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Liquid chromatographic method for the determination of ganciclovir and/or acyclovir in human plasma using pulsed amperometric detection

Satoshi Kishino[a], Yoh Takekuma[a], Mitsuru Sugawara[a], Tsuyoshi Shimamura[b], Hiroyuki Furukawa[b], Satoru Todo[b], Katsumi Miyazaki,[a,*]

[a]*Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060-8648, Japan*

[b]*First Department of Surgery, School of Medicine, Hokkaido University, Kita-15-jo, Nishi-7-chome, Kita-ku, Sapporo 060-8638, Japan*

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Abstract

We have developed a simple, rapid and highly sensitive method for determining plasma concentrations of ganciclovir and/or acyclovir by using reversed-phase chromatography followed by pulsed amperometric detection. A linear relationship between the amount of ganciclovir (0.05–10 μg/ml plasma) or acyclovir (0.1–20 μg/ml plasma) and peak height ratio was obtained. The relative standard deviations of all standard curves were greater than or equal to 0.999. The limits of detection for ganciclovir and acyclovir quantitation were 10 ng/ml and 50 ng/ml (signal/noise >3), respectively. Daily fluctuations of plasma standard curves (n=5) for the ganciclovir and acyclovir samples were small, with relative standard deviations (RSD) of 3.3 and 4.5% (n=5), respectively. The intra-assay precision for the ganciclovir and acyclovir samples were 6.9 (n=5) and 5.5% (n=5), respectively. Inter-assay precision of ganciclovir (n=3) and acyclovir (n=3) ranged from 2.6 to 6.8% and 3.5 to 5.0%, respectively. Using this method, the pharmacokinetics and removal of ganciclovir during continuous hemodiafiltration (CHDF) in a liver transplant recipient being treated for severe cytomegalovirus infection was investigated. The mean (±SD) ratio of ganciclovir concentrations at the inlet and outlet of the dialyzer (C_{outlet}/C_{inlet}) was 0.56±0.09. The areas under the curves of ganciclovir up to 12 h postdosing ($AUC_{0\rightarrow12}$) at the inlet and outlet of the dialyzer were 12.54 μg h/ml and 7.16 μg h/ml, respectively. The ultrafiltrate of ganciclovir was 16.6 mg. The terminal elimination half-life ($T_{1/2}$) of ganciclovir during CHDF was 3.6 h. These results demonstrate that CHDF effectively removes ganciclovir. Until formal guidelines have been established, ganciclovir or acyclovir dosage should be adjusted according to the results of monitoring of plasma drug concentration. The method described here is suitable for clinical monitoring of plasma ganciclovir or acyclovir levels in solid organ transplant recipients and for use in studies involving pharmacokinetics.

Keywords: Ganciclovin; Acyclovir

1. Introduction

Ganciclovir and acyclovir are potent antiviral drugs that have shown activity against herpes vi-

*Corresponding author. Tel.: +81-11-706-5680; fax: +81-11-756-1505.

E-mail address: katsumim@med.hokudai.ac.jp (K. Miyazaki,).

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ruses, including Epstein–Barr virus and cytomegalovirus [1,2]. Ganciclovir is an effective drug and should be considered for us as a first-line therapy in immunocompromised patients with cytomegalovirus infections [3–6]. Acyclovir is a nucleoside analog commonly used in the treatment of herpes virus infection [7,8].

Some high-performance liquid chromatographic (HPLC) methods for analysis of ganciclovir or acyclovir in plasma have been described. Campanero et al. [9] and Page et al. [10] reported that the limit of quantitation of plasma or serum ganciclovir using an HPLC method with UV detection was 50 ng/ml. On the other hand, Smith et al. [11] reported an HPLC method with UV detection for quantitation of acyclovir that has a minimum detection limit of 12 ng/ml. Such HPLC–UV methods have been used in pharmacokinetic studies on ganciclovir or acyclovir [12–15]. In solid organ transplant recipients, a wide variety of drugs were, however, administered. These HPLC–UV methods are often limited by one or more factors, such as potential interference from co-eluting matrix constituents or time-consuming extraction procedures. In order to overcome these problems, we have developed a simple, rapid and highly sensitive method for determining plasma or serum concentrations of ganciclovir and/or acyclovir by using reversed-phase chromatography followed by pulsed amperometric detection. Using this method, the pharmacokinetics and removal of ganciclovir during continuous hemodiafiltration (CHDF) in a liver transplant recipient being treated for severe cytomegalovirus infection was investigated. The plasma acyclovir concentrations in a liver transplant recipient during CHDF under therapy for prophylaxis of herpes virus infection were also measured using this method.

