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Short communication

Determination of acyclovir in human plasma by high-performance liquid chromatography

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Abstract

A selective and sensitive isocratic high-performance liquid chromatographic method for the analysis of acyclovir in human plasma was described. Acid deproteinisation was used as sample treatment. Mean analytical recoveries were higher than 94% at low and high concentrations. The quantification limit was 0.1 mg/l for a plasma volume of 500 μl and precision study exhibits coefficients of variation lower than 5%. The method is suitable for therapeutic monitoring of acyclovir concentrations in organ-transplant recipients.

Keywords: Acyclovir

1. Introduction

Acyclovir, 9-(2-hydroxyethoxy)methyl guanine (Fig. 1), is a nucleoside analog with antiviral activity against herpes viruses [1]. This drug is an effective agent in the treatment of herpes virus infections [2] and may also used in the prophylaxis of cytomegalovirus infections in immunocompromised patients [3,4].

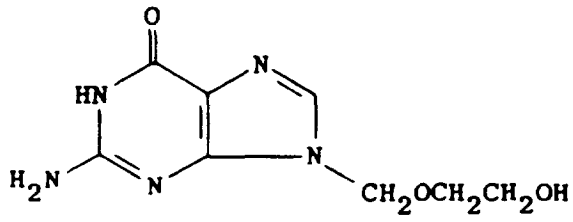

Fig. 1. Structure of acyclovir.

Some HPLC methods for the analysis of acyclovir in plasma, serum or urine has been published [5–10]. However, these analytical methods require the use of an ion-pairing agent [5–7], column thermostating [8,9] and fluorimetric detection [9] or are limited by the lack of sensitivity [10].

In this paper we report a sensitive, isocratic reversed-phase method for the determination of acyclovir in plasma samples from organ-transplant recipients receiving intravenous administration of acyclovir.

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2. Experimental

2.1. Reagents

Acyclovir was purchased from Welcome (Paris, France). Orthophosphoric acid, perchloric acid and potassium dihydrogenphosphate were supplied from Merck (Nogent-sur-Marne, France). Biorad drug-free serum was used for calibration (Biorad, Ivry-sur-Seine, France).

2.2. Apparatus and chromatographic conditions

A Model 420 pump equipped with a Model 430 variable-wavelength detector (Kontron, St. Quentin les Yvelines, France), a Model 712 Wisp sample processor (Waters, St. Quentin les Yvelines, France) and a D 2000 Chromato-integrator (Merck) was used. The column (150×4.6 mm I.D.) was packed with Hypersil ODS, 3 μm (Touzart et Matignon, Les Ulis, France). The isocratic mobile phase consisted of 0.02 mol/l potassium dihydrogenphosphate, pH 3.5. The flow-rate was 1.5 ml/min and the detector was set at 254 nm. All analyses were performed at ambient temperature.

2.3. Standards

A stock solution of acyclovir was prepared at a concentration of 25 mg/ml in water. This solution was diluted in water to obtain appropriate working standard solutions and was stable at −20°C for 1 month.

2.4. Sample collection and treatment

Blood samples (5 ml) were collected in heparinized tubes and centrifuged without delay at low temperature (4°C). Plasma samples were stored at −20°C until analysis. A 50-μl volume of 35% perchloric acid was added to 500 μl of plasma. After mixing, sample was centrifuged at 1500 *g* for 15 min at 4°C. Then 20 μl of the supernatant was injected onto the column.

2.5. Recovery and precision

Analytical recovery of acyclovir was determined by adding known concentrations of compounds to plasma and comparison of peak heights with those obtained by direct injection of standards.

Within-day, between-day precision and accuracy were determined by ten replicate analysis of drug free serum, spiked with known concentrations of acyclovir.

