

hope of improving their endurance capacity and recovery during training and competition (1-3). Like endogenous erythropoietin (Epo) the main action of rHuEpo is to stimulate the proliferation and the differentiation of erythroid progenitor cells in bone marrow and their evolution into mature erythrocytes (4,5) (Fig. 1). The Epo induced effects persist at significative values several

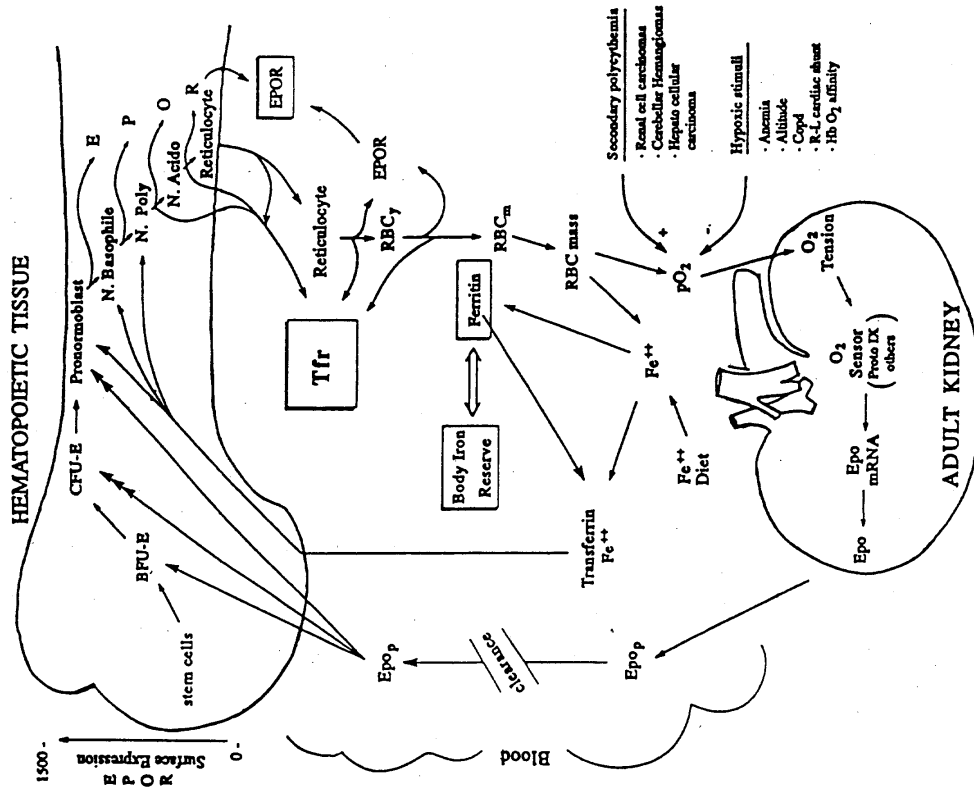


Fig. 1. Schematic representation of erythropoiesis. BFU-E: burst forming unit-erythroid; CFU-E: colony forming unit-erythroid; EPOR: erythropoietin receptors; RBC: red blood cells.

days after the last intake of the hormone, even if the Epo blood and urine concentrations become no more quantifiable. Recently, Gareau *et al.* (6) have shown that rHuEpo induces a delayed increase of serum soluble transferrin receptors (sTfr) which is an index of both tissue iron deficiency and expanded erythroid progenitor mass and a delayed decrease in ferritin (fr) concentration which is a measure of body iron storage. sTfr have been expressed in relation to fr, thus giving the serum sTfr/fr index. The authors concluded that concomitant changes in hematocrit and in the ratios sTfr/fr could reflect rHuEpo abuse. These changes in sTfr and fr persist several days after the last intake of rHuEpo while Epo serum concentrations have essentially returned to their baseline values. In a recent paper, Bressolle *et al.* (7) used a population pharmacodynamic approach to relate serum Epo concentrations to the effect of rHuEpo on sTfr, fr, and on the ratio sTfr/fr. Serum Epo concentration-time profile was compatible with a one-compartment open model and zero-order input rate (8) and the concentration-effect relationship was best described using the sigmoid E_{max} model (7). This effect-compartment ("link") model is used when a drug requires time to exert its pharmacological effect and the response is not immediately linked to the plasma drug concentration. This approach assumes that the rate of onset and offset of effect is governed by the rate of drug distribution to and from a hypothetical "effect site" (9). Recently, basic indirect response models characterized by either inhibition or stimulation of the response variable have been proposed (10,11) and widely discussed (12,13).

