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ORIGINAL ARTICLE

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Plasma and cerebrospinal fluid pharmacokinetics of gemcitabine after intravenous administration in nonhuman primates

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Abstract *Purpose:* Gemcitabine (dFdC) is a difluorine-substituted deoxycytidine analogue that has demonstrated antitumor activity against both leukemias and solid tumors. Pharmacokinetic studies of gemcitabine have been performed in both adults and children but to date there have been no detailed studies of its penetration into cerebrospinal fluid (CSF). The current study was performed in nonhuman primates to determine the plasma and CSF pharmacokinetics of gemcitabine and its inactive metabolite, difluorodeoxyuridine (dFdU) following i.v. administration. *Methods:* Gemcitabine, 200 mg/kg, was administered i.v. over 45 min to four nonhuman primates. Serial plasma and CSF samples were obtained prior to, during, and after completion of the infusion for determination of gemcitabine and dFdU concentrations. Gemcitabine and dFdU concentrations were measured using high-performance liquid chromatography (HPLC) and modeled with model-dependent and model-independent methods. *Results:* Plasma elimination was rapid with a mean $t_{1/2}$ of 8 ± 4 min (mean ± SD) for gemcitabine and 83 ± 8 min for dFdU. Gemcitabine total body clearance (Cl_{TB}) was 177 ± 40 ml/min per kg and the Vd_{ss} was 5.5 ± 1.0 l/kg. The maximum concentrations (C_{max}) and areas under the time concentration curves (AUC) for gemcitabine and dFdU in plasma were 194 ± 64 μM and 63.8 ± 14.6 μM·h, and 783 ± 99 μM and 1725 ± 186 μM·h, respectively. The peak CSF concentrations of gemcitabine and dFdU were 2.5 ± 1.4 μM and 32 ± 41 μM, respectively. The mean CSF:plasma ratio was 6.7% for gemcitabine and 23.8% for dFdU. *Conclusions:* There is only modest penetration of gemcitabine into the CSF after i.v. administration. The relatively low CSF exposure to gemcitabine after i.v. administration suggests that systemic administration of this agent is not optimal for the treatment of overt leptomeningeal disease. However, the clinical spectrum of antitumor activity and lack of neurotoxicity after systemic administration of gemcitabine make this agent an excellent candidate for further studies to assess the safety and feasibility of intrathecal administration.

Key words Gemcitabine · Nonhuman primates · Plasma pharmacokinetics · Cerebrospinal fluid pharmacokinetics

Introduction

Gemcitabine, (2′2′-difluorodeoxycytidine, dFdC), a difluorinated deoxycytidine analogue (Fig. 1), has structural and metabolic properties similar to those of cytarabine. Cytarabine, one of the most commonly used agents for the treatment of hematologic malignancies, is also utilized in the treatment and prevention of CNS leukemias and lymphomas. Unlike cytarabine, gemcitabine has demonstrated marked antitumor activity against a wide range of solid tumors including those that have a predilection for leptomeningeal spread such as non-small-cell lung cancer and breast cancer [2, 3, 5, 6, 12, 16, 18]. Like cytarabine, which is rapidly deaminated by cytidine deaminase to form the inactive metabolite ara-U, gemcitabine is also rapidly deaminated to an inactive uridine metabolite, 2′-difluoro-2′,2′-deoxyuridine (dFdU) [1, 19].

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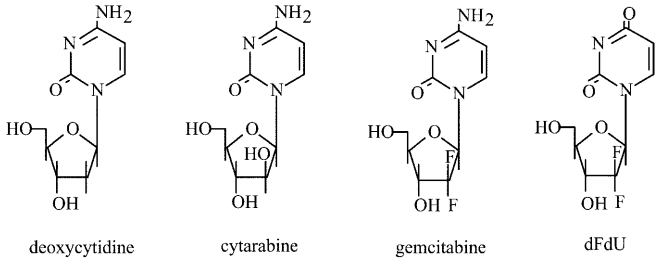

Fig. 1 Chemical structures of deoxycytidine, cytarabine (arabinosyl cytosine), gemcitabine (2′-2′-difluorodeoxyctidine), and dFdU

Although the plasma and intracellular pharmacokinetics of gemcitabine have been described in children and adults [1, 10, 11, 17], there are not any detailed studies of the pharmacokinetics of gemcitabine in the CSF. In order to characterize the CSF penetration of gemcitabine, we studied the plasma and cerebrospinal fluid pharmacokinetics of gemcitabine in a nonhuman primate model that is highly predictive of the CSF penetration in humans [13].

