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Bioequivalence Studies of two Formulations of Acyclovir Sodium Tablets

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The objective was to compare the bioavailability of two tablet dosage forms of acyclovir in human volunteers. Serum levels of acyclovir was determined by using high performance liquid chromatography. The pharmacokinetic parameters were estimated following the oral administration of a single dose (200 mg) tablet of the drug to 12 healthy volunteers. All volunteers received a single oral dose of the medication in a two period, two way cross-over design. The difference between the formulations were statistically insignificant. In all the cases the relative bioavailability of the test was found to be in the range of 83-106 %. No adverse reactions were observed during the entire study. The test formulation manufactured by Cadila Laboratories Pvt. Ltd., was found to be bioequivalent to the reference product.

ACYCLOVIR is an antiviral agent used for harpes 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]. Acyclovir is widely distributed into the body fluids and tissues in several animal species[4]. Acyclovir 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[5].

MATERIALS AND METHODS

Materials

Acyclovir and paracetamol were obtained from Cadila Laboratories Ltd., Ahmedabad, India. The reference formula (R) was Zovirax tablets (Batch No. 1K138) manufacturered by Wellcome Medical Division, Burroughs Wellcome Inc., Canada and the test formula (T) was acyclovir sodium tablets (Batch No. 4001) formulated by Cadila Laboratories Ltd., India. Methanol was of HPLC grade. Sodium octane sulphonic acid was purchased from Sigma Chemicals, USA. 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 Samples were withdrawn at 15, 30 and 45 min intervals and replaced with a fresh medium.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 (HPLC) instrument by the method

* For Correspondence

Table 1: Intra- and Inter-day Precision of Acyclovir 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	0.60	-2.0
0.5	0.523	0.03	4.33	4.6
2.0	2.27	0.22	1.50	13.5
Interday				
0.1	0.11	0.002	0.86	10.0
0.5	0.506	0.042	5.45	1.20
2.0	1.96	0.10	7.10	-2.0

Table 2 : Mean Pharmacokinetic Parameters (Mean ± S.D) of Two Formulations of Acyclovir

Parameter	Reference	Test
$AUC_{0\to t}$	3.35±0.17	3.47±0.18
$AUC_{0\to\infty}$	4.16±0.31	4.06±0.38
C_{max}	0.42±0.02	0.39±0.01

Table 3 : Analysis of variance (ANOVA) for $AUC_{0\to t}$

Source	d.f	SS	MS	F
Subjects	11	4.78	0.44	
Period	1	11.55	11.55	$F_{1,10} = 0.11$
Treatment	1	0.001	0.001	$F_{1,10} = 0.001$
Error	10	1.08	0.11	
Total	23	17.41		
$F_{1,10} = 4.96$				

Table 4 : Analysis of Variance (ANOVA) for $AUC_{0\to\infty}$

Source	d.f	SS	MS	F
Subjects	11	208.68	18.97	
Period	1	0.23	0.23	$F_{1,10} = 0.001$
Treatment	1	9.49	9.49	$F_{1,10} = 0.392$
Error	10	241.44	24.14	
Total	23	459.64		
$F_{1,10} = 4.96$				

proposed by Molokhia et al[6] with slight 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 x 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.

Table 5 : Analysis of Variance (ANOVA) for C_{max}

Source	d.f	SS	MS	F
Subjects	11	0.03	0.003	
Period	1	0.02	0.02	$F_{1,10} = 0.90$
Treatment	1	0.002	0.002	$F_{1,10} = 1.38$
Error	10	0.006	0.0006	
Total	23	0.058		
$F_{1,10} = 4.96$				

Table 6. Relative Bioavailability and Westlake's 95% Confidence Intervals (CI) o Test Formulation in Comparison with reference Formulation

Parameter	Relative Bioavailability	Westlake's 95% CI
$AUC_{0\rightarrow t}$	98%	88.0-109.7 %
$AUC_{0\rightarrow\infty}$	83%	81.9-106.7 %
C_{max}	106%	89.4-109.5 %

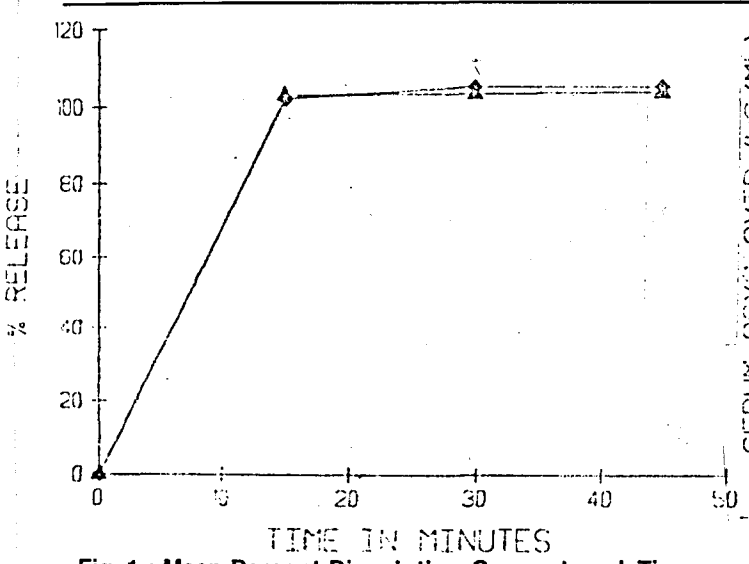

Fig. 1 : Mean Percent Dissolution Curve at each Time Point for the Reference (▲) and Test (◆) Tablets

