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Short communication

Determination and pharmacokinetics of acyclovir after ingestion of suspension form[1]

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Abstract

The study describes, simple, precise, sensitive and accurate HPLC assay with spectrofluorimetric detection for the determination of acyclovir in human plasma. The method was linear over a range 25–1200 ng ml^{-1}. The average yield in this method exceeded 80%. Limits of quantitation and detection were 25 and 10 ng ml^{-1}, respectively. On the basis of reported method, a single-dose of pharmacokinetics on 24 men, in two doses (200 and 400 mg) of acyclovir suspension has been investigated. Pharmacokinetic parameters obtained from both doses of the drug were compared. The linearity of acyclovir pharmacokinetics in the investigated dose ranges has been confirmed.

Keywords: Acyclovir; Determination in human plasma; HPLC with spectrofluorimetric detection; Pharmacokinetics study of suspension forms

1. Introduction

Acyclovir, a synthetic purine nucleoside derived from guanine, demonstrates strong and selective activity against herpes simplex and varicella zoster virus. Several methods for the assay of acyclovir in serum or plasma have been described including radioimmunoassay (RIA) [1,2], enzyme-linked immunosorbent assay [3] and high performance liquid chromatography (HPLC) [4–9].

Although immunological techniques appear, in general, more sensitive, they have number of significant disadvanages. These including the length of time ($\sim$24 h) to obtain final quantitative results, the large number of steps in procedure, and the need to develop antiserum [1,2] and/or monoclonal antibodies [3]

Therefore, the HPLC technique has been widely used, particularly in pharmacokinetic investigations as well as for therapeutic drug monitoring purposes. Nevertheless, commonly published HPLC methods determine acyclovir with quantita-

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tive limit of concentration 100 ng ml^{-1} [5–7]. Hence, these methods are not capable to determine acyclovir in plasma with sufficient sensitivity after administration of a single standard dose of acyclovir in the pharmacokinetic investigation.

More recently, two reversed phase HPLC methods with fluorescence detection have been published, which have a detection limit of 10 ng ml^{-1} [4] and 30 ng ml^{-1} [9], respectively. The first of these methods employed a strong acidic mobile phase, that could lead to the rapid deterioration of the stationary phase, and hence the shortening of the column life. In the second method, the mobile phase was modified with 0.02 M of disodium hydrogen orthophosphate and 60% perchloric acid in order to achieve pH 2.5.

The pharmacokinetics of acyclovir after ingestion of either tablet or solution is well documented [10–12]. However, there is lack of pharmacokinetic data after ingestion of suspension. In the present paper, we describe a simple, sensitive and highly selective HPLC method which, by its greater speed, appears to be of particular interest for pharmacokinetic studies of various forms of the drug. Moreover, using this method, pharmacokinetics of acyclovir on healthy volunteers after administration of two different doses of suspension have been investigated.

2. Experimental

2.1. Apparatus and reagents

The HPLC system used was a Kontron Instruments (Zurich, Switzerland) chromatograph, equipped with a computer system for aquisition and integration of the data (D-450, v. 3.3). The apparatus were consisted of a solvent pump (model 420), spectrofluorimetric detector (model SFM 25) and column Supelcosil™ LC 18 DB (Supelco, Bellefonte) 7.5 × 4.6 mm i.d., 3 μm particles. As the mobile phase a compsition of acetonitrile/glycine buffer 100 mM, pH 2.3 (3:97 v/v) was used. Measurements were carried out at a flow rate of 1.0 ml min^{-1} in ambient laboratory temperature.

Acyclovir was kindly supplied by Rosemont Pharmaceutials, (Leeds, UK) guanosine, internal standard was from Sigma (St. Louis, MO), acetonitril for chromatography was purchased from Merck (Darmstad, Germany) and perchloric acid, pure p.a. grade was obtained from Fluka (Buchs, Switzerland). Doubly distilled water was used throughout. Other reagents and solvents were pure p.a grade.

