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ORIGINAL ARTICLE

Pharmacokinetics of gemcitabine in tumor and non-tumor extracellular fluid of brain: an in vivo assessment in rats employing intracerebral microdialysis

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Abstract
Purpose Gemcitabine is a pyrimidine nucleoside analogue anticancer agent that has shown promising anti-tumor activity in several experimental models of brain tumor. However, the pharmacokinetic behavior of gemcitabine in the central nervous system, especially in brain tumors is currently not well understood. In this study we evaluated the gemcitabine brain extracellular fluid (ECF) in normal rats and in ECF obtained from tumor- and tumor-free regions of glioma-bearing rats, to better understand the availability of the drug to brain and brain tumors.
Methods The brain ECF pharmacokinetics of gemcitabine were investigated employing intracerebral microdialysis following intravenous administration of 10, 25 and 100 mg/kg doses in male Sprague–Dawley rats. In the second phase of the study, gemcitabine (25 mg/kg) was intravenously administered in rats implanted with C6 gliomas and ECF samples were simultaneously obtained from the tumor and tumor-free regions of the brain. Serial blood samples were obtained for evaluating the plasma pharmacokinetics of gemcitabine. Non-compartmental approach was employed for the analyses of the brain ECF and plasma pharmacokinetics of gemcitabine.
Results Following intravenous administration, gemcitabine rapidly distributed into rat brain. At doses equivalent to 10, 25 and 100 mg/kg, the brain ECF gemcitabine AUC (area under the plasma concentration—time curve measured over the last sampling time point) values were 2.46 ± 0.7, 3.20 ± 1.1, and 9.06 ± 3.0 μg h/ml, respectively. The brain ECF concentrations of gemcitabine declined in parallel with plasma concentrations. At the three doses evaluated, the relative brain distribution coefficient ($AUC_{brainECF}/AUC_{plasma}$) of gemcitabine ranged from 0.07 to 0.09 suggesting limited gemcitabine availability to brain tissues. Studies on C6 glioma-bearing rats revealed that following an intravenous dose of 25 mg/kg, the AUC values in the tumor-free and tumor-brain regions were 4.52 ± 2.4, and 9.82 ± 3.3 μg h/ml, respectively. Thus, the AUC of gemcitabine in the tumor ECF was on average 2.2-fold greater than the corresponding value in the tumor-free ECF of the brain. Plasma pharmacokinetics of gemcitabine remained unaltered in tumor-bearing animals, when compared to plasma pharmacokinetics in healthy animals.
Conclusions Our findings suggest that the overall brain exposure to gemcitabine is likely to be low as evident from the relative brain distribution coefficient of <0.1. However, the exposure is likely to be considerably higher in the brain tumor relative to tumor-free regions of the brain. The higher drug levels in brain tumor compared to the non-tumor region may facilitate selectively higher cytotoxicity against brain tumor cells.

Keywords Gemcitabine · Brain · ECF · Pharmacokinetics · Glioma · Microdialysis

Introduction

Gemcitabine (2′,2′-difluorodeoxycytidine, dFdC) (Gemzar®) is a deoxycytidine analogue anticancer agent with a broad spectrum of activity against several solid tumors. It acts by inhibiting DNA synthesis via competitive incorporation of its triphosphate metabolite into the growing DNA strand [11]. Its relatively low toxicity profile as evident from various clinical trials has made it a much desirable

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candidate to be used either as a single agent and/or as an adjuvant in the treatment of various malignancies. Gemcitabine is currently approved as a first-line treatment for patients with locally advanced (nonresectable stage II or stage III) or metastatic (stage IV) adenocarcinoma of the pancreas. It is also indicated in combination with cisplatin for the first-line treatment of patients with inoperable, locally advanced (stage IIIA or IIIB), or metastatic (stage IV) non-small cell lung cancer and with paclitaxel for the first-line treatment of patients with metastatic breast cancer after failure of prior anthracycline-containing adjuvant chemotherapy. Gemcitabine may also have a potential role in the treatment of central nervous system malignancies both as a cytotoxic agent as well as a potent radiosensitizer as evident from its activity against human brain tumor cell lines [4, 5, 13]. Its potential clinical use is further underscored by lack of observed neurotoxicity. However, there is significant ambiguity regarding its efficacy against brain tumors in clinical studies [1, 6, 8, 12, 16, 17]. Furthermore, there is poor understanding of penetration of gemcitabine across blood brain barrier (BBB) and blood–brain tumor barrier (BTB). It is also difficult to estimate the propensity of gemcitabine to cross blood–brain barrier based on its physicochemical properties. While it is a small molecular weight (263.2 Da) drug with negligible protein binding, it is a relatively hydrophilic compound and little is known about the process of gemcitabine transport into brain.

