Synergism between BCNU and irradiation in the treatment of anaplastic gliomas

An in vivo study using the avian sarcoma virus-induced glioma model


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The therapeutic effects of irradiation, BCNU, or combined irradiation and BCNU were studied in the avian sarcoma virus (ASV)-induced glioma model in rats. Whole-head orthovoltage radiation therapy was given in six equal fractions over 2 weeks, and BCNU was administered intraperitoneally as a single dose of 10 mg/kg. Two series of experiments were performed in order to duplicate the results. In Series 1, the median survival times of the experimental groups, in days after randomization, were as follows: control group (no treatment), 69; group receiving 2000 rads, 84 (p < 0.05); group receiving BCNU, 80.5 (p < 0.1); and group receiving 2000 rads + BCNU, 112 (p < 0.001). In Series 2, the median survival times were: control group, 73.5; group receiving 2300 rads, 85 (p < 0.01); group receiving BCNU, 92.5 (p < 0.025); and group receiving 2300 rads + BCNU, 123.5 (p < 0.001). In both series, combined therapy was significantly better than either radiation or BCNU alone. This is the first time that a synergistic effect of BCNU and irradiation has been reported in an in vivo brain-tumor model and supports the clinical use of this combination in the treatment of malignant gliomas.

KEY WORDS • experimental brain tumor • avian sarcoma virus-induced glioma • radiotherapy • BCNU • chemotherapy

P RIMARY malignant brain tumors persist as one of the yet incurable forms of human cancer. The life of the patient from diagnosis to death is not infrequently accompanied by significant disability resulting from the devastating neurological deficits caused by focal brain destruction. While the need for effective control of this disease is obvious, current therapy protocols offer at best a median survival time of only 52 weeks.20

Of the various experimental neoplasms that have been used in the investigation of therapeutic protocols for brain tumors,16,18 the one that most closely resembles the human anaplastic glioma is the glioma induced by the avian sarcoma virus (ASV) in mammals. This intracranial tumor is autochthonous, can be induced in 100% of animals, causes death of the host at predictable times, lends itself well to study in small mammals such as rats, and histologically resembles human anaplastic astrocytomas.5-6,20 The ASV-induced glioma model has been used in rats to study chemotherapy and chemoimmunotherapy regimens,1,13,20 and this report describes its use in evaluating radiotherapy and combined chemotherapy and radiotherapy protocols.

Materials and Methods

Neonatal CDF (inbred strain Fischer 344) rats were inoculated intracerebrally by percutaneous admin-
istration of 2 μl of ASV suspension on Day 5 (Day 1 = day after birth). Injections were made into the right hemisphere with a short No. 30 needle designed to place the inoculum near the lateral ventricular wall. The rats were then given randomly to foster mothers, so that each mother had the same number of young to nurse. On Day 30 the young rats were weaned and placed two to a cage with water and autoclaved rat chow ad libitum. At the time of weaning the rats were randomized into experimental groups of approximately 20 animals, with equal numbers of males and females. The groups were as follows:

1. Controls (no treatment)
2. Radiation therapy
3. BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) chemotherapy
4. Radiation therapy plus BCNU chemotherapy

Another group of animals was killed on Day 30 for histological verification of tumor induction.

Two series of experiments were performed, with the same four experimental groups in each series, in order to duplicate the results. In Series 1, irradiation of animals was performed using a Siemens orthovoltage therapy unit, having a half value layer (HVL) of 1.6 mm Cu, operated at 250 kilovolt peak (KVP) and 15 mA at a target brain distance of 50 cm with filters of 1/2 Cu and 1 Al. The dose rate to the brain at the midline was 110 rads per minute. Radiation was started 7 days after randomization into experimental groups, and a total dose of 2000 rads was given in six fractions of 333 rads over 2 weeks. The animals were anesthetized before irradiation with intraperitoneal sodium pentobarbital 30 mg/kg body weight. Each animal was individually restrained and the whole body, excluding the head, was shielded with two layers of lead sheets, 1.5 and 3 mm thick. This procedure and an accurate collimation of the field helped to avoid incidental radiation exposure to regions below the neck. Eight rats were irradiated at a time.

In Series 2, irradiation was performed using an orthovoltage therapy unit with an HVL of 1.15 mm Cu operated at 200 KVP and 15 mA at a target brain distance of 50 cm with filters of 1/2 Cu and 1 Al. The dose rate to the brain at the midline was 84 rads per minute. As in Series 1, radiation therapy commenced 7 days after randomization, but a total dose of 2300 rads was given in six fractions of 383 rads over 2 weeks. The animals were not anesthetized before irradiation, but each animal was restrained individually in a plexiglass holder especially designed for this purpose (Fig. 1). Each holder was covered with lead sheets to shield the body of the animal except for the head, and four rats were irradiated simultaneously (Fig. 2).

