臨 床 藥 理 學 會 誌 第20卷 第2號 2012
J Korean Soc Clin Pharmacol Ther 2012;20(2):135-144

Comparison of Pharmacokinetics and Safety of Two Formulations of Letrozole (2.5 mg) in Healthy Male Volunteers

[1]Department of Clinical Pharmacology and Therapeutics, Asan Medical Center, Seoul, Korea, [2]University of Ulsan College of Medicine, Seoul, Korea, [3]Pharmacokinetic and Pharmacogenetic Laboratory, Clinical Trial Center, Asan Medical Center, Seoul, Korea, [4]Department of Pharmacology and Clinical Pharmacology & Therapeutics, Seoul National University College of Medicine and Hospital, Seoul, Korea, [5]Clinical Trials Center, Pusan National University Hospital, Busan, Korea, [6]Department of Clinical Pharmacology, Busan Paik Hospital, Busan, Korea, [7]Department of Clinical Pharmacology and Therapeutics, Samsung Medical Center, Seoul, Korea

Yook-Hwan Noh[1], Kyun-Seop Bae[1,2], Sang-Heon Cho[4], Sangmin Choe[5], Jong-Lyul Ghim[6], Jin Ah Jung[7], Un-Jib Kim[1], Seok-Joon Jin[1], Hyun-Jung Park[3], Jung-Chul Kim[3], Hyeong-Seok Lim[1,2]

=Abstract=

Background: Letrozole is an oral non-steroidal inhibitor of the aromatase enzyme, which has proven to be a useful drug against breast cancer.

Methods: This single-dose, randomized 2 × 2 crossover study was conducted in healthy male volunteers. Participants of each sequence group (each 13 volunteers for sequence group) received, in randomized sequence, a single oral 2.5-mg dose of generic letrozole (test) or branded letrozole (reference). Each treatment period was separated by a 5-week washout period. Blood samples were collected for up to 312 hours after drug administration, and drug concentrations were determined using validated LC/MS-MS. Pharmacokinetic properties were obtained using noncompartmental analysis. Drug tolerability was assessed throughout the study, using measurements of vital signs, physical examination, clinical chemistry testing, EKG, and interviews.

Results: A total of 26 subjects completed the study. The geometric mean ratios (90% CI) of C_{max} and AUC_{last} were 0.92 (0.85 - 0.99) and 1.01 (0.97 - 1.04), respectively. No serious AEs were reported, and there were no clinically significant differences between test and reference groups.

Conclusion: The findings from this study suggest bioequivalence between two formulations of letrozole in healthy male volunteers. The safety profile of two formulations had similar characteristics.

Key words: Letrozole, Pharmacokinetics, Safety, Bioequivalence, Healthy volunteers

This research was sponsored by Yuhan Corporation (Seoul, Korea) and by a grant from the Korean Healthcare Technology R&D Project, Ministry of Health, Welfare & Family Affairs, Korea (grant no. A070001). This study was designed for the sponsor by the authors and then was contracted to the authors for completion. The authors have indicated that they have no other conflicts of interest with regard to the content of this article.

교신저자: 임형석
소 속: 울산대학교 의과대학 임상약리학교실 및 서울아산병원 임상약리학과
주 소: 서울특별시 송파구 올림픽로43길 88 번지 (우 138-736)
전화번호: 02-3010-4613, 팩스: 02-3010-4623, E-mail: mdlhs@amc.seoul.kr
접수일자: 2012. 07. 11. 수정일: 2012. 09. 20. 게재확정일: 2012. 09. 25.

INTRODUCTION

Letrozole is an orally active, reversible non-steroidal imidazole-based inhibitor of the aromatase enzyme,[1] which has proven to be a useful drug against breast cancer, both in the metastatic and in the adjuvant and recently neoadjuvant settings.[2] Pharmacokinetic studies of letrozole have been conducted in healthy volunteers and in breast cancer patients. After oral administration, letrozole is rapidly and completely absorbed (mean absolute bioavailability of 99.9 %) and distributed to tissues. It has a large volume of distribution at steady-state (1.87 L/kg; range 1.47 - 3.24) and ~60 % is bound to plasma proteins, mainly to albumin (55 %). The terminal $t_{1/2}$ of letrozole is 42 hours. The terminal $t_{1/2}$ was observed to be longer and AUC greater in patients with breast cancer than in healthy volunteers, possibly due to reduction in metabolic clearance. The major route of elimination of letrozole is metabolism by CYP450 isoenzymes (CYP 3A4 and CYP 2A6) into an inactive carbinol metabolite. Steady-state concentrations of letrozole are reached after 2-6 weeks and maintained for long periods with no evidence of drug accumulation. No significant drug interactions have been reported for letrozole.

