Bioequivalence Study of Generic Acyclovir Compared with the Brand Name Acyclovir®

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Abstract

The bioequivalence study of 200-mg generic acyclovir was conducted in healthy males. The reference Zovirax® and the test Zevin® were administered as a single oral dose after an overnight fast in a two-period, crossover design separated by 1 week. Serial blood samples were collected over a period of 24 hours. Plasma acyclovir concentrations were determined by HPLC and the pharmacokinetic parameters were analyzed by non-compartmental analysis.

Results : The $t_{1/2}$ for the test (4.5 ± 2.4 h) and Zovirax® (3.9 ± 2.6 h) were comparable. The analysis of variance was carried out and the median T_{max} (1.50 h) for the test was not statistically difference from Zovirax®. The mean (90% CI) of the $AUC_{0-\infty}$ and the C_{max} ratios for $(\frac{Test}{Reference})$ were 0.95 (0.83 - 1.09) and 0.95 (0.83 - 1.10), respectively. These values fell within the bioequivalence criteria of 0.80 - 1.25, thus it was concluded that Zevin® and Zovirax® were bioequivalence.

Key word : Acyclovir, Bioequivalence, Zevin®, Zovirax®

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Acyclovir is a guanosine nucleoside analogue with activity against herpes viruses[1,2]. The activity of acyclovir is highly selective since it has the affinity for the viral thymidine kinase encoded by herpes viruses to convert acyclovir to its monophosphate form. Thereafter, after converting to the active triphosphate form, it inhibits viral DNA synthesis. The activity of acyclovir is greater against herpes simplex virus (HSV) compared to vericella zoster (VZV)[1,2]. Oral acyclovir is effective for the treatment of primary and recurrent genital HSV[2]. Moreover, oral acyclovir given daily as prophylactic treatment for 4 months to 10 years to patients with frequent recurrent genital herpes have been shown to prevent or reduce the frequency and severity of the disease in greater than 95 per cent of patients[3]. Nevertheless, a prolonged course of acyclovir in immuno-compromised patients may result in a selection of resistant virus and failure of treatment despite continuation of acyclovir[4,5]. For herpes zoster, it has been shown to reduce the prevalence of postherpetic neuralgia and the time of healing of the lesion [2]. Acyclovir is generally well tolerated. Adverse effects of oral acyclovir such as nausea, vomiting, diarrhea, headache and rashes have occasionally been reported[2].

Acyclovir is partially absorbed from the gut. Its oral bioavailability ranges from 10 per cent - 30 per cent and decreases with increasing dosage[6,7]. Peak plasma concentrations average 0.4 to 0.8 mg/ml after 200-mg and 1.6 mg/ml after 800-mg doses [6]. A multiple-dose study in healthy subjects showed that the average peak plasma concentrations (μg/ml) were 0.83, 1.21 and 1.61, after oral doses of 200, 400 and 800 mg, respectively[2]. There was no effect of food on the absorption of acyclovir. The drug is mainly excreted unchanged by glomerular filtration and tubular secretion hence its half-life ($t_{1/2}$) and total body clearance depends on renal function. The $t_{1/2}$ of acyclovir in adults with normal renal function is about 3-4 hours (range 1.5-6 hours) and the $t_{1/2}$ increases to 20 hours in anuric patients[7,8]. Therefore, dosage adjustment is necessary for patients with creatinine clearance (CLcr) <50 ml/min and adjustment of dosage to 200-800 mg twice daily is recommended for patients with CLcr <10 ml/min[2].

In this study, the authors investigated the bioequivalence of 200-mg generic acyclovir manufactured by the Biolab Company, Bangkok, Thailand in comparison with the innovator Zovirax® after a single oral dose administration in healthy Thai male volunteers.

