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ORIGINAL RESEARCH ARTICLE

Comparison of the Pharmacokinetic–Pharmacodynamic Relationships of Two Darbepoetin Alfa Formulations in Healthy Male Volunteers

Seokuee Kim[1] · Taegon Hong[2] · Jae-Wook Ko[1] · Wooseong Huh[1,3] · Jung-Ryul Kim[1,4]

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Abstract

Objective This study compared the pharmacokinetic (PK), pharmacodynamic (PD), and safety properties of the test (CJ-40001) and reference (NESP®) versions of darbepoetin alfa following a single subcutaneous (SC) or intravenous (IV) administration in healthy male subjects.

Methods A single-blind, randomized, single-dose, two-period, two-intervention crossover study was conducted, with two separate parts consisting of SC or IV administration. In each period, either a test or reference product was administered via the SC or IV route. Serial blood samples for PK analysis and the reticulocyte, hematocrit, hemoglobin, and red blood cell counts for PD analysis were collected for up to 360 or 264 h after SC or IV administration, respectively. The PK and PD parameters were calculated using non-compartmental methods. The 90% confidence intervals of the geometric mean ratios for the PK and PD parameters between the two interventions were estimated. Safety and anti-drug antibody profile assessments were performed.

Results The mean darbepoetin alfa concentration–time profiles were comparable between the two products for SC and IV administration. Additionally, the PD and safety profiles were similar between the two products. Anti-drug antibody reactivity was negative for all samples from both intervention groups for SC and IV administration. The time-matched serum darbepoetin alfa concentration and the PD markers presented a counter-clockwise hysteresis, which suggests a time delay between the exposure and response.

Conclusion The test and reference darbepoetin alfa formulations had similar PK, PD, and safety profiles. Thus, it is expected that the two formulations are able to be used interchangeably in clinical settings.

ClinicalTrials.gov Identifier: NCT03542916.

Key Points

This is the first report on the pharmacokinetic and pharmacodynamic characteristics of CJ-40001, a biosimilar darbepoetin alfa.

Based on the comparable pharmacokinetics, pharmacodynamics, and safety profiles of the test and reference darbepoetin alfa, it is expected that they can be used interchangeably.

1 Introduction

Anemia is a common finding in patients with chronic kidney disease (CKD) and in those with cancer who are receiving multiple-cycle chemotherapy [1–6]. The anemia associated

✉ Wooseong Huh
wooseong.huh@samsung.com

✉ Jung-Ryul Kim
jungryul.kim@samsung.com

1 Department of Clinical Pharmacology and Therapeutics, Samsung Medical Center, Seoul, Republic of Korea

2 Department of Clinical Pharmacology, Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea

3 Division of Nephrology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

4 Department of Clinical Research Design and Evaluation, SAIHST, Sungkyunkwan University, Seoul, Republic of Korea

with CKD has a multifactorial pathology that includes relative erythropoietin (EPO) deficiency, uremic-induced inhibitors of erythropoiesis, shortened erythrocyte survival, and disordered iron homeostasis [2]. The etiology of chemotherapy-related anemia is also multifactorial, resulting from the myelosuppressive effects of chemotherapy and the direct effects on the renal tubules, decreasing the production of the EPO [7].

EPO is a glycoprotein hormone that regulates erythropoiesis and plays a crucial role in erythrocyte production and maturation [1, 8, 9]. EPO is mainly produced in the peritubular cells of the kidneys in adults and in hepatocytes in the fetus [1]. Hypoxia leads to an increase in EPO production, and EPO controls red blood cell (RBC) production [1, 10]. The management of CKD or chemotherapy-related anemia was revolutionized by the introduction of recombinant human EPO (rHuEPO), one of the key medications for these types of anemias [1, 2, 11–13]. However, according to the recommended use of rHuEPO, patients should receive the medicine two to three times a week via an invasive route [subcutaneous (SC) or intravenous (IV) injection] [14]. Difficulties relating to this type and frequency of administration led to the development of molecules with improved in vivo bioactivity that can reduce the frequency of administration. This research resulted in the discovery and development of the second-generation rHuEPO, darbepoetin alfa (NESP®, Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan), which can be administered less frequently than the first-generation rHuEPO [14, 15], and the US Food and Drug Administration (FDA) approved darbepoetin alfa for the treatment of CKD-associated anemia in 2001 [16].

The pharmacokinetic (PK) and pharmacodynamic (PD) relationship in reference darbepoetin alfa following intensive sampling has rarely been reported [17]. In addition, it has also not been reported whether such studies have been conducted under conditions where potential confounding factors were controlled. We therefore conducted a study of the PK–PD relationship and safety profile of test darbepoetin alfa (CJ-40001, CJ HealthCare Corp., Seoul, Korea) compared with that of the reference darbepoetin alfa (NESP®) given via two administration routes, SC and IV, in healthy volunteers.

2 Methods

2.1 Study Design

This study was conducted in accordance with the ethical principles of the 1964 Helsinki Declaration and the Korean Good Clinical Practices [18]. This study was a single-blind, randomized, single-dose, two-period, two-intervention crossover study, with two separate parts (Fig. 1). Before enrollment and any study-related procedures were performed, informed consent was obtained from all individual subjects included in the study. In each part, subjects who were eligible based on the inclusion/exclusion criteria were randomized to one of two sequence groups where they would each be administered a single dose of reference and test drug according to the sequence allocated to them.

