A Four-Hour Topotecan Infusion Achieves Cytotoxic Exposure throughout the Neuraxis in the Nonhuman Primate Model: Implications for Treatment of Children with Metastatic Medulloblastoma


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ABSTRACT

The purpose of this study was to define the length of topotecan (TPT) i.v. infusion necessary to attain a cytotoxic exposure for medulloblastoma cells throughout the neuraxis. In vitro studies of human medulloblastoma cell lines (Daoy, SJ-Med3) were used to estimate the length and extent of TPT systemic exposure associated with inhibition of tumor cell growth or the exposure duration threshold (EDT). We evaluated TPT systemic and cerebrospinal fluid (CSF) disposition in six male rhesus monkeys (8–12 kg) that received TPT 2.0 mg/m² i.v. as a 30-min or 4-h infusion. Plasma and CSF samples were assayed for TPT lactone by high-performance liquid chromatography, and the CSF exposures were compared with the estimated EDT. Results of the in vitro studies defined an EDT as a TPT lactone concentration of >1 ng/ml for 8 h (IC₅₀) daily for 5 days. The mean ± SD for systemic clearance (CLsys), penetration into fourth ventricle (%CSF₄v), and penetration into lumbar space (%CSF₅₆) were similar for the 30-min and the 4-h infusions. At a TPT lactone systemic exposure (AUCₚ₅) of 56.7 ± 19.9 ng/ml-h, time above 1 ng/ml in the fourth ventricle was 1.4-fold greater for a 4-h infusion compared with a 30-min infusion. At a TPT lactone AUCₚ₅ of 140 ng/ml-h, the 4-h infusion achieved the desired TPT exposure throughout the neuraxis (lateral and fourth ventricles and lumbar space), whereas the 30-min infusion failed to achieve it in the lumbar space. In conclusion, prolonging TPT i.v. infusion from 30-min to 4-h at a targeted AUCₚ₅ achieves the EDT throughout the neuraxis and represents an alternative method of TPT administration that will be tested prospectively in patients with high-risk medulloblastoma.

INTRODUCTION

Embryonal CNS tumors such as medulloblastoma have a high propensity to disseminate through the subarachnoid space (1–3). Children with localized medulloblastoma and who have a complete surgical excision followed by CSI and adjuvant chemotherapy have a 5-year event-free survival of 65–75% (4–7). However, children with metastatic disease at diagnosis who are treated with a similar approach have a 5-year event-free survival of 35–45%. In addition, infants with medulloblastoma have a poor prognosis because the majority of them have metastatic disease at diagnosis (1, 2). Recently published national trials have attempted to delay CSI in infants by using chemotherapy (2, 3). Unfortunately, a majority of infants with metastatic disease have disease progression when receiving chemotherapy and then must receive CSI. Because of the potential CNS toxicities associated with CSI, including intellectual and neuroendocrine abnormalities, alternative treatment approaches must be explored (8–10). The occurrence of metastatic disease throughout the neuraxis suggests that the CSF is an important source of drug exposure. Intra-thecal chemotherapy is an attractive method to achieve cytotoxic exposure in the neuraxis. However, practical limitations such as the frequency of administration, problems associated with administration in small children, risk of infection, and CSF-flow dynamics in patients with ventricular-access devices have limited the widespread use of this approach (1).

Systemic administration of chemotherapy is an alternative approach to achieve cytotoxic exposure in the neuraxis. This approach will circumvent the problems associated with intra-thecal administration mentioned above. The prototypical agent

Received 4/22/98; revised 7/8/98; accepted 7/10/98.
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1 Supported by USPHS awards CA23099, CA63512, Cancer Center Support Grant CA21765, and by American Lebanese, Syrian Associated Charities (ALSAC).

