Pharmacokinetic and Pharmacodynamic Comparison of Two Recombinant Human Erythropoietin Formulations, PDA10 and Eprex, in Healthy Korean Male Volunteers: A Randomized, Double-Blinded, Single-Dose, Two-Period Crossover Study

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ORIGINAL RESEARCH ARTICLE

Pharmacokinetic and Pharmacodynamic Comparison of Two Recombinant Human Erythropoietin Formulations, PDA10 and Eprex, in Healthy Korean Male Volunteers: A Randomized, Double-Blinded, Single-Dose, Two-Period Crossover Study

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Abstract

Background and Objectives A new biosimilar human recombinant epoetin alfa product (PDA10) has been developed by PanGen Biotech Inc., Korea. This study was planned to demonstrate the pharmacokinetic and pharmacodynamic comparability of PDA10 to an existing epoetin alfa (Eprex) after a single intravenous administration to healthy adult male volunteers.

Methods A randomized, double-blinded, single-dose, crossover study was conducted in 30 subjects. The subjects were assigned randomly to one of two sequence groups, and single doses of 100 IU/kg PDA10 or Eprex were administered intravenously on each of 2 treatment days separated by a 4-week washout period. Plasma erythropoietin concentrations were measured using an enzyme-linked immunosorbent assay and the pharmacokinetic parameters of the two treatments were compared. The time course and area under the effect curve (AUEC) of absolute reticulocyte counts were used as surrogate parameters for the pharmacodynamic evaluation. Adverse events (AEs) were recorded.

Results A total of 30 subjects were enrolled, and 27 completed the study. The geometric mean ratios (PDA10/Eprex) of erythropoietin for maximum plasma concentration (C_{max}) and area under the plasma concentration–time curve to the last measurable concentration (AUC_{0-last}) after intravenous administration of 100 IU/kg were 1.00 (90 % confidence interval [CI] 0.96–1.05) and 0.96 (90 % CI 0.93–1.00). The absolute reticulocyte counts of PDA10 and Eprex were similar, as determined from the maximum reticulocyte count and $AUEC_{0-last}$ values. Treatment-emergent AEs were mild and occurred in seven subjects.

Conclusion PDA10 and Eprex met the regulatory criteria for bioequivalence with respect to their pharmacokinetic profiles and pharmacodynamic actions.

Key Points

PDA10 and Eprex were bioequivalent with respect to their pharmacokinetic profiles and pharmacodynamic actions.

PDA10 and Eprex were well tolerated in healthy adult male volunteers.

1 Introduction

Erythropoietin is an essential endogenous hormone for proliferation, differentiation, and maturation of red blood cells (RBCs) in bone marrow [1]. Erythropoietin is released by the interstitial cells of the renal cortex in response to low blood oxygen concentration [2]. Because erythropoietin is mainly produced in the kidney, patients with chronic renal failure (CRF) have impaired erythropoietin production, resulting in anemia.

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Epoetin alfa was approved for correcting anemia in adults with CRF based on its ability to increase RBCs to a target level and maintain the increase over time as well as decrease the need for RBC transfusion support [3]. Moreover, epoetin alfa is effective for correcting chemotherapy-induced anemia and can be used for patients at risk for perioperative transfusions with anticipated significant blood loss [4–6].

Recently, a new formulation of epoetin alfa (PDA10) has been developed in Korea as a biosimilar product with comparable potency and safety to the original epoetin alfa product, Eprex. The PDA10 active ingredient is a recombinant human epoetin alfa produced from Chinese hamster ovary cells (CHO-DG44) using recombinant DNA technology and serum-free suspension culture techniques (PDA10 Investigator's Brochure, PanGen Biotech Inc., Suwon, Korea).

The aim of this study was to determine the comparability of the investigational product, PDA10, to the original product, Eprex, in terms of potency and safety by comparing their pharmacokinetic/pharmacodynamic characteristics in healthy adult male volunteers.

2 Methods

2.1 Subjects

Male volunteers aged 20–45 years and within 20 % of ideal body weight were eligible to participate. All subjects were determined to be healthy by a physical examination, medical history, and routine laboratory tests of hematology, blood chemistry, urinalysis, and 12-lead electrocardiogram (ECG) performed within 4 weeks prior to the first administration of study drug. Volunteers with a history of hepatic, renal, respiratory, hematologic, cardiovascular, or endocrine disorders; those with known hypersensitivity to any biologic agent; those who reported use of other drugs that might interfere with the study results within 14 days prior to the study; and those with a history of drug abuse were excluded from the study.