Healthy male adults aged 19–55 years with a body mass index of 19–28 kg/m^2 were eligible. Subjects who showed at least one of the following clinical laboratory results were excluded: blood hemoglobin (Hb) value < 13 or > 17 g/dL; blood reticulocyte (Ret) value more than the upper reference limit (2%); and/or blood vitamin B_{12} (211 pg/mL), ferritin (22 ng/mL), or transferrin (200 mg/dL) value less than the lower reference limit. Major exclusion criteria were the

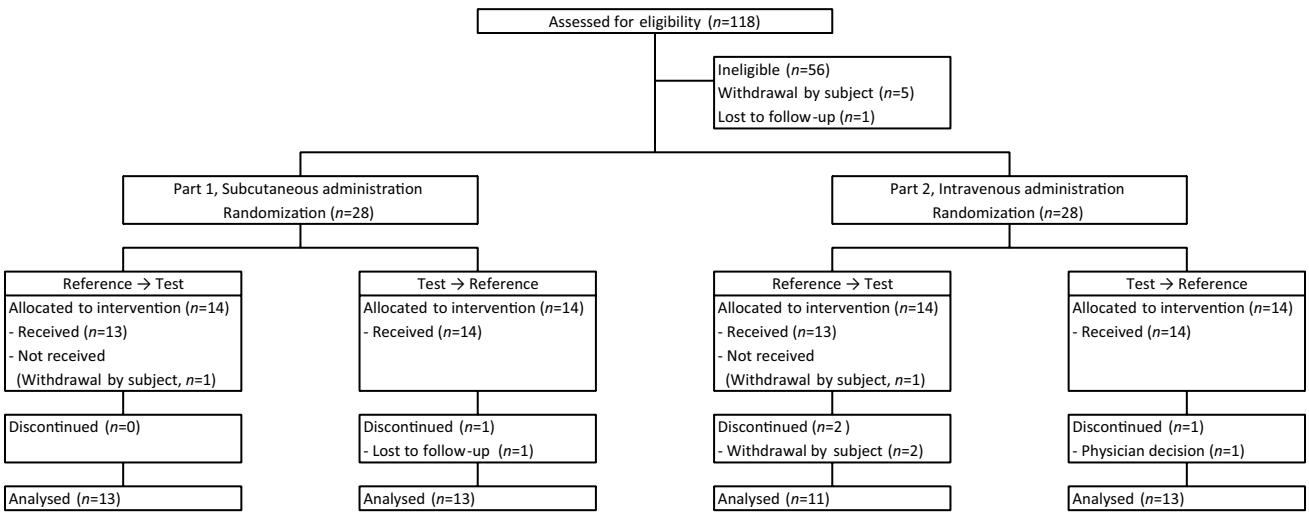

Fig. 1 Study design and subject disposition

following: history of drug abuse; history of major illness; positive status for HIV, syphilis, or hepatitis B or C; and clinically abnormal laboratory and 12-lead electrocardiography (ECG) safety parameters.

The intra-subject variability of the maximum serum concentration (C_{max}) and the area under the concentration–time curve (AUC) to the last measurable concentration (AUC_{last}) was assumed to be 20% and 15%, respectively, based on the previous report of darbepoetin alfa [19]. With a sample size of 20, a difference of 20% in the log-transformed PK parameters could be detected with a 90% test power at a significance level of 0.05. The total number of subjects for each part was 28 (14 subjects per sequence group), which could accommodate an approximate dropout rate of 25% [20].

The enrolled subjects were randomly allocated to one of the two sequences and received a single SC or IV injection of 60 µg of either the test drug (CJ-40001; CJ HealthCare Corp.) or the reference drug (NESP® Prefilled Syringe 60; Kyowa Hakko Kirin Co., Ltd.) after 10 h of fasting (Fig. 1). Depending on the part, the study drug was administered via the SC or IV route on the upper arm. A washout period of 35–49 days (representing at least a five-fold terminal elimination half-life ($t_{½}$) of darbepoetin alfa) was placed between the two periods to allow sufficient elimination of the drug administered in the previous period [19]. Blood samples for the PK assessment were obtained at predose and at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 168, 216, 264, and 360 h postdose in each period in part 1 and at predose and 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 168, 216, and 264 h postdose in each period in part 2. For the PD assessment, the Ret count (%), hematocrit (Hct; %), Hb (g/L), and RBC count (10^6/mm^3) were measured at predose and 24, 36, 48, 72, 96, 120, 168, 216, 264, and 360 h postdose in part 1 and at predose and 24, 36, 48, 72, 96, 120, 168, 216, and 264 h postdose in part 2. We assessed the anti-drug antibody (ADA) formation after darbepoetin alfa administration only in period 1. By doing this, we could rule out the carry-over effect in immunogenicity assessment.

2.2 Bioanalytical Methods

Serum darbepoetin alfa concentrations were quantified by a validated enzyme-linked immunosorbent assay (ELISA) method with a Quantikine® IVD® ELISA, a human EPO immunoassay kit (R&D Systems Inc., Minneapolis, MN, US; Lot number: 323465) [21, 22]. The quantification of EPO was made without distinction between endogenous EPO and darbepoetin alfa. The calibration standard samples of seven different concentrations (except the blank sample) constructed the calibration curve. Calibration curves of the test and reference showed linearity between 0.156 and 5 µg/L, and the lower limit of quantification for darbepoetin alfa was 0.156 µg/L. Samples expected to exceed the upper limit of the linear range were diluted before analysis with specimen diluent of the enzyme immunoassay kit. The intra- and inter-assay precision was 1.8% and 9.9%, respectively. The back-calculated concentration ranged from 84.0 to 105.8% at the lower limit of quantification (0.156 ng/mL), 98.8 to 101.6% at the upper limit of quantification (5 ng/mL), and 94.9 to 105.9% at the other concentrations.

To measure the ADA levels, the bridging ELISA method was used. The assay system utilized a biotin-labeled drug (test), a fluorescein-labeled drug, and an anti-fluorescein antibody. First, the positive control in human serum (100 µL), the biotin-labeled drug (100 µL), and the fluorescein-labeled drug (100 µL) were incubated simultaneously for 1 h in a polypropylene 96-well plate at 37 °C with shaking and then incubated overnight at 4 °C. This solution was then transferred to a streptavidin-coated microtiter plate, blocked with phosphate-buffered saline with Tween® 20 tablets, pH 7.4 (Takara Bio, Inc., Shiga, Japan) with 0.5% bovine serum albumin, and incubated for 1 h at room temperature. After removing the solution and washing, anti-fluorescein antibody and 3,3′,5,5′-tetramethylbenzidine were reacted on a plate.

