Pharmacokinetics of Novel Erythropoiesis Stimulating Protein Compared with Epoetin Alfa in Dialysis Patients

IAIN C. MACDOUGALL,* STEPHEN J. GRAY,† ORLAITH ELSTON,* CORMAC BREEN,* BARBARA JENKINS,† JEFF BROWNE,‡ and JOAN EGRIE‡
**Department of Renal Medicine, King's College Hospital, London, United Kingdom; †Amgen Limited, Cambridge, United Kingdom; and ‡Amgen, Inc., Thousand Oaks, California.*

Abstract. Novel erythropoiesis stimulating protein (NESP) is a hyperglycosylated analogue of recombinant human erythropoietin (Epoetin) which has an increased terminal half-life in animal models. The aim of this study was to extend these observations to humans. Using a double-blind, randomized, cross-over design, the single-dose pharmacokinetics of Epoetin alfa (100 U/kg) and an equivalent peptide mass of NESP were compared following intravenous bolus in 11 stable peritoneal dialysis patients. This was followed by an open-label study to determine the single-dose pharmacokinetics of an equivalent peptide mass of NESP by subcutaneous injection in six of these patients. The mean terminal half-life for intravenous NESP was threefold longer than for intravenous Epoetin (25.3 *versus* 8.5 h), a difference of 16.8 h (95% confidence interval, 9.4 to 24.2 h, $P = 0.0008$). The area under the serum concentration–time curve was significantly greater for NESP (291.0 ± 7.6 ng · h per ml *versus* 131.9 ± 8.3 ng · h per ml; mean ± SEM; $P < 0.0005$), and clearance was significantly lower (1.6 ± 0.3 ml/h per kg *versus* 4.0 ± 0.3 ml/h per kg; mean ± SEM; $P < 0.0005$). The volume of distribution was similar for NESP and Epoetin (52.4 ± 2.0 ml/kg *versus* 48.7 ± 2.1 ml/kg; mean ± SEM). The mean terminal half-life for subcutaneous NESP was 48.8 h. The peak concentration of subcutaneous NESP was approximately 10% of that following intravenous administration, and bioavailability was approximately 37% by the subcutaneous route. The longer half-life of NESP is likely to confer a clinical advantage over Epoetin by allowing less frequent dosing in patients treated for anemia.

Recombinant human erythropoietin (Epoetin) has been used for the treatment of renal and other anemias since it was first licensed nearly a decade ago (1,2). It is most commonly administered by subcutaneous or intravenous injection two or three times a week, and its efficacy, particularly for the anemia of renal failure, is undisputed.

Novel erythropoiesis stimulating protein (NESP) is a hyperglycosylated analogue of Epoetin that stimulates erythropoiesis by the same mechanism as the endogenous hormone. To create NESP, two extra N-linked carbohydrate addition sites were introduced into the primary sequence of Epoetin using site-directed mutagenesis. NESP has five N-linked carbohydrate chains, whereas both Epoetin and the endogenous hormone have three (3). Previous work (4) demonstrated that the sialic acid-containing carbohydrate of erythropoietin determines its serum half-life and *in vivo* activity. NESP was designed to test the hypothesis that creating a molecule with a higher sialic acid content than that occurring naturally would result in it having a longer half-life.

In animal models, NESP has been shown to have a longer terminal half-life and greater *in vivo* biologic activity than Epoetin, allowing it to be administered less frequently to obtain the same biologic response (3). The aim of this first human study was to compare the pharmacokinetics of intravenously administered NESP and Epoetin in dialysis patients, and to characterize the pharmacokinetics, including bioavailability, of subcutaneously administered NESP in the same group of patients.

Materials and Methods

Patients

Eleven patients with end-stage renal failure who were stable on continuous ambulatory peritoneal dialysis (CAPD) were included in the study. There were seven men and four women with a mean age of 55 yr (range, 27 to 75 yr) and a median duration on CAPD of 1 yr (range, 0.5 to 6 yr). Causes of renal failure were diabetic nephropathy (four patients), polycystic kidney disease (three patients), hypertensive nephropathy (one patient), and bilateral small kidneys (three patients). No patient had previously been treated with Epoetin or received intravenous iron therapy in the 2 mo before the study. All patients gave written informed consent, and the study was approved by the King's Healthcare Research Ethics Committee. The mean hemoglobin before study was 10.9 g/dl (SD 1.1), and the median serum ferritin was 138 μg/L (range, 65 to 323).

