The Comparative Bioavailability of a Generic and the Innovator Fluconazole Preparations in Healthy Thai Volunteers

SUPANIMIT TEEKACHUNHATEAN, M.D.*,
MALEEYA MANOROT, M.D.*,
AMPHAWAN APISARIYAKUL, Ph.D. (Pharmacol)*,
NOPPAMAS ROJANASTEIN, M.D.*,
CHAICHAN SANGDEE, Ph.D. (Pharmacol)*,
UDOMCHAI AJAYUTPHOKIN, M.S. (Pharmacol)*

Abstract

We studied the pharmacokinetics and compared the oral bioavailability of the "generic" (Biozole®, Biolab Company, Thailand) and the "innovator" (Diflucan®, Pfizer Incorporation, U.S.A.) fluconazole preparations in 12 healthy Thai volunteers. A 200 mg single oral dose of each preparation was given to the subjects in a randomized double-blind 2-period crossover design with 2 weeks washout period. Blood samples were collected just before and at 0.5, 1, 2, 2.5, 3, 4, 24, 48, 56 and 72 hours after drug administration. Serum fluconazole concentrations were determined by using high performance liquid chromatography. Individual concentration-time profiles and the pharmacokinetic parameters were analyzed by the noncompartmental pharmacokinetic method [TOPFIT, a pharmacokinetic data analysis program]. The pharmacokinetic parameters (T_{max}, C_{max}, V_d, Cl) of fluconazole in Thai healthy volunteers were comparable to those values observed in Caucasian subjects. The relative bioavailability of the generic Biozole® was 102.38 ± 9.79 per cent of Diflucan®. The means and 90 per cent confidence intervals (90% CI) of the [Biozole®/ Diflucan®] ratio of AUC_{0-72}, AUC_{0-inf} and C_{max} were 1.02 (0.98-1.06), 0.99 (0.95-1.03) and 1.13 (1.03-1.25), respectively. These values were well within the acceptable bioequivalence ranges of 0.8-1.25 proposed by the US FDA. The means and 90 per cent CI of T_{max} differences [Biozole®- Diflucan®] were -0.46 [(-1.03)-(0.12)]. This value was outside the stipulated bioequivalence range of ± 0.41 h (± 20% of the T_{max} of the reference formulation). Nevertheless, the T_{max} difference was not expected to be related to the differences in safety and efficacy of the drug. Hence, Biozole® and Diflucan® were bioequivalent with respect to the extent of absorption (AUC), and the C_{max}, and could be used interchangeably.

* Clinical Pharmacology Division, Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

Fluconazole is a synthetic antifungal bistriazole developed by Pfizer, Inc. and has been marketed as Diflucan®. The insertion of 2 fluoride atoms into its chemical structure increases its polarity and hydrosolubility. Thus, allowing the drug to be available in either oral or parenteral form[1]. Fluconazole is currently approved for the therapy of candida infections including oropharyngeal, esophageal and systemic candidiasis[2,3]. It is also effective in acute therapy and suppression of relapse cryptococcal meningitis[3,4]. In addition, it has been used for the treatment of severe fungal infections in AIDS, immunocompromised hosts and cancer patients[3]. Oral fluconazole is rapidly absorbed with bioavailability of approximately 90 per cent[1]. The bioavailability is not altered by food or gastric acidity[5]. Oral administration of single dose fluconazole results in peak plasma concentrations within 2 hours[6]. Fluconazole is widely distributed in tissues and body fluids including cerebrospinal fluid (CSF)[4], saliva[2] and vaginal secretion[6]. Plasma protein binding is low (11 to 12%). Fluconazole is excreted predominantly unchanged (more than 66%) in the urine[7,8]. The half-life of fluconazole is about 30 hours[4] and is greatly prolonged in pateints with renal insufficiency[9].

