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The pharmacokinetics and pharmacodynamics of recombinant human erythropoietin in haemodialysis patients

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1 The pharmacokinetics of and therapeutic response to recombinant human erythropoietin (rcEPO) were studied in 12 patients under chronic haemodialysis on a thrice weekly intravenous rcEPO treatment scheme. The kinetics of rcEPO were also assessed after a subcutaneous injection during the initial period and during maintenance treatment. RcEPO was measured in plasma by radioimmunoassay.

2 After the first i.v. dose plasma erythropoietin concentrations were best described by a monoexponential disposition function with a mean (± s.d.) elimination half-life of 5.4 ± 1.7 h. The volume of distribution was 70 ± 5.2 ml kg^{-1} and the clearance was 10.1 ± 3.5 ml h^{-1} kg^{-1} ($n = 12$).

3 After 3 months of continuous therapy, the plasma half-life of rcEPO decreased by 15% ($P < 0.05$, mean half-life during steady state: 4.6 ± 2.8 h), while mean clearance and volume of distribution remained constant.

4 After the first s.c. injection the mean (± s.d.) absorption time was 22 ± 11 h and systemic availability was 44 ± 7%.

5 Changes in haemoglobin concentrations were described by a linear additive dose-response model, defined by an efficacy constant (K_{eff}) and the mean erythrocyte lifetime (MRT_{Hb}). The sample mean (± s.d.) K_{eff} was 0.043 ± 0.017 g dl^{-1} Hb per 1000 units rcEPO and MRT_{Hb} was 10.02 ± 1.75 weeks. The net effect of rcEPO treatment was described by the area under the unit-dose-response curve (AUEC) with a mean (± s.d.) value of 0.45 ± 0.23 g dl^{-1} weeks.

6 RcEPO clearance showed a significant positive correlation ($r^2 = 0.41$) with the effectiveness of rcEPO therapy, as measured by the parameters K_{eff} or AUEC.

Keywords recombinant erythropoietin pharmacodynamic modelling pharmacokinetics renal anaemia haemodialysis

Introduction

Erythropoietin is a protein of 165 amino acids four of which are glycosylated (Browne *et al.*, 1986) and a molecular weight of 34,000 daltons. It is now available in a recombinant form (rcEPO) with a specific activity of about 200,000 units mg^{-1} (corresponding to 150 fmol per unit).

In adult humans, over 90% of erythropoietin is synthesised by kidney cells, where its secretion is stimulated by oxygen deficiency. Erythropoietin exerts its effects by binding irreversibly to erythrocyte progenitor cells in the red bone marrow and the spleen. This results in the differentiation and release of reticulocytes into the blood stream within about 8 days, leading to an increase of blood haemoglobin and consequently an increased oxygen supply to the kidneys, thereby decreasing the stimulation of ethythropoietin production (Fisher, 1988).

Being a macromolecule, erythropoietin is essentially confined to the vascular space and is excreted in urine to a negligible degree. Removal of terminal sialic acid residues from the carbohydrate moiety increases its affinity to liver cell receptors thereby accelerating its uptake and subsequent degradation (Spivac & Hogans, 1985).

RcEPO is especially effective in patients who cannot produce sufficient erythropoietin because of renal disease. It also seems useful in anaemic patients suffering from rheumatoid arthritis, neoplastic disease, acquired

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immunodeficiency (especially during azidothymidine treatment) and in certain myelodysplastic disorders. Its use to increase the yields of autologous transfusions is under investigation and, recently, its abuse as a doping agent in competitive sports has been reported (Faulds & Sorkin, 1989; Gurland *et al.*, 1991). In this study we sought to describe the pharmacokinetics of rcEPO and to develop a pharmacodynamic model of its effect on erythrocyte count and haemoglobin concentration.

Methods

Study design

This investigation was an open single and multiple dose study in 12 haemodialysis patients, for whom rcEPO treatment was indicated therapeutically (Table 1). All patients had blood haemoglobin concentrations below 10 g 100 ml^{-1}. Patients with iron or vitamin deficiency were not included. None of the patients had received transfusions prior to the study for correction of their anaemia. During the first 8 weeks, the same dose of 50 units kg^{-1} of rcEPO (ERYPO™, Cilag, Switzerland) was administered to all patients thrice weekly on completion of the haemodialyses. After 8 weeks, the doses were adjusted individually according to the haemoglobin level. Target blood haemoglobin levels were 100 to 110 g l^{-1}, since higher levels are often associated with arterial thrombosis and occlusion of the haemodialysis shunt. Haematological and routine clinical chemistry values were determined weekly during the first four months of treatment. Trough concentrations of rcEPO (prior to dialysis, 45 h after i.v. injection) were also determined weekly and the kinetics were studied in detail after four rcEPO injections.

The first injection of rcEPO which the patients received was given subcutaneously after the haemodialysis session. Blood samples were drawn predose and at 0.5, 2.0, 5.0, 12, 24, 36, 45, and 165 h. No further rcEPO was given during this period.

At 168 h after the first s.c. injection, the patients received their first intravenous injection on completion of the routine haemodialysis. Blood samples following intravenous injection over 1 min were taken from a forearm vein opposite to the injection site predose and at 5, 10 and 30 min and 1, 3, 5, 12, 24 and 45 h (and 48 h, after dialysis) after injection. One week (or, in 3 patients 2 weeks) following this intravenous injection of rcEPO regular therapy was started as described above.

A second i.v. study during maintenance therapy (again with doses of 50 units kg^{-1}) was performed after at least 3 months of continuous rcEPO treatment with the same sampling times as in the first i.v. study.

A second s.c. study was carried out 1 week after the second i.v. study using the same dose and sampling times as in the first s.c. study.

