Pharmacokinetics of Intrathecal Gemcitabine in Nonhuman Primates

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ABSTRACT

Purpose: Gemcitabine is an excellent candidate for regional therapy. We quantified cerebrospinal fluid (CSF) and plasma concentrations of gemcitabine and its inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU), in nonhuman primates given intrathecal gemcitabine.

Experimental Design: Three nonhuman primates received 5 mg of gemcitabine via lateral ventricle. CSF was sampled from the fourth ventricle in all of the animals and the lumbar space in one, and one had plasma sampled. One animal had ventricular CSF sampled after receiving 5 mg intralumbar gemcitabine. Gemcitabine and dFdU were measured by high-performance liquid chromatography. Three additional animals had 5 mg intralumbar gemcitabine administered weekly for 4 weeks and were monitored for toxicity.

Results: At 37°C in vitro, gemcitabine was stable in CSF. Ventricular delivery of gemcitabine produced peak ventricular CSF gemcitabine concentrations of 297 ± 105 μg/ml. After 6 h, the concentrations were <0.03 μg/ml. Intrathecal gemcitabine was rapidly and extensively converted to dFdU. CSF dFdU concentrations increased to 82 μg/ml at 1 h and then declined to very low values by 24 h. After intraventricular administration, CSF gemcitabine and dFdU areas under the curve (AUC) were 251 ± 85 and 249 ± 88 μg/ml × h. Intralumbar gemcitabine produced lower ventricular CSF gemcitabine and dFdU concentrations than did intraventricular gemcitabine. The plasma gemcitabine AUC associated with 5 mg of intraventricular gemcitabine was 2 mg/ml × h, which was >200-fold lower than the CSF gemcitabine AUC in the same animal. Transient CSF pleocytosis was the only toxicity observed.

Conclusions: Our results demonstrate a large pharmacokinetic advantage of intrathecal gemcitabine and support a planned Phase I clinical trial of this dosing strategy.

INTRODUCTION

The leptomeninges are an increasingly common site of recurrence for various malignancies (1–4), in part, because of the limited penetration of most systemically administered anticancer drugs across the blood-CSF barrier (5–7). Direct intrathecal administration of anticancer drugs, either into the ventricles through an intraventricular reservoir or into the thecal sac through lumbar puncture, is one approach to circumventing this problem of the meninges being a pharmacological sanctuary (2–4, 7). However, the effectiveness of this form of regional chemotherapy is restricted, in part, because only a limited number of anticancer drugs can be safely administered into the CSF (2–4, 7).

Gemcitabine (2′, 2′-difluorodeoxycytidine), a deoxycytidine analogue antimetabolite structurally related to 1-β-d-arabinofuranosylcytosine, has documented activity in a wide variety of common human neoplasms, including pancreatic (8–10), non-small cell lung (11–16), small cell lung (16–18), breast (19, 20), bladder (21–23), and testicular carcinomas (24, 25). It also has activity in lymphoma (26–28), ovarian cancer (29, 30), and head and neck cancer (31). Systemically administered gemcitabine crosses the intact blood-CSF barrier poorly (32). The total body clearance of gemcitabine approaches 5 liters/min (33, 34), which approximates cardiac output and is among the highest known clearance of any antineoplastic agent.

The pharmacological advantage of regional delivery can be calculated from the equation (35):

\[
\text{Relative advantage} = \frac{\text{Total body clearance}}{\text{Rate of delivery or absorption into the systemic circulation}}
\]

Therefore, the large value for the total body clearance of gemcitabine predicts there would be a large pharmacological advantage associated with its local delivery. This, combined with its broad spectrum of antitumor activity and a lack of irritative properties makes it an attractive candidate for intrathecal therapy. We used a unique, nonhuman primate model, which was designed for studying pharmacokinetics of drugs in CSF and which has proven to be predictive of human pharmacokinetics.
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Plasma samples were stored at −110°C and ammonium acetate (enzyme grade) were obtained from Calbiochem (San Diego, CA). Tetrahydrouridine was obtained from Calbiochem (San Diego, CA). Control nonhuman primate CSF (San Diego, CA).