A clear understanding of the concentrations of gemcitabine attained and its time course in brain tumor tissue is important to assess the role of gemcitabine in the treatment of primary and metastatic brain tumors and optimizing its dosing regimen. Thus, in this study we assessed the brain and brain tumor pharmacokinetics of gemcitabine using the in vivo microdialysis technique. Pharmacokinetics of gemcitabine was first characterized following intravenous administration in healthy rats at doses bracketing the clinically relevant doses. Subsequently, employing simultaneous microdialysis of the tumor and ''normal'' brain regions of glioma-implanted rats, the differences in the availability and time course of gemcitabine were compared. The technique of intracerebral microdialysis allowed temporal resolution and provided site-specific information of the analytes with high sensitivity and selectivity [15].

Materials and methods

Chemicals and supplies

Gemcitabine (2′,2′-difluoro deoxycytidine; dFdC) hydrochloride was a generous gift from Eli Lilly (Indianapolis, IN). The drug was reconstituted for intravenous administration in bacteriostatic 0.9% sodium chloride (Abbott Laboratories, Abbott park, IL) to a final concentration of 38 mg/ml. Tetrahydrouridine, a deoxycytidine deaminase inhibitor was obtained from Calbiochem (San Diego, CA). Cannulation materials for the implantation of venous catheters were purchased from Plastics One Inc., (Roanoke, VA) and VWR scientific (Batavia, IL). Dialysis membrane with a 13,000 MW cut-off and an outside diameter of 210 μm was purchased from Spectrum laboratories, CA. HPLC grade solvents were obtained from Fisher Scientific (Pittsburgh, PA). Concentric-style microdialysis probes were constructed as described elsewhere [18].

Animals

Male Sprague–Dawley rats (250–300 g) were obtained from Charles River Laboratories (Wilmington, MA). The animals were housed individually in a room maintained at 72°C, 55% relative humidity with a 12/12 h light/dark cycle and provided access to standard laboratory chow ad libitum. All procedures were in strict adherence to the ''Principles of laboratory animal care'' guidelines (NIH publication No. 85-23, revised 1985) and were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Tumor cell line

C6 glioma cell line, an *N*-nitrosomethylurea induced rat tumor was obtained from ATCC (Manassas, VA). The cell line was maintained in F12-K nutrient mixture (Invitrogen, Carlsbad, CA) fortified with 2 mM L-glutamine and adjusted to contain 15% horse serum plus 2.5% fetal bovine serum. The cells were grown to confluence in a humidified atmosphere containing 5% CO_2 at 37°C. For implantation into animals, the tumor cells were harvested using a 0.05% trypsin-EDTA solution and the cells were then suspended in fresh medium to obtain a final concentration of 6×10^6 cells/10 μl.

Guide cannula implantation

Rats were anesthetized and secured in a stereotaxic apparatus. The skull was exposed and bregma was identified. The coordinates for striatum were identified as 1.2 mm anteroposterior, 3.1 mm lateral from the bregma according to the atlas of Paxinos and Watson (The rat brain in stereotaxic coordinates; Academic Press, 1986) and a hole was drilled through the cranium, dorsal to the striatum. A guide cannula for the implantation of microdialysis probe was placed into it. The cannula was secured to the skull with screws and cranioplastic cement and was capped with a dummy stylet. The animals were allowed to recover for a

period of 48 h before the commencement of the microdialysis experiments.

Tumor implantation procedure

Rats were anesthetized with an injection of ketamine and xylazine (87/13 mg/kg, i.p.). The coordinates for striatum in both hemispheres of the brain were identified according to the atlas of Paxinos and Watson (1.2 mm anteroposterior and ±3.1 mm lateral from bregma) and a hole was drilled on each side through the cranium, dorsal to the striatum. A guide cannula was then lowered into the hole and secured to the skull with screws and cranioplastic cement. A Hamilton syringe containing 6×10^6 tumor cells in 10 µl was then slowly lowered into the striatum of the left hemisphere to a depth of 5 mm. The cells were then slowly released over a period of 2 min and then the syringe was left in place for 5 min to allow the tumor cells to completely dislodge from the syringe. The striatum of the right hemisphere was left tumor-free to serve as a control. The tumor was allowed to grow for a period of 12 days before drug administration and sampling. Venous catheters were introduced 2 days prior to the experiment.