Materials and methods

Drugs

Gemcitabine hydrochloride for injection was provided by Eli Lilly and Company (Indianapolis, Ind.) as 1-g vials. Vials were reconstituted with 25 ml sterile normal saline to a final concentration of 38 mg/ml, and the appropriate dose was infused over 45 min via a peripheral venous catheter. Bulk gemcitabine and dFdU for standard curves were also provided by Eli Lilly and Company. Tetrahydrouridine (THU; Sigma Chemical Company, St. Louis, Mo.) was diluted with sterile water to a final concentration of 1 m*M*. The internal standard, 2′-deoxycytidine (2′-dC; Sigma Chemical Company) was diluted with methanol to a final concentration of 165 μ*M*.

Animals

Four adult male rhesus monkeys (*Macaca mulatta*) weighing 10.7 to 13.6 kg were used for these pharmacokinetic studies. The animals were fed Lab Diet 5045 twice daily and were group-housed in accordance with the Guide for the Care and Use of Laboratory Animals [14]. Blood samples were drawn through a catheter placed in either the femoral or saphenous vein contralateral to the site of drug infusion, or through an indwelling catheter in the jugular vein. CSF samples were drawn from a subcutaneous Ommaya reservoir attached to an indwelling Pudenz catheter with its tip in the fourth ventricle [13]. The reservoir was pumped four times before and after each CSF sample collection to ensure adequate mixing with ventricular CSF.

Experiments

Gemcitabine, 200 mg/kg, was administered i.v. over 45 min. Blood was collected into heparinized tubes containing 50 μl 1 m*M* THU to prevent ex vivo deamination. Blood was collected prior to the dose, at 20 min into the infusion, at the end of the infusion and at 5, 15 and 30 min and 1, 1.5, 2, 4, 6, 8, 24 and 48 h after the infusion in all four animals. Samples were also collected at 72 h in three animals. Plasma was prepared immediately by centrifugation at 8600 *g* at 4 °C for 5 min. CSF was collected into tubes containing 20 μl 1 m*M* THU. CSF was collected prior to the infusion, at 20 min into the infusion, at the end of the infusion and at 10 and 30 min and 1, 1.5, 2, 4, 6, 8, 24 and 48 h after the infusion in all four animals, and 72 h after the infusion in two animals. Plasma and CSF samples were stored at –80 °C until the day of analysis.

Sample analysis

Gemcitabine and dFdU levels in plasma and CSF were measured using a modification of a recently described reverse-phase HPLC assay [20]. Prior to assay, 20 μl of 165 μ*M* 2′-dC internal standard was added to 200 μl of sample. Acetonitrile (1 ml) was then added to each plasma sample, followed by centrifugation (11,750 *g* for 10 min). The resulting supernatants were transferred to glass tubes and dried under a gentle flow of nitrogen. The residues were reconstituted in 200 μl mobile phase, and 100 μl was injected onto the HPLC system described below. After the addition of internal standard (20 μl 2′-dC/200 μl CSF) as described above, CSF samples were injected directly onto the HPLC system.

The HPLC system (Waters Associates, Milford, Mass.) consisted of a Waters 600 Controller, a Waters 717 Plus autosampler, and a Waters 490E programmable multiwavelength UV detector with a reverse-phase Luna 5μC18 (150×4.60 mm) analytical column (Phenomenex, Torrance, Calif.), preceded by a Waters Symmetry C18 (3.9×20 mm) guard column. The mobile phase consisted of 50 m*M* ammonium acetate (pH 5.0) and acetonitrile (96.5:3.5 v/v) at a flow rate of 1 ml/min. Gemcitabine and 2′-dC were monitored at 275 nm and dFdU was monitored at 265 nm. The lower limit of quantitation for gemcitabine in plasma and CSF was 0.5 μ*M*. The lower limits of quantitation for dFdU in plasma and CSF were 1 μ*M* and 0.5 μ*M*, respectively. Standard curves in plasma and saline were prepared for each experiment by the addition of known amounts of gemcitabine and dFdU. Standard curves were linear ($r^2 \geq 0.99$) over the range 0.5 to 50 μ*M* for gemcitabine and the range 0.5 to 1000 μ*M* for dFdU.

