Chiang Mai Medicall Journal 2008;47(3):97-103.

Original article

BIOEQUIVALENCE STUDY OF THE BIOSIMILAR RECOMBINANT HUMAN ERYTHROPOIETIN ALPHA (RENOGEN®) AND EPREX® AFTER SUBCUTANEOUS ADMINISTRATION

Noppamas Rojanasthien, M.D., Rapheephorn Khatsri, M.S., Nutthiya Hanprasertpong, M.D., Puongtip Kunanusorn, Ph.D.

Department of Pharmacology, Faculty of Medicine, Chiang Mai University

Abstact To determine the bioequivalence of two formulations of recombinant human erythropoietin alpha (epoetin alpha 4000-IU), the two formulations; Renogen® and the Reference, Eprex® were administered to 18 healthy Thai male volunteers as a single subcutaneous dose according to a randomized two-way crossover design. Serial blood samples were collected over a period of 96 hours. The pharmacokinetic parameters were analyzed by noncompartmental analysis, and bioequivalence analysis (ANOVA) was carried out using logarithmically transformed data of the AUC, Cmax and untransformed Tmax. The elimination half-life of the test product (28.7 h) and the reference (31.1 h) were comparable. The median Tmax of the test product (12.0 h, range 4-15 h) was slightly slower than that of the reference (11.0 h, range 8-15 h). The ANOVA showed no statistically significant differences between the AUC and Cmax values or between the test and the reference preparations. The mean (90% CI) for the ratios Test/Reference for AUC_{0-t}, $AUC_{0-\infty}$ and Cmax were 1.03 (0.97-1.10), 1.01 (0.96-1.06) and 0.99 (0.90-1.08), respectively, within the bioequivalence range of 0.80-1.25. The study concluded that the test product is bioequivalent to the reference. **Chiang Mai Medical Journal 2008;47(3):97-103.**

Keywords: bioequivalence, recombinant human erythropoietin alpha, Renogen®, Eprex®

Erythropoietin (EPO) is a glycoprotein growth factor that stimulates erythrocyte proliferation and differentiation. Endogenous EPO is produced in response to tissue hypoxia by the kidney.[1] Once released, EPO distributes to the bone marrow, where it binds to a receptor on the surface of committed erythroid progenitors and is internalized to stimulate a rapid expansion of erythroid progenitors as well as inducing the release of reticulocytes. In normal subjects, the ranges of plasma EPO levels varied from 3.3-13.5 IU/L (mean 6.70 IU/L),[2] 6-32 IU/L[3] and 10-30 IU/L.[1] However, changes in plasma EPO concentration are known to exhibit a circadian rhythm. EPO was found at its lowest mean level at 08:00 hours and had increased by 42% at 16:00 hours it

Address requests for reprints: Noppamas Rojanasthien, M.D., Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand: E-mail:nrojanas@mail.med.cmu.ac.th
Received 20 October, 2008, and in revised form 12 November, 2008.