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The abbreviations used are: CNS, central nervous system; TPT, topotecan; CSI, craniospinal irradiation; CSF, cerebrospinal fluid; EDT, exposure duration threshold; AUC, area under the plasma concentration-time curve.
Cytotoxic TPT Exposure in Neuraxis with 4-Hour Infusion

TPT has relatively high penetration into the CSF (30–40%) in children administered on protracted schedule (Ia camptothecin analogue and topoisomerase I interactive agent, medulloblastoma and have high penetration into the CSF. TNP', for use in systemic therapy should demonstrate activity against cell lines. The concentration and duration of TN' lactone exposure to TN' that produced the inhibition of cell growth of two representative medulloblastoma cell lines. The concentration and duration of TPT lactone exposure that produced 99% inhibition of cell growth (IC99) in vitro were used to define the EDT and the target exposure of TPT in the CSF of the nonhuman primate. The nonhuman primate model was used to determine the effect of prolonging the TPT i.v. infusion from 30 min to 4 h on TPT CSF disposition and maintenance of the EDT in the lateral and fourth ventricles and the lumbar space. The results of this study have direct implications for the use of TPT in the treatment of children with medulloblastoma.

MATERIALS AND METHODS

Cell Lines and Chemicals. The Daoy and SJ-Med3 pediatric medulloblastoma cell lines have been described in detail (13). Each cell line was grown as a monolayer in DMEM (BioWhitaker, Walkersville, MD) supplemented with 15% fetal bovine serum (Hyclone, Logan, UT). All of the experiments were done with cells in logarithmic growth. Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated.

Growth Inhibition Assays. The methods used for growth inhibition assays have been described previously (13). Briefly, Daoy or SJ-Med3 cells were seeded in 35-mm tissue culture dishes and allowed to attach overnight. TPT was added as the lactone form with equilibration between the lactone and hydroxy acid forms occurring within 1–2 h after adding the drug. At equilibrium, ~30% of TPT was in the lactone form, and this remained constant for the duration of the experiment.4 The concentrations of TPT lactone that are indicated on the vertical axis in Figs. 3 and 4 are the minimum lactone concentrations for the duration of drug exposure. Aliquots of stock solutions to achieve final concentrations of TPT lactone from 0.0012–120 ng/ml were added to triplicate dishes. Cells were exposed to TPT for 4 h/day (4q24h), 8 h/day (8q24h), and 24 h/day (continuous) for 5 consecutive days. The number of cells remaining in each dish on day 7 was determined by harvesting cells with trypsin and counting with a Coulter Multisizer II (Coulter, Hialeah, FL) cell counter. Results are expressed as number of cells in drug-treated cultures divided by number of cells in vehicle-treated controls (% survival). IC50 was calculated using GraphPad Prism software. The IC99 was defined as the TPT concentration at which intact cells could not be detected by visual inspection or by cell counter.

Drug Formulation and Administration. TPT was obtained from the National Cancer Institute Division of Cancer Treatment (Bethesda, MD) as a lyophilized light yellow powder containing 5 mg of TPT base, 60 mg of mannitol, and 25.5 mg of tartaric acid. TPT was reconstituted with 5 ml of sterile water for injection, USP (Baxter, Deerfield, IL), and further dilutions were made in 5% dextrose in water, USP (Baxter, Deerfield, IL). TPT (2.0 mg/m²) was given using a syringe infusion pump (Medfusion model 2010, Medex Inc., Duluth, GA) to the nonhuman primate as a 30-min or 4-h i.v. infusion through a peripheral venous catheter.

Nonhuman Primate Model. Six adult male rhesus monkeys (Macaca mulatta) weighing 8–12 kg (0.40–0.52 m²) were used in these experiments. All of the procedures were approved by the Animal Care and Use Committees at St. Jude Children’s Research Hospital and the University of Tennessee, Memphis. The animals were fed Teklad 20% Monkey Diet (Harlan Teklad, Madison, WI) and housed in accordance with the Guide for the Care and Use of Laboratory Animals (OPRR, NIH, Rockville, MD). Animals were monitored closely after surgical procedures and during drug studies for any neurological complications. Animals were administered TPT no more frequently than once a week.

