Plasma and Cerebrospinal Fluid Pharmacokinetic Study of Topotecan in Nonhuman Primates

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Abstract

Topotecan, a water soluble semisynthetic analogue of camptothecin, is a topoisomerase I inhibitor that has recently entered phase II clinical trials. Topotecan has shown significant preclinical activity in refractory murine tumors and in human tumor xenograft models. In addition, objective antineoplastic activity has been observed in recent adult phase I clinical trials. Topotecan is unstable in solution and is rapidly and spontaneously converted to a less active open ring form which predominates at physiological pH. This study was undertaken to better define the pharmacokinetic behavior of this highly unstable compound in both plasma and cerebrospinal fluid (CSF) and to measure the degree of CSF penetration of this novel antineoplastic agent.

Three nonhuman primates with indwelling Ommaya reservoirs received 10 mg/m² i.v. topotecan administered as a 10-min infusion. Frequent plasma and CSF samples were obtained and immediately extracted and assayed with a reverse phase high performance liquid chromatography assay to quantitate the concentration of topotecan (lactone). Samples were then acidified and re-injected to quantitate total drug (lactone ring plus open ring).

Peak plasma concentrations of topotecan ranged from 0.27 to 0.45 μM. Plasma disappearance of the lactone ring was biexponential with a distribution half-life (t1/2β) of 22 ± 5 min and an elimination half-life (t1/2β) of 1.3 ± 0.1 h. Total body clearance of topotecan was 72.1 ± 15.8 liters/h/m². The volume of distribution at steady state was 88.6 ± 33.2 liters/m². Peak CSF concentrations of topotecan occurred at 30 min following drug administration and ranged from 0.044 to 0.074 μM. CSF disappearance paralleled that in plasma. The mean ratio of the area under the CSF concentration-time curve to that in plasma was 0.32 (range, 0.29 to 0.37).

The mean CSF penetration of topotecan exceeds 30%, which is significantly greater than the penetration of most structurally similar chemotherapeutic agents. The impact of chemotherapy on the survival of patients with primary or metastatic central nervous system malignancies is very limited. Therefore, this novel antineoplastic agent is an excellent candidate for further study in patients with high risk or refractory central nervous system tumors.

Introduction

Topotecan [(S)-9-dimethylaminomethyl-10-hydroxycamptothecin hydrochloride, SK&F 104864-A, NSC 609699] (Fig. 1), a camptothecin analogue which inhibits topoisomerase I, is currently undergoing extensive phase II clinical evaluation. Topoisomerase I is an intranuclear enzyme which relaxes supercoiled DNA by creating single strand breaks which are subsequently religated by this enzyme (1). Topoisomerase I inhibitors, like topotecan, produce cytotoxicity by stabilizing the covalent complex between topoisomerase I and DNA which results in enzyme linked DNA breaks that cannot be religated in the presence of drug (2, 3).

Topotecan has demonstrated preclinical activity against several refractory murine tumors including B16 melanoma, colon carcinomas 38 and 51, and multidrug resistant p388 leukemia (2). Significant preclinical activity, including cures, have also been observed following topotecan in mice with rhabdomyosarcoma and osteosarcoma xenografts (4). In addition, in phase I studies of topotecan objective complete and partial responses have been observed in patients with non-small cell lung cancer, cisplatin resistant ovarian carcinoma, small cell lung cancer, and metastatic colorectal cancer (5–8).

Topotecan is unstable in solution and undergoes spontaneous hydrolysis at physiological pH to a less active open ring species (Fig. 1). The hydrolysis is pH dependent with the equilibrium favoring the open-ring form at a pH > 7.0 and the lactone form in acidic conditions. Pharmacokinetic studies of topotecan have been performed in adult and pediatric patients (7–12) but have been limited by the instability of the parent drug in solution. In addition, the CSF penetration of this novel antineoplastic agent has not been characterized. The CSF penetration of camptothecin appears to be negligible. CSF camptothecin levels were not detected in the limited number of patients in whom CSF samples were obtained during phase II trials of this drug (13). In this study the plasma and CSF pharmacokinetics of topotecan in nonhuman primates are examined.

Materials and Methods

Drugs. Topotecan (hydrochloride salt, adjusted to pH 3 to 4) was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD) in 5-mg vials which were reconstituted in 2 ml of sterile water. The appropriate dose of drug was further diluted with 12.5 ml of 5% dextrose which was administered over 10 min through either a peripheral venous or central venous catheter.