Assay methods

Plasma erythropoietin was measured by radioimmunoassay as described by Eckardt *et al.* (1988). A polyclonal rabbit antibody raised against human recombinant erythropoietin was kindly provided by Dr P. Hirth, Boehringer Mannheim, FRG. Tracer rcEPO (Amgen, USA), radioiodinated to a specific activity of 23 teraBq $mmol^{-1}$, was purchased from Amersham (Braunschweig, FRG). The 2nd international EPO standard at the WHO (erythropoietin enriched from human urine) was used as reference standard (Annable *et al.*, 1972). Routine calibrations were performed using the recombinant EPO standard produced by Amgen and purchased from Amersham (code: ARN6050). Binding curves were identical to those obtained with the WHO standard and the ERYPO™ preparation given to the patients. All incubations were carried out in phosphate buffered (10 mM, pH 7.5) saline (150 mM) with 0.1% (w/v) bovine serum albumin (BSA). BSA (20 µl 30% w/v) was pipetted into 5 ml polystyrene tubes, followed by 100 µl of the standards or plasma samples and mixed with 100 µl of diluted antibody. After 24 h of incubation at 4° C, 100 µl of tracer solution was added and left to stand at 4° C for a further 24 h. This late addition of the tracer was found to improve assay sensitivity. The antibody bound tracer was then separated using

Table 1 Patient characteristics and relevant blood chemistry results

Patient	*Sex*	*Age (years)*	*Initial weight (kg)*	*Aetiology of renal failure*	*Plasma aluminium (µM)*	*Ferritin (initial) (ng ml^{-1})*	*Alkaline phosphatase (u l^{-1})*
1	F	44	57	tubulo-interstitial nephropathy	0.52	57	62
2	F	53	62	tubulo-interstitial nephropathy	0.37	63	240*
3	M	52	47	glomerulonephritis	0.33	242	130
4	M	58	59	tubulo-interstitial nephropathy	0.29	81	230*
5	F	66	51	diabetic nephropathy	0.36	58	77
6	M	56	51	diabetic nephropathy	0.84	56	35
7	F	75	63	glomerulonephritis	0.68	76	111
8	F	37	50	diabetic nephropathy	0.57	50	137
9	F	57	48	tubulo-interstitial nephropathy	1.27*	34	108
10	F	47	79	tubulo-interstitial nephropathy	3.51*	31	190
11	F	79	45	tubulo-interstitial nephropathy	1.11*	179	120
12	M	60	81	glomerulonephritis	0.36	106	77

*Outside the normal range. All patients studied had two inactive kidneys, except number 12, who had an additional inactive kidney transplant. Serum transaminase activities were within the normal range in all patients.

cellulose particles coated with a second antibody (Sac-cel™, IDS Co., Boldon, UK). 300 µl of this precipitating reagent was added, mixed and after 1 h of incubation, the samples were centrifuged, supernatants decanted and the antibody-bound tracer counted. Concentrations were read from a standard curve using the spline interpolation algorithm of the 'Ria-calc' program from LKB (Bromma, Sweden).

The limit of determination was 4 units l^{-1}. The intra-assay coefficient of variation was 4.5% between 16 and 250 units l^{-1}. The inter-assay coefficient of variation was 6% between 15 and 140 units l^{-1} without dilution of the sample, and 7% between 100 and 1400 units l^{-1} after 1:10 dilution.

Pharmacokinetic analysis

Endogenous erythropoietin (C_{basal}) and administered rcEPO cannot be differentiated by the radioimmunoassay. Therefore, the predose C_{basal} values were regarded as constant over the periods of kinetic analyses and were subtracted when calculating pharmacokinetic parameters. Corrected plasma erythropoietin concentrations (C_{iv}) following i.v. injection of dose D were best described by a monoexponential function, while use of biexponential input functions resulted in overparametrisation according to the statistical criterion of Imbimbo *et al.* (1989).

Deconvolution of the subcutaneous data using prescribed input functions, namely rectangular pulse functions of variable duration and mono- and biexponential input functions (Cutler, 1978), indicated that a monoexponential input function best described absorption from the injection site, as judged by the criterion of Imbimbo *et al.* (1989). Hence, values of the absorption rate constant (k_a) and its reciprocal, the mean input time (MIT) were derived. The extent of systemic availability after s.c. injection was calculated from the ratio of AUC values after s.c. and i.v. injection.

Least squares nonlinear regression analysis of the data was performed with the BMDP™ derivative free program 'AR' (Dixon, 1987). Since analysis of the precision of the EPO radioimmunoassay showed a virtually constant coefficient of variation in the range between 10 to 1500 u l^{-1} (in the concentrations above 150 u l^{-1} after 1:10 dilution), iterative reweighting was performed with the reciprocal of the square fitted concentrations, i.e. a constant coefficient of variation was used as variance model for the intraindividual residual error.

Pharmacodynamic analysis

The change in blood haemoglobin was used as a surrogate index of the therapeutic effect of rcEPO. Haemoglobin, erythrocyte count, and hematocrit changed in parallel after administration of rcEPO in all patients. The per-dose haemoglobin level (Hb_{basal}) was assumed to be at steady state, representing a constant response to a constant rate of production of endogenous erythropoietin. A Bateman-function was used to describe the change in Hb following a single rcEPO dose. In the equation below, Hb(t) is the response following the superposition of the effects of several consecutively administered rcEPO doses:

$$Hb(t) = Hb_{basal} + \sum_{j=1}^{n} dose_j \, K_{eff} \left(e^{-\frac{t_j}{MRT_{Hb}}} - e^{-\frac{t_j}{MIT_{Hb}}} \right) \quad (1)$$

where: $t_j = time - t_{dose_j} - t_{lag}$

t_j is the time within the respective dosage interval (τ_j), that is the actual time minus the administration time (t_{dose_j}) of the j'th dose ($dose_j$), minus the lag-time between each dose and the respective Hb increase. Lag-time was around 8 days, as indicated by the appearance of reticulocytes in the blood and the increase in blood haemoglobin concentration. MRT_{Hb} is the mean haemoglobin (or erythrocyte) survival time and MIT_{Hb} is the mean onset time of the effect. MIT_{Hb} is analogous to the reciprocal of the rate constant defining transfer of drug from the pharmacokinetic to the effect compartment of classical pharmacokinetic-pharmacodynamic models (Holford & Sheiner, 1982). The two parameters t_{lag} and MIT_{Hb} could not be determined with sufficient precision since haematologic data were collected only weekly and therefore, both of these parameters were estimated and fixed at 1 week and 2 days, respectively. K_{eff} is a constant describing the efficacy of a unit dose of rcEPO in the stimulation of erythrocyte production. As an alternative to the linear model (equation 1), a saturable effect model was applied by replacing K_{eff} (in equation 1) by:

$$K_{eff} = K_{eff,max} \Big/ \left(1 + \frac{ED_{50}}{dose_j} \right) \quad (2)$$

where $K_{eff,max}$ is the maximum efficacy and ED_{50} is the dose associated with half maximum effect (Holford & Sheiner, 1982).

