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Intrathecal 5-fluorouracil in the rhesus monkey

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Summary. Because meningeal spread of both leukemia and solid tumors remains a difficult therapeutic problem, there is a compelling need to develop new agents for intrathecal administration. 5-Fluorouracil (5FU), an active anticancer agent, penetrates into the central nervous system to some degree following intravenous dosing. Significant systemic toxicity, however, is associated with this route of administration. Therefore, the pharmacokinetic behavior of 5FU following its intrathecal administration was studied in a rhesus monkey model. After a 10-mg intraventricular dose, the disappearance of the drug from ventricular cerebrospinal fluid was monoexponential, the half-life being 51 min; the area under the concentration-time curve (AUC) being greater than 18 mM h^{-1}; and the peak ventricular 5FU concentrations ranging between 10 and 15 mM. After a 1-mg intralumbar dose, the AUC was 1235 μM h^{-1}. No toxicity was observed following intraventricular administration of 5FU. After intralumbar administration of either a 10-mg or a 1-mg dose, however, local toxicity was observed in the lumbar spinal cord. These findings suggest that intrathecal administration of 5FU is not presently a feasible means of achieving cytotoxic cerebrospinal fluid concentrations.

Introduction

Despite advances in the chemotherapy of leukemia and solid tumors, meningeal spread of malignant diseases remains a difficult therapeutic challenge in both children and adults. Following systemic administration, some chemotherapeutic agents may penetrate effectively into the central nervous system when given in high doses, but systemic toxicity often limits this approach. Administration of agents directly into the cerebrospinal fluid (intrathecally) results in very high drug concentrations at the target site and circumvents systemic toxicities. The intrathecal approach, however, is limited by the paucity of drugs that have been proven to be safe and effective when delivered by this route. Methotrexate, cytarabine, and thiotepa have been used to treat menigeal malignancy. However, intrathecal therapy is usually confined to the administration of single agents due to the limited number of available drugs, whereas combination therapy has been demonstrated to be superior in the treatment of systemic disease. Thus, there is a compelling need to develop new agents for intrathecal use.

5-fluorouracil (5FU) is a rationally synthesized halogenated pyrimidine analog [6] with activity against a variety of solid tumors, including breast cancer, which has a propensity to metastasize to the central nervous system [5]. We have studied the cerebrospinal fluid (CSF) pharmacokinetics of 5FU in the rhesus monkey following systemic administration and determined that CSF penetration was highly schedule-dependent [9]. After a 20-min intravenous infusion of 520 mg/m^2, the CSF:plasma ratio was 48% and the peak CSF concentration was 70 μM; when the same dose was given over 4 h, the CSF:plasma ratio was 20% and the peak CSF concentration was 15 μM. These CSF 5FU concentrations approximate the in vitro inhibitory concentrations described for a number of human and murine cell lines [10, 19]. However, the relatively favorable CSF penetration of 5FU after its intravenous administration must be balanced against systemic toxicities such as myelosuppression and gastrointestinal tract toxicity, which are dose-limiting for 5FU [5].

Because of the success of other antimetabolites following their intrathecal delivery and because the antitumor spectrum of 5FU includes a number of solid tumors, we investigated the pharmacokinetics and feasibility of intrathecal administration of 5FU in a nonhuman primate model.

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Materials and methods

Animals. Adult male rhesus monkeys ranging in weight from 6 to 12 kg were used in this study. The animals were fed NIH Open Formula Extruded Nonhuman Primate Diet twice daily and were individually housed in accordance with the Guide for the Care and Use of Laboratory Animals [2].

Drug administration. 5FU was obtained from Lyphomed (Rosemont, Ill.) in vials containing 1 g 5FU/20 ml solution supplemented with NaOH for pH adjustment. This formulation was further diluted with Elliott's B solution, a physiologic buffer, for intrathecal administration. The pH of a 10-mg dose prepared in this manner is 9.0 and that of a 1-mg dose is 7.0.

