A Pediatric Phase I and Pharmacokinetic Study of Spirohydantoin Mustard


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ABSTRACT

A pediatric Phase I and pharmacokinetic study of the lipophilic alkylating agent spirohydantoin mustard (SHM) was conducted in 23 patients. The dose-limiting toxicity of SHM was neurological with disorientation, delirium, or hallucinations occurring in 9 of 23 patients. These symptoms were partially reversible and preventable with physostigmine. In 17 patients who were evaluable for response to treatment (14 of whom had central nervous system malignancies), no objective tumor responses were observed. Pharmacokinetic evaluation of SHM revealed a $t_{1/2}$ of 1.7 ± 0.7 min, $t_{1/2}$ of 16 ± 8.3 min, and total body clearance of 2134 ± 735 ml/min/m². Measurable peak plasma levels were less than 40% of that which produces cytotoxicity in vitro against monolayer cultures of rat 9L brain tumor. Over 90% of SHM was protein bound, greatly limiting the free drug available for central nervous system penetration. SHM cerebrospinal fluid to plasma ratios were less than 0.047. The above suggests that in spite of its lipophilicity, SHM may not reach clinically significant levels in the central nervous system at clinically tolerable doses.

INTRODUCTION

SHM (spiromustine, NSC 172112) (Fig. 1) is one of several hydantoin-containing nitrogen mustard drugs designed to cross the blood-brain barrier and to possess activity against CNS tumors (1). The rationale for its synthesis was based on prior observations which had shown that higher levels of diphenylhydantoin were detectable within brain tumor tissue than in surrounding normal brain (2). This suggested that substituted hydantoins might act as carrier molecules to aid the penetration of antineoplastic agents into the CNS. In SHM, a pentamethylene-substituted hydantoin serves as the carrier for a bis(2-chloroethyl)ethylamine alkylating moiety.

In preclinical studies, SHM showed both in vitro and in vivo antitumor activity (3). In vitro cytotoxicity was observed in the rat 9L brain tumor, and in vivo activity against murine B16 melanoma, colon 26 tumor, P388 leukemia, and the MX-1 mammary tumor xenograft were observed. Additionally, SHM showed significant activity in mice implanted with an intracranial ependymoblastoma cell line.

This report presents the results of a multiinstitutional pediatric Phase I trial of SHM in which plasma and CSF pharmacokinetics as well as toxicity and clinical response to this agent were evaluated.

MATERIALS AND METHODS

Patient Eligibility. Patients between 3 and 21 yr of age with malignancies refractory to conventional therapy were eligible for entry into this trial. The only exception to a requirement for prior histological documentation of malignancy in these patients was in individuals with brain stem or deep midline CNS tumors. In such cases the clinical diagnosis of tumor along with CT scans or MRI documentation of the original lesion and subsequent response to treatment were required for entry. All patients were required to have an Eastern Cooperative Oncology Group performance level of 3 or greater and a life expectancy of at least 8 wk. A complete physical and neurological exam and measurement of all palpable and radiographically (including CT scan and MRI) visible lesions were also required prior to entry.

Prior to starting each course of SHM, patients were required to have recovered from the toxic effects of antineoplastic therapy and to have adequate hepatic (bilirubin less than 2 mg/dl and serum transaminases less than 1.5 × normal) and renal function (creatinine less than 1.5 mg/dl or creatinine clearance greater than 60 ml/min/1.73 m²) as well as a normal coagulation profile, serum electrolytes, and uric acid. Patients with solid tumors (without bone marrow involvement) were required to have adequate peripheral blood counts (granulocytes greater than 1,500/mm³, platelets greater than 100,000/mm³). The use of steroids to control the symptoms of increased intracranial pressure and other standard supportive care measures were not limited.

All patients or their legal guardians signed a document of informed consent consistent with Federal and local institutional guidelines stating that they were aware of the investigational nature of this trial.