The purpose of the present study was to compare two pharmacodynamic approaches to relate serum Epo concentrations to the effect of rHuEpo on sTfr, and fr, the "indirect effect model" proposed by Jusko and Ko (10) and Dayneka *et al.* (11) and the "effect compartment" proposed by Sheiner *et al.* (9).

METHODS

Pharmacokinetic Analysis

The data used in this report have been published previously (8). rHuEpo (Recombinant human Epo alpha, Eprex®, Cilag AG, France) was administered subcutaneously at the dose of 200 units/kg each morning on Days 0, 2, 4, 7, and 10 to 18 healthy male athletes (19 to 26 years). Epo pharmacokinetics can be described using a linear, one-compartment model with zero-order input.

Population Pharmacodynamic Analysis

For the pharmacodynamic (PD) modeling approach, the EM-fit computer program (14) was used to estimate the population PD parameters.

This is a new implementation of the estimation-maximization EM algorithm (15). The individual pharmacokinetic parameters required in the calculation process for the estimation of the population PD parameters were fixed to the ones estimated in the pharmacokinetic (PK) analysis. The intersubject variability was assumed to be log-normally distributed and the residual variance term was assumed as homoscedastic.

Two pharmacodynamic modeling approaches have been used; (i) the effect compartment approach and (ii) the indirect effect model approach.

Compartment Model Approach

In this approach the effect compartment is modeled as an extra compartment linked to the central one by a first-order process.

The sigmoid E_{max} equations that were applied to data are (7): for data modeling of sTfr

$$y = E_0 + \frac{E_{max} \cdot C_E^\gamma}{C_E^\gamma + EC_{50}^\gamma} \quad (1)$$

for data modeling of fr

$$y = E_0 - \frac{E_{max} \cdot C_E^\gamma}{C_E^\gamma + EC_{50}^\gamma} \quad (2)$$

Where C_E is the serum Epo concentration at the effect site; E_0 represents the baseline effect prior to rHuEpo administration; E_{max} is the asymptotic maximum expected sTfr and fr levels; EC_{50} is the drug concentration when the effect is $E_0 \pm 50\%$ of E_{max} ; and γ is the sigmoidicity factor (reflecting the steepness of the curve).

Indirect Effect Model Approach

The basic premise of this approach is that a measured response (R) to a drug may be produced by indirect mechanism, (i) the factors controlling the input or production (k_{in}) of the response variable may be either inhibited or stimulated or (ii) the determinants of loss (k_{out}) of the response variable may be either inhibited or stimulated.

The rate of change of the response over time with no drug present can be described by

$$\frac{dR}{dt} = k_{in} - k_{out} \cdot R \quad (3)$$

where k_{in} represents the zero-order constant for production of the response and k_{out} defines the first-order rate constant for loss of the response. As stationarity is assumed, the response variable (R) begins at a baseline value

(R_0), changes with time following drug administration, and returns to (R_0). Thus

$$k_{in} = k_{out} \cdot R_0 \quad (4)$$

which reduces the number of parameters in the model.