Drug administration and pharmacokinetic sampling

On the morning of the experiment, concentric style microdialysis probes were inserted through the guide cannulae of the tumor and non-tumor sides of the brain. The length of the dialysis membrane for the striatum was 4.5 mm for normal rats and 2.5 mm for the tumor-bearing rats. The probes were perfused with simulated interstitial fluid at a slow input rate of 1.8 µl/min, in order to facilitate an optimal temporal resolution and higher recovery of gemcitabine. The dialysis fluid was composed of Dulbecco's phosphate-buffered saline containing 1.2 mM $CaCl_2$ and 5 mM glucose. After an equilibration period of 1.5 h, gemcitabine was injected intravenously. Normal rats ($n = 9$ per dose) received a single bolus dose of 10, 25 or 100 mg/kg of gemcitabine and tumor-bearing rats ($n = 9$) each received a clinically relevant bolus dose of gemcitabine (25 mg/kg). Early microdialysate samples were collected at the end of 5, 10, 20 and 30 min post-dose in normal rats dosed with 10 and 100 mg/kg gemcitabine and at the end of 15 and 30 min post-dose in the normal and tumor-bearing rats dosed with 25 mg/kg bolus dose of gemcitabine. Subsequent samples were collected every 30 min for 8–10 h and stored at –80°C until analyzed. For experiments conducted in normal rats, blood samples (200 µl) were collected via a catheter placed in the femoral vein, at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, and 8 h following drug administration. In the tumor-bearing rats, blood samples (200 µl) were collected using the jugular catheter at 0.5, 1, 1.5, 3, 5 and 8 h following drug administration. Blood samples were collected into heparinized collection vials containing tetrahydrouridine (10 µl of 10 mM THU or 0.5 mM final concentration). The blood samples were centrifuged immediately at a speed of 3,000 rpm for 10 min. The plasma was separated and stored at –80°C. The extraction of plasma samples was performed using a slight modification of a previously published procedure [10]. Briefly, 15% isopropanol in ethyl acetate (2 ml) was added to plasma samples (100 µl) and gently vortexed for 20 s. The samples were then centrifuged at 3,000 rpm for 15 min. The organic phase was transferred into glass vials and dried under nitrogen. The dried samples were reconstituted in mobile phase and analyzed using HPLC.

In vivo recovery analysis of gemcitabine

In vivo recovery of gemcitabine was estimated by determining the loss of the drug in vivo by reverse dialysis technique. Following drug administration and sampling, the blank dialysis buffer was replaced with a buffer containing gemcitabine (50 µM; with tetrahydrouridine). Following an equilibration period of 1 h, dialysis samples were collected every 30 min for at least 1 h. The in vivo recovery was then calculated as the % loss of gemcitabine from the probe inlet into the brain, assuming identical flux of molecules in both directions across the dialysis membrane.

$$\%\ \text{in vivo recovery of gemcitabine} = [(C_{\text{inlet}} - C_{\text{outlet}})/C_{\text{inlet}}] \times 100,$$

where, C_{inlet} and C_{outlet} were the concentrations of gemcitabine in the perfusate (inlet) and dialysate (outlet) fluids, respectively.

Histological evaluation

At the end of the experiments, the animals were decapitated. The brain was carefully removed and stored in 4% buffered formaldehyde solution at 4°C for at least 3 days. The tissues were then embedded in paraffin, sectioned and stained with Hematoxylin-Eosin and examined by light microscopy to determine the placement of the probe and tumor histology.

HPLC analysis of gemcitabine in dialysate and plasma samples

The analysis of gemcitabine was done using a slight modification of a previously published procedure [14]. The mobile phase consisted of ammonium acetate buffer (0.1 M) [with acetic acid (0.05%) and triethylamine to

adjust the pH to 7.0] and acetonitrile (98:2 *v*/*v*). The mobile phase was pumped at a flow rate of 0.8 ml/min using a Waters 510 pump. Gemcitabine was eluted on a μBondapak C18 column, with a 5 μm particle size packing (Alltech, Deerfield, IL) coupled with a guard column of similar properties. A Waters 486 variable wavelength UV/VIS detector coupled to millennium software was used for detecting and integrating the sample peaks, respectively. The lower limit of quantitation for gemcitabine using this method of analysis was 6 ng/ml for the microdialysate samples and 30 ng/ml for the plasma samples. The intra- and inter-day precision of the analyses was <15%. The accuracy was within ±12%.