Study Design. Patients were treated with i.v. bolus doses of SHM once weekly for 3 wk. Three weekly doses constituted one course of therapy, and courses were repeated at 28-day intervals. The starting dose was 4.5 mg/m² and drug escalations to 5.5, 6.5, 7.5, 8.5, 9.5, and 11.5 mg/m² were carried out once at least three patients evaluable for toxicity had been accrued at the prior dose level. Patients were allowed to escalate to the next highest dose level if they had successfully completed 2 courses at the prior dose. Only one escalation was allowed in an individual patient. Patients were monitored weekly with complete blood counts, neurological evaluations, and measurement of any palpable lesions. Radiographic and CT or MRI scans were repeated at the end of each course of therapy. Individual patients were removed from the study if they experienced unacceptable toxicity or objective disease progression after one or more courses of therapy. The trial was terminated as soon as dose-limiting toxicity was identified.

Drug Formulation and Administration. SHM was obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, as sterile hyphothesized powder in 10-mg vials. The drug was dissolved in 1 ml of anhydrous N,N-dimethylacetamide and this solution subsequently added to 100 ml of 10% fat emulsion (Intralipid; Cutter) with constant swirling to ensure proper mixing and partition into the lipid phase. Because of the instability of SHM in aqueous media (10% loss in Intralipid solution within 49 min), drug preparation and administration were required to be completed within 30 min (4). For all patients in whom pharmacokinetic studies were performed, the mixing and delivery of the drug were completed within 6 to 10 min.

Pharmacology Studies. SHM was measured in plasma and CSF using a gas chromatographic assay with thermionic detection (5). Blood samples representing 13 separate doses of SHM in six individual patients were obtained for pharmacokinetic analysis. Venous blood was drawn into 10-ml heparinized tubes prior to drug administration and at predetermined times following i.v. bolus administration. When spiromustine was given as a short i.v. infusion, timed blood samples were drawn into 10-ml heparinized tubes prior to drug administration and at predetermined times following i.v. bolus administration. When spiromustine was given as a short i.v. infusion, timed blood samples were drawn into 10-ml heparinized tubes prior to drug administration and at predetermined times following i.v. bolus administration. When spiromustine was given as a short i.v. infusion, timed blood samples were drawn into 10-ml heparinized tubes prior to drug administration and at predetermined times following i.v. bolus administration. When spiromustine was given as a short i.v. infusion, timed blood samples were drawn into 10-ml heparinized tubes prior to drug administration and at predetermined times following i.v. bolus administration.
slurry for 15 sec, after which the chilled blood was centrifuged at 1100 \times g for 2 min in a DynaCup table top centrifuge (Clay Adams; Becton, Dickinson and Co., Parsippany, NJ) to obtain plasma. A 1.0-ml aliquot of chilled plasma or CSF was subsequently transferred to a stoppered 13-ml glass centrifuge tube, and 10 \mu l of 1.57 \times 10^{-5} M (508 ng) 3-[2-[bis(2'-chloroethyl)amino]ethyl]-5,5-diethylhydantoin (1) in dimethylformamide was added as an internal standard. After mixing, the sample was then extracted with 9.0 ml of a 7:3 (v/v) hexane/toluene solution by vortexing for 2 min. The sample-solvent mixture was centrifuged for 2 min at 1100 \times g and 8.0 ml of the top organic layer were removed and transferred to a clean 13-ml glass centrifuge tube. At this point SHM had been stabilized by extraction into an organic solvent and subsequent sample preparation could be carried out in the laboratory. The organic extract was concentrated to approximately 1 ml volume with dry nitrogen on an analytical evaporator (N-Evap; Organon, South Berlin, MA) before being transferred to a 2-ml conical glass vial. The solution was evaporated to dryness, and the residue was reconstituted with 40 \mu l toluene. A 1.0-\mu l aliquot of this toluene solution was then analyzed by wide-bore fused silica capillary gas chromatography as previously described (5).

Standard curves for SHM in plasma were prepared for each patient’s samples by addition of known amounts of SHM to the corresponding pretreatment plasma. These spiked standards were processed in the same manner as above. Appropriate plasma standard curves were constructed using least-squares regression for each day’s analysis. These curves typically covered the range, 0–1000 ng/ml, and possessed correlation coefficients greater than 0.995. The limit of quantitation (signal to noise = 5) for this assay was 10 ng/ml, although smaller amounts of SHM could be detected. The plasma standard curve was also used for the CSF samples since prior laboratory observations had demonstrated that when pooled CSF from several patients was used, the standard curve produced was virtually identical to those produced in plasma.