The different time course of the Epo plasma concentrations and the effects on sTfr and fr suggests the presence of an indirect relationship between Epo concentrations and effect (Fig. 2). However, the average lag time of about 50 hr between the first intake of rHuEpo and the onset of the

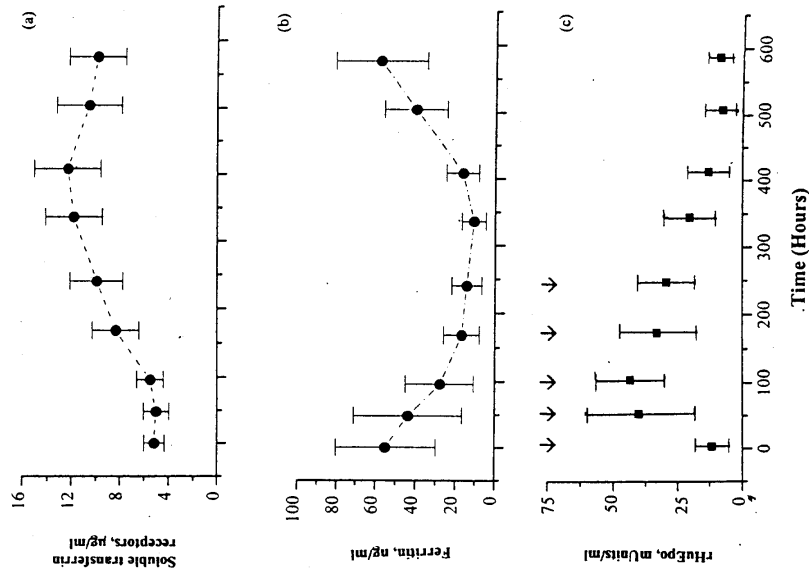


Fig. 2. Mean (\pm SD) effect on sTfr (a) and fr (b) and serum concentrations of erythropoietin (c) after repeated subcutaneous doses (200 U/kg^{-1}) of recombinant human erythropoietin to athletes. Days of injections (\downarrow).

measurable effects indicates that the Epo plasma concentrations are not directly responsible for the changes in the rate of production and in the rate of loss of the response.

To cope with such a delay, a delay function was incorporated into the model to describe the relationship between the Epo plasma concentrations and the endogenous mediators affected by the drug (C_m) and responsible for the effects on sTfr and fr

$$\frac{dC_m}{dt} = k_{e0}(C_p - C_m) \quad (5)$$

where C_m is the Epo concentration in a biophase compartment responsible for the effect, k_{e0} is a delay rate constant and C_p the Epo plasma concentration.

The available information concerning the mechanism of action of Epo suggests the use of models where the sTfr levels increase and where fr levels decrease due to the stimulation of the erythropoiesis according to the stimulation function, $S(t)$

$$S(t) = 1 + \frac{E_{\max} \cdot C_m}{C_{m50} + C_m} \quad (6)$$

Where C_{m50} represents the mediator concentration producing 50% of maximum stimulation at effect site and E_{\max} represents the maximum effect attributed to the drug.

The following models describing processes that results from stimulation of the factors controlling drug response were used

sTfr model

$$\begin{array}{c} \xrightarrow{k_{in}} \boxed{\text{Response}} \xrightarrow{k_{out}} \\ \quad \quad \quad (R) \\ \xleftarrow{C_{m50}} \end{array} \quad \frac{dR}{dt} = k_{in} \left(1 + \frac{E_{\max} \cdot C_m}{C_{m50} + C_m} \right) - k_{out} \cdot R \quad (7)$$

fr model

$$\begin{array}{c} \xrightarrow{k_{in}} \boxed{\text{Response}} \xrightarrow{k_{out}} \\ \quad \quad \quad (R) \\ \xleftarrow{C_{m50}} \end{array} \quad \frac{dR}{dt} = k_{in} - k_{out} \left(1 + \frac{E_{\max} \cdot C_m}{C_{m50} + C_m} \right) \cdot R \quad (8)$$

Statistical Analysis

To check the error model assumptions and the distribution on the estimated population pharmacodynamic parameters, the program estimates the

expected responses ($Y_{\text{predicted}}$) for each individual in the population and computes appropriate statistical tests to evaluate the distribution properties of the differences between the predicted and the observed data. To assess the posterior distribution properties of the residuals, the t test was used to compare the mean with 0; the Kolmogorov-Smirnov test was used to compare the sampled distribution to the expected one [$N(0, 1)$] (16).

