

Anargyros Xenocostas · Wing K. Cheung
Francis Farrell · Cindy Zakszewski · Marian Kelley
Andrzej Lutynski · Michael Crump · Jeffrey H. Lipton
Thomas L. Kiss · Catherine Y. Lau · Hans A. Messner

The pharmacokinetics of erythropoietin in the cerebrospinal fluid after intravenous administration of recombinant human erythropoietin

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Abstract Objectives: Erythropoietin (EPO) was originally described as a regulator of erythropoiesis. Recently, synthesis of EPO and expression of the EPO receptor (EPO-R) have been reported for the central nervous system (CNS). The potential use of EPO to prevent or reduce CNS injury and the paucity of information regarding its entry into the human CNS led us to examine the pharmacokinetics (PK) of recombinant human EPO (r-HuEPO) in the serum and cerebrospinal fluid (CSF).

Methods: Four patients with Ommaya reservoirs were enrolled to facilitate serial CSF sampling. R-HuEPO was given intravenously (IV) in single doses of 40,000 IU or 1,500 IU/kg and in multiple doses of 40,000 IU daily for 3 days.

Results: The EPO concentrations in the CSF increased after a period of slow equilibration. Linear first-order distribution kinetics were observed for serum and CSF. The concentration of EPO in the CSF was proportional to the serum concentration of EPO and the permeability of the blood-brain barrier (BBB), as determined by the albumin quotient ($Q_A = [\text{albumin}]_{\text{CSF}}/[\text{albumin}]_{\text{serum}}$).

A rise in the CSF concentration was seen as early as 3 h after IV administration. Peak levels (C_{max}) were reached between 9 h and 24 h. After a single dose of 1,500 IU/kg, the C_{max} in the CSF ranged from 11 mIU/ml to 40 mIU/ml, and the ratios of CSF/serum C_{max} ranged from 3.6×10^{-4} to 10.2×10^{-4} . The terminal half-life ($t_{1/2}$) values of EPO in serum and CSF were similar. The $t_{1/2}$ of r-HuEPO in the CSF ranged from 25.6 h to 35.5 h after a single dose of 1,500 IU/l. Using these parameters a PK model was generated that predicts the concentration-time profile of EPO in the CSF.

Conclusions: We report that r-HuEPO can cross the human BBB and describe for the first time the PK of EPO in the CSF after IV administration. Our data suggest that the concentration-time profile of EPO in the CSF can be predicted for individual patients if the serum concentration of EPO and the Q_A are known. This information may be useful in the design of clinical trials to explore the potential therapeutic effects of EPO during CNS injury.

Keywords Erythropoietin · Blood–brain barrier · Cerebrospinal fluid · Neuroprotective agents

A. Xenocostas (✉)
Hematology Division, Rm 2760,
London Health Sciences Centre/University of Western Ontario,
LHSC, Westminster Site, 800 Commissioners Road East,
London, ON, N6A 4G5, Canada
E-mail: anargyros.xenocostas@lhsc.on.ca
Tel.: +519-685-8500
Fax: +519-685-8477

W. K. Cheung · F. Farrell · C. Zakszewski · M. Kelley
Johnson and Johnson Pharmaceutical Research and Development,
Raritan, NJ, USA

C. Y. Lau
Ortho Biotech, Toronto, ON, Canada

A. Lutynski · M. Crump · J. H. Lipton · T. L. Kiss
H. A. Messner
Bone Marrow Transplant Program, Princess Margaret Hospital/
University Health Network and the Department of Medicine,
University of Toronto, Toronto, ON, Canada

