building block of compartmental systems is a homogenous compartment with transfer rates (intercompartmental or elimination) given by an autonomic (non-memory) function of the amount or concentration of the drug in the compartment, for example first-order transfer/elimination and Michaelis–Menten elimination.

Open-loop behaviour of flow-through systems

In contrast, the system analysis-based recirculatory system considered in this work is based on a more general building block (see Figure 1), namely a flow-through system (FTS), where the drug is dispersed in a non-homogenous way in the FTS due to the heterogeneous cardiovascular network and the heterogeneous nature of the tissues that make up the FTS. Flow is generally considered as mass transport of drug due to fluid flow and/or other processes, for example diffusion. The rate of transfer of drug out from the FTS is a memory-dependent process, since it depends on the history of the drug input to the FTS. Furthermore, linearity for the basic FTS unit is defined in a more general way by a convolution relationship between the rate of drug input to the FTS, $f_{in}(t)$, and the rate of drug output from the FTS, $f_{out}(t)$:

$$f_{out}(t) = \int_{-\infty}^{t} UR(u) f_{in}(t-u) du \equiv UR(t) \times f_{in}(t) \qquad (1A)$$

The unit response UR(t) of a FTS (Eqn 1A) defines the basic disposition kinetics of the FTS unit. The unit response function UR(t) encapsulates the two essential fundamental disposition components of the FTS, namely distribution and elimination. This linear drug disposition of a FTS has its origin at the molecular level in terms of a stochastic independent transport/elimination of the drug molecules (Cutler 1978; Veng-Pedersen 1988a, b, c, d, 1991, 2001). The UR(t) may be broken down into two components: (i) an extraction fraction, E, which represents the probability that a drug molecule that enters the FTS will be eliminated (i.e. does not appear as part of the output); and (ii) the transit time density function (p.d.f.), g(t), which represents the distribution component:

$$UR(t) = (1 - E)\ g(t) \qquad (2A)$$

The transit time density function, g(t), provides the mean transit time (MTT):

$$MTT = \int_{0}^{\infty} t g(t) dt \qquad (3A)$$

Accordingly, if the MTT is large, it means that the affinity of the drug to stay in the FTS is large. The degree of accumulation of drug in the FTS is therefore related to the MTT, which depends only on g(t). Thus, g(t) determines the distribution or drug affinity properties for the FTS.

The body may be considered kinetically as being composed of a number of FTS connected partly in series and partly parallel in such a way that the transfer of drug and other blood-borne substances can be recycled through the system. Mathematically, such systems can be suitably treated in a two-step derivation, by first deriving the open-loop characteristics of the system and then closing the loop.

In order to define the open-loop system one needs to decide on a suitable breaking point for breaking the closed loop and creating an open-loop system. For example, in Figure 1A, the point 'P' represents such a point.

Connection of FTS

Getting the open-loop input–output characteristics of multiple-connected FTS is facilitated by the following simple relationships.

Serial connection

$$UR_{12}(t) = UR_1(t) \times UR_2(t) = (1 - E_{12})\ g_{12}(t) \qquad (4A)$$

where:

$$g_{12}(t) = g_1(t) \times g_2(t) \qquad (5A)$$

$$E_{12} = E_1 + E_2 - E_1E_2 \qquad (6A)$$

Thus, two FTS connected serially can be 'lumped together' and treated exactly as a single FTS.

Parallel connection

If the fraction of the input $f_{in}(t)$ that goes to FTS_1 and FTS_2 is w_1 and $w_2 = 1 - w_1$ respectively, then:

$$UR_{12}(t) = w_1 UR_1(t) + w_2 UR_2(t) = (1 - E_{12})\ g_{12}(t) \qquad (7A)$$

where:

$$g_{12}(t) = w_1 g_1(t) + w_2 g_2(t) \qquad (8A)$$

$$E_{12} = w_1 E_1 + w_2 E_2 \qquad (9A)$$

Thus, irrespectively of the complexity of the connections of the individual FTS, an open-loop system can be reduced or 'lumped together' exactly to form a single FTS with a property given by Equations 1A and 2A.