increased to the highest level by 60% at 20:00 hours.[2] The recommended sampling time for EPO level is between 08:00-12:00 hours. The average EPO level in patients with chronic renal failure (creatinine clearance < 40 mL/mim) is usually low, due to loss of functional tissues and cells responsible for EPO production.[4] The anemia of chronic renal failure is due to inability of the kidney to produce adequate amounts of EPO as well as reduction in red cell survival. Nowadays, treatment of anemia from chronic renal failure has been revolutionized by the availability of recombinant human erythropoietin and renal failure patients will most likely respond to treatment with the exogenous EPO. Exogenous EPO for clinical use is produced by recombinant DNA technology and named recombinant human erythropoietin (rHuEPO) or epoetin. Epoetin alfa, epoetin beta, epoetin gamma, and epoetin omega are recombinant human erythropoietins derived from a cloned human erythropoietin gene.[5] All have the same 165 amino acid sequence, but differ in the glycosylation pattern. Epoetin-alpha and epoetin-beta are available for clinical use in Thailand. The efficacy of epoetin may depend on the route of administration. Clinical studies have demonstrated that the subcutaneous (SC) route offers a few advantages over intravenous (IV) administration. For instance, SC administration is more convenient, as it does not require any venous access. When compared to the IV route, SC administration significantly prolongs the higher levels of serum erythropoietin, thus sustaining the stimulation of erythropoiesis.[6] Although the bioavailability of SC administration is lower than IV, (bioavailability of 30-36% for epoetin alpha),[4,7] a weekly dosage of epoetin required for therapeutic effect has been reported as 15-50% lower through the SC route when compared with IV administration.[8] The dose-sparing effect has been attributed to the extended half-life after SC administration, resulting in a sustained stimulation of erythroid progenitor cells [half-lives for epoetin alpha after IV vs SC routes were 4-11 h vs 19-25.3 h].[5] Furthermore, a sudden drop in circulating erythropoietin levels after IV administration may cause lysis of young erythrocytes. The adverse effects reported in renal failure patients included headache, hypertension, and seizures. The dose of rHuEPO must be adjusted to obtain a gradual rise in hematocrit level, over a 2- to 4- month period, until final hematocrit levels of 33% to 36% are reached. Subcutaneous administration may cause redness, swelling, or itching at the site of injection. Pure red cell aplasia (PRCA) associated with neutralizing antibodies to endogenous EPO has been reported in chronic renal failure patients with long-term use of rHuEPO.[9] The majority of cases were observed after subcutaneous administration of epoetin-alpha, once human serum albumin had been removed from the formulation.[10] Although the global incidence of this phenomenon is relatively low, rHuEPO should be discontinued and the patient should not be switched to another product, as anti-erythropoietin antibodies may cross-react with other epoetin.

Recently rHuEPO alpha has been developed and marketed in Thailand. Since subcutaneous administration of different preparations of rHuEPOs may result in different pharmacokinetic profiles, this study investigated the pharmacokinetics of the test product in comparison with Eprex®.

Objective

To compare the bioequivalence of the test product (Renogen®) and Eprex® after subcutaneous administration at an equal dose in healthy Thai male volunteers.

Materials and methods

Eighteen healthy Thai male volunteers aged between 19-29 years old (mean 22.89± 3.18 years old and 23.00±1.87 years old for the T-R and R-T sequence, respectively) with a body mass index within 18.8–24.6 kg/m^2 (mean 20.30±1.18 years old and 21.46±1.79 years old for the T-R and R-T sequence, respectively) participated in this study. They were in good health on the basis of medical history, physical examination and routine blood test. Volunteers with known contraindication or hypersensitivity to the epoetin component were excluded as well as those with known hypersensitivity to human albumin products and mammalian cell-derived products. Volunteers with a known history of drug abuse, alcohol consumption and cigarette smoking were also excluded. The study was approved by the Research Ethics Committee of Chiang Mai University, Thailand and all volunteers signed the informed consent form prior to participation in the study.

Study drugs

Reference product: Eprex® 4000 IU (manufactured by Vetter Pharma GmbH. Ravensburg, Germany, imported by Jassen-Cilag Ltd., Bangkok, Lot No: 6CSTH00, Mfd 03/2006, Exp 08/2007).

Test product: Renogen® 4000 IU (manufactured by Shenzhen SciProGen Biopharmaceutical Co., Ltd. Yayuan Rd. Petrochemical Industry Zone Longgang District, Shenzhen, People's Republic of China, imported by Great Eastern Drug Co., Ltd., Bangkok, Lot No: 20060405, Mfd 27/04/2006, Exp 04/2008).

Method of drug administration and blood sample collections

The study was a randomized, two-period, two-sequence, crossover study. On the study day, the volunteers received a single dose subcutaneous administration of either the test product or reference on the upper arm at 09:00 hours. The wash out period between each study visit was 21 days, after which the volunteers were crossed over to receive the alternative product. On each study day, a total of 12 blood samples (3 mL each) were collected at predose (0 hour) and 1, 2, 4, 8, 12, 15, 18, 24, 48, 72 and 96 h after dose administration. The plasma sample was separated promptly and immediately freeze at -70 ^{o}C until assay. Erythropoietin concentration in plasma was determined by the ELISA method using a Quantikine® IVD® EPO Kit (R&D System Inc. MN, USA).