The individual data sets were fitted including all haemoglobin concentrations values and all erythrocyte counts, using the reciprocal of the haemoglobin content of the erythrocyte (Hb_E) as the fitted proportionality parameter between haemoglobin and erythrocyte count:

$$Number_{Erythrocytes}(t) = Hb(t) \frac{1}{Hb_E} \quad (3)$$

These pharmacodynamic relationships assume that the total blood volume remains constant. Although this may not be valid in all patients, it is supported by data from Cotes *et al.* (1989). The area under the unit-(rcEPO)-haemoglobin-concentration-time-curve (AUEC) was calculated from:

$$AUEC = \int_0^\infty Hb(t)\, dt = dose \, K_{eff} \, (MRT_{Hb} - MIT_{Hb}) \quad (4)$$

This has units of g Hb 100 ml^{-1} weeks.

In this analysis, numerical superposition of all single dose-response curves was applied in the evaluation by nonlinear regression analysis using a FORTRAN sub-

routine with the BMDP program AR. However, when using the derived parameters for predictions, this can be replaced by the analytical solution for prediction of the concentrations at steady state, if doses and dose intervals (τ) remain constant. For estimation of the endogenous erythropoietin formation rates from the rcEPO pharmacodynamic parameters, equation 1 was differentiated for dose.

Population statistics and statistical tests

The measured data are presented nonparametrically as medians and range. Significances of differences in pharmacokinetic data between different modes of administration and between single and multiple dose treatment were determined by using the Wilcoxon-matched paired signed rank test.

Precision of the individual parameter estimates is given in Tables 2 and 3 as the asymptotic standard error as calculated by the BMDP program AR. Pharmacokinetic population parameters, i.e. population means and their variances, were determined by the global two stage algorithm (GTS) described by Steimer *et al.* (1984).

Results

Pharmacokinetics of rcEPO following intravenous injection

Individual and mean plasma concentrations of rcEPO following the initial i.v. and s.c. doses are shown in Figure 1 and corresponding pharmacokinetic parameters after initial and maintenance doses are summarized in Table 2. Values of the initial volume of distribution were slightly greater than the expected volumes, possibly reflecting some binding of erythropoietin to the vascular endothelium and blood cells.

Like volume of distribution, systemic clearance was unchanged on continuous treatment. Values averaged about 10 ml h^{-1} kg^{-1} which, if multiplied by the basal erythropoietin level, indicates an endogenous erythropoietin formation rate in these patients of about 2000 u per week. The mean elimination half-life of rcEPO after i.v. injection of 5 h was shortened by 15% during maintenance treatment compared with initial dosage ($P < 0.05$). Two of the 12 patients appeared as outliers with half-lives around 10 h and, on renanalysis 3 months later, one of these still had an exceptionally long elimination half-life. Scrutiny of the routinely determined biochemical parameters, clinical data and patients' history only indicated a slightly elevated serum alkaline phosphatase activity in both of these patients. No accumulation of plasma rcEPO was evident during thrice weekly i.v. dosage, as illustrated by the constancy of weekly trough concentrations shown in Figure 2.

Absorption of erythropoietin after s.c. injection was prolonged, as indicated by MIT values of 7–70 h. Thus, 'flip-flop' kinetics applied and trough levels were in all cases greater than pretreatment levels after 45 h. The extent of systemic availability of the s.c. dose ranged from 28 to 100% (mean 44%).

Pharmacodynamics

During the first 8 weeks, all patients received 50 u kg^{-1} rcEPO thrice weekly. After 8 weeks of this treatment, median haemoglobin increased from 6.6 g 100 ml^{-1} (range: 5.2–7.2) to 9.5 g 100 ml^{-1} (range: 6.8–10.3)

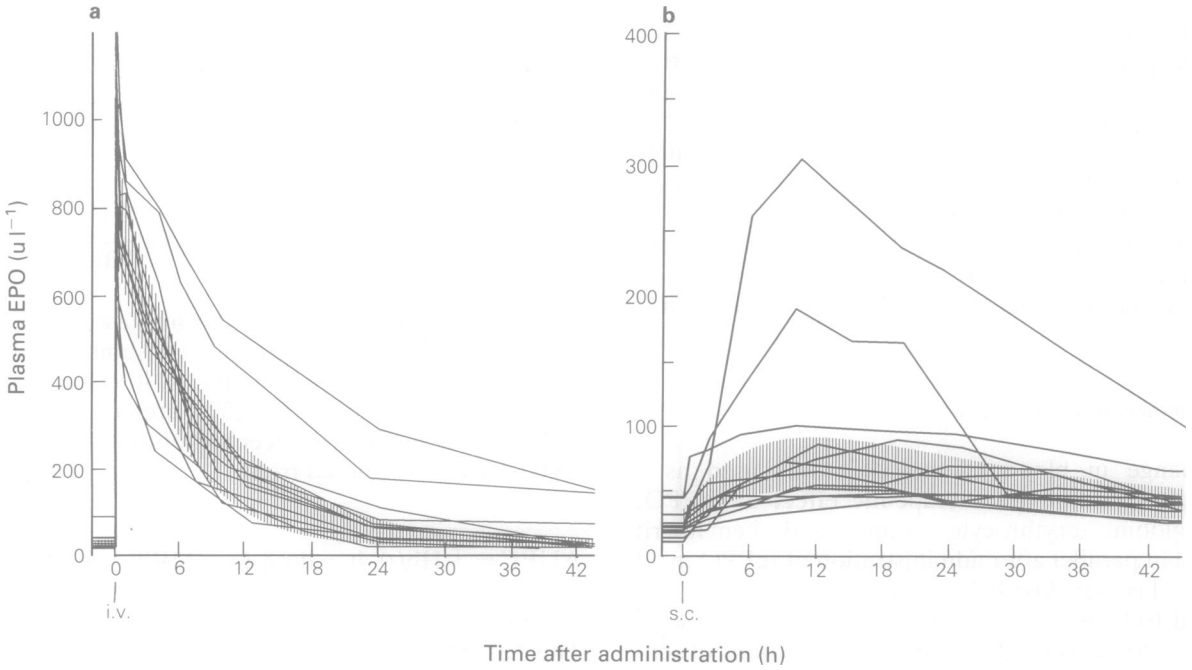

Figure 1 Plasma concentrations of rcEPO after i.v. injection on initial treatment (a) and after s.c. injection (b). The shaded areas represent the population mean (± s.d.) as calculated from the mean parameters and their variances. The two outlying curves in both plots represent data from the same individuals.

