Bioequivalence of Clozapine Tablets

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Abstract

Objective To perform a bioequivalence study of clozapine tablets between Clozaril® tablet (Novartis), the innovator product, and Clopaze® tablet (Pharminar, Thailand).

Method The study was performed in 12 healthy male volunteers for a single 100 mg dose of clozapine tablet. Randomized cross over design was used. Blood samples were collected before and after drug administration for 24 hours and determined for plasma clozapine concentration by HPLC method.

Results The results of the bioequivalence study of 100 mg clozapine tablets showed a high variation in pharmacokinetic parameters of both Clozaril® and Clopaze® similar to those reported elsewhere. When statistics were tested as stated in USP23 guideline for bioequivalence study, 90% confidence interval of the log of ratio of C_{max}, AUC_{24hr} and AUC_{inf} between Clopaze® and Clozaril® were within the range of 0.80 - 1.25.

Conclusion It can be indicated that the 100 mg Clozaril® and Clopaze® tablets used in this study are bioequivalent to each other. **J Psychiatr Assoc Thailand 2000; 45(3): 221-227.**

Key words : bioequivalence, clozapine

Introduction

Clozapine, a dibenzodiazepine derivative (piperazine-substituted tricyclic antipsychotic agent), is prescribed for treatment of refractory schizophrenia or for the patients who can not tolerate the extrapyramidal adverse effects of conventional antipsychotic medications[1]. Clozaril®, the innovative drug, was marketed in Thailand in the dosage of 25 and 100 mg/tablet. As well as the fact that it is quite expensive, the information on the pharmacokinetics of clozapine in Thais is insufficient. The information regarding bioequivalence study between Clozaril® and Clopaze®, product made in Thailand, will be helpful for physicians, pharmacists, and drug consumers for appropriate selection of drug. Confidence in therapeutic efficacy will be enhanced.

Materials and methods

The bioequivalence study between Clozaril® and Clopaze® tablets was approved by the Human Experimentation Committee, Research Institute for Health Sciences (RIHES), Chiang Mai University. The study was performed at Suanprung Psychiatric hospital, Chiang Mai. Plasma clozapine analysis and data analysis were executed at the Biopharmacy Research Unit, Faculty of Pharmacy, Chiang Mai University.

Volunteers: Volunteers were 12 healthy Thai males; their average age, weight, and height were 21.4 ± 1.2 years (range 18 - 24), 59.4 ± 5.8 kg (range 53 - 70), and 168 ± 7 cm (range 153 - 175), respectively. Their body mass indices were within the range of 18-24 kg/m^2. A physical examination as well as all clinical and routine laboratory evaluation tests for all volunteers, such as complete blood count, blood urea nitrogen, and liver function test, were within medically acceptable limits. None of them had a history of alcoholism, smoking, hepatic disease, active peptic ulcer disease and renal insufficiency. Before joining the study, all volunteers were informed of the details of the study, and signed a consent form. All volunteers were free to leave the study at any time.

Study design: A randomized, double blind, two-period crossover design was used with one week washout period. All subjects, physician and drug analyst were blinded. The innovator and test products, in the dosage of 100 mg/tablet, are Clozaril® (Lot No. 119, Novartis) and Clopaze® (Lot No. AK21801, Pharminar), respectively. These two clozapine tablets had already passed the content uniformity test.

One week before and during the period of the study, all volunteers had took no medicine and consumed no alcoholic beverages. Food had been abstained from 8.00 pm the night before the study. One tablet of either Clozaril® or Clopaze®, following the randomly assigned order, was taken by each volunteer at 8.00 am with 200 ml water.

Ten milliliters of blood samples were taken at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12 and 24 hour(s) after drug taking and, then centrifuged to separate plasma within half an hour. Plasma samples were stored at -48 Ī C. The plasma samples were analysed for clozapine content within one week of storage.