2. Experimental

2.1. Materials and sample preparation

Ganciclovir and acyclovir were purchased from Sigma (St. Louis, MO, USA). Potassium dihydrogenphosphate was purchased from Wako (Osaka, Japan). All other reagents were of the highest grade available commercially and were used without further purification.

The study was performed in the intensive care unit (ICU) of the Department of Medicine at Hokkaido University Hospital. Two living liver transplant recipients (one male and one female) were enrolled in this study. The Ethics Committee of Hokkaido University Hospital approved this study, and written consent was obtained from the two patients participating in the trial.

Solid-phase extractions (SPE) were performed with a novel polymeric reversed-phase sorbent, Sep-Pak C_{18} extraction cartridge (Waters). SPE cartridges were conditioned with 1 ml of methanol and 1 ml of distilled water. A plasma sample (0.5 ml) was loaded onto the column to remove interfering compounds and washed with 1 ml of distilled water. Ganciclovir and acyclovir were eluted from the sorbent with 1 ml of 10% methanol, and the solution was evaporated to dryness in vacuo. The residue was dissolved in mobile phase, and the solution was then injected into the HPLC system.

2.2. Calibration standards

Stock solution of ganciclovir (100 μg/ml) and acyclovir (100 μg/ml) were prepared by dissolving appropriate amounts in distilled water. The stock standards of ganciclovir and/or acyclovir were diluted with water, giving working solutions that ranged from 0.5 to 100 μg/ml. These working standards were further diluted in drug-free human plasma to give plasma concentrations of ganciclovir (0.05, 0.1, 0.5, 1, 5 and 10 μg/ml) and acyclovir (0.1, 0.5, 1, 5, 10 and 20 μg/ml). Stock and working solutions of ganciclovir and acyclovir were stable for at least 1 month without observable degradation when stored at −20 °C.

2.3. Analytical methods

Ganciclovir and acyclovir concentrations were determined as follows. An HPLC system (L-6200, Hitachi, Tokyo Japan) equipped with an HP1049A electrochemical detector (Hewlett-Packard, Waldbronn, Germany) was used, and a reversed-phase column (ERC-ODS-1161, 250 mm× 6.0 mm I.D.) was used. The column was heated to 55 °C. The

flow-rate was 1.0 ml/min and pressure was approximately 50 kg/cm^2. The mobile phase was 20 m*M* KH_2PO_4, and the reagent solution in the detection was 0.6 *M* NaOH. The peak was measured by pulsed amperometric detection using a gold working electrode and integrated with a Hitachi D-2500 data processor. The following pulse potentials and durations were used: $E_1 = 0.05$ V ($t_1 = 300$ ms); $E_2 = 0.65$ V ($t_2 = 100$ ms); $E_3 = -0.95$ V ($t_3 = 300$ ms). The response time was set to 8 s.

2.4. Hemodiafiltration technique

Vascular access was obtained by inserting a double-lumen catheter (10 Fr, Quintron®, Mahurkar, Bethel, USA) into a femoral vein. Continuous venovenous hemodiafiltration was performed using a cellulose triacetate hollow fiber dialyzer of 1.5 m^2 (FB-150F, Nipro, Osaka, Japan). The dialysate (Sublood-B®, FUSO, Osaka, Japan) flow-rate was held constant at 1000 ml/h, and blood flow was maintained at 100 ml/min. Ultrafiltrate was constantly obtained at 2000 ml/h and replaced as clinically indicated by Sublood-B in the postdilutional method.

2.5. Pharmacokinetic analysis

Ganciclovir was administered as an intravenous infusion over 1 h at a dose of 200 mg every 12 h. Arterial blood samples were taken from points located before and after the hemofilter. The blood was centrifuged and the plasma was separated. All samples were frozen at −30 °C until analysis. The pharmacokinetic parameters were calculated with a two-compartment open model of a constant infusion rate. The plasma concentration–time data were fitted to the following equation

$$C_1 = C_1^{eq} \times [1 - (K_{el} - \beta)/(\alpha - \beta) \times e^{-at} - (a - K_{el})/(\alpha - \beta) \times e^{-\beta t}$$

where

$$C_1^{eq} = K_0/V_1 \times K_{el}$$

These equations indicate that the concentration, C_1, approaches an asymptotic concentration, C_1^{eq}, which is equal to the infusion rate divided by the clearance, $V_1 \times K_{el}$.

Pharmacokinetic analysis was performed using the nonlinear least-squares regression analysis program (MULTI) [16]. The elimination half-life ($t_{1/2}$) was determined from the terminal log–linear phase of the plasma concentration–time curve.