Although generic fluconazole preparations may be chemically equivalent to the innovator, the study of relative bioavailability is still required to determine whether the "generic" is bioequivalent to the "innovator". The purpose of this study is to investigate the pharmacokinetics of fluconazole and to compare the oral bioavailability of the "generic" (Biozole®) and the "innovator" (Diflucan®) preparations in healthy Thai volunteers.

MATERIAL AND METHOD

Subjects

Twelve healthy volunteers (6 women and 6 men), 21-38 years of age (mean ± SD = 25.83 ± 4.73 years) participated in the study. Their weight and height ranged from 42.5-65 kg (54.75 ± 8.05) and 154-177 cm (161.83 ± 5.83), respectively. All subjects were abstinent from any medications for at least 1 week prior to the study and were free from any medical illnesses or underlying diseases judged by physical examination and routine blood test including complete blood count with differential count, blood urea nitrogen and creatinine, and liver function test. Female subjects who were sexually active, had reliable methods of contraception and were not pregnant at the time of the study (comfirmed by a urine pregnancy test). All subjects signed a written consent form to participate in the study. The study was approved by the Medical Ethic Committee of the Chiang Mai University, Faculty of Medicine.

Study Design

This was a randomized, double-blind, 2-period crossover study. All subjects, the physician administering the assigned treatment and the technician who performed the drug analysis were blinded. Each subject was randomly assigned to receive a 200 mg capsule of either Diflucan® (lot number 612064107, purchased from the Maharaj Nakorn Chiangmai Hospital) or Biozole® (lot number FC200G/I, donated from the Biolab Company) in the morning after an overnight fast and remained without food at least 4 hours after the oral dose administration. Blood samples were collected immediately before and at 0.5, 1, 2, 2.5, 3, 4, 24, 48, 56 and 72 hours after the drug administration. The blood samples were allowed to clot at room temperature, then centrifuged at 2400 rpm for 6 minutes. The serum samples were kept overnight at 0-4°C and then were analyzed for fluconazole concentrations within 24 hours after blood sample collections. At least 2 weeks after the previous visit, subjects were crossed over to receive a different fluconazole preparation.

Drug Assay

Concentrations of fluconazole in the serum samples were quantified with the use of the high performance liquid chromatography (HPLC) technique[2,4]. The HPLC system consisted of a C_8 reversed-phase column (4.6 mm X 150 mm), an ISS-200 automatic sampler and a model 410LC pump connected to an LC-235 diode array detector. Column eluate was monitored at 260 nm wavelength. Peak areas of the chromatogram were integrated with the use of a model 2100 PC integrator. The isocratic mobile phase was a mixture of 0.02 M phosphate buffer in methanol (69:31, v/v), pH 6.85. Hexadecyltrimethyl-ammonium bromide (0.182 g%) was used as an ion-pare reagent in the mobile phase. The mobile phase was pumped through the column at 1 ml/min. The serum sample was prepared with the use of a solid phase

extraction column. The retention time for fluconazole was approximately 5.98 minutes with the lower limit of detection of 1 μg/ml. Fluconazole concentrations were determined from a calibration curve of the standard concentrations of fluconazole peak areas with the use of linear regression. The interday and intraday variations were 6.54 and 5.05 per cent, respectively.

Statistical Methods and Data Analysis

The serum fluconazole concentration-time curves were analyzed by non-compartmental model. Maximal serum concentration (C_{max}) and time to reach the peak concentration (T_{max}) were obtained directly by visual inspection of each subject's serum concentration-time profile. Other pharmacokinetic parameters including elimination half-life ($t_{1/2}$), plasma clearance (CL), volume of distribution (V_d), area under the concentration time curve from time 0 to 72 hours ($AUC_{0\text{-}72\ h}$) and area under the concentration time curve from time 0 to infinity ($AUC_{0\text{-}inf}$) were derived with the use of TOPFIT 2.0, a Pharmacokinetic and Pharmacodynamic Data Analysis System for the PC.

Paired Students' *t*-test was used to determine the statistical differences between the mean values of serum fluconazole concentrations of the two preparation over time.