Closing the loop

Closing the loop of the single FTS system results in an output from the FTS that contains recycled drug due to the additive feed-back on the input. The result is an output, $f_{out}(t)$, which is consistently larger than seen for the open-loop system. The input and output for a closed-loop system is similarly to the open-loop system related by a convolution expression:

$$f_{out}(t) = UR_c(t) \times f_{in}(t) \qquad (10A)$$

Using the Laplace transform, it is readily shown that the closed-loop unit response function $UR_c(t)$ is simply related to the open-loop unit response UR(t) functions by:

$$UR_C(t) = \phi(UR(t)) \quad (11A)$$

where $\phi()$ is a 'loop-closing operator' defined as:

$$\phi(x) = L^{-1}(L(x)/(1 - L(x))) \quad (12A)$$

where $L^{-1}()$ and L() denote the inverse Laplace and Laplace transform operator, respectively.

Loop 'break point' and point of sampling

Closing the loop provides the output rate $f_{out}(t)$ according to the above Equation 10A. If the point of sampling is not directly from the output site, then the rate must be propagated to the point of sampling, which may involve taking into account the effect of FTS that the drug must go through to get to the sampling point. However, if the point where the closed loop is broken to create the open-loop analysis is appropriately chosen relative to the point of drug sampling. then such propagation is not necessary and the analysis is simplified. The break point 'P' in Figure 1A is appropriately chosen in this way.

Assembling the system

The basic structure of the system considered in this work is summarized in Figure 1A. The intravenous input of the drug goes through the FTS (A) to reach the break point (P). From P, the drug goes through the heart (H) and lungs (L). Subsequently, the drug in the arterial blood is pumped out in the body and is considered to go through two parallel FTS, denoted S1 and S2. Venous sampling of drug is done from the combined output of S1 and S2. The combined output from S1 and S2 reaches the breakpoint P after it is augmented by the original input from the external intravenous input. The choice of the two parallel FTS S1 and S2 in the proposed model is related to the specific properties of the drugs considered here and the specific focus of the present PK analysis. Other FTS configurations are possible and may be more appropriate for other drugs. However, the same basic principles presented in this work are readily applied to other recirculatory systems.

To get the overall recirculatory behaviour of the system (Figure 1A) the above 'simplifying FTS lumping rules' were applied repeatedly as illustrated in the model contruct conversions A → B → C → D (Figure 1). First the heart–lung system is lumped into a FTS denoted HL. Next, the S1 and S2 FTS are lumped into a FTS denoted S1S2. Finally, the HL and S1S2 FTS are lumped into a single FTS denoted HL+S1S2. The transfer of drug from the venous input site through the FTS system denoted A to get to P is considered very rapid relative to other transfer rates. Accordingly, $UR_A(t)$ is approximated as a Dirac delta function and effectively eliminated from the model.

Using the above lumping rules (Eqns 4A–9A) and the specific break point P the combined rate of output from S1 and S2 is related to the system input rate by the following convolution:

$$f_{out}(t) = UR_C(t) \times f_{in}(t) \quad (13A)$$

where:

$$UR_c(t) = \phi(UR_{HL+S1S2}(t)) \quad (14A)$$

$$UR_{HL+S1S2}(t) = UR_{HL}(t) \times UR_{S1S2}(t) \quad (15A)$$

$$UR_{S1S2}(t) = w_1 UR_{S1}(t) + w_2 UR_{S2}(t) \quad (16A)$$

where $0 < w_1 < 1$ and $w_2 = 1 - w_1$ are the fractions split of the output from the HL FTS as input to S1 and S2 (Figure 1B). The concentration of drug at the sampling site, $c_{out}(t)$, is given by:

$$c_{out}(t) = K_{out} f_{out}(t) \quad (17A)$$

where K_{out} is a rate-to-concentration conversion factor, which depends on the cardiac output.

