A controlled study of efficacy of interstitial or external irradiation in a virus-induced brain-tumor model in rats

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In a controlled study of interstitial radiotherapy in the avian sarcoma virus (ASV)-induced glioma model in rats, prolongation of survival was demonstrated (p = 0.08 in Experiment 1 and p = 0.03 in Experiment 2) following mean dosages of 7582 to 9902 cGy 125I, when compared to nontreatment or to control studies with implantation of nonradioactive seeds. More significant (p = 0.02) prolongation of survival was demonstrated following external beam whole-head radiotherapy with nine fractions of 333 cGy, three times weekly over 3 weeks (total dose 3000 cGy). Survival was more prolonged when whole-head radiotherapy was begun 35 days following virus inoculation rather than at 71 days, probably reflecting a greater efficacy with smaller tumor targets.

KEY WORDS • brain neoplasm • avian sarcoma virus • interstitial radiotherapy • brachytherapy • radiation therapy • rat

The avian sarcoma virus (ASV)-induced intracerebral rat glioma model has been used extensively in the past to study various therapeutic approaches for the control of intracranial glioma growth.4,5,14-16,23-25 Our experience with the application of interstitial irradiation to this autochthonous glioma model in a controlled study, comparing this therapeutic modality to external beam radiotherapy, is reported here.

Materials and Methods

Tumor Induction

Gliomas were induced by intracerebral (right frontal) inoculation of neonatal (5 ± 2 days) F344 rats with 5 μl of Schmidt-Ruppin (Subgroup D) ASV suspension as described in previous publications.2 At 30 days of age, the rats were weaned and randomly assigned to experimental groups of 20 animals with an attempt to have equal numbers of males and females in each group. Histological evidence of brain tumor has been verified in the past by the age of 30 days.

Interstitial Brachytherapy

Each rat was anesthetized 71 days after virus inoculation and placed in a three-point headholder. The head was shaved and the right scalp was cleansed with iodine and alcohol. A parasagittal incision was made in the frontal region on the right and self-retaining scalp sutures were placed to expose the underlying bone. A small craniotomy was opened using a dental drill to burr around the periphery of the small flap, which was temporarily removed exposing the underlying dura. The dura was opened and the brain surface was cauterized at a site just posterior to the level of the coronal suture line. Two seeds were inserted separately into the brain perpendicular to the surface until each was approximately 1 mm beneath the surface of the brain. To accomplish seed placement, a trocar was inserted approximately 5 mm into the brain parenchyma perpendicularly to the brain surface. The trocar was mounted in a Vernier clamp that permitted relatively precise positioning of seed placement. After each seed was dropped into the trocar, the trocar stylus was carefully replaced as the trocar was withdrawn, the weight of the stylus pushing the seed into the cavity left by the trocar. The seeds were placed in a parasagittal position approximately 4 mm lateral to the midline and one in front of the other, 2 mm apart. The craniotomy bone flap was placed back into position and the scalp was closed over it.
Interstitial vs. external irradiation in ASV-induced tumor

Experimental Series 1

In the first experimental series, there were three groups of animals. Group I rats were control animals, undergoing no treatment after tumor induction. The rats in Group II underwent implantation into the right cerebral hemisphere of two interstitial radiotherapy seeds which were devoid of any radioactivity (treatment controls). Group III animals were treated in a manner similar to Group II except radioactive seeds, each containing 0.6 mCi of $^{125}$I activity, were inserted as described above. Seed implantation was performed at 71 days after virus inoculation in order to study treatment of intracranial tumors which are generally grossly visible by that time, rather than microscopic in size. The rats in each group were followed to death. Histological examination of brain sections (stained with hematoxylin and eosin) was carried out in Groups II and III, except when prevented by autolysis.

