Pediatr Nephrol (2002) 17:933–937
DOI 10.1007/s00467-002-0932-0

ORIGINAL ARTICLE

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Pharmacokinetics of darbepoetin alfa in pediatric patients with chronic kidney disease

Received: 9 August 2001 / Revised: 27 March 2002 / Accepted: 4 April 2002 / Published online: 14 September 2002

Abstract Darbepoetin alfa is a novel erythropoiesis-stimulating protein with a two- to threefold longer half-life than recombinant human erythropoietin (epoetin) in adult patients with chronic kidney disease (CKD). This randomized, open-label, crossover study was conducted to determine the pharmacokinetic profile of darbepoetin alfa in pediatric patients with CKD. Twelve patients 3–16 years of age with CKD were randomized and received a single 0.5 μg/kg dose of darbepoetin alfa administered intravenously (IV) or subcutaneously (SC). After a 14- to 16-day washout period, patients received an identical dose of darbepoetin alfa by the alternate route. After IV administration, the mean clearance of darbepoetin alfa was 2.3 ml/h per kg, with a mean terminal half-life of 22.1 h. After SC administration, absorption was rate limiting, with a mean terminal half-life of 42.8 h and a mean bioavailability of 54%. Comparison of these results with those from a previous study of darbepoetin alfa in adult patients indicated that the disposition of darbepoetin alfa administered IV or SC is similar in adult and pediatric patients, although absorption may be slightly more rapid in pediatric patients after SC dosing. The mean terminal half-life of darbepoetin alfa in this study was approximately two- to fourfold longer than that previously reported for epoetin in pediatric patients.

Keywords Darbepoetin alfa · Novel erythropoiesis-stimulating protein · Pharmacokinetics · Anemia · Chronic kidney disease

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Introduction

Recombinant human erythropoietin (epoetin) has been shown to increase hemoglobin and reduce the need for red blood cell transfusions in pediatric patients with chronic kidney disease (CKD) [1, 2]. However, administration of epoetin is often required two or three times weekly, which can be a burden for younger patients and their parents.

Previous work has indicated that there is a direct relationship between the degree of glycosylation, serum half-life, and in vivo biological activity of epoetin [3]. Darbepoetin alfa [novel erythropoiesis-stimulating protein (NESP)] is a glycoprotein that was designed by introducing five amino acid changes into the primary sequence of epoetin to create two additional *N*-linked carbohydrate addition sites. Consequently, darbepoetin alfa has five *N*-linked carbohydrate chains, whereas epoetin has three. Although both molecules stimulate erythropoiesis by the same mechanism, darbepoetin alfa is biochemically distinct from epoetin, with a different amino acid sequence and an increased molecular weight.

Results from a previous pharmacokinetic study of darbepoetin alfa conducted in adult patients with CKD indicated that the terminal half-life of darbepoetin alfa was approximately threefold longer than that of epoetin after intravenous (IV) administration (25.3 h versus 8.5 h, respectively) and approximately twofold longer

after subcutaneous (SC) administration (48.8 h versus 24 h, respectively) [4, 5, 6]. However, the pharmacokinetic profile of darbepoetin alfa has not yet been evaluated in the pediatric population. This randomized, open-label, crossover study was conducted to evaluate the pharmacokinetics of darbepoetin alfa in pediatric patients with CKD.

Patients and methods

Patients

The protocol and consent form were approved by the institutional review board for each study center, and written informed consent was obtained from the parent, legal guardian, and patient (where applicable) before initiation of study procedures. Patients 1–16 years of age with CKD who were receiving hemodialysis or peritoneal dialysis or not yet receiving dialysis {glomerular filtration rate (GFR) <30 ml/min per 1.73 m^2 as estimated by the Schwartz formula [7, 8]} were enrolled at five study centers. Patients were receiving epoetin before the study, but had not received epoetin within 7 days of the first injection of darbepoetin alfa. Additional entry criteria included a hemoglobin concentration ≥9.0 g/dl and adequate iron stores (transferrin saturation ≥20%). Patients were excluded if they had uncontrolled hypertension (diastolic blood pressure >90 mmHg), elevated liver enzymes (>2 times the upper limit of the normal range), hematological disease or hemoglobinopathies, or other disorders that could interfere with the response to darbepoetin alfa or epoetin.