Model simplification to overcome an identification problem

An analysis of the expression for $c_{out}(t)$ above reveals that $UR_{HL}(t)$ is not identifiable. The reason for this is related to the fact that both input and sampling are done on the venous side of the recirculatory system. To overcome this problem $UR_{HL}(t)$ is removed from the model estimation by lumping it together with $UR_{S1}(t)$ and $UR_{S2}(t)$ to form two new URs denoted $UR_{HLS1}(t)$ and $UR_{HLS2}(t)$:

$$UR_{HLS1}(t) \equiv UR_{HL}(t) \times UR_{S1}(t) \quad (18A)$$

$$UR_{HLS2}(t) \equiv UR_{HL}(t) \times UR_{S2}(t) \quad (19A)$$

Accordingly, this results in the following convolution expression for the measured response, $c_{out}(t)$:

$$c_{out}(t) = K_{out}\phi(w_1 UR_{HLS1}(t) + w_2\, UR_{HLS2}(t)) \times f_{in}(t) \quad (20A)$$

Equation 20A is the key equation that provides the simplified general linear systems analysis solution to the model structure shown in Figure 1A.

The next section extends this linear recirculation model (Eqn 20A) to consider nonlinearity in the kinetics and arrive at a similar nonlinear system analysis model.

Inclusion of nonlinearity in the heterogenous environment

Inclusion of nonlinearity in a recirculatory system can be done in basically two ways: externally and internally to the FTS that makes up the system. To include nonlinearity internally to a FTS requires assumptions of the specific structure of the FTS. Such structure assumptions may be difficult to justify and will be a departure from the system analysis principle that this work subscribes to of keeping

assumptions to a minimum, while mathematically still accounting for the functional kinetic behaviour, with due regard to the physiology of the system.

The alternative of applying nonlinearity externally to the FTS provides the opportunity to simply apply the many nonlinearity relationships that have been proposed in the compartmental setting, for example the Hill equation for nonlinear elimination. However, this external approach may not be justified if it is known that the nonlinearity resides in several tissues.

The FEBE nonlinear elimination model proposed in this work and described below is a compromise between an internal and external nonlinearity model.

FEBE nonlinear elimination model

If extraction of a drug is occurring within a tissue and this extraction is associated with a nonlinear saturable process then the extraction will depend on the rate of transit of drug through the tissue in such a way that higher rates of transits will result in a smaller fraction of the drug being extracted. Thus, for a constant volumetric blood perfusion rate, an increased drug level in the blood will result in a reduced extraction fraction (i.e. more drug passing through unchanged). As the drug increases toward the saturation level the extraction fraction will approach zero.

However, for drugs that are not very extensively extracted by the tissue/organ in the FTS, it is to be expected that as the drug level decreases toward zero the extraction fraction will not approach a value of one. Instead it will approach a fixed value, due to finite transit time through the FTS that provides insufficient time for complete elimination.

In the perfusion of a drug eliminating tissue there may be a concentration gradient of the drug from the input side to the output side of the tissue. Accordingly, to consider this complex system in a simplifying way, it is valuable to deal with a net eliminating concentration, being some average over this concentration drop. Translated to drug perfusion rate (amount/time) the net eliminating drug perfusion rate may be considered as some average over the rate drop between the input rate $f_{in}(t)$ and the output rate $f_{out}(t)$.