The present study was designed to compare the pharmacokinetic properties, safety profiles, and relative bioavailability of generic (test) and branded (reference) letrozole tablets in healthy male volunteers.

SUBJECTS AND METHODS

1. Subjects

All enrolled subjects were healthy, male, Korean volunteers aged 19 to 55 years, who were within 20 % of ideal body weight. None of the subjects had significant disorders as determined by medical history and physical examination that included assessment of vital signs, electrocardiography, and clinical laboratory tests (hematology, blood chemistry, urinalysis, and testing for HIV and hepatitis B virus surface antigens). None of the subjects had a history of alcohol or drug abuse, and all had negative urine test results for drugs of abuse (eg, amphetamines, barbiturates, cocaine, opioids, benzodiazepines) and alcohol. All laboratory tests other than pharmacokinetic (PK) analysis were performed at the Department of Laboratory Medicine, Asan Medical Center, which has been accredited by the Korean Association of Quality Assurance for Clinical Laboratories. PK analysis was performed by the Clinical Trial Center (CTC) at Asan Medical Center. The institutional review board of Asan Medical Center approved the study protocol, and all procedures were performed in accordance with the Good Clinical Practice guidelines[3] and the Declaration of Helsinki and its amendments.[4] All participants provided written, informed consent before the screening test for eligibility.

2. Study designs

There were 2 treatment periods separated by a 5-week washout period, which is more than 5 times the half-life of 42 hours determined in previous studies.[2] Twenty-six eligible men were randomly assigned to 2 sequence groups in order of passing the eligibility test using the 1:1 randomization method before initiation of the study. A table of random numbers generated by R version 2.7.2 (R Foundation for Statistical Computing, Vienna, Austria) was used to assign subjects to receive the test drug or the reference drug. The test drug was manufactured by Yuhan Corporation (Seoul, Korea). It was approved for marketing by Korean Food and Drug Administration as trade name of Peratra® (Yuhan Corporation, Seoul, Korea). The trademark of reference drug was Femara® (Norvatis, Basel, SWISS).

Participants were instructed not to take other medications, including over-the-counter and herbal products, from 2 weeks before study's onset (study drug administration day) to the end of the study and were asked to abstain from smoking and use of alcohol- and caffeine-containing foods and beverages from 3 days before the study's onset to the end of the study. All subjects were admitted to the CTC at Asan Medical Center 1 day before dosing and confined until discharge; they did not eat or drink anything other than water for at least 10 hours before drug administration. The study drug was given with 240 mL of water, and fasting continued for an additional 4 hours after drug administration. Subjects' mouths were examined by investigators after ingestion to guarantee swallowing, and results were recorded in written source documents. All subjects received a standard meal after the 4- and 9-hour time period after dosing. After all of the scheduled procedures in the first period were finished, about 24 hours after dosing, the subjects were discharged from the hospital and subsequently visited the CTC to assess the tolerability and PK properties of letrozole, given a 5-week washout period; the same procedure was repeated with the other formulation until the last follow-up, 35 days after dosing in the first period. Subjects returned approximately 7 days after the last visit for safety profile assessment. All dietary, smoking, and drug-herbal product restrictions were maintained throughout the study period.

3. Blood sampling

Blood for PK analysis was drawn via an indwelling intravenous catheter placed in the forearm and was transferred into a heparinized tube. The first 1 mL of blood was discarded, a 6 mL sample was collected, and the cannula was then flushed with 1 mL of normal saline to ensure patency. Samples were obtained before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 24, 48, 96, 168, and 312 hours after oral administration of 2.5 mg of letrozole. Samples were centrifuged at 1800 g for 8 minutes at 4 ℃, and the plasma was immediately placed into polypropylene tubes at -20 ℃ and then transferred to a freezer at -70 ℃ until analysis.