The 90 per cent confidence intervals (90% CI) of the mean ratio of AUC and C_{max} were parametric and calculated according to the method of Schuirmann and Bolton[37,38]. Statistic analysis was performed on the natural logarithm (ln) transformed data and the three-way ANOVA. Thereafter, using the variance estimate (VAR) obtained from the ANOVA, calculated the 90 per cent CI from the following formulation[10-12]:

$$(\mu_A - \mu_B) = (\bar{X}_A - \bar{X}_B) \pm t^{v}_{0.1} \sqrt{\frac{2VAR}{n}}$$

Where $\bar{X}_A$, $\bar{X}_B$ were the observed means of the ln transformed parameters (either C_{max} or AUC) of test product (Biozole®) and reference product (Diflucan®). VAR (or S^2) was the error variance obtained from the three-ways ANOVA (the residual mean square of a two-way crossover study). n was the number of subjects. $t^{v}_{0.1}$ was the tabulated two-tails *t* value for 90 per cent CI. v was the number of degrees of freedom of the mean square from the ANOVA. The next step was taking the antilogarithm of the CI, which would express the bioequivalence as a ratio of the test product (Biozole®) and the reference product (Diflucan®). The two preparations were considered to be bioequivalent if the 90 per cent CI (2-sided at the 5% level) of these ratios were within the bioequivalence range of 0.80-1.25[10,11].

The limits for the bioequivalence range of T_{max} were expressed as untransformed data (absolute differences) and the stipulated bioequivalence range of the T_{max} difference [T_{max} (Biozole®)-T_{max} (Diflucan®)] was ± 20 per cent of the T_{max} of the Diflucan® preparation[10,11].

The other pharmacokinetic perameters were expressed as mean ± SD. The relative bioavailability (F_{rel}) of the generic preparation was determined with the use of the formula: $AUC_{0\text{-}72}$ h (Biozole®)/$AUC_{0\text{-}72}$ h (Diflucan®)

RESULTS

Twelve healthy subjects completed this study without any serious adverse effects. The mean serum fluconazole concentrations over time following single oral dose of 200 mg of Diflucan® and Biozole® are shown in Table 1 and Fig. 1, respectively. There were no statistical differences between the mean values of serum fluconazole concentrations of the two preparations over time. The means of each pharmacokinetic parameter of the two formulations are shown in Table 2. Both formulations of fluconazole were rapidly absorbed after oral administration with the mean T_{max} of 2.04 ± 0.81 and 1.58 ± 1.28 h for Diflucan® and Biozole®, respectively. At this time point, the mean C_{max} of Diflucan® and Biozole® were 5.77 ± 1.10 and 6.67 ± 1.92 μg/ml, respectively. The mean values of $AUC_{0\text{-}72}$ and $AUC_{0\text{-}inf}$ for Diflucan® were 206.85 ± 38.48 and 279.05 ± 52.98 μg.h/ml, comparable to those values of 210.22 ± 35.10 and 275.79 ± 51.21 μg.h/ml for Biozole®. The average extrapolation fraction of the $AUC_{0\text{-}inf}$ were 26 per cent and 24 per cent for Diflucan® and Biozole®, respectively.

Table 3 illustrates 90 per cent CI and point estimate of [Biozole®/Diflucan®] of $AUC_{0\text{-}72}$, $AUC_{0\text{-}inf}$, and C_{max} as well as the T_{max} differences of [Biozole®-Diflucan®]. The mean and 90 per cent CI of [Biozole®/Diflucan®] of $AUC_{0\text{-}72}$, $AUC_{0\text{-}inf}$ and C_{max} were 1.02 [0.98-

Table 1. Mean serum concentrations of fluconazole over time after a single 200 mg oral dose of the innovator and generic preparations in 12 healthy Thai volunteers.

