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Bioequivalence Assessment of Lovrak (Julphar, UAE) Compared with Zovirax (Glaxo Wellcome, UK) – Two Brands of Acyclovir – in Healthy Human Volunteers

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ABSTRACT: Two studies were performed to assess the relative bioavailability of Lovrak (Julphar, UAE) compared with Zovirax (Glaxo Wellcome, UK) at the International Pharmaceutical Research Center (IPRC), Amman, Jordan. One study involved acyclovir tablets and the other acyclovir suspension. Each study enrolled 24 volunteers and in both studies, after an overnight fasting, the two brands of acyclovir were administered as a single dose on 2 treatment days separated by 1 week washout period. After dosing, serial blood samples were collected for a period of 16 h. Plasma harvested from blood, was analysed for acyclovir by an HPLC method with UV detection. Various pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$ and K_{elm} were determined from plasma concentrations for both formulations and found to be in good agreement with the reported values. AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were tested for bioequivalence after log-transformation of data. No significant difference was found based on ANOVA; 90% confidence intervals for the test/reference ratio of these parameters were found within the bioequivalence acceptance range 80%–125%. Based on these statistical inferences it was concluded that a Lovrak tablet is bioequivalent to a Zovirax tablet and that Lovrak suspension is bioequivalent to Zovirax suspension. Copyright © 2004 John Wiley & Sons, Ltd.

Key words: acyclovir; bioequivalence; pharmacokinetics; HPLC; Julphar

Introduction

The bioequivalence of two formulations of the same drug is concluded based on a lack of difference in the rate (C_{max}) and extent of absorption (AUC) especially in conventional drug formulations [1]. In the present study the bioequivalence of acyclovir formulations were evaluated by comparing those pharmacokinetic parameters derived from the plasma concentration of acyclovir.

Acyclovir is a synthetic purine nucleoside analogue with in vitro and in vivo inhibitory activity against human herpes viruses including herpes simplex types 1 (HSV-1) and 2 (HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) [2–4]. In cell cultures, acyclovir has the highest antiviral activity against HSV-1, followed in decreasing order of potency against HSV-2, VZV, EBV and CMV [5]. Acyclovir is indicated for the treatment of initial episodes and the management of recurrent episodes of genital herpes in certain patients and for the acute treatment of herpes zoster (shingles) and chickenpox (varicella) [5–9].

Upon oral administration, the reported bioavailability was in the range 10–30% with no effect

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of food [5], the peak concentration was achieved after 1.5–2.4 h [10]; 9%–33% was bound to protein, and distributed well to amniotic fluid, aqueous humor, cerebrospinal fluid, kidney, brain, spleen and liver [10–12]. It is metabolized in the liver to 9-carboxymethoxymethylguanine which does not have any antiviral activity [5]; 62%–91% was excreted in urine with a half-life of 2.5–3.3 h [5,6,10].

Many pharmacokinetics studies [2,4,10–12] on acyclovir have been reported in the literature giving an overview of the various expected pharmacokinetic parameters, with only a few of them focused on bioequivalence demonstration [13].

Objectives of the study

The purpose of this study was to compare the bioavailability (rate and extent of absorption) of generic formulations of acyclovir (Lovrak tablets/suspension, Gulf Pharmaceutical Industries, Julphar, UAE) relative to the reference formulation (Zovirax® tablets/suspension, Glaxo Wellcome, UK) in healthy volunteers by statistical analysis of the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} as recommended by the FDA.

Material and Methods

Study products

Study I: Acyclovir tablets. The test product was Lovrak – Acyclovir 800 mg tablet, batch no. 0014, Expiry date: 02/2004 from Gulf Pharmaceutical Industries – Julphar, United Arab Emirates.

The Reference product was Zovirax® – Acyclovir 800 mg tablet, batch no. D8344A, Expiry date 03/2004 from Glaxo Wellcome, UK.

Study II: Acyclovir suspension. The test product was Lovrak 200 mg/5 ml suspension, batch no. 0029, Expiry 04/2005 from Gulf Pharmaceutical Industries – Julphar, United Arab Emirates.