SUBJECTS, MATERIAL AND METHOD

Drug formulations:

Acyclovir preparations

Reference product: 200 mg Zovirax® (Glaxo Wellcome Operations, Greenford, UK, Lot/C A032642, Mfg. 08.2000 Exp. 08.2005)

Test product: 200 mg acyclovir manufactured by The Biolab Company Bangkok, Thailand, Lot T18496, Mfg. 21.8.2001 Exp. 21.8.2006 (brand name Zevin®)

Subjects

A total of 12 healthy nonsmoking male volunteers aged between 22-47 years old with a body mass index ranging from 18-24.6 were enrolled in this study. All were in good health on the basis of medical history, physical examination as well as normal blood test for hepatic and renal function. Subjects with known contraindication or hypersensitivity to acyclovir were excluded as well as those with a known history of drug abuse or alcohol consumer. No drug was allowed 1 month before and during the study period to avoid the effects of inducting or inhibiting hepatic metabolizing enzyme and the risk of drug interactions. The study was approved by the Research Ethics Committee of the Chiang Mai University, Thailand and all subjects signed the informed consent form prior to participating in the study.

Study design

The study was a single-dose, randomized, two sequence (Test-Reference, Reference-Test) crossover design with one-week washout period. Subjects were admitted to the Clinical Pharmacology Unit of the Faculty of Medicine, Chiang Mai University at 6:30 a.m. after an over night fast. Thereafter, subjects were administered either a single 200-mg dose of Zovirax® or generic acyclovir with 200-ml water at 7:00 a.m. Subjects were fasted and maintained in the up-right position for 2 hours after drug administration. Water and lunch were served at 2 hours and 4 hours afterward, respectively. The identical food and fluid intake were served during the two study periods. Subjects were required to refrain from drinking xanthines and caffeine beverages, alcohol or smoking in order to standardize experimental conditions.

Blood sample collection

Serial venous blood samples (10 ml in sodium-heparin tubes) were collected before drug administration and at 20 min, 40 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 15 and 24 hours afterward. The plasma were separated within 30 minutes at room temperature and stored frozen at -20°C until analysis.

Determination of the plasma acyclovir concentration

Plasma acyclovir concentrations were analyzed by a high performance liquid chromatography (HPLC) method[9]. Plasma samples were prepared for extraction and purification in 3 steps. First, 0.25 ml of plasma sample was measured into an eppendorf microcentrifuge tube and deproteinized by adding 10 µl of 70 per cent perchloric acid. Next, the mixture was vortexed for 30 seconds on a vortex mixer and centrifuged at 2,500 rpm for 25 min. Then, the supernatant was transferred into a new eppendorf microcentrifuge tube and 50 µl were injected into the HPLC system. The HPLC system comprised a Jasco PU-980 pump, a Jasco 821-FP spectrofluorometer detector (gain, x 100; attenuation, 32; band width, 18 nm) (Jasco, Tokyo, Japan) and a Hitachi D-2,500 chromato-integrator (Tokyo, Japan). The analytical column was a Genesis C18 (Jones Chromatography, UK) column (4 µm, 150 x 4.0 mm I.D.) fitted with a Finesse Stand Alone Guard Column Holder (Jones Chromatography, UK) containing Genesis C18 (4 µm, 1 cm x 4 mm). The mobile phase consisted of 2.5 per cent acetonitrile in 0.02 *M* disodium hydrogen orthophosphate adjusted to pH 2.5. The analysis was run at a flow-rate of 0.7 ml/min with the detector operating at an excitation wavelength of 270 nm and an emission wavelength of 380 nm. Acyclovir was clearly separated from plasma at the retention time of 8.5 minutes (Fig. 1). Blank plasma samples were spiked with acyclovir in a serial dilution to obtain a standard calibration curve of 62.5, 125, 250, 500, 1,000 and 2,000 ng/ml. Acyclovir concentrations were determined using a calibration curve of the peak height *versus* concentration of acyclovir with use of linear regression. The lower limit of detection was 50 ng/ml. Twelve quality control samples spiked at 3 different concentrations of acyclovir (80, 400 and 1,200 ng/ml) were analyzed for intraassay validation and on four separate days for interassay validation. The coefficient of variation (%CV) of intraday and inter-day assay variation were 1.96-2.07 per cent and 2.44-4.84 per cent, respectively. Assay recovery was determined by comparing the peak height of acyclovir after the plasma separation procedure with those spiked in 0.6 per cent acetic acid-water. The recovery was 96.96-98.75 per cent.