Statistical and data analysis

Maximal plasma concentration (Cmax, mU/mL) and time to reach the peak concentration (Tmax, h) were obtained directly by visual inspection of each volunteer's plasma concentration-time profile. The area under the plasma concentration-time curve (AUC) from time 0-infinity ($AUC_{0-\infty}$, mU.h/mL) and half-life ($t_{1/2}$, h) were determined by non-compartmental analysis. The slope of the terminal log-linear portion of the concentration-time profile was determined by least-squares regression analysis and used as the elimination rate constant (K_e). The elimination half-life was calculated as $0.693/K_e$. The AUC_{0-t} from time zero to the last quantifiable

point (C_t) was calculated using the trapezoidal rule, and extrapolated AUC from Ct to infinity ($AUC_{t-\infty}$) was determined as C_t/K_e. Total $AUC_{t-\infty}$ was the sum of AUC0-t + $AUC_{t-\infty}$. The calculation was performed by using the TopFit, pharmacokinetic data analysis program for PC.

An analysis of varience (ANOVA) was performed to determine the statistical differences of pharmacokinetic parameters ($AUC_{0-\infty}$, C_{max}, and T_{max}), which represented the extent and rate of drug absorption. Statistical analysis of AUC and Cmax was performed on the logarithmically (ln) transformed data.[10-11] The 90% confidence interval for the ratio of AUC as well as Cmax values of the test preparation over those of the reference product were estimated using the following equation:

$$90\ \%\ CI\ (\mu T\text{-}\mu R) = \left(\bar{X}_T - \bar{X}_R\right) \pm t^{v}0.1\sqrt{\frac{2S^2}{n}}$$

- XT andXR are the observed mean of the (ln) transformed parameters (either C_{max} or AUC) for the test product (T) and the reference (R).

- S2 is the error variance obtained from the ANOVA.

- n is the number of volunteers.

- $t^{v}0.1$ is the tabulated two-tail t value for 90% CI.

- v is the number of degrees of freedom of the error mean square.

The antilogarithm of the confidence interval (μT-μR) expressed the bioequivalence as a ratio of test and reference [μT/μR]. The bioequivalence acceptance criteria required that the 90% CI for the ratio Test/Reference of the $AUC_{0-\infty}$ and C_{max} fell within the interval of 0.8-1.25.[10-11]

Results and discussion

All volunteers completed the study without any serious adverse events. During the first visit, volunteer No. 14 received treatment (5 days of cephalexin at 250 mg qid, paracetamol at 500 mg) for fever and tonsilitis.

The mean baseline erythropoietin concentration (mU/mL) for the test [6.40±4.18 (range 3.79-21.99)] and reference [6.05±2.24 (range 2.77-13.37)] showed no statistically significant difference (p=0.1294) and was comparable to those values reported.[1-3] After subcutaneous administration, the erythropoietin plasma concentration increased slowly. The median Tmax of the test (12.0 h, range 4-15 h) was slightly slower than that of the reference (11.0 h, range 8-15 h), and the mean (90% CI) for Tmax differed (h) by 0.83 [(-0.59)–2.26] (bioequivalence range ± 2.06 h). The delay in absorption after the subcutaneous route resulted in a sustained erythropoietin concentration above baseline levels for 72 h in both preparations. The elimination of half-life, and the elimination

Table 1. Pharmacokinetic parameters of erythropoietin and 90% CI

Pharmacokinetic parameters	Test Product	Reference product	90% CI (BE range 0.80-1.25)
C_{max} (mU/mL)	74.08±17.00	75.35±19.66	0.99 (0.90-1.08)
AUD (mU.h/mL)	2706.52±381.60	2578.94±525.92	1.03 (0.97-1.10)
AUC (mU.h/mL)	3619.41±540.42	3075.01±571.01	1.01 (0.96-1.06)
T_{max} (h)	11.1±3.12 (12.0)	10.3±2.27 (11.0)	
$t_{1/2}$ (h)	28.7±4.88	31.1±10.79	

PK parameters present as mean ± SD; T_{max} present as [Mean ± SD (median)]