The clearance of ganciclovir was calculated as follows:

$$CL = Q_B \times (1 - Hct) \times (C_{in} - C_{out})/C_{in}$$

where CL is clearance, Q_B is blood flow-rate, Hct is hematocrit, C_{in} is plasma concentration before the hemofilter, and C_{out} is plasma concentration after the hemofilter. The area under the curve (AUC) was calculated from the start of drug administration to the time of the last measurable blood concentration using the trapezoidal rule.

3. Results and discussion

3.1. Analysis of ganciclovir and acyclovir

Fig. 1 shows typical chromatograms of ganciclovir and acyclovir obtained under the above-mentioned conditions. An assay performed on drug-free human plasma showed the absence of an interfering peak at the retention times of ganciclovir (6.26 min) and acyclovir (8.24 min) (see Fig. 1B). A representative chromatogram of a blank plasma sample spiked with ganciclovir (2.5 μg/ml) and acyclovir (1.0 μg/ml) is presented in Fig. 1C. A plasma sample obtained from a patient administered ganciclovir is shown in Fig. 1D (acyclovir as an internal standard). Ganciclovir concentration was determined with the addition of acyclovir as an internal standard. On the other hand, when acyclovir concentration was measured, ganciclovir was used as the internal standard.

Calibration curves were determined by least-squares linear regression analysis. A linear relationship between the amount of ganciclovir (0.05–10 μg/ml plasma) or acyclovir (0.1–20 μg/ml plasma) and peak height ratio was obtained. The RSD values of all standard curves were greater than or equal to 0.999. The limits of detection for ganciclovir and acyclovir quantitation were 10 ng/ml and 50 ng/ml (signal/noise>3), respectively. The daily fluctuation

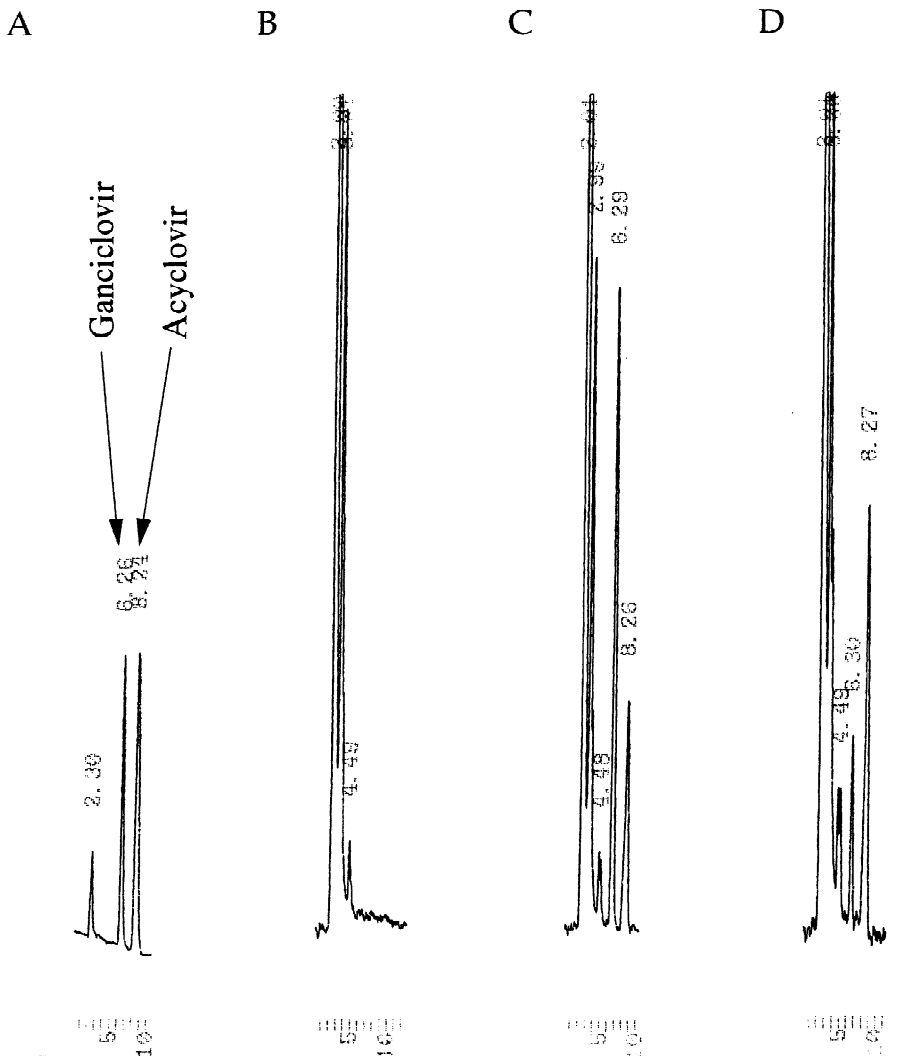

Fig. 1. Representative chromatograms of ganciclovir and acyclovir after treatment of standard and plasma samples. (A) Standard sample containing ganciclovir and acyclovir, (B) blank plasma, (C) human plasma with 2.5 μg/ml gangiclovir and 1.0 μg/ml acyclovir, (D) liver transplant patient being treated with ganciclovir.