Study Design

The study was performed in two phases. The first phase was a double-blind, randomized cross-over to compare the single-dose pharmacokinetics of Epoetin alfa (100 U/kg) with an equivalent peptide mass of NESP by intravenous bolus. Blood samples were taken before dosing, and at 5, 10, 15, and 30 min and 1, 2, 5, 8, 12, 16, 24, 30, 36,

Received November 10, 1999. Accepted May 7, 1999.
Correspondence to Dr. Iain C. Macdougall, Renal Unit, King's College Hospital, East Dulwich Grove, London SE22 8PT, United Kingdom. Phone: +44 171 346 6234; Fax No: +44 171 346 6472; E-mail: icm-kru@globalnet.co.uk
1046-6673/1011-2392
Journal of the American Society of Nephrology

48, 60, 72, and 96 h after dosing. Patients who had completed the first phase of the study were asked to participate in the second phase, which was an open-label study of the single-dose pharmacokinetics of NESP by subcutaneous injection. Patients were injected subcutaneously in the arm with the same dose of NESP as that used in the intravenous phase of the study. Blood samples were taken before dosing, and at 1, 2, 5, 8, 12, 16, 24, 30, 36, 48, 60, 72, 96, 120, and 168 h after dosing. There was a 28-d washout between each of the intravenous doses of Epoetin and NESP, and then an additional 28-d washout before the subcutaneous administration of NESP.

Pharmacokinetic Analyses

Serum levels of Epoetin and NESP were measured using the Quantikine IVD human erythropoietin enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The antibodies provided with the assay cross-react with both molecules. Levels of Epoetin and NESP were determined using Epoetin standard provided with the kit, or purified NESP from Amgen, Inc., respectively. The value obtained for each patient's predose sample was subtracted from the values obtained for the postdose samples before determination of pharmacokinetic parameters. Pharmacokinetic parameters for intravenous administration were estimated after adjustment for cross-over design effects and are expressed as mean ± SEM (5).

Results

Ten of the 11 patients completed the first (intravenous) phase of the study. One patient was withdrawn after receiving the first dose of study drug due to a change in dialysis modality from CAPD to hemodialysis. In all 10 patients, NESP had a longer terminal half-life ($t_{1/2,z}$) than Epoetin. The mean $t_{1/2,z}$ for NESP following intravenous administration was threefold longer than for Epoetin (25.3 *versus* 8.5 h), a difference of 16.8 h (95% confidence interval, 9.4 to 24.2 h; $P = 0.0008$) (Figure 1, Table 1). The area under the serum concentration–time curve (AUC) for NESP from 0 to 96 h was more than double that for Epoetin (291.0 ± 7.6 ng · h per ml *versus* 131.9 ± 8.3 ng · h per ml; mean ± SEM; $P < 0.0005$), and clearance, estimated using AUC from time 0 to infinity, was significantly lower for NESP compared with Epoetin (1.6 ± 0.3 ml/h per kg *versus* 4.0 ± 0.3 ml/h per kg; mean ± SEM; $P < 0.0005$). There was no significant difference in volume of distribution at steady state (V_d) between NESP and Epoetin (52.4 ± 2.0 ml/kg *versus* 48.7 ± 2.1 ml/kg; mean ± SEM). At 4 d (96 h) after the intravenous injection, mean levels of NESP were approximately 0.5 ng/ml above baseline, whereas those for Epoetin were only 0.02 ng/ml above baseline.

Table 1. Comparison of intravenous pharmacokinetic parameters for NESP and Epoetin alfa[a]

Parameter	NESP ($n = 11$)	Epoetin ($n = 10$)
$t_{1/2,z}$ (hours)	25.3 ± 2.2	8.5 ± 2.4
CL (ml/h per kg)	1.6 ± 0.3	4.0 ± 0.3
$AUC_{(0–96)}$ (ng · h per ml)	291.0 ± 7.6	131.9 ± 8.3
V_d (ml/kg)	52.4 ± 2.0	48.7 ± 2.1

[a] Results are given as mean ± SEM. NESP, novel erythropoiesis stimulating protein; $t_{1/2,z}$, terminal half-life; CL, clearance; AUC, area under the serum concentration–time curve; V_d, volume of distribution at steady state.

Six patients completed the second (subcutaneous) phase of the study. The mean $t_{1/2,z}$ of NESP following subcutaneous administration was 48.8 h (range, 33.5 to 68.0), which was twofold longer than that for the intravenous route in these six patients (mean difference, 24.9 h; 95% confidence interval, 8.5 to 41.3 h; $P = 0.01$) (Figure 2, Table 2). The mean time to maximum serum concentration (T_{max}) was 54.1 h (range, 36 to 72) after subcutaneous injection, and the mean maximum se-

