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Pharmacokinetic studies and LC-MS/MS method development of ganciclovir and dipeptide monoester prodrugs in sprague dawley rats

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Abstract

Ganciclovir (GCV) is utilized as an anti-herpetic agent. Reports from our laboratory have suggested that dipeptide ester prodrugs of GCV exhibit high affinity towards the oligopeptide transporter hPEPT1 and therefore seem to be promising candidates for the treatment of oral herpes virus infections. In this study, we have examined the bio-availability of a dipeptide prodrug of GCV after oral administration in jugular cannulated Sprague-Dawley rats. A new bio-analytical method was developed with Q-TRAP liquid chromatography tandem mass spectroscopy (LC-MS/MS) for simultaneous analysis of GCV, Valine-GCV (VGCV) and Tyrosine-Valine-GCV (YVGCV). Acyclovir (ACV) was used as an internal standard in the analysis. Area under plasma-concentration (AUC) time curves for total concentration of GCV after oral administration of YVGCV was found to be approximately 200% more than that of GCV following intestinal absorption. A complete conversion of the dipeptide prodrug (YVGCV) to parent compound, GCV, by hepatic first pass metabolism was evident due to the absence of intermediate metabolite VGCV and administered prodrug YVGCV. The dipeptide prodrugs of GCV exhibits higher systemic availability of regenerated GCV upon oral administration and thus seem to be promising drug candidate in the treatment of systemic herpes infections.

Keywords

Ganciclovir- dipeptide prodrugs; herpes; peptide transporter; oral dose; pharmacokinetics; method; LC-MS/MS

Introduction

Acyclovir (ACV) and Ganciclovir (GCV) are indicated in the treatment of primary and recurrent genital Herpes simplex virus (HSV) and other viral infections (Razonable 2011). ACV is a guanosine analogue approved by US Food and Drug Administration (FDA) for varicella zoster virus (VZV) treatment (Balfour et al. 1990; Wallace et al. 1992). It has a

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moderate bio-availability (< 30%) after oral administration. Intravenous (IV) administration demonstrated high tissue and fluid penetration. Topical ACV may be used for genital HSV infection but oral formulation is commonly recommended (Luby et al. 1984). It is cleared from systemic circulation mainly by glomerular filtration and tubular secretion. For severe cases such as HSV encephalitis, intravenous (IV) ACV administration is the first line of treatment which may cause intratubular precipitation of ACV crystals leading to reversible nephrotoxicity (De Clercq 1993; Bean and Aeppli 1985). Neurological symptoms such as delirium and seizures may occur in elderly patients with renal disorders (Perazella 1999; Hellden et al. 2006; Ernst and Franey 1998; Wade and Meyers 1983). Oral bio-availability of ACV was improved to ~54% with L-valyl ester prodrug of ACV (Valine-ACV or VACV) (Acosta and Fletcher 1997). After oral administration, VACV is almost completely hydrolyzed to ACV by first pass intestinal and hepatic metabolism. Serum ACV concentrations reach maximum in 1 to 3 h and serum levels were found to be much higher with VACV relative to ACV (Acosta and Fletcher 1997). Both, ACV and VACV act by similar mechanism where they are converted to ACV-triphosphate *via* catalysis by viral thymidine kinase (TK). ACV-triphosphate is incorporated into viral DNA chain which results in chain termination and prevents viral replication (Gnann et al. 1983).