Pharmacokinetic data analysis

Non-compartmental or model-independent analysis of gemcitabine concentration versus time data in plasma and brain ECF was performed employing WinNonlin version 3.1, (Pharsight, CA). The ECF concentrations of gemcitabine were time-averaged concentrations over the dialysate collection interval and were corrected for in vivo recovery. Area under the curve (AUC) was calculated employing the linear trapezoidal rule, as shown below:

$AUC_{0-tn} = \sum_{i=1}^{n}[(C_i + C_{i+1})/2] \times \Delta t$, where, C_i and $C_{i+1}$are the concentrations at time points t_i and t_{i+1}, respectively, and the time interval, $\Delta t = (t_{i+1} - t_i)$. Since the sampling interval of 30 min employed in our study may not facilitate temporal resolution required for precise determination of peak drug levels and elimination half life, only AUC_{0-t}, where t is the time corresponding to the last sample collection, was determined. For the sake of clarity while describing the AUC_{0-t} in brain and in glioma is only referred to as AUC hereafter. In case of plasma pharmacokinetics the total AUC (AUC_{0-inf}) was also measured and labeled accordingly. The extrapolated area under the curve from the last sampling time to infinity (AUC_{extr}) was calculated as $AUC_{extr} = C_{last}/\lambda_z$, where C_{last} and λ_z are the last measurable drug concentration and the terminal slope, respectively. The plasma pharmacokinetics analysis also included determination of C_{max} and elimination half-life.

Statistical analysis

The pharmacokinetic parameters of gemcitabine disposition in normal- and tumor-brain regions were evaluated for statistical differences employing single factor analysis of variance (ANOVA) followed by students *t*-test. A $P < 0.05$ was interpreted as the level of statistical significance.

Results

The brain ECF concentrations of gemcitabine in each animal were estimated by correcting the dialysate concentrations with the in vivo recovery value obtained from that particular animal. The average in vivo recovery of gemcitabine in healthy Sprague–Dawley rats was 13.6 ± 4.2%. The average in vivo recovery of gemcitabine in the tumor and non-tumor brain regions of the glioma-bearing rats were found to be 9.67 ± 3.4% and 11.48 ± 5.26%, respectively.

Following tumor implantation, all animals resumed normal activity with no loss of appetite or gross behavioral changes occurring during the 12-day period allowed for tumor growth. Histological evaluation of rat brains showed that 12 days following tumor implantation, a circular or ovoid tumor mass had formed at the site of inoculation. As shown in Fig. 1, the tumor was limited to the striatum of the left hemisphere (site of implantation), with no crossing of the neoplasm to the right hemisphere. In addition, microscopic examinations showed a narrow path formed by the passage of the microdialysis probe into the central part of the tumor, confirming the placement of the sampling probe into the tumor mass.

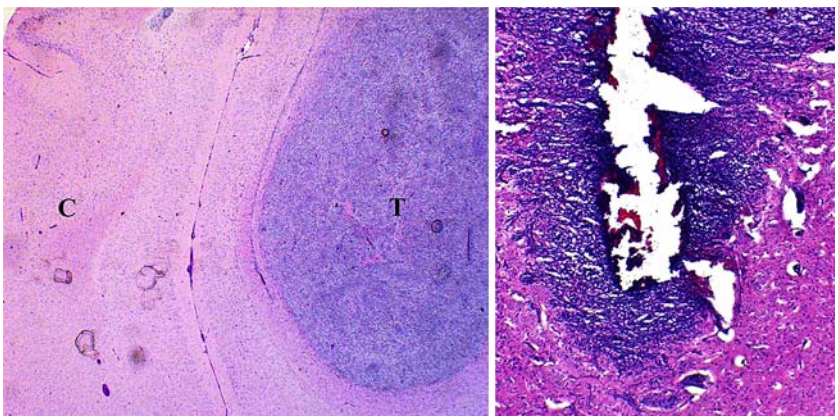

Fig. 1 Photomicrographs of brain from C6 glioma bearing rats. The *left panel* shows the formation of tumor (*T*) at the site of implantation, without any spread into the contra lateral hemisphere, which therefore serves as a control (*C*). The *right panel* shows the path formed by the insertion of a microdialysis probe into the tumor

The brain ECF concentrations (corrected for in vivo recovery) versus time (midpoint of dialysis collection interval) profiles of gemcitabine in normal rats and within the tumor- and tumor-free brain regions of the glioma-bearing rats following intravenous drug administration are shown in Figs. 2, 3, respectively. The brain ECF concentrations represent time-averaged concentrations over the dialysis collection interval. It was observed that gemcitabine distribution into brain following an intravenous bolus dose was rapid with drug levels detected at all initial time points.