2.2. Assay procedure

To 0.5 ml of plasma, 10 μl of the internal standard solution (100 μg ml^{-1} guanosine) were added. The sample was vortexed for 0.5 min., and then subsequently deproteinized by the addition of 12% perchloric acid. The mixture was shaken on vortex for 1 min and was then kept in ambient temperature for 10 min. After centrifugation, the supernatant was transferred to another tube, and aliquot of 20 μl was injected into chromatographic column. Excitation and emission wevelenght were set at 260 and 375 nm, respectively. Peak height ratios of the compound compared to the internal standard were taken. Using the slope and intercept calculated by linear regression analysis of the calibration curve data, made on each day of analysis, the concentration of acyclovir was calculated.

2.3. Subjects and procedure

The pharmacokinetic study was carried out crossover, on the group of 24 healthy volunteers, who were taking no concurrent medications. All gave prior informed written consent. After fasting overnight, each volunteer was administered orally with 200 or 400 mg of acyclovir in a form of suspension (200 mg per 5 ml and 400 mg per 5 ml Zovirax Glaxo–Wellcome). The first meal they had 4 h after drug administration. Blood samples were withdrawn into heparinised glas tubes prior to the dose and again at 0.25, 0.5, 0.75, 1.0, 1.5, 1.75, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0 and 24.0 h after drug ingestion. The samples were centrifugated immediately and the plasma harvested was stored at $-20°C$ prior to assay for acyclovir.

The pharmacokinetic parameters such as peak plasma concentration (C_{max}), time to peak (t_{max}), total area under the plasma concentration-time