Introduction

Erythropoietin (EPO) and the EPO receptor (EPO-R) are expressed in neuroepithelial tissues [1–6] and are upregulated in the central nervous system (CNS) during hypoxia and other causes of brain injury [7–9]. EPO protects neuronal cells exposed to hypoxia, glutamate or ultraviolet irradiation in vitro [6, 10, 11]. Intra-cerebroventricular administration of EPO in animal models of cerebral ischemia reduces the volume of tissue destruction [6, 8, 10, 12]. Systemically administered EPO crosses the blood-brain barrier (BBB) of rodents and protects against ischemic and traumatic brain injury, diminishes the severity and duration of experi-

mentally-induced autoimmune encephalitis and decreases the excitotoxicity of kainite [13]. A randomized study of patients with strokes caused by middle cerebral artery occlusion treated with recombinant human EPO (r-HuEPO) showed increased EPO levels in the cerebrospinal fluid (CSF) and demonstrated a significant clinical benefit [14]. In contrast, previous studies in neonates and one adult patient with a CNS tumor suggested that EPO does not cross the intact human BBB [7, 15].

To address this question, we investigated whether or not r-HuEPO can cross the BBB after IV administration in patients with Ommaya reservoirs that allowed for serial CSF sampling, and determined the pharmacokinetics (PK) of EPO in blood and CSF after single and multiple doses.

Methods

Patients and r-HuEPO administration

This study was approved by the Human Subjects Review Board of Princess Margaret Hospital and monitored by an independent safety committee. Four patients consented to participate in this study; their characteristics and the doses of r-HuEPO administered are summarized in Table 1. Epoetin alfa (Eprex) was obtained from Ortho Biotech, Canada, diluted in 12–15 ml saline and administered IV over a period of 3–5 min. The administration of rHuEPO was well tolerated, except in one patient who developed a femoral deep vein thrombosis (DVT) 5 days later.

Determination of EPO levels and calculation of the albumin quotient

Serum and CSF samples were stored frozen at -20°C until analysis. CSF specimens were obtained from the Ommaya reservoir after discarding twice the void

volume of the device (0.7 ml, Integra Neurosciences; NL 850-1212). Samples from each patient were tested simultaneously using the same reagents.

Serum and CSF concentrations of EPO were measured using a modified enzyme-linked immunosorbent assay (ELISA, R&D Systems Inc., Minneapolis MN, USA) [16]. The method was validated using standard concentrations of r-HuEPO, and the lower limit of quantification was defined at 7.8 mIU/ml for serum and 4.0 mIU/ml for CSF. Samples containing a concentration of EPO exceeding the upper range of the assay (250 mIU/ml) were diluted. The accuracy for serum and CSF determinations was established by repeated comparisons against a standard. The validation values obtained ranged from 83.6% to 116.1% for serum and 92.3% to 100.0% for CSF with intra-assay coefficients of variation (%CV) of $\leq 13\%$ and $\leq 8\%$, respectively.

To evaluate BBB permeability, the CSF/serum albumin ratio (albumin quotient: Q_A) was calculated according to Reiber [17]. Since albumin is exclusively synthesized outside of the CNS, the relative permeability of the BBB to macromolecules can be evaluated by the Q_A . The CSF albumin concentrations were measured by means of immunonephelometry using a Behring Nephelometer Analyser II and antiserum to human albumin (Dade Behring Marburg GmbH, Marburg, Germany).

PK analyses

Non-compartmental and compartmental analyses were conducted using WinNonlin software, Version 3.1A (Scientific Consulting Inc., NC, USA).

Non-compartmental analyses The following PK parameters were calculated by non-compartmental analyses using algorithms in the software: (i) peak serum concentration (C_{\max}): the observed maximum serum concentration; (ii) time to C_{\max} (t_{\max}): the time at which C_{\max} occurred; (iii) area under the serum concentration-time curve from time 0 to time infinity ($\text{AUC}_{0-\infty}$); (iv) terminal elimination half-life ($t_{1/2}$).