Time (h)	Serum concentration (μg/ml)				p value[b]
	Diflucan®		Biozole®		
0	0		0		
0.5	3.14	0.60[a]	4.83	1.00	0.21
1	4.51	0.46	5.76	0.51	0.08
2	5.42	0.32	5.40	0.33	0.87
2.5	5.44	0.31	5.54	0.38	0.56
3	5.38	0.30	5.21	0.31	0.35
4	5.11	0.31	5.20	0.31	0.56
24	3.27	0.18	3.31	0.16	0.72
48	2.08	0.14	2.15	0.12	0.55
56	1.78	0.09	1.71	0.10	0.50
72	1.37	0.08	1.28	0.09	0.07

[a] : Mean (SE)
[b] : Paired *t*-test

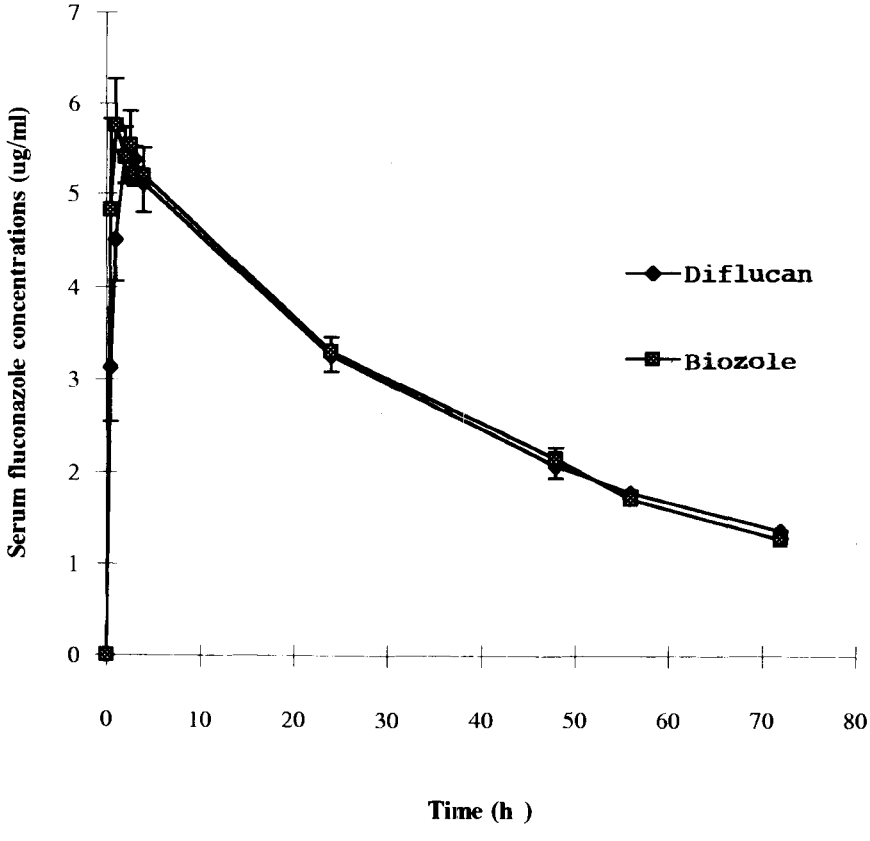

Fig. 1. Mean serum concentrations of fluconazole over time after a single 200 mg oral dose of the innovator and generic preparations in 12 healthy Thai volumteers. Data represent mean ± SEM.

Table 2. Pharmacokinetic parameters after a single 200 mg oral preparation of the innovator and generic fluconazole in 12 healthy Thai volunteers.

	Diflucan®		Biozole®	
C_{max} (μg/ml)	5.77	1.10[a]	6.67	1.92
T_{max} (h)	2.04	0.81	1.58	1.28
$t_{1/2}$ (h)	36.20	4.09	34.56	5.21
CL (ml/min/kg)	0.23	0.03	0.23	0.03
V_d (L/kg)	0.70	0.09	0.68	0.09
AUC_{0-72h} (μg.h/ml)	206.85	38.48	210.22	35.10
AUC_{0-inf} (μg.h/ml)	279.05	52.98	275.79	51.21
F_{rel} (%)			102.38	9.79[b]

a : Mean (SD)
b : Determined at the time 0-72 h

Table 3. Parametric 90% CI for the mean pharmacokinetic parameters of fluconazole 200 mg.