Monkeys. Three adult male rhesus monkeys (Macaca mulatta) ranging in weight from 6.9 to 9.7 kg were used in these experiments. The animals were fed NIH Open Formula Extruded Non-Human Primate Diet twice daily and group housed in accordance with the Guide for the Care and Use of Laboratory Animals (14). Blood samples were drawn through a catheter placed in either the femoral or the saphenous vein opposite from the site of drug administration.

Experiments. The plasma and CSF pharmacokinetics of topotecan were studied in 3 animals following an i.v. dose of 10 mg/m² administered over 10 min. Blood was collected in heparinized tubes prior to the dose and at 5, 15, 30 min, at 1, 1.5, 2, 3 h, and at 30- to 60-min intervals thereafter until parent drug could no longer be quantitated in the plasma. Plasma was separated immediately by centrifugation at 12,000 X g for 2 min in a rapid acceleration/ deceleration centrifuge. CSF samples were collected from an Ommaya reservoir at the same time points as plasma. The reservoir was pumped 4 times before and after each sample collection to ensure adequate mixing with ventricular CSF.

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2 The abbreviations used are: CSF, cerebrospinal fluid; HPLC, high performance liquid chromatography; AUC, area under the drug concentration-time curve; CL TB, total body clearance; Vdss, volume of distribution at steady state.
eluted from the column with methanol and an aliquot of the eluant was assayed. Topotecan and its open ring metabolite were detected by using a fluorescence detector (Applied Biosystems) at a λmax of 375 nm and a Aλ,λ of 470 nm (cutoff filter). Under these conditions, the open ring metabolite eluted with the solvent front and the lactone eluted at 4 min. The acidified eluant was injected after it had been at room temperature for at least 2 h. The concentration of the open ring hydrolysis product was obtained indirectly by subtracting the concentration of lactone in the unacidified sample from the total lactone in the acidified sample. CSF samples were analyzed for topotecan (lactone) by immediate direct injection of a 100-μl aliquot of CSF on the HPLC column. A second aliquot of CSF was acidified with 2% phosphoric acid in order to quantitate the total drug (lactone and open-ring species). The acidified eluant was injected after it had been at room temperature for at least 2 h. The concentration of the open ring hydrolysis product was obtained indirectly by subtracting the concentration of lactone in the unacidified sample from the total lactone in the acidified sample. CSF samples were analyzed for topotecan (lactone) by immediate direct injection of a 100-μl aliquot of CSF on the HPLC column. A second aliquot of CSF was acidified with concentrated phosphoric acid and the concentration of the open ring hydrolysis product was obtained as described above. Topotecan was detected by using a fluorescence detector (Applied Biosystems) at a λmax of 375 nm and a λmax 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 the monkey's plasma and CSF were prepared for each experiment by addition of known amounts of topotecan to plasma or CSF, respectively. Standard curves were linear (r² > 0.995) over a range of 0.002 to 1 μM. The lower limit of quantitation was 0.002 μM.

**Pharmacokinetic Analysis.** Plasma concentration versus time data from the topotecan bolus experiments were fit to mono- (n = 1) and biexponential (n = 2) equations:

\[
C(t) = \sum_{i=1}^{n} A_i e^{-\lambda_i t}
\]

using MLAB, a nonlinear curve fitting program, where C is the plasma concentration of topotecan at time t, A_i the coefficients, and λ_i the rate constants (16). Akaike’s information criterion (17) was used to determine which equation best fit the data. The half-life for each phase of elimination was calculated by dividing 0.693 by the rate constant (λ_i) for that phase. Noncompartmental methods were used to calculate other pharmacokinetic parameters. The AUC was derived by the linear trapezoidal method (18), and extrapolated to infinity by adding the quotient of the final plasma concentration divided by the terminal rate constant (λ_n). Total body clearance (Cl) was determined by dividing the dose by the AUC. The volume of distribution at steady state (Vss) was calculated by using the area under the moment curve (19). The fraction of drug penetrating into the CSF was derived from the ratio of the AUCs in CSF and plasma.

**Results**

**Plasma and CSF Pharmacokinetics.** Peak plasma concentrations of the lactone form of topotecan following an i.v. 10-min infusion of 10 mg/m² ranged from 0.27 to 0.45 μM. Plasma disappearance was best fitted by a biexponential equation with a mean t1/2α of 22 ± 5 min (range, 16–25 min) and a mean t1/2β of 1.3 ± 0.1 h (range, 1.2–1.4 h). Total body clearance was 72.1 ± 15.8 liters/h/m² (range, 55.1 - 86.4 liters/h/m²) and the Vdss was 88.6 ± 33.2 liters/m² (range, 52.6 - 188 liters/m²). Plasma disappearance of the total drug was also best fitted by a biexponential equation with a mean t1/2α of 41 ± 10 min (range, 30 - 50 min) and a mean t1/2β of 3.8 ± 1.6 h (range, 2.1 - 5.4 h). Total drug Cl was 28.9 ± 8.4 liters/h/m² (range 19.5 - 35.5 liters/h/m²) and the Vdss was 40.9 ± 13.1 liters/m² (range, 26.2 - 50.4 liters/m²). The plasma and CSF AUCs for both the lactone and the total drug following i.v. topotecan administration are shown in Table 1.