Clozapine analysis: Clozapine content was analyzed by a modified High Performance Liquid Chromatography[2]. Plasma 2.0 ml was pipetted and mixed with 500 µl of saturated tribasic sodium

phosphate using vortex mixer for 1 minute. Two milliliters of chloroform were added using vortex mixer for 5 minutes and then centrifuged at 4500 rpm for 10 minutes. Chloroform layer, 1.5 ml, was collected and then dried at 40 ĪC under low pressure (HBI Vortex-Evaporator®, Buchler Instruments, USA). Residue was dissolved with 200 µl mobile phase composed of 500 ng/mL indomethacin using as an internal standard. Then, 100 µL of the solution was injected onto a reverse phase Hypersil® ODS column (C18, 250 x 4.6 mm, 5 µm) connecting with a Hewlett Packard Series 1100 HPLC system (G1356AA, Germany) equipped with UV detecter. The mobile phase was phosphate buffer pH 3.52 : Acetonitrile (50:50) using with a flow rate of 1.5 mL/min. The UV detection was performed at 240 nm.

Standard curves were performed by preparing standard clozapine (Batch 981106, Zhejiang Wenling Pharmaceutical Factory, China) in plasma with the concentration of 20, 50, 100, 200, 500 ng/ml. Previous mentioned extraction process was performed and injected into the HPLC system.

Linearities of the standard curves were found in the range from 20 - 500 ng/ml (r^2>0.9950). Intraday and interday variation were determined before being used for the analysis of plasma clozapine content by performing of 3 standard curves daily for 3 days. The relative standard deviations at each concentration were less than 10%. Recovery percentage of the analysis method at high, medium and low concentration were more than 95%. The lower limit of detection was less than 20 ng/mL. However, standard curves were performed every day of analysis.

Data analysis: Pharmacokinetic parameters were determined. C_{max} and T_{max} were taken from the raw data. AUC was determined using trapezoidal rule. Elimination rate constant (k_e) was determined from the slope of the last portion of the log concentration - time curves and subsequently elimination half-life ($t_{1/2}$) was also calculated.

Statistical methods: Bioequivalence testing comprised of assessment with respect to the rate (C_{max}) and extent (AUC_{24hr} and AUC_{inf}) of clozapine absorption[4]. The division of Bioequivalence of the United State of America has employed a testing procedure termed the two one-sided tests procedure to determine whether average values for pharmacokinetic parameters measured after administration of the test and reference products are comparable. This procedure involves the calculation of a 90% confidence interval for the ratio of the product averages. The C_{max}, AUC_{24hr} and AUC_{inf} were analyzed statistically by logarithmically transformed. The 90% confidence interval for the difference in the means of the log-transformed data was calculated using the following equation[3-5]:

$$90\% \text{ confidence interval} = \triangle \pm t_{0.10,\, v} \sqrt{EMS(2/n)}$$

Where $\triangle$ is a difference in means of log transformed pharmacokinetic parameters (C_{max} or AUC_{24hr} or AUC_{inf}) between the test product and the reference, $t_{0.10,\, v}$ is the tabulated two-tail t value for a 90% confidence interval, v is a degree of freedom of the error mean square obtained from the ANOVA table, EMS is the error mean square from the ANOVA table and n is the number of subjects. Antilogarithm of the calculated confidence interval will yield an exact confidence interval for the ratio. For the bioequivalence between the test and reference

products, USP 23 required that a 90% confidence interval of the ratio of means of the pharmacokinetic parameters, ie. C_{max}, AUC_{24hr} and AUC_{inf} must be in the range of 0.80 -1.25[4].

Results

All volunteers who took either Clozaril® or Clopaze® slept within 30 minutes after the dose. Side effect, sialorrhea, was similarly found for both products in about 50% of the volunteers. They woke up around 7.00 pm for dinner, and continued sleeping until the next morning.