Table 1 Patient characteristics and erythropoietin(EPO) dosing used in study. *Dex* dexamethasone, *MTX* methotrexate, *NHL* non-Hodgkin lymphoma, *Pred* prednisone

Cases	Diagnosis	Recent CNS therapy ^a	Concurrent steroid therapy	Dose of r-HuEPO	Weight (kg)	Duration of PK study (h)	Albumin quotient ^b
Patient 1, age 51 years, female	Plasma cell leukemia	Cranial radiation Intrathecal MTX	Dex 24 mg QD Dex 2 mg QD	667 IU/kg (40,000 IU)	60	24	0.000681
				1,500 IU/kg (90,000 IU)		24	0.000716
Patient 2, age 55 years, female	Metastatic breast cancer	None	Pred 25 mg QD Pred 10–5 mg QD	1,500 IU/kg (120,000 IU)	80	96	0.0021
				500 IU/kg (40,000 IU) q24 h x3 doses		168	0.00406
Patient 3, age 68 years, male	Relapsed NHL	Chemotherapy	None	1,500 IU/kg (142,500 IU)	95	96	0.0038
Patient 4, age 50 years, male	Anaplastic multiple myeloma	Spinal cord radiation	Dex 12 mg QD Dex 4 mg QD	1,500 IU/kg (90,000 IU)	60	96	0.00327
				667 IU/kg (40,000 IU) q24 h x3 doses		168	0.00230

^aTherapy given within 1 month of PK study

^bAlbumin quotient (Q_A) = [albumin in CSF]/[albumin in serum]. Normal range $\pm 2\text{SD} = 0.0018 - 0.0074$ [22]

Compartmental analyses A two-compartment open model (model #8 in the WinNonlin Software) was fitted to the serum concentration data.

PK model A PK model was developed to describe the disposition characteristics of EPO in serum and CSF assuming: (i) first-order rate constants for all distribution and elimination processes; (ii) the rate of distribution of EPO from serum to CSF depends on the Q_A ; (iii) contribution of EPO in CSF is insignificant in the overall mass balance of EPO in the body. Parameters estimated from the two-compartment open model analyses were used as constants in the PK model: volume of distribution in the serum compartment (V_c), first-order rate constant from the tissue compartment to the serum compartment ($K_{2,1}$), first-order rate constant for the distribution phase in the serum EPO concentration-time plot (alpha), first-order rate constant for the terminal phase in the serum EPO concentration-time plot (beta). The PK model was fitted to single dose serum and CSF concentration data to estimate first-order rate constant from serum compartment to the CSF compartment (K_{in}), first-order rate constant from CSF compartment to the serum compartment (K_{out}), and apparent volume of distribution in the CSF compartment (V_{CSF}).

Results

Seven PK studies were performed on four patients. Their therapy and Q_A values are listed in Table 1. The CSF EPO concentrations reflected the serum EPO levels and the permeability of the BBB (Fig. 1A). The concentration of EPO in the CSF increased in all four patients after 1–3 h, peaked after approximately 24 h and declined bi-exponentially thereafter with similar terminal slopes in serum and CSF (Table 2). The serum and CSF EPO

concentration-time profiles after administration of three EPO doses reflected the IV dosing schedule (Fig. 1B). The EPO concentration in CSF peaked 4–10 h after each dose.

For patient 2, CSF samples were obtained from the Ommaya reservoir and by lumbar puncture (LP) 24 h after the third dose of EPO. The CSF sample obtained by LP showed increased cell numbers and 24-fold higher concentrations of EPO and albumin than the Ommaya reservoir derived sample (Table 3). The Q_A of the LP sample was elevated to 0.09860 (normal range $\pm 2SD = 0.0018-0.0074$) consistent with a localized increase in BBB permeability [17]. Thus, the increased EPO concentration in the LP sample appeared to reflect the concentration gradient of albumin. Magnetic resonance imaging scans demonstrated leptomeningeal enhancement over the conus and lumbar roots but no leptomeningeal or parenchymal brain metastases.

PK parameters

The PK parameters of EPO in serum and CSF of individual patients after a single IV dose are listed in Table 2. In the serum, after a single 1,500-IU/kg IV dose administration, the alpha (distribution) $t_{1/2}$ ranged from 2.48 h to 4.64 h and the beta (terminal elimination) $t_{1/2}$ ranged from 14.0 h to 25.1 h. A shorter $t_{1/2}$ beta value of 8.91 h was observed after a single 667-IU/kg dose for patient 1. Clearance of EPO in the patients ranged from 1.65 ml/h/kg to 3.59 ml/h/kg. Apparent volume of the central compartment (V_c) ranged from 23.2 ml/kg to 44.8 ml/kg, and approximated that of the plasma volume. Apparent volume of distribution at steady state (V_{ss}) ranged from 32.8 ml/kg to 73.2 ml/kg, suggesting confinement of EPO within the plasma circulation.