Table 2 Pharmacokinetic parameters describing the fate of recombinant human erythropoietin

	Treatment									
	Initial					*Maintenance*				
		intravenous		*subcutaneous*			*intravenous*		*subcutaneous*	
Patient	V ($ml\ kg^{-1}$)	*CL* ($ml\ h^{-1}\ kg^{-1}$)	$t_{1/2}$ (*h*)	*MIT* (*h*)	*F* (%)	V ($ml\ kg^{-1}$)	*CL* ($ml\ h^{-1}\ kg^{-1}$)	$t_{1/2}$ (*h*)	*MIT* (*h*)	*F* (%)
1	71.0 ±4.8	13.1 ±0.94	3.74 ±0.28	61.0 ±12.9	54.5 ±8.8	61.0 ±7.7	16.9 ±2.74	2.50 ±0.38	—	—
2	61.6 ±6.8	3.89 ±0.65	10.9 ±2.04	9.8 ±4.3	58.7 ±13.9	73.9 ±5.2	3.77 ±0.43	13.6 ±1.67	3.9 ±1.8	66.2 ±12.8
3	92.0 ±11.3	19.7 ±3.12	3.22 ±0.53	33.5 ±13.9	100 ±26.6	81.3 ±7.0	20.7 ±2.14	2.72 ±0.28	21.8 ±4.7	89.7 ±11.8
4	62.2 ±1.5	5.20 ±0.20	8.28 ±0.35	7.2 ±4.7	44.5 ±16.1	81.4 ±10.1	10.2 ±1.55	5.50 ±0.91	—	—
5	66.8 ±4.3	9.50 ±0.70	4.87 ±0.38	72.2 ±19.8	58.5 ±12.8	—	—	—	—	—
6	74.0 ±6.5	7.80 ±3.43	6.57 ±1.24	17.5 ±2.6	47.4 ±4.0	77.8 ±6.0	11.76 ±1.16	4.57 ±0.39	32.2 ±13.4	77.3 ±19.9
7	75.6 ±10.1	10.1 ±1.48	5.20 ±0.87	14.6 ±6.0	35.9 ±8.5	69.0 ±3.5	9.36 ±0.58	5.09 ±0.31	—	—
8	84.0 ±9.5	14.9 ±1.83	3.89 ±0.51	37.4 ±10.0	58.1 ±10.1	73.0 ±10.7	13.48 ±2.33	3.74 ±0.56	22.6 ±10.8	83.1 ±23.8
9	66.8 ±6.4	8.88 ±1.08	5.20 ±0.61	15.5 ±4.3	46.3 ±7.9	—	—	—	—	—
10	69.4 ±4.7	8.49 ±0.67	5.65 ±0.46	39.6 ±14.1	54.5 ±13.6	55.7 ±3.6	10.72 ±0.92	3.59 ±0.33	10.2 ±1.0	24.9 ±17.0
11	79.1 ±8.2	14.9 ±1.52	3.68 ±0.33	36.3 ±16.5	65.2 ±19.2		—	—	—	—
12	62.6 ±13.6	7.97 ±1.86	5.43 ±1.20	17.3 ±4.5	27.8 ±4.4	67.5 ±4.9	10.28 ±0.82	4.54 ±0.35	15.3 ±4.3	32.6 ±5.5
Mean*	69.8 ±5.2	10.09 ±3.54	5.40 ±1.7	21.5 ±11.3	43.7 ±6.7	69.5 ±6.7	11.11 ±4.9	4.64 ±2.80	13.3 ±6.0	53.8 ±21.7

*Population mean and standard deviations were determined by use of the 'global two stage method' (Steimer *et al.*, 1984). MIT = mean input time after subcutaneous injection (reciprocal of the absorption rate constant). Plasma half-lives were significantly shorter on maintenance therapy compared with first dose ($P < 0.05$, Wilcoxon-matched paired signed rank test) while the other parameters did not differ significantly.

(Table 3). After 8 weeks, weekly dose rates were individually adjusted to maintain a blood haemoglobin level of around 10 g 100 ml^{-1}.

To achieve an increase in blood haemoglobin of 3 g 100 ml^{-1} required rcEPO doses of between 400 and 4100 u kg^{-1}, achieved in a mean time of 9 (5–26) weeks. The mean maintenance doses were only slightly lower than the initial doses (Figure 3). Expressed alternatively, an rcEPO dose of 2000 u kg^{-1} resulted in a blood haemoglobin increase of 2.0–4.4 g dl^{-1} over a period of 14–24 weeks.

The model used to analyse the dose-response relationship allowed the data to be summarized by two constants, namely the efficacy coefficient (K_{eff}) and the survival time of haemoglobin in blood (MRT_{Hb}). The model assuming saturable efficacy (equation 2) was not statistically superior to the simpler linear model within the dose range studied. As illustrated by data from two representative patients (Figure 4), blood haemoglobin and erythrocyte counts increased in parallel on rcEPO stimulation. Thus, the mean erythrocyte haemoglobin concentration (Hb_E) remained constant during rcEPO therapy and its reciprocal could be used as a proportionality factor to relate blood haemoglobin and erythrocyte count. The mean value of K_{eff} was 0.043 g Hb dl^{-1} 1000 units (range: 0.018–0.071) and that of MRT_{Hb} was 10 weeks (range: 7.6–14) (Table 3). It is apparent from the coefficients of determination given in Table 3 and from the data shown in Figure 2 that the model equation describes the experimental data closely. The area under the unit-dose-effect-curve (AUEC), which is essentially the product of K_{eff} and MRT_{Hb}, may be calculated without curve-fitting to the model simply by application of the trapezoidal rule. This parameter summarizes the net response to a dose of rcEPO and may be a useful index of response in those patients in whom the data are insufficient to allow accurate determination of K_{eff} and MRT_{Hb}. A mean value of 0.44 mg Hb dl^{-1} weeks (range 0.25–0.99) was obtained. A significant positive correlation was found between rcEPO clearance and efficacy, as indicated by K_{eff} ($r = 0.64$; $P < 0.05$).