To be consistent with this simplifying principle, the following nonlinear model (denoted N), Equation 21A, is applied on the input side (Front end) and the output side (Back end) of the FTS:

$$f_{out}(t) = (1 - E_0 K_N/(K_N + f_{in}(t)))\ f_{in}(t) \equiv N(E_0, K_N, f_{in}) \tag{21A}$$

The pre- and post-FTS nonlinear elimination module denoted N in Figure 1E share the same parameters (E_0, K_N) for the given FTS (other combinations were tried as discussed below). The E_0 in Equation 21A is an extraction parameter that determines the magnitude of the extraction, while K_N is the nonlinear parameter that determines how much of this extraction is active relative to the input rate. The extraction goes from zero to a maximum of E_0 as K_N goes from zero to infinity or equivalently as $f_{in}(t)$ becomes increasingly smaller than K_N. Also, extraction goes towards zero as $f_{in}(t)$ goes toward infinity. Furthermore, the relationship and the whole system becomes linear when $f_{out}(t) = (1 - E_0)\ f_{in}(t)$, which occurs when the system operates in the so-called linear range, that is when $f_{in}(t) << K_N$. Thus, it is appropriate to call E_0 the linear extraction fraction corresponding to the linear form of Equation 21A:

$$f_{out}(t) = (1 - E_0)\ f_{in}(t) \equiv N(E_0, f_{in}) \tag{22A}$$

Transit time density model and drug extraction

The transit time distribution (p.d.f) of the linear component of the FTS was empirically modelled as a biexponential:

$$g(t) = \alpha\beta(\exp(-\alpha t) - \exp(-\beta t))/(\beta - \alpha) \equiv g(\alpha, \beta, t) \tag{23A}$$

while the extraction of the drug (Eqn 21A) was delegated to the pre- and post- nonlinear elimination modules 'N'. The input and output from the linear component of the FTS are given by:

$$f_{out}(t) = [\alpha\beta(exp(-\alpha t) - exp(-\beta t))/(\beta - \alpha)] \times f_{in}(t) \tag{24A}$$

The latter equation can be converted to ODE form by the following equations, subject to appropriate initial steady state conditions:

$$dy_1/dt = f_{in}(t) - \alpha y_1 \tag{25A}$$

$$df_{out}/dt = \alpha\beta y_1 - \beta f_{out} \tag{26A}$$

Model selection

A number of different recirculation models based on one FTS or two parallel FTS with and without FEBE nonlinearity modules, and with the two FSE sharing or not sharing the same disposition parameters were fitted to the C.E.R.A. and Epo data. The final model structure arrived at based on the Akaike information criteria was a model (Figure 1E, Table 1) consisting of two parallel, one linear and one nonlinear FTS sharing the same α and β in the linear component (HLS1 and HLS2; Figure 1E) of the FTS. The open-loop ODEs for this model are:

$$dy_1/dt = N(E_0, K_N, f_{in}(t)/2) - \alpha y_1 \tag{27A}$$

$$df_{out1}/dt = \alpha\beta y_1 - \beta f_{out1} \tag{28A}$$

$$dy_2/dt = N(0, f_{in}(t)/2) - \alpha y_2 \tag{29A}$$

$$df_{out2}/dt = \alpha\beta y_2 - \beta f_{out2} \tag{30A}$$

$$f_{out} = N(E_0, K_N, f_{out1}) + N(0, f_{out2}) \tag{31A}$$

which results in the following closed-loop final equations for the model (Figure 1E):

$$dy_1/dt = N(E_0, K_N, (f_{in}(t) + N(E_0, K_N, f_{out1}) + N(0, f_{out2}))/2) - \alpha y_1 \quad (32A)$$

$$df_{out1}/dt = \alpha\beta y_1 - \beta f_{out1} \quad (33A)$$

$$dy_2/dt = N(0, (f_{in}(t) + N(E_0, K_N, f_{out1}) + N(0, f_{out2}))/2) - \alpha y_2 \quad (34A)$$

$$df_{out2}/dt = \alpha\beta y_2 - \beta f_{out2} \quad (35A)$$

$$c_{out}(t) = K_{out}(f_{out1} + f_{out2}) \quad (36A)$$

Initial conditions, steady state and endogenous drug

Epo is produced as a hormone and is present in the body when the exogenous form (rHuEpo) is administered. Since the assay does not differentiate between the exogenous and endogenous form, it is necessary to include the endogenous kinetics in the analysis, that is to consider the total concentration of drug because that is what is being measured. Accordingly, the basal (endogenous) concentration of drug, c_{ss}, which is considered produced by a steady state production, is used to define the steady state exogenous drug production, f_{ss}