The Reference product was Zovirax® – 200 mg/5 ml suspension, batch no. A055316, Expiry 03/2005 from Glaxo Wellcome, UK.

Study subjects

Twenty four healthy adult male volunteers were enrolled in each study at Al-Mowasah Hospital, Amman, Jordan. The mean age was 22.8 ± 4.9 years with a range of 19–36 years and the mean body weight was 68.5 ± 9.8 kg with a range of 50–88 kg in the tablet study. Similar figures were calculated for the suspension study, namely a mean age of 23.9 ± 4.8 years with a range of 18.0–34.0 years and a mean body weight of 68.3 ± 6.8 kg with a range of 56–85 kg. The volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal and haematological diseases, as determined by their medical history, physical examination and routine laboratory tests (haematology, blood biochemistry and urine analysis). All subjects were negative for hepatitis B antigen. The volunteers were instructed to abstain from taking any drug including over-the-counter (OTC) for 2 weeks prior to and during the study period. The volunteers were informed about the risks and aim of the study by the clinical investigator and signed a written informed consent statement before entering the study. This study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of good clinical practice. The study protocol was approved by the Institutional Review Board (IRB) of Al-Mowasah Hospital, Amman, Jordan.

Drug administration and sample collection

In both studies after an overnight fasting (10 h) the subjects were given a single dose of either formulation (reference or test) of acyclovir. In study I, one tablet (800 mg) of either test or reference was given with 240 ml of water; in study II, 20 ml suspension (200 mg/5 ml) of either test or reference were given as a single dose with 240 ml of water. No food was allowed until 5 h after the dose administration. Water intake was allowed 2 h after the dose and then water, breakfast, lunch and dinner were given to all volunteers according to a time schedule. Volunteers were ambulatory during the study but prohibited from strenuous activity; they were under direct medical supervision at the study site. Approximately 10 ml blood samples for

acyclovir assay were drawn into heparinized tubes through indwelling cannula before (0 h) and at 0.33, 0.66, 1.0, 1.33, 1.66, 2.0, 2.5, 3.0, 3.5, 4, 5, 6, 7, 8, 10, 12 and 16 h after dosing. The blood samples were centrifuged at 4000 rpm for 10 min, plasma was separated and kept frozen at –20°C in coded polypropylene tubes. After a period of 7 days the study was repeated in the same manner to complete the crossover design.

Chromatographic conditions

Plasma samples were analysed for acyclovir according to a sensitive, selective and accurate HPLC method, which was developed and validated before the study according to the reported method [14]. All solvents used were of HPLC grade; while other chemicals and reagents were of analytical grade; acyclovir and guanosine (internal standard) were obtained from Julphar, UAE.

The HPLC system was from Shimadzu Kyoto, Japan, and it consisted of a solvent delivery pump (LCD-10A), a system controller (SCL-10A), an auto-injector (SIL-10A) and an UV-Vis detector (SPD-10A); integration was done using Class VP-5 software version 5.03. Chromatographic separation was performed using Nova-pak C_{18} (3.9×150 mm), 4.0 μm particle size, HPLC column (Waters, Ireland). The mobile phase consisted of 98.75% of 0.05 M potassium dihydrogen phosphate (pH 6.50 with 5.0 M KOH) and 1.25% methanol, and eluted at a flow rate of 0.8 ml/min. The effluent was monitored using a wavelength of 254 nm. The peak area were measured, and the peak area ratio of the drug to the internal standard and the concentration were calculated by Class VP-5 software (version 5.03) Shimadzu. Each analysis required a maximum of 14 min. The method was validated by following international guidelines [15].