2.2 Study Design

This was a single-center, randomized, double-blind, active-controlled, single-dose, two-period, two-sequence, two-treatment crossover study. Each period was separated by a 4-week washout based on the duration of effect from reticulocyte counts of recombinant human erythropoietin [7]. The subjects were randomized to either Sequence A (PDA10-Eprex) or Sequence B (Eprex-PDA10) according to a randomization table. The dose to be administered was calculated from the scheduled dose of 100 IU/kg and then rounded off to the nearest 500 IU.

After an overnight fast of 12 h, the subjects were administered single bolus doses of the formulations intravenously through an angiocatheter inserted into an antecubital vein at about 8 a.m. Blood samples (5 ml) for the erythropoietin assay were collected into a blood collection tube supplemented with anti-coagulant through an indwelling angiocatheter inserted into an antecubital vein of the contralateral arm just prior to the injection and 5, 15, 30 min, 1, 2, 4, 6, 8, 12, 24, and 48 h after administration.

Plasma was obtained by centrifugation at 750 g for 15 min at 4 °C and stored in cryotubes at −60 °C until plasma concentrations of the erythropoietin were measures. Blood samples (5 ml) for reticulocyte counts were taken before dosing and 3, 7, 14, 21, and 28 days after drug administration, and were analyzed within 24 h. These samples were stored at 4 °C until analysis. Blood samples (5 ml) for immunogenicity assessments were taken before dosing and 14 days after drug administration. The separated serum samples were kept at −60 °C until analysis.

This study protocol was approved by the Ethics Review Board of Ajou University Medical Center (Suwon, South Korea) in accordance with the ethical standards for human studies established by the Declaration of Helsinki and its amendments, and the applicable Good Clinical Practice guidelines. This study was registered with the Clinical Research Information Service (http://cris.nih.go.kr, identifier: KCT0001377). All volunteers were given detailed written and oral information about the study and were asked to provide written informed consent before being screened for eligibility.

2.3 Bioanalytical Methods

Plasma concentrations of erythropoietin were measured using the Quantikine IVD Human Epo Immunoassay kit (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. This method has been validated [8]. The lower limit of quantitation was 2.5 mIU/ml, and the standard-curve concentration was 2.5–200 mIU/ml. The method was linear within this range of concentrations, with a correlation coefficient >0.999. Precision, expressed as the % coefficient of variation, was <10.7 % and accuracy was 92.2–112.2 %.

Assays were performed using the following procedure. After 100 µl of erythropoietin assay diluent was loaded into each well, 100 µl of calibration standards and test solutions was added into the appropriate well. The wells were incubated for 1 h at room temperature with continuous shaking, and then the contents of each well were thoroughly aspirated. The wells were treated with erythropoietin conjugate, 400 µl, for 1 h at room temperature with continuous shaking and then washed four times with diluted wash buffer. Premixed substrate solution, 200 µl,

was added to each well, and the wells were incubated for 20 min at room temperature. Finally, 100 µl of stop solution was added, and then the optical density of each well was determined using a microplate spectrophotometer set to 450 nm.

Laboratory tests of blood chemistry, hematology, and urinalysis were performed using the routine clinical procedures in the Department of Laboratory Medicine, Ajou University Hospital, which is certified by the College of American Pathologists. All laboratory analyses were carried out in a blinded manner.

2.4 Pharmacokinetic and Pharmacodynamic Analysis

The pharmacokinetic analysis was performed by non-compartmental methods using Phoenix™ WinNonlin (Certara, L.P., Princeton, NJ, USA). Maximum plasma concentration (C_{max}) and time to C_{max} (t_{max}) were determined directly from the concentration–time data. The elimination rate constant (k_e) was estimated by linear regression of the data points included in the terminal phase of the log-linear plot of the concentration–time data, and the elimination half-life ($t_{½}$) was calculated as $0.693/k_e$. The area under the plasma concentration-time curve to the last measurable concentration ($AUC_{0–last}$) was calculated using the linear-log linear trapezoidal rule.

Pharmacodynamics were evaluated by changes in the reticulocyte count over time as described by European Medicines Agency guidelines [9]. Maximum reticulocyte count (E_{max}), time to E_{max}, and area under the effect curve ($AUEC_{0–last}$) were calculated.

