**In Vitro versus in Vivo Correlations of Chemosensitivity of Human Medulloblastoma**

Henry S. Friedman, S. Clifford Schold, Jr., Lawrence H. Muhlbauer, Thorir D. Bjornsson, and Darell D. Bigner


duke University Medical Center, Durham, NC 27710

**ABSTRACT**

An in vitro clonogenic assay was used to test the chemosensitivity of the human medulloblastoma cell line TE-671. Dose-response relationships for reduction in colony formation were generated for cyclophosphamide, vincristine, Adriamycin, 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC 409962), and 1,4-cyclohexadiene-1,4-dicarboxylic acid, 2,5-bis(1-aziridinyl)-3,6-dioxodiethyl ether (NSC 182986); and the in vitro drug dose at which there is a 75% reduction in the number of colonies in comparison to controls (ID75) were derived from these data. Methotrexate produced no colony reduction at any dose tested up to 1000 μg/ml. The in vitro results were compared to growth delays in s.c. TE-671 xenografts in athymic mice treated with the same agents. Agents with an ID75 less than assumed in vivo plasma drug concentrations were all active in vivo, whereas two of the three agents with an ID75 greater than assumed in vivo plasma drug concentrations demonstrated no in vivo activity. These results suggest that for these agents, the relationship between the ID75 of the drug and its in vivo concentration allows in vitro clonogenic assay results to agree with in vivo growth delay responses.

**INTRODUCTION**

Conventional therapy for medulloblastoma consists of surgical resection and subsequent whole neuraxis irradiation, yielding a 5-year survival rate of approximately 40 to 50% (5, 8). The role of adjuvant chemotherapy is undefined with discrepant results reported in 2 large studies. The European study compared conventional radiation with radiation and CCNU-vincristine, noting an advantage with the addition of chemotherapy (7). The Children's Cancer Study Group compared conventional radiation with disease and CCNU-vincristine-prednisone, and could find no difference in disease-free survival (15). Experimental chemo-therapeutic studies of medulloblastoma have been hampered by the lack of an in vitro or in vivo tumor model. We and others have reported the development of an in vitro and in vivo chemosensitivity model using the human medulloblastoma cell line TE-671 (17, 24, 35, 39) and preliminary therapeutic studies in athymic nude mice (18). We now report the in vitro chemosen-

1This work was supported by NIH Grants CA 11898, CA 32672, and PO1 NS20023-01; Duke Comprehensive Cancer Center Development Funds (CA 14236); and NS 20581.

2Recipient of an Association for Brain Tumor Research Fellowship in memory of Bryce Davis and an American Cancer Society Junior Clinical Faculty Fellowship. To whom requests for reprints should be addressed, at Department of Pediatrics, Division Hematology-Oncology, Duke University Medical Center, Durham, NC 27710.

* The abbreviations used are: CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC 79037); BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC 409962); AZQ, 1,4-cyclohexadiene-1,4-dicarboxylic acid, 2,5-bis(1-aziridinyl)-3,6-dioxodiethyl ether (NSC 182986); ID75, ID50, ID30 in vitro drug dose at which there is a 75, 50, or 10% reduction in the number of colonies in comparison to controls, respectively.

Received September 30, 1983; accepted July 10, 1984.

**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 (17).

**Clonogenic Assay.** Drug assays were performed after initial studies had established a linear relationship between the number of cells plated and the number of resulting colonies, allowing the selection of an optimal final drug concentration (5 x 10^6 cells/60-mm dish). For all experiments except with methotrexate, cells were grown to 80% confluence, mechanically harvested with a Pasteur pipet, washed, and resuspended in Eagle's minimal essential medium to a concentration of 1 x 10^7 cells/ml after passage through a 27-gauge needle. These cells were incubated for 1 hr at 37°C in room air with a drug or drug vehicle, washed twice, and resuspended in Richter's (26) zinc option minimal essential medium (Grand Island Biological Co., Grand Island, NY) supplemented with 20% heat-inactivated fetal calf serum to a concentration of 1 x 10^7 cells/ml. Two agarose-based solutions were prepared previously in a 44°C water bath. For the base layer, 1.4% agarose (SEA-KEM-ME; FMC Corp., Rockland, ME), 5X-zinc option, distilled H2O, fetal calf serum, and 5.5% sodium bicarbonate were mixed at a v:v ratio of 30:12:18:12:3, respectively, yielding a final agarose concentration of 0.56%. Three-mi aliquots were pipetted to each 60-mm Petri dish. For the top layer, the same reagents were mixed at a v:v ratio of 9.1:8:22:9:8:2, respectively, yielding a final agarose concentration of 0.26%. Two volumes of this solution were mixed with 1 volume of the cell suspension, achieving a final agarose concentration of 0.06%. Three-mi aliquots were pipetted to each 60-mm Petri dish. For the top layer, the same reagents were mixed at a v:v ratio of 9.1:8:22:9:8:2, respectively, yielding a final agarose concentration of 0.06%. Two volumes of this solution were mixed with 1 volume of the cell suspension, achieving a final agarose concentration of 0.17%. A final cell concentration of 5 x 10^6 cells/dish in 1.5 ml of this solution was plated over the base layer using 4 plates/drug or vehicle. For experiments with methotrexate, similarly harvested cells were grown in the continuous presence of methotrexate or vehicle at the same agarose concentrations. Plates were incubated for 14 days. Subsequently, the number of colonies (>50 cells) on each dish was counted using a Wild microscope (Heerbrugg, Switzerland). The mean percentage of colony formation was calculated from the number of colonies counted per dish/number of cells plated per dish. Response was assessed by the ratio of treated versus control colony formation.

