Effect of difluoromethylornithine on the antiglioma therapeutic efficacy of intra-arterial BCNU

ALAN R. COHEN, M.D., DENNIS D. PIETRONIGRO, PH.D., HUMBERTO CRAVIOTO, M.D., AND EUGENE S. FLAMM, M.D.

Departments of Neurosurgery and Neuropathology, New York University Medical Center, New York, New York

In an attempt to improve glioma management, an animal model was developed to evaluate the therapeutic efficacy of intra-arterial 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Furthermore, the model was used to study the antitumor activity of D,L-alpha-difluoromethylornithine (DFMO), a polyamine-biosynthesis inhibitor, used both as a single agent and in combination with intra-arterial BCNU.

An N-methylnitrosourea-induced gliosarcoma (T9) was transplanted stereotaxically into the right caudate nucleus of male Fischer 344 rats. Animals receiving a single low-dose (5 mg/kg) intracarotid injection of BCNU 9 days following tumor implantation had a 57% increase in life span compared with untreated control rats (p < 0.001). Intracarotid drug delivery was more effective than systemic (intraperitoneal) administration of the same dose of BCNU.

When given as a single agent, DFMO demonstrated dose-dependent effectiveness. As part of a combined regimen, DFMO enhanced the antitumor therapeutic activity of both systemic (intraperitoneal) and intra-arterial BCNU. Survival times of animals receiving combined DFMO and intra-arterial BCNU were almost double those of untreated controls, and were significantly better than survival times of animals receiving combined DFMO and intraperitoneal BCNU. These findings suggest methods to optimize current clinical chemotherapy for glioma.

KEY WORDS · chemotherapy · glioma · BCNU · difluoromethylornithine · rat

The single most effective drug currently available for glioma chemotherapy is 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), a small lipophilic alkylating agent. Recent clinical trials have suggested advantages of intra-arterial administration of this drug over the conventional intravenous route (WR Shapiro, et al., unpublished data, 1984). These studies, however, have been limited by two elements: the absence of an animal model to assess efficacy of intra-arterial therapy, and the presence of a hazardous toxicity appearing as delayed brain and eye necrosis.

This report describes a simple animal model for evaluating efficacy of intra-arterial therapy in intracerebral glioma-bearing rats. This model was extended to evaluate the antitumor activity of D,L-alpha-difluoromethylornithine (DFMO) both as a single agent and in combination with intra-arterial BCNU. Difluoromethylornithine is an irreversible enzyme-activated ("suicide") inhibitor of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis. Because polyamines occur in increased amounts in patients with a variety of neoplasms, and because DFMO blocks the synthesis of polyamines, there is considerable interest in using DFMO as a growth-inhibitor for neoplasms. Recently, DFMO was shown to potentiate in vivo cytotoxicity of systemic BCNU in animals bearing the 9L rat brain tumor, although it was inactive as a single agent. Since DFMO seems to have little associated toxicity, and since both DFMO and BCNU may have additive effects in destabilizing deoxyribonucleic acid (DNA), an investigation of the effects of combined treatment with DFMO and intra-arterial BCNU was undertaken.

Materials and Methods

Tumor Cell Line

The tumor cell line T9 was maintained in monolayer tissue culture in Falcon plastic flasks. Flasks contained Eagle's minimal essential medium supplemented with 20% newborn calf serum, non-essential amino acids, L-glutamine, and an antibiotic-antimycotic so-
lution containing penicillin, streptomycin, and amphotericin B. The cultures were incubated at 37°C in a high-humidity atmosphere with 5% CO₂. When cells formed a monolayer and became confluent, they were trypsinized (0.25% trypsin, 0.2% ethylenediaminetetraacetic acid) and subcultured. Aliquots of cells from various passages were kept frozen in liquid nitrogen with 10% glycerin.