The analyses of the hematologic parameters for PD assessment were conducted in the Department of Laboratory Medicine, Samsung Medical Center (SMC; Seoul, Republic of Korea). The Ret count, Hct, Hb, and RBC count were determined by flow cytometry using a Sysmex XE-2100 Hematology Autoanalyzer (Sysmex Corporation, Kobe, Japan).

2.3 Pharmacokinetic and Pharmacodynamic Analyses

The non-compartmental PK analysis of darbepoetin alfa was performed with Phoenix® WinNonlin® (version 7.1.0; Certara USA, Inc., Princeton, NJ, USA). The C_{max} and the time to C_{max} (t_{max}) were taken directly from the observed values. The AUC_{last} and the AUC from time zero to infinity (AUC_{∞}) were calculated by the linear-up/log-down method. The elimination rate constant (λ_z) was estimated by a linear regression of the log-linear decline of the concentration–time curve. The $t_{½}$ was calculated as $\ln 2/\lambda_z$. The clearance (CL) and apparent clearance (CL_{app}) were calculated by dividing the administered dosage by the AUC_{∞}. The volume of distribution (Vd) and apparent volume of distribution (Vd_{app}) were calculated by dividing the CL and CL_{app} by λ_z. Since the PK characteristics of darbepoetin alfa after SC or IV administration were

assessed in different subjects in separate parts of the study, we did not assess the bioavailability of darbepoetin alfa.

The time courses of the Ret count, Hct, Hb, and RBC count were investigated and compared between the test and reference drugs as PD markers. The maximum effect (E_{max}) and the time to E_{max} (t_{Emax}) were taken directly from the observed values. The AUC from time zero to the last point of quantifiable effect ($AUEC_{last}$) was calculated using the linear trapezoidal method.

2.4 Safety Analysis

All adverse events (AEs) were recorded on investigators' questionnaires or subjects' spontaneous reports. Physical examinations, vital-sign measurements, 12-lead ECGs, and laboratory testing (hematology, clinical chemistry, coagulation, and urinalysis) were performed at predefined, regular intervals throughout the studies and all clinically significant abnormal changes in the test results were reported as AEs. All AEs were assessed by the investigators with respect to severity, course, outcome, seriousness, and relationship to the study drug and were recorded regardless of the suspected relationship to the study drug. The clinical laboratory tests were performed by the Department of Laboratory Medicine at SMC. The laboratory is accredited by the College of American Pathologists, and quality control was ensured according to the internal standard operating procedures of SMC.

2.5 Statistical Analysis

Statistical analysis was carried out with SAS® Enterprise Guide® (version 7.1; SAS Institute Inc., Cary, NC, USA). The descriptive statistics of PK and PD variables for each analyte were summarized by intervention. The PK comparison was performed using the C_{max} and AUC_{last}. For the log-transformed PK parameters, linear mixed-effect analysis of variance was performed with fixed effects for the formulation, period, and sequence and a random effect for the subject nested for the sequence. The geometric mean ratio of the test to the comparator and the 90% confidence interval (CI) for each PK parameter was calculated. The test drug was considered to demonstrate a PK equivalence with the reference drug if the 90% CI for each PK parameter was within the range of 0.80–1.25. The PD parameters were log-transformed and then the significance of the difference between the two interventions was tested using the analysis of covariance (linear mixed model) including predose concentration as a covariate. For t_{Emax}, the significance of the difference between the two interventions was tested using the Wilcoxon signed rank test without log-transformation.

3 Results

3.1 Demographics

A total of 28 subjects were enrolled in both parts 1 and 2. A summary of the demographic data according to the study part is shown in Table 1. There were five and nine subjects who were smokers in parts 1 and 2, respectively, and the

Table 1 Demographic characteristics

Variables	Sequence		Total	*p* value[a]
	Reference → test	Test → reference		
Part 1: subcutaneous administration of darbepoetin alfa 60 μg				
Number of subjects	14	14	28	
Age (years)	32.64 ± 8.77	31.50 ± 7.29	32.07 ± 7.93	0.7107
Height (cm)	173.29 ± 4.66	173.67 ± 4.33	173.48 ± 4.42	0.7940
Weight (kg)	68.86 ± 6.36	68.24 ± 6.24	68.55 ± 6.19	0.8224
BMI (kg/m^2)	22.93 ± 1.88	22.62 ± 1.80	22.77 ± 1.81	0.6628
Part 2: intravenous administration of darbepoetin alfa 60 μg				
Number of subjects	14	14	28	
Age (years)	30.00 ± 7.50	29.07 ± 9.09	29.54 ± 8.19	0.7706
Height (cm)	171.41 ± 7.41	173.93 ± 5.68	172.67 ± 6.60	0.2868
Weight (kg)	67.09 ± 8.32	70.40 ± 7.76	68.75 ± 8.07	0.3216
BMI (kg/m^2)	22.81 ± 2.17	23.22 ± 1.65	23.02 ± 1.90	0.5783

Values are presented as the mean ± standard deviation

BMI body mass index

[a]Student's *t* test

mean (standard deviation) amounts of smoking were 7.0 (2.8) and 6.6 (3.5) cigarettes/day in parts 1 and 2, respectively. Demographic and other baseline characteristics were not significantly different between the two sequences in both parts. In part 1, there were two dropouts: one before and one after the investigational product administration. In part 2, there were four dropouts: one before and three after receiving the investigational product. Hence, 26 and 24 subjects completed the study in parts 1 and 2, respectively.

3.2 Pharmacokinetic Analysis

A summary of the PK parameters of darbepoetin alfa is presented in Table 2. The mean serum concentration–time profiles after SC or IV administration of the test or reference product are shown in Fig. 2.