The following agents were tested in vitro: BCNU; AZQ; methotrexate (NSC 740); vincristine (NSC 67574); Adriamycin (NSC 123127); and 4-hydroxy cyclophosphamide, the primary metabolite of cyclophosphamide active in vitro (kindly provided by Dr. M. Colvin, Johns Hopkins Oncology Center). All drug solutions were freshly prepared on the day of assay. AZQ, vincristine, and 4-hydroxy cyclophosphamide were dissolved in Eagle's minimal essential medium and serially diluted. BCNU was dissolved in absolute ethanol and serially diluted with Eagle's minimal essential medium. Mixtures (1:1) of drug solution and cell suspension were used to achieve a 1 ml cell suspension containing 10^6 cells with...
the desired final drug concentration.

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 x 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 ID75s and the ID50s by substituting 75 or 50% for percentage of colony formation and solving the regression equation for drug concentration.

Animals. Male or female athymic BALB/c mice (nu/nu genotype, 6 weeks or older) were used for all in vivo studies. Animals were maintained as described previously (9).

In Vivo Studies. Chemotherapy of the human medulloblastoma cell line growing s.c. in athymic nude mice was performed as described previously (18). Briefly, groups of 10 randomly assigned mice were treated i.p. with chemotherapeutic compounds when the median tumor volume as determined by caliper measurement exceeded 200 cu mm. The doses of compounds used were: BCNU, 75 mg/sq m for one dose; cyclophosphamide, 513.9 mg/sq m daily for 2 doses; vincristine, 7.31 mg/sq m for one dose; and methotrexate, 24 mg/sq m daily for 5 doses. These doses represented 75% of the calculated 10% lethal dose (18). For each experiment, one group of matching animals served as a control and received the drug vehicle on an identical schedule.

In vivo response was assessed by the median difference in days between the median of treated animals' and the median of control animals' tumors to reach a volume of 1000 cu mm. Statistical significance was determined by the Wilcoxon rank sum test.

All 6 agents tested in vitro were also tested in vivo; 4-hydroxycyclophosphamide, the primary metabolite of cyclophosphamide active in vitro, was used in the clonogenic assay to avoid the need for hepatic activation.

Pharmacokinetic Simulations. The time courses of expected drug concentrations were simulated using pharmacokinetic parameters which were either determined by us, i.e., for AZQ in athymic mice (30), or derived from data available in mice in published reports. The data for Adriamycin were in athymic mice (22), but in laboratory mice for cyclophosphamide (2), methotrexate (34), and vincristine (14). No data were available for BCNU in mice; data obtained in Fischer rats (23) were therefore used for that drug. The simulations were carried out using the appropriate coefficients and exponents adjusted for the doses used in our in vivo studies (20). The time-averaged plasma drug concentrations over the first hr, C1hr, were subsequently computed on the basis of the respective areas under the plasma concentration versus time curve (20).

RESULTS

The results of the in vitro dose-response curves are represented in Charts 1 and 2. Reduced colony formation was observed with increasing drug concentrations for all drugs except methotrexate. The calculated parameters of the dose-response relationships, i.e., slope, ID75, and ID50, are listed in Table 1. Excluding methotrexate, the dose-response curve was steepest for BCNU and shallowest for vincristine, but the ID75 and ID50 were lowest for Adriamycin and highest for BCNU. Also listed in Table 1 are the estimated in vivo plasma drug concentrations averaged over the first hr, and the results of the studies on the in vivo growth delays of implanted medulloblastoma after the drug treatments. Chart 3 shows pharmacokinetic simulations of the time course of expected drug concentrations in mice over the first hr after the doses used in the in vivo studies.

The 3 drugs (4-hydroxycyclophosphamide, Adriamycin, and AZQ) with ID75 < C1hr each produced a statistically significant growth delay in vivo. No documented in vivo growth delay was
In vitro and in vivo drug responses of a human medulloblastoma cell line (TE-671)

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>ID₅₀(μg/ml)</th>
<th>ID₇₅(μg/ml)</th>
<th>Cᵣ₃₅ (μg/ml)</th>
<th>Significance of in vivo drug activity (T-C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adriamycin</td>
<td>55.9</td>
<td>0.071</td>
<td>0.205</td>
<td>0.370</td>
</tr>
<tr>
<td>Vincristine</td>
<td>35.8</td>
<td>0.110</td>
<td>0.504</td>
<td>0.406</td>
</tr>
<tr>
<td>AZQ</td>
<td>50.8</td>
<td>0.590</td>
<td>1.83</td>
<td>2.16</td>
</tr>
<tr>
<td>4-Hydroxycyclophosphamide</td>
<td>48.0</td>
<td>0.550</td>
<td>8.15</td>
<td>Yes (9)</td>
</tr>
<tr>
<td>BCNU</td>
<td>69.9</td>
<td>3.52</td>
<td>7.79</td>
<td>No</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>1.56</td>
<td>No</td>
</tr>
</tbody>
</table>

* a = 0.666 hr⁻¹, b = 0.0914 hr⁻¹, Cᵣ₃₅ = 0.50 mg; cyclophosphamide, Cᵣ₃₅ = 41.0 μg/ml, K = 3.73 hr⁻¹.

