Cancer Chemother Pharmacol (1999) 43: 415–418

ORIGINAL ARTICLE

Baruti M. Serabe · Daryl J. Murry · Robert Dauser
Jed Nuchtern · John Durfee · Leticia McGuffey
Stacey Berg · Susan M. Blaney

Plasma and CSF pharmacokinetics of ganciclovir in nonhuman primates

Received: 8 May 1998 / Accepted: 25 September 1998

Abstract *Purpose*: The antiviral nucleoside analogue ganciclovir is a potent inhibitor of replication in herpes viruses and is effective against cytomegalovirus infections in immunocompromised patients. Ganciclovir is also used in cancer gene therapy studies that utilize the herpes simplex virus thymidine kinase gene (HSV-TK). The pharmacokinetics of ganciclovir in adults and children have been described previously but there are no detailed studies of the CNS pharmacology of ganciclovir. We studied the pharmacokinetics of ganciclovir in plasma and CSF in a nonhuman primate model that is highly predictive of the CSF penetration of drugs in humans. *Methods*: Ganciclovir, 10 mg/kg i.v., was administered over 30 min to three animals. Ganciclovir concentrations in plasma and CSF were measured using reverse-phase HPLC. *Results*: Peak plasma ganciclovir concentrations ranged from 18.3 to 20.0 μg/ml and the mean plasma AUC was 1075 ± 202 μg/ml · min. Disappearance of ganciclovir from the plasma was biexponential with a distribution half-life ($t_{1/2}\alpha$) of 18 ± 7 min and an elimination half-life ($t_{1/2}\beta$) of 109 ± 7 min. Total body clearance (Cl_{TB}) was 9.4 ± 1.6 ml/min/kg. The mean CSF ganciclovir AUC was 168 ± 83 μg/ml · min and the mean peak CSF concentration was 0.7 ± 0.3 μg/ml. The ratio of the AUCs in CSF and plasma was 15.5 ± 7.1%. *Conclusions*: Ganciclovir penetrates into the CSF following i.v. administration. This finding will be useful in the design of gene therapy trials involving the HSV-TK gene followed by treatment with ganciclovir in CNS or leptomeningeal tumors.

Key words Plasma · CSF · Pharmacokinetics · Ganciclovir · Nonhuman primates

Supported in part by the Clayton Foundation for Research (S.B. and S.M.B.) and Grant 57745 to the Pediatric Oncology Group from CTEP-Division Cancer Treatment/National Cancer Institute USPHS

B.M. Serabe · D.J. Murry · L. McGuffey
S. Berg · S.M. Blaney (✉)
Texas Children's Cancer Center,
Baylor College of Medicine,
6621 Fannin Street,
Houston, TX 77030, USA
Tel.: +1-713-770-4588; Fax: +1-713-770-4039

R. Dauser
Department of Neurosurgery,
Baylor College of Medicine,
Houston, TX 77030, USA

J. Nuchtern
Department of Surgery,
Baylor College of Medicine,
Houston, TX 77030, USA

J. Durfee
Center for Comparative Medicine,
Baylor College of Medicine,
Houston, TX 77030, USA

Introduction

The nucleoside analogue ganciclovir (9-[1,3-dihydroxy-2-propoxy)methyl]-guanine; DHPG) is a potent inhibitor of replication in viruses of the herpes family and is effective against cytomegalovirus (CMV) infection in immunocompromised patients. In the past decade, ganciclovir has been incorporated into many gene therapy studies in which mammalian cells are transduced with the herpes virus thymidine kinase gene (HSV-TK) using retroviral or adenoviral vectors. Ganciclovir is phosphorylated by HSV-TK to form ganciclovir triphosphate which causes DNA termination and cell death in transduced but not in normal host cells. Central nervous system (CNS) tumors are a primary target site for many preclinical and clinical gene therapy strategies. In order to characterize the CNS penetration of ganciclovir, we studied the plasma and cerebrospinal fluid (CSF) pharmacokinetics of ganciclovir in a nonhuman primate model that is highly predictive of CSF penetration in humans [3].