**Experimental Animals**

Male CDF rats (Fischer 344)* weighing 150 to 200 gm each were allowed to acclimate to our laboratory for 1 week prior to studies. They were given Purina rat chow and water ad libitum and were maintained six per cage in a room with a light-dark cycle (12 hours on, 12 hours off). Experiments were performed between 7 a.m. and 7 p.m.

**Intracerebral Tumor Implantation**

Prior to injection, cells were trypsinized, harvested, and suspended in growth medium that contained 0.5% agar. This technique tended to minimize extracranial tumor extension and promote the growth of intracerebral spheroids. Cells were counted in a hemocytometer, and cell viability was determined by the trypan-blue exclusion method. The suspension was incubated in a water bath maintained at 37°C. Immediately prior to implantation, cells were drawn up into a Hamilton microliter syringe equipped with a No. 25 needle. One million tumor cells in a volume of 10 μl were injected stereotaxically into the right cerebral hemisphere of each animal.

For tumor inoculation, animals were anesthetized on Day 0 with pentobarbital sodium (25 to 30 mg/kg) intraperitoneally and placed in a stereotaxic frame. The head was shaved, a midline longitudinal scalp incision was made, and the bregma identified. A dental drill was used to place a 1-mm burr hole 3 mm to the right of the bregma and 1 mm posterior to the coronal suture. Next, the Hamilton syringe was lowered to a depth of 5 mm from the dural surface and then withdrawn 1 mm. The purpose of this was to create a small pocket into which the cells could be injected. The needle bevel was positioned to face laterally to help avoid intraventricular injection. Cells were injected slowly over a period of 2 minutes. The needle was then withdrawn and the hole sealed with bone wax. The operative field was cleaned with povidone-iodine solution and the scalp was closed with clips. With this technique, tumors were reliably placed in the vicinity of the right caudate nucleus.

**Drugs**

*BCNU. A stock solution of BCNU (carmustine)† was prepared as 100 mg dissolved in 3 ml absolute ethanol and refrigerated at 4°C in a light-free vial. Prior to use, an aliquot was removed and diluted with sterile water to a concentration of 1 mg/ml in 3% ethanol. Whether administered intra-arterially or intraperitoneally, the drug was always prepared in the manner described above and given at a dosage of 5 mg/kg. For example, a 200-gm rat would receive 1 mg of BCNU in a volume of 1 ml of 3% ethanol in water, a dose of 5 mg/kg. This is a relatively low dose, and approximates 40% of the 10% lethal dose (LD₁₀) determined for intraperitoneal BCNU in rats. A dose of 5 mg/kg is equivalent to 30 mg/m sq for a rat. This, however, cannot be extrapolated to man because of significant differences in cross-species toxicity.

Stock solution was prepared in this fashion because refrigerated BCNU is unstable in aqueous form after only 3 hours and sooner if kept at room temperature. However, refrigerated dilute solutions of BCNU in 95% or absolute alcohol have been found to be stable for up to 3 months. The stability of the BCNU stock solution was checked routinely by analyzing the absorption peak at 232 nm on a Perkin-Elmer 552A spectrophotometer. No appreciable loss of activity occurred during the course of our experiments.

* Difluoromethylornithine. Difluoromethylornithine§ was administered to appropriate animals as the sole source of drinking water over a 20-day period from Days 5 to 24 following tumor implantation. A preliminary series of experiments on normal rats (each weighing 150 to 200 gm) was conducted to determine an appropriate oral drug dose. The DFMO was reconstituted in tap water as 1%, 2%, and 5% solutions (w/v). A 5% solution was not well tolerated, with most animals developing diarrhea and weight loss. Concentrations of 1% and 2% were well tolerated without clinical evidence of toxicity. Rats receiving 2% DFMO had an average daily consumption of 15 to 20 ml, or 1.5 to 2.0 gm/kg body weight. Therefore, DFMO concentrations of 1% and 2% were used in this experiment.

