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Comparative bioavailability of two tablet formulations of acyclovir in healthy volunteers

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Abstract. This investigation was carried out to evaluate the bioavailability of a new tablet formulation of acyclovir (400 mg), Clovir, relative to reference product, Zovirax (400 mg) tablets. The 2 brands were found to be similar in weight variation, disintegration time, dissolution, and assay as stipulated by the USPXXIII, as well as by the manufacturer. The bioavailability was carried out on 24 healthy male volunteers who received a single dose (400 mg) of the test (T) and the reference (R) products in the fasting state, in a randomized balanced 2-way crossover design. After dosing, serial blood samples were collected for a period of 16 hours. Plasma harvested from blood was analyzed for acyclovir by a sensitive and validated high-performance liquid chromatographic assay. The maximum plasma concentration (C_{max}), area under the plasma concentration-time curve up to the last measurable concentration (AUC_{0-t}), and to infinity ($AUC_{0-\infty}$), and the absorption rate ($C_{max}/AUC_{0-\infty}$) were analyzed statistically under the assumption of a multiplicative model. The time to maximum concentration (T_{max}) was analyzed assuming an additive model. The parametric confidence intervals (90%) of the mean values of the pharmacokinetic characteristics (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, $C_{max}/AUC_{0-\infty}$) for T/R ratio were in each case, well within the bioequivalence acceptable range of 80 – 125%. The test formulation was found bioequivalent to the reference formulation by the Schuirmann's two 1-sided t tests and by Wilcoxon Mann Whitney two 1-sided tests procedure. Therefore, the 2 formulations were considered to be bioequivalent.

Key words: acyclovir – comparative bioavailability – Clovir – Zovirox – healthy volunteers

Introduction

Acyclovir is a nucleoside analogue with antiviral activity in vitro against the herpes simplex viruses (HSV), Varicella zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV) and human herpes virus 6 (HHV-6) [O'Brien and Campoli-Richards 1989]. Acyclovir selectively inhibits DNA replication of herpes viruses, with low host cell toxicity. The antiviral is preferentially activated in infected cells, initial phosphorylation occurs via viral thymidine kinase, and acyclovir triphosphate (the active derivative obtained from the monophosphate via host cell enzymes) inhibits viral DNA polymerase more readily than the cellular enzyme, thus preventing viral replication [Readron and Spector 1991, Wagstaff et al. 1994].

The pharmacokinetic parameters of acyclovir following oral administration show generally high variability. The absorption of acyclovir from the gastrointestinal tract is slow and incomplete. Peak plasma levels are generally obtained 1.5 – 2.0 hours after administration and reported to be about 0.4 – 0.5 μg/ml or 0.6 – 0.75 μg/ml after a single dose of 200 mg or 400 mg, respectively [De Miranda and Blum 1983, Vergin et al. 1995]. The absolute bioavailability after oral administration is about 20% [Strauss et al. 1985]. About 80% of an oral dose is accordingly not absorbed but is excreted with the feces. The main excretory organ for acyclovir is the kidney [Laskin et al. 1982a]. After intravenous injection about 70% – 80% of the dose is renally eliminated as unchanged drug [Laskin et al. 1982b, Vergin et al. 1995]. The mean plasma elimination half-life determined in healthy adult is 2.9 ± 0.8 h [Laskin et al. 1982b, Spector et al. 1981].

In view of high interindividual variability of the pharmacokinetic parameters of acyclovir especially the incomplete absorption following oral administration, studies of the bioavailability of newly developed tablet formulation is deemed essential. The objective of this study is the determination of the bioavailability of a new commercial tablet formulation of acyclovir (Clovir) relative to a reference formulation (Zovirax). Bioequivalence of the 2 prod-

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ucts was assessed based on the plasma concentration data obtained following their administration to 24 healthy male volunteers in a balanced 2-way crossover design.