GCV is an acyclic 2'-deoxyguanosine (dGuo) analogue available in oral and parenteral formulations and indicated in the treatment of herpes simplex virus (HSV) infections (Martin et al. 1983; Smee et al. 1983). In vitro, GCV demonstrated to possess greater (~10 times) or nearly equal potency than ACV against cytomegalovirus, Epstein-Barr virus and HSV-1, HSV-2 and VZV (Karl B. Frank 1984). Oral GCV administration demonstrated poor bio-availability, ~5% (Nichols and Boeckh 2000). In cells infected with HSV, viral thymidine kinase phosphorylates GCV to the corresponding monophosphate (MP) i.e., ganciclovir monophosphate (GCV-MP) (Cheng et al. 1983). Cellular kinases then convert GCV-MP into the triphosphate (TP) (GCV-TP), which competitively inhibits DNA polymerase-catalyzed incorporation of dGuo-TP into DNA (Cheng et al. 1984). GCV-TP is incorporated into the end of a growing chain of viral DNA with simultaneous loss of pyrophosphate (Hamzeh et al. 1990; Hamzeh and Lietman 1991). GCV does not appear to serve as a chain terminator but is incorporated internally into DNA strands (Cheng et al. 1983). This mechanism causes a decrease in the rate of DNA synthesis and thereby disrupting viral replication (Cheng et al. 1984). The specificity of GCV renders it as an excellent substrate for viral kinases, whereas it is a poor substrate for endogenous mammalian kinases (Crumpacker 1996; Freeman and Gardiner 1996). The ability of GCV-TP to inhibit replication has stimulated a suicide gene therapy approach for the treatment of human tumors (Black et al. 1996; Freeman and Gardiner 1996; Moolten and Wells 1990; Smythe et al. 1995; Vile and Hart 1993). Transfection of herpes simplex virus thymidine kinase (HSVtk) gene into mammalian cells results in the efficient conversion of GCV to GCV-MP. As with virally infected cells, subsequent conversion to GCV-TP then provides a substrate to compete with dGuo-TP for incorporation into elongating DNA. Lower rate of DNA synthesis and subsequent incorporation of GCV-TP into DNA can inhibit host cell DNA replication. A critical component of this approach involves the ability to rapidly and specifically monitor plasma levels of GCV so that appropriate pharmacokinetic models can be constructed and optimal dosing strategies can be developed. Unlike ACV, GCV exhibits

excellent antiviral activity against the herpes family of viruses. It is the drug of choice for systemic and ocular human cytomegalovirus (HCMV) infections and HSV-1. Even though GCV has an advantage regarding antiviral activity, the molecule shows low permeability across various physiological barriers, including blood aqueous and blood retinal barriers, and intestinal epithelial cells. It also exhibits relatively low aqueous solubility.

A few attempts have been made in our laboratory to identify derivatives of GCV, especially for topical drug delivery to the eye. Lipophilic acyl ester prodrug strategy was utilized to improve corneal permeation (Tiruchera et al. 2002). Even though permeability across the cornea was improved, aqueous solubility of the compounds diminished. Similarly, dipeptide prodrugs of GCV were synthesized to treat various HSV ocular infections (Majumdar et al. 2005). Recently, nutrient transporters are being targeted for efficient drug delivery across cell membranes. Previously published reports from our laboratory have delineated such strategies (Majumdar et al. 2005; Cholkar et al. 2013). Peptide transporters (PEPT1, PEPT2), amino acid ($B^{0,+}$, $b^{0,+}$ and LAT), folate and other vitamin transporters as well as transferrin and insulin receptors are excellent targets for drug delivery. Peptide transporters (PEPT1 and PEPT2) have so far been the most widely utilized drug delivery targets. Several drugs including cephalosporins, VACV and VGCV are substrates of peptide transporters (Majumdar et al. 2005; Anand and Mitra 2002; Atluri et al. 2004). Oral bio-availabilities of valine linked antiviral agents have improved significantly (Wiltshire et al. 2005; Acosta and Fletcher 1997). Valine ester prodrugs, after oral administration, were broken down into their parent drugs by enzymes such as peptidases and esterases present in intestinal mucosa. Our earlier reports have shown that hydrophilic GCV is not a substrate for nucleoside/nucleobase transporters present on the cornea and its permeation across the rabbit cornea occurs primarily by passive diffusion (Majumdar et al. 2003). Peptide derivatives of hydrophilic molecules, such as GCV, may enhance both aqueous solubility and PEPT aided permeation across the cornea.