The measured SHM plasma concentration versus time curve was fit to a biexponential equation \( C_p = C_{p0} e^{-\alpha t} + C_{p1} e^{-\beta t} \) using MLAB, an iterative nonlinear least squares regression program (6). Pharmacokinetic parameters were then calculated using standard equations (7). The plasma AUC was determined by the use of the linear trapezoidal rule over the time range encompassing all measured levels and adding to it a sum representing the AUC from this last measured point extrapolated to infinity by using the term

\[
1.44 \times C_p \times t_{1/2} \beta
\]

where \( C_p \) is the last measured plasma concentration and \( t_{1/2} \beta \) the appropriate terminal-phase half-life. In the instance where SHM was given as a bolus dose and multiple CSF time points were measured, the CSF AUC was estimated by the linear trapezoidal rule with extrapolation to time 0 and to the first time point where the drug was no longer detectable. The CSF:plasma ratio was then determined by comparing the CSF AUC to the plasma AUC. For those cases in which SHM was given as a short infusion, the CSF:plasma ratio was estimated by comparing the CSF concentration at the end of infusion to the mean apparent plasma steady-state concentration.

SHM protein binding was evaluated in pooled fresh normal human plasma. Because of drug instability in plasma at ambient temperature, binding was determined at 3°C. Both pH 7.4 phosphate buffer containing 0.9% NaCl and plasma were spiked with spiromustine to a concentration of 1 \mu g/ml (approximating the highest measured plasma concentration), vortexed for 15 sec, and then incubated for 5 min. Four 1.0-ml aliquots of both buffer and plasma were centrifuged at 1100 \times g for 1 h in Centrifuge micro centrifugation systems (Amicon Corp., Danvers, MA). Ultrafiltrate volumes were determined and SHM was quantitated in the buffer and plasma ultrafiltrates, as well as in the plasma retentate and the spiked buffer and plasma which had not been ultrafiltered. Equivalent concentrations of SHM in the buffer and the corresponding ultrafiltrate indicated no membrane holdup during ultrafiltration. The percentage of protein binding was defined as

\[
100\% \times \frac{1 - F}{C_p}
\]

where \( F \) was the concentration in the plasma ultrafiltrate. A mass balance recovery study based on ultrafiltrate and retentate concentrations was used to check the above calculations.

RESULTS

Phase I Trial. Twenty-three patients (13 males and 10 females) ranging in age from 3.5 to 20 yr were accrued in this study and all were evaluable for toxicity.

Systemic toxicity was generally mild and consisted mostly of self-limited gastrointestinal symptoms such as nausea, emesis, diarrhea, and constipation. Severe hematological toxicity consisting of a platelet count of 16,000/mm³ occurred in a single individual 1 wk after the second SHM dose. However, this event is believed related to the effects of prior chronic nitrosourea therapy rather than to SHM.

Neurotoxicity was the only consistently observed toxic event associated with SHM and was dose limiting. Table 1 characterizes the symptoms associated with each grade of neurotoxicity, the number of patients at each grade, and the SHM dose. Twelve episodes of Grades I and II neurotoxicity occurred in 11 patients and in each instance there was spontaneous abatement of this toxicity within 12 h. These episodes occurred at all but the highest SHM dose studied (11.5 mg/m²) in which only Grade III toxicity was observed. Eleven episodes of Grade III neurotoxicity occurred in 9 patients, and such episodes were noted at every dose of SHM studied. In addition to those features listed, all patients developed mydriasis within 5 min of SHM administration. Xerostomia frequently followed mydriasis, and both symptoms were self-limited. The duration of mydriasis paralleled that of the other symptoms of neurotoxicity and its disappearance usually signaled that the end of other neurological symptoms was near.

As the number of patients accrued at any dose level of SHM is small, there did not appear to be any visible increase in the frequency of Grade III neurotoxicity with increasing SHM dose. Although the only patients to regularly demonstrate a duration of such toxicity beyond 24 h occurred at SHM doses beyond 8.5 mg/m², this modest trend toward increasing duration of toxicity with SHM dose is also unreliable because of limited patient accrual (see Table 1). In addition, there was no evidence of cumulative neurotoxicity or increased sensitivity to the neurotoxic effects of SHM with repeated drug exposure at the same or greater doses. A patient was equally likely to have a greater or lesser grade of neurotoxic symptomatology with subsequent SHM doses.

