Preclinical Antitumor Activity of Penclomedine in Mice: Cross-Resistance, Schedule Dependence, and Oral Activity against Tumor Xenografts in Brain

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ABSTRACT

Penclomedine is a di-2-picolinylpyridine derivative with p.o. antitumor activity in preclinical leukemia and solid tumor models. Described here are an in vivo cross-resistance profile of penclomedine, treatment schedule dependence studies, and studies exploring the effects of p.o. drug on human tumors xenografted into mouse brain. The latter studies exploited the apparent facile distribution of penclomedine to the central nervous system. Tumor models used included murine leukemia lines selected in vivo for acquired resistance to various antitumor drugs and the human mammary and lung tumor xenografts MX-1 and H82, respectively. The therapeutic effects of p.o. penclomedine against s.c. MX-1 and H82 xenografts were shown to be independent of treatment schedule. Therapeutic activity was comparable when p.o. and parenteral treatments were compared. Lines of P388 leukemia resistant to melphanal, cyclophosphamide, and Carmustine were cross-resistant to penclomedine in vivo. Leukemia lines resistant to antimetabolites, DNA binders/intercalators, and vincristine were not cross-resistant to penclomedine. Intracerebrally implanted MX-1 xenografts retained their sensitivity to p.o. penclomedine, and therapeutic activity was at least comparable to that of Carmustine, a drug known for its ability to cross the blood-brain barrier. These results demonstrate attributes of penclomedine that are relatively uncommon among currently available antitumor drugs and that are of interest for the anticipated clinical development of this drug.

INTRODUCTION

Penclomedine is an α-picolinyl(2-methylpyridine) derivative with clinically relevant antitumor activity in several experimental leukemia and solid tumor models, including advanced-stage human tumor xenografts (1, 2). In earlier studies (1), i.p. penclomedine induced regression of established s.c. CD8F1 murine mammary adenocarcinoma. Penclomedine p.o. induced modest tumor growth delays in established s.c. MCF-7 human mammary adenocarcinoma xenografts and in early-stage s.c. murine mammary adenocarcinoma 16/C. In standard screening models (i.p. tumor, i.p. drug, except as noted), penclomedine produced modest improvements in survival in L1210 leukemia, P388 leukemia, and M5076 fibrosarcoma. The drug exhibited no activity in the i.p. B16 melanoma, i.v. Lewis lung, or s.c. colon 38 models. Penclomedine was active both i.p. and p.o. in the subrenal capsule assay with the MX-1 human mammary carcinoma xenograft, and subsequent studies revealed that p.o. penclomedine was capable of inducing complete regressions of advanced-stage s.c. MX-1 xenografts and H82 small-cell lung carcinoma xenografts (1). These results prompted further studies.

Penclomedine was synthesized by Dow Chemical USA (Walnut Creek, CA) as a potential fungicide. Noteworthy attributes of penclomedine are its relatively novel chemical structure, its antitumor activity following p.o. administration, and its ability to produce complete regressions of selected, advanced-stage, s.c. human tumor xenografts. The extensive metabolism of penclomedine is being studied with a view toward identification of the form in which the compound exerts its antitumor effects (3). The cross-resistance profile reported here may provide some insight into mechanistic aspects of penclomedine action. Additional studies have been aimed at defining the extent and limitations of the antitumor activity of penclomedine. A preliminary account of this work has been presented (4).

MATERIALS AND METHODS

Animals, Tumors, and Drugs. Mice were young adult females of an appropriate strain, either BALB/c x DBA/2 F1 (hereafter called CD2F1) for the murine leukemias or athymic NCr-nu for the human tumor xenografts. These mice were obtained from various suppliers under contract with the Biological Testing Branch (Developmental Therapeutics Program, Division of Cancer Treatment, NCI). Each strain was housed in a separate barrier facility. CD2F1 mice were caged in stainless steel cages with hardwood bedding (Sani Chip; P. J. Murphy Forest Products Corp.). Diet consisted of Wayne Rodent Bilox (Teklad Premier, Inc.) and tap water ad libitum. With aseptic techniques, athymic mice were transferred to sterile, polycarbonate, filter-capped Microisolator cages (Lab Products, Inc.) containing sterile hardwood bedding. Athymic mice were fed Wayne Sterilizable Rodent Bilox and filter-sterilized water ad libitum. All manipulations of these mice were conducted in laminar-flow biosafety hoods. Temperature in the barrier facilities was maintained at 24 ± 1°C (SD). Relative humidity was 50 ± 10%. Lighting was operated automatically on 12-h light/dark cycles.