Pharmacokinetic parameter measurement

Maximal plasma concentration (C_{max}, ng/ml) and time to reach the peak concentration (T_{max}, h) were obtained directly by visual inspection of each subject's plasma concentration-time profile. The area under the plasma concentration-time curve (AUC) from time 0-infinity ($AUC_{0-\infty}$, ng.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 (Ct) was calculated using the trapezoidal rule. Extrapolated AUC from Ct to infinity ($AUC_{t-\infty}$) was determined as Ct/Ke. Total $AUC_{0-\infty}$ was the sum of AUC_{0-t} + $AUC_{t-\infty}$. The calculation was performed by TopFit, pharmacokinetic data analysis program for PC.

Statistical analysis

An analysis of variance (ANOVA) was used to determine the statistical differences of the C_{max}, and $AUC_{0-\infty}$. This procedure was referred to the confidence interval approach required by the US FDA (10-12). Statistic analysis of AUC and C_{max} were performed on a logarithmically (ln) transformed data. Thereafter, using the variance estimate (S^2) obtained from the ANOVA calculated the 90 per cent CI from the formulation:

$$90\%\ CI\ (\mu_T - \mu_R) = (\bar{X}_T - \bar{X}_R) \pm t^{v}_{0.1}\sqrt{\frac{2S^2}{n}}$$

- $\bar{X}_T$, $\bar{X}_R$ were the observed means of the (ln) transformed parameters (either C_{max} or AUC) for the test product (T) and the references (R).
- S^2 was the error variance obtained from the ANOVA
- n was the number of subjects.
- $t^{v}_{0.1}$ was the tabulated two-tail t value for 90% CI.
- v was the number of degree of freedom of the mean square from the ANOVA.