Goodness of fit of the nonlinear regression analysis of the pharmacodynamic data was judged by acceptance of the least complex equation or model that did not produce systemic deviations in the residuals and that provided the lowest Akaike criterion.

RESULTS

Pharmacokinetic Study

A preliminary independent PK analysis was done on the serum levels of Epo in order to estimate the individual pharmacokinetic parameters. Total clearance (CL), volume of the compartment (Vd), and the absorption duration time were used as model parameters, elimination half-life was derived from $\ln(2) \times Vd/CL$. The elimination half-life ranged from 23.2 to 114.9 hr with a mean ($\pm SD$) of 33.8 ± 19.2 hr. The total clearance and the volume of distribution, uncorrected for bioavailability, were 0.057 ± 0.016 L/hr per kg and 2.97 ± 1.43 L/kg, respectively; the average absorption duration time was 10.1 ± 1.0 . Large interindividual variability of PK parameters are observed.

Pharmacodynamic Study

The influence of the γ value on the predictive performances of the models was investigated by comparing the Akaike criterion, the changes in the log-likelihood values, the visual inspection of the fitted curves, and the residual plots versus time when γ was assumed to be fixed to 1 and estimated as a model parameter. The models retained for the final data analysis include γ as a model parameter for the compartmental model approach and for indirect data modeling of sTfr, while γ fixed does not produce a worse fit for indirect data modeling of fr.

Population PD parameters are given in Tables I and II for direct and indirect response models, respectively.

Figure 3 portrays the posterior individual fitting of sTfr for each subject. The curves in the upper left panel shows the pharmacodynamic profiles resulting from the use of Eq. (1) (compartment model approach). The sTfr values slowly increase with a maximum observed several days after the last

Table I. Population Pharmacodynamic Parameters Using the Effect Compartment Approach^a

	k_{e0} (h ⁻¹)	E_0^b	E_{max}^b	γ	EC_{50} (units/L)
sTfR					
M	1.27×10^{-3}	5.04	10.9	3.31	14.5
%CV	13.8	10.7	26.0	14.1	28.9
SD of residual error	0.96				
AIC	-1.63				
fr					
M	7.17×10^{-3}	57.5	44.6	4.27	15.1
%CV	31.4	32.4	34.7	38.9	23.3
SD of the residual error	39.7				
AIC	-3.80				

^aValues are mean (percentage coefficient of variation, % CV).

^bUnits: soluble transferrin receptor (sTfR), $\mu\text{g/ml}$; ferritin (fr), ng/ml; AIC, Akaike criterion.

Table II. Population Pharmacodynamic Parameters Using the Indirect Effect Model Approach^a

	C_{min30} (units/L)	k_{out} (hr ⁻¹)	R_0^b	E_{max}^b	γ	k_{e0} (h ⁻¹)
sTfR						
M	2.51	1.09	4.48	18.3	9.31	1.23×10^{-3}
%CV	31.1	15.6	12.5	15.3	24.0	17.1
SD of residual error	1.08					
AIC	-1.80					
fr						
M	0.64	2.24	59.8	15.5	1.0	20.8×10^{-3}
%CV	101	7.7	14.3	3.7	2.0	
SD of the residual error	62.5					
AIC	-3.78					

^aValues are mean (percentage coefficient of variation, % CV).

^bUnits: soluble transferrin receptor (sTfR), $\mu\text{g/ml}$; ferritin (fr), ng/ml; AIC, Akaike criterion.

drug administration. Thereafter, the response gradually returns to the baseline. According to the patient, large interindividual variations in the response occur. The curves in the upper right panel shows the PD profiles resulting from the use of Eq. (7) (stimulation of the factors controlling the production of the drug response). The goodness of fit is shown by the analysis of the scatterplot of the posterior predicted values versus the individual observed responses (lower panels). In all cases the mean value of residuals was not significantly different from zero (Student *t* test) and the Kolmogorov-Smirnov test showed that the residual distributions were not significantly different from a normal distribution $N(0, 1)$.