in plasma standard curves ($n=5$) was small, with a relative standard deviation (RSD) of 3.3%. The intra-assay precision for the ganciclovir and acyclovir samples were 6.9% ($n=5$) and 5.5% ($n=5$), respectively. Inter-assay precision was evaluated by comparing the data obtained on three validation days. Inter-assay precision of ganciclovir ($n=3$) and acyclovir ($n=3$) ranged from 2.6 to 6.8% and 3.5 to 5.0%, respectively. Recoveries of ganciclovir and acyclovir from spiked samples were determined by comparing the peak areas of plasma obtained from freshly prepared sample extracts at low, medium and high concentration levels. The recoveries of ganciclovir and acyclovir were found to be $>88\%$ ($n=3$) and $>90\%$ ($n=3$), respectively.

The procedure described was developed for therapeutic monitoring of ganciclovir or acyclovir in solid organ transplant recipients who are simultaneously being administered a wide variety of drugs, such as predonisone, fluconazole, vancomycin, amikacin,

acetoaminophen, omeprazole, tacrolimus. Under these chromatographic conditions, no endogenous sources of interference were observed in plasma. On the other hand, several ID_{50} (dose of antimicrobial agent required to inhibit growth of 50% of specific isolates) values of ganciclovir have been reported in the literature for specific strains of cytomegalovirus, ranging from 0.2 to 2.75 μg/ml [17,18]. The trough acyclovir concentrations for prophylaxis of herpes virus infection have been reported to vary greatly between individuals (range 0.39–4.51 μg/ml) [19,20]. Both the sensitivity and the precision of the method are good, and no interfering peaks are seen in plasma. This assay has provided a simple and reliable liquid chromatographic method for the determination of trough ganciclovir and/or acyclovir levels. The method described is suitable for clinical monitoring of plasma ganciclovir or acyclovir levels in solid organ transplant recipients and for use in studies involving pharmacokinetics.

3.2. Pharmacokinetic profile of ganciclovir or acyclovir in a liver transplant recipient during continuous hemodiafiltration

Plasma concentration–time profiles of ganciclovir in patient (female, 54 years old, primary biliary cirrhosis) and acyclovir in patient (male, 35 years old, primary sclerosing cirrhosis) at the inlet and outlet of the dialyzer during CHDF are shown in Figs. 2 and 3, respectively. The ganciclovir concentration peaked at 3.32 μg/ml at 1 h after the start

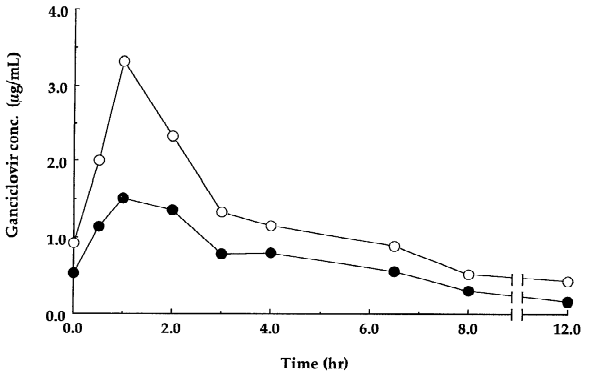

Fig. 2. Plasma concentration–time profiles of gancoclovir in patient at the inlet (○) and outlet (●) of the dialyser during continuous hemodiafiltration (CHDF).