Materials and methods

Drugs

Ganciclovir was purchased from Hoffman-La Roche (Nutley, N.J.) in 10-ml sterile vials, each containing ganciclovir sodium equivalent to 500 mg ganciclovir. Vials were reconstituted with 10 ml sterile water for injection. The appropriate dose of drug was further diluted in normal saline to a final concentration of 10 mg/ml or less prior to infusion via a peripheral or central venous catheter.

Animals

Three adult male rhesus monkeys (*Macaca mulatta*) ranging in weight from 8.5 to 10.4 kg were used in these pharmacokinetic studies. The animals were fed a Non-Human Primate Diet twice daily and group-housed in accordance with the Guide for the Care and Use of Laboratory Animals [4]. Blood samples were drawn from a catheter placed in either the femoral or the saphenous vein contralateral to the site of drug administration. CSF samples were drawn from a chronically indwelling Pudenz catheter attached to a subcutaneously implanted Ommaya reservoir [3]. The reservoir was pumped four times before and after each CSF sample collection to ensure adequate mixing with ventricular CSF.

Experiments

Three animals received an intravenous (i.v.) dose of ganciclovir 10 mg/kg, administered as a 30-min infusion. Blood was collected in heparinized tubes prior to administration, 15 min into the infusion, at the end of the infusion, and at 5, 15, and 30 min and at 1, 2, 4, 6, and 8 h following the completion of the infusion. CSF samples were collected from the Ommaya reservoir at the same time-points with the exception of the 8-h postinfusion sample. Blood samples were immediately centrifuged at 1500 rpm for 10 min. The plasma and CSF samples were stored at −80 °C until the day of analysis.

Sample analysis

Ganciclovir levels in plasma and CSF were measured using a modification of a recently described reverse-phase HPLC assay [6]. Acyclovir (Glaxo Wellcome, Research Triangle Park, N.C.), 5 μg/ml, was added to 500 μl plasma samples and 110 μl CSF as an internal standard. Plasma proteins were extracted by adding 500 μl 0.8 *M* perchloric acid (Aldrich Chemical Company, Milwaukee, Wis). After incubation on ice for 10 min the samples were centrifuged at 12000 *g* for 3 min. A 660-μl aliquot of the supernatant was mixed with 100 μl 2 *M* potassium phosphate buffer, pH 9.3 (Sigma Chemical Co., St. Louis, Mo), incubated on ice for 10 min, and then centrifuged at 12000 *g* for 3 min. The recovery of ganciclovir and acyclovir was 80 ± 9% and 91 ± 10%, respectively. Supernatant was transferred to an autosampler vial and maintained at 10 °C. CSF samples were directly injected onto the HPLC column. Standard curves in plasma and CSF were prepared for each experiment by the addition of known amounts of ganciclovir to plasma or mobile phase, respectively. Standard curves were linear ($r^2 > 0.995$) over the range 0.06 to 25 μg/ml ganciclovir. The lower limit of quantitation was 0.06 μg/ml.

The HPLC system consisted of a refrigerated autosampler, an automated gradient controller, and a programmable ultraviolet multiwavelength detector (Waters Corporation, Milford, Mass). The analytical column was a Beckman C-18 Ultrasphere ODS 5 μ, 4.6 mm × 25 cm (Rainin Instrument Co., Woburn, Mass). Ganciclovir and acyclovir were eluted isocratically with a mobile phase of 15 m*M* potassium phosphate, pH 2.88, with 1% acetonitrile at a flow rate of 1 ml/min for 12 min. The analytical column was washed with 100% acetonitrile for 5 min and then allowed to re-equilibrate with mobile phase for 10 min between samples. Ganciclovir and acyclovir were monitored by ultraviolet absorbance at 254 nm and had retention times of 11 and 14 min, respectively.

