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Original article

Pharmacokinetics of recombinant human erythropoietin applied subcutaneously to children with chronic renal failure

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Abstract. The single-dose pharmacokinetics of recombinant human erythropoietin (rHuEPO) given SC was investigated in 20 patients aged 7–20 years at different stages of chronic renal failure. In a pilot study we confirmed the lower bioavailability of the drug in 2 children when given SC compared with the IV route (24% and 43%, respectively). Following administration of 4,000 units/m^2, rHuEPO SC effective serum erythropoietin concentrations increased from a mean baseline level (±SD) of 23±13 units/l to a mean peak concentration of 265±123 units/l, which was reached after 14.3±9.4 h, followed by a slow decline until baseline values were attained at 72 h. Mean residence time was 30±9 h and mean elimination half-time 14.3±7 h. The single-dose kinetics of SC rHuEPO in children with different degrees of renal failure are comparable to those in adult patients. Possibly, the higher efficacy of SC rHuEPO in patients with renal anaemia compared with IV rHuEPO is related to its prolonged action.

Key words: Anaemia – Chronic renal failure – Erythropoietin – Pharmacokinetics

Introduction

Anaemia is a predictable complication of chronic renal failure (CRF) in children [1, 2]. Its mechanisms are complex, but insufficient production of erythropoietin (EPO) in relation to the degree of anaemia appears to be the most important factor. The gene for human EPO has been cloned and recombinant human EPO (rHuEPO) is available for clinical use [3]. The efficacy of EPO in correcting renal anaemia in adults and children [4–10] with end-stage renal disease has been demonstrated. However, the optimum route of administration and dosage of rHuEPO are still debated.

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After IV administration to adult patients with CRF, the elimination half-life ($t_{1/2}$) of rHuEPO is relatively short (5–12 h) compared with that following SC application [11–14]. This route may be preferable to the IV route, allowing less frequent application and injections at home. In adults with CRF maintained on SC rHuEPO, the drug appears to be more effective than when given IV, despite the lower bioavailability [11, 13, 15, 16]. For children, SC administration appears to be of special benefit in preterminal CRF and during continuous ambulatory peritoneal dialysis (CAPD) [4, 9], where a permanent vascular access is not available.

The pharmacokinetics of rHuEPO have been studied in adult patients with CRF [14, 17–19] since sensitive and specific assays for the hormone have become available [20]. In this paper we report the results of a single-dose pharmacokinetic study of SC rHuEPO in paediatric CRF patients selected for long-term treatment with the drug.

Patients and methods

Patients

Twenty paediatric patients with CRF were investigated. The study was approved by the local ethics committee. Informed consent was obtained from the patients or their parents. Table 1 presents the relevant clinical data. The primary kidney disorder was congenital or hereditary in 14 patients and acquired in 6 children. No child had previously been treated with rHuEPO. All patients were in a stable clinical condition without infection. Liver function tests were normal. The mean base haemoglobin level was 7.0±1.0 (range 5.2–9.0) g/dl.

At the time of the study, 6 patients were undergoing regular haemodialysis, 7 were being treated with CAPD and 7 were on conservative treatment [preterminal CRF, mean serum creatinine level 6.3 (range 3.2–12.0) mg/dl]. Dialysis was started at a mean of 28 (range 0.5–100) months prior to the pharmacokinetic investigation. In patients on CAPD, no peritonitis was observed in the 3 months prior to the start of rHuEPO therapy.

Study design

In a pilot study we compared the response of 2 patients with CRF on dialysis, a boy and a girl, both 15 years old, to a rHuEPO dose of 4,000

Table 1. Clinical characteristics of 20 paediatric patients with chronic renal failure receiving 4,000 units recombinant human erythropoietin (rHuEPO)/m²

	Mean ± SE	Range
Age (years)	12.7 ± 3.9	6.8 – 19.9
Weight (kg)	34.0 ±13.9	17.0 – 59.8
Height (cm)	140.6 ±24.0	111 –181
Body surface area (m²)	1.16± 0.34	0.68– 1.77

units/m² given as a SC injection in the thigh and 1 week later as an IV bolus. This pilot study allowed investigation of EPO distribution, bioavailability and other pharmacokinetic parameters. In the main study we gave the same dose of rHuEPO SC to the rest of the patients. rHuEPO was supplied as a sterile buffered solution (1 ampoule = 4,000 units/ml, Cilag, Sulzbach, Germany).