Our earlier work has demonstrated the utilization of peptide transporter for transcorneal delivery (Majumdar et al. 2005). In these studies, dipeptide monoester prodrugs of GCV were synthesized and their superior aqueous solubility and stability are described. Inhibition studies of radio labeled glycyl-sarcosine (gly-sar) and glycyl-proline (gly-pro) with these prodrugs demonstrated their affinity towards the peptide transporter on the cornea. The derivatives also demonstrated higher antiviral efficacies *in vitro* and *in vivo*, against HSV-1, HSV-2, VZV and HCMV relative to parent drug. In this report we describe a strategy to deliver GCV in the form of dipeptide prodrugs to enhance oral bio-availability which in turn would be very effective against systemic and genital herpes infections.

In this study, liquid chromatography–coupled mass spectrometry (LC–MS/MS) has been applied extensively to measure drug concentrations in biological matrices due to its sensitivity, selectivity and precision. To the best of our knowledge, no LC-MS/MS method has been reported in literature for simultaneous measurement of drug concentrations of GCV and GCV peptide prodrugs. The aim of the present study is to develop a selective and reproducible LC-MS/MS method for the quantitative estimation of GCV and GCV peptide prodrugs in biological fluids. This method is successfully applied to determine

pharmacokinetic behavior of GCV and GCV peptide prodrugs in jugular vein cannulated Sprague Dawley rats following oral administration.

Methods and Materials

GCV and acyclovir (ACV) were purchased from Sigma Chemical Co (St. Louis, MO) while valine-GCV (VGCV) and Tyrosine-Valine-GCV (YVGCV) were synthesized in our laboratory (Majumdar et al. 2005). HPLC grade methanol, acetonitrile, diethyl ether, dichloromethane, isopropyl alcohol and analytical grade and formic acid were procured from Fisher Scientific (New Brunswick, NJ). Ultrapure water from MilliQ-system (Millipore, Molshecin France) was used in all experiments and analysis. All chemicals were of HPLC grade and used as received without further purification.

Preparation of standard stocks and spike solutions

GCV, VGCV, YVGCV and ACV stock solutions were prepared at 1 mg mL⁻¹ in methanol. Calibration standards 0.50 – 1000 ng mL⁻¹ for GCV, 10.0 – 500 ng mL⁻¹ for both VGCV and YVGCV were prepared in plasma by spiking 2% of respective calibration curve dilutions. Acyclovir is used as an internal standard (IS). Twenty five microliter of 10 µg mL⁻¹ IS was spiked in the standards and plasma samples (Table 1). All samples were stored at -80° C until further use.

Procedure for sample preparation and extraction

Rat plasma samples were thawed at room temperature and vortexed. Samples of 0.1 mL were transferred into 1.5 mL polypropylene micro centrifuge tubes using calibrated pipettes, followed by the addition of 25 µL of 10.0 µg mL⁻¹ freshly prepared IS solution to all samples, except for the blank. These solutions were vortexed for 30 seconds. Samples were extracted by the addition of 1000 µL of isopropyl alcohol – dichloromethane (40:60 v/v) and then vortexed for approximately 2 min. After centrifugation at 12,000 rpm at 4° C for 25 min, 850 µL of organic layer was transferred to a pre- labeled fresh 1.5 mL poly propylene micro centrifuge tube and evaporated in the Speed Vac® at 35° C for 60 min.

The residue was reconstituted with 100 µL of mobile phase, vortexed for 30 seconds and transferred into a pre-labeled HPLC autosampler vial with silanized inserts. Fifteen microliters of the resulting solution was injected into LC-MS/MS for analysis.

In vivo Studies with Sprague-Dawley Rats

GCV (10 mg/kg) and dipeptide prodrug (YVGCV, at an equivalent dose of 10 mg/kg of GCV) were orally administered in jugular cannulated rats. Animals were fasted overnight (12–18 h) with free access to water. Freshly prepared drug solutions in water were administered by oral gavage (0.8 mL). Blood samples (200 l) were collected from jugular veins at predetermined time intervals over a period of 8 h. Heparinized saline (200 l) was injected to maintain a fairly constant fluid volume. Plasma was immediately separated by centrifugation and then stored at °80° C until further analysis. Prior to the analysis, plasma samples were thawed at room temperature and extracted as described earlier.