3.2.1 Part 1: Subcutaneous Administration

The serum concentrations of darbepoetin alfa peaked at a median of approximately 24 h for the test and reference product after SC administration (Fig. 2a). The serum level of darbepoetin alfa decreased with similar elimination curves between interventions. The mean AUC_{last} and C_{max} values of the test product were 249.62 h·µg/L and 2.44 µg/L, respectively; the corresponding values of the reference product were 262.68 h·µg/L and 2.64 µg/L, respectively. The geometric mean ratios of the AUC_{last} and C_{max} between the test and reference product after SC administration were 0.9533 (90% CI 0.9110–0.9976) and 0.9326 (90% CI 0.8689–1.0009), respectively (Table 3). The differences between interventions of exposure after SC administration were not significant.

Table 2 Summary of pharmacokinetic variables for darbepoetin alfa

Variables	Test	Reference
Part 1: subcutaneous administration of darbepoetin alfa 60 µg		
Number of subjects	27	26
t_{max} (h)[a]	24.08 (12–48)	24.03 (8–48)
C_{max} (µg/L)	2.44 ± 0.74	2.64 ± 0.81
AUC_{last} (h·µg/L)	249.62 ± 56.30	262.68 ± 57.42
AUC_{∞} (h·µg/L)	294.93 ± 62.81	310.37 ± 79.18
$t_{½}$ (h)	134.17 ± 45.52	135.57 ± 62.53
CL_{app} (L/h)	0.21 ± 0.05	0.21 ± 0.05
Vd_{app} (h)	40.11 ± 13.33	37.86 ± 12.08
Part 2: intravenous administration of darbepoetin alfa 60 µg		
Number of subjects	25	26
C_{max} (µg/L)	17.78 ± 2.34	18.64 ± 3.80
AUC_{last} (h·µg/L)	466.86 ± 83.46	479.78 ± 86.92
AUC_{∞} (h·µg/L)	505.14 ± 99.72	516.30 ± 89.75
$t_{½}$ (h)	121.43 ± 59.75	119.05 ± 53.23
CL (L/h)	0.12 ± 0.02	0.12 ± 0.03
Vd (h)	20.45 ± 7.69	20.48 ± 9.77

Data are presented as the mean ± standard deviation unless stated otherwise

AUC_{∞} area under the concentration–time curve from time zero to infinity, AUC_{last} area under the concentration–time curve to the last measurable concentration, CL clearance, CL_{app} apparent clearance, C_{max} maximum serum concentration, $t_{½}$ terminal elimination half-life, t_{max} time to maximum observed serum concentration, Vd volume of distribution, Vd_{app} apparent volume of distribution

[a]Data presented as median (minimum–maximum)

3.2.2 Part 2: Intravenous Administration

The serum level of darbepoetin alfa decreased with similar elimination curves after IV administration between interventions (Fig. 2b). The arithmetic means of the darbepoetin alfa AUC_{last} value for the reference and test were 479.78 and 466.86 h·µg/L, respectively, and the arithmetic means of the darbepoetin alfa C_{max} value for the reference and test were 18.64 and 17.78 µg/L, respectively. The geometric mean ratios of the AUC_{last} and C_{max} between the test and reference product after IV administration were 0.9798 (90% CI 0.9205–1.0429) and 0.9701 (90% CI 0.8883–1.0594), respectively (Table 3). There was no significant difference in exposure between the two interventions.

3.3 Pharmacodynamic Analysis

The mean Ret counts increased up to 168 and 120 h after the SC and IV drug administration and then decreased until the last observed time (Fig. 3). In both parts, the $AUEC_{last}$ and E_{max} of the PD analytes were comparable between the test and the reference product (Table 4). Moreover, the time courses for the changes in PD analytes were similar between the test and reference drug (Table 4; Fig. 3). The time-matched PK and PD markers presented a counterclockwise hysteresis, which suggests a time delay between the change in serum concentration of darbepoetin alfa and the PD response (Fig. 4). From the timepoints at which both PK and PD were measured, the highest level of darbepoetin alfa concentration and Ret count were observed at 24 and 168 h after administration of darbepoetin alfa, respectively, in both part 1 and part 2.

3.4 Safety

A total of 54 subjects (part 1: 27, part 2: 27) received at least one dose of the test or reference drug. The treatment-emergent AEs that occurred in two or more subjects are shown in Table 5. No serious AE- and AE-related dropouts occurred during the study. All AEs were mild to moderate in severity and were recovered without sequelae. Frequencies of treatment-emergent AEs were similar between