The nonhuman primate model of systemic and CSF drug disposition was modified from that described by McCully and colleagues (Fig. 1; Ref. 14). The monkeys were premedicated 2 h before surgery with a single dose of dexamethasone (1 mg/kg i.v.), gentamicin (2 mg/kg i.v.), and ampicillin/sulbactam (30 mg/kg). After surgery, gentamicin (2 mg/kg i.m. every 12 h for 7 days), ampicillin/sulbactam (30 mg/kg s.c. every 12 h for seven days), dexamethasone (1 mg/kg s.c. twice daily for 3 days, and then tapered), and buprenorphine (0.01 mg/kg twice daily for the first 2 days after surgery) were administered. To promote patenty after surgical implantation of Ommaya reservoirs and catheters, they were flushed with 0.9% sodium chloride solution (Baxter, Deerfield, IL) every 3–4 h for the first 12 h. The flushes were continued twice daily for 3–5 days until CSF was clear of blood. Thereafter, Ommaya reservoirs and catheters were flushed twice weekly.

We obtained CSF samples from chronically indwelling Pudenz catheters (1.4-mm internal diameter, 1.9-mm outer diameter, 23-cm length, Heyer-Schulte NeuroCare, Pleasant Prairie, WI) that were placed in the lateral and fourth ventricles and attached to s.c.-implanted Ommaya reservoirs (1.5-cm flat-bottomed side-inlet reservoir, Heyer-Schulte NeuroCare Group, Pleasant Prairie, WI). Lateral ventricular catheters were placed as previously described (14). The placement of the fourth-ventricular catheters was modified from that described by McCully and colleagues (14). The Pudenz catheter was placed 1.5 cm into the fourth ventricle to avoid insertion into the aqueduct and to decrease fibrotic occlusion.

CSF samples were obtained from the lumbar space in duplicate studies from two animals via surgically placed catheters attached to s.c. implanted Ommaya reservoirs (1.5-cm flat-bottomed side-inlet reservoir, Heyer-Schulte NeuroCare Group). Lumbar catheters were placed by making a 6-cm incision.
sion over the L6 and L7 vertebrae, and muscles were retracted laterally. A laminectomy was performed at L7 to expose the dura. A 3-mm durotomy was performed, and the catheter was inserted into the thecal sac and passed cranially for 5 cm.

Blood samples were obtained from the s.c. implanted polysulfone and silicone vascular access port (3.3 cm by 1.2 cm, V-A-P™ model GPV-AC, Access Technologies) attached to a coated polyurethane catheter (size 5 french, Hydrocoat™, Access Technologies) surgically placed in the femoral vein. The vascular port was accessed using a Huber point needle, flushed with 10 ml of 0.9% sodium chloride, and then filled with heparin (1000 units/ml). The vascular access port was flushed with 0.9% sodium chloride, followed by a heparin lock (1000 units/ml) twice per week.

**Sample Collection and Analysis.** When TPT was administered as a 30-min i.v. infusion, plasma and fourth ventricle CSF samples were obtained before and 0.25, 0.5, 1, 1.5, 3, and 6 h after the end of the infusion. When TPT was administered as a 4-h i.v. infusion, plasma and fourth ventricle CSF samples were obtained before and 0.5 and 1.5 h after the start of the infusion, before the end of the infusion, and 0.5, 1, 2, 4, and 6 h after the end of the infusion. In a single nonhuman primate given a 4-h infusion, lateral ventricular CSF samples were obtained 0.5 and 1.5 h after the start of the infusion, before the end of the infusion, and 0.5, 2, and 4 h after the end of the infusion. Lumbar CSF samples were obtained at the same times as plasma and fourth ventricle samples in a single nonhuman primate after a 30-min infusion and in two nonhuman primates after a 4-h infusion.

For each time point, 3 ml of blood was collected from a site (femoral vein catheter) contralateral to the site of i.v. administration and placed in a heparinized tube (12, 15, 16). Within 2 min after collection, the blood sample was processed and stored at −70°C until analyzed by high-performance liquid chromatography. For each time point, 300 μl of CSF was collected from the Ommaya reservoir, centrifuged in a microfuge for 2 min at 10,000 rpm, and then processed (12, 15, 16).

TPT undergoes reversible pH-dependent hydrolysis between the active-lactone and inactive-hydroxy acid forms. In the present study, TPT lactone and total (sum of lactone and hydroxy acid) concentrations in plasma and CSF samples were measured by an isocratic high-performance liquid chromatography assay using a fluorescence detector (Shimadzu RF551, Columbia, MD; Refs. 12, 16–18). Calibration curves for plasma and CSF samples were constructed using single-donor human plasma and pooled human CSF, respectively. The minimum detectable TPT lactone and total concentration in the plasma and CSF was 0.25 ng/ml.