4. Measurement of letrozole plasma concentration

All plasma samples were handled and analyzed by the CTC at Asan Medical Center using validated bioanalytical methods.[5,6] Plasma letrozole concentrations were determined using validated high-performance liquid chromatography (HPLC; Symbiosis, Spark Holland Instruments, Emmen, The Netherlands) coupled with tandem mass spectrometry (MS/MS; API4000, ABSciex Inc., Foster City, CA). Liquid-liquid extraction used ethyl ether from Fisher Scientific Korea Ltd. (Seoul, Korea) and employed a method developed in the analytic laboratory of CTC at Asan Medical Center. Anastrozole was used as an internal standard for analytes, which was provided by Yuhan Corporation. A 2.0 × 50 mm Shiseido MG 3μm column (Shiseido Co., Ltd., Tokyo, Japan) was used for chromatographic separation. The mobile phase consisted of a mixture of acetonitrile purchased from J. T. Backer, Inc. (Phillipsburg, NJ) and water [60:40, v/v] with 0.1 % acetic acid from Merck Korea Ltd. (Seoul, Korea). The flow rate of mobile phase was 0.2 mL/min at room temperature, and the injection volume was 10 μL. The tandem mass spectrometry system was operated with electrospray ionization in the positive ion mode. This method had a linear quantifiable range of 0.1 to 50 ng/mL and a r^2 of 0.999 or better. Intra-day precision ranged from 1.63 % to 3.77 %, and inter-day precision ranged from 0.67 % to 6.51 %. Accuracy was 88.10 % to 97.34 %. The analyte was stable in human plasma following 3 freeze-thaw cycles, in plasma after storage for 24 hours at room temperature, in stock solution after storage for 6 hours at room temperature, and in the HPLC autosampler after storage at 4 ℃ for 24 hours.

5. Pharmacokinetic assessments

The change in plasma concentration of letrozole in each subject was analyzed by noncompartmental PK analysis. Noncompartmental PK analysis was performed with WinNonlin® version 5.2 (Pharsight Corporation, Mountain View, CA).[7] Plasma maximum concentration (C_{max}) and time to Cmax (t_{max}) were determined directly from the observed values. The terminal elimination rate constant (λ_z) was estimated by linear regression of the decline in the log10 plasma concentration of letrozole over time. The $t_{1/2\beta}$ was calculated for each subject as $\ln(2)/\lambda_z$. The individual AUC from time zero to the specified time after dosing (AUC_{last}) was calculated using the linear trapezoidal rule. The $AUC_{0-\infty}$ was calculated as $AUC_{last} + C_{last}/\lambda_z$, where C_{last} was the last measurable concentration.

6. Safety assessments

Adverse events (AEs) were monitored by asking subjects general health-related questions at the scheduled physical examinations that were performed at the study onset and throughout the study. Vital signs (seated blood pressure, heart rate, respiratory rate, axillary temperature) were recorded at screening, predose baseline, 24, 48, 96,

168, and 312 hours after dosing and follow-up visit. An electrocardiogram examination was performed for screening. Clinical blood tests were also performed at regular intervals and included hematologic analysis (red blood cell count, hemoglobin, hematocrit, platelet count, white blood cell count), blood chemistry (electrolytes, hepatic enzymes, renal function parameters, fasting blood glucose, albumin, cholesterol, triglycerides, phosphorus, lactic dehydrogenase, uric acid), and urinalysis. The clinical importance of AEs and the likelihood that AEs were related to the study drug were assessed by unmasked investigators throughout the study. The AEs were evaluated as 'not-related' if the assessed AEs were the frequently reported ones in other aromatase inhibitors.

7. Statistical analysis

The demographic characteristics of enrolled subjects were summarized using descriptive statistics. The PK properties of letrozole have relatively low between-subject variability (unpublished previous study results, 2008). Hence, we calculated that a sample size of 10 was needed to provide >80 % power to detect a geometric mean ratio of AUC or C_{max} of ≥1.20 between the test and reference formulations. We used a sample size of 26, considering the potential for dropouts occurring during the study, since the Korean Food and Drug Administration requires at least 24 subjects as the minimum sample size for bioequivalence study.[3] ANOVA was performed on the natural logarithm-transformed AUClast and C_{max} values, and a P value of <0.05 was considered significant. The ANOVA model considered sequence, period, and treatment as fixed factors and subject within a sequence as a random factor. Geometric mean ratios of test and reference values for AUC_{last} and C_{max} were calculated. The formulations were assumed bioequivalent if the 90 % CIs for the geometric mean ratios of the test formulation to the reference formulation were 0.8 to 1.25.[8] Statistical analyses were performed using WinNonlin® version 5.2 (Pharsight Corporation) and R version 2.7.2 (R Foundation for Statistical Computing).