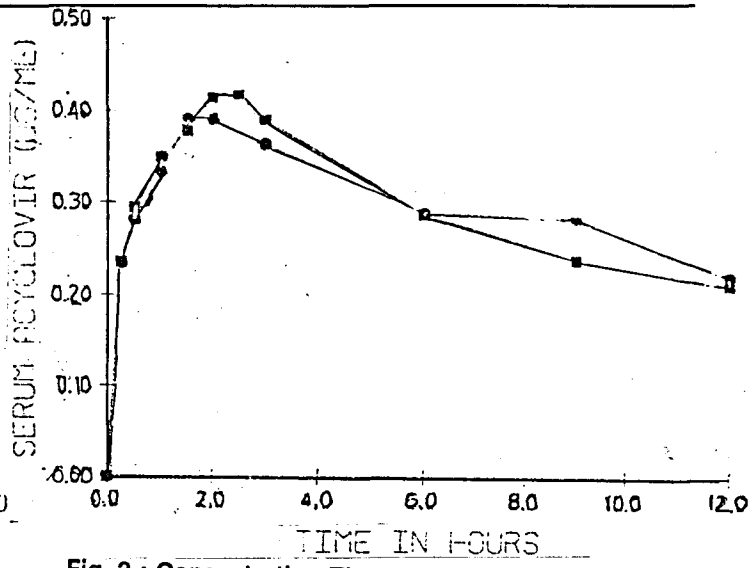

Fig. 2 : Concentration-Time Curve Representing the Mean Concentration at Each time Point for the reference (□) and Test (○) Tablets in Human Volunteers

Preparation of serum samples

To 1 ml of serum (spiked or subject's serum), 25µl 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 acyclovir concentration in the test serum samples.

Recovery and reproducibility

Serum samples were spiked with known concentration of acyclovir 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 volunteer's serum samples on different days to ensure the reproducibility of the analysis and to calculate inter - and intra-day variation. The control samples were stored under the same conditions as that of volunteer's samples.

Pharmacokinetics and bioavailability study

The bioavailability study was conducted using two formulations containing 200 mg Acyclovir sodium tablets. Twelve healthy male volunteers, all were students of L. M. College of Pharmacy, Ahmedabad, India participated in the study. All volunteer's received a single oral dose of the medication in a two period, 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 one week between the two periods. Blood samples were centrifuged and serum was collected for analysis.

The area under the concentration-time curve upto 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[7].

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 parameters, their mean and standard deviation is given in Table 2. Figure 1 shows the dissolution profile of reference and test formulations. Figure 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 acyclovir over the entire period of blood collection in all the volunteer's and more than 85% recovery was obtained by this method. Acyclovir was well tolerated by the subjects. No adverse effects were observed in any of the subjects during and after the study.

Table 3, 4, and 5 shows the results of ANOVA for $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ and C_{max} respectively. The results of ANOVA showed no significant difference between the formulations with respect to formulation and sequence of administration.

The bioavailability of the test formulation relative to the reference was found by the test/reference ratio for the mean $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$ 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 83-106%. Table 6 also shows the Westlake's confidence intervals which are in the range between 82-110 %. Thus, the study indicates that the test formulation is not significantly different compared to the reference formulation.

REFERENCES

1. Richards, DM, Carmine, AH, Brogden, RM, Heel, RC, Speight, TM and Avery, GS. **Drugs,** 1983, 26, 378.
2. Bridgen, D, Fowle, A and Rosling, A. In Collier, LH and Oxford, J, Eds. Developments in Antiviral Therapy, Academic Press, London, 1980, 53.
3. Van Dyke, RB, Straube, R, Large, K, Hintz, M, Spector, S and Connors, JD. Twenty second Interscience Conference on Antimicrobial Agents and Chemotherapy, Florida, 1982, 414.
4. de Miranda, P, Krasny, HC, Page, DA and Elion, GB. **J. Pharmacol. Exp. Ther.,** 1981, 219, 309.
5. Laskin, OL, **Clin. Pharmacokinet.,** 1983, 8, 187.
6. Malokhia, AM., Niazy, EM, El-Hoofy, SA and EL-Dardari, ME. **J. Liq. Chromatogr.,** 1990, 13, 981.
7. Steinijans, VW and Diletti, E. **Eur. J. Clin. Pharmacol.,** 1983, 24, 127.