Fig. 1 **A** Predicted (solid line) and observed concentrations of erythropoietin (EPO) in serum (circles) or cerebrospinal fluid (CSF) (triangles) in patient 1 who received a single 667 IU/kg (open symbols) and 1,500 IU/kg (closed symbols) IV doses and in patients 2, 3 and 4 who received a single 1,500 IU/kg IV dose of Eprex. **B** Predicted (solid line) and observed concentrations of EPO in serum (circles) or CSF (triangles) in patient 2 who received three 500 IU/kg (40,000 IU) Eprex doses every 24 h and in patient 4 who received three 667 IU/kg (40,000 IU) Eprex doses every 24 h

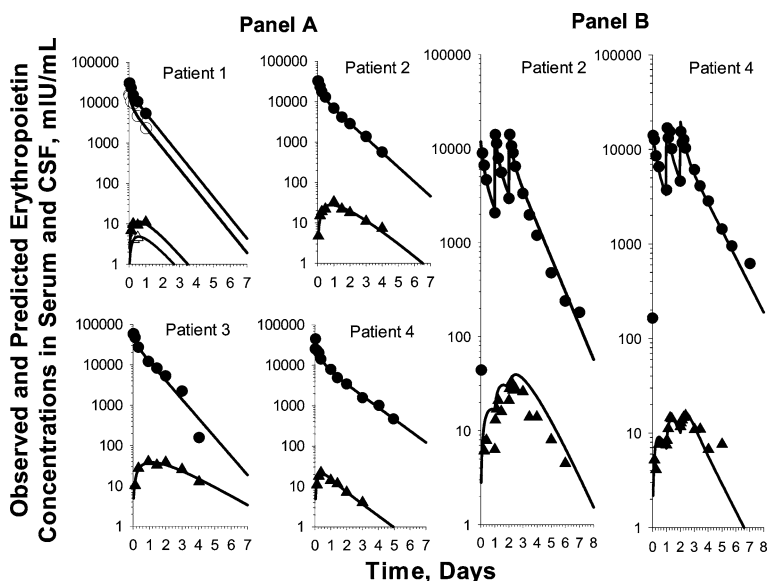


Table 2 Pharmacokinetic parameters of erythropoietin in the serum and cerebrospinal fluid (CSF). C_{max} peak serum or CSF concentration, T_{max} time at which C_{max} occurred, V_c apparent volume of distribution of the central compartment, V_{ss} apparent volume of distribution at steady-state, CL clearance, $t_{1/2}$ terminal elimination half-life, Alpha $t_{1/2}$ half-life of the distribution phase, Beta

$t_{1/2}$ half-life of the terminal elimination phase, $AUC_{0-Tlast}$ area under the serum or CSF concentration–time curve from time zero to the time of last quantifiable sample at Tlast, $AUC_{0-\infty}$ area under the serum or CSF concentration–time curve from time zero to time infinity, ND not determined