CSF topotecan concentrations peaked at 30 min and ranged from 0.044 to 0.074 μM for the lactone and 0.065 to 0.097 μM for the total drug. CSF disappearance paralleled that in plasma. The mean ratio of the area under the CSF concentration-time curve to that in plasma was 0.32 (range, 0.29 to 0.37) for the lactone and 0.24 (range, 0.22 to 0.27) for total drug. A representative concentration-time profile of the lactone form of topotecan in plasma and CSF is shown in Fig. 2.

The hydrolysis of topotecan to the open ring form was rapid. Greater than 60% of total drug in plasma was in the open ring form by

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**Table 1. Pharmacokinetic parameters following a 10-mg/m² i.v. bolus dose of topotecan in 3 animals**

<table>
<thead>
<tr>
<th>Monkey</th>
<th>AUC CSF (μM-h)</th>
<th>AUC Plasma (μM-h)</th>
<th>CSF:plasma</th>
<th>AUC CSF (μM-h)</th>
<th>AUC Plasma (μM-h)</th>
<th>CSF:plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH955</td>
<td>0.1029</td>
<td>0.361</td>
<td>0.28</td>
<td>0.249</td>
<td>1.123</td>
<td>0.22</td>
</tr>
<tr>
<td>CH940</td>
<td>0.0904</td>
<td>0.239</td>
<td>0.37</td>
<td>0.185</td>
<td>0.684</td>
<td>0.27</td>
</tr>
<tr>
<td>CH843</td>
<td>0.0854</td>
<td>0.275</td>
<td>0.31</td>
<td>0.145</td>
<td>0.617</td>
<td>0.24</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.0926 ± 0.009</td>
<td>0.292 ± 0.063</td>
<td>0.32 ± 0.05</td>
<td>0.193 ± 0.052</td>
<td>0.808 ± 0.275</td>
<td>0.24 ± 0.03</td>
</tr>
</tbody>
</table>

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anticancer drugs. For example, CSF levels of etoposide are less than 5 to 10% of simultaneously drawn plasma levels (21), and doxorubicin, another highly protein bound antineoplastic agent, is not detectable in CSF (22).

The poor prognosis of patients with primary or metastatic central nervous system malignancies is in part related to the lack of effective chemotherapeutic agents that penetrate the blood-brain barrier. Topotecan is a new anticancer drug with a novel mechanism of action. The results of the present study document that topotecan has substantial CNS penetration, and suggest that it should be evaluated in patients with central nervous system tumors.

Discussion

The plasma and CSF pharmacokinetics of topotecan were studied in a nonhuman primate model which has previously been predictive of CSF drug penetration in humans (20). The plasma disappearance of topotecan in the model was biexponential as has been observed in humans; however, the mean CL/F in nonhuman primates (72 liters/h/m²) was significantly faster than in children (27 liters/h/m²) (9). The more rapid clearance rate in nonhuman primates (mean t₁/₂β= 1.3 h) compared with humans (mean t₁/₂,β of approximately 3 h (8, 9, 11). The mean Vd, in nonhuman primates (89 liters/m²) is comparable to that observed in humans (69 liters/m²) (11).

Lactone hydrolysis in both species is rapid with greater than 60% conversion to the less active open ring form in plasma within 30 min after a 10-min infusion in nonhuman primates and greater than 40% conversion to the open ring form within 15 min following a 1-h infusion in humans (8, 9).

The mean CSF:plasma ratio of the lactone form of topotecan exceeds 30% which is significantly greater than the penetration of structurally similar antineoplastic agents. For example, the CSF penetration of camptothecin appears to be negligible, primarily as a result of its extensive protein binding. Greater than 97% of camptothecin is protein bound (13) and therefore minimal free drug is available to penetrate into the CSF. Measurement of the degree of topotecan protein binding is complicated by its rapid spontaneous hydrolysis at physiological pH. However, preliminary studies suggest that the protein binding of topotecan is minimal (<20%).

Fig. 3. Representative concentration-time curves of topotecan total drug and lactone from a single animal (No. 955) in plasma following a 10-mg/m² i.v. 10-min infusion.

References

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