Human tumor lines were obtained from the NCI tumor repository (Frederick, MD). Frozen cells were thawed, cultured in RPMI 1640 with 10% heat-inactivated fetal bovine serum (HyClone), and harvested for s.c. implantation into athymic mice. The resulting solid tumors were then maintained by serial in vivo passage of trocar fragments (about 30 mg each). These lines were periodically restarted from frozen stocks to maintain relatively consistent passage numbers over the course of this work. Murine leukemias P388/L and L1210/L (parent, drug-sensitive, or wild-type lines) were obtained from the same source and propagated according to standard NCI protocols (5). Leukemia sublines expressing acquired resistance to 10 clinically useful antitumor drugs were developed at Southern Research Institute as described previously (6).

Penclomedine was supplied by the Pharmaceutical Resources Branch (Developmental Therapeutics Program, Division of Cancer Treatment, NCI). For injection into mice, penclomedine was prepared fresh daily as a suspension in aqueous hydroxypropyl cellulose (Klucel). A parenteral formulation was also provided by the Pharmaceutical Resources Branch. This was a 20% oil emulsion (30 mg of penclomedine/ml) that was diluted with 5% aqueous dextrose for injection. All dosages were administered to groups of CD2F1 mice on the basis of their average body weight. Athymic mice were treated on the basis of their individual body weights. Treatment routes and schedules are indicated in the tables. Mice were observed daily for survival. Other observations (e.g., tumor measurements) were recorded as indicated in the text and tables.
and have been described previously (1).

Each experiment included multiple dosages of penclomedine ranging from nontoxic to frankly toxic dosages. Results are reported here in terms of optimal, nontoxic dosages (less than or equal to the 10⁻¹₀ lethal dose) selected from these complete dose responses. Typically, each dosage group consisted of 10 mice. Tumorous control groups generally were untreated. All experiments were repeated at least once for confirmation unless otherwise noted.

Schedule Dependence. Different treatment schedules (and routes; see text) were compared simultaneously in single, internally controlled experiments in accordance with the methods described by Schabel et al. (7). In solid tumor models, comparisons were based on clinically relevant end points, namely, T — C; complete regressions, time to treatment failure, and tumor-free survivors. Tumor growth delay was calculated as the difference in the time (days) required for control and treated tumors to reach a selected evaluation size (7). The magnitude of T — C may be affected by treatment duration, which may differ between treatment schedules. Where necessary, this pitfall was avoided by relating T — C to net cell kill at the end of treatment as described by Lloyd (8). Because several experiments produced many complete regressions, the time to treatment failure, as defined in the tables, was used to describe the durations of these responses.

Cross-Resistance in Vivo. An in vivo cross-resistance profile was developed by comparing the activity of penclomedine against a parent ("wild type") sensitive leukemia and against a subline developed for resistance to a specific drug (6). The rationale and experimental design for this approach have been described in detail (9). Each of the 20 experiments reported here included a range of 4 penclomedine dosages, although data are presented for optimal, nontoxic dosages only. Dying mice were necropsied, and if death was judged to be nonleukemic based on the absence of ascites or splenomegaly, it was considered likely to have been drug induced. In each experiment, additional tumorous groups were treated with a range of dosages of the appropriate drug to confirm the resistance of the drug-resistant subline used. Moreover, a resistant leukemia was compared directly in each experiment to the drug-sensitive parent leukemia from which resistant sublines were derived, and the parallel groups of mice were treated identically with a single drug preparation. Experiments were repeated at least once for confirmation. Each of these experiments also included 6 groups of mice bearing initial tumor burdens ranging from 10⁸ to 10³ cells (by serial dilution). These "titration" groups provided the basis for an assessment of therapeutic response as described by Schabel et al. (7). Therapeutic response was based on the median day of death of dying mice. Long-term (45- to 60-day) survivors were excluded from calculations of the percentage of increased life span and from estimates of treatment-induced changes in tumor burden (7). If an optimal dosage of penclomedine effected a net cell kill that was at least 2 log₁₀ units less in a drug-resistant subline than the cell kill in the parallel sensitive-leukemia, that drug-resistant subline was judged to be cross-resistant to penclomedine.

i.e. Tumor Models. MX-1 mammary adenocarcinoma and U251 glioblastoma xenografts were implanted i.e. into athymic mice. Implants of 10⁶ cells in 0.03 cm³ were injected into the right hemisphere with a 0.25-inch 25-gauge needle. Titrations of i.e. implants of 10⁴ to 10⁵ cells permitted estimation of a doubling time and calculation of cell kill for each tumor model (7).