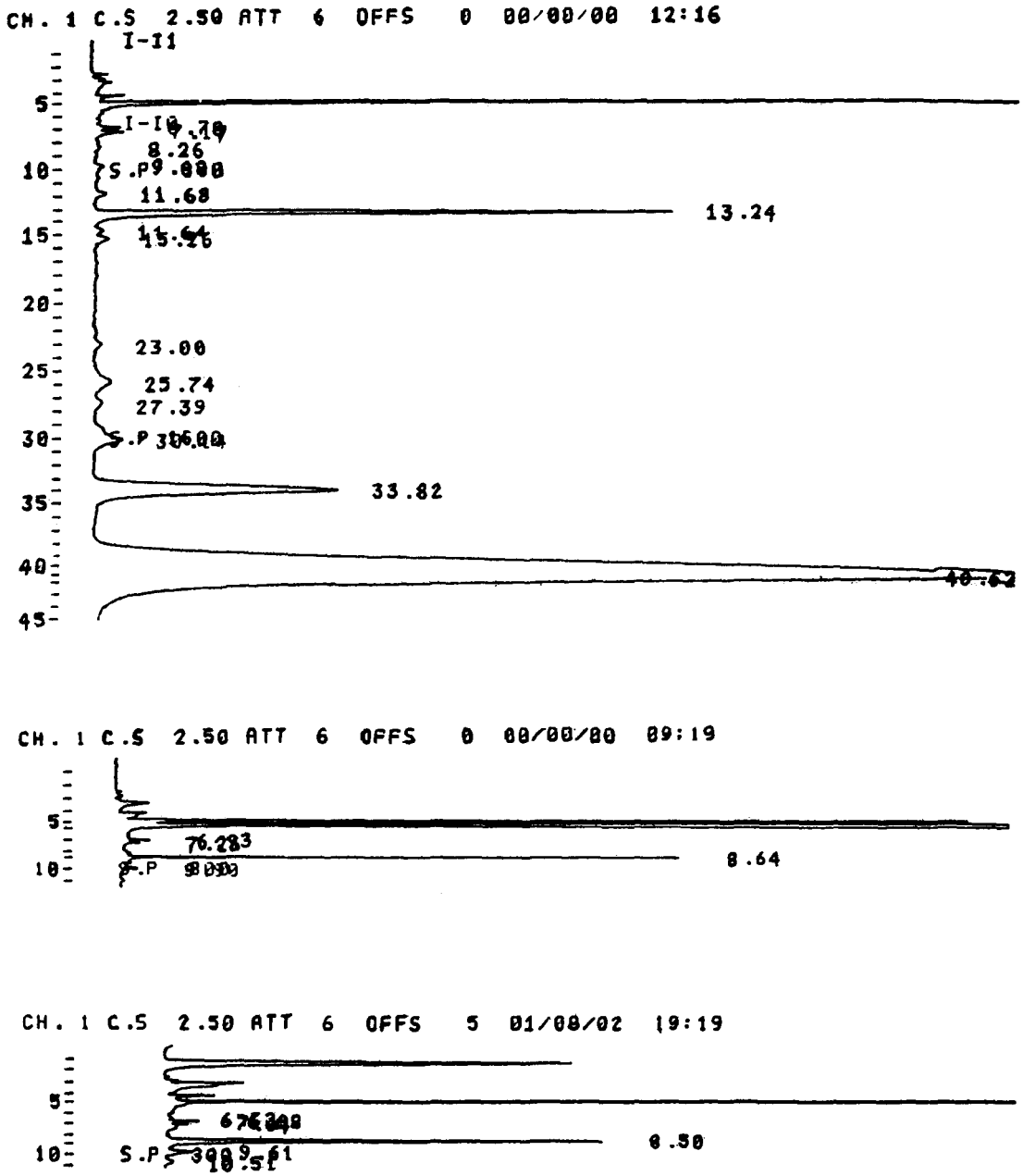

Fig. 1. Chromatograms of acyclovir in plasma.

(A) Blank plasma.
(B) Plasma spiked with 400 ng/ml of acyclovir.
(C) Volunteer plasma containing 332.3 ng/ml of acyclovir 40 mins after oral administration of acyclovir. (Acyclovir, RT = 8.5 mins).