Subjects, materials and methods

Subjects

Twenty-four healthy male adult volunteers participated in the study. Their mean age (± SD) was 36.3 ± 7.5 years with a range of 21 – 50 years, body weight of 77.9 ± 8.7 kg with a range of 62 to 92 kg, and height of 173.5 ± 6.1 cm with a range of 160 – 185 cm. On the basis of medical history, clinical examination, and laboratory investigation (hematology, blood biochemistry, and urine analysis), no subject had a history or evidence of hepatic, renal, gastrointestinal or hematologic deviations, or any acute or chronic diseases or drug allergy. The volunteers were asked to abstain from taking any drug including over-the-counter (OTC) for at least 2 weeks prior to and during the study. Informed consent was obtained from the subjects after explaining the nature and purpose of the study. The study protocol was approved by King Khalid University Hospital, College of Medicine Research Center (CMRC), King Saud University, Riyadh, Saudi Arabia.

Study design and blood sampling

The study design was a single dose, 2-treatment, 2-period, 2-sequence crossover with a 1-week washout period between phase I and phase II dosing. Subjects were randomly divided into 2 equal groups and assigned to 1 of the 2 sequences of administration. Each subject received a single dose of 400 mg tablet of either brand with 240 ml of water after an overnight fast for at least 10 hours. Subjects were allowed to eat a standard breakfast at 4 h, lunch at 8 h, and dinner at 12 h after drug administration. Beverages and food containing caffeine were not permitted over the entire course of the study. Volunteers were ambulatory during the study but were prohibited from strenuous activity. Multiple blood samples (7 ml) were collected in evacuated glass tubes (heparinized vacutainers, Becton and Dickinson, Rutherford, NJ, USA) through an indwelling cannula placed in the forearm vein before (0 hour) and at 0.50, 1.0, 1.50, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 14.0, and 16.0 hours post-dosing. The plasma was then separated after centrifugation and stored frozen at –20° C pending analysis.

Analysis of plasma samples

The concentrations of acyclovir in plasma were measured by modification of Nebringer and Koel [1993] HPLC method. The assay involved ultrafiltration (Ultrafree-MC 5000 NMWL filter unit, Millipore, Bedford, MA, USA) step prior to ion-pair reversed phase liquid chromatography, using guanosine as internal standard. The drug and internal standard were eluted from a Novapak C18 column at ambient temperature with a mobile phase consisting of 5% methanol and 95% 1-octanesulfonic acid (0.05 M) in 0.1 M potassium dihydrogen phosphate buffer adjusted to an apparent pH 2.8, at flow rate of 1.0 ml/min. The effluent was monitored by fluorescence detector set at excitation and emission wavelengths of 260 and 375 nm, respectively. The standard curves for the analyte in plasma were generated daily and were linear ($r > 0.995$) in the range of 10 – 1,000 ng/ml over the entire period of the study. The limit of quantitation of acyclovir in plasma is 10 ng/ml. Intrarun coefficients of variation (CVs) ranged from 4.10% – 9.56% ($n = 18$) and interrun Cvs ranged from 4.08% – 10.84% ($n = 20$) at 6 different concentrations (15, 50, 80, 90, 200, and 600 ng/ml). The absolute recoveries ranged from 70.15% to 103.08% and relative recoveries from 96.15% to 104.28% at the 6 different concentrations. The test samples from the dosed volunteers were always analyzed along with standard and quality control samples. All specimens used to study precision and bias were interspersed with clinical specimens during analysis.

Standard in vitro tests of acyclovir tablets

Both the test formulation (Clovir, T, tablets, 400 mg, lot No. 11075, Saudi Pharmaceutical Industries and Medical Appliances Corporation (Spimaco), Saudi Arabia) and the reference formulation (Zovirax, R, tablets, 400 mg, lot No. H1934A, The Wellcome Foundation Ltd., England) were examined for conformation to compendial standards and the manufacturer specifications for hardness, friability, weight variation, disintegration, dissolution, and assay.

Pharmacokinetic analysis

The pharmacokinetic characteristics for acyclovir were determined from the plasma concentration-time data. The maximum plasma concentrations (C_{max}) and time to reach maximum plasma concentrations (T_{max}) were obtained directly by inspection of the individual drug plasma concentration time data, and were used as measures of rate of absorption. The area under the plasma concentration time curve up to the last time (t) showing a measurable concentration (C_t) of the analyte (AUC_{0-t}) was determined by using the linear trapezoidal rule. Truncated areas under the plasma concentration-time curve up to different time points (such as AUC_{0-2}, AUC_{0-4}, AUC_{0-6} ... AUC_{0-16}) were also determined by using the same procedure. The apparent elimination rate constant (K_{el}) was calculated by the technique of least-squares regression from the data of