Physostigmine has been observed to abolish or greatly reduce the severity of the intense anticholinergic symptoms which characterized SHM neurotoxicity (8, 9). Based on this observation we gave physostigmine (2 mg/m² i.m. or i.v., followed
by 1 mg/m²/h as an infusion) to 6 of 9 patients who demonstrated Grade III neurotoxicity after receiving SHM. In each of these instances a dramatic reduction in the intensity of neurotoxic symptoms (to Grade I or II level) occurred within 15 to 20 min. Following this, patients often slept as long as 12 h. In 3 of the above 6 patients, prophylactic physostigmine was given prior to or with 7 subsequent doses of SHM and no further episodes of Grade III neurotoxicity occurred in these individuals. No attempt was made to rechallenge these patients to subsequent doses of SHM without giving physostigmine. In most patients, physostigmine was stopped at approximately 12-h intervals to assess their residual toxic manifestations. In some, further physostigmine was unnecessary while in others, Grade III symptoms reappeared within 1–2 h and physostigmine was restarted.

Because of the lack of significant systemic toxicity, the maximum tolerated dose of SHM in this trial was defined as that dose which produced Grade III neurotoxicity requiring the administration of physostigmine for greater than 24 h in 2 of 3 patients. Based on this, the maximal tolerable dose of SHM in this trial was judged to be 9.5 mg/m² weekly for three doses.

Table 2 lists the diagnosis of the 23 patients entered onto this study; of these 17 were evaluable for response after completing at least 1 full 3-wk course of SHM. Fourteen of these patients had primary CNS malignancies and one a meningeal lymphoma. Stability of previously advancing clinical symptomatology was noted in 2 patients for periods of 2 and 3.5 mo, although their CT scans did not demonstrate any evidence of response.

Pharmacology Studies. For the six patients who had bolus dose SHM plasma kinetics determined, the Cp versus time curve could be adequately described by a biexponential equation, representing a 2-compartment open model which was subsequently used to calculate the half-lives and other pharmacokinetic parameters listed in Table 3. Fig. 2 shows a representative

Table 3 Pharmacokinetic parameters of bolus dose spiromustine

<table>
<thead>
<tr>
<th>Patient (mg/m²)</th>
<th>t½α (min)*</th>
<th>t½β (min)</th>
<th>AUC (ng/ml/min)</th>
<th>CL (ml/min/m²)*</th>
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* t½α, half-life of initial distribution phase; t½β, elimination half-life for terminal phase.
* Mean ± SD.

Table 4 Pharmacokinetic parameters of spiromustine infusion

<table>
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<tr>
<th>Patient (mg/m²)</th>
<th>Infusion length (min)</th>
<th>Css (ng/ml)*</th>
<th>AUC (ng/ml/min)</th>
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</table>

* Css, apparent steady-state plasma concentration.
* CL, apparent total body clearance.

Cp versus time curve indicating that spiromustine plasma levels fall rapidly until they are undetectable 1 h after drug administration.

The validity of the above pharmacokinetic model and the first order extrapolation for the AUC calculation from time 0 to the first time point was confirmed in three patients who also received SHM as a short (15-min) i.v. infusion. For these patients the apparent steady-state Cp was used to calculate drug clearance and compared with the clearance calculated from a corresponding bolus dose (Tables 3 and 4). The similarity of the clearance in both instances corroborated the above pharmacokinetic model.

SHM was not readily measurable in CSF. In five of the nine instances where CSF samples were obtained following a bolus dose of SHM, the drug could not be detected. When spiromustine could be detected, levels were very low and often at or below the limit of quantitation (10 ng/ml) of our assay. In all three instances in which SHM was given as a short infusion, low levels of the drug were detectable in CSF. The CSF concentrations at the time closest to the end of infusion were used to estimate the extent of CNS drug penetration (Table 5).

Protein binding studies at 3°C revealed only that 9 ± 3% of the total spiromustine concentration could be measured in the
plasma ultrafiltrate. No losses of compound were observed in the ultrafiltration units indicating that SHM is highly protein bound (91% at 3°C) in human plasma. These values can be readily extrapolated to 37°C since the temperature coefficient of protein binding is minimal (10).

DISCUSSION

As observed by others (8, 11), the dose-limiting toxicity of SHM is neurological and anticholinergic in nature, consisting of mydriasis, xerotonia, and variable degrees of lethargy, confusion, and in some instances, hallucinations. The neurotoxicity did not appear to be cumulative with the treatment schedule used in this trial, although cumulative neurotoxicity has been observed in adult trials where a daily ×3 treatment schedule was used (8, 9).