** Chart 3. Pharmacokinetic simulations of the time course of expected drug concentrations over the first hr after administration to athymic mice. **

** Table 1. Parameters of in vitro dose-response curves and results of in vivo drug studies. **

** DISCUSSION **

Our use of an in vitro clonogenic assay has demonstrated the dose response of the human medulloblastoma cell line TE-671 to 6 antineoplastic agents. These results provide analysis of the cellular sensitivity of the cell line to these drugs, allowing correlation with observed or predicted in vivo plasma drug levels and therapeutic efficacy. Published criteria for determining appropriate in vitro drug concentrations predictive of in vivo activity include the average drug concentration over 1 hr at the peak of the plasma clearance curve (4), one-tenth peak plasma drug concentration (37), and a sensitivity index based on the area under the cell survival curve (29). Chang (12) has recently demonstrated in a pancreatic adenocarcinoma model the relationship between the ID₇₅, calculated from the log dose-response curve, and the in vivo plasma drug concentration that was estimated from either human pharmacokinetic data (1) or the 10% lethal dose in animal studies (38). Our data suggest that, for this established human medulloblastoma tumor line, drugs with average in vivo plasma drug concentrations over the first hr higher than the in vitro ID₅₀ or ID₇₅ will demonstrate an in vivo antitumor activity as measured by significant tumor growth delay.

The present findings are of potential clinical significance, since not only do they indicate that results of the in vitro clonogenic assays provide information as to whether a given drug shows an effect, but they also indicate what approximate range of drug concentrations has to be achieved in vivo. When making comparisons between in vitro and in vivo antitumor drug concentrations, however, numerous factors must be considered, e.g., protein binding, tissue distribution, mechanism of action, and the pharmacokinetics of the drug. In the present study, we used the estimated average in vivo plasma concentrations for the comparisons. Other pharmacokinetic parameters need to be evaluated, and precise in vitro/in vivo correlation will require measure-
ments of achievable murine drug plasma levels and ideally also of tumor drug levels. AZQ was the only drug in the present study with a measured in vivo plasma drug concentration, and this drug, active in vivo, demonstrated an ID50 lower than the average in vivo plasma drug concentration over the first hr. Nevertheless, further studies will need to demonstrate the overall relationship between parameters of the in vitro dose-response curve and measured (rather than estimated) in vivo plasma drug concentration.

The most important function of an in vitro assay is accurate prediction of in vivo response. Salmon et al. (29) and Von Hoff et al. (37) have shown a good correlation between decreased colony formation of biopsy derived cells in short-term culture and a patient's tumor response, especially in predicting resistance. Nevertheless, as indicated by Selby et al. (32) "although a predictive value of negative test equal to 90 percent seems impressive, it appears less so when the prevalence of drug resistance is 80 percent." Direct correlations between in vitro and in vivo experimental models with established cell lines and stable transplantable tumors allow easier analysis, although all chosen indexes of correlation must by design be arbitrary compared to clinical situations. Previous work has demonstrated the significant in vitro sensitivity-in vivo response correlations observed in human melanoma (21, 36), human pancreatic carcinoma (4, 11, 13), and human colon adenocarcinoma (40). Nevertheless, many human tumor models reflecting a range of types of human cancer may need to be tested to ensure that significant in vitro-in vivo correlations exist.

It is possible that the in vitro/in vivo model described here may allow screening of agents with efficacy in the therapy of medulloblastoma, but this remains to be established. In the absence of randomized Phase III clinical trials showing the unequivocal value of single or combination adjuvant drug therapy, it is difficult to relate our in vitro-in vivo results with TE-671 to clinical results with medulloblastoma. Nevertheless, in Phase II trials with cyclophosphamide, Allen and Helson (3) have shown responsiveness of medulloblastoma, but this remains to be established. In the absence of significant in vitro sensitivity-in vivo response correlations observed in human melanoma (21, 36), human pancreatic carcinoma (4, 11, 13), and human colon adenocarcinoma (40). Nevertheless, many human tumor models reflecting a range of types of human cancer may need to be tested to ensure that significant in vitro-in vivo correlations exist.

REFERENCES


18. Ghotbali, M. W., Velasco, M. E., and Ross, E. R. Binding of neurons specific endtoside (NE) antibodies to mammalian cerebellums and human medulloblas-
In Vitro versus in Vivo Correlations of Chemosensitivity of Human Medulloblastoma

Henry S. Friedman, S. Clifford Schold, Jr., Lawrence H. Muhlbaier, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/11/5145

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.