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Bioequivalence Studies of Two Formulations of Famciclovir Tablets by HPLC Method

K.V. SUBRAHMANYAM*, P. MOHANRAJ, P. SANDHYARANI, V.S. SARAVANAN and N. GOPAL
Department of Pharmaceutical Analysis, Nandha College of Pharmacy
Erode-638 052, India
E-mail: subhashguy@yahoo.co.in

The objective was to compare the bioavailability of two tablet dosage forms of famciclovir in rabbits. Serum levels of famciclovir was determined by using high performance liquid chromatography. The pharmacokinetic parameters were estimated following the oral administration of a single dose (250 mg) tablet of the drug to 12 healthy rabbits. All rabbits received a single oral dose of the medication in a two period, two way cross-over design. The difference between the formulations were statically insignificant. In all the cases the relative bioavailability of the test was found to be in the range of 83-105 %. No adverse reactions were observed during the entire study. The test formulation manufactured by Cipla Lab., was found to be bioequivalent to the reference product.

Key Words: Bioequivalence, Famciclovir, Serum, HPLC.

INTRODUCTION

Famciclovir is an antiviral agent used for herpes simplex virus type-1 and type-2[1]. Absorption of acyclovir from the gastrointestinal tract is variable and incomplete[2,3]. It is estimated that 30-40 % of an oral drug is absorbed. The time to reach peak concentrations in plasma is approximately 1.5 to 2 h after an oral dose[2]. Famcilovir is excreted principally in urine through glomerular filtration and tubular secretion, with only a small percent of the dose being oxidized to 9-carboxy methoxy ethyl guanine[4].

EXPERIMENTAL

Famciclovir and paracetamol were obtained from Cipla Laboratories Ltd., Daman, India. The reference formula (R) was Famtrex manufactured by Cipla. The test formula (T) was famciclovir tablets formulated by Cipla, FDC, Laboratories Ltd., India. Methanol was of HPLC grade. Sodium octane sulphonic acid was purchased from Sigma chemicals. All other chemicals were of reagent grade.

Dissolution studies: Dissolution testing was carried out using the USP paddle method (Electolab TDF-06, India). The dissolution medium consisted of 0.1 N hydrochloric acid, pH 1.2 and the dissolution volume was 900 mL. Samples were withdrawn at 15, 30 and 45 min intervals and replaced with a fresh medium. Samples were filtered, diluted and analyzed directly at 245 nm using a Hitachi 2000 spectrophotometer.

Chromatographic conditions: The analysis was performed using a high-pressure liquid chromatography instrument by the method proposed by Molokhia *et al.*[5] with modification. The mobile phase consisted of 7 % methanol:water containing 5 mM sodium sulphonic acid with a final pH of 2.5. The flow rate was 1.5 mL/min. Samples were injected into column (Pecosil ODS 10 µm, size 250 cm × 4.6 mm, Perkin Elmer, USA) and the absorptivity of the mobile phase was monitored at 254 nm using a variable wavelength UV detector (Perkin Elmer UV/Visible spectrophotometer detector LC 290). The output was recorded on an integrator (PE Nelson Model 1020, Perkin Elmer, USA). The detection limit of the method was 0.05 µg/mL.

Preparation of serum samples: To 1 mL serum (spiked or subject's serum), 25 µL of paracetamol solution (2.5 µg/mL) as internal standard and 1.5 mL of methanol was added and vortex-mixed for 5 min. To this solution 100 mL of 40 % trichloro acetic acid was added to precipitate the serum proteins and again vortex mixed for 5 min and centrifuged at 5000 rpm for 20 min. The supernatant was separated and 40 µL was injected into HPLC instrument for analysis.

Standardization and calibration: Pooled spiked serum which contained 0.05-10 µg/mL was used to construct the calibration curve. Peak height ratios were plotted against concentration. The calibration curves were used to calculate famciclovir concentration in the test serum samples.

Recovery and reproducibility: Serum samples were spiked with known concentration of famciclovir and used as quality control samples. Controls were chosen at 0.5, 2 and 5 µg/mL and they were used to calculate extraction recovery. Controls were analyzed on the same day to calculate interday variation. They were injected along with rabbit's serum samples on different days to ensure the reproducibility of the analysis and to calculate inter and intra-day variation.