Calculation of Pharmacokinetic Parameters

All relevant pharmacokinetic parameters were calculated with noncompartmental analyses of plasma concentration-time curves following oral administration of GCV and dipeptide prodrug. A pharmacokinetic software package Phoenix WinNonlin v6.1, (Pharsight, Mountain View, CA) was employed to calculate primary and secondary pharmacokinetic parameters. Maximum plasma concentrations (C_{max}) were obtained from the plasma concentration vs time curves and the area under the plasma concentration time curves (AUC_{last} and $AUC_{infinity}$) were determined by the linear-log linear trapezoidal method with extrapolation, in Phoenix WinNonlin. The slopes of the terminal phase of plasma profiles were estimated by log-linear regression and the terminal rate constant (λ) was derived from the slope. The terminal plasma half-lives were calculated from the equation $t_{1/2} = 0.693/\lambda$. Clearance (Cl/F) and mean residence time (MRT) were calculated as ratio of dose and $AUC_{infinity}$, and area under the first moment curve (AUMC)/ $AUC_{infinity}$, respectively. The total concentration parameters were calculated by adding the concentrations of the administered drug and the regenerated intermediates in terms of GCV.

Liquid-Chromatographic operating conditions

Chromatographic analysis was carried out on API 3200 triple quadrupole linear ion Qtrap mass spectrometer. Prominence Series of Shimadzu ultrafast liquid chromatographic (UFLC) system consists of Shimadzu 20 M Vp Binary pump, Shimadzu SIL 20 AM autosampler (Shimadzu Scientific Instruments, North America) with a reversed phase Xterra C8 column (50×4.6 mm i.d, $5\mu m$, Waters, USA). Isocratic mobile phase composed of 85 % v/v of acetonitrile in water containing 0.05 % v/v of formic acid was pumped at a rate of 0.2 mL min^{-1} .

Mass spectrometer operating conditions

MDS Sciex API 3200 Triple Quadrupole linear QTrap mass spectrometry (Applied Biosystems/MDS Sciex, Foster City, CA) system interfaced by turbo ionspray (TIS) with positive ion source in MRM mode was applied for detection. Ultra high pure nitrogen served as collision activated dissociation (CAD) at 4 psi. Nebulizer and turbo gas were optimized at 40 and 50 psi respectively. TIS source temperature was maintained at 2000°C , with source voltage and dwell time optimized at 5200V and 200 milliseconds, respectively. Mass dependent parameters were tuned and optimized for GCV, VGCV and YVGCV Parent and daughter ions obtained by direct infusion mode ($20 \mu\text{L min}^{-1}$) with built-in Harvard infusion syringe pump were optimized.

Statistical Analysis

All experiments were conducted at least in triplicate, and results are expressed as mean $\pm$ SD. Student's t-test was applied to detect statistical significance between the parameters of the prodrug and GCV, and $p < 0.05$ was considered to be statistically significant. Statistical comparisons between the parameters of the prodrug and drug were performed using the analysis of variance (SPSS for Windows, release 10.0.7; SPSS Inc., Chicago, IL).

Results

Mass spectrometer operation condition for GCV, VGCV and YVGCV

In order to optimize electrospray ionization condition for GCV, VGCV and YVGCV, full scan mass spectra were acquired in the positive ion mode. During a direct infusion experiment, the mass spectra for GCV, VGCV, YVGCV and the internal standard (ACV) revealed peaks at mass to charge ratio (m/z) 256.20, 355.40, 518.50 and 226.3, respectively, as protonated molecular ions $[M+H]^+$. The most stable abundant fragment ion observed in each product MS/MS spectrum was at m/z 152.3 for GCV, 338.3 for VGCV, 367.5 for YVGCV and 152.20 for IS. These spectra were achieved by optimizing the collision energies at 18.0 V, 30.0 V and 18.0 V respectively as shown in Figs 1 - 4. Quantitative determination was performed in MRM scan positive ion mode with the following mass transitions: 256.20→152.30 for GCV, 355.4→338.3 Val-GCV, 518.5→367.5 for Tyr-Val-GCV, and 226.20→152.20 for ACV (Fig 1- 4).