3. Results and discussion

A chromatogram of a drug-free serum spiked with acyclovir at a concentration of 5.0 mg/l is shown in Fig. 2. Acyclovir was eluted at an absolute retention time of 9.8 min and no interference with related endogenous compounds such as, uric acid, hypoxanthine, xanthine, guanine, guanosine or with commonly prescribed drugs in transplant recipients such as

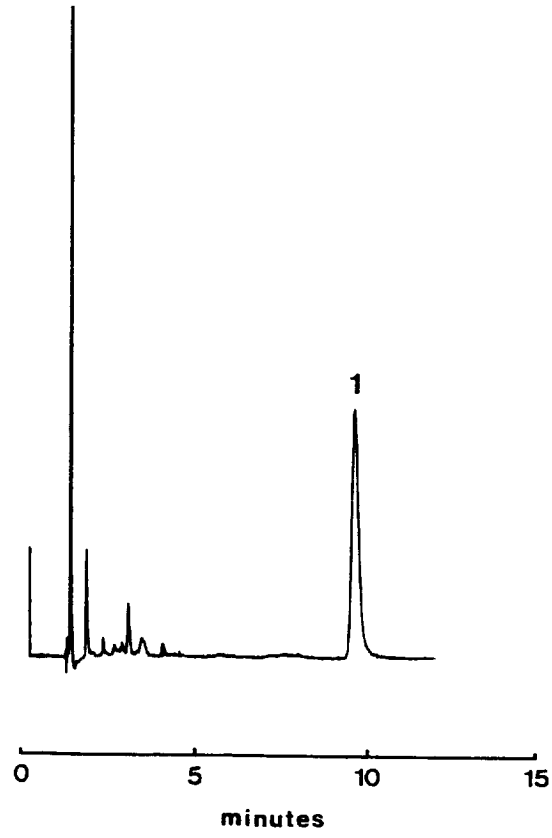

Fig. 2. Chromatogram of a plasma sample spiked with 5.0 mg/l of acyclovir. Chromatographic conditions are described in Section 2.2. Absorbance unit: 64 mV full scale.

azathioprine, ciclosporine, prednisone, dexamethasone, ganciclovir and some over-the-counter medications (furosemide, bumetamide, vancomycine, pristinamycine, salicylic acid, cimetidine, ranitidine, pyostacine, 6-mercaptopurine, phenobarbital) was found.

The relationship between the peak height and the concentration in the range 0.1 to 50 mg/l was linear up to 50 mg/l with correlation coefficient greater than 0.998. The regression equation was $y=5675x+0.007$. Mean analytical recovery (mean±S.D., $n=6$) from plasma was 96±6% and 94±4% at concentrations of 2.0 and 10.0 mg/l, respectively. The minimum detectable amount defined as a signal-to-noise ratio of 4 was found to be 2 ng and the quantification limit was 0.1 mg/l with a relative standard deviation (R.S.D.) less than 15% for a 500-μl sample volume. With regard to quantification limit of 0.01 mg/l (injection volume: 130 μl) reported in a previous study [6], the limit of the method described here can also be reduced to 0.02 mg/l by increasing the injection volume from 20 μl to 100 μl. However, low injection volume (20 μl) contribute to a long lifetime of the column: ca. 600 samples were injected into the column without any deterioration of its performance. Furthermore, a quantification limit of 0.1 mg/l is sufficient to perform pharmacokinetic studies as shown in Fig. 3. The within-day and between-day relative standard deviation were lower than 2.0% at a concentration of 10.0 mg/l and less than 6.0% at a concentration of 2.0 mg/l as shown in Table 1.

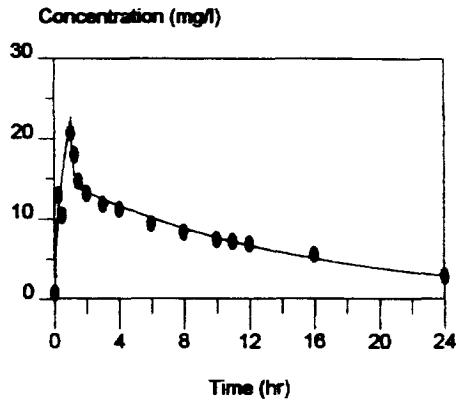

Fig. 3. Concentration–time profile of acyclovir from a heart-transplant patient following 1-h i.v. infusion of 10 mg/kg of acyclovir.

Table 1
Precision and accuracy

Concentration added (mg/l)	Concentration found (mg/l)	R.E. (%)	R.S.D. (%)
Within-day			
2.0	1.96	3.7	−2.0
10.0	10.03	0.9	0.3
Between-day			
2.0	2.01	4.2	0.5
10.0	10.04	1.2	0.4

R.S.D. is relative standard deviation, R.E. is relative error (concentration found−concentration added)×100/(concentration added).