For intraventricular administration, a 10-mg dose of 5-FU at a final concentration of 12.5 mg/ml was injected into the fourth ventricle via an Ommaya reservoir [15]. The reservoir was then flushed slowly with 1 ml Elliott's B solution. For intralumbar administration, the drug was diluted in Elliott's B solution to a final volume of 1 ml and delivered by lumbar puncture followed by a 0.5-ml Elliott's B flush. Catheter placement was verified by the free flow of CSF.

Pharmacokinetics. The pharmacokinetic behaviour of 5FU was studied in three rhesus monkeys following a 10-mg intraventricular dose and in a single, different animal following a 1-mg intralumbar dose. CSF samples (0.3 ml) were drawn through an Ommaya reservoir that was pumped three to four times before and after sampling or through a temporary lumbar catheter. In the animals receiving a 10-mg intraventricular dose, ventricular CSF was sampled before dosing and at 0.5, 1.5, 2, 3, 4, 6, 8, 12, and 24 h following the dose. In addition, lumbar CSF was obtained from one animal prior to dosing and at 2, 4, 6, and 8 h after the dose. In the animal receiving a 1-mg intralumbar dose, lumbar CSF was obtained before dosing and at 0.5, 2, 4, 6, and 8 h after the dose. Samples were frozen immediately and stored at –20° C until anlayzed.

The 5FU concentration was measured using a modification of a previously described high-performance liquid chromatographic (HPLC) assay [11]. In brief, 10–50 µl CSF was injected directly onto a Beckman Resolve column (8 mm × 10 cm) in a Waters Radial Compression Module and was eluted isocratically with 0.05% acetic acid at 1 ml/min. The retention time was 8.5 min.

Concentration-time data from the 1-h infusion experiments were fitted to both monoexponential ($n = 1$) and biexponential ($n = 2$) equations with MLAB [12] 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 γ_i is the rate constant. Aikake's information criterion was used to determine the best-fit equation [20]. The half-life of elimination was calculated by dividing 0.693 by the rate constant. Other pharmacokinetic parameters were calculated using model-independent methods.

Toxicology. Following the single 10-mg intraventricular dosing of 5FU in three animals for pharmacokinetic evaluation as described above, 5FU was given weekly for up to 8 weeks via the lumbar route to evaluate the potential toxicity of chronic dosing. This study did not attempt to evaluate the toxicity of long-term 5FU administration. Each animal was treated at only one dose level (10 or 1 mg); animals that received intraventricular 5FU did not subsequently undergo intralumbar drug treatment.

Results

Pharmacokinetics

The disposition of 5FU in the ventricular and lumbar CSF following a 10-mg intraventricular dose is shown in Fig. 1.

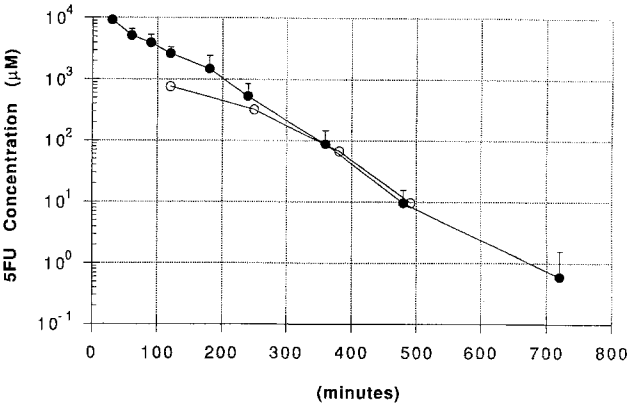

Fig. 1. 5FU concentrations in ventricular (●) and lumbar (○) CSF following a 10-mg intraventricular dose

Table 1. Pharmacokinetic parameters of 5FU in ventricular and lumbar CSF after intraventricular administration of a 10-mg dose

Animal number	Peak concentration (mM)	$t_{1/2}$ (min)	AUC (mM h^{-1})	Cl (ml/min)
835	14.4	51.1	18.7	0.07
970	15.3	55.2	21.5	0.06
8548	12.6	45.4	14.5	0.09
Mean ventricular	14.1	50.6	18.2	0.072
835 (lumbar)	2.6	51.1	5.06	0.03

Disappearance was monoexponential, the half-life being 51 min; the clearance, 0.07 ml/min; and the area under the concentration-time curve (AUC), 18 mM h^{-1} (Table 1). Following a 1-mg intralumbar dose, the clearance of 5FU from lumbar CSF was 0.10 ml/min, the half-life was 38 min, and the AUC was 1235 µM h^{-1}.