Precision and accuracy

Accuracy was determined by analyzing multiple calibration curve samples (0.5 – 1000.0 ng mL⁻¹) with methods presented in Table 1. Accuracy for GCV (90.8 – 107.0 %), VGCV (97.2 – 105.3 %) and YVGCV (85.2 – 112.4 %) was observed for calibration curve standards. Linearity of GCV, VGCV and YVGCV was obtained over the concentration range of 0.5 -1000 and 10.0 - 500.0 ng mL⁻¹ respectively, in rat plasma at pH=7.2 (Table 1). Linear regression, best fit $1/x^2$ “weighting” factor mean correlation coefficient (r) were calculated to be 0.9995, 0.9981 and 0.9895 for GCV, VGCV and YVGCV, respectively. The recovery for YVGCV, VGCV and GCV were, obtained 78-85%, 80-92% and 78-95%, respectively. Figure 5 shows the chromatogram for simultaneous determination of GCV, YVGCV, VGCV and IS. The recovery protocol and recovery calculations are described in our previous published articles (Earla et al. 2012; Earla et al. 2014).

Pharmacokinetic studies in rats

Plasma pharmacokinetics of GCV (Fig 6) and its prodrug YVGCV (Fig 7) were studied in male sprague-dawley rats. Experiments were conducted for 8 h after drug administration at a dosage of 10 mg/kg of GCV and its molar equivalent amount of YVGCV. Blood samples were collected, processed and analyzed with a LC-MS/MS spectrometry method developed in our laboratory. The YVGCV and its amino acid metabolite VGCV were not found in the blood at any sampling time point as expected. GCV dipeptide was found to be completely and rapidly metabolized by enzymes present in the plasma Fig 7. Ganciclovir was analyzed in both the control and test experiments (Fig 6 and 7). Comparison studies demonstrated YVGCV to generate better pharmacokinetic properties than GCV. Plasma mean total exposure (AUC_{last} and $AUC_{infinity}$) for regenerated GCV following the administration of YVGCV were found to be ~1.73 fold and ~2.34 fold higher respectively, relative to GCV. Plasma mean peak exposures (C_{max}) for regenerated GCV in YVGCV group was ~2.03 fold higher than control GCV group. Terminal elimination half-life ($t_{1/2}$), mean residence time (MRT), apparent volume of distribution(V_z) and clearance (Cl) for regenerated GCV, from YVGCV prodrug, was similar to GCV control group. Pharmacokinetic parameters

determined for GCV and YVGCV after oral administration to rats is presented in Tables 2 and 3.

Discussion and conclusions

Recovery of GCV and its monoester peptide prodrugs from rat plasma samples by liquid-liquid extraction procedure resulted in clean samples with consistent and high extraction efficiency. We have investigated various sample extraction methods, including protein precipitation and liquid-liquid extraction with different organic solvents (acetonitrile, methanol, isopropyl alcohol, perchloric acid, trichloro acetic acid, ethyl acetate, methyl (t)-butyl ether, dichloromethane, hexane and diethyl ether and their combinations at various ratios). Isopropyl alcohol-dichloromethane combination efficiently precipitated proteins and extracted analytes from the plasma. Though the sample extraction was efficient with acetonitrile and methanol, results were found to be inconsistent. Extraction of analytes with a mixture of isopropyl alcohol- methyl (t)-butyl ether also gave high recovery for VGCV and YVGCV but low recovery for GCV.