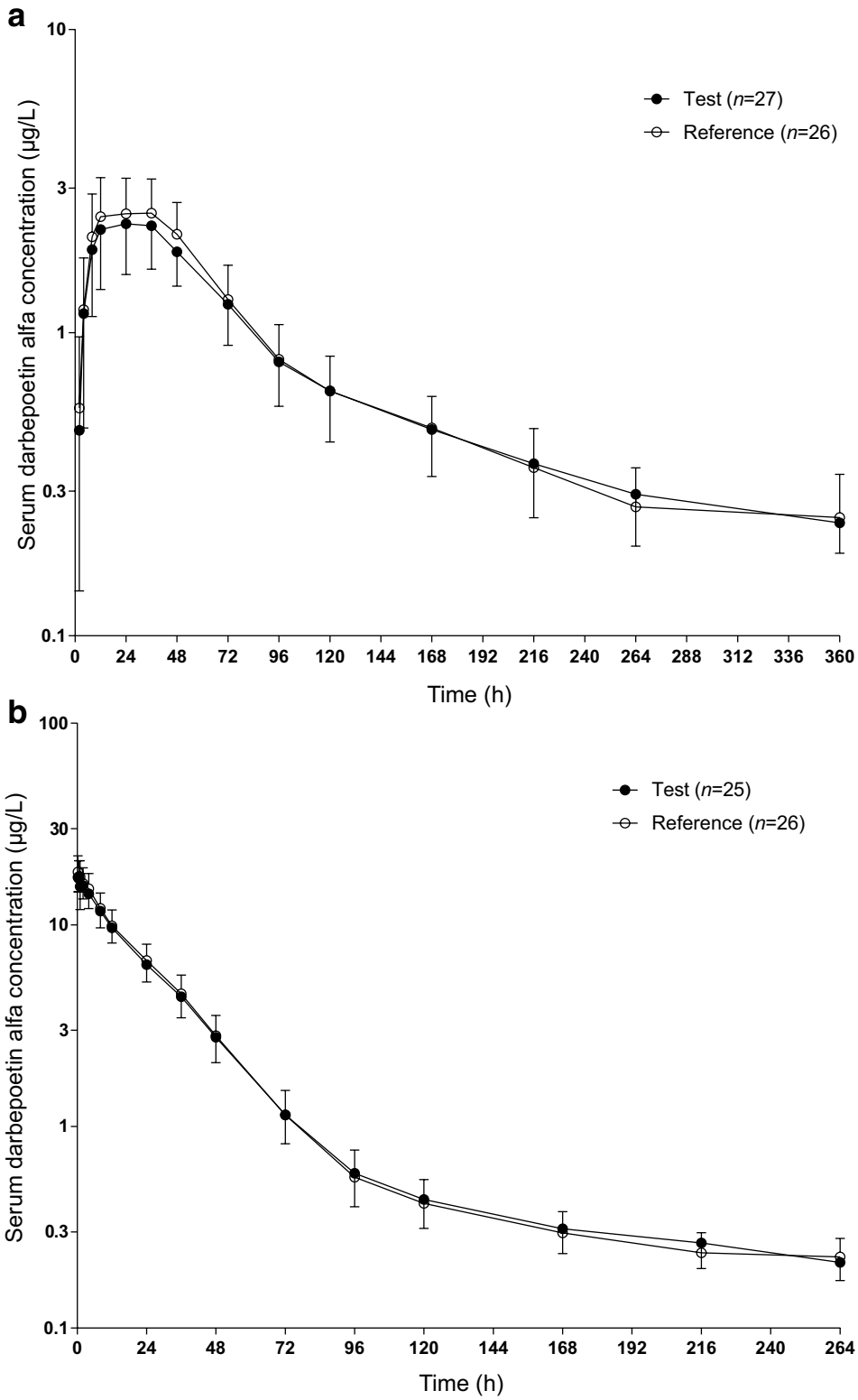

Fig. 2 Mean serum concentration–time profiles of darbepoetin alfa after **a** subcutaneous and **b** intravenous administration of the test or reference formulation. Note: bars represent standard deviations

the test and reference drug in parts 1 ($p = 0.4152$) and 2 ($p = 0.2528$). Clinical laboratory evaluation of hematology, blood chemistry, and urinalysis throughout the study showed no unexpected changes that could be attributed to the test and reference drugs. There was no clinically significant finding in the vital signs, ECGs, and physical examination results in all interventions. The ADA reactivity was negative for all samples from both intervention groups regardless of

Table 3 Pharmacokinetic comparison of darbepoetin alfa between interventions

Variables	Geometric least square mean (CV)		Geometric mean ratio (90% confidence interval)[a]
	Test	Reference	
Part 1: subcutaneous administration of darbepoetin alfa 60 μg			
Number of subjects	27	26	
AUC_{last} (h·μg/L)	241.13 (22.56)	252.95 (21.86)	0.9533 (0.9110–0.9976)
C_{max} (μg/L)	2.35 (30.17)	2.52 (30.69)	0.9326 (0.8698–1.0009)
Part 2: intravenous administration of darbepoetin alfa 60 μg			
Number of subjects	25	26	
AUC_{last} (h·μg/L)	457.93 (17.88)	467.36 (18.12)	0.9798 (0.9205–1.0429)
C_{max} (μg/L)	17.97 (13.16)	18.52 (20.38)	0.9701 (0.8883–1.0594)

AUC_{last} area under the concentration–time curve to the last measurable concentration, *C_{max}* maximum serum concentration, *CV* coefficient of variation

[a]Transformed back to the original scale after statistical analysis using log-transformed data