The AUC of gemcitabine in the ECF collected from the normal rats as well from the tumor and non-tumor regions of glioma-bearing Sprague–Dawley rats, determined using non-compartmental analysis are summarized in Table 1.

The relative brain distribution coefficient is calculated as ($AUC_{brain\ ecf}/AUC_{plasma}$).

As evident from the data in Table 1, with an increase in the dose of gemcitabine by 2.5- and 10-fold, respectively, in the normal Sprague–Dawley rats there was approximately 1.3 and 3.6-fold increase in the AUC values of gemcitabine brain ECF levels. Likewise, at 25 and 100 mg/kg doses, the plasma AUC_s were 1.6 and 3.6-fold higher than those at 10 mg/kg doses. A comparison of the relative brain distribution coefficients of gemcitabine calculated as the ratio of gemcitabine AUC in the brain ECF to that of plasma, indicated that the ratio was similar (approximately 0.07–0.09) across the doses employed.

With respect to the tumor-bearing animals, intravenous administration of gemcitabine (25 mg/kg) resulted in considerably higher levels and exposure to unbound gemcitabine in brain tumor compared to tumor-free brain region (Fig. 3). The AUC of gemcitabine in the tumor brain ECF was on average 2.2-fold greater than the corresponding values in the tumor-free ECF of the brain ($P < 0.05$).

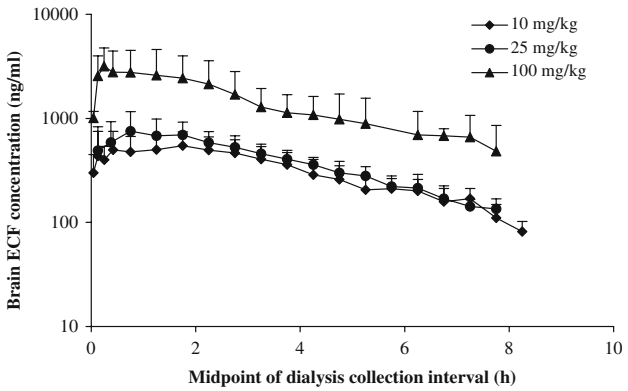

Fig. 2 Rat brain extra cellular fluid (*ECF*) concentration versus time profiles of gemcitabine following intravenous bolus doses of 10, 25 and 100 mg/kg. Data points represent the mean ± SD obtained from $n = 9$ rats

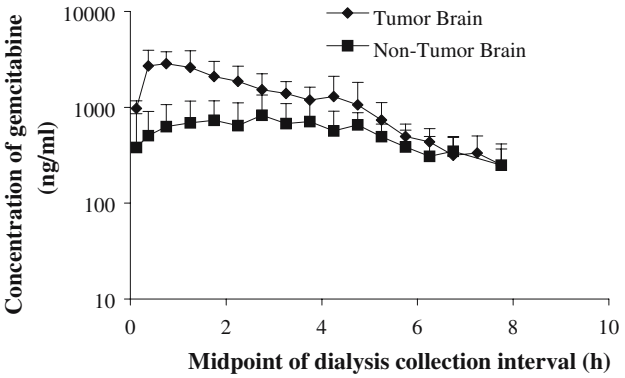

Fig. 3 Concentration versus time profiles of gemcitabine in tumor-bearing and tumor-free regions of the rat brain following intravenous administration of a 25 mg/kg bolus dose. Each point represents the mean ± SD of values obtained from $n = 9$ rats

The plasma pharmacokinetic profiles of gemcitabine in normal and tumor-bearing Sprague–Dawley rats are represented in Fig. 4. The plasma gemcitabine concentration versus time plots in normal rats at the three doses employed (10, 25 and 100 mg/kg) are shown in Fig. 4a. A comparison of the plasma concentration time profile of gemcitabine in normal versus tumor bearing rats at 25 mg/kg level is illustrated in Fig. 4b.