For the determination of serum EPO concentrations, blood samples were taken from an indwelling venous catheter immediately before and 10, 20, 30 and 60 min and 2, 3, 4, 5, 8, 12, 16, 18, 20, 24, 30, 36, 48 and 72 h (in some children 96 h) after rHuEPO application. The centrifuged serum samples were stored at –20° C until analysis. In haemodialysed patients the drug was administered immediately after termination of the session and blood was analysed only during dialysis-free intervals. The radioimmunoassay used for EPO estimation had been described previously [20]. It has a lower detection limit of 5 units/l with an interassay coefficient of variation (CV) of 6.7% for an EPO concentration of 44 units/l. In normal children below 15 years of age, serum EPO levels determined by the same assay range between 7 and 47 units/l (mean 18.8 units/l); in adolescents up to the age of 20 years they are between 13 and 36 units/l (mean 21 units/l) [21].

Data analysis

Pharmacokinetic parameters were estimated using standard methods. Both model-independent and model-dependent approaches were used for the pharmacokinetic analysis of data obtained from the two modes of rHuEPO administration. The base level of serum EPO was subtracted from the measured EPO concentration profile before a pharmacokinetic model was fitted to the concentration versus time data.

Pharmacokinetic modelling. Following an IV bolus injection, several one-, two- and three-compartment open models (COM) using exponential functions were applied to describe EPO serum concentrations. After SC administration, a one-COM with first-order or zero-order input was applied for prediction of the EPO concentration profile where TKO is the unbiased estimation of the time at which peak concentration occurred (t_{max}) [22]. All non-linear regression analyses for the estimation of the kinetic parameters were performed by MKMODEL, an extended least squares modelling programme developed by Holford [23].

Total clearance (C) was estimated by non-compartmental analysis from the area under the serum concentration versus time curve (AUC) according to the equation: $C = f \times dose/AUC$, where f is the fraction of the dose absorbed (bioavailability). Mean residence time (MRT), i.e. the average time one molecule of EPO remains in the central compartment,

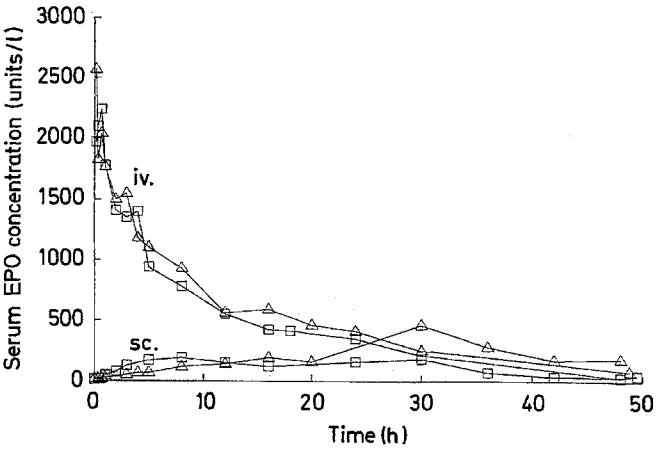

Fig. 1. Serum concentration of erythropoietin (*EPO*) versus time profile after IV and SC application of 4,000 units recombinant human EPO (rHuEPO)/m² in 2 adolescents treated with continuous ambulatory peritoneal dialysis (△) and haemodialysis (□), respectively

was calculated by using the equation: MRT = AUMC/AUC, where AUMC is the model-independent area under the first moment curve.

The model-independent AUC up to the last measured serum concentration was estimated by using the linear trapezoidal rule with extrapolation to infinity (∞), determined by using the ratio of the least measured serum concentration over the terminal rate constant (LM/lz); lz was estimated by unweighed linear regression of log Cp (units/l) versus time for the last 3 or 4 data points. $t_{1/2}$ was defined as ln2/lz (= 0.683/lz).

Statistics. Mean standard deviation and CV were determined by using standard equations [24].