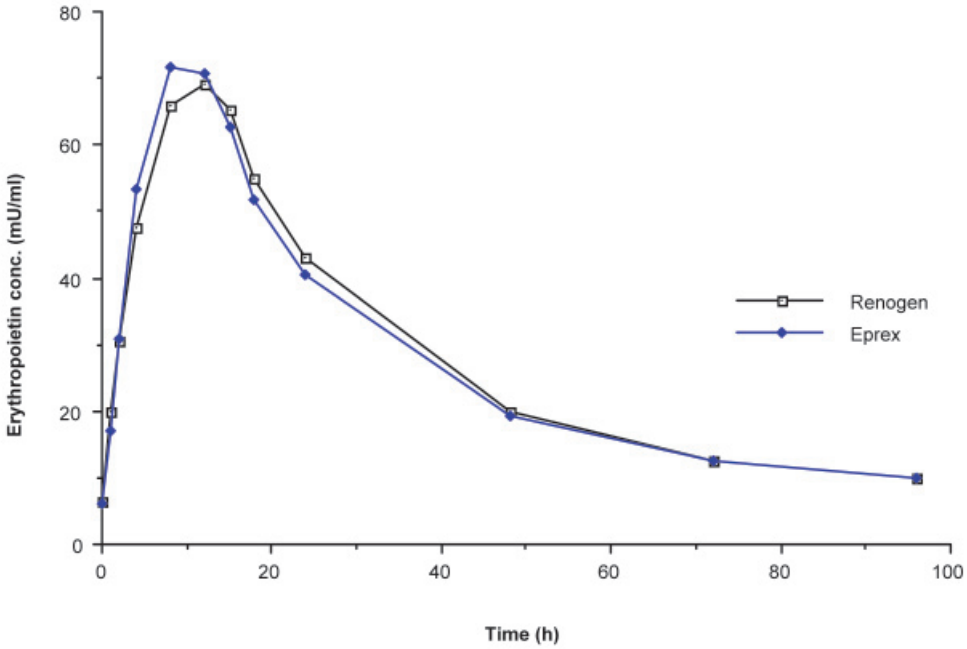

Figure 1. Mean plasma concentration-time profiles after single subcutaneous administration of 4000 IU Renogen® (-□ -) and Eprex® (-♦-)

rate constant of the test (28.69 h, 0.0244 h-1) and reference (31.09 h, 0.0242 h-1) were similar and comparable to those values reported [average t1/2 20 h, range 6.94-48.27 h].[13] The mean plasma concentration-time curves of the test and reference product were also comparable (Fig 1). The relative bioavailability (Frel) calculated from Cmax, AUC_{o-t} and $AUC_{o-\infty}$ of Renogen®/Eprex® was 101%, 104% and 102%, respectively. From the ANOVA table, the intra-subject coefficient of variation (%CV) estimated from S2 obtained from the ANOVA after logarithmic transformation, for the AUC_{o-t}, $AUC_{o-\infty}$ and Cmax, was 11%, 8% and 16%, respectively. According to the nomograms and tables of Diletti,[13] the power of test obtained from this study for AUC_{o-t}, $AUC_{o-\infty}$ and Cmax was more than 90% for the sample size of 18. The average Cmax (mU/mL) of Renogen® (74.08) and Eprex® (75.35) showed no significant difference, with a mean (90% CI) of the ratios of 0.99 (0.90-1.08). Similarly, the extent of absorption in both preparations was bioequivalent based on the mean (90% CI) of 1.03 (0.97-1.10) and 1.01 (0.96-1.06) for the ratios of the AUC_{0-t}, and $AUC_{0-\infty}$, respectively. We concluded that Renogen® was bioequivalent to Eprex®, with respect to the rate and extent of absorption.

References

1. Graber SE, Krantz SB. Erythropoietin and the Control of Red Cell Production. Ann Rev Med 1978;29:51-66.
2. Wide L, Bengtsson C, Birgegard G. Circadian rhythm of erythropoietin in human serum. Br J Haematol 1989;72:85-90.
3. Krzyzanski W, Jusko WJ, Wacholtz MC, Minton N, Cheung WK. Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after multiple subcutaneous doses in