RESULTS

1. Study subjects

This bioequivalence study was conducted in 26 adult Korean male volunteers. Table 1 shows the demographic characteristics of all enrolled subjects. Physical examinations before study onset indicated that all subjects were healthy.

Table 1. Demographics of 26 subjects in letrozole bioequivalence study

Characteristics	Mean	SD
Age, years	28.1	4.4
Height, cm	174.3	5.3
Weight, kg	69.1	7.2
BMI, kg/m^2	22.7	1.8

* Abbreviations: BMI, body mass index, SD standard deviation.

Table 2. Pharmacokinetic results of the two letrozole formulations

Pharmacokinetic parameters	Yuhan Letrozole (Test)	Femara® (Reference)
	Mean ± SD	Mean ± SD
$AUC_{0\text{-last}}$, ng·hr/mL	2069.15 ± 805.43	2079.69 ± 879.11
C_{max}, ng /mL	35.18 ± 6.75	38.33 ± 6.86
t_{max}*, hr	1.5 (1 ~ 4)	1.5 (0.5 ~ 4)
V_z/F, L	93.7 ± 19.1	94.9 ± 14.4
CL/F, L/hr	1.3 ± 0.5	1.3 ± 0.5
$t_{1/2\beta}$, hr	56.95 ± 29.22	58.97 ± 33.43

* median (range).

There were no withdrawals during the study period.

2. Safety properties

There were no serious or unexpected AEs during the study. A total of 21 AEs were reported (14 using the test formulation, 7 using the reference formulation) in 9 of the 26 participants. These AEs were abdominal discomfort (n=2), nausea (n=1), dizziness (n=1), asthenia (n=1), ankle sprain (n=1), abnormal laboratory test values (n=8) in test formulation, and upper respiratory infection (n=2), diarrhea (n=1), headache (n=1), abnormal laboratory test values (n=3) in reference formulation, respectively. Abnormal laboratory test values were 1 “blood bilirubin increased”, 1 “blood triglyceride increased”, 1 “blood creatine kinase increased”, 1 “blood lactate dehydrogenase increased”, 1 “blood aspartate transaminase increased”, 1 “blood alanine transaminase increased”, 1 “blood calcium increased”, 1 “urinary occult blood positive” in test formulation, and 1 “blood bilirubin increased”, 1 “blood triglyceride increased”, 1 “blood creatine kinase increased” in reference formulation, respectively. In all cases, subjects experienced full recovery. All AEs were mild and were considered unrelated to the administration of the study drugs.

3. Pharmacokinetic properties

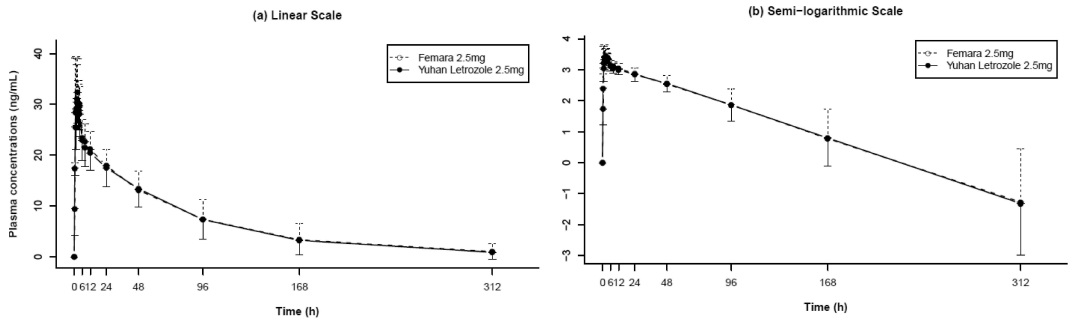

Figure 1. Plasma letrozole concentration (mean and standard deviation) versus time after a single 2.5 mg oral dose in 26 healthy male subjects.