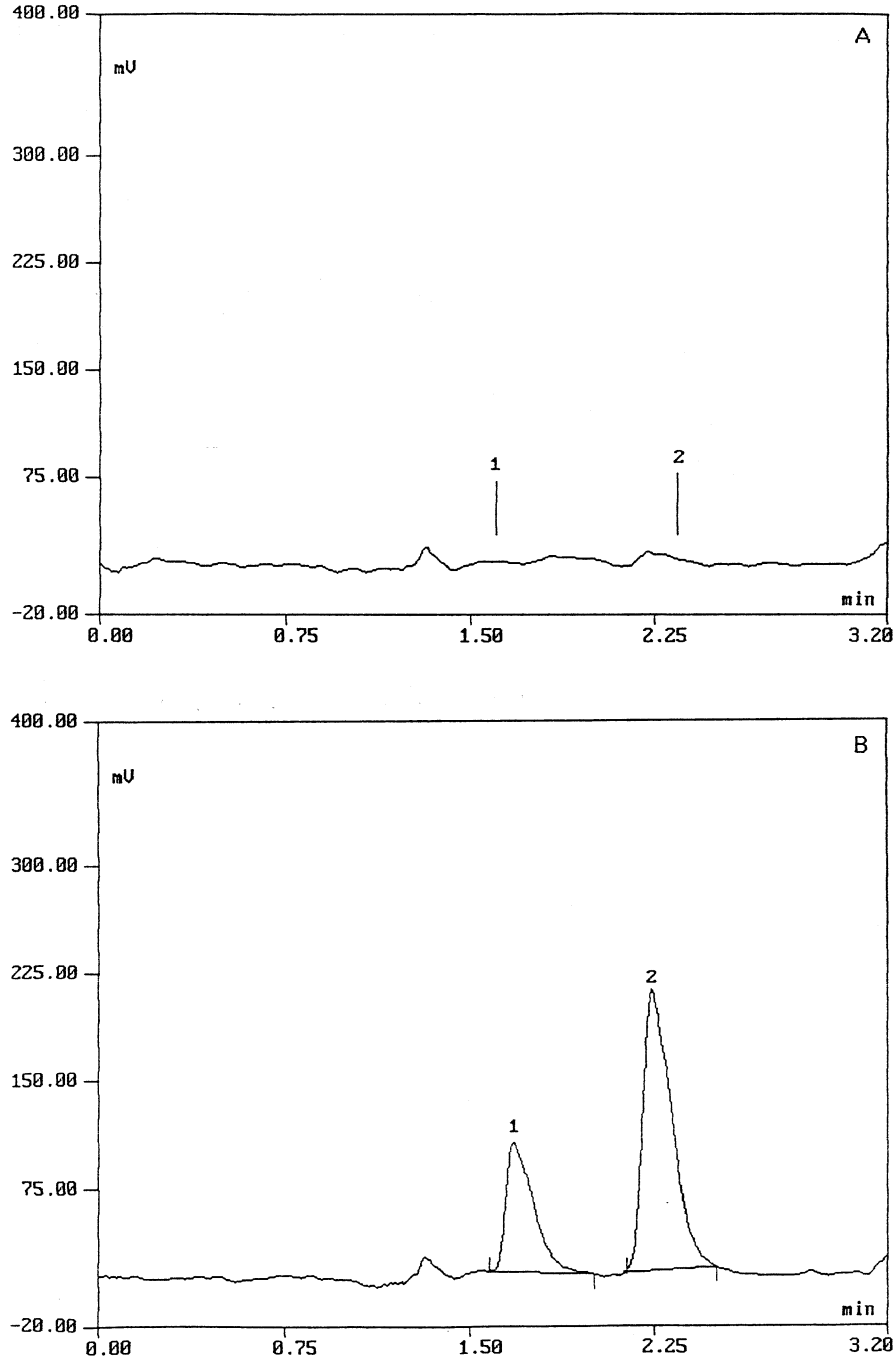

Fig. 1. Chromatograms of serum sample: drug-free (A), and spiked by 300 ng ml^{-1} of acyclovir and 1000 ng ml^{-1} of internal standard (B) (1—acyclovir, 2—internal standard).

Table 1
Limit of quantitation, precision and inaccuracy during one analytical run

Replicates/parameter	Nominal concentration (ng ml^{-1}) and peak height ratio (phr)							
	25	50	100	200	400	600	900	1200
	Back-calculated concentrations (ng ml^{-1})							
1	29.2[a]	54.8	112.9	191.5	418.3	640.4	969.8	1244.3
2	23.3[a]	58.7	106.0	174.5	497.7	525.6	833.0	1386.5
3	26.9[a]	53.3	99.3	175.1	374.9	595.6	935.4	1202.1
4	—	56.1	—	181.7	417.4	607.2	897.2	1263.0
5	22.5[a]	—	99.2	191.0	392.6	622.0	884.9	1184.7
6	29.7[a]	41.0	121.3	—	395.8	596.4	910.7	1241.1
Mean	26.32	52.78	107.74	182.76	416.12	597.87	905.17	1253.6
SD	3.31	6.88	9.46	8.25	43.19	39.26	46.49	71.26
CV (%)	12.6	13.0	8.8	4.5	10.4	6.6	5.1	5.7
Bias (%)	5.3	5.6	7.7	-8.6	4.0	−0.4	5.7	4.5

Reference calibration curve; $n = 8$; regression equation, linear; x-coefficient, 0.000235; intercept, 0.00452; correlation, 0.99596.
[a] Reference calibration curve; $n = 8$; regression equation, linear; x-coefficient, 0.000236; intercept, 0.00269; correlation, 0.99829.

curve (AUC), as well as elimination parameters such as elimination rate constant (K_{el}), half life ($t_{0.5}$) and total clearance (Cl), were obtained for each subject separately and then mean values were calculated.

2.4. Validation of analytical method

The concentrations used were based on the range expected during pharmacokinetic investigations. For the within-day precision and inaccuracy (bias), pools of plasma was spiked with acyclovir working standards to obtain concentrations of: 50, 100, 200, 400, 600, 900 and 1200 ng ml^{-1}. Between-day validation was calculated for three concentrations (100, 200, 400 ng ml^{-1}), corresponding to quality control samples of the method. The samples were preparated before experiment, stored in the same conditions as samples taken from volunteers, and were assessed over the current made calibration curve. The limit of quantitation was calculated from the six independent made replications at concentration 25 ng ml^{-1}. Specificity of the assay was determined on the basis of different plasma samples.