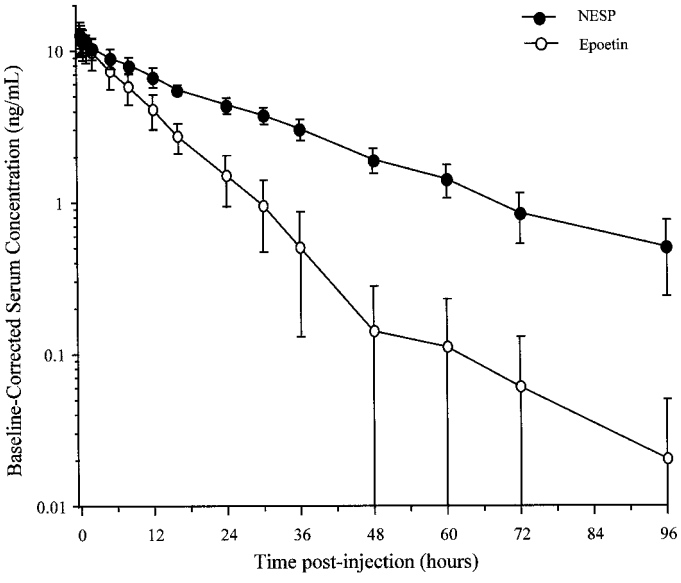

Figure 1. Comparison of intravenous pharmacokinetic profiles of NESP ($n = 11$) and Epoetin alfa ($n = 10$). Mean ± SD.

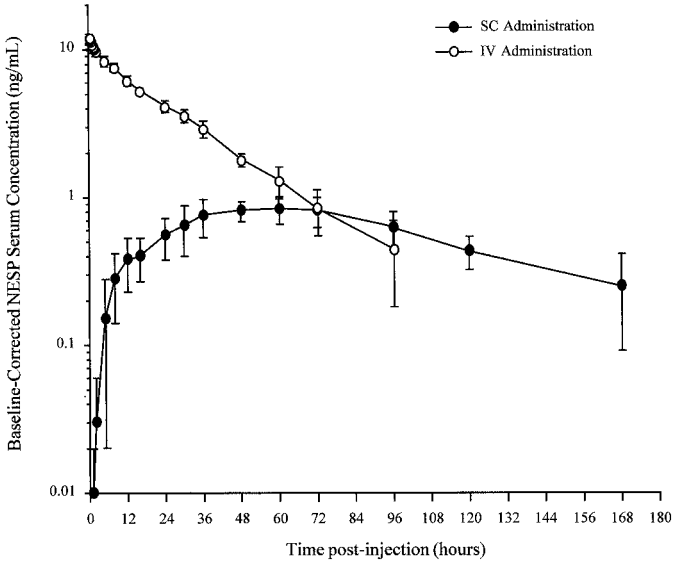

Figure 2. Comparison of subcutaneous ($n = 6$) and intravenous ($n = 6$) pharmacokinetic profiles of NESP in patients that received both treatments. Mean ± SD.

Table 2. Subcutaneous pharmacokinetic parameters for NESP[a]

Parameter	NESP ($n = 6$)
$t_{1/2,z}$ (hours)	48.8 ± 5.2
C_{max} (ng/ml)	0.94 ± 0.1
T_{max} (hours)	54.1 ± 5.1
$AUC_{(0-\infty)}$ (ng · h per ml)	108.2 ± 11.0
Bioavailability (%)	36.9 ± 3.0

[a] Results are given as mean ± SEM. C_{max}, maximum serum concentration; T_{max}, time to maximum serum concentration. Other abbreviations as in Table 1.

rum concentration (C_{max}) was approximately 10% of that for the equivalent intravenous dose. At 7 d (168 h) after the subcutaneous injection, mean levels of NESP were approximately 0.3 ng/ml above baseline. Bioavailability was estimated for each patient from the ratio of AUC from time 0 to infinity (∞) following subcutaneous and intravenous administration. The mean bioavailability of NESP was 36.9% (SEM, 3.0) by the subcutaneous route.

There were no safety concerns during the study; no reported adverse events were considered to be related to Epoetin or NESP administration.

Discussion

This is the first report on administration of NESP to humans. The results of this single-dose pharmacokinetic study show that NESP has approximately a threefold longer terminal half-life than Epoetin following intravenous administration (mean 25.3 *versus* 8.5 h) and a two- to threefold decrease in clearance. The volume of distribution at steady state for both molecules was approximately equivalent to plasma volume. These results are consistent with the findings from animal studies with NESP (3), which also showed approximately a threefold longer terminal half-life following intravenous administration, a threefold decrease in clearance, and a similar volume of distribution when compared with Epoetin.