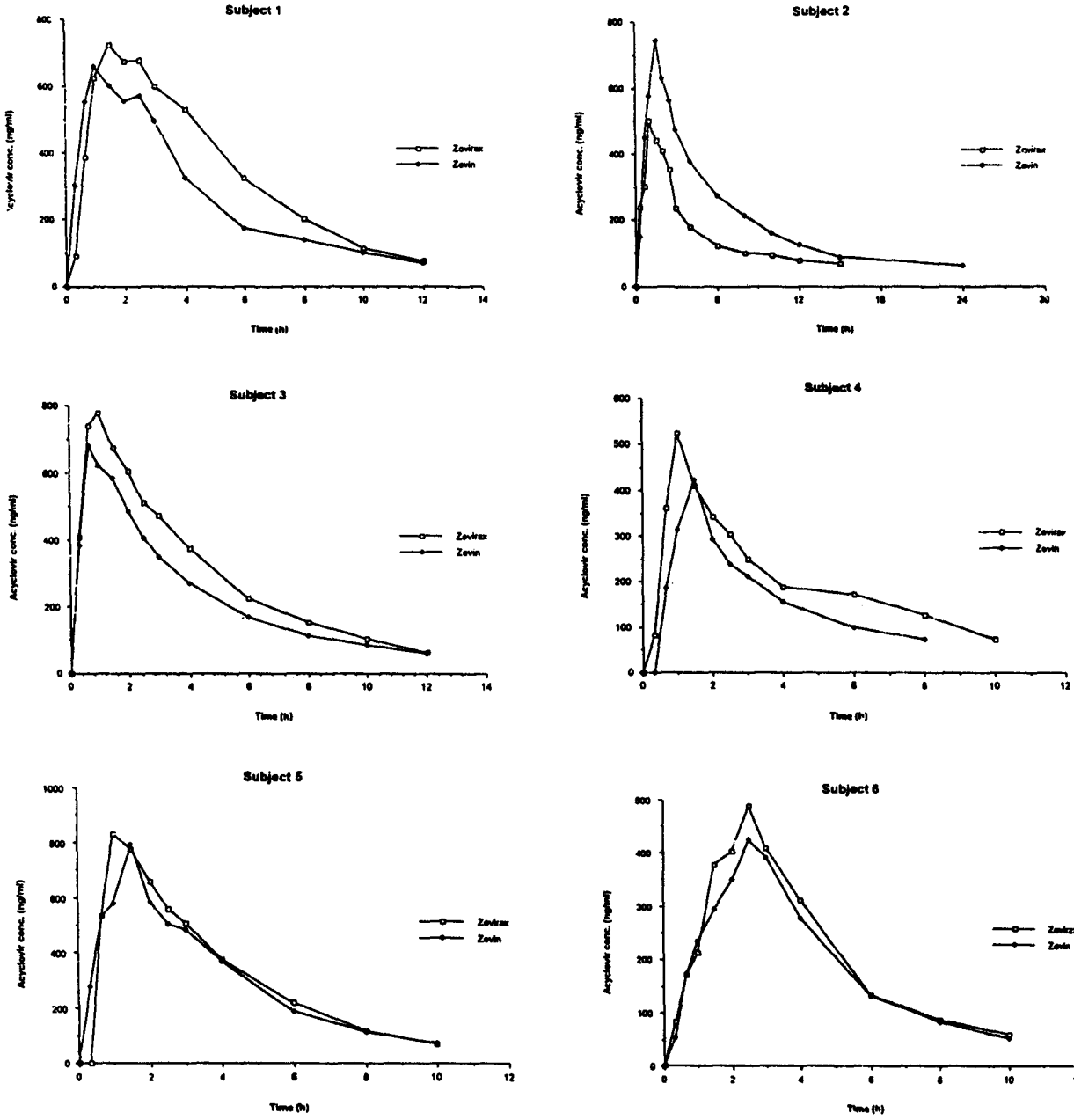

Fig. 2. Pairwise presentation of plasma concentration-time profiles after a single oral administration of 200 mg Zovirax® (-❑-) and the test (-◆-) in an individual subject.

The antilogarithm of the confidence interval of $\mu_T - \mu_R$ would express the bioequivalence as a ratio of the test and the reference (μ_T/μ_R). The bioequivalence acceptance criteria required that the 90 per cent CI for the ratio μ_T/μ_R of the $AUC_{0-\infty}$ and C_{max} fell within the interval of 0.8-1.25[10-12]. An analysis of T_{max} difference (T_{max} Test- T_{max} Reference) was expressed as untransformed data and the