Pharmacokinetics and bioavailability study

The bioavailability study was conducted using two formulations containing 250 mg famciclovir tablets. Among 12 healthy rabbits, each rabbit received a single oral dose of the medication in a two way cross over design.

Blood samples were withdrawn at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 6.0, 9.0 and 12.0 h. The wash-out period was 1 week between the two periods. Blood samples were centrifuged and serum was collected for analysis.

The area under the concentration-time curve up to last time interval ($AUC_{0\to t}$) and extrapolated to infinity ($AUC_{0\to\infty}$) were calculated by trapezoidal method. The maximum serum concentration (C_{max}) was directly obtained from concentration-time curve.

Analysis of variance (Anova) was performed on the pharmacokinetic parameters for the formulations. Westlake's 95 % confidence intervals for the bioavailability ratio between test and reference formulations were calculated[6].

RESULTS AND DISCUSSION

The reproducibility of the assay methodology adopted in this study is evident from the data given in Table-1. Both the products met the specification for content uniformity and dissolution. A summary of pharmacokinetic parameter, their mean and standard deviation is given in Table-2. Fig. 1 shows the dissolution profile of reference and test formulations. Fig. 2 shows the concentration time data of reference and test tablets. The HPLC method employed in this study was sensitive enough to monitor serum levels of famciclovir over the entire period of blood collection in all the rabbits and more than 85 % recovery was obtained by this method. Famciclovir was well tolerated by the subjects. No adverse effects were observed in an of the subjects during and after the study.

TABLE-1
INTRA- AND INTER-DAY PRECISION OF FAMCICLOVIR ASSAYS IN SERUM AT CONCENTRATION 0.1, 0.5 AND 2.0 µg/mL SERUM

	Mean (n = 10)	SD	CV (%)	Bias (%)
Intraday				
0.1	0.098	0.001	0.62	-2.00
0.5	0.532	0.030	4.31	4.70
2.0	2.250	0.210	1.49	13.40
Interday				
0.1	0.110	0.002	0.84	10.00
0.5	0.504	0.041	5.45	1.21
2.0	1.950	0.110	7.10	-2.00

Tables 3-5 shows the results of Anova for $AUC_{0\to t}$ and $AUC_{0\to\infty}$ and C_{max}, respectively. The results of Anova showed no significant difference between the formulations with respect to formulation and sequence of administration.

TABLE-2
MEAN PHARMACOKINETIC PARAMETERS (MEAN ± SD) OF TWO FORMULATION OF FAMCICLOVIR

Parameter	Reference	Test
$AUC_{0\to t}$	3.34 ± 0.16	3.46 ± 0.16
$AUC_{0\to\infty}$	4.17 ± 0.30	4.05 ± 0.37
C_{max}	0.41 ± 0.02	0.38 ± 0.01

TABLE-3
ANALYSIS OF VARIANCE (ANOVA) FOR $AUC_{0\to t}$

Source	d.f.	SS	MS	F
Subjects	11	4.750	0.430	
Period	1	11.540	11.540	$F_{1, 10} = 0.110$
Treatment	1	0.001	0.001	$F_{1, 10} = 0.001$
Error	10	1.090	0.110	
Total	23	17.420		
$F_{1, 10} = 4.95$				

TABLE-4
ANALYSIS OF VARIANCE (ANOVA) FOR $AUC_{0\to\infty}$

Source	d.f.	SS	MS	F
Subjects	11	207.68	18.96	
Period	1	0.23	0.23	$F_{1, 10} = 0.001$
Treatment	1	9.39	9.48	$F_{1, 10} = 0.393$
Error	10	241.43	24.14	
Total	23	459.54		
$F_{1, 10} = 4.95$				

TABLE-5
ANALYSIS OF VARIANCE (ANOVA) FOR C_{max}

Source	d.f.	SS	MS	F
Subjects	11	0.030	0.0030	
Period	1	0.020	0.0200	$F_{1, 10} = 0.90$
Treatment	1	0.002	0.0020	$F_{1, 10} = 1.37$
Error	10	0.006	0.0006	
Total	23	0.057		
$F_{1, 10} = 4.95$				