Results

SC versus IV rHuEPO disposition

Figure 1 and Table 2 demonstrate the differences in the disposition and pharmacokinetics of rHuEPO when given to the same 2 patients by the SC and later by the IV route. Following the IV bolus injection, the serum EPO concentration versus time profiles were best characterized by a two-COM according to the results of MKMODEL fit statistics (Log Likelihood, Schwartz Criterion, WSSR) as demonstrated by using MODELTEST [23].

When the drug was given by the SC route, the apparent peak serum concentration (c_{max}) was delayed and much lower when compared with the IV application. The slow EPO release from the SC tissue stores produced an EPO

Table 2. Comparison of pharmacokinetic parameters after application of 4,000 units rHuEPO/m² by the IV and the SC route to 2 patients on dialysis aged 15 years

Patient	Application	c_{max} (units/l)	AUC∞ (units/l per hour)	C (l/h)	Vd (l)	$t^1/_2\,\beta$ (h)	f (%)
1 (female,	IV	2,225	20,187	0.277	0.0657	10.8	100
weight 38.8 kg)	SC	176	4,820	1.162	0.1111	3.0	23.9
2 (male,	IV	2,554	24,309	0.279	0.0484	10.9	100
weight 58.6 kg)	SC	438	10,468	0.649	0.1552	15.4	43.1

Vd, Volume of distribution; f, bioavailability; c_{max}, peak serum concentration; AUC, extrapolated area under the serum concentration versus time curve; C, clearance; $t_{1/2}$, half-life

Table 3. Pharmacokinetic data of 4,000 units rHuEPO/m² given SC to 20 paediatric patients with chronic renal failure[a]

Non-compartmental analysis		
c_{max}	(units/l)	265.4 ±123.5
t_{max}	(h)	14.3 ± 9.4
AUC ∞	(units/l per hour)	7,684 ± 3,141
AUC-24 h	(units/l per hour)	3,731 ± 1,603
C/f	(l/h)	0.6709 ±0.3699
One-COM with zero-order input		
t_{lag}	(h)	0.55± 0.83
TKO	(h)	14.1 ± 9.4
MRT	(h)	29.7 ± 9.2
$t^1/_2$	(h)	14.3 ± 7.2

t_{max}, Time at which peak concentration occurred; t_{lag}, lag time; TKO, unbiased estimation of t_{max}; MRT, mean residence time

[a] Mean ± SD

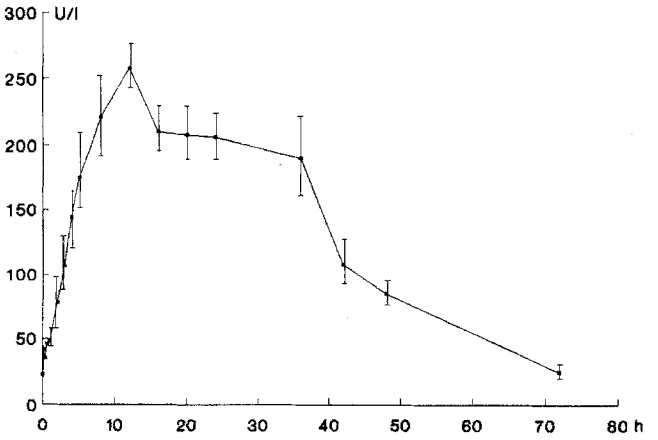

Fig. 2. Serum EPO concentration versus time profile after SC application of 4,000 units rHuEPO/m² in 20 paediatric patients with chronic renal failure (mean ± SEM)

concentration-time profile resembling a one-COM with a zero-order input into the central compartment. The SC rHuEPO disposition in the 2 patients presented in Fig. 1 was not as smooth as expected, but rather discontinuous. After c_{max} was reached, EPO serum levels decreased but rose again between 20 and 30 h after application.

SC rHuEPO disposition and kinetics in 20 patients with CRF

Serum EPO base levels in the 20 patients tested were within the range of healthy subjects (mean 23±13 units/ml) except in 1 subject (70 units/l). After SC administration of 4,000 units rHuEPO/m², the mean serum EPO concentration increased slowly with a mean lag time (t_{lag}) of 0.55 h until a mean c_{max} of 265 units/l was reached, with a mean t_{max} of 14.3 h (Table 3, Fig. 2). Subsequently, it decreased to 205 and 78 units/l after 24 and 48 h, respectively; 72 h after administration the mean serum EPO level almost reached the basal value (25 vs. 23 units/l, $n = 5$). In a few patients, slightly elevated EPO concentrations were observed up to 96 h. Additional pharmacokinetic results are presented in Table 3. No significant differences were found between the pharmacokinetic data of young and older children or between patients with preterminal and end-stage CRF.