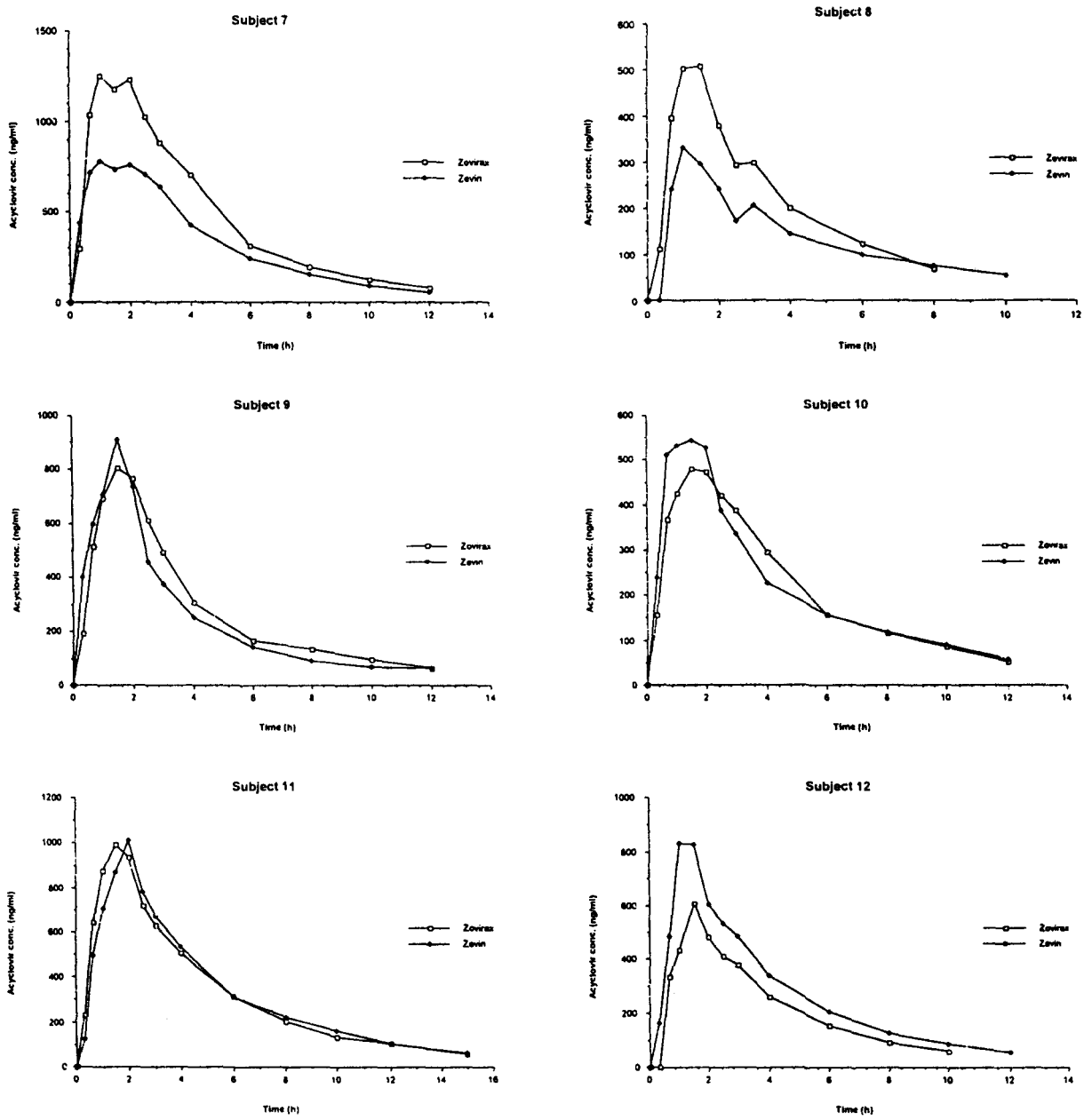

Fig. 2. Pairwise presentation of plasma concentration-time profiles after a single oral administration of 200 mg Zovirax® (-❑-) and the test (-♦-) in an individual subject.

stipulated bioequivalence range of difference was ± 20 per cent of the T_{max} of the reference formulation.

RESULT AND DISCUSSION

All subjects completed the study without any serious adverse effects. Pairwise individual plasma

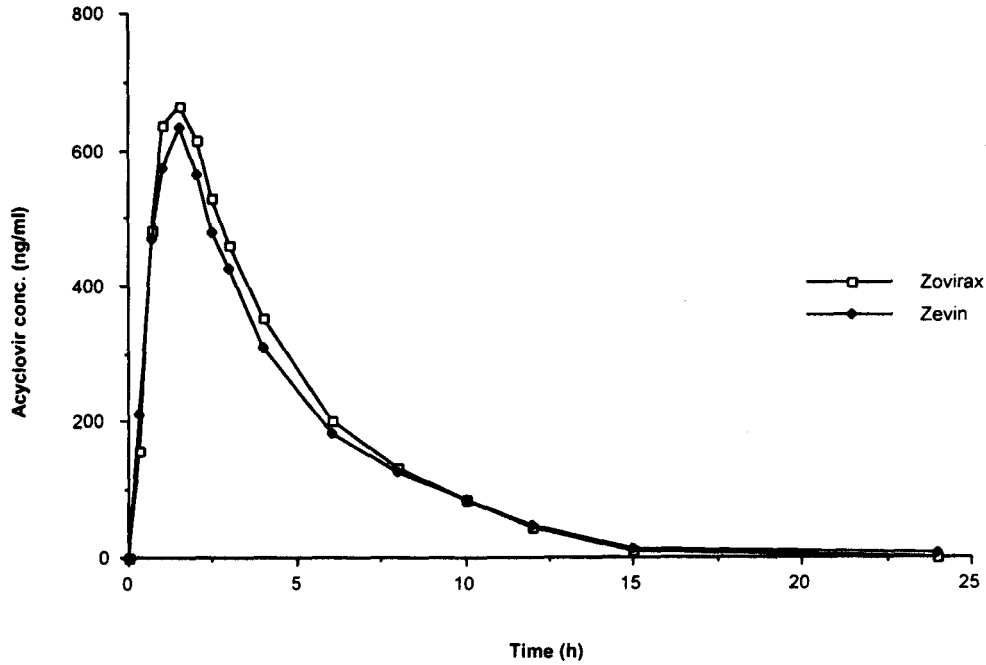

Fig. 3. Mean plasma concentration-time profiles after a single oral administration of 200 mg Zovirax® (-❑-) and the test Zevin® (-♦-).