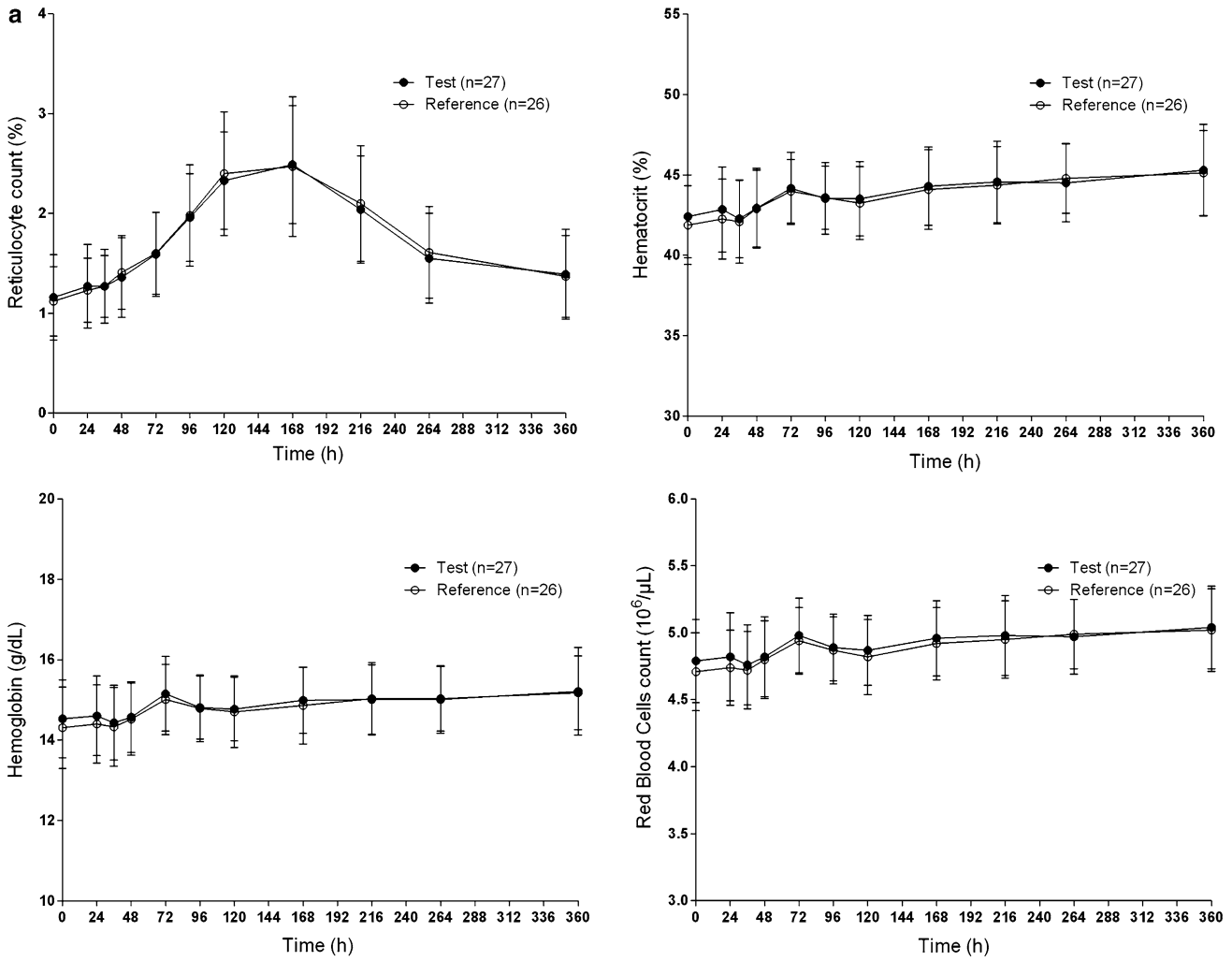

Fig. 3 Mean pharmacodynamic variable–time profiles of darbepoetin alfa after **a** subcutaneous and **b** intravenous administration of the test or reference formulation. Note: bars represent standard deviations

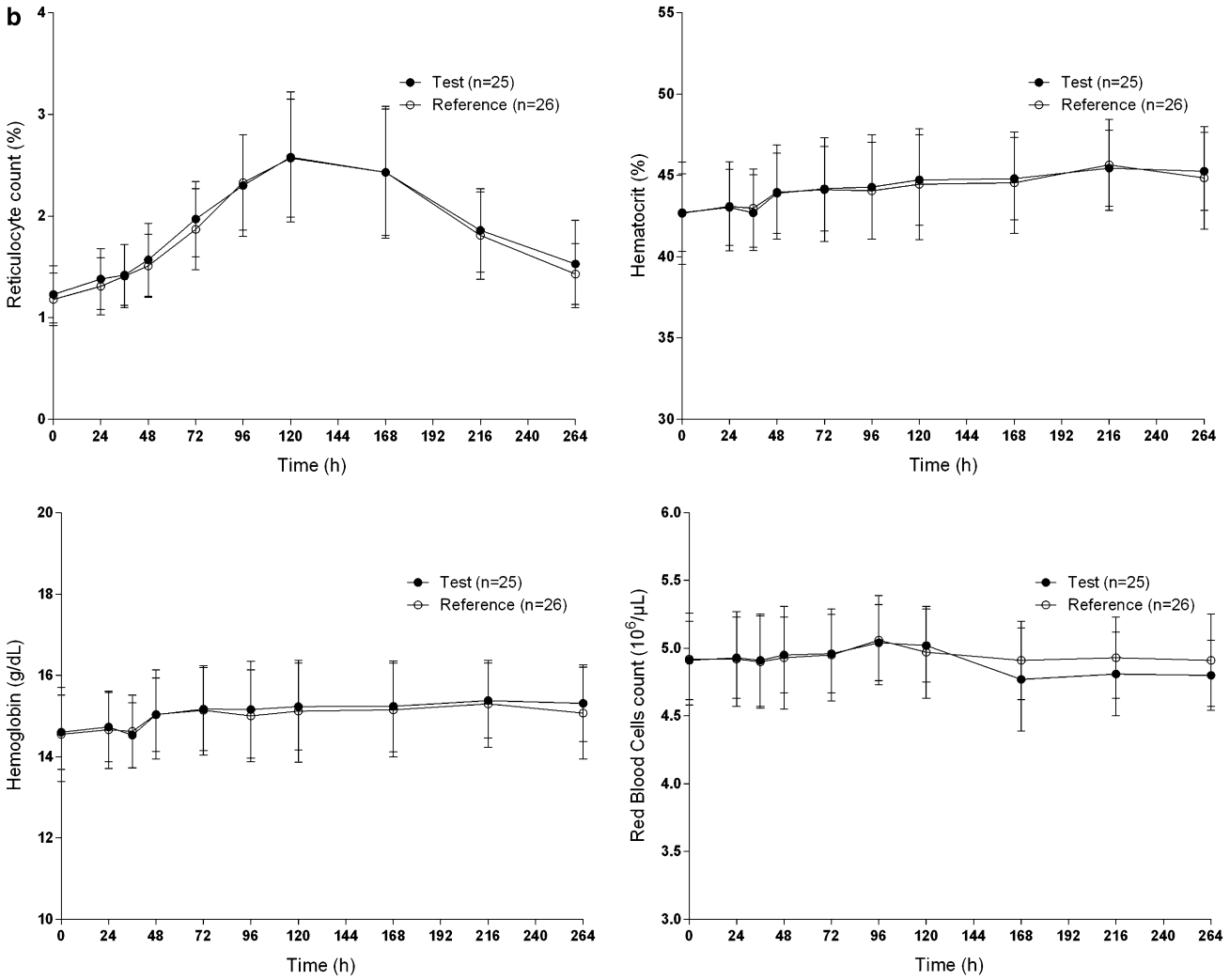

Fig. 3 (continued)

administration route. Administration of the test drug given as a single SC or IV injection was safe and well-tolerated in healthy male subjects and the frequency of AEs was similar to that in the reference drug.

4 Discussion

This study was designed to evaluate the PK, PD, and safety profiles of test and reference darbepoetin alfa given as a single SC or IV injection in healthy male subjects. The test darbepoetin alfa exhibited comparable safety, PK, and PD profiles to the reference drug in both administration routes.