As has been found for Epoetin, serum levels of NESP increase slowly after subcutaneous administration, reaching a peak at a mean of 54 h. This time to peak concentration is longer than has been observed for Epoetin (usually about 16 to 24 h) (6–11), and this is likely to be due to the increased molecular size of NESP (38,000 *versus* 30,400 daltons). The mean terminal half-life of NESP was 48.8 h following subcutaneous administration, which is nearly double that by the intravenous route (25.3 h). This is due to the fact that the subcutaneous half-life represents a balance between absorption from the injection site and elimination from the circulation. Previous clinical studies have shown extremely variable results for the terminal half-life of Epoetin following subcutaneous administration, but the data suggest that the terminal half-life of NESP is at least twofold longer than that of Epoetin by this route (6–11). The bioavailability of subcutaneous NESP was 37%, which is at least as great as that reported for subcutaneous Epoetin (6–11).

Although the underlying mechanism is not known, research has indicated that the sialic acid-containing carbohydrate moieties of erythropoietin have a significant effect on serum clearance, and that serum clearance is the primary determinant of *in vivo* biologic activity (4,12). Increasing the sialic acid-containing carbohydrate of erythropoietin decreases serum clearance, thereby increasing the terminal half-life and *in vivo* activity. In animal models, NESP has increased potency compared with Epoetin and can be administered less frequently to obtain the same increase in hematocrit. For example, in normal mice, NESP was nearly 20-fold more potent than Epoetin when each was administered once weekly (3). The data presented here confirm the longer terminal half-life of NESP in anemic dialysis patients. A single dose of subcutaneous NESP equivalent to 100 U/kg Epoetin was able to maintain mean serum concentrations at least threefold above baseline for up to 7 d, a level considered sufficient to stimulate erythropoiesis in anemic patients (13). It is likely that the longer half-life of NESP will confer a clinical advantage over Epoetin by allowing less frequent dosing in patients treated for anemia, possibly allowing dosing every 1 or 2 wk. Studies are under way to investigate the clinical efficacy of NESP for the treatment of anemia in dialysis patients.

Acknowledgments

This study was supported by a grant from Amgen, Inc. (Thousand Oaks, CA). We thank James Matcham and Elizabeth Beaumont for statistical input to the study design and analysis of data; Annette Lloyd and Breege Traynor for study coordination; Anne Heatherington for determination of pharmacokinetic parameters; Jatinder Harchowal and Claudette Allerdyce for pharmacy support; and Georgina Chandler, Sue Begg, and Peter Ellis for nursing support.

References

1. Winearls CG, Oliver DO, Pippard MJ, Reid C, Downing MR, Cotes PM: Effect of human erythropoietin derived from recombinant DNA on the anaemia of patients maintained by chronic haemodialysis. *Lancet* I: 1175–1178, 1986
2. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW: Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. *N Engl J Med* 316: 73–78, 1987
3. Egrie JC, Dwyer E, Lykos M, Hitz A, Browne JK: Novel erythropoiesis stimulating protein (NESP) has a longer serum half-life and greater *in vivo* biological activity compared to recombinant human erythropoietin (rHuEPO) [Abstract]. *Blood* 90: 56a, 1997
4. Egrie JC, Grant JR, Gillies DK, Aoki KH, Strickland TW: The role of carbohydrate on the biological activity of erythropoietin. *Glycoconjugate J* 10: 263, 1993
5. Hills M, Armitage P: The two-period cross-over clinical trial. *Br J Clin Pharmacol* 8: 7–20, 1979
6. Macdougall IC, Roberts DE, Neubert P, Dharmasena AD, Coles GA, Williams JD: Pharmacokinetics of recombinant human erythropoietin in patients on continuous ambulatory peritoneal dialysis. *Lancet* I: 425–427, 1989

7. Boelaert JR, Schurgers ML, Matthys EG, Belpaire FM, Daneels RF, De Cre MJ, Bogaert MG: Comparative pharmacokinetics of recombinant erythropoietin administered by the intravenous, subcutaneous and intraperitoneal routes in continuous ambulatory peritoneal dialysis patients. *Perit Dial Int* 9: 95–98, 1989
8. Macdougall IC, Roberts DE, Coles GA, Williams JD: Clinical pharmacokinetics of epoetin (recombinant human erythropoietin). *Clin Pharmacokinetics* 20: 99–113, 1991
9. Halstenon CE, Macres M, Katz SA, Schnieders JR, Watanabe M, Sobota JT: Comparative pharmacokinetics of epoetin alfa and beta. *Clin Pharmacol Ther* 50: 702–712, 1991
10. Faulds D, Sorkin EM: Epoetin (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, 1989
11. Flaharty KK: Clinical pharmacology of recombinant human erythropoietin (r-HuEPO). *Pharmacotherapy* 10: 9S–14S, 1990
12. Varki A: Biological roles of oligosaccharides: All of the theories are correct. *Glycobiology* 3: 97–130, 1993
13. Korbett SM: Anemia and erythropoietin in hemodialysis and continuous ambulatory peritoneal dialysis. *Kidney Int* 43: S111–S119, 1993