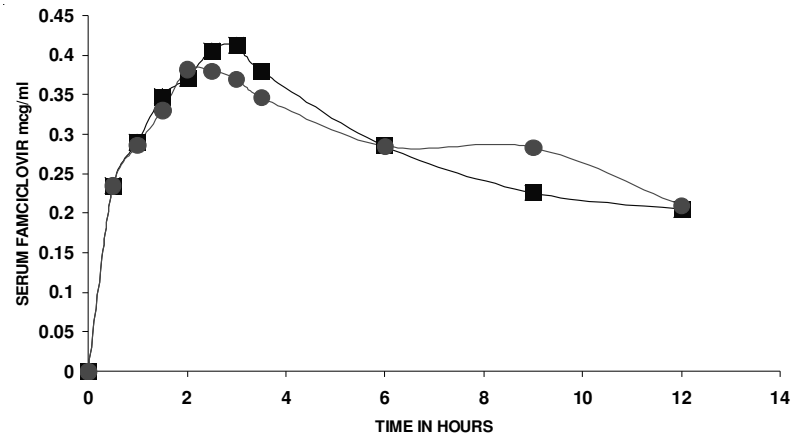

Fig. 1. Concentration-time curve representing the mean concentration at each time point for the reference (■) and test (●)tablests in rabbits

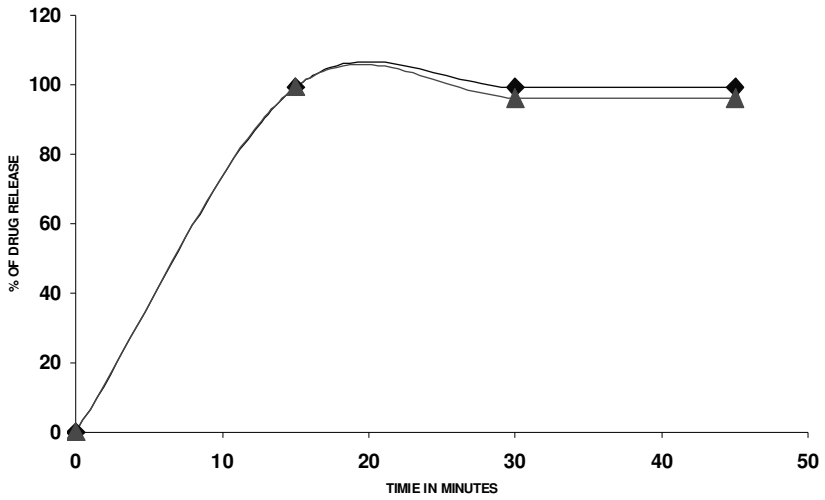

Fig. 2. The mean per cent dissolution curve at each time point for the reference (▲) and test (◆) tablets

The bioavailability of the test formulation relative to the reference was found by the test/reference ratio for the mean $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$ and C_{max} which is shown in Table-6. In all the cases the relative bioavailability of the test was found to be in the range of 83-105 %. Table-6 also shows the Westlake's confidence intervals which are in the range between 81-109 %. Thus, the study indicates that the test formulation is not significantly different compared to the reference formulation.

TABLE-6
RELATIVE BIOAVAILABILITY AND WESTLAKE'S 95 % CONFIDENCE INTERVALS (Cl) TEST FORMULATION IN COMPARISON WITH REFERENCE FORMULATION

Parameter	Relative bioavailability (%)	Westlake's 95 % Cl
$AUC_{0\rightarrow t}$	98	88.5-109.6 %
$AUC_{0\rightarrow \infty}$	82	81.9-106.5 %
C_{max}	105	89.4-109.7 %
Parameter	Reference	Test
$AUC_{0\rightarrow t}$	3.34 ± 0.17	3.46 ± 0.18
$AUC_{0\rightarrow \infty}$	4.15 ± 0.31	4.06 ± 0.38
C_{max}	0.43 ± 0.02	0.38 ± 0.01

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