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Intrathecal administration of topotecan in nonhuman primates

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Abstract The cerebrospinal fluid (CSF) pharmacokinetics of topotecan were studied in a nonhuman primate model following intraventricular administration of 0.1 mg. Lactone and total drug concentrations were measured using a reverse-phase HPLC method with fluorescence detection. The mean peak concentrations of lactone and total drug in ventricular CSF were 83 ± 18 μM and 88 ± 25 μM, respectively. CSF drug elimination of the lactone was bi-exponential with a terminal half-life of 1.3 h. The mean clearance from ventricular CSF was 0.075 ml/min for the lactone and 0.043 ml/min for total drug. The ventricular CSF drug exposure (AUC) to lactone was 450-fold greater following intraventricular administration of 0.1 mg topotecan than after systemic intravenous administration of a 40-fold higher dose (10 mg/m^2). Peak lumbar concentrations ($n = 1$), which occurred 2 h after intraventricular drug administration, were 0.98 μM and 2.95 μM for the lactone and total drug, respectively. A transient CSF pleocytosis was observed in one animal following intraventricular topotecan administration and in one animal following intralumbar topotecan administration. No other acute or chronic neurologic or systemic toxicities were observed following a single intraventricular dose or weekly ($\times 4$) intralumbar topotecan. Compared with systemic topotecan, intrathecal administration provided a significant pharmacokinetic advantage in terms of CSF drug exposure and did not produce any significant neurotoxicity in a nonhuman primate model. Intrathecal topotecan should be evaluated clinically as a potential alternative therapy for refractory meningeal tumors.

Key words Topotecan · Meningeal malignancies Intrathecal

Introduction

The meninges, protected by the blood–brain barrier from the cytotoxic effects of systemic anticancer chemotherapy, are a unique site of recurrence for certain malignancies. One strategy to circumvent this pharmacologic sanctuary is direct intrathecal administration of anticancer drugs. However, there are currently a limited number of agents that have been demonstrated to be safe when administered by this route. This paucity of safe and effective intrathecal drugs frequently forces the clinician to rely on single-agent therapy for the treatment of meningeal disease, despite the fact that combination chemotherapy has been shown to be superior to monotherapy for the treatment of systemic malignancy. Thus, there is an urgent need to develop new agents for intrathecal administration.

Topotecan is a new water-soluble topoisomerase I inhibitor, which demonstrates a high degree of antitumor activity in a broad spectrum of murine tumors and in human tumor xenograft models [10, 11]. Antitumor activity also has been observed in phase I studies of topotecan [6, 9, 14, 15]. Following a short intravenous infusion in nonhuman primates, topotecan has been shown to penetrate into the cerebrospinal fluid

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(CSF) to a significant degree and has not been associated with neurotoxicity [4]. Based on the promising preclinical and clinical antitumor activity of topotecan, its novel mechanism of action, and the lack of neurologic toxicity in both nonhuman primates and humans following systemic administration, further preclinical pharmacokinetic and toxicity studies were performed to determine the feasibility of intrathecal topotecan administration.

Materials and methods

Drugs

Topotecan (hydrochloride salt, adjusted to pH 3–4) was supplied by the Division of Cancer Treatment, NCI (Bethesda, Md) in 5-mg vials which were reconstituted in 2 ml of normal saline. The drug was further diluted with normal saline to a final concentration of 0.1 mg/ml. The drug solution (1 ml) was injected into the Ommaya reservoir, after which the reservoir was pumped four to six times to ensure adequate mixing throughout the ventricular system.

Monkeys.

Six adult male rhesus monkeys (*Macaca mulatta*) weighing 6.2–11.1 kg were used in these experiments. The animals were fed Open Formula Extruded Non-Human Primate Diet twice daily and group-housed in accordance with Guide for the Care and Use of Laboratory Animals [8]. Ventricular CSF samples were obtained from a chronically indwelling fourth ventricular catheter attached to a subcutaneously implanted Ommaya reservoir [13]. Lumbar CSF samples were obtained from an indwelling temporary lumbar catheter in one animal. Blood samples were drawn through a catheter placed in either the femoral or the saphenous vein.