Study design

This was a randomized, open-label, crossover study. After a 1-week screening period, eligible patients were randomized to receive a single 0.5-μg/kg dose of darbepoetin alfa administered IV or SC. After a 14- to 16-day washout period, patients received an identical dose of darbepoetin alfa by the alternate route. The randomization was generated by computer program and was stratified by age group (1–6, 7–11, and 12–16 years).

A darbepoetin alfa dose of 0.5 μg/kg was selected because it allowed for a direct comparison with a previous pharmacokinetic study of darbepoetin alfa conducted in adult patients with CKD [4]. In addition, previous clinical experience with darbepoetin alfa has indicated that doses of 0.45–0.75 μg/kg are safe and effective for the treatment of anemia in the CKD population. Based on peptide mass, 0.5 μg/kg of darbepoetin alfa is approximately equivalent to 100 U/kg of recombinant human erythropoietin.

IV doses were administered as a bolus within 15 s, and SC doses greater than 1 ml in volume could be divided into two injections (delivered in rapid succession). A pre-dose blood sample for the determination of baseline erythropoietin concentrations was collected within 30 min of the first study drug administration. In addition, a pre-dose blood sample was drawn on the day of the second darbepoetin alfa injection for the determination of pre-dose levels. For the evaluation of darbepoetin alfa pharmacokinetics after IV administration, blood samples were collected 5 min, 30 min, and 1, 2, 5, and 8 h after dosing, once between 24 and 48 h after dosing, once between 72 and 96 h after dosing, and at 168 h after dosing. After SC administration, blood samples were collected 6, 24, 34, 48, 58, 72, and 96 h after dosing; 2 additional samples were taken at least 16 h apart between 120 and 168 h after dosing. Sample times for IV and SC dosing were optimized for each route to ensure proper characterization of the individual profiles. Blood sample volumes were intentionally minimized (1.5 ml per sample) for this pediatric population. As darbepoetin alfa is a protein therapeutic, serum for measurement of possible antibody formation to darbepoetin alfa was collected before each darbepoetin alfa dose and at the end of the study.

Pharmacokinetic analysis

Serum samples were analyzed using the Quantikine in vitro diagnostics epoetin alfa enzyme-linked immunosorbent assay (ELISA) kit (R and D Systems, Minneapolis, Minn., USA). The standard curve was constructed using darbepoetin alfa, and quality controls ensured individual assay quality. In validation tests, the intra-assay precision ranged from 1% to 4% for spiked samples and from 2% to 4% for clinical serum samples. Interassay precision ranged from 5% to 7% for spiked samples. The assay range was 5 ng/ml (upper limit of quantitation) to 0.078 ng/ml (lower limit of quantitation), and the detection limit of the assay was 0.005 ng/ml. Serum concentrations of darbepoetin alfa were corrected for baseline endogenous erythropoietin or epoetin concentrations (which cross-react with the assay), as quantified against the darbepoetin alfa standard curve, by direct subtraction. Cross-reactivity of recombinant human growth hormone in the ELISA was assessed at therapeutically relevant concentrations and was verified to be negligible.

The pharmacokinetic analysis was performed using conventional noncompartmental methods [9]. Single-dose pharmacokinetic parameters were estimated for each subject after both IV and SC dosing, as appropriate. For estimation of the terminal phase, at least the last three data points that consistently decreased and were greater than or equal to twice the limit of quantitation in the darbepoetin alfa assay were included in the extrapolation. After IV dosing, peak concentration at time zero ($C_{0\ by}$ linear back-extrapolation), initial volume of distribution (V_o=dose/C_o), area under the serum concentration-time curve from time zero to infinity (AUC), clearance (CL=dose/AUC), terminal half-life ($t_{1/2}$=ln 2/λ_z, where λ_z is the terminal rate constant), and volume of distribution at steady state (V_{ss}) were calculated. After SC dosing, peak serum concentration (C_{max}) and the time at which C_{max} was observed (T_{max}), AUC, and $t_{1/2}$ were estimated. Bioavailability ($F=AUC_{SC}/AUC_{IV}$) after SC dosing was estimated for each subject for whom both IV and SC profiles could be evaluated; if necessary, corrections for dose changes were made.