	Patient 1		Patient 2		Patient 3	Patient 4	
Dose of r-HuEPO	667 IU/kg (40,000 IU)	1,500 IU/kg (90,000 IU)	1,500 IU/kg (120,000 IU)	500 IU/kg/dose (40,000 IU q24 h-3 doses)	1,500 IU/kg (142,500 IU)	1,500 IU/kg (90,000 IU)	667 IU/kg/dose (40,000 IU q24 h-3 doses)
Serum	Non-Compartmental analyses						
C_{max} (mIU/ml)	15,639	30,552	32,380	8,923* 14,032* 14,066*	58,759	44,909	14,028* 16,873* 15,394*
$AUC_{0-Tlast}$ (mIU·h/ml)	157,927	309,641	534,315	500,083	956,563	605,980	830,025
Tlast (h)	24.5	24	96	168	95	119	168.5
$AUC_{0-\infty}$ (mIU·h/ml)	186,932	401,483	551,533	506,962	961,126	623,388	859,546
$t_{1/2}$ (h)	8.70	11.9	21.1	26.3	20.1	25.8	33.0
	Compartmental analyses (2-Compartment open model)						
Alpha $t_{1/2}$ (h)	0.502	2.48	3.46	ND	2.85	4.64	ND
Beta $t_{1/2}$ (h)	8.91	14.0	20.1	ND	15.4	25.1	ND
CL (ml/h/kg)	3.59	3.59	2.78	ND	1.65	2.49	ND
V_c (ml/kg)	36.4	44.2	42.5	ND	23.2	44.8	ND
V_{ss} (ml/kg)	45.5	64.2	68.0	ND	32.8	73.2	ND
CSF	Non-Compartmental analyses						
C_{max} (mIU/ml)	4.6	11	33	7.9* 21.0* 33.0*	40.2	22	7.6* 14.5* 15.3*
T_{max} (h)	12.5	24	24	9.83 30 52	22.5	9	11 30.25 57
$t_{1/2}$ (h)	ND	ND	34.1	29.6	30.5	25.6	35.5
$AUC_{0-Tlast}$ (mIU·h/ml)	38.3	215	1664	2051	2779	789	1185
$AUC_{0-\infty}$ (mIU·h/ml)	ND	ND	2,023	2,243	3,339	937	1,574
Tlast (h)	ND	12.5	96	144	97	72.27	120.25

*Peak level achieved in the serum or CSF after each dose

The EPO levels observed in the CSF appeared to depend on both the serum concentration of EPO and the permeability of the BBB as measured by the Q_A . The first-order distribution kinetics suggest that single rather than split IV doses are preferable to achieve a rapid rise of EPO in the CSF. The C_{max} was approximately two to three times greater after one 1,500-IU/kg dose than after three daily doses of 500–667 IU/kg in patients 2 and 4 (Table 2).

PK model

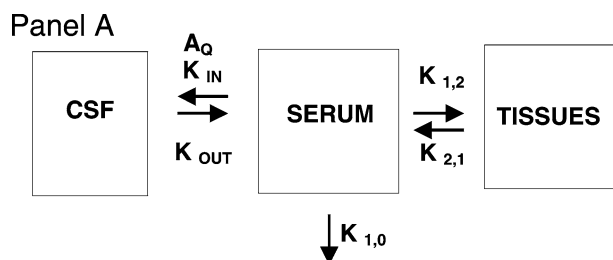
Compartmental and non-compartmental analyses identified the serum concentration of EPO and the Q_A as the two principal determinants for the EPO concentration–time profile in the CSF. Using these two variables, we constructed a PK model with the assumptions described in the methods (Fig.2A). The estimated parameters for the PK model are listed in Fig.2B. The predicted con-

Table 3 Comparison of cerebrospinal fluid (CSF) samples obtained from Ommaya reservoir or by lumbar puncture (LP) in patient 2 after three IV doses of erythropoietin (EPO). $[EPO]_{LP}$ and $[EPO]_{Ommaya}$ are concentrations of EPO in samples of CSF ob-

tained by LP or from the Ommaya reservoir, respectively. $Q_{A,LP}$ and $Q_{A,Ommaya}$ are albumin quotients determined using the albumin concentration from samples of CSF obtained by LP or from the Ommaya reservoir, respectively. NA not applicable

Samples(time after first EPO dose)	[EPO] mIU/ml	Ratio of $[EPO]_{LP}/[EPO]_{Ommaya}$	[Albumin mg/l]	Albumin quotient (Q_A) ^a	Ratio of $Q_{A,LP}/Q_{A,Ommaya}$	WBC×10 ⁶ /l
CSF from Ommaya (71.5 h)	26	24.2	150	0.00406	24.3	1
CSF from LP (72.5 h)	629		3,650	0.09860		9
Serum (71.5 h)	3,327	NA	37,000	NA	NA	7,100