For fr, Fig. 4 shows the PD profiles resulting from Eq. (2) (sigmoid E_{max} model, upper left panel) and from Eq. (8) (stimulation of the factors controlling the loss of response, upper right panel). The lower panels show

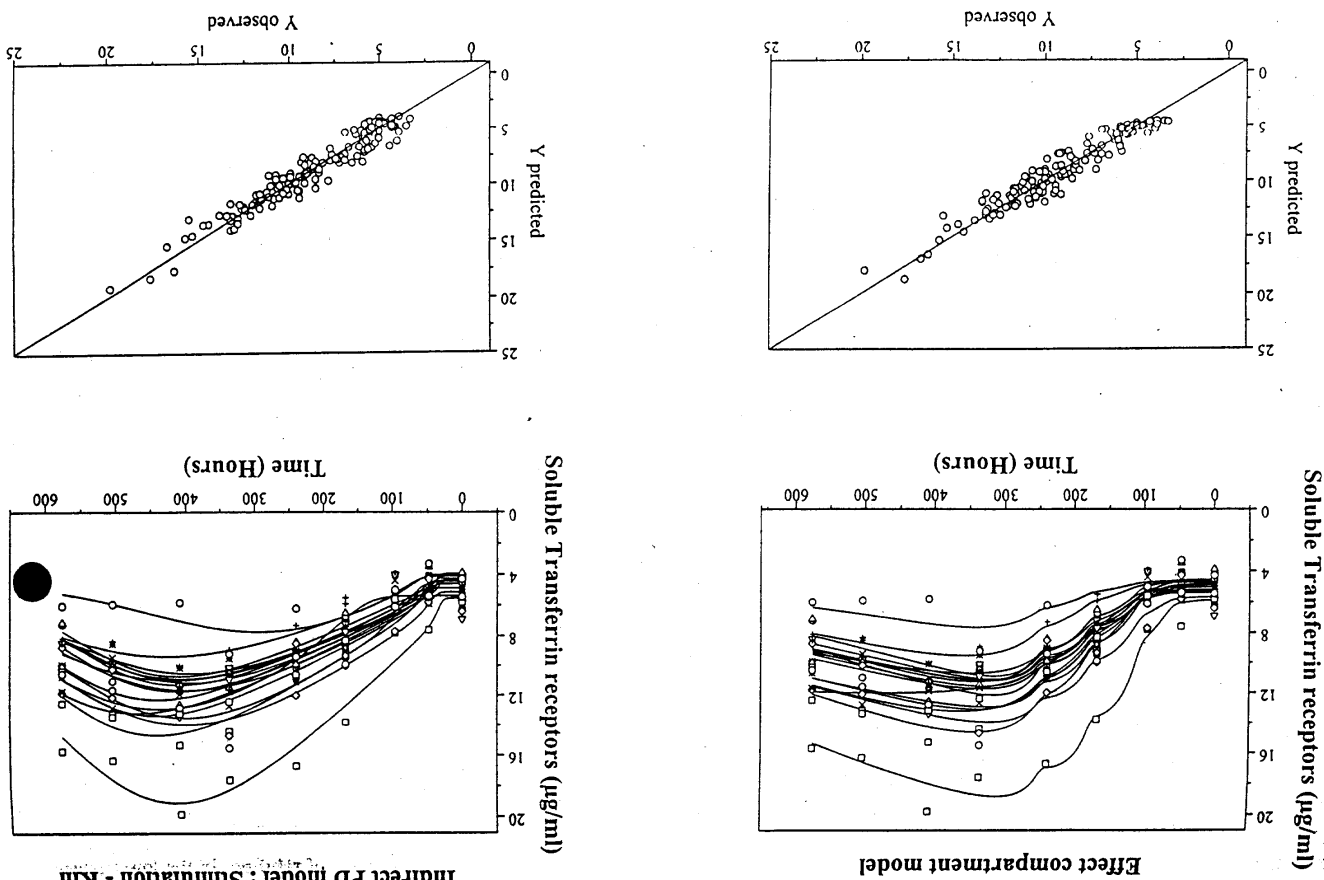


Fig. 3. Pharmacokinetic/pharmacodynamic posterior individual fitting of soluble transferrin receptors as index of the therapeutic effect of rHuEpo. In the lower panels, the lines are drawn with an intercept of 0 and a slope of 1.

the scatter plot of posterior predicted values versus the observed responses. The relationship between the effect parameters and the serum Epo concentration in the effect compartment (C_E) presented in Fig. 5 corroborates the choice of the effect compartment model.