For the PKs, the CIs of the geometric mean ratios (test to reference) for C_{max} and AUC_{last} fell within the acceptance criteria of 0.80–1.25 with both SC and IV administration. The inter-subject variabilities of PK variables for the test drug were similar to those for the reference drug in both administration routes. The $t_{½}$ of darbepoetin alfa was longer following SC injection than following IV injection for the test drug, which is consistent with the previously reported results for the reference product [19, 23]. The $t_{½}$ of darbepoetin alfa after SC administration reflects flip-flop kinetics [23]. In this study, the $t_{½}$ of the reference darbepoetin alfa following IV injection was 119.05 h, which is four times more than that of a previous report (25.3 h) [19]. This study employed more protracted sampling periods (up to 360 h) than the previous study (96 h) and the $t_{½}$ calculated from data with the same last timepoint (96 h) as the previous study is 20.4 h. The $t_{½}$ of darbepoetin alfa in healthy subjects after SC administration is not reported. For reference, the $t_{½}$ was reported to be 48.8 h in dialysis patients with blood sampling up to 168 h after SC administration of darbepoetin alfa [24]. Meanwhile, since the quantification of EPO was made without distinction between endogenous EPO and darbepoetin alfa, correction to a baseline value was

Table 4 Summary of pharmacodynamic variables for darbepoetin alfa between interventions

Variables	Test	Reference	*p* value[a]
Part 1, subcutaneous administration of darbepoetin alfa 60 µg			
Number of subjects	27	26	
Reticulocyte count			
$AUEC_{last}$ (h·%)	623.5 (23.7)	628.9 (24.3)	0.5159
E_{max} (%)	2.45 (22.6)	2.47 (26.2)	0.6802
t_{Emax} (h)[b]	168.0 (95.9–168.7)	168.0 (119.7–168.9)	0.3426
Hematocrit			
$AUEC_{last}$ (h·%)	15,866 (5.07)	15,833 (4.80)	0.6694
E_{max} (%)	46.1 (5.24)	45.8 (4.39)	0.4557
t_{Emax} (h)[b]	168.0 (95.9–168.7)	264.1 (72.0–361.2)	0.9696
Hemoglobin			
$AUEC_{last}$ (h·g/dL)	5371 (5.37)	5353 (5.49)	0.5707
E_{max} (g/dL)	15.6 (5.58)	15.5 (5.04)	0.9713
t_{Emax} (h)[b]	263.9 (0.0–360.9)	264.1 (24.0–361.2)	0.6238
Red blood cell count			
$AUEC_{last}$ (h·10^6/µL)	1776 (5.34)	1767 (5.20)	0.9433
E_{max} (10^6/µL)	5.15 (5.58)	5.11 (4.83)	0.4708
t_{Emax} (h)[b]	263.5 (24.0–360.9)	264.6 (72.0–361.2)	0.7127
Part 2: intravenous administration of darbepoetin alfa 60 µg			
Number of subjects	25	26	
Reticulocyte count			
$AUEC_{last}$ (h·%)	510.2 (19.5)	500.5 (20.0)	0.0735
E_{max} (%)	2.65 (18.6)	2.60 (21.3)	0.4007
t_{Emax} (h)[b]	120.1 (72.0–168.8)	120.1 (96.2–168.6)	0.7174
Hematocrit			
$AUEC_{last}$ (h·%)	11,735 (5.19)	11,705 (6.39)	0.6236
E_{max} (%)	46.3 (5.47)	46.2 (6.36)	0.7122
t_{Emax} (h)[b]	215.9 (48.0–264.3)	215.6 (36.0–264.5)	0.6151
Hemoglobin			
$AUEC_{last}$ (h·g/dL)	3995 (6.11)	3970 (6.99)	0.3561
E_{max} (g/dL)	15.7 (6.53)	15.7 (7.06)	0.6233
t_{Emax} (h)[b]	168.1 (48.1–264.3)	120.1 (0.0–264.5)	0.7063
Red blood cell count			
$AUEC_{last}$ (h·10^6/µL)	1304 (5.31)	1302 (6.50)	0.5796
E_{max} (10^6/µL)	5.13 (5.50)	5.12 (6.54)	0.2600
t_{Emax} (h)[b]	168.8 (48.1–264.3)	191.9 (24.0–264.5)	0.6418

Data are presented as the geometric least square mean (coefficient of variation) unless specified otherwise

$AUEC_{last}$ area under the effect–time curve from time zero to the last point of quantifiable effect, E_{max} maximum effect, t_{Emax} time to maximum effect

[a]Linear mixed model with intervention, period, sequence, group, and sequence-nested subject effect and predose value as a covariate for $AUEC_{last}$ and E_{max}, while the Wilcoxon signed rank test was the source for t_{Emax}

[b]Data presented as median (minimum–maximum)

plausible for PK analysis. However, in this study, baseline correction was not carried out because most baseline values were below the lower limit of quantification.

It is recommended that darbepoetin alfa be administered once a week or every 2 weeks for patients with CKD on dialysis and with 4-week intervals for patients with CKD not on dialysis [25, 26]. The Hct, Hb, and RBC counts that reflect erythropoiesis increased up to 360 h, while the Ret counts recovered to baseline levels after an initial incremental increase following SC injection of darbepoetin alfa. Following IV injection, Hct, Hb, and RBC counts increased for 216 h and then decreased thereafter, while the Ret