Pharmacokinetic analysis

Postinfusion gemcitabine concentration versus time data from plasma and CSF were fitted to monoexponential and biexponential equations with MLAB, (Civilized Software, Bethesda, Md.) using the formula:

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

where C is the drug concentration at time t, A_i is the intercept, and λ_i is the rate constant. Aikake's information criterion was used to determine which equation best fitted the data [21]. All other pharmacokinetic parameters were determined using model-independent methods. The area under the concentration-time curve (AUC) was determined by the linear trapezoidal method and extrapolated to infinity using the terminal rate constant [9]. Clearance was determined by dividing dose by AUC. The half-life for each phase of elimination was calculated by dividing 0.693 by the rate constant (λ_i) for that phase. The steady-state volume of distribution (Vd_{ss}) was calculated from the area under the moment curve [15]. CSF penetration was determined by dividing the AUC in CSF by the AUC in plasma (AUC_{CSF}:AUC_P).

Evaluation for toxicity

Clinical laboratory studies including complete blood counts, electrolytes (including Ca^{++}, Mg^{+} and phosphorus), liver function tests, and renal function tests were obtained on a weekly basis for a minimum of 3 weeks after the gemcitabine infusion. Animals were also observed on a daily basis for a minimum of 3 weeks postinfusion for any evidence of behavioral change.

Results

Gemcitabine was well tolerated, without significant hematologic or other organ toxicity, after a single i.v. infusion of 200 mg/kg. The plasma and CSF concentration-time profiles of gemcitabine and dFdU are shown in Fig. 2. The disappearance of gemcitabine from plasma following an i.v. infusion over 45 min was best described by a monoexponential equation. The maximum plasma concentrations (C_{max}) for gemcitabine and dFdU were 194 ± 64 μM, and 783 ± 99 μM, respectively (mean ± SD; Tables 1 and 2). The C_{max} of gemcitabine in CSF was 2.5 ± 1.4 μM, with an AUC of 3.8 ± 0.9 μM·h. The CSF C_{max} of dFdU was 32 ± 41 μM, with an AUC of 399 ± 199 μM·h. Gemcitabine was not detectable from 2.5 h after dosing, whereas, dFdU was still present in the CSF at 11 h. The AUC_{CSF}:AUC_P was 7% for gemcitabine and 24% for dFdU.

Gemcitabine was rapidly eliminated from plasma with a $t_{1/2}$ of 8 ± 4 min compared with a $t_{1/2}$ of 83 ± 8 min for dFdU. The plasma AUC was 63.8 ± 14.6 μM·h for gemcitabine and 1725 ± 186 μM·h for dFdU. The CL_{TB} of gemcitabine was 177 ± 40 ml/min per kg, and the Vd_{ss} was 5.5 ± 1.0 l/kg.

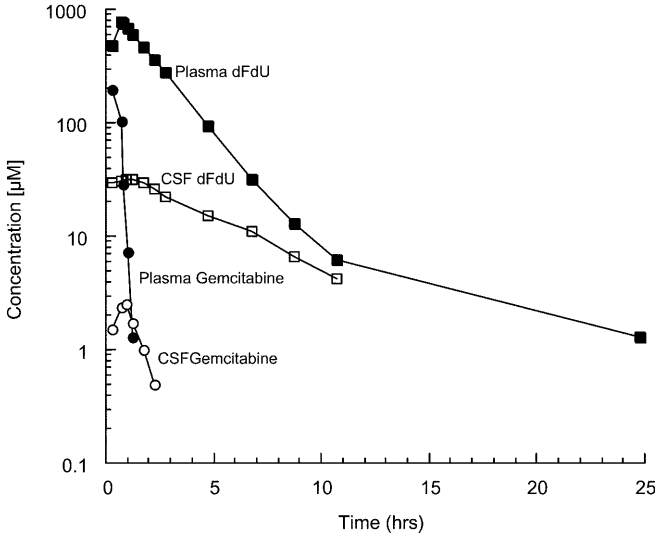

Fig. 2 Representative plasma and CSF concentration-time profile of gemcitabine and dFdU following 45 min i.v. infusion of gemcitabine to nonhuman primates