**Pharmacokinetic Analysis.** A three-compartmental model (Fig. 2) was fit using maximum likelihood estimation to TPT plasma and fourth ventricular concentrations after a 30-min and a 4-h infusion (ADAPT II; Ref. 19). Model parameters estimated included the volume of the central compartment ($V_1$), elimination rate constant from the central compartment ($k_{10}$), intercompartmental rate constants ($k_{12}$, $k_{21}$), rate constant describing TPT penetration into the ventricular CSF ($k_{13}$), and elimination rate constant from the ventricular CSF ($k_{31}$), rate constant describing distribution from the fourth ventricle to the lumbar space ($k_{41}$), and elimination rate constant from the lumbar space ($k_{43}$). Total volume of the CSF was fixed at 23 ml, and volume of the lumbar space was fixed at 2 ml.
2540 Cytotoxic TPT Exposure in Neuraxis with 4-Hour Infusion

Fig. 3 Growth inhibition curves for Daoy medulloblastoma cell lines by TPT. Cells were plated in 35-mm dishes at an appropriate density and allowed to attach overnight, and TPT was added to cell culture medium as described in “Materials and Methods.” Cells were exposed for 4 (●), 8 (▲), and 24 (●) h/day for 5 consecutive days. Each point represents the mean of duplicates. A representative experiment of five experiments is shown.

Fig. 4 Growth inhibition curves for SJ-Med3 medulloblastoma cell lines by TPT. Cells were plated in 35-mm dishes at an appropriate density and allowed to attach overnight. TPT was added to cell culture medium as described in “Materials and Methods.” Cells were exposed for 4 (●), 8 (▲), and 24 (●) h/day for 5 consecutive days. Each point represents the mean of duplicates. A representative experiment of three experiments is shown.

Fig. 5 TPT lactone concentration-time profiles in the plasma, fourth ventricular CSF, and lumbar CSF after a TPT dose of 2.0 mg/m² administered over 30 min. Individual data points and best-fit line of the data are represented for TPT lactone concentrations in the plasma (●, - - -), fourth ventricular CSF (▲, ——), and lumbar CSF (●, ——).

Fig. 6 TPT lactone concentration-time profiles in the plasma, fourth ventricular CSF, and lumbar CSF after a TPT dose of 2.0 mg/m² administered over 4 h. Individual data points and best-fit line of the data are represented for TPT lactone concentrations in the plasma (●, - - -), fourth ventricular CSF (▲, ——), and lumbar CSF (●, ——).

RESULTS

Growth Inhibition Assays. Growth inhibition curves for Daoy and SJ-Med3 human medulloblastoma cell lines are presented in Fig. 3 and 4, respectively. Data in Fig. 3 show that the TPT lactone IC50 of Daoy cells exposed to TPT continuously or for 8 h/day (8q24h) for 5 days was 0.3 and 0.2 ng/mL, respectively. However, when Daoy cells were...
exposed to TPT for only 4 h/day for 5 days, the IC_{so} increased 3-fold to 0.8 ng/ml. Similarly, data in Fig. 4 show that the TPT lactone IC_{so} for SJ-Med3 cells exposed to TPT continuously or for 8 h/day for 5 days was 0.6 and 0.7 ng/ml, respectively. The IC_{so} increased 5-fold to 3.2 ng/ml for SJ-Med3 cells exposed for 4 h/day for 5 days. The data in Fig. 3 and 4 also reflect the steep TPT lactone exposure-response relationship as suggested from our studies of these tumors grown as xenografts (11). For example, doubling the exposure of TPT from 4 h to 8 h each day resulted in a 5-fold reduction in IC_{so} for SJ-Med3 cells. In Daoy cells exposed to TPT continuously or 8 h/day, the IC_{so} for TPT lactone was estimated as 1.2 ng/ml, only 3-fold higher than the IC_{so}. In SJ-Med3 cells exposed to drug continuously or 8 h/day, the IC_{so} for TPT (2.5 ng/ml) was also approximately 3-fold higher than the IC_{so} for this cell line. We conclude that the exposure of medulloblastoma cells to TPT for 8 h/day for 5 consecutive days produced a greater inhibition of tumor cell growth than did 4-h exposures. From these data, we propose that a CSF concentration of TPT lactone ≥ 1 mg/ml for 8 h/day would be associated with a better antitumor effect and will, therefore, serve as the target EDT in the CSF for our TPT regimen in children with high-risk medulloblastoma.