Parameters	[Biozole®/Diflucan®] ratio	
	Mean	90% CI
AUC_{0-72h} (μg.h/ml)	1.02	0.98-1.06
AUC_{0-inf} (μg.h/ml)	0.99	0.95-1.03
C_{max} (μg/ml)	1.13	1.03-1.25

Parameters	Difference between [Biozole®/Diflucan®]	
	Mean	90% CI
T_{max} (h)	(-0.46)	(-1.03)-(0.12)

1.06], 0.99 [0.95-1.03] and 1.13 [1.03-1.25], respectively. These values were well within the acceptable bioequivalence range of 0.80-1.25[36]. The mean and 90 per cent CI of the T_{max} difference were -0.46 and [(-1.03)-(0.12)], respectively, which was outside the acceptable range.

DISCUSSION

The bioequivalence testing of Biozole® and Diflucan® following single oral administration of 200 mg doses were conducted in a randomized two-way crossover design. The result demonstrated that the mean ratios [Biozole®/Diflucan®] of the AUC_{0-72}, AUC_{0-inf} and C_{max} were close to 1 and their 90 per cent CI were within the international bioequivalence range of 0.80-1.25. Therefore, it could be implied that the two products were bioequivalent with respect to the extent of drug absorption and C_{max}. However, the parametric point estimate of the difference of T_{max} [Biozole®- Diflucan®] of (-0.46) h and 90 per cent CI of (-1.03) - (0.12) h implied that T_{max} of Biozole® was significantly shorter than Diflucan®, since this value was outside the bioeqivalence range of ±0.41 h (±20% of the T_{max} of Diflucan®).

In this study, C_{max} and T_{max} were estimated from the highest concentration measured and the time of its occurrence. Since the accuracy of the T_{max} was limited by discrete blood sampling schedule and the serum concentration-time curve was quite flat near the peak, the value of T_{max} chosen may not be a good representative of the actual value. Nevertheless, the T_{max} difference which occurred between the two products may be explained by the presence of different excipients and manufacturing process which could affect the

rate of drug dissolution *in vitro* and drug absorption *in vivo*. Another reason was the variations in absorption rate due to physiological conditions such as gastric emptying time and intestinal transit time. Although, the average T_{max} of Biozole® and Diflucan® were statistically different between the two formulations (1.58 ± 1.28 *vs* 2.04 ± 0.81 h). These values were within the average T_{max} values reported in the literature (1-3 h)[9,13].

Other pharmacokinetic parameters such as $t_{1/2}$, V_d(L/kg), CL(ml/min/kg), AUC_{0-72} and AUC_{0-inf} were comparable between the two products. The average V_d reported in this study was slightly smaller than those values reported in the literature (0.71 L/kg)[14]. On the other hand, the C_{max} values of fluconazole from our study were also higher than the averaged values reported in other studies (4.70 μg/ml)[13]. The reason may be due to the smaller volume of distribution of our subjects compared to Caucasian subjects. The averaged Cl of the two products reported here was less than the values reported in the literature (0.27 ± 0.070 ml/min/kg)[1]. The averaged $t_{1/2}$ of Diflucan® and Biozole® in this study was slightly longer than the values reported in other studies (32 ± 5 h)[8].

SUMMARY

The bioequivalence testing demonstrated bioequivalence of the two brands concerning the extent of absorption (AUC_{0-72}, AUC_{0-inf}) and C_{max}. The time to reach the peak (T_{max}) of Biozole® was shorter than Diflucan®, and was out of the bioequivalent range. However, since the difference of T_{max} is usually not related to the differences in safety and efficacy of the drug, the bioequivalence with respect to the AUC and C_{max} might imply that the generic Biozole® may be used interchangeably with Diflucan® when the cost-effectiveness is concerned.