Table 1. Comparison of acyclovir pharmacokinetic parameters after oral administration of 200 mg Zevin® (T) and Zovirax® (R).

Subject No	Tmax* (h)		Cmax (ng/ml)		AUC (ng.h/ml)		F_{rel} (%)	$T_{1/2}$(h)	
	T	R	T	R	T	R		T	R
1	1.00	1.50	658.71	725.80	3,632.57	4,372.75	83.07	4.55	2.83
2	1.50	1.00	744.54	499.47	5,726.94	3,573.80	160.25	10.80	12.20
3	0.67	1.00	681.66	777.18	3,168.04	3,857.38	82.13	4.07	3.29
4	1.50	1.00	423.94	523.57	1,675.69	2,378.28	70.46	3.24	3.24
5	1.50	1.00	789.60	831.37	3,338.29	3,462.09	96.42	2.61	2.45
6	2.50	2.50	423.81	487.70	2,109.42	2,365.71	89.17	2.91	3.37
7	1.00	1.00	775.02	1,246.18	4,144.13	6,038.57	68.63	2.70	2.91
8	1.00	1.50	330.78	507.20	1,686.18	2,056.83	81.98	4.36	2.56
9	1.50	1.50	907.88	802.37	3,655.72	3,644.27	100.31	7.45	3.83
10	1.50	1.50	543.01	480.36	2,922.87	2,835.98	103.06	4.21	3.89
11	2.00	1.50	1,010.69	991.14	5,198.29	5,145.93	101.02	3.77	3.87
12	1.00	1.50	831.23	607.11	3,550.27	2,489.57	142.61	3.17	2.73
Mean	1.50	1.50	676.74	706.62	3,421.85	3,518.43	98.26	4.49	3.93
SD	0.53	0.43	209.24	238.82	1,309.45	1,211.91	27.53	2.37	2.65
% CV	35.14	28.87	30.92	33.80	38.27	34.44	28.02	52.88	67.47
Min	0.67	1.00	330.78	480.36	1,675.69	2,056.83	68.63	2.61	2.45
Max	2.50	2.50	1,010.69	1,246.18	5,726.94	6,038.57	160.25	10.80	12.20

Tmax presented as median values

concentration-time profiles of Zovirax(and the test product as well as their mean plasma concentration-time profiles are depicted in Fig. 2 and 3, respectively. The pairwise concentration-time profiles of the test and the reference were relatively similar. The mean plasma concentration-time curves of Zovirax® and test product were relatively comparable although the peak acyclovir concentration of the test was

Table 2. Parametric 90% CI of the mean pharmacokinetic parameters ($AUC_{0-\infty}$, C_{max} and T_{max}) of the test Zevin® / the reference Zovirax®.

PK parameters	Mean	90% CI	Acceptable range
$AUC_{0-\infty}$ $(\frac{Test}{Reference})$	0.95	0.83 - 1.09	0.80 - 1.25
C_{max} $(\frac{Test}{Reference})$	0.95	0.83 - 1.10	0.80 - 1.25
T_{max} (Test - Reference)	0.01	(-0.21) - 0.24	± 0.28

slightly lower than that of Zovirax®. Table 1 compares individual calculated pharmacokinetic parameters (Cmax, Tmax, $AUC_{0-\infty}$ and t1/2) of Zovirax® and test product. The median time to reach the maximal concentration (Tmax) for the test (1.5 h, range 0.7-2.5 h) was identical to that of Zovirax((1.5 h, range 1.0-2.5 h) and was consistent with those previously reported by other authors[13,14]. However, the average C_{max} (ng/ml) of the test was slightly lower than that of Zovirax® (676.7 *vs* 706.6). The relative bioavailability (F_{rel}) calculated from $AUC_{0-\infty}$ of Test/Reference was 98.26 per cent and the mean elimination half-lives ($t_{1/2}$, h) for the test [4.49 ± 2.37 (range 2.61-10.80)] and Zovirax® [3.93 ± 2.65 (range 2.45-12.20)] were comparable and were within the range of previous reports[7,8]. All these parameters of test and the reference Zovirax® did not differ significantly. Bioequivalence analysis showed that the mean (90% CI) of the $AUC_{0-\infty}$ and C_{max} ratios [μ_T/μ_R] for Test/Zovirax® were 0.95 (0.83-1.09) and 0.95 (0.83-1.10), respectively. These values fell within the satisfying bioequivalence criteria as shown in Table 2. Furthermore, the median (90% CI) of T_{max} difference was 0.01 h [(-0.21)-0.24] which was within the acceptable range of ± 0.28 h.