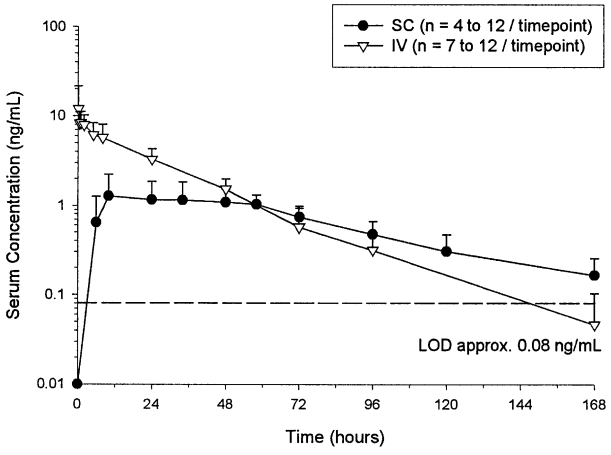
Of the 12 patients who received both IV and SC darbepoetin alfa, sufficient data were available for the extrapolation of 10 IV and 8 SC concentration-time profiles. Extrapolation was not possible where five or fewer data points were available, or samples were not collected beyond 72 h. For the 8 SC profiles for which extrapolation could be performed, 7 IV profiles could also be fully evaluated.

Results

Thirteen patients were randomized to the IV→SC group (n=7) or the SC→IV group (n=6). One patient had a GFR exceeding the inclusion criteria of <30 ml/min per 1.73 m^2 and was withdrawn from the study before receiving darbepoetin alfa. Thus, pharmacokinetic samples were collected for 12 patients (6 in each group). Demographic and baseline characteristics are presented for these patients in Table 1. All patients had been receiving epoetin before entry into the study.

A summary of noncompartmental pharmacokinetic parameters after IV and SC administration of darbepoetin alfa is provided in Table 2 and Fig. 1. After IV administration, darbepoetin alfa serum concentrations declined in a biphasic manner, with quantifiable concentrations up to at least 96 h after dosing. The mean (±SD) terminal half-life was 22.1±4.8 h. The mean volume of distribution at steady state was 80.9±32.5 ml/kg.

After SC administration, darbepoetin alfa serum concentrations increased slowly to peak concentrations between 10 and 58 h after dosing (mean T_{max}=36.2±



Note: Not all patients had data available at all time points

Fig. 1 Mean (SD) baseline-corrected darbepoetin alfa serum concentration-time profiles after intravenous and subcutaneous administration (log scale). *LOD* Limit of detection

Table 1 Patient demographic and baseline characteristics

	Total patients (*n*=12)
Sex: male	6 (50%)
Mean age (years)[a]	11.0±4.7
Age group (years)	
1–6	2 (17%)
7–11	4 (33%)
12–16	6 (50%)
Weight (kg)[a]	35.3±18.3
Mode of dialysis	
Hemodialysis	9 (75%)
Peritoneal dialysis	1 (8%)
None	2 (17%)
Hemoglobin (g/dl)[b]	11.0 (9.1–13.4)

[a] Mean±SD
[b] Median (range)

14.1 h). The mean peak serum concentration of darbepoetin alfa after SC administration (C_{max}=1.3±0.6 ng/ml) was approximately eightfold lower than after IV administration (C_0=10.9±4.6 ng/ml). However, serum concentrations were quantifiable for a longer period of time after SC dosing (approximately 168 h) than after IV dosing (Fig. 1). The washout period of 14 days was sufficient to allow concentrations to reduce to baseline values. Concentrations after SC administration declined in a monophasic manner. The mean terminal half-life of darbepoetin alfa after SC administration (42.8±23.0 h) was approximately twofold longer than after IV administration, indicating that there is ongoing darbepoetin alfa absorption during this interval, and absorption may be rate limiting. Exposure to darbepoetin alfa, as measured by $AUC_{(0-\infty)}$, was approximately twofold lower after SC administration than after IV administration, and the estimated mean bioavailability after SC dosing was 54%±14.3%. There were no safety concerns during the study, and no antibody formation to darbepoetin alfa was detected for any patient.