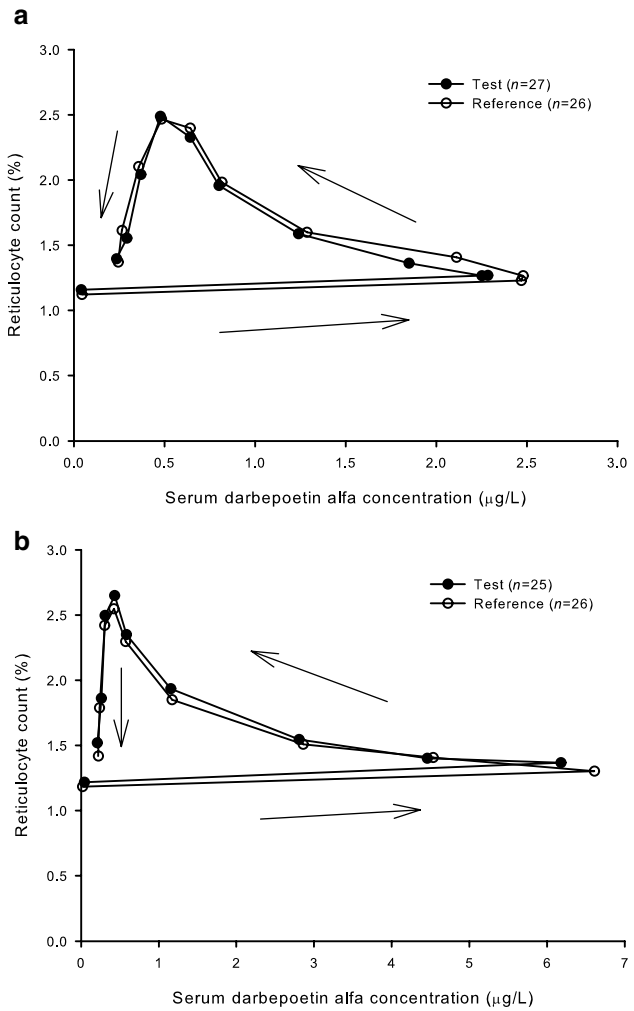

Fig. 4 Relationship between mean serum darbepoetin alfa concentration and reticulocyte count after **a** subcutaneous and **b** intravenous administration of the test or reference formulation. Note: the symbols represent the mean pharmacokinetic–pharmacodynamic relationship at predose and 24, 36, 48, 72, 96, 120, 168, 216, 264, and 360 h after subcutaneous administration, and at predose and 24, 36, 48, 72, 96, 120, 168, 216, and 264 h after intravenous administration

count–time profile was similar to that of the SC route. This may result from slow absorption along with the long duration of SC administration, which leads to sustained drug exposure and persistence of the effect. However, IV administration of darbepoetin alfa also showed an erythropoietic effect over sufficient time in this study. By examining the response after administration, in this study we again confirmed the appropriateness of the darbepoetin alfa regimens, including the IV route recommendation, for patients on hemodialysis [25].

Intensive PK–PD studies of darbepoetin alfa in healthy subjects have not been reported. However, we verified the relationship and time-dependent changes in the PK–PD relationship of darbepoetin alfa, revealing it to be a counter-clockwise hysteresis loop. This characteristic of rHuEPO had already been reported in previous studies [22, 27]. The counter-clockwise hysteresis implies that there is a delay in equilibrium between the serum drug concentration and the PD effect [28] related to the maturation of normoblasts in bone marrow (3–7 days) [29–31].

The PKs and PDs of darbepoetin alfa are well-established in patient populations and healthy subjects. Thus, regarding assessment of rHuEPO biosimilars, PK and PD results with sufficient evidence to support a conclusion of no clinically meaningful differences may make a comparative efficacy study unnecessary in cases where there is a meaningful correlation between the PK and PD results and clinical effectiveness [32]. Although there are some patients who are resistant to rHuEPO, the cause of this is well-known and it has been established that rHuEPO shows a dose-proportional erythropoietic effect in about 90% of anemia patients [33, 34]. Because of this, in general, the PK and erythropoetic responses to rHuEPO in patients with CKD are not expected to differ from those in healthy subjects [35, 36]. For reference, the PK/PD assessments in healthy subjects were considered the most perceptive in identifying the PK and PD response characteristics for a proposed biosimilar of epoetin alfa [37]. Furthermore, there is a known correlation between the Hb response and the improvement in quality of life after administration of darbepoetin alfa [38]. This study provides good evidence to enable prediction of the efficacy and effectiveness in patients receiving darbepoetin alfa with regards to Hb response and quality of life.

Administration of a single dose of test and reference darbepoetin alfa was safe and well-tolerated in healthy male subjects. Observed AEs were consistent with the AEs reported in the prescribing information of NESP® and no unexpected AEs were found following the administration of the test.

5 Conclusion

The test darbepoetin alfa had similar PK and PD characteristics as the reference drug and the safety and immunogenicity profile was similar between the two interventions. In terms of PDs, the serum concentration of darbepoetin alfa and erythropoietic response showed hysteretic relationships that were comparable between the two interventions. The similarities of the PK and PD properties and the safety

Table 5 Treatment-emergent adverse events reported in two or more subjects between interventions

Symptoms and signs	Test	Reference
Part 1: subcutaneous administration of darbepoetin alfa 60 μg	$n=27$	$n=26$
Back pain	1 (1)	2 (2)
Headache	1 (1)	2 (2)
Injection-site bruising	3 (3)	
Nasopharyngitis	1 (1)	4 (4)
Neutrophil count decreased	2 (2)	2 (2)
Part 2: intravenous administration of darbepoetin alfa 60 μg	$n=25$	$n=27$
Blood creatine phosphokinase increased	2 (2)	1 (1)
Headache	2 (2)	
Nasopharyngitis	2 (2)	2 (2)
Neutrophil count decreased	2 (2)	
Myalgia	1 (1)	1 (1)

Data presented as the number of subjects (number of events)

profiles of the test and reference drug imply that they can be used interchangeably.

Author contributions This study was designed and conducted by TH, J-WK, WH, and J-RK. The sponsor was not involved in the study design or in the analysis and interpretation of the data. All of the co-authors participated in either writing or reviewing the manuscript.

Compliance with Ethical Standards

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Conflict of interest Seokuee Kim, Taegon Hong, Jae-Wook Ko, Wooseong Huh, and Jung-Ryul Kim declare that they have no conflict of interest.

Ethical approval The study protocol was reviewed and approved by the health authorities from the Ministry of Food and Drug Safety of South Korea and the Institutional Review Board of Samsung Medical Center. Written informed consents were obtained from all subjects included in this study.

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