The BCNU was dissolved in an ethanol-water solution and injected intraperitoneally in a single dose of 10 mg/kg body weight (75% of the L.D10) on the seventh day after randomization. In animals receiving combined chemotherapy and radiation therapy, the BCNU was administered 1 to 2 hours before the first radiation treatment.

Animals were followed until death, at which time a partial autopsy was performed. The brains were examined grossly and then fixed in 10% formalin for subsequent histological examination. The percentage of survivors for each group of rats was plotted against time, and the results were compared by Wilcoxon rank sum statistical analysis, and the Mann-Whitney U-test for large sample approximation.
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### TABLE 1

Survival data for all experimental groups

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>No. of Rats</th>
<th>Range of Survival (days)</th>
<th>Median Survival (days)</th>
<th>Experimental/Control (%)</th>
<th>p Value for Comparison with Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>20</td>
<td>22-107</td>
<td>69</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>irradiation</td>
<td>19</td>
<td>34-134</td>
<td>85</td>
<td>123</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BCNU</td>
<td>20</td>
<td>52-128</td>
<td>80.5</td>
<td>117</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>BCNU &amp; irradiation</td>
<td>24</td>
<td>55-186</td>
<td>113.5</td>
<td>164</td>
<td>&lt;0.001</td>
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<tr>
<td>Series 2</td>
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</tr>
<tr>
<td>control</td>
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<td>45-145</td>
<td>73.5</td>
<td>—</td>
<td>—</td>
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<tr>
<td>irradiation</td>
<td>19</td>
<td>14-129</td>
<td>85</td>
<td>116</td>
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<tr>
<td>BCNU</td>
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<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Experimental group median survival/control group median survival × 100.
†p value was obtained by pair-wise comparison of survival curves.

### Results

In Series 1, deaths related to the anesthetic occurred in three rats, one in the radiation group and two in the combined therapy group. In Series 2, two rats in the combined therapy group were lost when their cages opened accidentally during transportation to the radiotherapy unit. These animals were excluded in assessing the results.

#### Survival Data

Survival data (expressed as time from randomization into experimental groups until death) for the experimental groups are shown in Table 1 and Fig. 3. In each series of experiments, the group treated with radiation alone had significantly increased survival times when compared to controls, whereas BCNU therapy was associated with significantly improved survival in one of two experiments. In both series, combined therapy with BCNU and radiation significantly prolonged survival not only when compared to controls but also when compared to either therapy alone (p < 0.01).

#### Pathology

All rats which were killed at the time of randomization had microscopic periventricular gliomas. In animals receiving no treatment, 2000 rads, BCNU, or 2000 rads and BCNU, large intracerebral neoplasms were present at the time of death, except for three of the 38 untreated rats in whom the tumor was microscopic. In Series 2, seven of 19 rats receiving 2300 rads were found to have little or no tumor at the time of death; in four of these animals there was destruction of white matter, which may represent a radiation effect (Fig. 4), but in three cases no intracranial lesion was identified. Of the 16 animals receiving BCNU and 2300 rads, two had little or no tumor at autopsy, and the cause of death was not apparent.

### Discussion

The effect of radiation therapy on brain tumors has been studied infrequently in animals. Netsky, et al., found a “significant” but not always permanent decrease in the growth rate of a subcutaneously transplanted mouse ependymoblastoma after 3000 rads and 5000 rads of orthovoltage irradiation given as single doses to the tumor area in unanesthetized animals. Edelman, et al., reported improved survival of mice with intracranially transplanted ependymoblastomas, treated with whole-head orthovoltage radiation at a total of 1100 to 1840 rads given in five equal fractions over 2 weeks in unanesthetized animals. With the same irradiation schedule, total doses of 2200 to 3000 rads were curative, while 3500 rads caused death from radiation damage to extracranial structures. Using the intracerebral mouse ependymoblastoma model, Tator, et al., noted no significant effect on survival with single doses of 400 rads whole-brain orthovoltage irradiation, improved survival with 800 rads, and toxicity (early death) with 1200 or 1600 rads given as single doses to anesthetized animals.

The relevance to the therapy of human anaplastic gliomas of the above studies, as well as other studies in which chemotherapy protocols have been investigated using this model, is uncertain, since the transplanted ependymoblastoma is very different histologically from the usual human glioma and may respond differently to irradiation and chemotherapeutic agents. Indeed, Leith, et al., by measuring the fraction of surviving clonogenic cells after single doses of radiation (up to 3000 rads) to the heads of anesthetized rats bearing intracerebrally transplanted 9L gliosarcomas, found that these tumors were relatively radioresistant compared to the mouse ependymoblastoma. They suggested that the effects of radiotherapy in the gliosarcoma model more closely paralleled those in human gliomas.
Rosenblum followed up this work with an in vivo survival study, in which he demonstrated prolonged survival of rats with the intracerebral 9L gliosarcoma after a single dose of 2000 rads given as whole-brain irradiation.