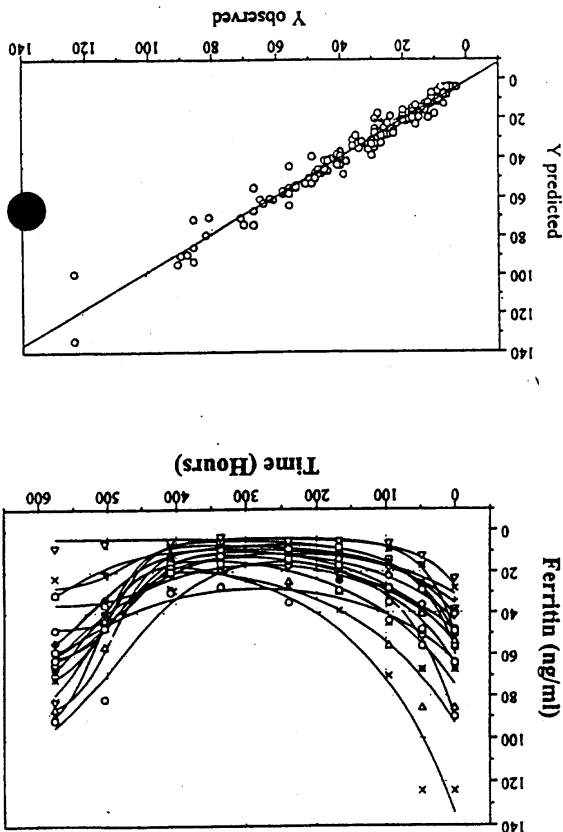
Additional fittings, using the Models 7 and 8 with C_p in the place of C_m , have been done to confirm the importance of the use of C_m concentrations in the biophase compartment. The comparison of the predicted values to the observed ones, the analysis of the residual distribution, and the plot of the prediction versus the observed values reveal that the use of C_m produces better fittings. A real improvement in the predictions is observed during the first 50-80 hr after the intake of rHuEpo when the Epo plasma concentrations reach high values and no measurable change is observed on the marker values, particularly for sTfr (see Fig. 2).

DISCUSSION

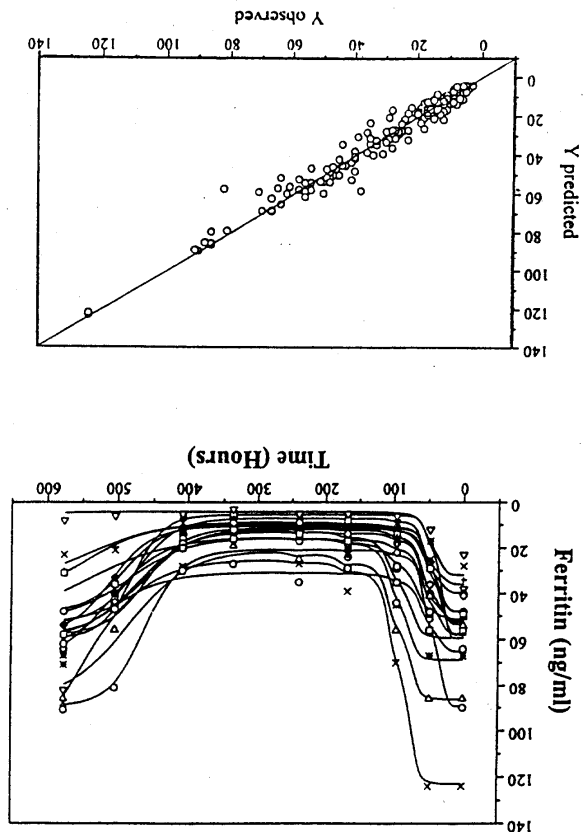
There is no real difference in the descriptive features of the two models used. The Akaike criterion obtained with the two modeling approaches are very close. Moreover, the visual inspection of the fitted curves generated by the two approaches indicates that the results found are equivalent.