**TPT Lactone Plasma and CSF Pharmacokinetics.**

Representative TPT lactone plasma, fourth ventricular CSF, and lumbar CSF concentrations after a 30-min and a 4-h infusion are presented in Figs. 5 and 6, respectively. Pharmacokinetic parameters for TPT lactone are similar after a 30-min and a 4-h infusion and are summarized in Table 1. The average TPT lactone penetration into the fourth ventricle was similar between the two infusion rates (see Table 1). Because of the relatively small volume of the lateral ventricle in the nonhuman primate model, we were only able to obtain CSF samples from a single subject after a 4-h infusion. The average ± SD ratio of lateral to fourth ventricle TPT lactone was 0.02 ± 0.01 (range, 0.01-0.02), and, as expected, the ratio of TPT lactone AUC_{lum}/AUC_{CSF} for a 4-h infusion was 1.0. The average ± SD times above a TPT lactone concentration of 1 mg/ml in the fourth ventricle after a 30-min and a 4-h infusion were 4.8 ± 1.1 h (range, 3.8-6.0 h) and 6.9 ± 1.2 h (range, 5.5-8.5 h), respectively. In addition, the ratios of lactone AUC_{CSF}/AUC_{lum} after a 30-min and a 4-h infusion were 0.36 ± 0.07 and 0.35 ± 0.02, respectively. The penetration of TPT lactone in the lumbar space after a 30-min (n = 1) and a 4-h infusion (n = 2) was 0.12 and 0.11 ± 0.01, respectively. The k_{ab}, k_{4-1}, CL_{lum}, and AUC_{lum} for TPT lactone were 0.02 ± 0.02 h^{-1}, 3.8 ± 4.1 h^{-1}, 0.018 ± 0.0010

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**Table 1** Pharmacokinetic parameters for topotecan lactone are presented after 30-min (n = 5) and 4-h (n = 5) infusions and are summarized as the mean ± SD and median (range)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>30-min infusion</th>
<th>4-h infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_{10}</td>
<td>h^{-1}</td>
<td>0.92 ± 0.33</td>
<td>1.7 ± 0.71</td>
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<td>k_{12}</td>
<td>h^{-1}</td>
<td>0.34 ± 0.23</td>
<td>1.4 ± 1.4</td>
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<td>k_{31}</td>
<td>h^{-1}</td>
<td>0.74 ± 0.38</td>
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<tr>
<td>V_{CSF}</td>
<td>liter/m²</td>
<td>45.8 ± 17.4</td>
<td>24.2 ± 10.9</td>
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<tr>
<td>CL_{CSF}</td>
<td>liter/h²</td>
<td>38.7 ± 15.8</td>
<td>34.2 ± 12.0</td>
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<tr>
<td>AUC_{CSF}</td>
<td>ng/ml·h</td>
<td>53.9 ± 26.5</td>
<td>56.3 ± 13.9</td>
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<td>k_{13}</td>
<td>h^{-1}</td>
<td>0.0017 ± 0.0010</td>
<td>0.0037 ± 0.0027</td>
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<tr>
<td>V_{lum}</td>
<td>liter/m²</td>
<td>2.9 ± 1.1</td>
<td>3.4 ± 2.3</td>
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<tr>
<td>CL_{lum}</td>
<td>liter/h²</td>
<td>0.15 ± 0.06</td>
<td>0.18 ± 0.11</td>
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<tr>
<td>AUC_{lum}</td>
<td>ng/ml·h</td>
<td>23.7 ± 13.0</td>
<td>25.4 ± 13.4</td>
</tr>
<tr>
<td>Penetration into 4th ventricle</td>
<td></td>
<td>0.43 ± 0.03</td>
<td>0.42 ± 0.13</td>
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</tbody>
</table>