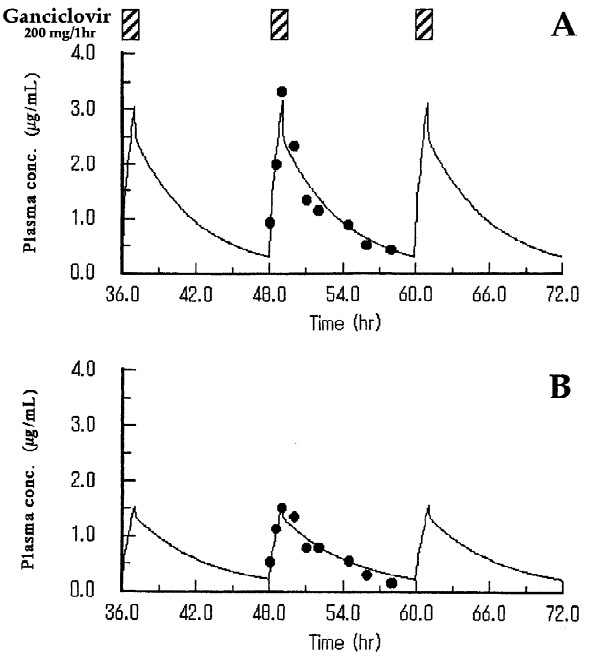

Fig. 3. Plasma concentration–time profiles of acyclovir in patient at the inlet (○) and outlet (●) of the dialyser during continuous hemodiafiltration (CHDF).

of the infusion. Trough concentrations immediately before and at 12 h after the start of drug administration during CHDF were 0.93 and 0.43 μg/ml, respectively. The mean (±SD) ratio of ganciclovir concentrations at the inlet and outlet of the dialyzer (C_{outlet}/C_{inlet}) was 0.56±0.09. The areas under the curves of ganciclovir up to 12 h postdosing ($AUC_{0\rightarrow12}$) at the inlet and outlet of the dialyzer were 12.54 μg h/ml and 7.16 μg h/ml, respectively. The ultrafiltrate of ganciclovir was 16.6 mg. The mean clearance was 26.61±5.51 ml/min. The concentration–time profiles at the inlet and outlet of the dialyzer and simulation curves and values of pharmacokinetic parameters are shown in Fig. 4 and Table 1, respectively. The terminal elimination half-life ($t_{1/2}$) of ganciclovir during CHDF was 3.6 h. These results demonstrate that CHDF effectively removes ganciclovir, which can be expected given its low molecular mass (255), water solubility (3 mg/ml) and low protein binding (average of unbound drug: 99%).

Cytomegalovirus infection occurs in immunocompromised hosts, such as organ transplant patients and

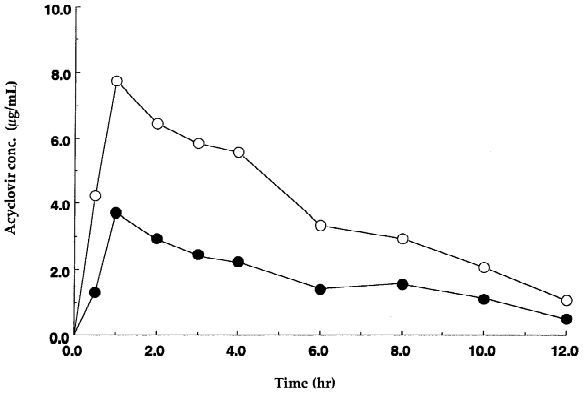

Fig. 4. Simulation curves and plasma ganciclovir concentrations at the inlet (A) and outlet (B) of the dialyser during continuous hemodiafiltration (CHDF).

patients with acquired immunodeficiency syndrome, and in critically ill patients [21]. Cytomegalovirus infections are a major cause of mortality in organ transplant recipients [22]. Ganciclovir, the first effective antiviral drug for treatment of cytomegalovirus diseases in humans, is frequently used to treat cytomegalovirus infections in critically ill patients [23]. On the other hand, acyclovir is commonly used in the treatment of herpes virus infection [7,8]. It is also used in the prophylaxis of cytomegalovirus infections in immunocompromised patients [24]. In critically ill patients, CHDF has been proved to be a convenient extracorporeal technique to manage renal failure and subsequent fluid overload. However, little is known about the influence of CHDF on drug removal and pharmacokinetics. Therefore, the results of this study should be useful for optimizing drug therapy in patients on CHDF. Until formal guidelines have been established, ganciclovir or acyclovir dosage should be adjusted according to the results of monitoring of plasma drug concentration.

Table 1
Pharmacokinetic parameters of ganciclovir and acyclovir at the inlet and outlet of the dialyzer during continuous hemodiafiltration (CHDF)

Pharmacokinetic parameters		Ganciclovir		Acyclovir	
		Inlet	Outlet	Inlet	Outlet
K_{12}	(1/h)	23.16	30.94	0.87	0.16
K_{21}	(1/h)	3.39	4.65	11.46	0.85
V_c	(l)	9.02	19.16	22.11	54.53
V_{ss}	(l)	73.38	146.77	23.79	64.79
K_{el}	(1/h)	1.65	1.36	0.16	0.15
$t_{1/2}$	(h)	3.60	4.11	4.62	5.71

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