Experimental Chemotherapy of Human Medulloblastoma with Classical Alkylators

Henry S. Friedman, O. Michael Colvin, Susan M. Ludeman, S. Clifford Schold, Jr., Victoria L. Boyd, Lawrence H. Mulhauber, and Darell D. Bigner

ABSTRACT

Seven classical alkylators were tested for activity against the continuous human medulloblastoma cell line TE-671 grown in vitro and as s.c. and intracranial xenografts in athymic mice. Drugs tested included melphalan, cyclophosphamide (4-hydroperoxycyclophosphamide in vitro), iphosphamide (4-hydroperoxycyclophosphamide in vitro), phylketocyclophosphamide, phylketophosphamide, Asta Z 7557, and thiotriethylphosphoramide. All agents were active, with melphalan demonstrating the most activity in vitro and in vivo. Comparative studies of cyclophosphamide and phylketocyclophosphamide revealed partition coefficients (log P) of 0.73 and >1.69, respectively, and cyclophosphamide exhibited the most cytotoxic activity in vitro and in vivo. Further studies comparing cyclophosphamide and iphosphamide, respectively (23). All agents were active, with melphalan demonstrating the most activity in vitro and in vivo. Further studies comparing cyclophosphamide and phylketocyclophosphamide demonstrated the greater lipophilicity and toxicity (following i.p. administration) of the former as well as the potential means to therapeutically exploit these differences.

INTRODUCTION

Chemotherapeutic intervention for medulloblastoma has not contributed significantly to the successful treatment of this tumor. Chemotherapy has produced well documented responses in recurrent disease (1–5) but has only provided a minimal increase in disease-free survival for patients with advanced disease (6, 7). This low efficacy of chemotherapy may be a consequence of the heterogeneity of this tumor (8–11), cellular drug resistance, or poor drug delivery to the tumor site. The selection of chemotherapeutic agents for medulloblastoma (and glioma) has in large part been based on the presumed need to choose low molecular weight lipophilic compounds able to effectively penetrate to the intracranial tumor site. However, this presumption, based in large part upon anatomical studies in normal brain demonstrating capillary tight junctions devoid of fenestrae (12) and the therapy of intracranial L1210 leukemia in essentially normal brain (13–15), may not reflect the barrier disruption present in a brain tumor (16, 17). Equal considerations need to be directed toward the selection of agents highly cytotoxic to brain tumors (independent of molecular weight or lipophilicity), since even their partial delivery to the intracranial site may have greater therapeutic ramifications than the greater delivery of less active low molecular weight lipophilic compounds. The most rational approach might well be the identification of highly cytotoxic agents, with subsequent efforts devoted to increasing their delivery to the tumor site.

We have described previously the establishment and utilization of our model to study medulloblastoma, and subsequent studies have indicated significant efficacy of the classical alkylators cyclophosphamide and melphalan against the human medulloblastoma cell line TE-671 growing s.c. and intracranially in athymic mice (18–21). We now extend these observations and report the in vitro and in vivo sensitivity of TE-671 to seven classical alkylators. Agents studied included melphalan (NSC 8806), cyclophosphamide (NSC 26271) (4-hydroperoxycyclophosphamide in vitro), iphosphamide (NSC 10924) (4-hydroperoxycyclophosphamide in vitro), thio-TEPA (NSC 6396), the 4-thiocylophosphamide derivative Asta Z 7557 (22), and phylketocyclophosphamide and phylketophosphamide, two novel analogues of the aldehydic metabolites of cyclophosphamide and iphosphamide, respectively (23). All agents were active, with melphalan demonstrating the most activity in vitro and in vivo. Further studies comparing cyclophosphamide and thio-TEPA demonstrated the greater lipophilicity and toxicity (following i.p. administration) of thio-TEPA, as well as the potential means to therapeutically exploit these differences.

MATERIALS AND METHODS

Cell Line. The human medulloblastoma cell line TE-671 established from a cerebellar tumor (24) was grown in cell culture as described previously (18).

Clonogenic Assay. All drugs assays were performed as described previously, utilizing a 1-h drug incubation with a final cell concentration of 2.5 × 10⁴ cells/dish (20). Response was assessed by the ratio of treated versus control colony formation.