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**Table 2** Pharmacokinetic parameters for topotecan total are presented after 30-min (n = 5) and 4-h (n = 5) infusions and are summarized as the mean ± SD and median (range)

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<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>30-min infusion</th>
<th>4-h infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_{10}</td>
<td>h^{-1}</td>
<td>0.60 ± 0.43</td>
<td>1.5 ± 0.79</td>
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<tr>
<td>k_{12}</td>
<td>h^{-1}</td>
<td>0.20 ± 0.14</td>
<td>6.4 ± 5.8</td>
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<tr>
<td>k_{31}</td>
<td>h^{-1}</td>
<td>2.1 ± 0.15</td>
<td>2.1 ± 2.0</td>
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<tr>
<td>V_{CSF}</td>
<td>liter/m²</td>
<td>20.6 ± 10.3</td>
<td>9.9 ± 2.8</td>
</tr>
<tr>
<td>CL_{CSF}</td>
<td>liter/h²</td>
<td>9.9 ± 5.0</td>
<td>13.7 ± 5.6</td>
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<tr>
<td>AUC_{CSF}</td>
<td>ng/ml·h</td>
<td>243.7 ± 142.6</td>
<td>166.3 ± 66.1</td>
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<tr>
<td>k_{13}</td>
<td>h^{-1}</td>
<td>0.0026 ± 0.0019</td>
<td>0.0084 ± 0.0056</td>
</tr>
<tr>
<td>V_{lum}</td>
<td>liter/m²</td>
<td>2.9 ± 1.6</td>
<td>3.7 ± 2.1</td>
</tr>
<tr>
<td>CL_{lum}</td>
<td>liter/h²</td>
<td>0.13 ± 0.07</td>
<td>0.19 ± 0.10</td>
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<tr>
<td>AUC_{lum}</td>
<td>ng/ml·h</td>
<td>84.2 ± 57.8</td>
<td>61.7 ± 33.0</td>
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<tr>
<td>Penetration into 4th ventricle</td>
<td></td>
<td>0.34 ± 0.06</td>
<td>0.36 ± 0.05</td>
</tr>
</tbody>
</table>
The ratio \( \frac{AUCLUM}{AUC4h} \) after a 30-mm infusion was 0.56.

TN' penetration into the CSF over a wide range of TN' doses and \( \frac{AUCLUM}{AUC4h} \) of TN' total \( \frac{AUCLUM}{total AUC4h} \) and 0.08 ± 0.07 h, 1.5 ± 1.2 h, 0.0030 ± 0.0029 L/h/m², respectively. The ratio of TN' total \( \frac{AUCLUM}{total AUC4h} \) after a 30-mm and a 4-h infusion were 0.24 and 0.18, respectively.

**TPT Total Plasma and CSF Pharmacokinetics.** Pharmacokinetic parameters for TPT total are similar after a 30-min and a 4-h infusion and are summarized in Table 2.

As with the TPT lactone the average TPT total penetration into the fourth ventricle was similar between the two infusion rates (see Table 2). The ratio of TPT total \( \frac{AUCLUM}{total AUC4h} \) after a 4-h infusion was 1.0.

The penetration of TPT total in the lumbar space after a 30-min and a 4-h infusion were 0.21 and 0.25, respectively. The mean ± SD \( k_{lat}, k_{40}, CLUM, \) and \( AUCLUM \) for TPT total were 0.08 ± 0.07 h⁻¹, 1.5 ± 1.2 h⁻¹, 0.0030 ± 0.0029 L/h/m², and 24.5 ± 2.5 ng/ml/h, respectively. The ratio of TPT total \( \frac{AUCLUM}{total AUC4h} \) after a 30-min infusion was 0.56. The ratio of TPT total \( \frac{AUCLUM}{total AUC4h} \) after a 4-h infusion was 0.66.

**Toxicity.** No hematological or nonhematological toxicity was observed after either the 30-min or the 4-h i.v. TPT infusion in the nonhuman primate model.