healthy subjects. Eur J Pharm Sci 2005;26:295-306.
4. Fehr T, Ammann P, Garzoni D, et al. Interpretation of erythropoietin levels in patients with various degrees of renal insufficiency and anemia. Kidney Int 2004;66:1206-11.
5. Deicher R, Horl WH. Differentiating factors between erythropoiesis-stimulating agents: a guide to selection for anaemia of chronic kidney disease. Drugs 2004;64:499-509.
6. Ashai NI, Paganini EP, Wilson JM. Intravenous versus subcutaneous dosing of epoetin: a review of the literature. Am J Kidney Dis 1993;22(Suppl 1):23-31.
7. Besarab A. Optimizing anaemia management with subcutaneous administration of epoetin. Nephrol Dial Transplant 2005;(Suppl 6):10-5.
8. National Kidney Foundation. IV. NKF-K/DOQI Clinical Practice Guidelines for Anemia of Chronic Kidney Disease: update 2000. Am J Kidney Dis 2001;37(1 Suppl 1):S182-238.
9. Macdougall IC. Antibody-mediated pure red cell aplasia (PRCA): epidemiology, immunogenicity and risks. Nephrol Dial Transplant 2005;20(Suppl 4): 9-15.
10. Criteria and Guideline for the Bioequivalence Study of Generic Drugs. Drug Control Division, Food and Drug Administration Thailand, Ministry of Public Health, 2000.
11. Nation LR, Sansom NL. Bioequivalence requirements for generic products. Pharmaco Ther 1994; 62:42-55
12. Halstenson CE, Macres M, Katz SA, et al. Comparative pharmacokinetics and pharmacodynamics of epoetin alfa and epoetin beta. Clin Pharmacol Ther 1991;50:702–12
13. Diletti E, Hauschke D, Steinijans VW. Sample size determination for bioequivalence assessment by means of confidence intervals. Int J Clin Pharmacol Ther Toxicol 1991;1:1-8.

การศึกษาชีวสมมูลของยา RECOMBINANT HUMAN ERYTHROPOIETIN ALPHA 2 ตำรับเมื่อให้โดยการฉีดเข้าใต้ผิวหนัง

นพมาศ โรจนเสถียร, พ.บ., รพีพร ขัติศรี, ว.ทม., ณัฐิยา หาญประเสริฐพงษ์, พ.บ., พวงทิพย์ คุณานุสรณ์, Ph.D.

ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

บทคัดย่อ การศึกษาชีวสมมูลของยา recombinant human erythropoietin alpha ขนาด 4000-IU 2 ตำรับ ระหว่างยา Renogen® และยาต้นแบบ Eprex® ในอาสาสมัครชายไทยสุขภาพดี 18 ราย โดยอาสาสมัครแต่ละรายจะได้รับยาทั้ง 2 ตำรับ โดยการฉีดเข้าใต้ผิวหนังครั้งเดียวตามแผนการศึกษาแบบสุ่มสลับชนิด 2 ทาง ทำการเก็บตัวอย่างเลือดตามเวลาที่กำหนดนาน 96 ชั่วโมง นำระดับยาในเลือดไปคำนวณหาค่าพารามิเตอร์ทางเภสัชจลนศาสตร์และทดสอบชีวสมมูลโดยวิธีทางสถิติ ผลการศึกษาพบว่าค่าครึ่งชีวิตของยาทดสอบ (28.7 ชั่วโมง) มีค่าใกล้เคียงกับยาต้นแบบ (31.1 ชั่วโมง) และเวลาที่ความเข้มข้นของยาสูงสุดในพลาสมาของยาทดสอบ (12 ชั่วโมง) จะช้ากว่ายาต้นแบบ (11 ชั่วโมง) เล็กน้อย และค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ที่ใช้ในการกำหนดชีวสมมูลของยาทั้งสองไม่แตกต่างกัน โดยมีช่วงค่าความเชื่อมั่นที่ร้อยละ 90 ของอัตราส่วนของพื้นที่ใต้โค้งของกราฟความเข้มข้นของยาในพลาสมาถึงเวลาสุดท้ายของการเก็บตัวอย่างเลือด (AUC_{0-t}) และถึงเวลาอนันต์ ($AUC_{0-\infty}$) และความเข้มข้นสูงสุดของยาในพลาสมาเท่ากับ 1.03 (0.97-1.10), 1.01 (0.96-1.06) และ 0.99 (0.90-1.08) ตามลำดับ ซึ่งอยู่ในช่วงชีวสมมูล 0.80-1.25 การศึกษานี้จึงสรุปว่ายาทั้งสองตำรับมีชีวสมมูลกันทั้งในด้านปริมาณและอัตราเร็วของการดูดซึมยา **เชียงใหม่เวชสาร 2551;47(3):93-107.**

คำสำคัญ: ชีวสมมูล, Renogen®, Eprex®