2.5 Tolerability

Adverse events were spontaneously reported by the subjects or solicited by non-leading questioning by the investigators. Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE) was used for the grading of AEs. Blood pressure and heart rate were measured in the sitting position with an automated device (OMRON M5, OMRON Healthcare, Dalian, China) after a 3-min rest at baseline and 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h after each administration of study drug. Physical examination, clinical laboratory tests, and ECG were performed at baseline, 2, 4, 7, and 11 days after each administration of study drug and 2 weeks after administration of the last study drug.

2.6 Statistical Analysis

Descriptive statistics were calculated for the demographic data and pharmacokinetic parameters. Baseline levels of erythropoietin, RBCs, hemoglobin, hematocrit, and absolute reticulocyte count between formulations were compared using the paired t test. The point estimate and 90 % confidence interval (CI) for the geometric mean ratios (PDA10/Eprex) for C_{max} and $AUC_{0–last}$ values were calculated by analysis of variance (ANOVA) using a mixed-effects model to compare the PDA10 and Eprex pharmacokinetic parameters. Treatment, sequence, and period were used as fixed effects, and subjects nested within sequence were used as a random effect. The point estimate and 90 % CI for the geometric mean ratios (PDA10/Eprex) for E_{max} and $AUEC_{0–last}$ values were calculated by ANOVA as described above for the pharmacokinetic analyses to compare the PDA10 and Eprex pharmacodynamic parameters. Formulations were considered to meet the US FDA regulatory criteria for bioequivalence if the 90 % CIs for the treatment ratios were 80–125 % [10]. The statistical analysis was performed using SAS software ver. 9.3 (SAS Institute Inc., Cary, NC, USA).

3 Results

3.1 Demographics

A total of 30 healthy subjects (mean age 23.7 years, range 22–36; height 175.3 cm, range 166–184; weight 69.4 kg, range 60–86) were enrolled, and 27 subjects completed the study. Three subjects withdrew informed consent. The demographic characteristics of all subjects are shown in Table 1.

3.2 Pharmacokinetics

No significant difference was found in the baseline erythropoietin concentration between formulations. The mean (±standard deviation [SD]) baseline plasma erythropoietin concentrations were 9.76 (±4.37) mIU/ml for Eprex and 9.66 (±4.13) mIU/ml for PDA10 ($p = 0.932$).

After a single intravenous administration of 100 IU/kg Eprex or PDA10, plasma erythropoietin concentrations reached a maximum at 5 min for both formulations (Fig. 1). Mean (±SD) C_{max} Eprex and PDA10 values were 2518.50 (±269.30) mIU/ml and 2531.21 (±272.50) mIU/ml; and the $AUC_{0–last}$ values were 17,094.51 (±2141.31) mIU·h/ml and 16,464.51 (±1872.40) mIU·h/ml, respectively. In addition, estimated mean (±SD) Eprex and PDA10 clearance values were 0.41 (±0.06) l/h and 0.42 (±0.04) l/h. The geometric mean ratios of the PDA10 and Eprex formulations were 1.00 (90 % CI 0.96–1.05) for C_{max} and 0.96 (90 % CI 0.93–1.00) for $AUC_{0–last}$, with no apparent differences between formulations in maximum plasma drug concentrations or overall drug exposure (Table 2).

Table 1 Demographic characteristics of the healthy adult male volunteers

Variables	Statistics	Eprex/PDA10 ($n = 16$)	PDA10/Eprex ($n = 14$)	Total ($n = 30$)
Age (years)	Mean (SD)	23.4 (1.2)	24.1 (3.6)	23.7 (2.6)
	Median	23.5	23.0	23.0
	Range	22.0–26.0	22.0–36.0	22.0–36.0
Height (cm)	Mean (SD)	176.0 (4.4)	174.6 (3.3)	175.3 (3.9)
	Median	176.5	174.5	176.0
	Range	166.0–184.0	169.0–180.0	166.0–184.0
Weight (kg)	Mean (SD)	69.6 (6.4)	69.1 (6.4)	69.4 (6.3)
	Median	70.5	67.5	68.0
	Range	60.0–85.0	63.0–86.0	60.0–86.0