the last 4 – 7 points of each plasma concentration time curve. The $AUC_{0-\infty}$ values (express the magnitude of absorption) were determined by adding the quotient of $*C_t$ and the appropriate Kel to the corresponding AUC_{0-t}, that is:

$$AUC_{0-\infty} = AUC_{0-t} + *C_t/K_{el}$$

where $*C_t$ is the estimated last plasma concentration. The sampling period covered more than 95% of total AUCs for both brands T and R. The apparent elimination half-life ($t_{1/2}$) of acyclovir in plasma was calculated by using the following equation:

$$t_{1/2} = (\ln 2)/K_{el}$$

The ratio $C_{max}/AUC_{0-\infty}$ was also computed and was used as a measure for the rate of absorption.

Statistical analysis

The 2-way analysis of variance (ANOVA) for crossover design was used to assess the effect of formulations, periods, sequences and subjects within sequence on logarithmically transformed data of AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, $C_{max}/AUC_{0-\infty}$, K_{el} and $t_{1/2}$ and truncated AUCs parameters. The ANOVA for T_{max} was carried out on the untransformed data. Sequence effects were tested against the mean square term for subjects within sequence. All other main effects were tested against the mean square error term. Parametric 90% confidence intervals based on the ANOVA of the mean T/R ratios of AUC parameters, C_{max} and $C_{max}/AUC_{0-\infty}$ were computed under the assumption of a multiplicative model. Parametric 90% confidence intervals for the characteristic T_{max} was performed under the assumption of additive model and the equivalence range was expressed in absolute differences of the mean T – R. Nonparametric confidence interval was also performed [Hauschke et al. 1990]. In addition, bioequivalence between the 2 formulations was also assessed by Schuirmann's two 1-sided t tests [Schuirmann 1987] and by means of nonparametric Mann Whitney Wilcoxon tests procedure [Hauschke et al. 1990]. Plots of residuals versus predicted and univariate analyses were performed for the AUCs and C_{max} to screen for extreme outliers and departure from normality. For all analyses effects were considered statistically significant if the probability associated with F was < 0.05. All analyses of the data were performed with the statistical software package SAS using the GLM procedure (Statistical Analysis System, SAS Institute, Inc., Cary, NC, USA).

Results and discussion

Table 1 shows the results of the in vitro tests for the test and the reference tablet formulations of acyclovir. The results show that both products met the specifications for hardness, friability, weight variation, disintegration time, dissolution, and assay as stipulated by the USPXXIII, as well as by the manufacturer.

Table 1 In vitro results for tablet formulations of acyclovir

Test	Clovir (T)	Zovirax (R)
Weight variation (USP XXIII)	conform	conform
Hardness (Kp)	15.3	15.75
Friability (USP XXIII)	0.03% conform	0.1% conform
Average weight (mg)	595.8	518.3
(CV %)	1.86	0.63
Disintegration (min)*	14.0	12.0
Dissolution**	conform	conform
Mean % dissolved	100.1	101.8
(CV %)	1.8	0.4
Assay (HPLC)	101.5%	103.8%

* = disintegration time recommended by the manufacturer is 15 min, the test was performed on 18 tablets from each brand and they conform according to USP XXIII criteria for disintegration of uncoated tablets, ** = apparatus 2, 100 rpm, medium 0.1 N HCl, time: 30 min, NLT 90%, dissolution was performed on 24 tablets of each product and each tablet passed the requirements in both products

Acyclovir was well tolerated by the subjects. Unexpected incidents that could have influenced the outcome of the study did not occur. All volunteers who started the study continued to the end and were discharged in good health.

Both formulations of acyclovir were readily absorbed from the gastrointestinal tract of the volunteers. Acyclovir was measurable at the first sampling time (0.5 h) in 20 volunteers following administration of the 2 brands. Plasma concentrations at the last sampling period (16 h), were below 10 ng/ml (lower quantifiable limit) in 2 subjects following administration of the reference formulation and in 3 subjects following administration of the test formulation. The mean plasma concentration time curves for the 2 brands are demonstrated in Figure 1. It can be seen that the mean plasma concentration time profile from brand T and R are almost superimposed. Sixteen ANOVA's were performed to compare acyclovir plasma concentrations produced by the 2 formulations at each sampling time (e.g. 0.5, 1.0, 1.5 h etc.). There was no statistical difference between the 2 formulations at the 16 time points.