SUMMARY

The authors conducted the bioequivalence testing of 200-mg oral preparations of acyclovir manufactured by the Biolab Company, Bangkok, Thailand, in comparison with Zovirax® in 12 healthy Thai male volunteers. The result demonstrated that the mean Test/Reference ratio of the $AUC_{0-\infty}$ was close to 1 and its 90 per cent CI was within the bioequivalence range of 0.80-1.25. In addition, the mean ratio Test/Reference (90% CI) of the C_{max} was 0.95 (0.83-1.10). This value was within the bioequivalence range of 0.80-1.25. The median (90% CI) of T_{max} difference was 0.01 h [(-0.21)-0.24] overlapped the stipulated the bioequivalence range of ± 0.28 h. The result of this study indicated that the generic and innovator acyclovir preparations were bioequivalent with respect to the rate and extent of drug absorption.

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การทดสอบชีวสมมูลของยาสามัญอะซัยโคลเวีย เปรียบเทียบกับยาต้นแบบ

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การศึกษาชีวสมมูลของยาอะซัยโคลเวีย ขนาด 200 มิลลิกรัมในอาสาสมัครชายไทยสุขภาพดี เปรียบเทียบระหว่างยาต้นแบบโซไวแร็ก® กับยาทดสอบเซวิน® ที่ผลิตโดยบริษัทไบโอแลบ จำกัด ประเทศไทย อาสาสมัครแต่ละคนได้รับการสุ่มไขว้เพื่อรับประทานยา ทั้งยาต้นแบบและยาทดสอบ โดยรับประทานยาหนึ่งครั้งหลังจากงดน้ำและอาหาร ระยะเวลาการศึกษาห่างกัน 1 สัปดาห์ ตัวอย่างเลือดจะเก็บตามเวลาที่กำหนดในเวลา 24 ชั่วโมง หลังจากรับประทานยา และนำไปตรวจวัดหาความเข้มข้นของยาอะซัยโคลเวีย โดยวิธีโครมาโตกราฟฟี ชนิดของเหลวสมรรถนะสูงและประเมินค่าทางเภสัจลนศาสตร์โดยวิเคราะห์แบบ non compartment ผลการศึกษาพบว่า ค่าเฉลี่ยของค่ากึ่งชีวิตของยาทดสอบ (4.5 ± 2.4 ชั่วโมง) และยาต้นแบบ (3.9 ± 2.6 ชั่วโมง) มีค่าใกล้เคียงกัน การวิเคราะห์ทางสถิติโดยใช้อะโนวา พบว่า ค่าเฉลี่ยเวลาที่ความเข้มข้นของยาสูงสุดในเลือดของยาทดสอบและยาต้นแบบ (1.50 ชั่วโมง) มีค่าไม่ต่างกันทางสถิติและค่าเฉลี่ย (ช่วงความเชื่อมั่นร้อยละ 90) ของอัตราส่วน (ยาทดสอบ/ยาต้นแบบ) ของพื้นที่ใต้กราฟที่เวลา 0 ถึงอสงไขยและความเข้มข้นสูงสุดของยาในเลือดมีค่าเท่ากับ 0.95 (0.83–1.09) และ 0.95 (0.83–1.10) ตามลำดับ ซึ่งอยู่ในช่วงของชีวสมมูลที่ยอมรับคือ 0.80–1.25 จากการศึกษาครั้งนี้สรุปได้ว่ายาทดสอบและยาต้นแบบมีชีวสมมูลเท่าเทียมกัน

คำสำคัญ : ชีวสมมูล, อะซัยโคลเวีย

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