Table 3 Response measures after administration of rcEPO

Patient	Time on dialysis (months)	Initial Hb ($g\ dl^{-1}$)	Initial Haematocrit (%)	8 weeks Hb ($g\ dl^{-1}$)	8 weeks Haematocrit (%)	Dose/δHb3 ($u\ kg^{-1}$)	Time of δHb3 (weeks)	K_{eff} ($gHb\ dl^{-1}\ 1000\ u^{-1}$)	MRT_{Hb} (weeks)	Hb_E* (pg)	AUEC ($g\ dl^{-1}\ weeks^{-1}$)	$r^2 \cdot 100$ (%)
1	5	6.4	19	9.7	29	1400	9	0.042 ±0.004	9.08 ±0.79	30.7 ±0.7	0.420 ±0.034	79.6
2	105	5.2	18	6.8	22	4087	26	0.023 ±0.005	13.03 ±3.02	27.1 ±0.8	0.279 ±0.052	72.3
3	4	5.7	18	8.3	26	1570	10	0.062 ±0.003	8.72 ±0.72	29.6 ±0.4	0.59 ±0.02	94.7
5	10	6.7	21	8.7	26	2315	14	0.029 ±0.003	8.33 ±1.0	30.4 ±0.5	0.28 ±0.03	80.6
6	167	6.8	22	8.8	27	2000	11	0.033 ±0.004	8.76 ±1.14	33.0 ±0.6	0.31 ±0.03	78.7
7	37	6.4	20	9.5	27	910	6	0.043 ±0.008	8.25 ±2.27	28.9 ±0.76	0.38 ±0.08	65.7
8	2	6.5	20	10.3	32	1220	7.5	0.069 ±0.005	13.5 ±1.5	29.6 ±0.47	0.79 ±0.05	84.1
9	46	7.2	23	10	31	1380	9.2	0.074 ±0.006	13.9 ±1.33	29.6 ±0.43	0.99 ±0.08	82.1
10	7	6.9	22	10	32	1560	8	0.031 ±0.003	7.68 ±1.01	29.0 ±0.47	0.26 ±0.03	86.0
12	68	6.2	21	9.1	28	3270	16	0.018 ±0.002	13.9 ±2.6	28.5 ±0.49	0.25 ±0.02	87.0
Median:	41.5	6.6	21	9.5	27	1560	9.6	Mean 0.042 s.d. 0.016	10.02 1.75	29.2 2.6	0.445 0.232	87.0

δHb3 = a 3 mg dl^{-1} increase in blood haemoglobin concentration over the pretreatment value. Fitted parameters are given with their standard deviations.
*Hb_E = haemoglobin content of an erythrocyte, determined over the whole period for each individual by nonlinear regression analysis.
r^2 = Coefficient of determination for predicted *vs* observed haemoglobin concentration given as % of Hb concentration variance explained by the model.

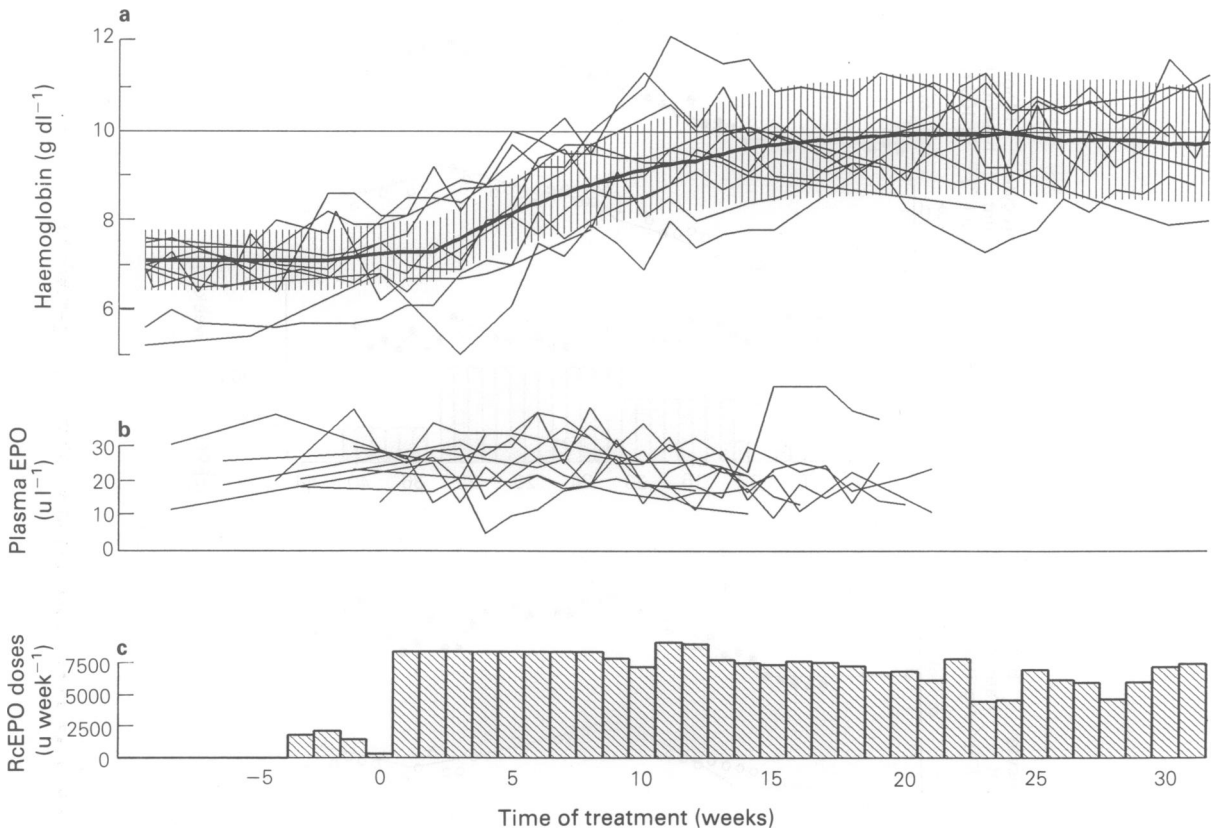

Figure 2 a) Individual haemoglobin time courses and mean response (bold line, calculated using equation 1 with the population parameters given in Table 3). b) Plasma erythropoietin concentrations (45 h post i.v. injection) determined at weekly intervals. c) rcEPO dosage.