The following agents were tested in vitro: melphalan (NSC 8806); 4-hydroperoxycyclophosphamide; 4-hydroperoxycyclophosphamide; Asta Z 7557; thio-TEPA (NSC 6396); phylketocyclophosphamide; and phylketophosphamide. All drug solutions were freshly prepared immediately prior to the assay. Melphalan was dissolved in 0.1 N HCl and serially diluted in Eagle's minimal essential medium. Thio-TEPA and Asta Z 7557 were dissolved in Eagle's minimal essential medium and serially diluted to desired concentrations. 4-Hydroperoxycyclophosphamide, 4-hydroperoxycyclophosphamide, 4-hydroperoxycyclophosphamide, and phylketocyclophosphamide, and phylketophosphamide were all dissolved in DMSO (2 mg/ml) and serially diluted in Eagle's minimal essential medium. Mixtures (1:1) of drug solution and cell suspension were used to achieve a 1-ml cell suspension containing 10⁶ cells with the desired final drug concentration.

Melphalan and iphosphamide were provided generously by the Developmental Therapeutics Program of the National Cancer Institute. 4-Hydroperoxycyclophosphamide was synthesized in the laboratory of Dr. O. M. Colvin. 4-Hydroperoxycyclophosphamide, phylketocyclophosphamide, and phylketophosphamide were synthesized in the laboratory of Dr. S. Ludeman by Dr. V. L. Boyd. Thio-TEPA was commercially available. Asta Z 7557 was generously provided by Dr. P. Hilgard, Asta-Werke Co. (Bielgard, W. Germany).

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3 The abbreviations used are: thio-TEPA, thiotriethylphosphoramide; DMSO, dimethyl sulfoxide; ID₅₀, ID₇₀, ID₉₀, in vitro drug dose at which there is a 50, 75, or 90% reduction in the number of colonies in comparison to controls, respectively.
Calculation of in Vitro Parameters. Parameters of the dose-response curves were derived by linear regression analysis of the relationship between percentage of colony formation (treated colonies/control colonies \( \times 100 \)) and log drug concentration. To obtain the individual percentage of colony formation values for the different drug concentrations, the number of colonies for each treated dish was divided by the mean number of colonies of the control dishes. The slope value of each log dose versus response curve was then used to compute the ID_{50}, ID_{75}, and ID_{90} by substituting 90, 75, or 50% for percentage of colony formation and solving the regression equation for drug concentration, with confidence limits for the regression concentration.

Animals. Male or female athymic BALB/c mice (nu/nu genotype, 6 weeks or older) were used for all in vitro studies. Animals were maintained as described previously (25).

Tumor Line. The human medulloblastoma cell line TE-671 was grown s.c. and intracranially in athymic nude mice as described previously (21).

Tumor Transplantation. S.c. and intracranial tumor transplantation was performed as described previously (19).

Tumor (s.c.) Measurement. Tumors s.c. were measured every 3 to 4 days with vernier calipers (Scientific Products, McGaw Park, IL) until the volume exceeded 2000 mm\(^3\). Width and length in mm were measured, and volume was calculated by the formula: \( W^2 \times L / 2 \), where \( W \) is width and \( L \) is length (25).

Drug Toxicity. The lethal toxicity of individual drugs was assessed by probit analysis (26). A minimum of 4 doses with 10 animals/dose was used to calculate the 10% lethal dose of each drug. The dose used in the experiments was 100% of the calculated 10% lethal dose administered as a single i.p. injection in a volume of 90 ml/m\(^2\). The doses were: melphalan, 71.3 mg/m\(^2\); cyclophosphamide, 1391 mg/m\(^2\); phenylketocyclophosphamide, 279.9 mg/m\(^2\); ifosphamide, 2014.9 mg/m\(^2\); phenylketophosphamide, 672.8 mg/m\(^2\); Asta Z 7557, 1641.8 mg/m\(^2\); and thiop-TEPA, 61.8 mg/m\(^2\). Melphalan was administered in 16.7% DMSO, phenylketocyclophosphamide and phenylketophosphamide in 33% DMSO, and cyclophosphamide, ifosphamide, Asta Z 7557, and thio-TEPA in 0.9% NaCl solution.

Tumor (s.c.) Therapy. Groups of 8 to 10 randomly assigned mice were treated i.p. with chemotherapeutic compounds according to the previously described dosages and schedule when the median tumor volume exceeded 200 mm\(^3\). One group of matching animals served as a control and received the drug vehicle.

Response was assessed by mean treated versus control tumor volume (\( T/C \)), the difference in days between the median of treated individual animals and the median of control animals' tumors to reach a volume of 5 times the initial treatment volume (\( T - C \)), and treated versus control tumor regressions. P-values \( \leq 0.01 \) were considered significant. Statistical significance was assessed as described previously (19).