One patient who had Grade III neurotoxicity lasting 60 h at an SHM dose of 8.5 mg/m² was extremely obese and her SHM dose was calculated on the basis of actual weight. Because brain weight does not vary as much as body weight, we suspect that this patient may have been relatively overdosed and suffered a longer duration of neurotoxicity than would have otherwise occurred had she been dosed on the basis of ideal body weight. Unfortunately, no SHM levels are available in this patient. However, a similar episode of toxicity in an obese patient has been observed in one adult SHM trial (8).

The SHM maximum tolerated dose of 9.5 mg/m² weekly times 3 established in our trial reflects the use of physostigmine both acutely or prophylactically to treat episodes of Grade III neurotoxicity. Even though the mechanism of the apparent protective effect of physostigmine is unknown, similar observations regarding the capacity of this drug to reduce the duration and intensity of neurotoxicity and to extend the maximum tolerated dose have been reported by others (8, 9). Although it has been reported that physostigmine has no effect on the severity of SHM-induced neurotoxicity in mice (11), the SHM doses given were at least an order of magnitude greater on the basis of body surface area than the doses used in current Phase I trials (8, 9, 11) and were lethal in greater than ½ of these animals.

Although clinical and CT evidence of tumor response has been observed in one recent adult study (8), we and others (11) have not observed any significant responses. Even so, the decision to pursue a Phase II evaluation of an agent should not be made solely on the basis of such observations, as Phase I trials are not designed to measure therapeutic response. However, the unacceptably high incidence of Grade III neurotoxicity throughout the entire range of SHM doses studied (9 of 24 courses), the surprisingly low CSF penetration of SHM, and the lack of SHM levels in a range that has demonstrated in vivo cytotoxicity raise concern regarding the potential value of SHM in a Phae II setting or beyond.

The highest measurable plasma value of SHM observed in this study, 1920 ng/ml, occurred in a patient treated at 11.5 mg/m² and was associated with significant neurotoxicity. This value is no more than 40% of the 5–10 μg/ml SHM concentration which produced only minimal in vitro cytotoxicity in the very sensitive monolayer cultures of rat 9L brain tumor (12). Further, the level achieved in this patient was the result of a dose that represents less than 6% of the total 38 mg/m² (12.5 mg/kg) daily ×5 dose that was optimal in vivo in the mouse ependymoblastoma model (1).

While the neurotoxicity of SHM suggests that this agent (or a neurotoxic metabolite) does indeed cross the blood-brain barrier in humans, the magnitude of such penetration appears to be quite small, probably reflecting the high degree of protein binding of SHM and marked aqueous instability. The greatest CSF level observed in this study was 23 ng/ml, reflecting a CSF to plasma ratio of only 4.7%. In the three other instances (see Table 5) where SHM was detectable in CSF, the levels and CSF:plasma ratios were even lower. (In five of nine courses given to 3 patients in whom CSF was sampled, SHM was undetectable.) When these levels are contrasted with those which are minimally active in the rat 9L tumor cultures, human CSF levels are 200–500 times smaller.

Although it is difficult to directly apply in vitro and in vivo antitumor data from animal tumors to the clinical situation in humans, the great differences in the clinically tolerable doses of SHM in humans and those effective in vivo in the murine ependymoblastoma as well as the differences between in vitro cytotoxic levels and those obtained in plasma and CSF cannot be ignored. The above data suggest that the brain parenchymal levels of SHM which are achievable at clinically tolerable doses in humans are likely to be quite limited and generally below levels which produce significant antitumor activity.

ACKNOWLEDGMENTS

The clinical pharmacy skills of Paul Jarosinski and the secretarial support of Mariann Waldbillig are gratefully acknowledged.

REFERENCES


Table 5 Relative availability of spiromustine to the CSF

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose (mg/m²)</th>
<th>Administration route</th>
<th>CSF:plasma ratio (%)</th>
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<td>Infusion</td>
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<tr>
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<tr>
<td>5</td>
<td>11.5</td>
<td>Infusion</td>
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* Calculated from estimated CSF AUC and trapezoidal plasma AUC.
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