Experimental Series 2

The second experimental series was comprised of five groups of rats. Group IV served as control animals, undergoing no treatment after tumor induction. Group V and VI rats were treated with implants as described in the above paragraph for Experiment 1. Group VII rats were subjected to external beam radiotherapy to the whole head. The irradiation source was a $^{60}$Co teletherapy unit with a high-voltage level of 1.5 mm Cu operated at 200 kilovolt peak and 15 mA at a target brain distance of 50 cm with filters of $\frac{1}{2}$ Cu and 1 Al. The dose rate to the brain at the midline was 84 cGy/min. These treatments were begun 35 days following virus inoculation (“early” radiotherapy) with fractions of 333 cGy per day on Mondays, Wednesdays, and Fridays for 3 weeks. The therapy was administered without anesthesia, using Plexiglas rat restrainers as previously described. This is the usual radiotherapy treatment that has been shown to be efficacious when begun at this stage of tumor growth. In order to compare interstitial radiotherapy (begun at 71 days), a group of rats (Group VIII) was treated exactly as described for Group VII, except that external beam radiotherapy was begun at 71 days (“late” radiotherapy). The total cumulative dose for each of these groups was 3000 cGy in nine fractions over 3 weeks. As in Experiment 1, the rats in each group were followed until death, with histological examinations of the brains of Groups V and VI, unless prevented by autolysis.

Survival Time

Kaplan-Meier survival curves were calculated for each group of rats by plotting the proportion surviving versus time in days (Fig. 1). The significance of difference between untreated control and treated groups was determined by Wilcoxon rank-sum statistical analysis.

Fig. 1. Kaplan-Meier survival curves for each group in Experiment 1.

Fig. 2. Upper and Center: Radiographs of a rat head showing the typical location of the seeds (lateral view, upper; mentovertex view, center). Lower: Coronal section of a rat brain showing a seed in place. Time of death and brain sectioning was 90 days after implantation.
Results

Experimental Series 1

In this initial experiment, the median survival times (Fig. 1) following intracranial virus inoculation for the three groups were: 136 days for Group I (control group), 122 days for Group II (nonradioactive seeds), and 158 days for Group III (radioactive seeds). As compared to control results, the survival time of the rats in Group III approached significance (p = 0.08). For purposes of computing the total dose of interstitial irradiation delivered to these rats, isodose curves were calculated and were applied to the survival time of each rat following implantation. The average width of the implanted rat brains was 16 mm. Presuming that the irradiated field of interest encompassed the maximum width of the right hemisphere (8 mm), the minimum dose rate of radiation would be 4.09 cGy/hr. The individual total dose of irradiation to the 20 rats in Group III ranged from 0 (one rat died after randomization and prior to implantation) to 36,347 cGy, which represents a mean dose of 9902 cGy and a median dose of 7565 cGy.

The location of radioactive seeds at the time of brain examination following death is illustrated in Fig. 2. Histological examination of the brains of 17 rats in Group II revealed eight glioblastomas multiforme, seven anaplastic astrocytomas, and two oligodendrogliomas. Histological examination of the brains of 13 rats in Group III was possible, with tumors classified as follows: seven mixed anaplastic gliomas, one glioblastoma, one oligodendroglioma, one astrocytoma, and one sarcoma. Precise histological changes attributable to radiation could not be detected, whereas the seed sites were evident (Fig. 3). There did not appear to be any correlation of histological type and survival time.

Experimental Series 2

The median survival times for the rats in this experiment are shown in Table 1: 116 days for Group IV (control group), 122 days for Group V (nonradioactive seeds), 134 days for Group VI (radioactive seeds), 150 days for Group VII (early radiotherapy), and 140 days for Group VIII (late radiotherapy). There were significant increases in the survival of rats in Group VI (radioactive seeds), Group VII (early external radiotherapy), and Group VIII (late external radiotherapy) compared to untreated control rats. Isodose curve parameters similar to those used in Experiment I showed that the range of radiation therapy for individual rats in Group VI was from 13 to 21,884 cGy, a mean of 7582 cGy and a median of 7123 cGy for the group as a whole.

Histological examination was possible in 13 brains of the rats in Group V, and the tumors were classified as follows: eight astrocytomas, two oligodendrogliomas, one mixed anaplastic glioma, one ganglioglioma (Fig. 4), and one sarcoma. Histological examination was possible in 11 rats in Group VI, revealing four mixed anaplastic gliomas, four astrocytomas, two glioblastomas, and one sarcoma. As in Experiment 1, there was no correlation of histological type with survival time. Although seed implantation defects in the tissue could be identified, no histological changes could be determined in the nonautolyzed brains in this limited postmortem examination material that distinguished Group V from Group VI rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Therapy</th>
<th>Survival (days)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>control (no treatment)</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>nonradioactive seeds</td>
<td>122</td>
<td>0.27</td>
</tr>
<tr>
<td>VI</td>
<td>radioactive seeds</td>
<td>134</td>
<td>0.03</td>
</tr>
<tr>
<td>VII</td>
<td>3000 rads, external beam (early)</td>
<td>150</td>
<td>0.02</td>
</tr>
<tr>
<td>VIII</td>
<td>3000 rads, external beam (late)</td>
<td>140</td>
<td>0.02</td>
</tr>
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* Significance of difference, compared to Group IV.
Interstitial vs. external irradiation in ASV-induced tumor