**Design of TPT Treatment Regimens for Children with Medulloblastoma.** We have shown that TPT systemic disposition in children is linear and that TPT dose can be adjusted to achieve a target plasma systemic exposure, defined as a TPT lactone single-day AUC (26, 27). Moreover, we have shown that TPT penetration into the CSF over a wide range of TPT doses and AUCs in children with medulloblastoma is linear. In addition, data from the present study show that TPT penetration into the CSF is linear in the nonhuman primate. Thus, we performed simulation studies to determine the plasma TPT AUC required to achieve the target EDT throughout the neuraxis. The results of these studies showed TPT lactone plasma AUC of 140 ng/ml/h was required to achieve the EDT in the fourth ventricle and lumbar space. Representative simulations of TPT lactone concentration-time profiles in the fourth ventricle after a 30-min and a 4-h infusion are presented in Fig. 7. The average ± SD of time above 1 ng/ml in the fourth ventricle for a 30-min and a 4-h infusion was 7.4 ± 1.7 h (range, 5.5–10.5 h) and 9.2 ± 1.0 h (range, 8.0–11.0 h), respectively. Representative simulations of TPT lactone concentration-time profiles in the lumbar space after a 30-min and a 4-h infusion are presented in Fig. 8. The average ± SD of time above 1 ng/ml in the lumbar CSF studies after a 30-min and a 4-h infusion was 5.0 ± 1.1 h (range, 3.5–7.0 h) and 7.5 ± 0.92 h (range, 6.1–8.8 h), respectively. Thus, TPT targeted to a plasma AUC of 140 ng/ml/h using a 4-h infusion achieves the EDT of 1 ng/ml throughout the neuraxis for nearly 8 h, whereas the 30-min infusion achieves the EDT only in the fourth ventricle.

**DISCUSSION**

The efficacy and toxicity associated with the treatment of leptomeningeal disease in children with medulloblastoma and other embryonal tumors are a major clinical challenge. Although the CSF disposition of TPT has been evaluated in the nonhuman primate model (28) and in children with CNS malignancies (12), this is the first report evaluating TPT systemic exposure and schedule optimization to achieve a target cytotoxic EDT throughout the subarachnoid space. Our use of data from tissue culture studies and the nonhuman primate model to develop a rational treatment schedule is a novel strategy to develop anticancer agents in the treatment of primary and metastatic CNS malignancies. Our results comparing a 30-min and a 4-h infusion indicate a 4-h infusion at a daily targeted lactone plasma AUC of 140 ng/ml/h achieves the EDT, defined as a TPT lactone concentration of ≥1 ng/ml in the CSF for 8 h, in the lateral and fourth ventricles and in the lumbar space. The importance of these data are underscored by the need to develop new anticancer agents or novel i.v. administration strategies that may reduce our present reliance on irradiation and the problems associated with intrathecal drug administration.

Two sources of drug exposure, CSF for metastatic seeding and plasma for the primary tumor mass, have been postulated to be important in the treatment of children with high-risk medul-
loblastoma. Results of our tissue culture studies suggested that the TPT lactone concentration should be maintained above 1 ng/ml for 8 h (IC50) throughout the neuraxis. Although we do not suggest that in vitro studies precisely predict in vivo response, the in vitro EDT was used as a guide to determine whether effective exposure of TPT could be achieved in the neuraxis of the nonhuman primates. In the nonhuman primate model, administration of TPT as a 4-h infusion at a daily lactone systemic exposure of 140 ng/ml/h achieved the EDT in the CSF of the fourth ventricle and the lumbar space, whereas the 30-min infusion did not achieve the EDT in the lumbar space. Importantly, the neuraxis, specifically the lumbar space, is a primary site of metastatic spread in patients with medulloblastoma and other CNS malignancies (1, 4, 5). Moreover, at the plasma TPT AUC necessary to attain the EDT in the lumbar space (i.e., 140 ng/mlh), we have observed complete responses in mice bearing Daoy and SJ-Med3 pediatric medulloblastoma xenografts (11).