Discussion

Both monophasic and biphasic serum concentration profiles have been described in adults following the IV administration of rHuEPO [13, 14, 17, 18, 25–27]. In a recent paper describing the pharmacokinetics of IV rHuEPO given to nine children with end-stage renal disease, a monophasic profile was described; however, the curves presented in that paper suggest rather a two-COM [28]. Curve fitting of the IV data from our children with CRF demonstrated that the two-COM is the most appropriate model.

In contrast, SC administration of rHuEPO resulted in a completely different drug disposition and the pharmacokinetics were characterized by a lower c_{max}, a smaller AUC and a prolonged $t_{1/2}$. For the interpretation of these data one should be aware that, strictly speaking, pharmacokinetic parameters resulting from IV and SC administration cannot be compared, because prolonged drug release from the SC tissue into the central compartment influences both disposition and kinetics of the drug. In addition, we often observed a discontinuous drug disposition after SC injection as if, for some reason, the drug transfer to the central compartment had been transiently interrupted. We believe that the one-COM with zero-order input is able to predict adequately the serum EPO concentration-time course after SC administration.

Our results are similar to the pharmacokinetic data obtained by Boelaert et al. [12] after SC rHuEPO administration in adult patients on CAPD; in the latter study, twice our dose, i.e. 300 units/kg (vs. 133 units/kg in our series), was given to adult CAPD patients, resulting in mean AUC and c_{max} values which were about twice as high as in our patients. Macdougall et al. [11], who gave an rHuEPO dose (120 units/kg) similar to that used in our study, observed a comparable mean AUC, but mean c_{max} was only 176 units/l and mean EPO levels remained above the baseline up to 96 h. Stockenhuber et al. [27], who injected 100 units rHuEPO/kg to adult patients on dialysis, reported c_{max} and AUC values less than half of those observed in this study. In these studies, however, $t_{1/2}$ was similar to our findings. In other pharmacokinetic studies, where smaller doses (40–50 units/kg) of SC rHuEPO were given to adult patients [14, 25, 29] or children [28], c_{max} was relatively low and t_{max} was sometimes delayed up to 24 h. It should be stressed that our results obtained in CRF patients aged 7–20 years cannot be translated to younger children.

The absolute bioavailability found in our pilot study after SC rHuEPO administration was in the range reported in adult patients with end-stage renal disease (21%–36%) [11, 14, 27]. In a recent paediatric study, it ranged between 21% and 60% with a mean of 40%. The low bioavailability of rHuEPO compared with other hormones given SC (such as growth hormone [30]) may be due to the large size of the EPO molecule or to the presence of peptidases in the skin preventing absorption of the drug [31].

No significant difference was found in the decay of serum EPO between our patients with moderate (preterminal) CRF and those with end-stage renal disease. This confirms the results of Kindler et al. [13] who investigated

the pharmacokinetics of IV EPO in adult patients with various degrees of CRF and concluded that EPO elimination occurs mainly through non-renal mechanisms.

Our study demonstrates that the single-dose pharmacokinetics of rHuEPO in paediatric patients with CRF are similar to those observed in adult patients. Although the prolonged presence of high serum EPO levels after SC rHuEPO injection do not necessarily indicate a sustained stimulatory effect on the bone marrow, recent clinical studies suggest that the rHuEPO requirement is less with the same dose given by the SC compared with the IV route [14, 15]. Our experience suggests that a single SC dose of 150 units rHuEPO/kg applied once a week is sufficient to produce and maintain the required target haemoglobin levels in most children with CRF [10]. It remains to be investigated if a more frequent SC administration without changing the total dosing rate results in a higher efficacy of the drug and whether $t_{1/2}$ is progressively shortened by repeated administration, as found in patients receiving EPO by the IV route [17, 32].

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