Recovery of the method was quantified at three different concentrations (100, 200, 600 ng ml^{-1}). The recovery of internal standard, in concentration used in experimental procedure, have also been carried out (Table 3). This was made comparing peak heights from plasma samples after analytical procedure at each concentration with the peak heights obtained from direct injected working standard on chromatography column. The same method was used to assess the recovery of internal standard. The stability of investigated compound, stored in three concentrations (200, 400, 600 ng ml^{-1}), after each of the three freeze-thaw cycles, during 1 month, have also been controled.

3. Results and discussion

Typical chromatograms of blank plasma (A) and plasma spiked with 300 ng ml^{-1} of acyclovir and 1000 ng ml^{-1} of internal standard are shown in Fig. 1. As can be seen, no interference was found in the region of investigated compounds.

The linearity of the method was confirmed with precision and inaccuracy below 15% over a range of 25–1200 ng ml^{-1}. These concentrations cover a whole range of bioanalysis parameters including C_{max}. The limit of quantitation, precision and inaccuracy during one analytical run are presented in Table 1. The limit of quantitation, defined as the lowest concentration level which is measured precisely and accurately with %CV and

Table 2
Precision and accuracy-between days

No	Parameters of calibration curves			Nominal concentrations (ng ml^{-1})					
	Correlation	Slope	Intercept	100 Back-calculated concentrations	%	200	%	400	%
1	0.9944	0.0016	0.0258	111.5	11.5	220.6	10.3	432.4	8.1
				91.3	−8.7	—		383.3	−4.2
2	0.9921	0.0017	0.1376	109.4	9.4	188.2	−5.9	441.4	10.4
				—		169.0	−15.5	—	
3	0.9931	0.0014	−0.0029	92.9	−7.1	181.0	−9.5	356.4	−10.9
				—		177.0	−11.5	402.5	0.6
4	0.9924	0.0015	0.0315	100.2	0.2	172.0	−14.0	419.0	4.8
				100.4	0.4	204.9	2.5	425.0	6.3
5	0.9928	0.0016	0.0263	92.1	−7.9	222.5	11.3	438.5	9.6
				77.3	−22.7	177.6	−11.2	444.5	11.1
6	0.9948	0.0014	0.0507	114.4	14.4	179.8	−10.1	397.5	0.6
				99.0	−1.0	204.5	2.3	381.1	−4.7
n				10		11		11	
Mean				98.85		190.65		411.05	
SD				11.15		19.22		29.01	
CV (%)				11.3		10.1		7.1	
Bias (%)					−1.2		−4.7		2.9

%bias less than 15% was 25 ng ml^{-1}. A detection limit of 10 ng ml^{-1} was obtained at a signal to noise ratio 3:1. Precision of the assay, calculated as a coefficient of variance for within-day variability, ranged from 4.5% for 200 ng ml^{-1} to 13.0% for 50 ng ml^{-1}. Inaccuracy of the method for within-day variability ranged from −0.4% for 600 ng ml^{-1} to −8.6% for 200 ng ml^{-1}. Table 2 lists precision and accuracy in between-day tests. As can be seen, precision ranged from 7.1% for 400 ng ml^{-1} to 11.3% for 100 ng ml^{-1}, while inaccuracy ranged from −1.2% for 100 ng ml^{-1} to −4.7% for 200 ng ml^{-1}. As it was shown in Table 3, the mean recoveries for three different concentrations of acyclovir (100, 200, 600 ng ml^{-1}) as well as the internal standard (1000 ng ml^{-1}), calculated in six replications, exceeded 80% in each case. Freeze-thaw stability tests, made three times during 1 month, in three different concentrations, confers stability of acyclovir during these operations.