Intrathecal Gemcitabine was administered at a dose of 5 mg in 1 ml of 0.9% NaCl, and dFdU concentrations were determined by visual inspection, and area under the concentration versus time curves for gemcitabine and dFdU in CSF, and plasma were calculated noncompartimentally using the LAGran computer program (41).

RESULTS

In Vitro Stability of Gemcitabine in CSF. When incubated for up to 24 h at a concentration of 10 µg/ml in CSF at 37°C, gemcitabine underwent no detectable in vitro decomposition or conversion to dFdU.

In Vivo Pharmacokinetics. In the three animals given intraventricular gemcitabine, peak fourth ventricular CSF gemcitabine eluted at 13 min, and area under the concentration versus time curves for gemcitabine and dFdU in CSF, and plasma were calculated noncompartimentally using the LAGran computer program (41).

The HPLC system used consisted of a Hewlett Packard 1090L HPLC (Hewlett Packard, Palo Alto, CA) fitted with a Waters (Milford, MA) Symmetry C18 (5 µm, 4.6-mm inner diameter × 250 mm) column and a Waters Symmetry guard column. Gemcitabine, dFdU, and internal standard were eluted with a mobile phase that consisted of 50 mm ammonium acetate (pH 5):acetonitrile [97.5:2.5 (v/v)] and was pumped at 1 ml/min. Column eluate was monitored at 280 nm with a Waters 440 absorbance detector, and detector output was integrated with Chrom Perfect chromatography software (Justice Laboratory Software, Palo Alto, CA), so as to integrate the area under the gemcitabine, dFdU, and internal standard peaks. Gemcitabine and dFdU concentrations were determined by calculating the ratio of gemcitabine to dFdU peak area to that of the respective internal standard in each sample and comparing that ratio to a concomitantly performed standard curve that included concentrations between 0 and 50 µg/ml. Under these conditions, gemcitabine eluted at ~7.7 min, dFdU eluted at ~13 min, and internal standard eluted at ~31.1 min. The lower limit of quantitation was 0.03 µg/ml, and the assay was linear between 0 and 50 µg/ml.

Pharmacokinetic Analysis. Peak concentrations of gemcitabine and dFdU were determined by visual inspection, and area under the concentration versus time curves for gemcitabine and dFdU in CSF, and plasma were calculated noncompartimentally using the LAGran computer program (41).

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citabine concentrations were 309, 397, and 187 μg/ml, respectively (Fig. 1A). Gemcitabine concentrations in ventricular CSF subsequently declined monoexponentially and were not detectable beyond 6 h after gemcitabine administration (Fig. 1A). The decay constants of gemcitabine in the CSF of these three animals were 1.28, 1.46, and 0.90 h⁻¹, corresponding to half-lives of 0.54, 0.47, and 0.77 h, respectively. dFdU appeared rapidly in the CSF (Fig. 1B). After intraventricular administration of gemcitabine, peak CSF dFdU concentrations of 85, 114, and 58 μg/ml occurred at 1–2 h and then declined rapidly (Fig. 1B). However, at 24 h, CSF dFdU concentrations still remained above the lower limit of quantitation of the assay used in these studies (Fig. 1B). The decay constants associated with the decline in CSF dFdU concentrations were 0.22, 0.29, and 0.17 h⁻¹, respectively. In the one animal given intraventricular gemcitabine and in whom lumbar, as well as ventricular, CSF samples were obtained, there was evidence of gemcitabine distribution from ventricular to lumbar CSF (Fig. 2).