Prolonging the TPT infusion from 30-min to 4-h increased by 1.4-fold the time for which the TPT lactone exposure was above a concentration of 1 ng/ml in the fourth ventricular CSF. We have published data previously (12) that showed that the median CSF TPT lactone CSF penetration increased from 0.29 to 0.42 when the infusion time was increased from 24 to 72 h. However, because of wide interpatient variability in CSF penetration, this difference was not statistically significant. In the present study, we found that the 30-min and the 4-h infusions had similar systemic clearance, percent of CSF penetration, rate of CSF penetration (k13), and elimination from the CSF (k31). Thus, the increased length of time that the TPT lactone concentration was above 1 ng/ml could be attributed to prolonging the duration of penetration into the CSF. The TPT CSF disposition after a 30-min and a 4-h infusion were consistent with the results of Blaney et al. (28) in the nonhuman primate, and in our previous clinical study of TPT in pediatric patients with primary CNS malignancies (12).

Drug distribution in the CNS follows the flow of CSF (29, 30). The similar exposure of TPT in the lateral and fourth ventricles (1.1 ± 0.16) is consistent with the presence of choroid plexus, and the close proximity of the lateral and fourth ventricles. The ratio of TPT concentrations in the lumbar space and fourth ventricle (0.51 ± 0.40) is consistent with the relatively distant proximity of the lumbar space and the fourth ventricle, and is similar to the distribution ratio of TPT that we reported in patients (12) and the distribution ratio reported for other chemotherapy agents (e.g., methotrexate; Refs. 22, 31, 32).

The nonhuman primate is a useful model to evaluate drug disposition in the neuraxis based on the CNS anatomy similar to that of humans, the ability to place chronically indwelling ventricular and lumbar catheters, and the ability to perform repeated sampling in an unanesthetized state (28, 32–34). However, the use of this model to predict CSF drug disposition in the presence of CNS malignancies may have limitations (14, 28, 31). CNS tumors may disrupt the blood-brain barrier, alter CSF flow, and subsequently change drug distribution within the CSF, but in the healthy nonhuman primate model, the blood-brain barrier is intact. However, we found that the CSF penetration of TPT into the ventricular and lumbar CSF was comparable to what we have observed in children with primary CNS malignancies. In addition, we have collected a limited number of paired ventricular and lumbar TPT concentrations in children with medulloblastoma, and our results were consistent with the data reported herein.

The three- and four-compartment models adequately described TPT disposition in the plasma, fourth ventricle, and lumbar space. Our pharmacokinetic model has three advantages compared with the models proposed by Sung et al. (35) and Baker et al. (12): (a) generation of parameter estimates after doses and administration schedules used clinically; (b) sampling from three areas within the CSF; and (c) model estimation of TPT disposition in the lumbar space. The pharmacokinetic model by Sung et al. was derived from the results of a study in which TPT was administered as a 10-min i.v. infusion at a dose of 10 mg/kg (20 mg/m2). However, the maximum-tolerated dose of TPT as a 30-min infusion in the treatment of pediatric malignancies has been reported to be 2 mg/m2 (36). The present model incorporated plasma concentrations along with data from ventricular and lumbar CSF and, because of this, may more accurately reflect TPT CSF disposition in children than previously published models. Thus, the model would have utility in prospectively designing TPT treatment regimens for patients with primary and metastatic CNS malignancies.

The clinical relevance of this study is underscored by the need to develop novel treatment strategies and schedules of i.v. administration that may reduce or delay the need for irradiation, and may preclude the need for intrathecal administration. In fact, we have used this pharmacokinetic model to develop a regimen of i.v. TPT for an ongoing Phase II study in children with high-risk medulloblastoma. TPT is given as a 4-h infusion for 5 consecutive days with the TPT dosage pharmacokinetically adjusted to attain a target plasma TPT lactone systemic exposure (i.e., 140 ng/mlh). Serial ventricular CSF samples are obtained to determine the length of time for which the TPT lactone concentration is above 1 ng/ml. Furthermore, although the duration of drug exposure may need to be modified for specific tumors, the pharmacokinetic principles documented here are applicable to a more general design of i.v. treatment regimens for CNS malignancies.

ACKNOWLEDGMENTS

We thank Suzan Hanna, Audrey Smith, Yuri Yanishevski, and Dr. Enrique Serment for technical support.

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A. J. Gajjar, unpublished observations.


A four-hour topotecan infusion achieves cytotoxic exposure throughout the neuraxis in the nonhuman primate model: implications for treatment of children with metastatic medulloblastoma.

W C Zamboni, A J Gajjar, T D Mandrell, et al.


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