The plasma pharmacokinetics of the normal and tumor-rats are summarized in Table 2.

It appears from Fig. 4a and the assessed terminal half life values that the systemic elimination characteristics of gemcitabine did not change with change in dose.

Moreover, the plasma pharmacokinetics of gemcitabine in tumor bearing rats appeared to be similar to that in normal rats at an equivalent dose of 25 mg/kg. This indicated that the presence of brain tumor did not alter the plasma pharmacokinetics of gemcitabine. Thus, our findings suggest that despite the absence of any changes in the plasma pharmacokinetics of gemcitabine due to the presence of a brain tumor, the brain *tumor* distribution coefficient, $AUC_{tumor\ brain}/AUC_{plasma}$ of gemcitabine was higher (0.186), compared to the *non-tumor* brain distribution coefficient, $AUC_{tumor\text{-}free\ brain}/AUC_{plasma}$ (0.085), indicating an altered permeability of gemcitabine into brain tumors due to a differential uptake across the BTB and BBB.

Discussion

In a previously published study, Kerr et al. [7] observed that the mean CSF: plasma concentration ratio for gemcitabine is 6.7%, indicative of overall poor penetration to brain tissues. Thus, the apparent low availability of gemcitabine to the brain tissue may limit its use in the treatment of the CNS malignancies. This is underscored by clinical

Table 1 Brain ECF and plasma pharmacokinetics (Mean ± SD) of gemcitabine from normal (n = 9) and tumor-bearing (n = 7) Sprague-Dawley rats, as obtained by non-compartmental analysis of the concentration versus time data

	Dose (mg/kg)	$AUC_{brain\ ecf}$ (μg h/ml)	AUC_{plasma} (μg h/ml)	Relative brain distribution coefficient
Normal rats	10	2.46 ± 0.7	29.0 ± 5.68	0.084
	25	3.20 ± 1.1	49.0 ± 15.8	0.065
	100	9.06 ± 3.0	104.0 ± 26.7	0.087
Tumor- bearing rats				
Tumor-free region	25	4.52 ± 2.4	52.7 ± 14.8	0.085
Brain tumor region	25	9.82 ± 3.3*	52.7 ± 14.8	0.186

* indicates $P < 0.05$ compared to tumor-free region

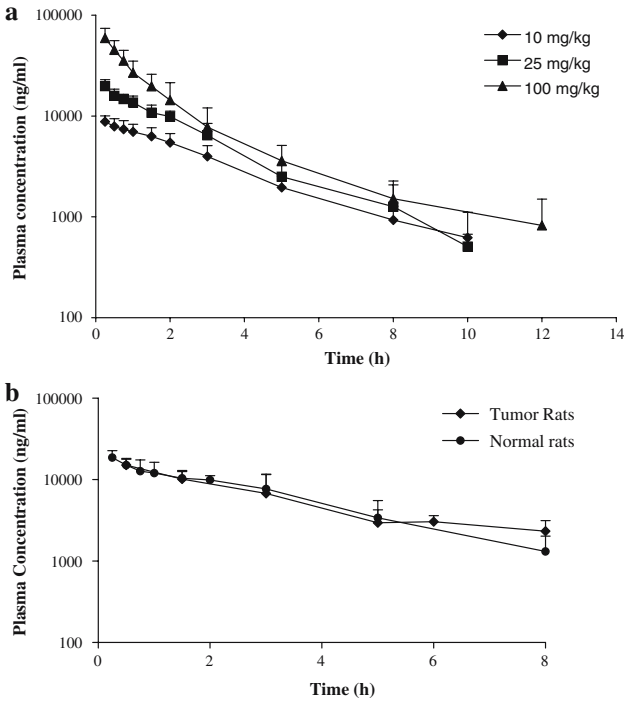

Fig. 4 **a** Plasma concentration versus time profiles of gemcitabine in normal rats following intravenous bolus doses of 10, 25 and 100 mg/kg; values represent mean ± SD obtained from n = 9 rats. **b** Concentration versus time profiles of gemcitabine disposition in the plasma of brain tumor-bearing and normal (control) rats, following intravenous administration of a 25 mg/kg bolus dose. Each point represents the mean ± SD of values obtained from n = 7 rats

reports and case studies, where gemcitabine was observed to have poor efficacy in the treatment of brain tumors. Our study was undertaken to better understand the brain as well as brain tumor disposition of gemcitabine following intravenous administration of the drug.