Intracranial Tumor Therapy. Groups of 10 randomly assigned mice were treated i.p. with chemotherapeutic compounds according to the previously described dosages and schedule on Day 12 after tumor implantation. One group of 10 animals served as a control and received the drug vehicle. Assessment of response was the comparison of median survival time and long-term survivors (>60 days) between treated and control groups. Statistical significance was assessed as described previously (19).

Studies Comparing Cyclophosphamide with Phenylketocyclophosphamide Partition Coefficients. A 14 mm solution of cyclophosphamide monohydrate or 4-hydroperoxycyclophosphamide in water (8 mg/2 ml) was vortexed (5 mm) with an equal volume (2 ml) of octanol. After standing (5-10 min), the layers were separated, and each was analyzed by \(^{31}P\)-nuclear magnetic resonance spectroscopy, using an identical set of acquisition and display parameters (0.15 ml D\(_2\)O or DMSO-d\(_6\) was added to each layer as a nuclear magnetic resonance lock signal). The acquisition parameters included a 5-kHz spectral window, 8192 data points, a \( \pi / 2 \) pulse of 20 \( \mu \)s, low-power proton decoupling, and a pulse recycle time of 2 s. The free induction decay signal that was obtained after 1000 pulses was exponentially multiplied so as to result in an additional 0.97 Hz of line broadening in the frequency-domain spectrum. The signal intensities (peak height \( \times \) width at half-height) given after 1000 pulses were used to determine the relative concentration of cyclophosphamide or 4-hydroperoxycyclophosphamide in the octanol and water layers. Phenylketocyclophosphamide and phenylketophosphamide were treated as above, except that they were first dissolved in octanol (10 mg/2 ml; 14 mm solution) and then extracted with water.

Murine Plasma Activity following Cyclophosphamide or Phenylketocyclophosphamide. Groups of 20 mice were given injections i.p. of either cyclophosphamide or phenylketocyclophosphamide at the 10% lethal dose and sacrificed 30 min later. The animals' blood, obtained by direct cardiac puncture using heparinized syringes, was pooled into two separate samples.

The plasma was separated and used in a clonogenic assay against TE-671 grown in cell culture. A final 1:1 mixture of plasma and cell suspension was incubated and handled as already described. Controls were 1:1 mixtures of cell suspensions and plasma obtained in a similar fashion from mice that had received an equal i.p. injection of drug vehicle.

Hematological Toxicity Studies. Groups of 60 mice were given injections i.p. of either cyclophosphamide or phenylketocyclophosphamide at the 10% lethal dose. A random selection of 4 mice from each group was bled and sacrificed on days 1, 3, 5, 7, 8, 12, 15, and 20, and the blood samples were placed in microvet CB1000 microtubes (Sarstedt, W. Germany). Complete blood counts were run on an ELT-8 ds (Ortho Diagnostic Systems, Westwood, MA) and WBC differentials were performed by one of the authors (H. S. F.). Four mice receiving no drug were bled and sacrificed on day 0, and their complete blood counts and WBC differentials were used as baseline values.

RESULTS

Clonogenic Assay. The results of the in vitro dose-response curves are represented in Figs. 1 and 2. Reduced colony formation was observed with increasing drug concentrations for all drugs tested. The calculated parameters of the dose-response relationships, i.e., ID_{50}, ID_{75}, ID_{90} are listed in Table 1. Melphalan produced more cell kill, on a molar basis, than the other agents tested, with an ID_{50}, ID_{75}, and ID_{90} of 0.9, 1.9, and 3.1 \( \mu \)M, respectively.

Drug Toxicity. Twenty deaths in 280 tumor-bearing animals

Fig. 1. In vitro dose-response curves showing the chemosensitivity of the human medulloblastoma cell line TE-671. 95% confidence limits.
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Table 1 In vitro sensitivity of TE-671

<table>
<thead>
<tr>
<th>Drug</th>
<th>ID_{50} (μM)</th>
<th>ID_{75} (μM)</th>
<th>ID_{90} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melphalan</td>
<td>0.9</td>
<td>1.9</td>
<td>3.1</td>
</tr>
<tr>
<td>4-Hydroperoxycylophosphamide</td>
<td>1.9</td>
<td>6.3</td>
<td>12.8</td>
</tr>
<tr>
<td>Phenylketocylophosphamide</td>
<td>0.8</td>
<td>2.3</td>
<td>4.6</td>
</tr>
<tr>
<td>4-Hydroperoxycylophosphamide</td>
<td>5.4</td>
<td>12.6</td>
<td>20.8</td>
</tr>
<tr>
<td>Phenylketophosphamide</td>
<td>1.9</td>
<td>7.4</td>
<td>17.0</td>
</tr>
<tr>
<td>Asta Z 7557</td>
<td>3.2</td>
<td>5.2</td>
<td>12.8</td>
</tr>
<tr>
<td>Thio-TEPA</td>
<td>20.3</td>
<td>53.1</td>
<td>94.6</td>
</tr>
</tbody>
</table>