Toxicity

The animals that received a single 10-mg intraventricular dose of 5FU tolerated the drug well, showing no sign of neurotoxicity. However, two animals that received 10-mg intralumbar doses exhibited the acute onset of bilateral hind-limb paralysis, one at 4 days after the fifth weekly dose and the other at 10 weeks after the injection of a single dose (intrathecal 5FU was stopped in the second animal when toxicity was observed in the first). Subsquently, the dose of intrathecal 5FU was reduced to 1 mg and further toxicology studies were performed. A single animal receiving 1 mg weekly via the intralumbar route developed the same clinical signs 18 days after completing a course of eight weekly injections.

On necropsy, the spinal cords of the three animals with hind-limb paralysis demonstrated severe necrosis of the ventral horn of the sacral spinal cord in one animal, ballooning degeneration of myelin sheaths in the spinal nerve radicles of the lumbar cord and cauda equina in another, and both demyelination of the lumbar and sacral cord and ventral horn necrosis in the third (Fig. 2). No abnormality was seen in the cerebella of these animals.

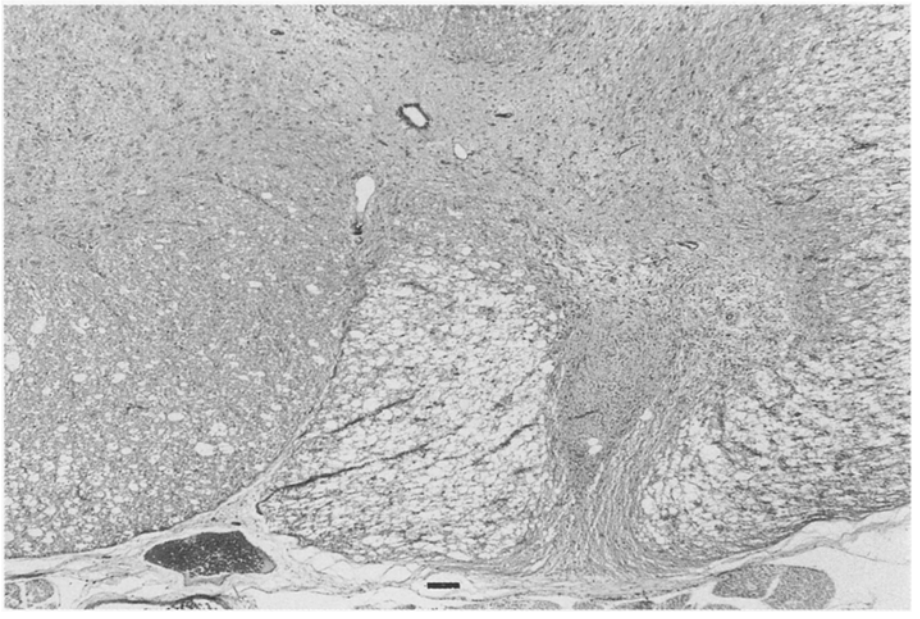

Fig. 2. Lumbar spinal cord of a rhesus monkey following intralumbar injection of 5FU. Note the severe unilateral necrosis of the ventral horn and the myelin degeneration of white fiber tracts. H&E stain. Bar = 100 μm

Discussion

Pharmacokinetics studies demonstrated an advantage for regional administration of 5FU. The mean AUC value for 5FU in ventricular CSF after the intraventricular administration of a 10-mg dose was over 18 mM h^{-1}, and the peak concentration was 10–15 mM. The corresponding AUC in the lumbar space was 5.06 mM h^{-1}, and the peak level was 760 μM at 2 h after administration. Thus, the ratio of ventricular: lumbar AUC was approximately 0.3. In contrast, the ventricular CSF AUC achieved after an intravenous bolus dose of 520 mg/m^2 in the same model was 221 μM h^{-1} [9]. Therefore, the ventricular AUC was 80 times greater following intrathecal administration of 2% of the systemic dose. Even after a 1-mg dose, the AUC was more than 5-fold that achieved following intravenous dosing.