$$f_{in}(t) = f_{ss} + f_{in,ex}(t) \quad (37A)$$

A novel, heuristic approach was developed that provided an easy steady state solution defining the initial conditions. The procedure is simply to iteratively make use of the open-loop ODEs (Eqns 27A–31A), and for a fixed (current estimate) of f_{ss} as only primary input to repeatedly feed back the output to the input until convergence. This process produces the corresponding c_{ss} value.

Terminal elimination half life (t½)

For low drug concentrations the systems becomes linear. The Laplace transform of the linear ODEs results in a polynomial determinant for the eigenvalues. The terminal half-life t½ is obtained from the largest (least negative) eigenvalue (λ_{max}) of the denominator polynomial:

$$t½ = \ln(2)/(-\lambda_{max}) \quad (38A)$$

Disposition mean residence time (MRT)

The disposition mean residence time is only defined as a parameter if the system is linear. Thus, the MRT referred to here is the average time a drug molecule is present in the systemic circulation when the system is in the linear operation range:

$$MRT = MTT \times (1 - E)/E^2 \quad (39A)$$

This expression is simply the product of the MTT and the average number of times that a drug molecule goes through the system before being eliminated.

Linear clearance (CL)

From a simple Laplace transform analysis of the system operating in the linear range it is evident that the clearance (linear clearance) based on dose/AUC principles is given by the following expression:

$$CL = E/(K_{out} \times (1 - E)) \quad (40A)$$

Linear elimination rate parameter (K_L)

As discussed above, the present system analysis-based recirculation model framework is fundamentally different from classical compartmental modelling in several ways. Due to the heterogeneous nature of the FTS units that make up the structure of the model, first-order rate parameters, so abundantly present in conventional compartmental modelling, are absent in the current PK modelling framework. However, one may define parameters of a similar dimension (i.e. having units of reciprocal time) and of similar kinetic significance. The following parameter, which will be denoted simply as the linear elimination rate parameter, is such a parameter:

$$K_L = E/MTT \quad (41A)$$

The word 'linear' in the name refers to the system being described as linear (or operating in the linear range) and is not to be interpreted as a first-order (linear) rate parameter. The linear elimination rate parameter simply encapsulates the two most important individual parameters determining the elimination of the drug in a recirculation system, namely the single pass extraction fraction and the MTT. Clearly, the drug elimination will increase with an increase in the extraction fraction and also with a decrease in the mean transit time.

Volumes of distribution in heterogeneous FTS-based system

As outlined in the Results and Discussion above, the volume of distribution concept is difficult to deal with for heterogeneous FTS-based systems. Nevertheless, the following two volume-type parameters can be defined, which may be of similar usefulness to volume terms that are used in compartmental kinetics when comparing the PK of drugs.

Apparent steady state volume (V_{ss})
An apparent steady state volume of distribution:

$$V_{ss} = CL/K_L \quad (42A)$$

Residence volume (V_{res})

$$V_{res} = MTT/K_{out} \quad (43A)$$

Peak recirculation time (t_{peak})

The peak recirculation (t_{peak}) time is the time following a bolus intravenous administration that it takes to reach a maximum

concentration at the sampling site. It was determined numerically during the numerical integration of the model ODEs.

Nonlinearity index

It is difficult to judge the significance of a nonlinear parameter in a kinetic model unless the magnitude of the parameter is looked at relative to the magnitude of the variables in the proper functional context. The nonlinear elimination parameter K_N (Eqn 21A) operates in the context of the expression $K_N/(K_N + f_{in}(t))$. Thus, the nonlinearity experienced on an individual basis, and the significance of K_N in each case, may be judged from the ratio $K_N/\max(f_{in}(t))$, which is denoted the nonlinearity index.