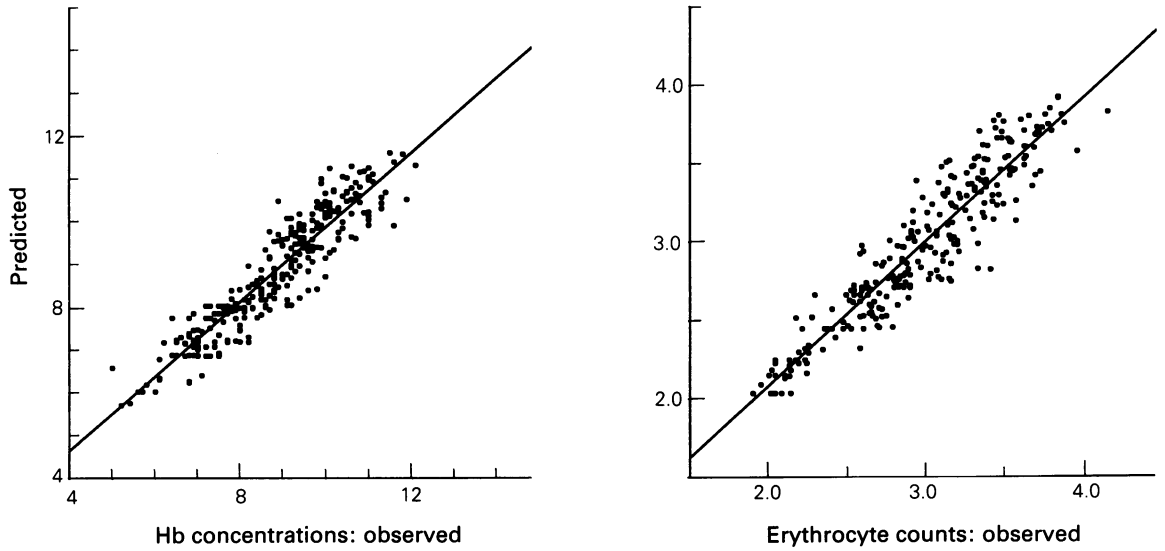

Figure 3 Relationship between the measured and predicted individual blood haemoglobin concentrations (g dl^{-1}) (left plot, $r^2 = 0.87$) and erythrocyte counts (mio μl^{-1}) (right plot, $r^2 = 0.86$).

Discussion

The values of rcEPO clearance determined in this study were comparable with those reported in other studies in which a similar dose was used (Flaharty *et al.*, 1990; Kampf *et al.*, 1989; Lim *et al.*, 1989; MacDougall *et al.*, 1989a,b; McMahon *et al.*, 1990; Neumayer *et al.*, 1989; Urabe *et al.*, 1988). However, a survey of the literature indicates that rcEPO clearance decreases about 4-fold over the dose-range of 5–100 u kg^{-1}. In addition, others have noted a time-dependent increase in rcEPO clearance over a few days after initiation of treatment (McMahon *et al.*, 1990). Lim *et al.* (1989) also found that the plasma half-life of rcEPO decreased from 7.7 to 4.6 h with time. Although no change in clearance with time was noted in our study, the half-life did show a

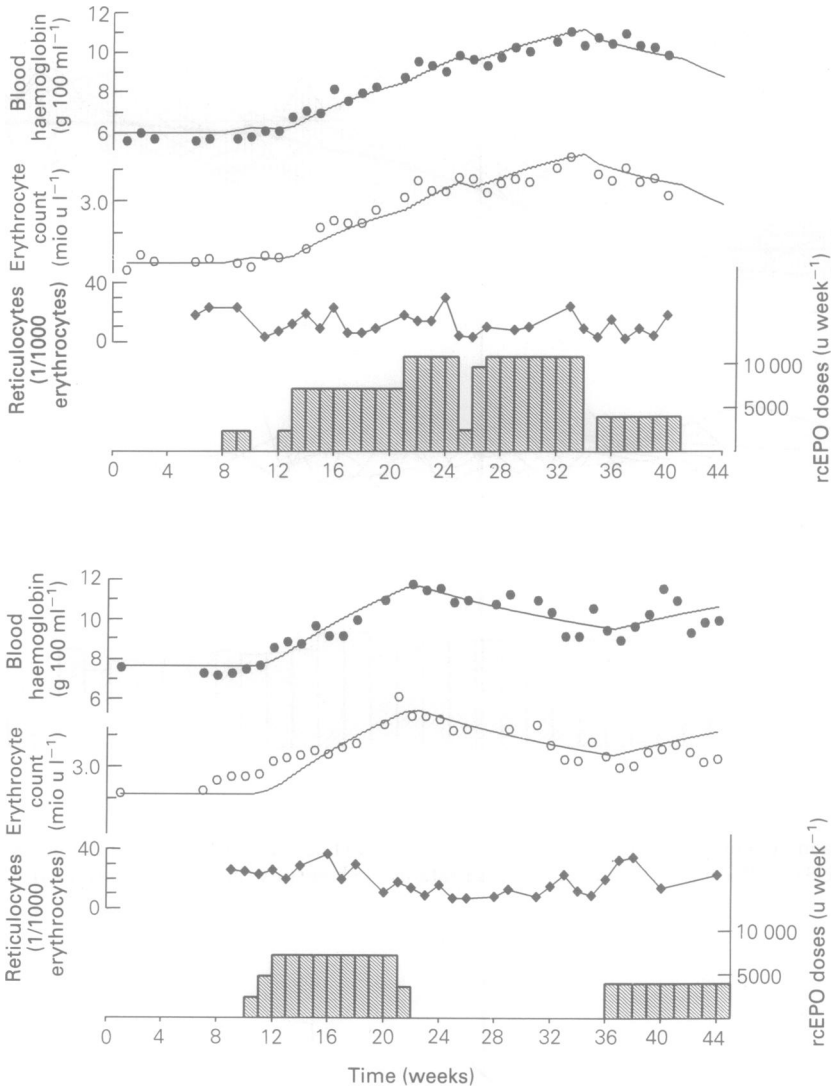

Figure 4 The time courses of blood haemoglobin (●), erythrocyte count (○), and reticulocyte count (◆) in response to the rcEPO dose regime (depicted as a bar plot) in patients 9 (a) and 3 (b). Haemoglobin and erythrocyte counts were fitted simultaneously with the same parameter values of K_{eff} and MRT_{Hb} with a constant Hb_E value serving as proportionality factor between the two measures. The bold lines are the model estimates of the time courses.