Despite exceedingly high concentrations of gemcitabine and its inactive dFdU metabolite in the CSF of monkeys given intraventricular gemcitabine, plasma concentrations of both gemcitabine and dFdU were orders of magnitude lower (Fig. 3). When the area under the CSF gemcitabine concentration versus time curve (414 μg/ml h) associated with intraventricular administration versus the area under the plasma gemcitabine versus time curve (2.0 μg/ml h) in the same animal, there was a >200-fold higher exposure in the CSF, thus confirming the pharmacokinetic advantage of regional administration.

Data from the one animal in whom ventricular concentrations of gemcitabine and dFdU were measured after both intraventricular and intralumbar administration of gemcitabine dem-
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The ventricular CSF gemcitabine area under the concentration-time curve produced by intralumbar administration of gemcitabine fulfills each of these requirements and, therefore, represents an excellent candidate for intrathecal administration. The desirable characteristics of such new agents would include: demonstrated activity against tumor types known to metastasize to the meninges; a low likelihood of producing local toxicities; and a high total body clearance, which would translate into a substantial pharmacokinetic advantage for local or regional therapy.

Gemcitabine is known to undergo extensive metabolism by deoxycytidine deaminase to the inactive metabolite dFdU, thereby accounting for the majority of its systemic clearance. Although gemcitabine proved to be stable in vitro and in control nonhuman primate CSF, each of the in vivo studies described provided evidence of extensive conversion of gemcitabine to dFdU within the intrathecal space. It is unclear whether this metabolism occurs in the meninges or in the brain parenchyma, although immunohistochemical staining of these tissues for cytidine deaminase could help address this question. The data presented confirm the predicted large pharmacokinetic advantage associated with intrathecally administered gemcitabine. The studies presented in the current manuscript substantiate the assumptions made in undertaking these studies. Gemcitabine is known to undergo extensive metabolism by deoxycytidine deaminase to the inactive metabolite dFdU, thereby accounting for the majority of its systemic clearance.

A transient CSF pleocytosis, a common finding after intrathecal drug administration in the model used in these studies, was observed in all of the animals after intralumbar gemcitabine. The baseline CSF WBCs for animals 1, 2, and 3 were 50, 30, and 20 cells/μl, respectively. Peak CSF WBCs in all three of the animals occurred in week 2 and were between 110 and 130 cells/μl. There were no clinically significant changes in blood counts or serum chemistries after intra-CSF gemcitabine administration. No other significant acute or chronic neurological or systemic toxicities were observed after chronic intralumbar gemcitabine administration.

DISCUSSION

Metastasis of malignant cells to the meninges is associated with an exceedingly poor prognosis. The ability of the blood-CSF-barrier to limit access of systemically administered anticancer chemotherapy to the meninges facilitates the survival of microscopic subclinical disease, which might otherwise be eradicated, and greatly reduces the efficacy of systemically administered antineoplastic chemotherapy against more extensive, clinically evident disease. One strategy to circumvent this pharmacological sanctuary is direct intrathecal administration of anticancer drugs. However, the limited number of agents available for such administration provides an ongoing incentive to develop new agents that might prove more effective or that might be combined with other agents already available for intrathecal administration. The desireable characteristics of such new agents would include: demonstrated activity against tumor types known to metastasize to the meninges; a low likelihood of producing local toxicities; and a high total body clearance, which would translate into a substantial pharmacokinetic advantage for local or regional therapy.

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administration of gemcitabine in the present studies prompted us to perform subsequent chronic toxicity studies, which confirmed the lack of acute or chronic toxicity associated with repeated intraventricular administration of gemcitabine. These encouraging results, when combined with confirmation of the predicted great pharmacokinetic advantage associated with intrathecal administration of gemcitabine and the known spectrum of activity of gemcitabine against a variety of human neoplasms, has allowed us to design and initiate a Phase I clinical trial of this strategy.

ACKNOWLEDGMENTS

We greatly appreciate the excellent clerical assistance of Ezekiel McCuffy and the encouragement and constructive criticisms of Drs. Robert Dedrick and Jerry Collins.

REFERENCES


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