Animals receiving combination therapy of DFMO along with intra-arterial BCNU or its diluent vehicle (3% ethanol) presented a unique problem. Following pentobarbital anesthesia for carotid artery catheterization, animals had virtually no oral intake for 24 hours and sometimes longer. Therefore, on Day 9 (the day of carotid catheterization), rats in the combination-therapy group received a parenteral supplement of DFMO. This DFMO supplement was given intraperitoneally as a total dose of 100 mg/kg over 24 hours in divided 6-hourly doses, the first of which was given 10 minutes prior to carotid injection.

**Intra-Arterial Injections**

All intra-arterial therapy was administered as a single

---

* Rats obtained from Taconic Farms, Germantown, New York.
† BCNU supplied by Bristol Laboratories, Syracuse, New York.
‡ Spectrophotometer, Model 552A, manufactured by Perkin-Elmer, Delft, The Netherlands.
§ DFMO supplied by Merrell Dow Research Institute, Cincinnati, Ohio.
Effect of DFMO on antiglioma efficacy of BCNU injection on Day 9. Rats were anesthetized with pentobarbital and atropine and the right carotid arterial system was exposed under an operating microscope. The distal external carotid artery (ECA) was occluded along with its branches, as was the pterygoplaceine artery, which in rats is a major extracranial branch of the cervical internal carotid artery (ICA). A PE 10 catheter was inserted into the ECA and passed retrograde to the bifurcation to permit injection into the ICA without interrupting the ICA circulation. The carotid injection (BCNU or vehicle) was administered slowly over 10 minutes via an infusion pump. When the injection was finished the catheter was removed, the ECA was occluded with a bipolar electrocoagulator, and the incision was closed in a single layer with a nylon suture.

Treatment

Following T9 tumor implantation on Day 0, animals received either no treatment at all or various combinations of BCNU and DFMO. The BCNU was given to appropriate animals as a single injection on Day 9, either intra-arterially or intraperitoneally. One group received a single dose of intra-arterial BCNU (5 mg/kg) into the right ICA. Another group received an intracarotid injection of the same volume of diluent vehicle (3% alcohol) without BCNU. A final group received the same dose of BCNU systemically, by intraperitoneal injection.

The DFMO was given to appropriate animals as a 1% or 2% solution as the sole source of drinking water for 20 days (Days 5 to 24 following tumor implantation). Those animals receiving combination therapy of DFMO along with an intra-arterial BCNU injection received supplemental intraperitoneal DFMO on the day of the carotid injection (Day 9) as described. The experimental paradigm is outlined in Table 1.

Evaluation

The efficacy of these different therapeutic regimens was compared using length of survival as an endpoint. Animals still alive 90 days following tumor implantation were considered long-term survivors and were sacrificed and autopsied. All other animals were autopsied at the time of death. Gross inspection was made of the lungs, liver, and kidneys, and the brain was removed at the time of death. Gross inspection was made of the brain or eye toxicity.

Discussion

This implantation model has resulted in establishment of intracerebral tumor in greater than 99% of cases, with an operative mortality of less than 1%. To observe the rate of tumor growth, we sacrificed a group of animals at different times following intracerebral tumor inoculation. These animals had received no experimental treatment. As early as 5 days following implantation, tumor was clearly visible adjacent to the needle track, and tended to grow as an intracerebral spheroid.

The tumor was firm, grayish, and well circumscribed but not encapsulated. It was densely cellular with numerous mitoses and a variable degree of endothelial proliferation. Little necrosis was apparent until the tumor grew to be very large. Pathologically, it has remained a highly anaplastic gliosarcoma.

Tumor size was not significantly different among animals receiving the various treatments. Death seemed to occur when the tumor reached a critical size. At the time of death, 95% of animals had tumors with a mean coplanar diameter of at least 5 mm. Examination of H & E-stained tumor sections revealed no remarkable differences between animals in different groups. There was no evidence of systemic metastases. At the 5-mg/kg dose of BCNU used, there was no evidence of brain or eye toxicity.