Combination radiotherapy and chemotherapy has only recently been investigated in experimental brain-tumor systems. Theoretically, enhancing interactions between cancer chemotherapeutic agents and radiation can be classified into three types. First, there may be a synergistic effect involving tumor and normal tissues equally, as occurs with DNA-binding agents such as actinomycin D and adriamycin. Such drugs would not be expected to improve the therapeutic effect of irradiation. Second, there may be an additive effect in those tissues in which the...
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chemotherapeutic agent has a toxic effect by itself, as exemplified by the potentiation of methotrexate damage to the central nervous system by irradiation. Third, the chemotherapeutic agent and irradiation may exhibit a synergistic effect which is more pronounced in tumor than in normal tissues. This type of interaction might be of therapeutic benefit and has been demonstrated for bleomycin, cyclophosphamide, and BCNU in studies comparing the potentiation by these drugs of radiation effect in esophagus, lung, and intestine with that noted in the EM T6 mammary tumor in mice. Of these three drugs, the one most useful in the treatment of brain tumors has been BCNU. Wharam, et al., reported that the synergistic effect on the mouse EM T6 tumor that was noted when BCNU (8 mg/kg) was given 2 hours prior to irradiation (200 to 2400 rads) was associated with a flattening of the shoulder of the radiation dose-response curve, which suggests that pretreatment with BCNU might inhibit repair of radiation-induced sublethal damage in the tumor. Wheeler, et al., using cultured rat gliosarcoma cells, found that low doses of BCNU given before radiation in vitro could act as a radiosensitizer and increase cell kill by irradiation, and they, like Wharam, et al., noted that BCNU decreased the shoulder of the radiation dose-response curve. Mealey, et al., reported that combinations of BCNU and radiation therapy in vitro produced more cell kill of human glioblastomas in culture than either modality of treatment used singly. In the only reported in vivo study involving brain tumors, Thomson, et al., using the intracerebral mouse ependymoblastoma model, failed to demonstrate potentiation of radiation (2000 to 2500 rads) effect by BCNU (10 to 30 mg/kg). Combined irradiation and BCNU did prolong survival of the tumor-bearing mice, but no more so than BCNU alone. In an unreported in vivo study, Rosenblum, using the 9L glioma in rats, found a synergistic effect of BCNU, 13.3 mg/kg as a single dose, and whole-brain irradiation of 2000 rads as a single dose, with the BCNU being given 6 hours before, simultaneously with, or 6 hours after, irradiation. Prolongation of survival time was most marked when BCNU followed radiotherapy.

In our present study the effects of radiotherapy and chemotherapy with BCNU on survival times have been investigated for the first time in the ASV-induced rat glioma model. These tumors are autochthonous and histologically resemble human anaplastic astrocytomas more than any other previously studied model. Results of studies with this model could have considerable relevance to the human glioma situation. The striking finding in the current investigation was the marked synergism between BCNU and fractionated radiation therapy, when BCNU, 10 mg/kg body weight was given intraperitoneally as a single dose 1 to 2 hours before the first of six fractions of orthovoltage whole-brain irradiation, the total radiation dose being either 2000 rads or 2300 rads in 2 weeks. The synergism between BCNU and irradiation seems to parallel the treatment results for human anaplastic gliomas, particularly with reference to the findings of the Brain Tumor Study Group. Median survival time following surgical resection of glioblastoma was 52 weeks for patients treated with BCNU and radiation compared to 36 weeks for patients treated with radiation alone, 18 weeks for patients treated with BCNU alone, and 14 weeks for patients treated with the best conventional care but no chemotherapy or radiotherapy.

It was of particular interest that a number of rats treated with 2300 rads died after an effective increase in life expectancy, without a significant intracranial tumor mass. The precise cause of death cannot be determined from this survival study. However, future studies, in which animals are killed and perfused at various intervals after radiotherapy, should permit detailed pathological examination of the tissues, free from autolytic artifacts, so as to define particularly any toxic effects on the central nervous system resulting from therapy.

In view of the finding of synergism between BCNU and irradiation in the ASV glioma model, we plan to study the effects of different doses of radiation, alone and in combination with single or multiple doses of

Fig. 4. Deep subcortical white matter and internal capsular region in a rat that received 2300 rads. The empty spaces represent destruction of white matter. H & E, × 100.
BCNU. Furthermore, since Wheeler, et al.,27 have documented, in a cell culture system, marked potentiation of radiation effect by BCNU given in low doses, with the degree of cell kill being dependent on the timing of BCNU in relation to irradiation, further studies in our laboratory will attempt to confirm their findings in the in vivo situation. The model will also be used to evaluate radiosensitizers, such as mitomidazole, and other combinations of radiation therapy with chemotherapeutic and/or immunotherapeutic agents such as levamisole and bacillus Calmette-Guérin cell walls attached to oil.

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References


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