Our main purpose was not only to find a "reasonable" model suitable to describe the changes on the sTfr and fr consequent to the prolonged intake of rHuEpo in a population of individuals but also to show how, with an understanding of the mechanism of action of Epo, stimulation process

Fig. 4. Pharmacokinetic/pharmacodynamic posterior individual fitting of ferritin as index of the therapeutic effect of rHuEpo. In the lower panels, the lines are drawn with an interval of 0 and a slope of 1. These are drawn with an interval of 0 and a slope of 1.



Indirect PD model : Stimulation - Kout



Effect compartment model

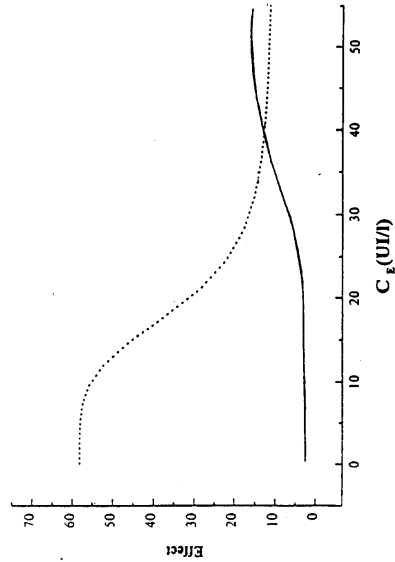


Fig. 5. Relationship between the effect parameters and the serum rHuEpo concentration in the effect compartment (C_E). fr (---); sTfr (—).

an understanding of the mechanism of action of Epo, stimulation process of production and loss rates could govern the time course of changes on the marker values. For this reason we decided to use indirect model.

The comparisons of the fixed and random population parameters estimated using the two approaches reveal a lot of similarities. The mean upper response limits (equal to $1 + E_{\max}$ in the indirect stimulation models and $E_0 \pm E_{\max}$ in the compartmental ones) were of 19.3 and 15.9 with a random effect of 15 and 26%, respectively, for the sTfr and were of 16.5 and 12.9 with a random effect of 4 and 35%, respectively, for the fr. The mean basal values and their random effects were also very close for the two computational methods and for the two markers. Likewise, k_{e0} computed by the two modeling approaches was very close. Finally the CE_{50} (the concentration giving 50% of the $E_0 \pm E_{\max}$ in the compartmental approach) and the C_{m50} (the concentration in the biophase giving 50% of the stimulation in the rate or production or loss in the indirect model) were of the same order of magnitude for the two markers when the same computational approach was used.

The indirect model is based on mechanistic interpretation of the relationship of drug concentrations and effects. The correct use of such modeling approach requires some inference about the pharmacological properties and the mechanism of action of the drug. The indirect model operates best when the response variable immediately reflects the production or loss process. When such a immediate relationship is not established, delay functions may be needed between the response variable and the endogenous receptor, mediator, or enzyme affected by the drug. This seems the case for our data.

The effect model is closer to a descriptive modeling approach and it supplies an empirical description of the dynamic process. This approach may be useful to describe and simulate PK/PD relationships especially when the biochemical and physiological mechanisms implied in the drug action regulation are not precisely known.

In any case the use of the indirect model seems to be more relevant in pharmacodynamic modeling to understand the potential mechanism of action of Epo. On the bases of the assumptions underlying this modeling approach, it would be extremely useful to measure the parameters describing the mechanism of erythropoiesis shown in Fig. 1 and to relate such a value to the one estimated in our model. The agreement of these parameters would definitely "validate" the choice of the indirect model. Unfortunately, we do not have this information.

The purpose of this study was to evaluate pharmacokinetic-pharmacodynamic relationships using an experimental design which mimics the potential dosage regimen used by athletes for doping. Obviously, an exhaustive evaluation of the dose-response relationship requires the use of increasing

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