Experiments

The CSF and plasma pharmacokinetics of topotecan were studied in three animals following an intraventricular dose of 0.1 mg. Ventricular CSF was collected immediately prior to and at 5 min, 30 min, and 1, 1.5, 2, 3, 4, 6, and 8 h after the dose. The reservoir was pumped ten times before and after each sample collection to ensure adequate mixing with ventricular CSF. Lumbar CSF samples were collected in one animal at 1, 2, 3, 4, 6, 8, and 10 h. Blood samples were collected in two animals immediately before and at 5 min, 30 min, and 1, 1.5, 2, and 3 h after the dose. Plasma was separated immediately by centrifugation at 12000 *g* for 2 min in a rapid acceleration/deceleration centrifuge.

Three additional animals received a 0.1 mg intralumbar dose of topotecan weekly for 4 weeks to determine if there was acute or cumulative toxicity (systemic or neurologic) following administration of multiple intrathecal doses. Lumbar CSF was obtained for cell counts and protein and glucose concentrations prior to each drug dose and weekly for 2–4 weeks following the last intralumbar dose. Complete blood counts and serum chemistries were determined at the same time. The animals were closely observed for any other evidence of neurologic or systemic toxicity.

Sample analysis

In solution, topotecan (lactone) is unstable and undergoes reversible hydrolysis to a less-active hydroxy acid (open-ring) form. At physiologic pH, the equilibrium favors the hydroxy acid form. Topotecan concentrations (total drug and lactone) were measured in plasma and CSF using a recently described reverse phase HPLC assay [5]. CSF samples were diluted with mobile phase to bring the concentration within the range of the standards and analyzed for lactone by immediate direct injection of 20–100 µl aliquots onto the high-performance liquid chromatography (HPLC) column. A second aliquot of CSF was acidified with concentrated phosphoric acid in order to quantitate the total drug (lactone and open-ring species). The acidified sample was injected after it had been at room temperature for at least 2 h. Plasma samples were immediately extracted using activated Fisher PrepSep C18 columns. Topotecan was eluted from the column with methanol. The sample was analyzed for lactone by immediate injection of an aliquot of the eluant onto the HPLC column. The remaining cartridge eluant was acidified with 2% phosphoric acid in order to quantitate the total drug (lactone and open-ring species) as described above. Topotecan was detected using a Spectroflow 980 fluorescence detector (Applied Biosystems, Ramsey, N.J.) at a λ_{ex} of 375 nm and a λ_{em} of 470 nm (cutoff filter). Under these conditions, the open-ring metabolite eluted with the solvent front and the lactone eluted at 4 min. Standard curves in monkey CSF and plasma were prepared for each experiment by addition of known amounts of topotecan to plasma or CSF, respectively. Standard curves were linear ($r^2 > 0.995$) over the range 0.002–1 µ*M*. The lower limit of quantitation was 0.002 µ*M*.

Pharmacokinetic Analysis

The half-lives of the lactone and total drug were estimated by nonlinear regression analysis using the MLAB program [12]. Noncompartmental methods were used to calculate other pharmacokinetic parameters. The area under the drug concentration versus time curve (AUC) was derived by the linear trapezoidal method [7], and extrapolated to infinity. CSF clearance following intraventricular topotecan was calculated by dividing the dose by the AUC.

Results

Pharmacokinetics

The mean peak levels of lactone and total drug in the ventricular CSF following intraventricular administration of a 0.1 mg topotecan dose were 83 ± 18 µ*M* and 88 ± 25 µ*M*, respectively. Lactone and total drug were rapidly eliminated from the CSF. The mean clearance of lactone from the ventricular CSF was 0.075 ml/min (range 0.076–0.121 ml/min) and total drug clearance was 0.043 ml/min (range 0.061–0.087 ml/min). The CSF disappearance of both lactone and total drug following intraventricular topotecan (Fig. 1) was bi-exponential with a mean terminal half-life of 1.3 h (range 1.1–1.6 h) for the lactone and 1.8 h (range 1.2–2.6 h) for total drug. The values of pharmacokinetic parameters for the lactone and total drug following an intraventricular dose are shown in Table 1.