Extraction with a combination of isopropyl alcohol- dichloromethane (40: 60 v/v) further improved accuracy and reproducibility (precision). The recovery for GCV, VGCV and YVGCV were between 78% and 95%. The extraction efficiency for analytes in plasma are discussed in our previously published article (Earla et al. 2014). The precision and accuracy results indicate ruggedness and reproducibility of the method. Finally, a solution of isopropyl alcohol- dichloromethane (40: 60 v/v) was added for extraction of pharmacokinetic samples because of its consistency and ruggedness.

Although it is not yet possible to cure herpes virus infections, the management of genital herpes infections has improved considerably since the introduction of acyclo-guanosine based antiviral drugs in the early 1980s. The incidence of genital herpes infections caused by HSV-1 and 2 has increased significantly in the past 20 years (Fleming et al. 1997). Although genital herpes is self-limiting in healthy adults, the disease is painful and distressing, with severe psychosocial impact (Manne and Sandler 1984; Goldmeier et al. 1988). Acyclovir was the first effective antiviral drug approved for widespread use and is still extensively prescribed, particularly in the treatment of immuno-competent patients with genital HSV disease (Perry and Faulds 1996). Although ACV is a well-tolerated and effective antiviral agent, its bio-availability after oral administration is very low. As a result, up to five times dosing per day is often necessary for the management of genital HSV infections. VACV enhances the bio-availability of ACV due to the recognition of VACV by intestinal peptide transporter hPEPT1, which mediates transport across intestinal epithelium to blood. In this study, we have utilized hPEPT1 to increase the oral bio-availability of GCV intended to treat genital herpes infections with dipeptide prodrugs. In an earlier article we have reported the aqueous solubility, chemical and enzymatic stability of the prodrug. Dipeptide prodrugs have exhibited high aqueous solubility and chemical stability (Majumdar et al. 2005). In a similar trend, GCV prodrugs were converted to parent drug by esterases and peptidases present in the intestine and blood. An intermediate, VGCV, was not generated in blood indicating the complete breakdown of ester bond and regeneration of parent drug, GCV by esterases.

Mean peak exposures ($C_{\max}$) of regenerated GCV after oral administration of prodrug YVGCV was found to be approximately two times of GCV. Similarly, mean total exposures (AUC_{last} and AUC_{infinity}) of regenerated GCV following the administration of prodrug YVGCV were ~ 1.73 and ~ 2.03 times than GCV (Table 2 and 3). Terminal elimination rate constants and plasma half lives in both test and control rats were found to be very similar as expected, since the regenerated GCV will be eliminated by the same passive elimination process as the parent drug. The mean value of $T_{\max}$ for test rats after oral administration of YVGCV was found to be approximately half the mean value of control rats which indicates that the prodrug was rapidly absorbed into the blood compared to GCV. This rapid absorption is probably due to active transport across the intestinal barrier through PEPT. Our earlier studies suggested that peptide prodrugs of GCV were found to be substrates of PEPT on the corneal epithelium (Anand and Mitra 2002). YVGCV also has higher lipophilicity (Majumdar et al. 2005) compared to the relatively hydrophilic GCV, a factor that may be responsible for rapid absorption across the intestinal epithelium. Since the peptide prodrugs also exhibited higher aqueous solubility (Majumdar et al. 2005), these can also be given at higher doses compared to GCV. In conclusion, YVGCV exhibited better absorption and higher plasma concentration compared to GCV. This prodrug can be a substitute for GCV intended to treat both systemic and genital herpes infections, especially in immune compromised patients.

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Figure 1. LC-MS/MS spectrum of ganciclovir (GCV) and its respective ions produced by MRM method. The major ion of GCV was found to be 152.3

Figure 2. LC-MS/MS spectrum of Valine-ganciclovir (VGCV) and its respective ions produced by MRM method

Figure 3. LC-MS/MS spectrum of Tyrosine-Valine-ganciclovir (YVGCV) and its respective ions produced by MRM method

Figure 4. LC-MS/MS spectrum of acyclovir (ACV) and its respective ions produced by MRM method

Figure 5. Chromatogram showing different elution time for acyclovir (ACV), ganciclovir (GCV) and its prodrugs (valine-GCV (VGCV) and Tyrosine-Valine-GCV (YVGCV)) simultaneous

Figure 6.