Fig. 2. In vitro dose-response curves showing the chemosensitivity of the human medulloblastoma cell line TE-671. 95% confidence limits.

were attributed to drug toxicity with the following distribution: melphalan, 1 of 40; cyclophosphamide, 4 of 40; phenylketocylophosphamide, 0 of 40; iphosphamide, 2 of 40; phenylketophosphamide, 8 of 40; Asta Z 7557, 5/40; and thio-TEPA, 5 of 40. Mean nadir weight loss was: melphalan, 15.7%; cyclophosphamide, 17.6%; phenylketocylophosphamide, 17.7%; iphosphamide, 12.6%; phenylketophosphamide, 19.5%; Asta Z 7557, 10.7%; and thio-TEPA, 17.0%.

s.c. Tumors. The response to chemotherapy is illustrated in Figs. 3 and 4 and summarized in Table 2. There was a statistically significant response to all 7 alkylators. Melphalan produced the longest growth delays of 20.9 of 19.5 days in duplicate trials, as well as a 100% incidence of tumor regressions. No cures resulted from the administration of any of the drugs. No control animals demonstrated any tumor regressions.

Intracranial Tumors. All agents were active against intracranial xenografts, with a percentage of increase in median survival of 13—64% ((median survival treated animals — median survival control animals)/median survival control animals x 100) compared to controls (Table 3). Melphalan produced the largest increases in median survival, 64, 42, and 64% in triplicate trials (Fig. 5). Three animals were long term survivors (two treated with melphalan and one with phenylketocylophosphamide). All other animals, control and treated, with the exception of 13 toxic early deaths, died with massive intracranial tumors.

Partition Coefficients (P). The log Ps for cyclophosphamide, 4-hydroperoxycylophosphamide, phenylketocylophosphamide, and phenylketophosphamide were 0.73, 0.77, >1.69, and >1.69 respectively.

Murine Plasma Activity. The treated/control percentage of colony formations was 0.0 and 0.2% for plasma from cyclophosphamide-treated animals and 40.0 and 20.0% for plasma from phenylketocylophosphamide-treated animals in duplicate trials.

Hematological Toxicity Studies. The serial mean values for hematocrit, WBC count, platelet count, and absolute neutrophil count were shown in Table 4. Cyclophosphamide and phenylketocylophosphamide both produced a significant leukopenia, although the associated neutropenic nadir as lower and of longer duration in the cyclophosphamide-treated animals.

DISCUSSION

Advances in the treatment of medulloblastoma have reflected improvements in neurological technique and the use and refinement of radiation therapy (6). Chemotherapeutic intervention has had only a limited influence on the natural history of this tumor, producing at best a modest improvement in the disease free survival of patients with advanced disease (6, 7). Further improvements in therapy must reflect the more effective utilization of chemotherapy, since the use of additional radiotherapy is limited by considerations of unacceptable neurotoxicity.
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studies suggesting significant in vivo efficacy for the classical alkylators cyclophosphamide and melphalan (19, 21). The present studies extend this work, exploring the in vitro and in vivo efficacy of seven classical alkylators against TE-671, demonstrating activity for all agents tested. This activity is, in itself, interesting, although one might have predicted that all additional agents chosen would be effective in light of their ultimate conversion to a phosphoramid mustard. However, differences in their transport or physical properties make several agents potentially attractive as therapeutic alternatives in the treatment of medulloblastoma.

Parameters influencing the delivery of a drug to the intracranial site are multifactorial and include lipophilicity (as quantitated by partition coefficients), ionization, molecular size, and pharmacokinetic considerations such as blood flow, plasma clearance, and protein binding (27). A rational approach to the therapy of central nervous system tumors must address not only the agent’s intrinsic cytotoxicity but the effective delivery of the agent to the intracranial site. Although a lipophilic agent achieves greater access to the intracranial site than does a nonlipophilic compound of similar molecular weight (28) the use of a minimally active lipophilic compound may be less effective than use of a markedly active compound with relatively less penetration. The optimal approach would be to identify active compounds and subsequently attempt modifications enhancing delivery without compromising cytotoxicity. The activity of cyclophosphamide in the treatment of medulloblastoma in the laboratory (19) and in the clinic (4) led us to pursue the possibility that the differences among a series of classical alkylators might allow enhanced drug delivery while preserving cytotoxic activity.