Simultaneous lumbar CSF samples were measured in one animal following intraventricular topotecan (Fig. 2). Lumbar topotecan concentrations peaked at 0.98 µ*M* for the lactone and 2.95 µ*M* for total drug. The

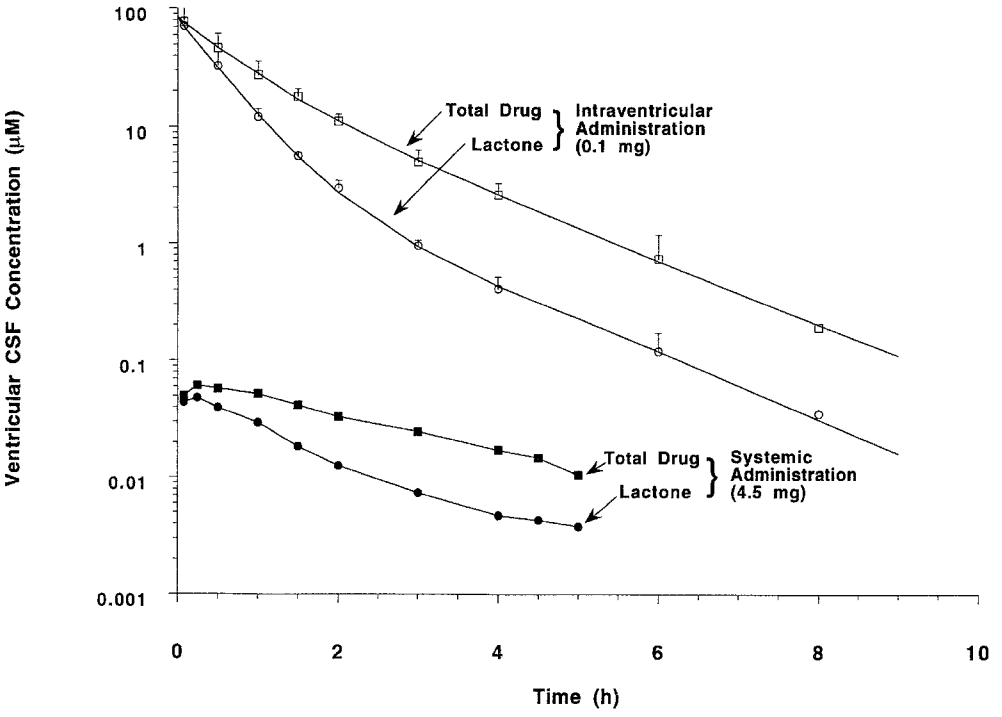

Fig. 1 Ventricular CSF concentration–time curves of topotecan, total drug (□) and lactone (○), following an intraventricular dose of 0.1 mg (*Points* Geometric mean from three animals; *bars*, SD) Ventricular CSF concentrations of topotecan, total drug (■) and lactone (●), following intravenous administration of a 10 mg/m^2 (total dose 4.5 mg) dose in a single animal are also shown.

Table 1 Pharmacokinetic parameters for intraventricular topotecan (0.1 mg) in three animals (*T.D.* Total drug)

Monkey	AUC Lactone (μM · h)	AUC T.D. (μM · h)	AUC Lactone/T.D.	Clearance Lactone (ml/min)	Clearance T.D. (ml/min)	$t_{1/2}\alpha$ Lactone (h)	$t_{1/2}\beta$ Lactone (h)	$t_{1/2}\alpha$ T.D. (h)	$t_{1/2}\beta$ T.D. (h)
141	60.7	116	0.52	0.061	0.031	0.35	1.6	0.50	1.6
X940	41.8	66.7	0.63	0.087	0.055	0.33	1.1	0.65	1.2
625	48.4	84.3	0.57	0.076	0.043	0.37	1.1	0.69	2.6
Mean ± SE	50.3 ± 9.6	89 ± 25	0.57 ± 0.06	0.075 ± 0.013	0.043 ± 0.012	0.35 ± 0.02	1.3 ± 0.3	0.61 ± 0.1	1.8 ± 0.7