Table 2 Darbepoetin alfa noncompartmental pharmacokinetic parameters after intravenous and subcutaneous administration [$t_{1/2}$ terminal half-life, C_0 concentration at time zero (maximum IV concentration), $AUC_{(0-\infty)}$ area under the curve from zero to infinity, V_0 initial volume of distribution, V_{SS} volume of distribution at steady state, T_{max} time to maximum concentration, C_{max} maximum concentration, V_Z/F relative volume of distribution associated with the terminal phase]

Parameter (units)	Mean±SD	*n*
IV administration		
$t_{1/2}$ (h)	22.1±4.8	10
C_0 (ng/ml)	10.9±4.6	10
$AUC_{(0-\infty)}$ (ng.h/ml)	233±56	10
V_0 (ml/kg)	51.6±13.7	10
V_{SS} (ml/kg)	80.9±32.5	10
Clearance (ml/h per kg)	2.3±0.6	10
SC administration		
$t_{1/2}$ (h)	42.8±23.0	8
Bioavailability (%)	54.2±14.3	7
T_{max} (h)	36.2±14.1	10
C_{max} (ng/ml)	1.3±0.6	10
$AUC_{(0-\infty)}$ (ng.h/ml)	122±20	8
V_Z/F (ml/kg)	268.3±144.7	8
Relative clearance (ml/h per kg)	4.3±0.6	8

Discussion

This is the first report on the administration of darbepoetin alfa to pediatric patients with CKD. The results of this study indicate that the terminal half-life of darbepoetin alfa is approximately twofold longer after SC administration than after IV administration in this patient population. Exposure over time was approximately twofold higher with IV dosing than with SC dosing, resulting in an estimated bioavailability of 54% after SC administration.

The design of this study is similar to that of a previous pharmacokinetic study conducted in adult patients with CKD [4], allowing a comparison of the pharmacokinetic profile of darbepoetin alfa between the adult and pediatric populations. In the adult study, patients received a single IV dose of 0.5 μg/kg darbepoetin alfa or 100 U/kg epoetin and were crossed over to receive the alternate therapy after a 28-day washout period. A subset of patients also received a single SC dose of 0.5 μg/kg darbepoetin alfa after an additional 28-day period. Overall, the mean half-life of darbepoetin alfa when administered by IV or SC routes was similar in pediatric and adult patients (Table 3 and Fig. 2). After SC administration, the mean bioavailability of darbepoetin alfa was slightly higher in pediatric patients, and the earlier T_{max} suggests that darbepoetin alfa may be absorbed at a slightly more rapid rate in pediatric patients compared with adult patients after SC dosing.