Discussion

Interstitial radiotherapy (brachytherapy) has been utilized for treatment of recurrent gliomas for over four decades, beginning with radium sources8,22 and later including radioactive phosphates,3,12,26 radioactive gold,10,11,27 radioactive iridium,11,18,21 radioactive helium/neon,1 and radioiodine.5-11 The large clinical series of 33 cases of glioblastoma and 43 cases of other gliomas treated with radioiodine implants by Gutin, et al.,9 had impressive postimplantation survival times of 67 and 78 weeks, respectively. More recently, smaller numbers of patients have been given interstitial radiotherapy as part of initial management using neutron sources alone,20 radioiodine implants in conjunction with external beam radiotherapy,13,28 or radioactive iridium implants in conjunction with external beam radiotherapy plus 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) chemotherapy.17 Although the technology of implantation has become sophisticated and efficacy is apparent, the issues of timing of this therapy and its use in conjunction with or in place of other modalities remain to be defined more precisely. Some of these questions may need investigation in suitable model systems.

In a study of 9L brain tumor cells transplanted into F344 rats, Gutin, et al.,9 described an increased length of survival (%ILS) following low-dose removable 125I brachytherapy (28%), a more impressive increased survival with BCNU chemotherapy (67%), and a synergistic increased survival with combined brachytherapy and BCNU (167%), compared with nontreated control groups. Gliomas induced by ASV, used in the present study, are similar histologically to human gliomas and a dose-response effect of this tumor to external beam irradiation has been reported.25 Vascular damage and secondary coagulative necrosis of the brain was never found histologically in past experiments using external beam radiotherapy in tumor-bearing rats, and cerebral blood vessels were unremarkable.25 For purposes of the current experiments, the total cumulative external beam radiation dose of 3000 cGy was selected because a consistent therapeutic benefit of this dose has been demonstrated in several previous studies24,25 coupled with a lesser apparent oral toxicity of this dose to irradiated rats.

These experiments demonstrated no significant survival benefit derived from implantation of nonradioactive seeds (implanted controls), whereas there was an increase in survival in rats implanted with radioactive seeds (p = 0.08 in Experiment 1 and p = 0.03 in Experiment 2). When compared to 125I seed-implanted rats, tumor-bearing rats treated with 3000 rads of external beam radiation beginning 71 days following virus inoculation had a slightly greater extended survival (p = 0.02). When external beam radiation was administered 35 days after virus inoculation, the median survival was somewhat greater than when the external beam radiation therapy was delayed until 71 days. This finding probably reflects a greater benefit of radiation therapy upon smaller tumor volumes.

Due to the fact that animals were permitted to live until spontaneous death, the ability to analyze the histology carefully was prevented in some cases by autolysis. Precise histological study was not the purpose of this study design. However, it was possible to ascertain that seed sites could often be identified, that there were no obvious unique histological features to brains containing radioactive seeds, and that the histopathology of the tumors was similar to that previously described by Copeland, et al.,2 with exception of the discovery of one ganglioglioma (Fig. 4). Necrotizing and calcifying changes following brachytherapy have been described by Ostertag, et al.,19 in a noncontrolled study of this virus-induced brain tumor model in dogs.

Conclusions

In this autochthonous, ASV-induced glioma model in rats, 125I interstitial radiotherapy (mean dose 7582 to 9902 cGy) provided an increase in survival time compared to untreated controls as well as controls implanted with nonradioactive seeds. However, this increase in survival time was not quite as great as with external beam radiotherapy to the whole head to a total dose of 3000 rads, which itself was most effective when administered earlier rather than later following virus inoculation. Thus, quite high radiation doses generated with 125I brachytherapy did not result in survival times as significantly different from control results as those achieved by lower doses delivered externally.

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References


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