RESULTS

On the basis of complete regressions and tumor-free survivors, initial results (Table 1) suggested that p.o. dosing with penclomedine on multiple treatment days might offer a therapeutic advantage compared with a single treatment day regimen. On consideration of the range of doses causing at least 50% complete regressions, a split-dose regimen (every 6 h, for four doses) was less effective than single-dose treatment (data not shown). This difference seemed to be due to the higher total dose tolerated with a single treatment, because both regimens

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>Optimal dosage (mg/kg/dose)</th>
<th>Dose intensity (mg/kg/day)</th>
<th>Complete regression (days)</th>
<th>Time to treatment failure (days)</th>
<th>Tumor-free survivors (day 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose</td>
<td>1000</td>
<td>1000</td>
<td>10/10</td>
<td>&gt;37</td>
<td>6</td>
</tr>
<tr>
<td>Every 6 h for 4 doses</td>
<td>167</td>
<td>668</td>
<td>8/10</td>
<td>26</td>
<td>7</td>
</tr>
<tr>
<td>Every 4 days for 3 doses</td>
<td>445</td>
<td>148</td>
<td>10/10</td>
<td>&gt;29</td>
<td>10</td>
</tr>
<tr>
<td>Daily for 9 days</td>
<td>88</td>
<td>88</td>
<td>10/10</td>
<td>&gt;29</td>
<td>10</td>
</tr>
</tbody>
</table>

* Treatment began on day 14; median tumor sizes per group on day 14 ranged from 282 to 504 mg. Untreated control tumors exhibited a median time of 21 days to reach 1500 mg.

** Less than or equal to the 10⁻¹₀ lethal dose, selected from a range of dosages.

† Number of days from last treatment to earliest recurrence after complete regression.

produced seven to eight complete regressions at a total dose of 445 mg/kg. Subsequent experiments using a human tumor xenograft less sensitive to penclomedine (Table 2) indicated that although daily doses might be superior to intermittent treatment (Experiment 1, 4 of 10 complete regressions and longer tumor growth delay in treatment failures), daily doses (for 9 days) were not superior to a single dose (Experiment 2, regressions and tumor growth delays are similar).

If therapeutic response is interpreted as a function of dose intensity (10, 11), these experiments (Tables 1 and 2) reveal no remarkable difference in therapeutic response over a 10-fold range of dose intensity. Skipper (11) has indicated that dose intensity correlates better with therapeutic response in terms of cures or nearness to cure (as in the present case) than does total dose. It may be concluded that in MX-1 and H82 xenografts, penclomedine activity is not schedule dependent. A study with a parenteral formulation of penclomedine (Table 3) revealed that antitumor activity observed with p.o. administration was not remarkably different when parenteral routes of administration were used. However, parenteral penclomedine was somewhat more potent than p.o. penclomedine (Table 3), suggesting some reduction of bioavailability by p.o. administration. Additional studies will be required to determine whether complete regressions of MX-1 xenografts are more durable and perhaps result in cures following p.o. penclomedine.

It was of interest to determine the in vivo cross-resistance profile of penclomedine because this information may influence patient selection for clinical trials or interpretation of responses (or lack of response) in previously treated patients or may provide insight into the mechanism(s) of action of penclomedine by analogy to drug classes that exhibit similar profiles. In the latter context, the data must be interpreted with caution. For example, alkylating agent-resistant lines seemed to be cross-resistant to penclomedine (Table 4). However, alkylating agent-resistant lines are not necessarily cross-resistant to alkylating agents (6, 12).