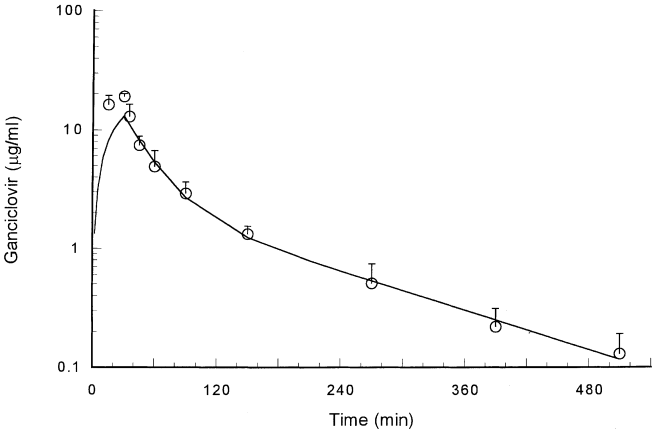

Fig. 1 Plasma concentration versus time profile of ganciclovir following i.v. ganciclovir 10 mg/kg administered over 30 min. Values are the geometric means from three animals (*bars* SD)

Pharmacokinetic analysis

Plasma concentration versus time data following i.v. administration of ganciclovir over 30 min were fited to mono- ($n = 1$) and biexponential ($n = 2$) equations:

$$C(t) = \sum_{i=1}^{n} A_i e^{-\lambda_i \cdot t}$$

using MLAB (Civilized Software, Bethesda, Md.), a nonlinear curve-fitting program, where C is the plasma concentration of ganciclovir at time t, A_i the intercept, and λ_i the rate constants. Akaike's information criterion [11] was used to determine which equation better fitted the data. All other pharmacokinetic parameters were calculated by using noncompartmental methods. The AUC was derived by the linear trapezoidal method and extrapolated to infinity using the terminal elimination rate constant [2]. The total body clearance was calculated by dividing the dose by the AUC. The volume of distribution at steady state (Vd_{ss}) was calculated using the area under the moment curve [8]. The CSF penetration was determined by dividing the AUCs in CSF (AUC^{CSF}) by the AUCs in plasma (AUC^{P}).

Results

Plasma and CSF pharmacokinetics

The disappearance of ganciclovir from the plasma following an i.v. infusion over 30 min (Fig. 1) was best described by a biexponential equation. Ganciclovir was rapidly eliminated from the plasma with a mean $t_{1/2}\alpha$ of 18 min (range 11 to 24 min) and a mean $t_{1/2}\ \beta$ of 109 min (range 103 to 116 min). The mean plasma AUC to infinity was 1112 ± 170 μg/ml · min (range 919 to 1236 μg/ml · min) and the mean plasma AUC to the last time-point was 1075 ± 163 μg/ml · min (range 893 to 1209 μg/ml · min). The mean plasma clearance was 9.4 ml/min per kg (range 8.1 to 11.2 ml/min per kg). The pharmacokinetic parameters for each animal are shown in Table 1.

Table 1 Pharmacokinetic parameters following administration of ganciclovir 10 mg/m^2 i.v. over 30 min in three animals

Animal	Cl_{TB} plasma (ml/min/kg)	Vd_{ss} (l/kg)	AUC plasma (μg/ml·min)	AUC CSF (μg/ml·min)	CSF:plasma[a]	$t_{1/2}$ (min)	
						α	β
JØ	8.9	13.6	1123	83.3	.073	11	103
L976	11.2	8.9	893	173	.192	24	109
J128	8.1	8.2	1209	249	.201	19	116
Mean ± SD	9.4 ± 1.6	10 ± 2.9	1075 ± 163	168 ± 83	.155 ± .071	18 ± 7	109 ± 7

[a] Ratio of the AUC $_{\rightarrow tlast}$ CSF to AUC $_{\rightarrow tlast}$ plasma