tendency to decrease, indicating that in some patients the volume of distribution of rcEPO may have decreased. This may be a consequence of the increase in haematocrit during rcEPO treatment.

Subcutaneous injection of rcEPO is preferred increasingly over i.v. administration because patients can perform these injections themselves and the dose-(cost)-effect relationship is more favourable (Bommer *et al.*, 1988). The latter may be due to the prolonged elevation of plasma rcEPO concentration following this mode of administration. The mechanism of s.c. absorption of rcEPO is unclear since the vascular endothelium is poorly permeable to large glycoproteins. Access through pores or transport via the lymphatic system seems likely.

Pharmacodynamics

The efficacy of rcEPO is usually assessed from the increase in blood haemoglobin over an arbitrary time interval (Faulds & Sorkin, 1989). However, this only allows comparisons when a fixed dosing schedule is maintained. In contrast, the approach presented here utilizes information over the whole treatment time course, summarizing it in the two constants K_{eff} and MRT_{Hb}, which describes the production and destruction of erythrocytes. K_{eff} reflects the effectiveness of the drug in stimulating erythrocyte release and the individual's ability to respond to it. The positive correlation between K_{eff} and the decrease in rcEPO may reflect a higher extraction of rcEPO by bone marrow in those patients with superior response. Alternatively, those patients with higher rcEPO clearance may be healthier and, therefore, respond better to treatment.

Calculation of MRT_{Hb} in rcEPO treated patients, as described here, may provide a cheap and safe alternative to current radiochemical methods for estimating erythrocyte survival time (e.g. ^{51}chromium *ex vivo* labelling of erythrocytes). Under a constant dose scheme, MRT_{Hb} is the main parameter responsible for the time span until the new steady state level of haemoglobin is achieved. MRT_{Hb}, as calculated here, will of course be affected by blood losses during haemodialyses and blood sampling.

The mean onset time of effect (MIT_{Hb}) could not be determined with sufficient precision in this study and was approximated at 2 days. Its inclusion in the model seemed necessary since the onset of the effects of rcEPO is delayed. MIT_{Hb} is an empirical constant that corresponds partly to the (reciprocal of the) transfer rate constant from the plasma into the bone marrow (Holford & Sheiner, 1982).

It should be emphasized that the linear pharmacodynamic model that we have used is largely empirical and cannot be extrapolated to doses far outside those which we have studied. This limitation is evident when extrapolating the therapeutic effects of rcEPO down to the endogenous release rate of erythropoietin. This resulted in a population mean of the endogenous erythropoietin release rate of 21,300 units per week, a value which seems unrealistically high and incompatible with the erythropoietin formation rates extrapolated from rcEPO clearance. In addition, on initiation of therapy, some patients showed a higher response than expected from the curves determined by regression analysis of all data points of the respective individual. Indeed, a recent study (Uehlinger *et al.*, 1992) demonstrated saturability in rcEPO efficacy in an analysis of rcEPO population pharmacodynamics. However, in their study nonlinearity was evident especially at higher doses than given in our study. The simple model used by us offered a reasonable description of the overall time-course of response, and may be useful in designing optimal dosing schedules. Using the pharmacodynamic parameters derived in this study it is predicted that a rise in blood haemoglobin of 4 g 100 ml^{-1} would require continuous administration of 42 units kg^{-1} rcEPO. In the absence of a loading dose, the new steady state should be achieved in about 35 weeks. The model that we have derived might also be valuable in comparing different formulations of rcEPO.

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References

Annable, L., Cotes, P. M. & Mussett, M. V. (1972). The second international reference preparation of erythropoietin, human, urinary, for bioassay. *Bulletin Organization Monde Santé*, **47**, 99–112.

Bommer, J., Ritz, E., Weinreich, T., Bommer, G. & Ziegler, T. (1988). Subcutaneous erythropoietin. *Lancet*, **ii**, 406.

Browne, J. K., Cohen, A. M., Egrie, J. C., Lai, P. H., Lin, F.-K., Strickland, T., Watson, E. & Stebbing, N. (1986). Erythropoietin: gene cloning, protein structure, and biological properties. *Cold Spring Harbor Symposia on Quantitative Biology*, **60**, 693–702.

Cotes, P. M., Pippard, M. J., Reid, C. D. L., Winearls, C. G., Oliver, P. O. & Royston, J. P. (1989). Characterization of the anaemia of chronic renal failure and the mode of its correction by a preparation of human erythropoietin (r-HuEPO). An investigation of the pharmacokinetics of intravenous erythropoietin and its effects on erythrokinetics. *Quart. J. Med.*, **70**, 113–137.

Cutler, D. J. (1978). Numerical deconvolution by least squares: use of prescribed input functions. *J. Pharmacokin. Biopharm.*, **6**, 227–241.

Dixon, W. J. (ed.) (1987). *BMDP Biomedical computer programs*. Berkeley, California: University of California Press.

Eckardt, K.-U., Kurtz, A., Hirth, P., Scigalla, P., Wieczorek, L. & Bauer, C. (1988). Evaluation of the stability of human erythropoietin in samples for radioimmunoassay. *Klin. Wochenschr.*, **66**, 241–245.

Faulds, D. & Sorkin, E. M. (1989). Epoietin (recombinant human erythropoietin) A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in anaemia and the stimulation of erythropoiesis. *Drugs*, **38**, 863–899.

Fisher, J. W. (1988). Pharmacologic modulation of erythropoietin production. *Ann. Rev. Pharmac. Tox.*, **28**, 101–122.