Melphalan was the most active agent studied in vitro and in vivo. Although small differences in growth delay or survival prolongation produced by 2 agents may not be biologically significant, these results are provocative. Despite similar activity to cyclophosphamide (M, 261,000, log P 0.73) against relatively permeable s.c. xenografts (29) and a considerably lower partition coefficient, melphalan (M, 306,000, log P = -1.70) (30) produced equivalent if not greater prolongations of median survival in animals bearing intracranial xenografts. This
Table 3  Chemotherapeutic response of TE-671 growing intracranially in athymic mice

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Drug</th>
<th>Median survival (days)</th>
<th>P-value</th>
<th>Long term (&gt;60 days) survivors</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Melphalan-1</td>
<td>59 (24–75+)s</td>
<td>0.007</td>
<td>2</td>
<td>NSs</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>36 (25–59)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Melphalan-2</td>
<td>54 (38–72+)</td>
<td>0.001</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>38 (29–52)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cyclophosphamide-1</td>
<td>35 (34–38)</td>
<td>0.001</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Phenylketocyclophosphamide-1</td>
<td>32 (28–37)</td>
<td>0.003</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>27 (23–35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Cyclophosphamide-2</td>
<td>39.5 (36–44)</td>
<td>&lt;0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Asta Z 7557-1</td>
<td>36 (34–39)</td>
<td>&lt;0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Phenylketocyclophosphamide</td>
<td>34.5 (33–41)</td>
<td>0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30.5 (22–37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Asta Z 7557-2</td>
<td>35 (29–38)</td>
<td>&lt;0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Iphosphamide-1</td>
<td>38 (32–42)</td>
<td>&lt;0.001</td>
<td>0</td>
<td>NS</td>
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<td></td>
<td>Phenylketoiphosphamide-1</td>
<td>37 (31–43)</td>
<td>&lt;0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30 (25–32)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Iphosphamide-2</td>
<td>38 (31–48)</td>
<td>&lt;0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30 (22–35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Phenylketoiphosphamide-2</td>
<td>45 (38–54)</td>
<td>&lt;0.001</td>
<td>0</td>
<td>NS</td>
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<td>Cyclophosphamide-3</td>
<td>49 (45–54)</td>
<td>&lt;0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Thio-TEPA-1</td>
<td>49 (33–54)</td>
<td>0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Thio-TEPA-2</td>
<td>49 (31–54)</td>
<td>0.007</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>34.5 (25–45)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Melphalan-3</td>
<td>37 (32–52)</td>
<td>&lt;0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Cyclophosphamide-4</td>
<td>37 (31–55)</td>
<td>&lt;0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Thio-TEPA-3</td>
<td>29 (24–40)</td>
<td>0.001</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>22.5 (17–26)</td>
<td></td>
<td></td>
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* Numbers in parentheses, range.

Table 4  Mean hematological values following injection of cyclophosphamide or phenylketocyclophosphamide into athymic mice

<table>
<thead>
<tr>
<th>Day</th>
<th>Hcta</th>
<th>WBCa</th>
<th>ANCc</th>
<th>PLTd</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>43.1</td>
<td>1.4</td>
<td>877</td>
<td>699</td>
</tr>
<tr>
<td>1</td>
<td>53.4</td>
<td>1.4</td>
<td>1302</td>
<td>803</td>
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<tr>
<td>3</td>
<td>52.6</td>
<td>0.1</td>
<td>100</td>
<td>709</td>
</tr>
<tr>
<td>5</td>
<td>48.6</td>
<td>0.2</td>
<td>200</td>
<td>289</td>
</tr>
<tr>
<td>7</td>
<td>51.7</td>
<td>0.6</td>
<td>540</td>
<td>234</td>
</tr>
<tr>
<td>8</td>
<td>45.2</td>
<td>1.4</td>
<td>1315</td>
<td>760</td>
</tr>
<tr>
<td>12</td>
<td>50.5</td>
<td>3.0</td>
<td>2587</td>
<td>450</td>
</tr>
<tr>
<td>15</td>
<td>48.8</td>
<td>2.1</td>
<td>2058</td>
<td>761</td>
</tr>
<tr>
<td>20</td>
<td>47.6</td>
<td>1.3</td>
<td>1107</td>
<td>807</td>
</tr>
</tbody>
</table>

a Hct, hematocrit (%).

b WBC, WBC count (×10⁹/liter).

c ANC, absolute neutrophil count (×10⁹/liter).

d PLT, platelet count (×10⁹/liter).

with known activity against TE-671 (34).