Beginning several days before death, most tumor-bearing animals exhibited a characteristic symptom complex. This began with rapid weight loss, piloerection, and grooming impairment. Following this came

Table 1

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Day 0*</th>
<th>Day 5†</th>
<th>Day 9‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>T9</td>
<td>—</td>
<td>IA BCNU</td>
</tr>
<tr>
<td>3</td>
<td>T9</td>
<td>—</td>
<td>IA ETOH</td>
</tr>
<tr>
<td>4</td>
<td>T9</td>
<td>—</td>
<td>IP BCNU</td>
</tr>
<tr>
<td>5</td>
<td>T9</td>
<td>DFMO 1%</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>T9</td>
<td>DFMO 2%</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>T9</td>
<td>DFMO 1%</td>
<td>IA BCNU</td>
</tr>
<tr>
<td>8</td>
<td>T9</td>
<td>DFMO 2%</td>
<td>IA BCNU</td>
</tr>
<tr>
<td>9</td>
<td>T9</td>
<td>DFMO 2%</td>
<td>IA ETOH</td>
</tr>
<tr>
<td>10</td>
<td>T9</td>
<td>DFMO 2%</td>
<td>IP BCNU</td>
</tr>
</tbody>
</table>

* T9: 1 million gliosarcoma cells inoculated stereotaxically into the right caudate nucleus of male Fischer rats.
† DFMO: Difluoromethylornithine, reconstituted as 1% or 2% solution, was administered to appropriate animals as the sole source of drinking water from Days 5 to 24. Groups 7 to 10 received intraperitoneal DFMO supplementation (100 mg/kg over 24 hours in divided 6-hourly doses on Day 9). This was necessary because of negligible oral intake in the period just following carotid catheterization.
‡ IA = intra-arterial injection, given into the right internal carotid artery over 10 minutes on Day 9 via an external carotid artery catheter inserted retrograde to the bifurcation. IP = intraperitoneal; BCNU = 1,3-bis(2-chloroethyl)-1-nitrosourea, 5 mg/kg; ETOH = 3% ethanol, the diluent vehicle for BCNU.
TABLE 2
Survival data for intracerebral-glioma-bearing rats*

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>No. of Rats</th>
<th>Median Day of Survival</th>
<th>No. of 90-Day Survivors</th>
<th>% Increase in Life Span</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>22</td>
<td>21</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>IA BCNU</td>
<td>13</td>
<td>33</td>
<td>1</td>
<td>57.1</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>3</td>
<td>IA ETOH</td>
<td>11</td>
<td>22</td>
<td>0</td>
<td>4.8</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>IP BCNU</td>
<td>6</td>
<td>28</td>
<td>0</td>
<td>33.3</td>
<td>NS</td>
</tr>
<tr>
<td>5</td>
<td>DFMO 1%</td>
<td>12</td>
<td>23</td>
<td>0</td>
<td>9.5</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>DFMO 2%</td>
<td>11</td>
<td>27</td>
<td>1</td>
<td>28.5</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>7</td>
<td>DFMO 1% + IA BCNU</td>
<td>8</td>
<td>29</td>
<td>0</td>
<td>38.1</td>
<td>NS</td>
</tr>
<tr>
<td>8</td>
<td>DFMO 2% + IA BCNU</td>
<td>14</td>
<td>41</td>
<td>1</td>
<td>95.2</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>9</td>
<td>DFMO 2% + IA ETOH</td>
<td>10</td>
<td>31</td>
<td>2</td>
<td>47.6</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>10</td>
<td>DFMO 2% + IP BCNU</td>
<td>6</td>
<td>33</td>
<td>0</td>
<td>57.1</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

* IA = intra-arterial (internal carotid artery); IP = intraperitoneal; BCNU = 1,3-bis(2-chloroethyl)-1-nitrosourea, 5 mg/kg; DFMO = difluoromethylornithine; ETOH = 3% ethanol diluent vehicle for BCNU; NS = not significant. DFMO 2% + IA BCNU yielded a 24.2% increased life span vs. BCNU alone (p < 0.05); DFMO 2% + IA ETOH was not significantly better than DFMO 2% alone.