The present method was used for monitoring acyclovir concentration in plasma samples from organ transplant recipients. Representative chromatograms of a blank plasma and a plasma sample from a heart-transplant patient receiving 10 mg/kg i.v. infusion over 1 h are shown in Fig. 4.

Peak plasma concentration of acyclovir was found to be 21 mg/l. Typical steady state C_{max} and C_{min} values after 1 h intravenous infusion of 10 mg/kg of acyclovir were 20 mg/l and 1 mg/l, respectively [11]. Acyclovir being predominantly eliminated by the kidneys, renal impairment affects plasma concentrations and rate of elimination. Acyclovir is generally well tolerated but nephrotoxicity and neurotoxicity have been reported following intravenous administration of acyclovir in patients with renal impairment [12,13]. These effects have been related to high peak plasma concentrations. With regard to multi-organ failure, especially severe renal failure which can occur after transplantation, monitoring of acyclovir concentrations may be recommended in transplant patients.

The method presented here is simple, precise and sensitive enough for the therapeutic monitoring and pharmacokinetic studies of acyclovir. Moreover, we should like to point out that this analytical method may be also used for analysis of another antiviral drug, ganciclovir [14] and represents a potential tool for drug monitoring during prophylactic antimicrobial regimens recommended in the care of organ transplant recipients [3].

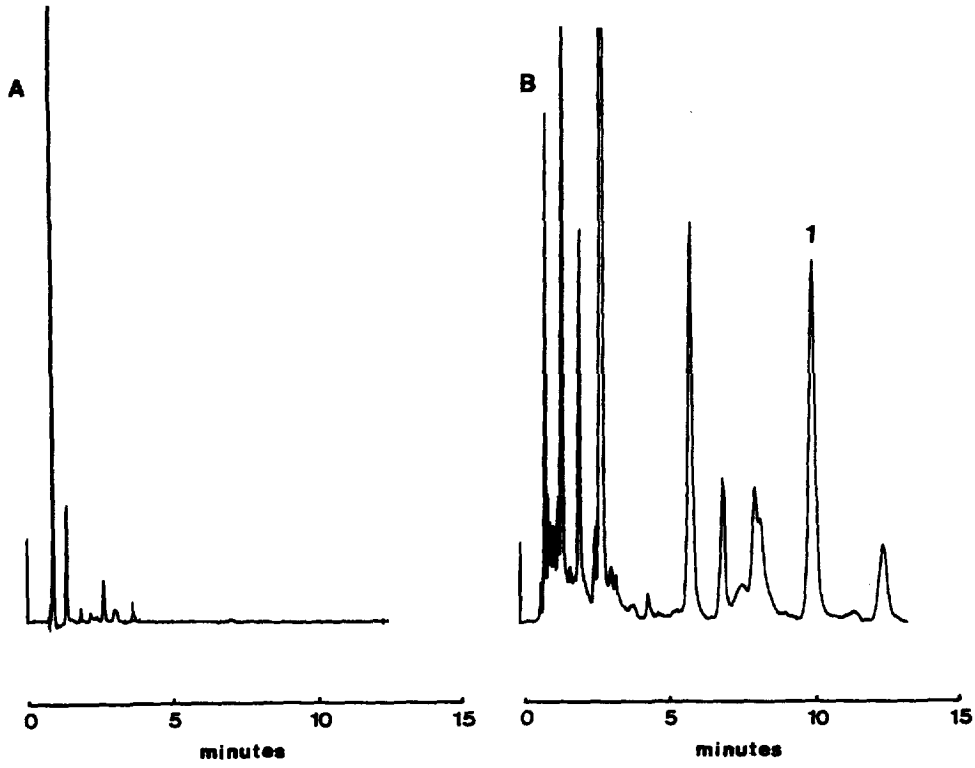

Fig. 4. Representative chromatograms of (A) blank plasma and (B) plasma sample collected at the end of a 10 mg/kg i.v. infusion over 1 h of acyclovir from a transplant patient: (1) acyclovir, 21.0 mg/l. Absorbance unit: 128 mV full scale.

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