Average clozapine concentration - time curves of Clozaril® and Clopaze® tablets are demonstrated in Figure 1. Biphasic clozapine blood levels for both Clozaril® and Clopaze® tablets were seen in most volunteers.

Pharmacokinetic parameters, ie. AUC_{24hr}, AUC_{inf}, C_{max}, T_{max}, are collated in Table 1. AUC_{24hr} were 3015.05 ± 608.83 and 2793.22 ± 740.33 ng.hr/ml and AUC_{inf} were 3755.74 ± 837.28 and 3366.97 ± 1036.28 ng.hr/ml for Clozaril® and Clopaze®respectively. Average C_{max} were 308.31 ± 89.65 ng/mL and 315.75 ± 81.98 ng/mL for Clozaril® and Clopaze®,respectively. Average elimination rate constant was 0.08 ± 0.02 hr^{-1} and 0.07 ± 0.02 hr^{-1} for Clozaril® and Clopaze®, respectively. T_{max} varied highly for the data both from those taking Clozaril® (142.75 ± 133.59 minutes) and Clopaze® (100.00 ± 69.28 minutes).

90% confidence interval of the ratio of AUC_{24hr}, AUC_{inf} and C_{max} between Clopaze® and Clozaril® were 0.91-1.01, 0.88-0.98 and 0.97-1.24 respectively.

Discussion

Biphasic clozapine blood levels which were seen in most volunteers for both Clozaril® and Clopaze® tablets might be expected since the volunteers were in the fasting condition and slept for about 12 hours. This characteristic has been noted before[6] and it was found in many other
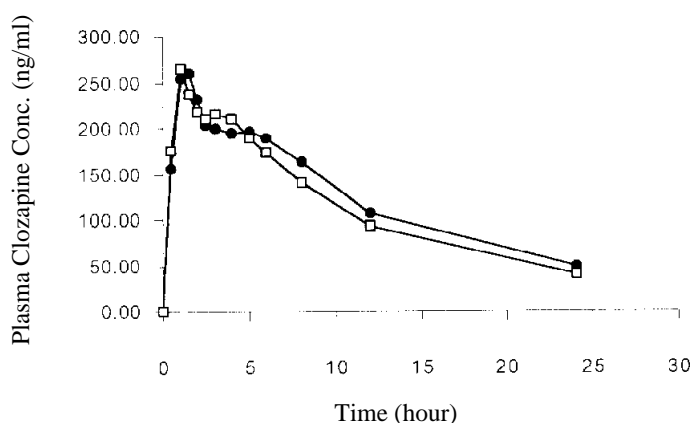

Figure 1 Average plasma clozapine concentration at various sampling times. (●) Clozaril® (□) Clopaze® (n = 12)

Table 1   Pharmacokinetic parameters of Clozaril® and Clopaze® (n=12)

Pharmacokinetic parameters	Clozaril®	Clopaze®	90% Confidence Interval of the ratio
AUC_{24hr} * (ng.hr/ml)	3015.05 ± 608.83	2793.22 ± 740.33	0.91-1.01
AUC_{inf} *(ng.hr/ml)	3755.74 ± 837.28	3366.97 ± 1036.28	0.88-0.98
C_{max} * (ng/ml)	315.75 ± 81.98	308.31 ± 89.65	0.97-1.24
T_{max} (min)	142.75 ± 133.59	100.00 ± 69.28	
k_e (hr^{-1})	0.07 ± 0.02	0.08 ± 0.02	
$t_{1/2}$ (hr)	10.09 ± 2.67	9.21 ± 2.09	

* Log data transformation

antipsychotic agents[7,8].

High variation in all pharmacokinetic parameters agree with the high interindividual variation which had been previously reported[9-12]. The biphasic blood level was possibly the reason for the high variation of both Clozaril® and Clopaze®. Additionally, the considerable variation of plasma levels of clozapine had been reported (about fivefold to eightfold), not only among the patients receiving the same daily doses, but also in individual patients over time[8]. Variation in the absorption of the drug was a suggested reason rather than variation in the hepatic metabolism and clearance.