(Received for publication on March 31, 1998)

REFERENCES

1. Debruyne D, Ryckelynck JP. Clinical pharmacokinetics of fluconazole. Clin Pharmacokinet 1993; 24: 10-27.
2. Force RW, Nahata MC. Salivary concentrations of ketoconazole and fluconazole: implications for drug efficacy in oropharyngeal and esophageal candidiasis. Ann Pharmacother 1995; 29: 10-5.
3. Fromtling RA. Fluconazole (Diflucan®): a new antifungal triazole. Drugs Today 1990; 26: 547-56.
4. Foulds G, Brennan DR, Wajszczuk C, et al. Fluconazole penetration into cerebrospinal fluid in humans. J Clin Pharmacol 1988; 28: 363-6.
5. Washton H. Reveiw of fluconazole: a new triazole antifungal agent. Diag Microbiol Infect Dis 1989; 12: 229S-3S.
6. Houang ET, Chappatte O, Byrne D, Macrae PV, Thorpe JE. Fluconazole levels in plasma and vaginal secretions of patients after a 150-milligram single oral dose and rate of eradication of infection in vaginal candidiasis. Antimicrob Agents Chemother 1990; 34: 909-10.
7. Humphreys MJ, Jevons S, Trabit MH. Pharmacokinetic evaluation of UK-49858, a metabolically stable triazole antifungal drug in animals and humans. Antimicrob Agents Chemother 1985; 28; 648-53.
8. Shiba K, Saito A, Miyahara T. Pharmacokinetic evaluation of fluconazole in healthy volunteers. Jpn Antibiotics 1989; 42: 17-30.
9. Toon S, Ross CE, Gokat R, Rowland M. An assesment of the effects of impaired renal function and haemodialysis on the pharmacokinetic of fluconazole. Br J Clin Pharmacol 1990; 29: 221-6.
10. Nation LR, Sansom NL. Bioequivalence requirements for generic products. Pharmacol Ther 1994; 62: 41-55.
11. Sauter R, Steinijans VW, Diletti E, Bohm A, Schultz HU. Presentation of results from bioeqivalence studies. Int J Clin Pharmacol Ther Toxicol 1992; 30 (Suppl. 1): S7-S30.
12. Schuirmann DJ. A comparison of the two one-side tests procedure and the power approach for assessment the equivalence of average bioavailability. J Pharmacokinet Biopharm 1987; 15: 657-80.
13. Farrow PR, Faulkner JK, Brammer KW. The pharmacokinetics of fluconazole. Symposium on Fluconazole: A Novel Advance in the Therapy for Systemic Fungal Infections. Dorado, Puerto Rico, Oct 8-9. Abstract no. 14-199, 1988.
14. Jezequel SG. Fluconazole: Interspecies scaling and allometric relationships of pharmacokinetic properties. J Pharm Pharmacol 1994; 46: 196-9.

การเปรียบเทียบไบโออะไวลาบิลิตีของยาฟลูโคนาโซลชนิดรับประทานที่ผลิตจากบริษัทต้นตำรับกับบริษัทอื่นในอาสาสมัครชาวไทยสุขภาพดี

ศุภนิมิต ทีฆชุณหเถียร, พ.บ., นพมาศ โรจนเสถียร, พ.บ.*, มาลียา มโนรถ, พ.บ.*, ชัยชาญ แสงดี, ปร.ด.*, อัมพวัน อภิสริยะกุล, ปร.ด.*, อุดมชัย อัจยุตโภคิน, วท.ม.**