Plasma drug log-concentration vs Time profile of ganciclovir (GCV) (Blue diamonds) after oral administration of 10 mg/kg dose of GCV in rats. Data points presented are in Mean $\pm$ S.D. Number of animals used, n=3

Figure 7.

Plasma drug log-concentration vs Time profile of regenerated ganciclovir (GCV) from YVGCV (Blue diamonds) after oral administration of an equivalent to 10 mg/kg of GCV in rats. Data points presented are in Mean $\pm$ S.D. Number of animals used, n=4

Table 1
GCV and its prodrug(s) calibration curve standards calculated value(s) and percentage accuracy(s)

Nominal Conc. (ng mL ⁻¹)	Calculated Conc. (ng mL ⁻¹)				Percentage accuracy	
	GCV	Val-GCV	Tyr-Val-GCV	GCV	Val-GCV	Tyr-Val-GCV
0.50	0.53			107.0		
1.00	1.00			99.6		
10.00	10.14	9.90	9.90	101.4	99.6	99.0
50.00	53.19	50.85	50.85	106.4	101.0	101.7
100.00	102.37	112.36	112.40	102.4	105.3	112.4
250.00	238.06	212.94	213.00	95.2	98.5	85.2
500.00	454.23	508.98	509.00	90.8	97.2	101.8
1000.00	948.94			94.9		

GCV: Ganciclovir; Val-GCV: Valine-ganciclovir prodrug; Tyr-Val-GCV: Tyrosine- valine-ganciclovir prodrug

LOQ for GCV: 0.50 ng mL⁻¹; Val-GCV: 10.0 ng mL⁻¹ and Tyr-Val-GCV: 10.0 ng mL⁻¹

ULOQ for GCV: 1000.00 ng mL⁻¹; Val-GCV: 500.0 ng mL⁻¹ and Tyr-Val-GCV: 500.0 ng mL⁻¹

Percentage of coefficient of variation (% CV): within 7.3 % for GCV; 11.2 for Val-GCV and 16.2 for Tyr-Val-GCV

Table 2

Plasma pharmacokinetic parameters of GCV after oral administration at a dose of 10 mg/kg. Values presented as Mean $\pm$ S.D.

pk parameters	Plasma GCV (Mean $\pm$ S.D.)
AUClast (min*ng mL ⁻¹)	31214 $\pm$ 4951
AUCinfinity (min*ng mL ⁻¹)	32988 $\pm$ 4839
Cmax (ng mL ⁻¹)	220 $\pm$ 110
Tmax (min)	65 $\pm$ 35
λ_z (min ⁻¹)	0.0065 $\pm$ 0.0011
t _{1/2} (min)	108 $\pm$ 19
Cl (mL min ⁻¹)	77 $\pm$ 11
Vz (mL)	12121 $\pm$ 3918
AUMC (min*min* ng mL ⁻¹)	26164881 $\pm$ 28635541
MRT (min)	137 $\pm$ 47

Table 3

Plasma pharmacokinetic parameters of GCV after oral administration of Tyr-Val-GCV equivalent to dose of 10 mg/kg of GCV. Values presented as Mean $\pm$ S.D.

pk parameters	Regenerated GCV in plasma (Mean $\pm$ S.D.)
AUClast (min*ng mL ⁻¹)	54115 $\pm$ 16747
AUCinfinity (min*ng mL ⁻¹)	77177 $\pm$ 2920
Cmax (ng mL ⁻¹)	448 $\pm$ 47
Tmax (min)	30 $\pm$ 14
λ_z (min)	0.0054 $\pm$ 0.0014
t _{1/2} (min)	133 $\pm$ 35
Cl (mL min ⁻¹)	79 $\pm$ 3
Vz (mL)	15017 $\pm$ 3419
AUMC (min*min*ng mL ⁻¹)	21943245 $\pm$ 7636512
MRT (min)	148 $\pm$ 20