For many drugs, transport into brain may involve active transport processes at the level of blood–brain barrier or the blood-CSF barrier. Under these circumstances drug concentrations obtained through cerebrospinal fluid sampling may not truly represent drug concentrations at more distant target sites such as brain tumors. In this regard, intracerebral microdialysis technique offers an alternative means for obtaining more predictive drug concentrations at the target site. This technique allows sampling of drug concentrations from extracellular fluid (ECF) of specific regions of the brain including from tumors, thereby providing relevant information regarding the concentrations of drug at the site of action. The relative distribution coefficient of 0.07–0.09 (i.e. 7–9%) observed in our studies are consistent with the aforementioned findings of Kerr et al. and corroborate that the penetration of gemcitabine into the brain tissues is limited.

A significant finding of our study was that the uptake of gemcitabine was markedly higher in brain tumors than in the tumor-free hemisphere of the rat brain, indicating a more permeable tumor vasculature. By combining a well-characterized brain tumor model, with a validated sampling technique such as intracerebral microdialysis, we were able to evaluate the brain tumor pharmacokinetics of gemcitabine. To the best of our knowledge, this study represents the first attempt at utilizing the intracerebral microdialysis procedure to evaluate the uptake and disposition of gemcitabine in an in vivo brain tumor model.

Our observations with gemcitabine are in agreement with similar observations made with other anticancer agents such as methotrexate and cisplatin that also show differential uptake into brain tumor tissue [2, 3, 9]. We first employed a range of gemcitabine doses (10–100 mg/kg) that brackets the clinically relevant dose to assess whether the extent of distribution of gemcitabine into the brain was dose dependent. As indicated earlier, the overall extent of gemcitabine distribution into brain relative to plasma as indicated by the relative brain distribution coefficient remained unchanged over the tenfold dose range. We then compared the ECF AUCs in brain tumor and non-tumor regions at a gemcitabine dose level of 25 mg/kg. A comparison of the brain ECF pharmacokinetics of gemcitabine in normal rats and in the non-tumor hemisphere of glioma-bearing rats revealed no significant differences. A differential uptake across normal- and tumor-brain barriers appears to be responsible for the increased tumor concentrations of gemcitabine observed in the current in vivo brain tumor model. In vitro studies conducted in our lab show the mean IC_{50} value of

Table 2 Plasma Pharmacokinetics (Mean ± SD) of gemcitabine in normal (n = 9) and tumor-bearing (n = 7) Sprague-Dawley rats, as obtained by non-compartmental analysis of the concentration versus time data

	Dose (mg/kg)	C_{max} (μg/ml)	AUC_{0-t} (μg h/ml)	AUC_{0-inf} (μg h/ml)	$T_{1/2}$ (h)
Normal rats	10	9.71 ± 1.11	29.0 ± 5.68	32.60 ± 7.32	2.17 ± 0.46
	25	21.92 ± 4.40	49.0 ± 15.8	53.35 ± 16.4	1.82 ± 0.37
	100	78.71 ± 22.50	104.0 ± 26.7	114.70 ± 29.08	1.75 ± 0.40
Tumor-bearing rats	25	20.3 ± 3.6	52.7 ± 14.8	60 ± 17.6	2.2 ± 0.54

gemcitabine in C6 glioma cell line to be 0.66 ± 0.15 μg/ml (MTT assays, data not shown). Since the gemcitabine peak concentration at a dose of 25 mg/kg in the brain tumor ECF (3.14 μg/ml) were significantly higher than those in non-tumor ECF (0.97 μg/ml) of tumor-bearing rats or the normal brain ECF of healthy rats (0.84 μg/ml), it appears that therapeutic concentrations of gemcitabine may be achieved and maintained for a longer duration of time in brain tumors. Thus, this differentially higher uptake in brain tumors may facilitate higher cytotoxicity of gemcitabine against brain tumor cells.

In summary, our studies demonstrate that the brain ECF: plasma ratio of gemcitabine exposure in healthy rats is in the range of 7–9%. However, in animals implanted with brain tumors, the uptake of gemcitabine was found to be significantly increased, with a brain tumor ECF to plasma ratio of gemcitabine approximately 18%. Further studies employing various doses and clinically relevant dosing regimens are warranted. Given the observed differentially higher gemcitabine levels observed in brain tumor compared to tumor-free region of the brain, our studies support further investigation of gemcitabine in the treatment of brain neoplasms.

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