Figure 1 shows the mean plasma concentration -time curves of the 2 formulations following a single 2.5-mg dose, and Table 2 shows the values of the PK parameters for the test and reference formulations. Both formulations had very similar AUC, C_{max}, and t_{max} values.

ANOVA test indicated no significant effect of sequence, period, or formulation on the PK results (data are not shown). The point estimates for the geometric mean ratios of the test and reference formulations were 1.01 (90 % CI, 0.93 - 1.04) for AUC_{last} and 0.92 (90 % CI, 0.85 - 0.99) for C_{max} (Table 3). This meets the Korean regulatory criteria for assuming bioequivalence.[8)]

DISCUSSION

The current study of healthy male volunteers indicated that a 2.5-mg generic formulation of letrozole met the regulatory requirements for assuming bioequivalence to the reference formulation. Overall, both formulations were well tolerated, and there were no serious AEs. There were also no significant differences in the safety profiles between the test and reference formulations.

The terminal elimination half-life of letrozole was about 58 hours in this study, and this value is a little longer than previous study value (42 hours) but the washout period of 5 weeks in this study is appropriate to exclude the carryover effect. The t_{max} of both letrozole formulations was about 1.5 hours from single dose administration, and it is similar with other reported values (about 2 hours).[9)]

The AEs were predominantly mild in intensity, and no serious AEs were reported. The headache, dizziness, vomiting, elevated blood alanine transaminase, and elevated total blood bilirubin reported in the present study are frequently reported with other aromatase inhibitors.[2,10)]

This study was conducted in healthy male subjects, although letrozole is prescribed mostly to postmenopausal women. This study was focused on the comparative bioavailability of the 2 formulations. We assumed that there was no formulation-gender interaction, which means that although there are differences in the PK properties of letrozole between females and males, these differences apply in the same way in both formulations. Notably, bioequivalence studies for other drugs mainly for women were also conducted in male subjects. Our comparison of 2 letrozole formulations enrolled subjects using strict selection criteria, randomly assigned the subjects to different treatment sequences, and

Table 3. Geometric mean ratios and 90 % confidence intervals for AUC_{last} and C_{max} in test (Yuhan Letrozole) and reference (Femara®) formulations in healthy male volunteers

Pharmacokinetic Results	Geometric Mean Ratio (Test/Reference)	90% Confidence Intervals for Geometric Mean Ratio
AUC_{last}	1.01	0.97 ~ 1.04
C_{max}	0.92	0.85 ~ 0.99

employed a 2×2 crossover design. These procedures reduced the potential confounding effects on the evaluation of the 2 formulations. Thus, our study results reflect the characteristics of the formulation itself; we can reasonably assume that the comparative bioavailability in this study would also be applicable to female patients. In conclusion, the results of this study suggest that the test (Peratra®; Yuhan Corporation) and reference (Femara®; Norvatis) formulations had similar PK characteristics and similar plasma-concentration time profiles. The test formulation of letrozole met the Korean regulatory criteria for assuming bioequivalence to reference formulation in AUC and C_{max}.

ACKNOWLEDGEMENTS

The authors acknowledge the in-house support provided by the CTC at Asan Medical Center (Seoul, Korea). The sponsor had no role in the study design, conduct of the study procedures, data analyses, or data interpretation. The sponsor monitored the studies during the whole clinical trial period.

All of the authors agreed on the contents of the manuscripts, and the corresponding author made the final decision regarding its submission. Drs. Bae, Lim, Noh, Cho, Ghim, Jung, Kim and Jin contributed to the study design, data interpretation, and data collection. Dr. Noh contributed to the data analyses, literature search, figure creation, study design, data collection, data interpretation, and manuscript writing. Dr. Lim reviewed the manuscript. HJ Park and JC Kim analyzed the PK sample concentrations.