Impaired placing responses, epistaxis, dacryohemorrhhea with occasional proptosis, stupor, a variable contralateral hemiparesis or paraparesis (the lower extremities were involved more often than the upper), a brief period in which animals were moribund, and death.

Survival data are presented in Table 2. Twenty-two control animals (Group 1) survived for a median of 21 days following tumor implantation. There were no long-term survivors in this group and all but one animal died within 27 days. The 13 animals receiving a single intra-arterial injection of BCNU on Day 9 (Group 2) lived a median of 33 days. This represents a 57.1% increase in life span (ILS) (p < 0.001). Another group of 11 animals that received a single intra-arterial injection of vehicle only (3% ethanol, Group 3) survived for a median of 22 days. This was not significantly different from the survival time of untreated animals. In the six Group 4 animals that received the same dose of BCNU systemically (intraperitoneally), median survival time was 28 days. This difference did not reach statistical significance when compared to treated control rats. At the 5-mg/kg dosage we used, however, intra-arterial BCNU was significantly better than intraperitoneal BCNU (p < 0.01).

Treatment with oral DFMO 1% as a single agent (Group 5, 12 rats) did not significantly increase median survival time; however, in 11 Group 6 rats, DFMO 2% produced a 28.5% ILS compared to untreated controls (p < 0.001). When DFMO 1%, which was ineffective alone, was used in combination with intra-arterial BCNU (Group 7, eight rats), it failed to significantly modify the effects of intra-arterial BCNU. The combination of DFMO 2% and intra-arterial BCNU (Group 8, 14 rats), however, nearly doubled the median survival time, producing an ILS of 95.2% compared with untreated controls (p < 0.001). This regimen produced a 24.2% ILS over and above that of intra-arterial BCNU alone (p < 0.05). Along the same lines, the addition of DFMO 2% to a dose of intraperitoneal BCNU that was clinically ineffective alone produced a 57.1% ILS (p < 0.01) in six Group 10 rats. This was the same relative ILS as that achieved by intra-arterial BCNU alone.

The number of long-term survivors (animals alive at 90 days) in this study was small. When these animals were sacrificed and autopsied, all had evidence of tumor growth. Although various treatments significantly lengthened survival time, the overwhelming majority of animals ultimately succumbed to their tumors. It is of interest that long-term survivors were found only in groups that had received either intra-arterial BCNU (5 mg/kg) or oral DFMO 2% as part of their therapeutic regimen (Groups 2, 6, 8, and 9).

The operative mortality rate for animals undergoing carotid catheterization was quite high (25%). Most deaths were related to intraoperative hypoxia, attributed to the unpredictable effects of intraperitoneal pentobarbital anesthesia. Pathologically, all operative deaths occurred in animals bearing small tumors (less than 2 to 3 mm in size). Nevertheless, we cannot exclude drug toxicity as a contributing factor, in that operative deaths were more common among animals receiving combined DFMO and intra-arterial BCNU than they were among those receiving intra-arterial BCNU alone, and were least among those receiving the intra-arterial diluent vehicle (ethanol) alone.

Discussion

These findings demonstrate that intra-arterial BCNU can increase survival times of animals with implanted intracerebral glioma. This effect is statistically significant when compared to survival times of animals receiving no treatment and animals receiving systemic (intraperitoneal) BCNU. Although Yamashita, et al., found an increased survival in T1 tumor-bearing rats treated with intracarotid 1-(4-amino-2-methyl-primidine-5-yl)-methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU), the present report is the first to
Effect of DFMO on anti-glioma efficacy of BCNU

our knowledge to demonstrate a therapeutic effect of intra-arterial BCNU in an animal model.