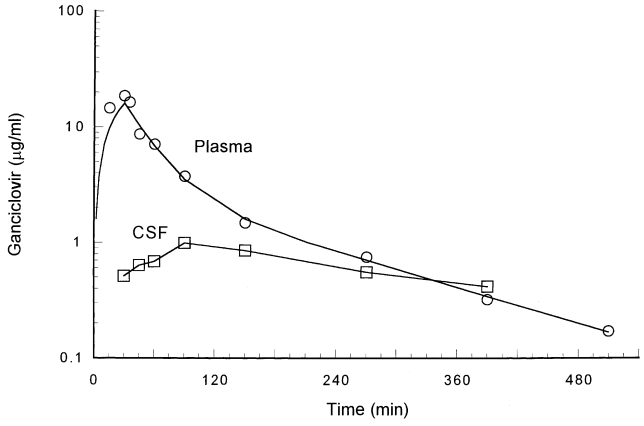

Fig. 2 Representative concentration-time curves from a single animal (animal J128) for ganciclovir in plasma and CSF following an i.v. dose of 10 mg/kg administered over 30 min

Ganciclovir was detected in the CSF after i.v. administration with a peak level occurring 30 min following completion of the infusion. The mean peak CSF level was 0.66 μg/ml (range 0.30 to 0.99 μg/ml). The mean CSF ganciclovir AUC was 168 μg/ml · min (range 83.3 to 249 μg/ml · min). The penetration of ganciclovir into the CSF, as represented by the ratio of the AUC^{CSF} to the AUC^{P}, was 15.5 ± 7.1%. A representative concentration versus time profile of ganciclovir in plasma and in CSF after an i.v. ganciclovir dose is shown in Fig. 2.

Toxicity

No hematological or other organ toxicity was observed following a single i.v. ganciclovir dose of 10 mg/kg.

Discussion

Our results show that the elimination from plasma in a nonhuman primate was biexponential with a clearance of 9.4 ml/min per kg (~184 ml/min per m^2) and a terminal half-life of 109 min following i.v. administration of a 10 mg/kg dose. In comparison, studies in adult humans after doses of 2.5 to 5 mg/kg have demonstrated that elimination is biexponential with a clearance of 4.2 ml/min per kg (~155 ml/min per m^2) [7] and a terminal half-life of 102 to 216 min [1, 7]. Thus, the plasma pharmacokinetic parameters of ganciclovir after i.v. administration are similar in nonhuman primates and humans.

There is limited data about the CSF penetration of ganciclovir in humans. Fletcher et al. measured CSF ganciclovir concentrations at a single time-point in three adult patients who were receiving an i.v. dose of 2.5 mg ganciclovir every 8 to 12 h. The CSF concentrations ranged from 0.5 to 0.62 μg/ml between 0.25 and 5.7 h following drug administration. These CSF concentrations were 24 to 67% of the concurrent model-predicted plasma concentrations [1]. However, comparison of concurrent CSF and plasma time-points often leads to inaccurate estimates of overall CSF drug exposure. For example, in our study CSF ganciclovir concentrations range from 3% to greater than 100% of the simultaneous plasma concentrations, reflecting the fact that the clearance of agents from the CSF may be slower than the corresponding plasma clearance. The overall CSF drug exposure relative to plasma exposure is better estimated by comparing the CSF and plasma AUCs. In our study, the CSF to plasma AUC ratio for ganciclovir following a short i.v. infusion was approximately 16%.

In in vitro CNS tumor models of rat or human glioma cells transduced with a retrovirus vector and exposed to 0.1 μ*M* (0.025 μg/ml) ganciclovir, DNA synthesis has been shown to be inhibited within 1 h and cell survival to be decreased by 50% in 5 h [12]. In addition, transduced rat glioma cells demonstrate dose-dependent sensitivity to ganciclovir. The survival rate was 15% to 20% following exposure to 0.1 μM (0.025 μg/ml) ganciclovir in one cell line versus no survival after exposure to 30 μM (7.5 μg/ml) [9]. However, using colony formation assays, the same authors have reported that the survival rate was 30% following exposure to a dose of 3 μ*M* (0.75 μg/ml) [9]. In our studies, CSF concentrations exceeded 0.6 μg/ml in two of the three animals and were greater than 0.025 μg/ml for at least 6 h in all three animals. Thus, following a dose of 10 mg/kg in the nonhuman primate, concentrations of ganciclovir that are cytotoxic to transduced cells in some in vitro systems can be achieved without evidence of systemic or CNS toxicity.