SD standard deviation

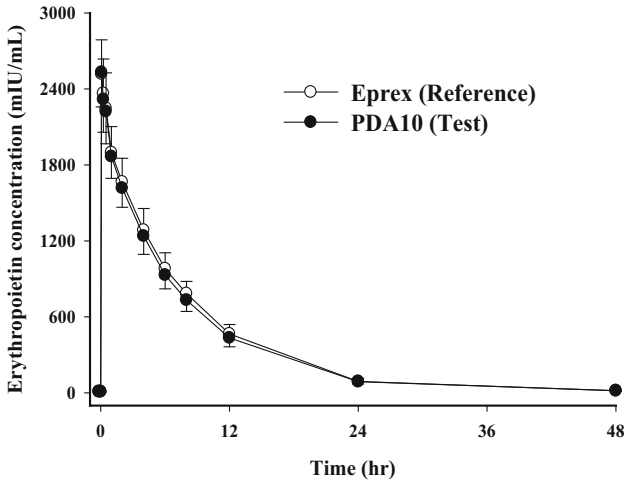

Fig. 1 Mean (SD) plasma erythropoietin concentrations over time after a single intravenous administration of 100 IU/kg Eprex or PDA10 to 27 healthy adult male volunteers

3.3 Pharmacodynamics

No significant differences were detected in the baseline levels of RBC, hemoglobin, hematocrit, or absolute reticulocyte count between the formulations. The mean (±SD) baseline RBC values of Eprex and PDA10 were 4.70×10^6/μl (±0.23) and 4.71×10^6/μl (±0.23) ($p = 0.929$); the hemoglobin values were 14.8 g/dl (±0.8) and 14.7 g/dl (±0.8) ($p = 0.900$); the hematocrit values were 43.3 % (±2.1) and 43.3 % (±2.0) ($p = 0.948$); and absolute reticulocyte counts were 55.5×10^3/μl (±15.4) and 57.5×10^3/μl (±16.8) ($p = 0.610$), respectively (Fig. 2).

After a single intravenous administration of 100 IU/kg Eprex or PDA10, absolute reticulocyte counts reached maximum values at a median of 7 days for both formulations. The mean (±SD) Eprex and PDA10 E_{max} values were 95.4×10^3/μl (±21.7) and 93.4×10^3/μl (±24.2); and those of $AUEC_{0-last}$ were 1773.6×10^3/μl × day (±404.0) and 1832.6×10^3/μl × day (±414.4), respectively. The geometric mean ratios of the PDA10 and Eprex formulation were 0.97 (90 % CI 0.90–1.05) for E_{max} and 1.03 (90 % CI 0.98–1.08) for $AUEC_{0-last}$, with no apparent differences between the formulations in maximum absolute reticulocyte count or $AUEC_{0-last}$ (Table 3).

3.4 Safety

A total of 30 subjects who received at least one dose of the randomized treatment were assessed for safety. During the

Table 2 Mean (standard deviation) pharmacokinetic parameters and geometric mean ratios of erythropoietin after a single intravenous administration of 100 IU/kg Eprex or PDA10 to 27 healthy adult male volunteers

Parameters	Unit	Eprex	PDA10	Geometric mean ratio, PDA10/Eprex (90 % CI)
$t_{½}$	h	6.72 (0.59)	6.94 (0.86)	
t_{max}	min	5	5	
C_{max}	mIU/ml	2518.50 (269.30)	2531.21 (272.50)	1.00 (0.96–1.05)
AUC_{0-last}	mIU·h/ml	17,094.51 (2141.31)	16,464.51 (1872.40)	0.96 (0.93–1.00)
AUC_{∞}	mIU·h/ml	17,261.86 (2158.28)	16,641.46 (1877.92)	
CL	l/h	0.41 (0.06)	0.42 (0.04)	

AUC_{∞} area under the plasma concentration–time curve from time zero to infinity, AUC_{0-last} area under the plasma concentration–time curve to the last measurable concentration, *CL* clearance, *CI* confidence interval, C_{max} peak plasma concentration, t_{max} time to C_{max}, $t_{½}$ elimination half-life