^aAlbumin quotient (Q_A)=[albumin in CSF]/[albumin in serum]



Panel B

Patient	K_{IN} (h^{-1})	K_{OUT} (h^{-1})	V_{CSF} (mL/kg)
Patient 1	0.004578	0.0686	1.756
Patient 2	0.003537	0.0406	2.363
Patient 3	0.001181	0.0220	1.260
Patient 4	0.002786	0.1060	2.513
N	4	4	4
Mean	0.003021	0.0593	1.973
SD	0.00143	0.0366	0.577
CV%	47.3	61.7	29.3

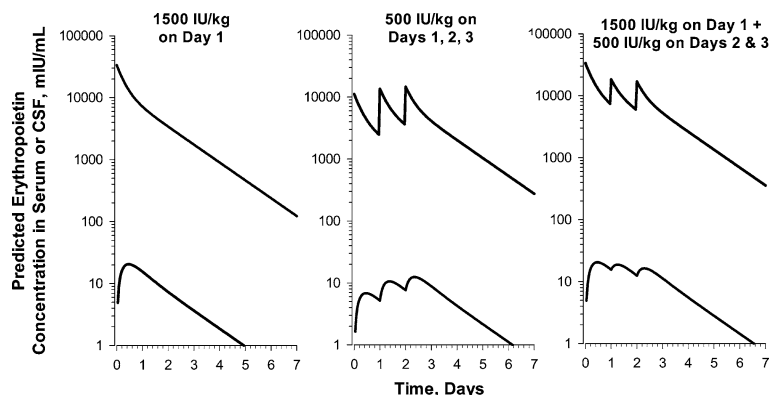
Fig. 2 **A** The erythropoietin (EPO) pharmacokinetic model for the distribution of EPO into the central nervous system. $K_{1,2}$ and $K_{2,1}$ represent the rate constants between the serum and tissue compartments. K_{in} and K_{out} represent the rate constants between the serum and CSF compartments. Partition into the CSF compartment is also a function of the albumin quotient (Q_A). $K_{1,0}$ represents the elimination rate constant from the serum compartment. **B** Estimated model parameter values of the pharmacokinetic model for EPO are provided for each patient in tabular form

centration-time profiles of EPO in serum and CSF are based on the estimated model parameters and are shown as the solid lines in Fig. 1A. For patient 1, the predicted concentration-time profiles in serum and CSF for the lower IV dose of 667 IU/kg are based on the model parameters estimated from the higher IV dose of 1,500 IU/kg in the same patient. There was a close agreement between the predicted profiles and observed data, suggesting that this PK model accurately describes the disposition kinetics of EPO in serum and CSF.

Discussion

To determine whether or not r-HuEPO crosses the human BBB after IV administration, we used relatively

Fig. 3 Predicted concentration-time profiles of erythropoietin (EPO) in serum (*upper line*) and in cerebrospinal fluid (CSF) (*lower line*) after a single 1,500 IU/kg IV dose (*left panel*), three daily 500-IU/kg IV doses (*middle panel*), and a single 1,500 IU/kg IV dose on day 1 followed by two daily 500-IU/kg IV doses on day 2 and day 3 (*right panel*). Single-dose PK parameters for patient 2 and a Q_A value of 0.00406 were used in the simulations



high doses of 1,500 IU/kg to mimic neuroprotective concentrations in animal studies. The dose-proportional increases in CSF concentrations of EPO suggest penetration of systemically administered r-HuEPO into the CNS.

The distribution of EPO into the CSF followed first-order kinetics, with serum concentration as the driving force, and depended on the permeability of the BBB as measured by the CSF/albumin ratio (Q_A). A PK model using these assumptions was able to describe the concentration-time profiles of EPO in the CSF. The predictive value of the model can be illustrated by simulations predicting how certain dosing regimens (such as an initial 1,500 IU/kg IV dose followed by two daily 500 IU/kg IV doses) can be used to rapidly increase and then maintain the concentration of EPO in CSF (Fig. 3).