90% confidence interval of the ratio of C_{max}, AUC_{24hr} and AUC_{inf} between Clopaze® and Clozaril® were in a range of 0.80-1.25 as those stated in USP 23. Therefore, bioequivalence can be indicated between Clopaze® and Clozaril®.

It should be noted that this finding was limited only for the lot used in the study. In addition, this study was designed as a single dose administration in healthy volunteers, therefore, long term use in patients should be considered regarding the therapeutic effect. However, a report has stated that, for clozapine, there is no correlation between blood concentration and its therapeutic effect[11]. Thus, when clozapine is used to treat patients, dose titration has to be carried out from a low dose to a high dose until the effective dose for each patient is acheived[1,10]. Therapeutic drug monitoring may also be performed for a rapid dosage regimen adjustment[1].

Conclusion

The results of the bioequivalence study revealed a high variation in pharmacokinetic parameters of both Clozaril® and Clopaze®, 100 mg/tablet, as had been reported elsewhere. When statistics were tested as stated in USP guideline for bioequivalence study, 90% confidence interval of the log of ratio of C_{max}, AUC_{24hr} or AUC_{inf} between Clopaze® and Clozaril® were within the range of 0.80-1.25. Therefore, it can be indicated that the 100 mg Clozaril® and Clopaze® tablets used in this study are bioequivalent to each other.

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บทคัดย่อ

วัตถุประสงค์　เพื่อศึกษาชีวสมมูลของยาเม็ดโคลซาปีนระหว่างผลิตภัณฑ์ต้นแบบคือ ยา Clozaril® ของบริษัท Novartis กับผลิตภัณฑ์ที่ผลิตในประเทศไทยคือ ยา Clopaze® ของบริษัท Pharminar

วิธีการศึกษา　ศึกษาในอาสาสมัครชายสุขภาพดี 12 คน โดยการให้อาสาสมัครรับประทานยาเม็ดโคลซาปีนในขนาด 100 มิลลิกรัม การศึกษาเป็นแบบสุ่มสลับ เก็บตัวอย่างเลือดของอาสาสมัครก่อนและหลังรับประทานยาที่เวลาต่าง ๆ ในช่วง 24 ชั่วโมงหลังการให้ยา และวิเคราะห์หาความเข้มข้นของยาในพลาสม่าโดยวิธี HPLC

ผลการศึกษา　พบว่าค่าตัวแปรทางเภสัชจลนพลศาสตร์ของยา Clozaril® และ Clopaze® ขนาด 100 มิลลิกรัม/เม็ด มีความแปรปรวนสูงคล้ายคลึงกับที่ได้มีการรายงานไว้แล้ว เมื่อนำไปทดสอบทางสถิติโดยวิธีที่กำหนดไว้ใน USP 23 พบว่า ค่าตัวแปรทางเภสัชจลนพลศาสตร์ของยาทั้งสองไม่แตกต่างกัน โดยมี 90% Confidence Interval ของอัตราส่วนของความเข้มข้นสูงสุดของยาในพลาสม่า (C_{max}) หรือพื้นที่ใต้เส้นโค้งของกราฟความสัมพันธ์ระหว่างความเข้มข้นของยาในพลาสม่ากับเวลาช่วง 24 ชั่วโมง (AUC_{24hr}) กับเวลาอนันต์ (AUC_{inf}) ในรูปลอการิทึมระหว่าง Clopaze® กับ Clozaril® อยู่ในช่วงที่ USP กำหนด (0.80-1.25)

สรุป ยา Clozaril® และ Clopaze® ในขนาด 100 มิลลิกรัม/เม็ด ที่ใช้ในการศึกษาครั้งนี้มีชีวสมมูลซึ่งกันและกัน **วารสารสมาคมจิตแพทย์แห่งประเทศไทย 2543; 45(3): 221-227.**

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