REFERENCES

1. Campos SM. Aromatase inhibitors for breast cancer in postmenopausal women. *Oncologist*, 2004;9(2):126-136.
2. Dellapasqua S, Colleoni M. Letrozole. *Expert Opin Drug Metab Toxicol*, 2010;6(2):251-259.
3. KFDA. Korea good clinical practice (KGCP) guidelines. http://clinicaltrials.kfda.go.kr/guide/laws/board_list.jsp?category_seq=12 [Online] (last visited on 07 July 2012).
4. Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects. http://www.wma.net/en/30publications/10policies/b3/ [Online] (last visited on 07 July 2012).
5. Marfil F, Pineau V, Sioufi A, Godbillon SJ. High-performance liquid chromatography of the aromatase inhibitor, letrozole, and its metabolite in biological fluids with automated liquid-solid extraction and fluorescence detection. *J Chromatogr B Biomed Appl*, 1996;683(2):251-258.
6. Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Quantification of carvedilol in human plasma by liquid chromatography using fluorescence detection: application in pharmacokinetic studies. *J Pharm Biomed Anal*, 2007;44(1):250-253.
7. Rowland M, Tozer TN. Clinical Pharmacokinetics and Pharmacodynamics: Concepts and Applications. 4th ed. Wolters, Kluwer/Lippincott, Williams & Wilkins, 2011.
8. KFDA. Bioequivalence guideline in Korea. Published by Korea Food and Drug Administration; http://www.kfda.go.kr/index.kfda?mid=226&pageNo=1&seq=139&cmd=v [Online] (last visited on

July 07 2012).
9. Lonning P, Pfister C, Martoni A, Zamagni C. Pharmacokinetics of third-generation aromatase inhibitors. *Semin Oncol*, 2003;30(4 Suppl 14):23-32.
10. Noh YH, Ko YJ, Cho SH, Ghim JL, Choe S, Jung JA, Kim UJ, Jin SJ, Park HJ, Song GS, Lim HS. Pharmacokinetic comparison of 2 formulations of anastrozole (1 mg) in healthy Korean male volunteers: a randomized, single-dose, 2-period, 2-sequence, crossover study. *Clin Ther*, 2012;34(2):305-313.

=국문초록=

건강한 성인 남성에서 두 가지 제형의 레트로졸정의 약동학 특성 및 안전성 비교

노육환[1], **배균섭**[1,2], **조상헌**[4], **최상민**[5], **김종률**[6], **정진아**[7], **김운집**[1], **진석준**[1], **박현정**[3], **김정철**[3], **임형석**[1,2]

[1]서울아산병원 임상약리학과, [2]울산대학교 의과대학 임상약리학교실, [3]서울아산병원 임상시험센터 약동약력연구실, [4]서울대학교 의과대학 약리학교실 및 서울대학교 병원 임상약리학과, [5]부산대학교병원 임상시험센터, [6]인제대학교 부산백병원 임상약리학과, [7]삼성서울병원 임상약리학과

배경: 레트로졸은 유방암 치료에 사용되는 경구용 비스테로이드성 aromatase 억제제이다.
방법: 이 연구는 건강한 성인 남성을 대상으로 무작위 배정, 단회 투여, 공개설계로서 두 치료군, 두 순서, 두 시기의 교차 시험법으로 진행되었다. 각 순서군에 13명씩 무작위 배정되어, 한군은 1기에 페마라®정(대조약)을, 2기에 유한 레트로졸정(시험약)을, 다른 한군은 반대 순서로 두 시기 사이에 약 5주의 휴약기를 가지고 각각 2.5 mg정 1정씩 투여 받았다. 약동학 분석을 위한 혈액 검체는 공복 상태에서 투약 후 312 시간까지 얻어졌다. 레트로졸의 혈장 내 농도는 liquid chromatography-tandem mass spectrometry를 이용하여 측정하였다. 안전성 평가는 활력징후, 문진 및 신체검사, 심전도, 진단검사실 검사와 이상반응 모니터링을 통하여 이루어졌다.
결과: 총 26명의 피험자가 연구를 완료하였고, 약동학 분석에 26명의 자료가 모두 사용되었다. C_{max}와 AUC_{last} 파라미터에 대한 시험약과 대조약의 기하평균비는 각각 0.92 (90 % 신뢰구간: 0.85 - 0.99)와 1.01 (90 % 신뢰구간: 0.97 - 1.04)였다. 보고된 중대한 이상반응은 없었으며, 임상적으로 유의한 변화도 관찰되지 않았다.
결론: 건강한 자원자에서 페마라®정과 유한 레트로졸정의 약동학 특성과 안전성은 유사하였다.

Key words: 레트로졸, 약동학, 안전성, 생동성, 건강한 자원자