In addition, it was shown that the polyamine biosynthesis blocker, DFMO, potentiates the anti-glioma cytotoxicity of BCNU. This may have particular importance in light of the widespread toxicity reported with the use of intra-arterial BCNU. This study shows that a systemically ineffective dose of BCNU (5 mg/kg) can be rendered clinically effective with the addition of oral DFMO. Furthermore, this therapeutic potentiation persists when BCNU is delivered directly into the carotid artery. Here the same dose of BCNU was clinically effective by itself, but the addition of DFMO further increased life span by 25%, and almost doubled survival times over those of untreated control rats.

The findings also demonstrate antitumor activity of DFMO as a single agent; this may be dose-related, as it was noted with DFMO 2% but not with DFMO 1%. This effect may have been amplified by our early initiation of DFMO treatment, beginning 5 days after tumor inoculation. At this time the tumor burden is quite low. Nevertheless we believe this is a true therapeutic and not prophylactic effect, based upon the demonstration of clearly well-established tumor at 5 days.

The choice of DFMO in glioma chemotherapy is based upon an attempt to manipulate polyamine synthesis, a metabolic pathway common to all actively dividing cells. In mammals, the amino acid ornithine is the sole substrate for polyamine biosynthesis, being converted to putrescine by the enzyme ornithine decarboxylase (ODC). Putrescine is then converted to the higher polyamines spermidine and spermine by the successive addition of aminopropyl moieties donated by decarboxylated S-adenosylmethionine. The decarboxylation of ornithine to form putrescine is the rate-limiting step in this pathway, and ODC is a highly inducible enzyme. Its half-life of about 11 minutes is the shortest reported for any mammalian enzyme. 43

The pathway for polyamine biosynthesis is shown in Fig. 1.

It is clear that polyamines are related to cell growth. They occur in highest concentrations in tissues actively synthesizing protein, and show increased turnover in rapidly growing tissues. 5,10,22,27,37,42,50,52,53 Since they are cationic, the polyamines have an affinity for negatively charged intracellular molecules and bind strongly to the polynucleotides. 11,15 It has been proposed that by reducing net charge, polyamines may promote the conformational stability of DNA. 52,57

Polyamines are present in the central nervous system (CNS) 23,41,47 and are increased in the cerebrospinal fluid (CSF) of patients with a variety of CNS malignancies. 31,40,59 It has been reported that the putrescine concentration in malignant gliomas correlates with the degree of malignancy. 17,18 Work in our laboratory has demonstrated selective uptake and metabolism of radiolabeled putrescine in experimental and human brain tumors. 34 This metabolism also appears to reflect the degree of anaplasia for gliomas.

The development of polyamine biosynthesis blockers has suggested a new approach to antineoplastic therapy. Since polyamines are a prerequisite for cellular growth and gliomas exhibit increased polyamine metabolism, it is reasonable to hypothesize that inhibition of polyamine synthesis might arrest tumor growth. Because ODC is the rate-limiting enzyme in the polyamine pathway, this is a logical site to attack. In 1974, alphamethylornithine was synthesized and found to competitively inhibit ODC. Although active in vitro, this agent was ineffective in vivo preparations. 48

In 1978, Metcalf, et al.,36 synthesized DFMO, a time-dependent, enzyme-activated, irreversible ("suicide") inhibitor of ODC. Figure 2 illustrates the structure of DFMO and its similarity to the substrate ornithine. Difluoromethylornithine is a relatively small molecule with a very short in vitro half-life of about 15 minutes, 46 with minimal toxicity in experimental animals even at very high doses. 24 It leads to a dramatic depletion of intracellular putrescine and spermidine but usually not of spermine, and can slow or halt cell division.