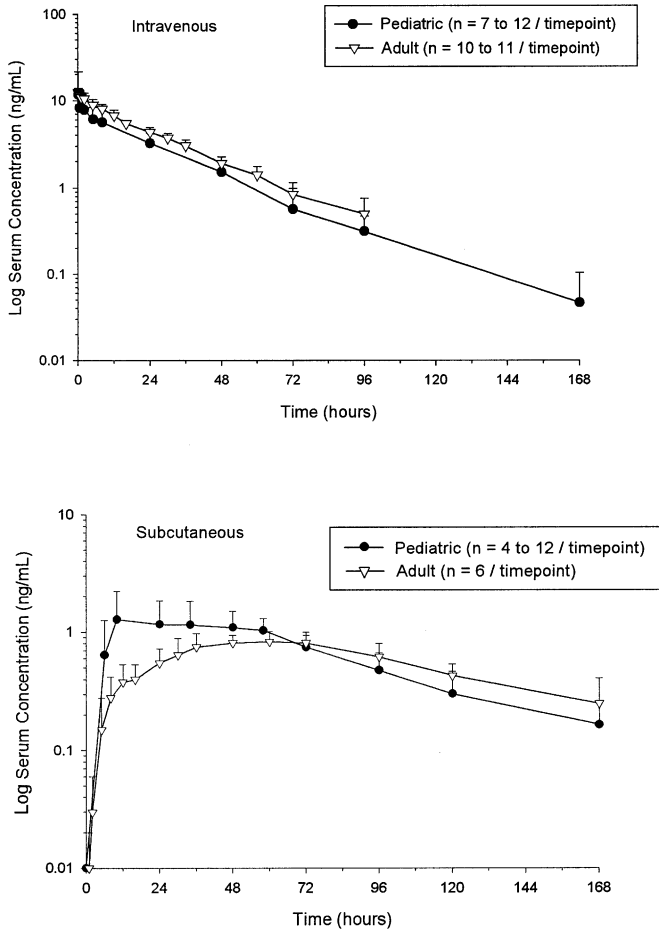


Fig. 2 Comparison of darbepoetin alfa pharmacokinetics between pediatric and adult patients (log scale). Data in adults are from Macdougall et al. [4]

Table 3 Comparison of darbepoetin alfa pharmacokinetic parameters in adult and pediatric patients

Parameter[a]	Pediatric	*n*	Adult[b]	*n*
IV administration				
$t_{1/2}$ (h)	22.1±4.8	10	25.3±7.3	11
Clearance (ml/h per kg)	2.3±0.6	10	1.6±1.0	11
SC administration				
$t_{1/2}$ (h)	42.8±23.0	8	48.8±12.7	6
Bioavailability (%)	54±14	7	37±7	6
T_{max} (h)	36.2±14.1	10	54.1±12.5	6

[a] Mean±SD
[b] Data from Macdougall et al. [4]

These results are consistent with previous pharmacokinetic studies conducted in pediatric patients receiving epoetin. Previous reports indicated that the mean terminal half-life of epoetin after IV administration to pediatric patients ranged from 5.6 to 10.9 h, with mean clearance values ranging from 6 to 10.1 ml/h per kg [10, 11, 12, 13]. After SC dosing, absorption was rate limiting, such that the mean terminal half-life ranged from 13.3 to 25.2 h, with bioavailability estimates ranging from 33% to 40% [10, 13, 14, 15]. Due to the wide range in values, comparison with literature estimates for adults is difficult. However, Evans et al. [10] concluded that, after IV dosing in pediatric patients, clearance was increased twofold and terminal half-life decreased by 30% to 86% compared with adults, and after SC dosing, bioavailability was increased twofold. Geva and Sherwood [11], however, concluded that the pharmacokinetics of epoetin in pediatric patients were similar to that of adults. Thus, the findings in the current study are similar to observations for epoetin with regard to the pharmacokinetics of darbepoetin alfa in children and adults.

The mean terminal half-life of darbepoetin alfa in this study was 42.8 h after SC administration and 22.1 h after IV administration, approximately two- to fourfold longer than the half-life of epoetin (SC 21.1 h, IV 5.6 h) in pediatric patients from a previous study [10]. Thus, the pharmacokinetic profile of darbepoetin alfa may allow for less frequent dosing relative to epoetin. In adult patients with CKD who were receiving dialysis, darbepoetin alfa administered IV once weekly maintained hemoglobin concentrations as effectively as epoetin administered IV three times weekly [16]. In addition, a randomized, comparative study of darbepoetin alfa and epoetin administered IV or SC found that 95% of dialysis patients who were receiving epoetin once weekly at baseline successfully maintained stable hemoglobin concentrations when switched to darbepoetin alfa administered once every 2 weeks [17].

The results of this single-dose study indicate that the pharmacokinetic profile of darbepoetin alfa administered IV or SC in pediatric patients with CKD is similar to that of adults, although the absorption of darbepoetin alfa may be slightly more rapid in pediatric patients after SC administration. A study to evaluate the efficacy and safety of darbepoetin alfa in pediatric patients is currently underway.

Acknowledgements This study was supported by Amgen (Thousand Oaks, Calif., USA). Results from this study were presented in abstract form at the 33rd Annual Meeting of the American S ociety of Nephrology in Toronto, Canada, October 2000. The authors wish to thank the study coordinators and patients at each of the participating centers.

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