Mechanisms proposed for the transport across the BBB include: (i) receptor-mediated transport; (ii) carrier-mediated transport; (iii) fluid phase endocytosis; (iv) non-specific or receptor-mediated adsorptive endocytosis; and (v) transmembrane diffusion [18]. Our data are consistent with first-order transmembrane transport of r-HuEPO or a similar non-receptor-mediated/non-saturable mechanism. The penetration of r-HuEPO into the CSF indicated by the $AUC_{CSF}:AUC_{serum}$ ratio ranges from 0.02% to 0.31% after single doses, similar to results in rats and macaques [19, 20]. The AUC_{CSF}/AUC_{serum} ratios were 0.089% (95% CI: 0.081–0.099) and 0.03–0.22%, respectively.

The Q_A values derived from Ommaya reservoir sampling did not exceed the normal range despite diagnoses of malignant CNS disease and treatments with chemotherapy and/or cranial irradiation. The use of corticosteroids may have contributed to the maintenance of BBB function of our patients [21–23]. Consistent with these observations, we showed a doubling of Q_A in one of the patients during prednisone taper (Table 1).

Local injury may facilitate the entry of systemically administered EPO into the CNS [14, 18]. Local meningeal infiltration or inflammation by metastatic disease was the probable cause of the marked increase in the concentration (24-fold) of EPO and albumin in CSF samples by LP in patient 2.

The half-life values of the elimination phase for EPO in serum and CSF were similar for each patient and

Table a Contribution and relationship of authors to supporting company

Author	Contribution and relationship with Ortho Biotech/ Johnson and Johnson
A.X.	Co-Principal investigator. Involved in all aspects of study. Invited speaker and medical monitor for Ortho Biotech, Canada.
W.K.C.	Pharmacokinetic analyses and modeling. Trial design. Interpretation of data and review of manuscript. Employee of Johnson and Johnson, USA.
F.F.	Analyses of samples. Employee of Johnson and Johnson, USA.
C.Z.	Validation of assay for EPO in serum and CSF Employee of Johnson and Johnson, USA.
M.K.	Validation of assay for EPO in serum and CSF Employee of Johnson and Johnson, USA.
A.L.	Patient recruitment and clinical care. No relationship with supporting company.
M.C.	Patient recruitment and clinical care. Review of manuscript. No relationship with supporting company.
J.H.L.	Interpretation of data and review of manuscript. No relationship with supporting company.
T.L.K.	Interpretation of data and review of manuscript. No relationship with supporting company.
C.L.	Trial design. Interpretation of data and review of manuscript. Employee of Ortho Biotech, Canada.
H.A.M.	Co-Principal investigator. Involved in all aspects of study. Invited speaker and advisory board member for Ortho Biotech, Canada.

ranged from 8.7 h to 35.5 h. The $t_{1/2}$ values of EPO in serum were longer than the previously published $t_{1/2}$ values of about 5 h [24]. Longer half-life values observed in patients 2, 3, and 4 in this study represent the elimination phase. The lower value for patient 1 reflects the combined distribution and elimination phase during the first 24 h.

The concentration of EPO in CSF required for neuroprotection in humans is not known. In the human stroke trial, EPO concentrations of approximately 15 mIU/ml in the CSF correlated with improved functional outcomes consistent with concentrations achieved in this study [14].

Doses of 1,500 IU/kg have been safely administered three times weekly for up to 3–4 weeks in normal volunteers [25]. A review of all controlled studies using r-HuEPO demonstrated that adverse events differ from indication to indication and generally reflect events associated with the underlying illness [26]. The risk of thrombotic events was not increased over baseline. However, a causal relationship between the administration of EPO and the observed DVT in our patient cannot be excluded.

In conclusion, we demonstrate for the first time, the distribution kinetics of EPO into the human CSF and present a PK model to describe the distribution and elimination of EPO in serum and CSF after single or multiple IV doses. It is hoped that these data will facilitate the design of future clinical trials to examine the role of EPO as a neuroprotectant.

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