In vivo cross-resistance was determined by using a model that was applied extensively by Schabel et al. (6) and that has undergone continued development in our laboratories (9, 13, 14). In Table 4 are presented results of optimal, nontoxic
low sensitivity of P388/0 or L1210/0 to penclomedine, a 2-log increase of a leukemia subline. The present interpretation has been suggested as the criterion for resistance or cross-resistant to penclomedine (Table 4). As indicated in "Materials and Methods," a 2-log decrease of a parent or wild-type, sensitive murine leukemia and the optimal treatment in each of a pair of independent, confirmatory experiments with each line. If, for example, penclomedine treatment was identical in all experiments; dosages were 120, 90, 67, and 50 mg/kg/dose. Each experiment included two arms: a sensitive parent tumor (P388/0 or L1210/0); and a drug-resistant tumor. In addition to penclomedine treatment, each arm included untreated mice bearing serially diluted tumor inocula (see "Materials and Methods") and tumor-bearing controls treated with the drug to which resistance had been selected. All tumor implants were i.p., and treated groups were implanted with 10⁶ cells (P388 lines) or 10⁵ cells (L1210 lines). All experiments were repeated for confirmation, and the data shown are for optimal dosages from each of two experiments.

Table 2 Dependence of therapeutic response on treatment schedule of p.o. penclomedine in advanced s.c. H82 small-cell lung carcinoma xenografts

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>Optimal dosage (mg/kg/dose)</th>
<th>Total dose (mg/kg)</th>
<th>Dose intensity (mg/kg/day)</th>
<th>Complete regressions</th>
<th>Tumor-free survivors</th>
<th>Tumor growth delay* (T - C, days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1^</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Every 4 days for 3 doses</td>
<td>297</td>
<td>891</td>
<td>99</td>
<td>0/10</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Daily for 9 doses</td>
<td>120</td>
<td>1080</td>
<td>120</td>
<td>0/10</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>540</td>
<td>60</td>
<td>4/10</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>Experiment 2^</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single dose</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>5/6</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>Daily for 9 doses</td>
<td>120</td>
<td>1080</td>
<td>120</td>
<td>3/6</td>
<td>2</td>
<td>27</td>
</tr>
</tbody>
</table>

^ Average of the difference in the median times (days) postimplant for treated (T) and control (C) tumors to reach each of two evaluation sizes.

^ Treatment began on day 12; median tumor sizes per group on day 12 ranged from 272 to 336 mg. Each dosage shown was less than or equal to the 10% lethal dose. Observation extended to day 64.

^ Treatment began on day 14; median tumor sizes per group on day 14 ranged from 126 to 170 mg. Each dosage shown resulted in one death in a group of six mice. Observation extended to day 57.

Table 3 Activity of parenteral formulation of penclomedine in advanced s.c. mammary MX-1 carcinoma xenografts

Mice were implanted s.c. with MX-1 tumor fragments and observed until tumor sizes reached 200–300 mg. Penclomedine emulsions were delivered by the three routes shown. The experiment included for comparison an identical arm treated with penclomedine suspensions (see "Materials and Methods"; data not shown).

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Dosage* (mg/kg/dose)</th>
<th>Complete regressions</th>
<th>Time to treatment failure^ (days)</th>
<th>Tumor-free survivors (day 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.o.</td>
<td>202</td>
<td>Toxic</td>
<td>&gt;45</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>10/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>10/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i.p.</td>
<td>135</td>
<td>Toxic</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>10/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>8/10</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>i.v.</td>
<td>135</td>
<td>Toxic</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>10/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>10/10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Daily treatment on days 11–15; median tumor sizes per group on day 11 ranged from 196 to 279 mg.

^ Number of days from last treatment (day 15) to earliest recurrence after complete regression.

Therapeutic response from a range of dosages in each of 20 experiments. Each experiment compared, on a log cell kill basis, the response of a parent or wild-type, sensitive murine leukemia and the response of a specifically drug-resistant leukemia to penclomedine. Log cell kill was estimated from internal bioassay of tumor cell populations increased by about 3 log units (P388 and L1210 lines). All experiments were repeated for confirmation, and the data shown are for optimal dosages from each of two experiments.

Table 4 Activity of penclomedine against murine leukemia sublines with acquired resistance to selected antitumor drugs