Sample preparation for HPLC injection

A volume of 100 μl of the internal standard working solution (guanosine 8.0 μg/ml) was added to 0.5 ml plasma sample. The samples were vortexed for 30 s, 100 μl of 1.0 M HCl was added then the samples were vortexed for 30 s and centrifuged at 13 200 rpm for 5 min. A solid phase extraction (SPE) technique was used; 1.0 ml of methanol was added to the SPE cartridge (Oasis MCX 1 cc, 30 mg, 30 μm) for conditioning, 1.0 ml of de-ionized water was added for equilibration, and then the plasma samples were loaded. 1.0 ml of methanol was added as a washing step (slow flow rate), 1.0 ml of 0.52% ammonium solution (NH_4OH in methanol) was added as an elution step (very slow flow rate). The eluent was evaporated to dryness in a water bath at 45°C and then the residue was reconstituted with 200 μl mobile phase, vortexed for 30 s and transferred to a microcentrifuge tube (1.5 ml), centrifuged at 13 200 rpm for 2 min. 100 μl aliquot sample was injected into a HPLC column, where acyclovir and internal standard were separated from endogenous substances.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by means of a model independent method using a Kinetica™ 2000 computer program [16]. The elimination rate constant (λ_Z) was obtained as the slope of the linear regression of the log-transformed plasma concentration values versus time data in the terminal phase. The elimination half-life ($T_{1/2}$) was calculated as $0.693/\lambda_Z$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t}+C_t/\lambda_Z$, where C_t is the last measurable concentration.

Statistical analysis

To assess the bioequivalence between the two formulations, AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were considered as the primary variables. Two-way analysis of variance (ANOVA GLM procedure; KineticaTM 2000 Computer program [16]) for crossover design was used to assess the effect of formulations, periods, sequences and subjects on these parameters. The difference between two related parameters was considered statistically significant for p-value equal to or less than 0.05. Parametric 90% confidence intervals [17] based on the ANOVA of the mean test/reference (T/R) ratios of *AUCs* and C_{max} were computed.

Results and Discussion

Acyclovir was well tolerated by the volunteers; unexpected incidents that could have influenced the outcome of the study did not occur. There was no drop-out and all volunteers who started the study continued to the end and were discharged in good health.

The described analytical method was proven sensitive and accurate for the determination of acyclovir in plasma. The retention times were 6.09 and 8.1 min for acyclovir and guanosine (internal standard), respectively. Under the described conditions, the limit of quantitation for acyclovir was 50 ng/ml and the relationship between the concentration and the peak area ratio was found to be linear within the range 50–1500 ng/ml of acyclovir. The intra-day accuracy of the method for acyclovir ranged from 96.73% to 109.40%, while the intra-day precision ranged from 3.69% to 6.90%. The inter-day accuracy ranged from 99.73% to 107.37%, while the inter-day precision ranged from 3.69% to 11.73%. The stability study showed that acyclovir is stable in plasma for 4 months when stored at -20°C.

Both formulations were readily absorbed from the gastrointestinal tract and acyclovir was measurable at the first sampling time (0.33 h) in some volunteers. The mean concentration-time profiles of the two studies are shown in the Figures 1 and 2 indicating that the mean plasma drug concentration profiles of the two brands were closely similar and superimposable. Peak concentrations were attained at 1.5–2.0 h after drug administration and then declined moderately and were detectable until the last blood sample. All calculated pharmacokinetic parameters were in good agreement with reported values [2,4,10–13].

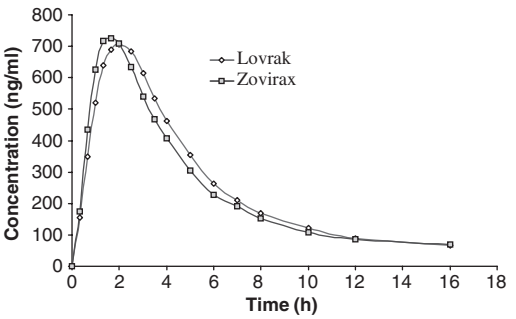

Figure 1. Mean plasma concentration of acyclovir tablets (800 mg) after oral administration of a single dose of two brands