The identification of cyclophosphamide and melphalan as active agents against TE-671 growing intracranially led us to seek agents with a phosphoramide mustard as the ultimate alkylating metabolite, but with a higher degree of lipophilicity. It was hoped that this would result in greater penetration to the intracranial tumor site with an increased antitumor effect against the xenografts. Phenylketocyclophosphamide and phenylketoiphosphamide, initially synthesized to determine the steric, electronic, and therapeutic consequences of C-4 substitution in cyclophosphamide and iphosphamide, met this criterion. These compounds, with a considerably higher partition coefficient (log P >1.69), do not undergo an enzyme-catalyzed oxidation with ultimate formation of aldophosphamide but instead form a phosphoramide mustard through an elimination reaction. Our studies indicated greater activity in vitro for these compounds but greater toxicity in vivo as well. This toxicity may reflect the importance of aldehyde dehydrogenase, which is not a result of the metabolism of cyclophosphamide to a less lipophilic product, since the partition coefficient of 4-hydroperoxycyclophosphamide (a model for the cyclophosphamide metabolite 4-hydroperoxycyclophosphamide) was similar (log P 0.79). A precise explanation for this activity of melphalan is not yet known, but it may reflect the facilitated transport (competitive with glutamine and other amino acids) melphalan demonstrates in tumor cells as well as normal brain (31–33). Studies in progress are currently evaluating the potential therapeutic modulation of melphalan's transport and cytotoxicity by methionine sulfoximine, a glutamine synthetase inhibitor.
The activity of melphalan is now being studied in a phase II study. These observations suggest that this decreased activity of the phenylketocyclophosphamide is due to rapid clearance of the agent, resulting in poor drug exposure to the tumor. It is therefore not surprising that both phenylketocyclophosphamide and cyclophosphamide were less active in the treatment of intracranial xenografts, despite their increased lipophilicity. Further studies will address the pharmacokinetics and toxicity of these compounds, exploring the potential benefits of intra-arterial administration. Documentation of short plasma half-life and high plasma extraction coefficients would provide an additional rationale to study intra-arterial delivery in athymic rats bearing TE-671 xenografts (38).

Iphosphamide and Asta Z 7557 were chosen for study to determine if their biological and pharmacological differences from cyclophosphamide might result in greater activity, particularly since it has been suggested that iphosphamide has greater clinical activity than cyclophosphamide in other cancers (39). Both compounds were less active than was cyclophosphamide/4-hydroperoxycyclophosphamide in vivo and in vitro, with neither agent appearing to offer more promise than the parent compound in this medulloblastoma model.

Thio-TEPA was chosen for study because it is the only classical alkylator currently delivered via the intrathecal route (40) and has demonstrated activity against medulloblastoma (41). The activity of thio-TEPA in vitro was quite lower than that of 4-hydroperoxycyclophosphamide, but it did demonstrate comparable activity to cyclophosphamide at an equitoxic dose in vitro, illustrating the importance of other parameters such as drug pharmacokinetics in relating in vitro and in vivo studies. These studies do suggest the potential utility of 4-hydroperoxycyclophosphamide delivered via the intrathecal route.

These studies, suggesting the potential role of the classical alkylators in the treatment of medulloblastoma, are limited by reliance on a single cell line, TE-671, to define drug sensitivity. Nevertheless, this still represents the best available system to study the therapeutic profile of human medulloblastoma. The recent establishment of two new human medulloblastoma cell lines, D283 Med (42) and Daoy (43), will allow generation of an alkylator sensitivity profile more representative of this heterogeneous tumor. Nevertheless, the current results with TE-671 are provocative and bear analysis in the clinical setting. The activity of melphanal is now being studied in a phase II protocol for patients with recurrent medulloblastoma. The effective translation of our laboratory data to the clinical setting is the ultimate goal of these studies.

REFERENCES

28. Levin, V. Relationship of octanol/water partition coefficient and molecular


Experimental Chemotherapy of Human Medulloblastoma with Classical Alkylators

Henry S. Friedman, O. Michael Colvin, Susan M. Ludeman, et al.


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