The reported method was applied to human pharmacokinetic investigations. We have examined the single-dose pharmacokinetics of acyclovir given as a suspension in two doses (200 and 400 mg) on a 24 volunteer group, in crossover studies. Fig. 2 shows mean plasma concentration of the drug after administration of 200 and 400 mg of suspension. On the basis of determined concentrations, pharmacokinetic parameters of acyclovir have been calculated and listed in Table 4.

Table 3
Recovery of acyclovir from human plasma

Compound	Recovery (ng ml^{-1})			
	100	200	600	1000
Acyclovir	90.2 ± 14.8%	84.8 ± 6.9%	84.1 ± 5.2%	NC
Internal standard	NC	NC	NC	81.8 ± 6.6%

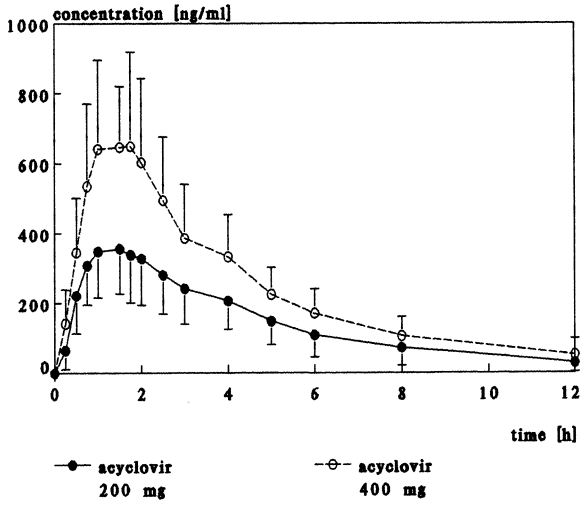

Fig. 2. Concentration-time curves of acyclovir after single oral dose of 200 mg and 400 mg drug suspension.

Acyclovir given in suspension form has been shown to be more rapidly absorbed than from tablets, reaching peak concentration after relatively short time ($t_{max\ 200} = 1.31 \pm 0.46$ h and $t_{max\ 400} = 134 \pm 0.39$ h). Double increase of the dose from 200 to 400 mg produce about two times increase of peak concentration ($C_{max\ 400}/C_{max\ 200} = 1.991$). However, the same increase of the acyclovir dose gave lower than two ratio of the area under the concentration–time curve ($AUC_{400}/AUC_{200} = 1.675$). Simultaneously, as it was shown in Table 4, lack of significant differences in parameters describing elimination of the drug from the body, i.e. elimination rate constant (K_{el}), half life ($t_{0.5}$) and total clearance (Cl) has been observed.

In order to compare the pharmacokinetics of acyclovir, given as a suspension in two doses, the calculations of C_{max} and AUC were standarized in relation to 1 mg of the dose. As it can be seen in Table 4, only the AUC value is slightly higher for 200 mg dose, but the difference is not statistically significant. Moreover, the value of C_{max} rises proportionally with the dose. Thus, it can be concluded, that the pharmacokinetics of acyclovir in dose range 200–400 mg is linear.

Table 4
Mean pharmacokinetic parameters and statistical analysis

Parameter	$D = 200$ mg	$D = 400$ mg	t-test
AUC (ng h ml^{-1})	1974 ± 956	3308 ± 1263	—
AUC/1 mg (ng h ml^{-1})	9.87 ± 4.78	8.00 ± 3.39	n.s.
C_{max} (ng ml^{-1})	420.8 ± 141.4	838.1 ± 259.2	—
C_{max}/1 mg (ng ml^{-1})	2.06 ± 0.73	2.10 ± 0.65	n.s.
t_{max} (h)	1.31 ± 0.46	1.34 ± 0.39	n.s.
K_{el} (h^{-1})	0.273 ± 0.138	0.259 ± 0.137	n.s.
$t_{0.5}$ (h)	3.33 ± 1.97	3.47 ± 1.99	n.s.
Cl/F (ml min^{-1})	127 ± 75	140 ± 55	n.s.

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