Difluoromethylornithine restricts in vitro growth in normal and neoplastic cells, 9,28,35,51 and this phenomenon can be reversed by the exogenous addition of polyamines. As a single agent, DFMO also inhibits growth of certain transplantable solid tumors in vivo. It produced a small increase in survival for mice inoculated with L1210 leukemia cells 18 and a dramatic

J. Neurosurg. / Volume 65 / November, 1986 675
inhibition of the subcutaneously implanted EMT-6 murine mammary tumor.29

Polyamine depletion induced by DFMO alters DNA conformation in 9L rat brain-tumor cells.31 Perhaps these conformational changes summate with the DNA-destabilizing effects of BCNU. In fact, DFMO decreases the cytotoxicity of other chemotherapeutic agents including cis-diaminedichloroplatinum(II) (cisplatin), 1-beta-D-arabinosylcytosine (ara-C), and aziridinylbenzoquinone (AZQ).2,33,34 Possibly the conformational changes produced by DFMO are unfavorable to these agents, but other mechanisms may be involved.

Our present investigation adds to the extensive work of Marton and coworkers29 on the role of DFMO in glioma chemotherapy. They found that DFMO as a single agent was cytostatic when added to cultured 9L rat brain-tumor cells.46 This effect was reversed by the addition of putrescine. As well, DFMO potentiated the cytotoxicity of BCNU to these tumor cells grown in both monolayer tissue culture20 and as multicellular spheroids.48 Combined therapy, however, had no effect on cells resistant to BCNU.36

In animals bearing the intracerebral 9L gliosarcoma or the murine glioma 26, DFMO was ineffective as a single agent but dramatically enhanced the antitumor effect of intraperitoneal BCNU.29 It makes sense that if DFMO potentiates the effects of systemically administered BCNU, it should do so for intra-arterial BCNU as well. Our results demonstrate this to be true.

Although Marton’s group29 reported “little or no efficacy” of DFMO as a single agent, their findings suggest that DFMO should be active alone, depending upon the size of the tumor burden. One of their therapeutic combinations which showed an additive effect was intraperitoneal BCNU followed by DFMO 8 days later. Since BCNU is such a short-acting drug, this effect is not likely to be due to an interaction between the two agents. Rather, as noted by the authors, DFMO-induced polyamine depletion probably acted to delay repopulation of tumor cells following the decreased tumor burden produced by BCNU cell kill. This finding, along with our observation of antitumor activity by DFMO alone, suggests the exciting possibility of using DFMO for glioma maintenance chemotherapy.

The present study, in which only a fixed 5-mg/kg dose of BCNU was used, leaves unresolved the matter of which route of BCNU delivery (arterial or venous) is the optimal one to use in combination with DFMO therapy. We have shown that DFMO can render a systemically ineffective dose of BCNU effective and improve efficacy of an already active intra-arterial dose of BCNU. By examining DFMO’s contribution to titrated dosages of both systemic and intra-arterial BCNU, one can determine quantitatively the optimal combination therapy. Such a determination would not be difficult using the present model and would, of course, have immediate clinical impact.

Conclusions

A simple model is described for evaluating the efficacy of intra-arterial BCNU therapy in intracerebral-glioma-bearing rats. Using this model it was demonstrated that animals treated with a single injection of intracarotid BCNU survived significantly longer than both untreated control rats and rats receiving the same dose of drug delivered systemically. As well, treatment with the polyamine biosynthesis inhibitor DFMO significantly increased survival times, and potentiated both systemically administered and intra-arterially administered BCNU. This model should be helpful in the planning of future clinical chemotherapy protocols for glioma.

Acknowledgments

We thank Myrna Retino and Frank Drnovsky for their technical assistance and Dr. Melvin Schwartz for helping with the statistical analysis of data.

References


A. R. Cohen, et al.
Effect of DFMO on anti-glioma efficacy of BCNU


42. Russell DH, Levy CC: Polyamine accumulation and biosynthesis in a mouse L1210 leukemia. Cancer Res 31:


Manuscript received May 29, 1985.
Accepted in final form April 17, 1986.

Address reprint requests to: Alan R. Cohen, M.D., Department of Neurosurgery, New York University Medical Center, 550 First Avenue, New York, New York 10016.