The truncated areas under the plasma concentration time curves were calculated from time zero to specific sampling time (AUC_{0-2}, AUC_{0-4}, AUC_{0-6} etc.) (cumulative AUC) according to the trapezoidal rule. The mean truncated AUC's for the specific sampling interval after administration of the 2 formulations are presented in Table 2. There was no statistically significant difference (ANOVA) between the 2 formulations in any of the 8 truncated AUCs. The parameters used to measure bioavailability were AUC_{0-t}, $AUC_{0-\infty}$, for the extent of absorption and T_{max}, C_{max}, and $C_{max}/AUC_{0-\infty}$ for the absorption rate and they were calculated in a model-independent manner.

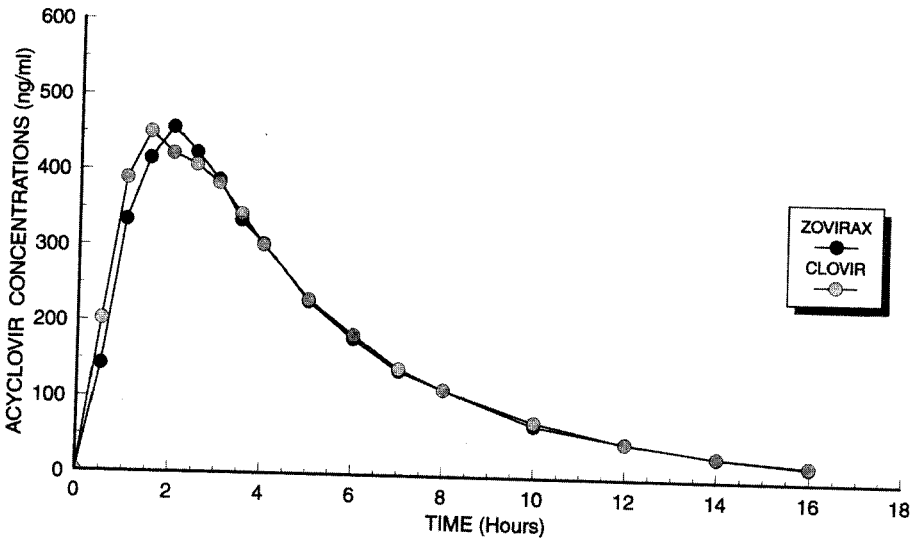

Fig. 1 Mean plasma concentration time profiles of acyclovir following oral administration of the 2 brands to 24 healthy male volunteers.

Table 2 Mean truncated area under the acyclovir plasma concentration-time curve for each formulations

Sampling interval (h)	Mean truncated AUC (CV %) (ng×h/ml) Test formulation	Reference formulation
0 – 2.0	609.77 (37.4)	548.30 (36.2)
0 – 4.0	1,358.12 (34.0)	1,311.61 (37.1)
0 – 6.0	1,832.22 (34.5)	1,799.98 (36.9)
0 – 8.0	2,122.84 (33.7)	2,066.87 (35.9)
0 – 10.0	2,310.74 (33.3)	2,250.14 (35.1)
0 – 12.0	2,413.53 (32.9)	2,366.63 (34.4)
0 – 14.0	2,509.49 (32.7)	2,445.30 (33.8)
0 – 16.0	2,559.29 (32.6)	2,494.39 (33.5)

Table 3 Mean pharmacokinetic characteristics for acyclovir after administration of the 2 formulations to 24 subjects

Parameter	Test formulation	Reference formulation
AUC_{0-t} (ng×h/ml)		
Geometric mean	2,436.70	2,361.61
Range	1,772.42 – 3,349.95	1679.25 – 3,321.26
$AUC_{0-\infty}$ (ng×h/ml)		
Geometric mean	2,537.92	2,465.38
Range	1,854.37 – 3,473.44	1,775.61 – 3,423.09
C_{max} (ng/ml)		
Geometric mean	500.85	477.95
Range	349.53 – 717.45	315.54 – 723.93
T_{max} (h)		
Mean	1.792	1.938
± SD	0.846	0.697
K_{el} (h^{-1})		
Geometric mean	0.219	0.214
Range	0.183 – 0.262	0.179 – 0.256
$t_{1/2}$ (h)		
Geometric mean	3.165	3.239
Range	2.643 – 3.790	2.707 – 3.875
$C_{max}/AUC_{0-\infty}$ (h^{-1})		
Geometric mean	0.198	0.194
Range	0.167 – 0.233	0.156 – 0.242