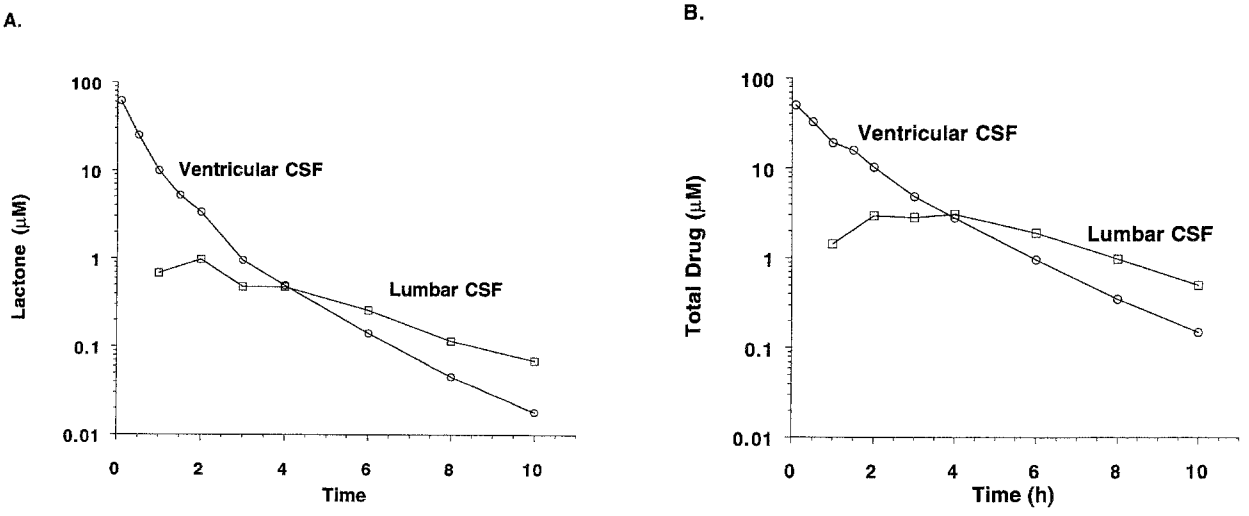

Fig. 2A,B Representative concentration versus time curves from a single animal (animal X940) in ventricular and lumbar CSF following a 0.1 mg intraventricular dose of topotecan. **A** lactone, **B** total drug

peak levels occurred at 2 h following intraventricular drug administration. The AUCs were 3.7 μM · h for the lactone and 18.0 μM · h for total drug. Lumbar CSF exposures to the lactone and total drug were 8.6% and 26.1%, respectively, of the ventricular CSF exposure. Plasma concentrations of topotecan (lactone and total drug) were below the lower limits of assay quantitation (0.002 μ*M*).

A transient CSF pleocytosis was observed in one animal following intraventricular topotecan administration and in another animal after the first intralumbar topotecan dose. A pleocytosis was not observed following subsequent intralumbar doses or in the other animals. Complete blood counts and serum chemistries were not affected by intraventricular or intralumbar topotecan. No other acute or chronic neurologic or systemic toxicities were observed in the 12-month period following intrathecal topotecan.

Discussion

The preclinical pharmacokinetics and toxicity of intrathecal topotecan were studied in a nonhuman primate model that has been successfully used for the preclinical development of other intrathecal agents [2, 3]. Despite the rapid clearance of topotecan from the CSF after an intraventricular dose, high concentrations of drug were achieved in both the ventricular and lumbar CSF. Plasma concentrations of topotecan were below the lower limits of assay quantitation, which is consistent with the lack of systemic toxicity. In addition, there was no acute or chronic neurotoxicity observed following administration of either a single intraventricular dose of topotecan or intralumbar topotecan administered weekly for 4 weeks. As in plasma, the hydrolysis of the lactone form (parent drug) of topotecan in the CSF was rapid with greater than 50% conversion to the less-active open-ring form within 90 min of drug administration. This is consistent with the finding that lactone CSF clearance was 2 times greater than CSF bulk flow (0.034 ml/min) [1], while total drug CSF clearance was only slightly greater than CSF bulk flow.

Comparison of the CSF exposure following intravenous topotecan administration versus intraventricular administration revealed that there was a significant pharmacokinetic advantage with intraventricular administration. The mean AUC in ventricular CSF was 450-fold greater for lactone and 350-fold greater for total drug following intraventricular topotecan administration, despite a 40-fold lower total dose (Fig. 1). Thus, intrathecal drug administration may potentially be a means to circumvent the toxicity associated with systemic drug administration.

Although the lumbar exposure to topotecan was lower than ventricular exposure in the single animal in which lumbar levels were measured, peak lactone concentrations were almost 1 μM and concentrations above 65 nM were maintained for at least 10 h. The exposure to lactone versus total drug (AUC lactone/AUC total drug.) was also higher in the ventricular CSF versus the lumbar CSF (63% versus 20%). These findings are consistent with diffusion of topotecan from the CSF and conversion of lactone to the hydroxyacid form of topotecan while the CSF circulates from the ventricular space to the lumbar space.

In conclusion, these preclinical studies suggest that intrathecal topotecan administration is feasible and achieves high concentrations of drug in the CSF without evidence of neurotoxicity. Phase I studies of intrathecal topotecan are planned.

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