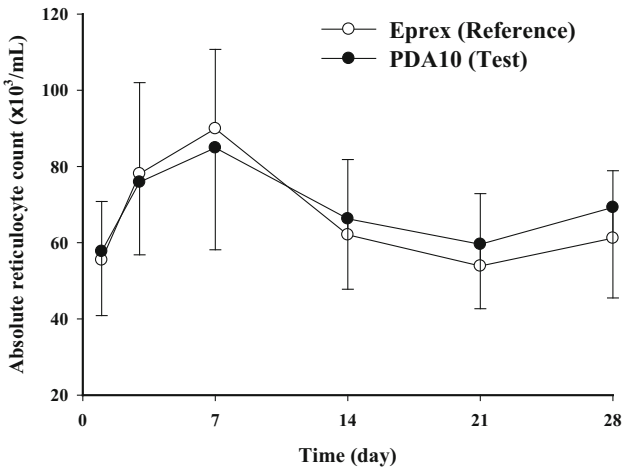

Fig. 2 Mean (SD) plasma absolute reticulocyte count over time after a single intravenous administration of 100 IU/kg Eprex or PDA10 to 27 health adult male volunteers

study, seven AEs were reported in the seven subjects, which were mild in severity. One case each of urticaria, increased creatine phosphokinase level, and abnormal liver function test were reported after administering PDA10. One case each of elevated blood pressure, myalgia, headache, and pyuria were reported after administering Eprex. No clinically significant changes were observed in the clinical laboratory test results, ECG findings, vital signs, or physical examinations.

4 Discussion

This study was performed to compare pharmacokinetic and pharmacodynamic data of PDA10 to an existing epoetin alfa (Eprex) after a single intravenous administration of 100 IU/kg to healthy adult male volunteers.

PDA10 and Eprex showed similar pharmacokinetic profiles, and the 90 % CIs of the C_{max} and AUC_{0-last} ratios fell within the acceptance range of 80–125 %, indicating that PDA10 and Eprex were bioequivalent with respect to the rate and extent of exposure to exogenous epoetin alfa. PDA10 and Eprex were also bioequivalent with respect to the maximum absolute reticulocyte count and $AUEC_{0-last}$.

The differences between the study drugs in baseline concentrations of endogenous erythropoietin were negligible, and they had little effect on the C_{max} and AUC_{0-last} results. Plasma concentration may be corrected with the baseline value in pharmacokinetic studies of an endogenous substance, such as erythropoietin [11, 12]. However, endogenous erythropoietin concentrations vary significantly over a 24-h period [13], so the accuracy of exogenous erythropoietin concentrations would be limited using baseline corrections with a single pre-dose value; thus, we performed the pharmacokinetic analysis without a baseline correction.

The pharmacodynamics of erythropoietin should be evaluated as part of pharmacokinetic studies. In single-dose studies, absolute reticulocyte count is the most relevant and recommended pharmacodynamic marker to assess erythropoietin activity [9]. Therefore, the duration of the effect on reticulocyte count due to erythropoietin rather than the elimination half-life of erythropoietin was considered when the washout period was determined.

The results of this study are limited by including only healthy adult male volunteers. Therefore, the study results cannot be generalized to female, elderly, or patient populations.

5 Conclusion

Our results indicate that PDA10 and Eprex were comparable with regards to pharmacokinetic/pharmacodynamic characteristics and safety profiles. The data derived from this study provide a basis for continued development into phase III therapeutic confirmatory trials.

Table 3 Mean (standard deviation) pharmacodynamic parameters and geometric mean ratios of the absolute reticulocyte count after a single intravenous administration of 100 IU/kg Eprex or PDA10 to 27 healthy adult male volunteers

Parameters	Unit	Eprex	PDA10	Geometric mean ratio, PDA10/Eprex (90 % CI)
t_{max}	day	7 (3–28)	7 (3–28)	
E_{max}	$\times 10^3/\mu l$	95.4 (21.7)	93.4 (24.2)	0.97 (0.90–1.05)
$AUEC_{0-last}$	$\times 10^3/\mu l \times$ day	1773.6 (404.0)	1832.6 (414.4)	1.03 (0.98–1.08)

AUEC$_{0-last}$ area under the effect–time curve to the last blood sampling, *CI* confidence interval, E_{max} maximum absolute reticulocyte count, t_{max} time to E_{max} presented as median (range)

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Compliance with Ethical Standards

Funding This study was sponsored and funded by PanGen Biotech Inc., Suwon, Korea.

Conflict of interest All authors have no conflicts of interest to declare.

Ethical approval All procedures in this study were in accordance with the 1964 Helsinki declaration and its amendments, and the Ethics Review Board of Ajou University Medical Center (Suwon, South Korea), which approved the study.

Informed consent All subjects were informed of the nature and purpose of the study and gave written informed consent to participate before any screening procedures.

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