The three animals that received a single 10-mg dose of 5FU by the intraventricular route showed no sign of toxicity. However, two animals that received the same dose by the intralumbar route and a single animal that received eight weekly 1-mg doses of intralumbar 5FU developed clinical signs of spinal cord toxicity and showed demyelination or local necrosis of neurons in the spinal cord in the region of 5FU instillation at necropsy. The localized nature of the pathology together with the finding that a single dose was sufficient to produce this toxicity in one animal suggests that the neurotoxicity observed resulted from the high local concentrations of 5FU produced by administration into the lumbar space.

Toxicity similar to that observed after intrathecal administration of 5FU in the monkeys has been observed in humans following the intrathecal administration of other antimetabolites. Spinal cord dysfunction ranging from transient pain or weakness to frank paraplegia has been reported following intrathecal administration of methotrexate or, less commonly, cytarabine. In cases in which an autopsy was performed, profound spinal cord demyelination was observed [1, 4, 17]. It has been suggested that in the case of methotrexate, toxicities may result from unusually prolonged elevations of CSF drug levels [1]. Both the clinical and the pathological features of methotrexate and cytarabine spinal cord toxicity in humans are similar to those we observed following intrathecal 5FU administration in the monkey. This similarity suggests that such spinal cord toxicity may be common to the class of antimetabolites in general rather than being a specific feature of 5FU.

The neurotoxicity observed in this study is clearly different from that seen in humans after high-dose systemic 5FU therapy, which is characterized by an acute cerebellar syndrome associated with dysmetria, ataxia, and, on occasion, a more global encephalopathy [8, 14, 16]. It has been postulated that 5FU toxicity results from the production of metabolites such as fluorocitrate that are toxic to the central nervous system by virtue of their inhibition of important enzymatic pathways [13]. However, the predominant metabolite of 5FU excreted in the urine is fluoro-β-alanine; no fluorocitrate has been detected [7]. Furthermore, several patients with familial dipyrimidine dehydrogenase deficiency, who were thus incapable of metabolizing 5FU, exhibited severe neurotoxicity after 5FU therapy [3, 18], suggesting that the toxicity was due to 5FU itself and not to the production of toxic metabolites. In the present study, neither cerebellar toxicity nor cerebral dysfunction was observed. It remains unclear as to whether the local toxicity observed following intralumbar instillation of 5FU might be produced by the same mechanism that causes the cerebellar and cerebral dysfunction that is sometimes seen after intravenous administration of this agent.

This study raises important concerns regarding the development of 5FU as an intrathecal agent. Although the pharmacokinetics studies demonstrate an advantage for intrathecal over intravenous dosing in terms of total exposure (AUC), both routes of administration result in peak CSF levels are well above the growth-inhibitory concentration for 50% of the cells (IC_{50}) reported for human leukemia

cell lines in vitro [10]. In addition, if the intrathecal dose of 5FU were lowered to 0.1 mg in the monkey, the predicted AUC derived from the pharmacokinetic parameters presented in this report would be 200 $\mu M\ h^{-1}$. This value is virtually identical to the AUC of 220 $\mu M\ h^{-1}$ obtained after a single intravenous bolus dose of 520 mg/m^2 in the monkey [9]. Thus, the advantage of intrathecal administration of 5FU in terms of increased regional exposure would be lost at a dose of 0.1 mg. Furthermore, although intrathecal administration circumvents systemic toxicity, the unexpected finding of spinal cord toxicity makes intralumbar administration unsafe. Alternate strategies for drug delivery, such as low-dose "concentration times time" administration via an Ommaya reservoir or continuous intraventricular infusion, might offer a means of circumventing the neurotoxicity resulting from high local peak drug concentrations. At present, however, intrathecal administration does not appear to be a feasible means of attaining potentially cytotoxic 5FU concentrations.

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