Penclomedine treatment was identical in all experiments; dosages were 120, 90, 67, and 50 mg/kg/dose. Each experiment included two arms: a sensitive parent tumor (P388/0 or L1210/0); and a drug-resistant tumor. In addition to penclomedine treatment, each arm included untreated mice bearing serially diluted tumor inocula (see "Materials and Methods") and tumor-bearing controls treated with the drug to which resistance had been selected. All tumor implants were i.p., and treated groups were implanted with 10⁶ cells (P388 lines) or 10⁵ cells (L1210 lines). All experiments were repeated for confirmation, and the data shown are for optimal dosages from each of two experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Optimal dosage (mg/kg/dose)</th>
<th>Approximate log10 change in tumor burden at end of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>P388/MTX</td>
<td>90</td>
<td>-1, -1</td>
</tr>
<tr>
<td>P388/ara-C</td>
<td>120</td>
<td>-1, 0</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>-1, 1</td>
</tr>
<tr>
<td>L1210/5-FUra</td>
<td>120</td>
<td>+2, +2</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>+2, +2</td>
</tr>
<tr>
<td>P388/ADR</td>
<td>90#</td>
<td>-1, 0</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0, 0</td>
</tr>
<tr>
<td>P388/VCR</td>
<td>90#</td>
<td>-2, 0</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0, 0</td>
</tr>
<tr>
<td>P388/AMSAs</td>
<td>90</td>
<td>0, 3</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>-1, -7</td>
</tr>
<tr>
<td>P388/DDP</td>
<td>90#</td>
<td>-1, 0</td>
</tr>
<tr>
<td></td>
<td>90#</td>
<td>0, 0</td>
</tr>
<tr>
<td>P388/L-PAM</td>
<td>90#</td>
<td>-1, 1</td>
</tr>
<tr>
<td></td>
<td>90#</td>
<td>-1, 2</td>
</tr>
<tr>
<td>P388/BCNU</td>
<td>67</td>
<td>0, 2</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>-1, 0</td>
</tr>
<tr>
<td>P388/CPA</td>
<td>120</td>
<td>-3, -1</td>
</tr>
<tr>
<td></td>
<td>90#</td>
<td>-4, -1</td>
</tr>
</tbody>
</table>

* Cell line designations: ara-C, 1-β-D-arabinofuranosylcytosine; 5-FUra, 5-fluorouracil; ADR, Adriamycin; DDP, cisplatin; L-PAM, melphalan; CPA, cyclophosphamide.

# Highest dosage studied; no toxicity observed.

As indicated in “Materials and Methods,” a 2-log differential has been suggested as the criterion for resistance or cross-resistance of a leukemia subline. The present interpretation may be appreciated better by noting that in spite of the relatively low sensitivity of P388/0 or L1210/0 to penclomedine, a 2-log differential between sensitive and some resistant lines was possible in these experiments. In general, the tumor burden among these resistant lines remained static during 5 days of penclomedine treatment (Table 4), while untreated control tumor cell populations increased by about 3 log units (P388 and L1210 doubling times, <0.5 day).
PRECLINICAL ANTITUMOR ACTIVITY OF PENCLOMEDINE

Table 5 Therapeutic response of i.c. implanted MX-1 mammary carcinoma xenografts to i.v. or p.o. penclomedine

Penclomedine treatment began on day 1 after i.c. implantation of 10^6 MX-1 cells into nude mice. Penclomedine was administered i.v. or p.o. daily for 5 days at the dosages shown. The median days of death of untreated control mice were day 18 in Experiments 1 and 3 and day 22 in Experiment 2. There were no 60-day survivors among untreated controls.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dosage (mg/kg/dose)</th>
<th>% of increase in life span</th>
<th>Approximate log_{10} change in tumor burden at end of treatment</th>
<th>60-day survivors/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (i.v.)</td>
<td>100</td>
<td>-24</td>
<td>Toxic</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>+131</td>
<td>-3.8</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>+76</td>
<td>-1.9</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>+69</td>
<td>-1.6</td>
<td>0/10</td>
</tr>
<tr>
<td>2 (p.o.)</td>
<td>135</td>
<td>-64</td>
<td>Toxic</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>+90</td>
<td>-2.3</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>+68</td>
<td>-1.6</td>
<td>0/5</td>
</tr>
<tr>
<td>3 (p.o.)</td>
<td>202</td>
<td>-56</td>
<td>Toxic</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>+183</td>
<td>-4.9</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>+155</td>
<td>-4.1</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>+86</td>
<td>-2.0</td>
<td>2/10</td>
</tr>
<tr>
<td>BCNU* (i.p.)</td>
<td>27</td>
<td>+33</td>
<td>-1.0</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>+16</td>
<td>-0.5</td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>+11</td>
<td>-0.3</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* Based on median day of death of dying mice and derived from groups of 10 mice each implanted i.c. with 10^6, 10^5, 10^4, or 10^3 MX-1 cells.