คณะผู้วิจัยได้ทำการศึกษาเภสัชจลนศาสตร์และเปรียบเทียบไบโออะไวลาบิลิตีของยาฟลูโคนาโซลชนิดแคปซูลที่ผลิตจากบริษัทภายในประเทศ (ไบโอโซล®, บริษัทไบโอแลบ จำกัด) กับที่ผลิตจากบริษัทต้นตำรับ (ไดฟลูแคน®, บริษัทไฟเซอร์ จำกัด) ในอาสาสมัครชาวไทยสุขภาพดีจำนวน 12 คน การศึกษาเป็นแบบ randomized, double-blind, 2-period crossover trial โดยอาสาสมัครแต่ละรายจะได้รับประทานยาฟลูโคนาโซลครั้งละ 200 มิลลิกรัม จำนวน 2 ครั้ง ซึ่งการศึกษาแต่ละครั้งจะมีระยะห่างกันอย่างน้อย 2 สัปดาห์ ตัวอย่างเลือดจะถูกเก็บก่อนที่อาสาสมัครได้รับยาและที่ 0.5, 1, 2, 2.5, 3, 4, 24, 48, 56 และ 72 ชั่วโมงหลังได้รับยา การตรวจวัดความเข้มข้นของยาฟลูโคนาโซลในซีรัมใช้วิธี HPLC (High performance liquid chromatrography) ความเข้มข้นของยาในซีรัมที่เวลาต่างๆ ของอาสาสมัครแต่ละคนจะถูกนำมาประเมินหาค่าทางเภสัชจลนศาสตร์โดยอาศัยการวิเคราะห์แบบ noncompartmental model ผลการศึกษาพบว่าค่าทางเภสัชจลนศาสตร์ส่วนใหญ่ของยาฟลูโคนาโซลเมื่อให้โดยการรับประทานจะมีความคล้ายคลึงกันระหว่างอาสาสมัครสุขภาพดีชาวไทยกับชาวต่างชาติ ไบโออะไวลาบิลิตีของยาไบโอโซล® มีค่าเท่ากับ 102.38 ± 9.79 เปอร์เซ็นต์เมื่อเทียบกับยาไดฟลูแคน® ค่าเฉลี่ยและช่วงระยะความเชื่อมั่น 90 เปอร์เซ็นต์ของอัตราส่วน [ยาไบโอโซล®/ยาไดฟลูแคน®] ของพื้นที่ใต้กราฟที่เวลา 0-72 ชั่วโมง, พื้นที่ใต้กราฟที่เวลา 0-เวลาที่ไม่สิ้นสุด และความเข้มข้นสูงสุดของยาในเลือดมีค่าเท่ากับ 1.02 (0.98-1.06), 0.99 (0.95-1.03) และ 1.13 (1.03-1.25) ตามลำดับ ค่าต่างๆเหล่านี้อยู่ในช่วงของชีวสมมูลที่ยอมรับได้คือ 0.8-1.25 แต่ค่าเฉลี่ยและช่วงระยะความเชื่อมั่น 90 เปอร์เซ็นต์ของความแตกต่างของเวลาที่ระดับยาสูงสุดในเลือด [ยาไบโอโซล®-ยาไดฟลูแคน®] มีค่าเท่ากับ -0.46 [(-1.03)-(0.12)] ซึ่งอยู่นอกช่วงของชีวสมมูลที่ยอมรับได้คือ ± 0.41 ชั่วโมง (± 20 เปอร์เซ็นต์ของเวลาที่ระดับยาสูงสุดในเลือดของยาต้นตำรับ) แสดงว่าเวลาที่ระดับยาสูงสุดในเลือดของไบโอโซล® สั้นกว่าไดฟลูแคน® อย่างไรก็ตาม ความแตกต่างดังกล่าวไม่น่าจะมีผลต่อประสิทธิภาพและความปลอดภัยในการใช้ยา จากการศึกษาครั้งนี้สรุปได้ว่ายาทั้งสองชนิดนี้มีชีวสมมูลกันในด้านปริมาณการดูดซึมยาและในด้านระดับยาสูงสุดในเลือด

* ภาควิชาเภสัชวิทยา, คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่, จ.เชียงใหม่ 50200