Table 3 shows the geometric mean values and the range for the above parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, and $C_{max}/AUC_{0-\infty}$) along with K_{el} and $t_{1/2}$. The pharmacokinetic characteristic T_{max} is presented as mean (± SD). The relative bioavailability of the generic formulation was found to be 108.9%, 108.5%, and 109.1% based on $AUC_{0\rightarrow t}$, $AUC_{0-\infty}$, and C_{max}, respectively.

Results of the ANOVA of the bioavailability data clearly indicated that there were no significant differences between formulations on any of the pharmacokinetic characteristics (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, K_{el}, $t_{1/2}$, and $C_{max}/AUC_{0-\infty}$). Neither was there any period and sequence effect on these parameters. However, there were significant intersubject variabilities on all parameters. This was expected in view of the wide intersubject variations in these parameters probably due to interindividual variabilities in drug clearances. The intraindividual variations in the AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, K_{el}, $t_{1/2}$ and $C_{max}/AUC_{0-\infty}$ estimated from the coefficients of variation as determined by ANOVA were 23.88%, 23.39%, 22.48%, 28.85%, 9.45%, 9.73%, and 12.57%, respectively.

Table 4 shows the parametric 90% confidence intervals of the mean values of the pharmacokinetic characteristics (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, K_{el}, $t_{1/2}$, and $C_{max}/AUC_{0-\infty}$) as well as the point estimates for T/R ratio assuming multiplicative model. Nonparametric confidence intervals were also included. The confidence limits for the mean AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, K_{el}, $t_{1/2}$, and $C_{max}/AUC_{0-\infty}$ indicated that these values are entirely within the bioequivalence accept-

Table 4 Parametric and nonparametric 90% confidence intervals for the mean pharmacokinetic characteristics of acyclovir formulations

Model: multiplicative	T/R point estimate	Confidence limits	Level of confidence
Parametric analysis*			
AUC_{0-t}	103.2	94.2 – 113.0	90
$AUC_{0-\infty}$	103.0	94.1 – 112.6	90
C_{max}	104.8	96.7 – 113.5	90
K_{el}	102.3	98.7 – 106.1	90
$t_{1/2}$	97.7	94.2 – 101.4	90
$C_{max}/AUC_{0-\infty}$	101.8	97.1 – 106.7	90
Nonparametric analysis**			
AUC_{0-t}	102.0	88.7 – 121.0	91.13
$AUC_{0-\infty}$	101.7	88.7 – 121.1	91.13
C_{max}	105.2	92.1 – 119.7	91.13
K_{el}	102.7	96.4 – 107.3	91.13
$t_{1/2}$	97.2	93.2 – 103.7	91.13
$C_{max}/AUC_{0-\infty}$	100.5	94.1 – 109.2	91.13
Model: additive	T – R (hours) Point estimate	Confidence limits	Level of confidence
Parametric analysis*			
T_{max}	0.146	–0.059 – 0.351	90
Nonparametric analysis**			
T_{max}	0.0	–0.25 – 0.25	91.13

* = two 1-sided t tests, ** = two 1-sided Wilcoxon tests

able range of 80 – 125%. With regard to the characteristic T_{max} untransformed data were used and the bioequivalence range was expressed in absolute difference instead of proportions (Table 4). It can be seen from Table 4 that the parametric point estimate of the difference (T – R) is 0.15 h and thus within the stipulated bioequivalence range of ± 0.39 h. The 90% confidence interval ranges from –0.06 to 0.35 h and the nonparametric confidence intervals ranged from –0.25 to 0.25 h, hence equivalence with respect to the rate of absorption can also be concluded. Further, the test formulation was found bioequivalent to the reference formulation by the Schuirmann's two 1-sided t tests and by Wilcoxon Mann Whitney two 1-sided tests procedure.

In conclusion, based on the pharmacokinetic and statistical results of this study, we can assume interchangeability of both preparation in clinical practice.

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