Flaharty, K. K., Caro, J., Erslev, A., Whalen, J. J., Morris, E. M., Bjornsson, T. D. & Vlasses, P. H. (1990). Pharmacokinetics and erythropoietic response to human recombinant erythropoietin in healthy men. *Clin. Pharmac. Ther.*, **47**, 557–564.

Fukuda, M. N., Sasaki, H., Lopez, L. & Fukuda, M. (1989). Survival of recombinant erythropoietin in the circulation: the role of carbohydrates. *Blood*, **73**, 84–89.

Gribben, J. G., Devereux, S., Thomas, N. S. B., Keim, M., Jones, H. M., Goldstone, A. H. & Linch, D. C. (1990). Development of antibodies to unprotected glycosylation sites on recombinant human GM-CSF. *Lancet*, **335**, 434–437.

Gurland, H. J., Moran, J., Samtleben, W., Scigalla, P. & Wieczorek, L. (eds) (1991). Erythropoietin in renal and non-renal anemias. *Contrib. Nephrol.*, **88**, 000–000.

Holford, N. H. G. & Sheiner, L. B. (1982). Kinetics of pharmacologic response. *Pharmac. Ther.*, **16**, 143–166.

Hughes, R. T., Cotes, P. M., Oliver, D. O., Pippard, M. J., Royston, P., Stevens, J. M., Strong, C. A., Tam, R. C. & Winearls, C. G. (1989). Correction of the anaemia of chronic renal failure with erythropoietin: pharmacokinetic studies in patients on haemodialysis and CAPD. *Contrib. Nephrol.*, **76**, 122–130.

Imbimbo, B. P., Imbimbo, E., Daniotti, S., Verotta, D. & Bassotti, G. (1988). A new criterion for selection of pharmacokinetic multiexponential equations. *J. pharm. Sci.*, **77**, 784–789.

Kampf, D., Kahl, A., Passlick, J., Pustelnik, A., Eckardt, K.-U., Ehmer, B., Jacobs, C., Baumelou, A., Grabensee, B. & Gahl, G. M. (1989). Single-dose kinetics of recombinant human erythropoietin after intravenous, subcutaneous and intraperitoneal administration. *Contrib. Nephrol.*, **76**, 106–111.

Lim, V. S., DeGowin, R. L., Zavala, D., Kirchner, P. T., Abels, R., Perry, P. & Fangman, J. (1989). Recombinant human erythropoietin treatment in pre-dialysis patients. *Ann. Int. Med.*, **110**, 108–114.

Lui, S. F., Chung, W. W. M., Leung, C. B., Chan, K. & Lai, K. N. (1989). Pharmacokinetics and pharmacodynamics of subcutaneous and intraperitoneal administration of recombinant human erythropoietin in patients on continuous ambulatory peritoneal dialysis. *Clin. Nephrol.*, **33**, 47–51.

MacDougall, I. C., Roberts, D. E., Neubert, P., Dharmasena, A. D., Coles, G. A. & Williams, J. D. (1989a). Pharmacokinetics of intravenous, intraperitoneal, and subcutaneous recombinant erythropoietin in patients on CAPD. *Contrib. Nephrol.*, **76**, 112–121.

MacDougall, I. C., Roberts, D. E., Neubert, P., Dharmasena, A. D., Coles, G. A. & Williams, J. D. (1989b). Pharmacokinetics of recombinant human erythropoietin in patients on continuous ambulatory peritoneal dialysis. *Lancet*, **i**, 425–427.

MacDougall, I. C., Roberts, D. E., Coles, G. A. & Williams, J. D. (1991). Clinical pharmacokinetics of Epoietin (recombinant human erythropoietin). *Clin. Pharmacokin.*, **20**, 99–113.

McMahon, F. G., Vargas, R., Ryan, M., Jain, A. K., Abels, R. I., Perry, B. & Smith, I. L. (1990). Pharmacokinetics and effects of recombinant human erythropoietin after intravenous and subcutaneous injections in healthy volunteers. *Blood*, **76**, 1718–1722.

Neumayer, H.-H., Brockmöller, J., Fritschka, E., Roots, I., Scigalla, P. & Wattenberg, M. (1989). Pharmacokinetics of recombinant human erythropoietin after s.c. administration and in long-term i.v. treatment in patients on maintenance hemodialysis. *Contrib. Nephrol.*, **76**, 131–142.

Rossum, J. M. van, deBie, J. E. G. M., van Lingen, G. & Teeuwen, H. W. A. (1989). Pharmacokinetics from a dynamical systems point of view. *J. Pharmacokin. Biopharm.*, **17**, 365–392.

Spivak, J. L. & Hogans, B. B. (1989). The *in vivo* metabolism of recombinant human erythropoietin in the rat. *Blood*, **73**, 90–99.

Steimer, J. L., Mallet, A., Golmard, J. L. & Boisvieux, J. F. (1984). Alternative approaches to estimation of population pharmacokinetic parameters: comparison with the nonlinear mixed-effect model. *Drug Metab. Rev.*, **15**, 265–292.

Stone, W. J., Graber, S. E., Krantz, S. B., Dessypris, E. N., O'Neil, V. L., Olsen, N. J. & Pincus, T. P. (1988). Treatment of the anaemia of predialysis patients with recombinant human erythropoietin: a randomized, placebo-controlled trial. *Am. J. med. Sci.*, **296**, 171–179.

Uehlinger, D. E., Gotch, F. A. & Sheiner, L. B. (1992). A pharmacodynamic model of erythropoietin therapy for uremic anemia. *Clin. Pharmac. Ther.*, **51**, 76–89.

Urabe, A., Takaku, F., Mizoguchi, H., Kubo, K., Ota, K., Shimizu, N., Tanak, K., Mimura, N., Nihei, H., Koshikawa, S., Akizawa, T., Akiyama, N., Otsubo, O., Kawaguchi, Y. & Maeda, T. (1988). Effect of recombinant human erythropoietin on the anaemia of chronic renal failure. *Int. J. Cell Clon.*, **6**, 179–191.

Verotta, D. (1990). Comments on two recent deconvolution methods. *J. Pharmacokin. Biopharm.*, **18**, 483–499.

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