The ability to achieve cytotoxic ganciclovir concentrations in the CSF following i.v. administration has important implications for the treatment of CNS tumors

with gene therapy. In most ongoing clinical trials, the HSV-TK-containing vector is delivered directly to the tumor via stereotactic surgery. Since ganciclovir cannot be administered directly into the tumor, the ability of the drug to penetrate the CNS is critical to the potential success of this approach. This is particularly important for strategies designed to treat neoplastic meningitis. The feasibility of intrathecal administration of HSV-TK-containing vectors followed by i.v. or intrathecal administration of ganciclovir has been demonstrated in rodents with 9L gliosarcoma [5, 10] and additional preclinical studies of this strategy in nonhuman primates are in progress. Our data provide preliminary evidence that adequate CSF concentrations may be attained after i.v. ganciclovir administration, potentially eliminating the need for frequent intrathecal injections. However, further in vitro studies are required to accurately determine the concentrations of ganciclovir required for cytotoxicity in a variety of CNS and leptomeningeal tumors.

References

1. Fletcher C, Sawchuk R, Chinock B, Miranda P de, Balfour H (1986) Human pharamcokinetics of the antiviral drug DHPG, Clin Pharmacol Ther 40: 281–286
2. Gibaldi M, Perrier, D (1982) Estimation of areas. In: Swarbrick J (ed) Pharmacokinetics, 2nd edn. Marcel Dekker, New York, pp 445–449
3. McCully C, Balis F, Bacher J, Phillips J, Poplack D (1990) A Rhesus monkey model for continuous infusion of drugs into cerebrospinal fluid. Lab Anim Sci 40: 520–525
4. National Research Council (1996) Guide for the care and use of laboratory animals. National Academy Press, Washington, D.C.
5. Oshiro E, Viola J, Oldfield E, Walbridge S, Bacher J, Frank J, Blaese R, and Ram Z (1995) Toxicity studies and distribution dynamics of retroviral vectors following administration of retroviral vector-producer cells. Cancer Gene Ther 2: 87–95
6. Page T, Sherwood C, Connor J, Tarnowski T (1996) Simple reversed-phase high-performance liquid chromatography quantitation of ganciclovir in human serum and urine. J Chromatorgr B 675: 342–346
7. Paul S, Dummer S (1992) Topics in clinical pharmacology: ganciclovir. Am J Med Sci 304: 272-277
8. Perrier D, Mayersohn M (1982) Noncompartmental determination of steady state volume of distribution for any mode of administration. J Pharm Sci 71: 372–373
9. Takamiya Y, Short P, Moolten F, Fleet C, Mineta T, Breakfield X, Martuza R (1993) An experimental model of retrovirus gene therapy for malignant brain tumors. J Neurosurg 79: 104–110
10. Viola J, Ram Z, Walbridge S, Oshiro E, Trapnell B, Tao-Cheng J, Oldfield E (1995) Adenovirally mediated gene transfer into experimental solid brain tumors and leptomeningeal cancer cells. J Neurosurg 82: 70–76
11. Yamaoka K, Nagakawa T, Uno T (1978) Application of Akaike's Information Criterion (AIC) in the evaluation of linear pharmacokinetic equation. J Pharmacokinet Biopharm 6: 165–174
12. Zerbe L, Hughes T, Josephs S, Davidson B, Shewach D (1995) Rapid cytotoxicity with ganciclovir following adenovirus or retrovirus transduction of glioma cells with herpes virus thymidine kinase. Proc Am Assoc Cancer Res 36: 423