Discussion

Progress in the treatment of many systemic malignancies has led to overall increases in progression-free survival. As a consequence, there has been an increase in the incidence of neoplastic meningitis from a variety of underlying solid tumors, including breast and lung carcinomas. Presumably this occurs because the CNS is a pharmacologic sanctuary that restricts entry of most anticancer agents across the blood-brain and blood-CSF barriers. Since the prognosis for patients with neoplastic meningitis is dismal, it is important to identify active anticancer agents that significantly penetrate into the CSF. Therefore, we studied the plasma and CSF pharmacokinetics of gemcitabine in a nonhuman primate model that has previously been predictive of CSF drug penetration in humans [13].

Gemcitabine disappeared rapidly from plasma in nonhuman primates due to metabolic conversion of parent drug to an inactive metabolite, dFdU. The terminal $t_{1/2}$ of 8 ± 4 min is similar to that reported in humans (6.7 to 8 min in adults and 11 to 25 min in children) [1, 11, 17]. Likewise, gemcitabine Cl_{TB} in nonhuman primates was approximately 2100 ml/min (177 ml/min per kg) compared with 2400 ml/min in

Table 1 Plasma and CSF pharmacokinetic parameters of gemcitabine after 45 min i.v. infusion to nonhuman primates (*P* plasma, *CSF* cerebrospinal fluid)

Animal	AUC_P (μM·h)	AUC_{CSF} (μM·h)	AUC_{CSF}:AUC_P	Plasma $t_{1/2}$ (min)	Vd_{ss} (l/kg)	Cl_{TB} (ml/min/kg)
J124	49.9	4.04	0.081	8	7.0	223
J128	55.6	4.50	0.081	8	5.9	200
J0	72.0	2.73	0.038	5	5.1	154
J86	81.5	n/a	n/a	14	4.7	136
Mean ± SD	63.8 ± 14.6	3.8 ± 0.9	0.067 ± 0.025	8 ± 4	5.5 ± 1.0	177 ± 40

Table 2 Plasma and CSF pharmacokinetic parameters of dFdU after i.v. gemcitabine administration over 45 min in nonhuman primates (*P* plasma, *CSF* cerebrospinal fluid)

Animal	AUC_P (μM·h)	AUC_{CSF} (μM·h)	AUC_{CSF}:AUC_P	Plasma $t_{1/2}$ (min)
J124	1596	239	0.150	88
J128	1605	527	0.328	84
J0	1845	271	0.147	88
J86	1974	648	0.328	71
Mean ± SD	1725 ± 186	399 ± 199	0.24 ± 0.10	83 ± 8

humans [11]. CSF gemcitabine pharmacokinetics in the nonhuman primate revealed that there was only modest penetration (7%) of gemcitabine into the CSF and relatively greater penetration (24%) of dFdU. These findings are consistent with those of previous studies that demonstrate that the nucleobase is a major determinant of the CSF penetration of some nucleosides [4, 7].

Grunewald et al. have demonstrated that accumulation of dFdCTP, the active intracellular metabolite of gemcitabine, in mononuclear cells and circulating leukemia cells is saturable at plasma gemcitabine concentrations in excess of 15–20 μM [1, 10]. In the nonhuman primate the C_{max} of gemcitabine in CSF was only 2.5 μM with an AUC of 3.8 $\mu M \cdot$h. Thus, gemcitabine exposure attained in the CSF following i.v. administration is probably not optimal for the treatment of leukemic neoplastic meningitis. However, because gemcitabine has demonstrated antitumor activity against leukemia and a wide variety of solid tumors, regional administration may have potential for achieving desired gemcitabine concentrations in the CSF. We have recently performed preclinical studies to evaluate the feasibility and pharmacokinetics of gemcitabine following intrathecal administration and will be initiating clinical studies evaluating this approach shortly [8].

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