DISCUSSION

Because of the observation of CNS side effects with parenteral administration (data not shown), it was expected that penclomedine was crossing the blood-brain barrier. Thus, the sensitive MX-1 mammary adenocarcinoma was modeled as an i.c. xenograft to assess the potential usefulness of penclomedine against tumor cells in the CNS. The results presented in Table 5 indicate that therapeutically active amounts of penclomedine reached the CNS after either p.o. or parenteral administration to mice. It was possible to reduce the MX-1 tumor burden in i.c. implanted mice by 2 to 5 log_{10} units [estimated by bioassay (Table 5)]. Therapeutic responses to penclomedine were somewhat better than could be obtained with an optimal regimen of BCNU, a clinical agent known for its ability to cross the blood-brain barrier. Optimization of the penclomedine treatment schedule for i.c. disease will require additional studies. Similar experiments were conducted with the U251 human glioblastoma in an attempt to model a primary CNS tumor. This xenograft was less sensitive to penclomedine than was MX-1 and exhibited little response when studied as an s.c. model in other experiments. However, a 1-log_{10} unit reduction of i.c. U251 tumor burden was achieved with i.v. treatment daily for 5 days (data not shown). With a view toward a more thorough investigation of this aspect, preliminary experiments were conducted to select a primary glioblastoma more sensitive to penclomedine than was U251. Mice bearing s.c. implants of SF 295 and XF 498 were treated with penclomedine p.o. daily for 5 days. There were modest, dose-dependent delays in SF 295 growth and marginal delays in XF 498 growth. SF 295 failed to respond to single-dose treatment with i.p. BCNU. In contrast, BCNU produced 30-day tumor growth delays of XF 498 xenografts (data not shown). These preliminary results were not deemed sufficiently encouraging to warrant evaluation of i.c. implants of these glioblastomas.

The antitumor activity of penclomedine was observed initially in the murine P388 leukemia model formerly used for drug screening by the NCI (1). In our earlier report we described the responses to penclomedine of several experimental tumors, including 4 breast tumor systems. In general, breast tumors were responsive in vivo, but non-breast tumor models indicated comparatively less antitumor activity or none at all (1). It would be premature to conclude that penclomedine exhibited a remarkable degree of tumor selectivity. Noteworthy among earlier observations was the activity of penclomedine following p.o. dosing (1).

The present studies were conducted to answer three questions relevant for planning and interpreting clinical trials. What treatment schedule might be preferred? What patients might be less likely to respond to penclomedine therapy due to acquired resistance to other chemotherapeutic agents? How useful might this apparently lipid-soluble compound be against primary or metastatic brain tumors?

The antitumor activity of p.o. administered penclomedine in 2 human tumor xenograft models suggests little if any dependence on treatment schedule. Pharmacokinetic characteristics of penclomedine, namely, slow and/or incomplete absorption following a p.o. dose in mice, an elimination half-life of about 1 h, and partitioning into deep (tumor tissue?) compartments (3), probably account for this observation. The drug is equally effective when administered i.p., i.v., or p.o. to mice.

Based on the in vivo cross-resistance profile presented here, patients' tumors suspected of having acquired clinical resistance to alkylating agents may exhibit cross-resistance to penclomedine. In contrast, the present data suggest that patients treated previously with MTX, 1-beta-D-arabinofuranosylcytosine, 5-fluorouracil, Adriamycin, vincristine, AMSA, or cisplatin may be fully responsive to penclomedine. Although cross-resistance data must be interpreted with caution when trying to answer questions about mechanisms of action (15), the in vivo cross-resistance profile of penclomedine is consistent with other data(4) that suggest alkylating agent-like characteristics for this drug. It is not apparent from the structure or from any definitive work to date what the mechanism of alkylation is.

The collateral sensitivity exhibited by P388/AMSA is interesting. P388/AMSA tends to exhibit cross-resistance to drugs typically associated with multidrug resistance, but it is sensitive to the other agents represented in Table 4(5). No other examples of collateral sensitivity are known for this tumor line. It would be useful to characterize the mechanism of cross-resistance displayed by this tumor system in more detail.

4 M. M. Ames, personal communication.
5 W. R. Waud and S. D. Harrison, Jr., unpublished data.
be of interest to study regimens combining AMSA and penclomedine.

Our data demonstrate that penclomedine enters the CNS in concentrations sufficient to elicit an antitumor response in a sensitive tumor (MX-1). This is a clinically important attribute exhibited by few antitumor drugs (16). However, assessment of the potential of penclomedine for therapy of primary brain tumors, i.e., delineation of the in vivo sensitivity of human brain tumor xenografts to penclomedine, will require further work.

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