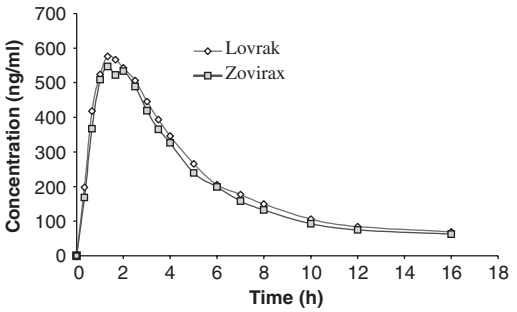

Figure 2. Mean plasma concentration of acyclovir 200 mg/5 ml suspension after oral administration of a single dose (20 ml) of the two brands

Table 1 shows the pharmacokinetic parameters for two studies. The extent of absorption is a key characteristic of a drug formulation, and therefore *AUC* is an important parameter for a comparative bioavailability (bioequivalence) study [18]. However, the other two parameters, C_{max} and T_{max}, are also important features of the plasma level profile and could affect the therapeutic use of a drug [18] and hence were also considered in the study. The relative bioavailability of Lovrak vs Zovirax is shown in Tables 2 and 3.

The mean and standard deviation of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the two products did not differ significantly, suggesting that the blood profiles generated by Lovrak are comparable to those produced by Zovirax. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in periods, formulations or sequence, having p value greater than 0.05. 90% confidence intervals also demonstrated that the ratios of AUC_{0-t}, $AUC_{0-\infty}$ or C_{max} of the two formulations lie within the FDA acceptable range of 80%–125%.

For T_{max} the parametric point estimate of difference (test–reference) was 0.31 h (Study I) and 0.33 h (Study II), and found to be within the acceptance limits ($\pm$ 20% of reference mean).

Table 1. Pharmacokinetic parameters of acyclovir formulations (mean ± standard deviation; $n = 24$)

Pharmacokinetic parameter	Study I Acyclovir tablets		Study II Acyclovir suspension	
	Lovrak (test)	Zovirax (reference)	Lovrak (test)	Zovirax (reference)
AUC_{0-t} (ng/ml.h)	3793.32 ± 1661.26	3693.20 ± 1631.68	3253.31 ± 1437.95	2937.12 ± 887.85
$AUC_{0-\infty}$ (ng/ml.h)	4259.39 ± 1674.50	4207.51 ± 1768.95	3621.57 ± 1513.90	3260.34 ± 918.41
C_{max} (ng/ml)	842.0 ± 287.33	824.79 ± 292.97	628.0 ± 252.13	607.0 ± 161.35
T_{max} (h)	1.74 ± 0.73	1.59 ± 0.50	1.56 ± 0.59	1.55 ± 0.60
$T_{1/2}$ (h)	4.87 ± 1.62	5.18 ± 1.87	3.84 ± 0.65	3.51 ± 0.64
K_{elim} (h)	0.1564 ± 0.0465	0.1528 ± 0.0591	0.1862 ± 0.04	0.2046 ± 0.04

Table 2. Relative bioavailabilities of reference and test formulations

Pharmacokinetic parameter	Study I Acyclovir tablets	Study II Acyclovir suspension
AUC_{0-t}	111.43%	110.26%
$AUC_{0-\infty}$	108.77%	110.19%
C_{max}	108.27%	103.47%

Table 3. 90% confidence intervals of log-transformed data

Pharmacokinetic parameter	Study I Acyclovir tablets formulation	Study II Acyclovir suspension formulation
AUC_{0-t}	86.8–119.5%	97.1–116.9%
$AUC_{0-\infty}$	87.1–116.7%	98.4–116.6%
C_{max}	90.8–115.5%	91.8–109.7%

Plasma levels may be used as surrogate parameters for clinical activity; therefore the results of this study suggest equal clinical efficacy of the two brands of acyclovir.

Conclusion

Statistical comparison of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} clearly indicated no significant difference between Lovrak and Zovirax. Based on the study results, it is concluded that Lovrak, manufactured by Gulf Pharmaceutical Industries, U.A.E. is bioequivalent to Zovirax, manufactured by Glaxo Wellcome, UK, and that both products can be considered equally effective in medical practice.

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