Radiosurgery and fractionated radiation therapy: comparison of different techniques in an *in vivo* rat glioma model

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To identify histological changes and effects on survival in rats harboring C6 gliomas, the authors compared radiosurgery to different fractionated radiation therapy regimens including doses of calculated biological equivalence. Rats were randomized to control (54 animals) or treatment groups after implantation of C6 glioma cells into the right frontal brain region. At 14 days, treated rats underwent stereotactic radiosurgery (35 Gy to tumor margin; 22 animals), whole-brain radiation therapy (WBRT) (20 Gy in five fractions; 18 animals), radiosurgery plus WBRT (13 animals), hemi-brain radiation therapy (85 Gy in 10 fractions; 16 animals) or single-fraction hemibrain irradiation (35 Gy; 10 animals). When compared to the control group (median survival 22 days), prolonged survival was identified after radiosurgery (*p* < 0.0001), radiosurgery plus WBRT (*p* < 0.0001), WBRT alone (*p* = 0.0002), hemibrain radiation therapy to 85 Gy (*p* < 0.0001), and 35-Gy hemibrain single-fraction irradiation (*p* = 0.004). Compared to the control group (mean tumor diameter, 6.8 mm), the tumor size was reduced in all treatment groups except WBRT alone. Reduced tumor cell density was exhibited in rats that underwent radiosurgery (*p* = 0.006) and radiosurgery plus WBRT (*p* = 0.009) when compared with rats in the control group, a finding not observed after any fractionated regimen. Increased intratumoral edema was identified after radiosurgery (*p* = 0.03) and combined treatment (*p* = 0.05), but not after fractionated radiation therapy or 35-Gy single-fraction hemibrain irradiation. In this animal model, the addition of radiosurgery significantly increased tumor cytotoxicity, potentially at the expense of radiation effects to regional brain. We found no difference in survival benefit or tumor diameter in animals that underwent radiosurgery compared to the calculated biologically equivalent regimen of 10-fraction radiation therapy to 85 Gy. The histological responses after radiosurgery were generally greater than those achieved with biologically equivalent doses of fractionated radiation therapy.

KEY WORDS • radiosurgery • brain neoplasm • glioma • radiation therapy

The increased use of stereotactic radiosurgery or stereotactic fractionated irradiation as an addition or alternative to conventional therapy for malignant brain tumors mandates investigation into the relative effects of these approaches. Use of the C6 rat glioma model is a valuable technique to evaluate the response of different radiation modalities on tumor biology, to study chemotherapeutic agents, and to analyze tumor growth patterns. We previously tested the effects of stereotactic radiosurgery alone using a frontal lobe C6 rat glioma model. In that initial study we examined animal survival after radiosurgery and studied the histological effects of various radiosurgical doses. Although we detected no statistical increase in animal survival after radiosurgery, treated tumors significantly regressed and had reduced cellular density. In the present study, we sought to evaluate the clinical and histological response of a growing malignant glioma after either high-dose stereotactic radiosurgery (margin dose 35 Gy), fractionated hemibrain radiation therapy to 85 Gy in 10 fractions (calculated to be biologically equivalent to radiosurgery), a single-fraction hemibrain regimen of 35 Gy, lower-dose fractionated whole-brain radiation therapy (WBRT) (20 Gy in five fractions), radiosurgery plus WBRT, or an untreated control group. We hypothesized that radiosurgery alone or in combination with WBRT would increase animal survival in comparison to no treatment or WBRT alone and that a histological correlate could be defined with the survival response. The treatment arms were chosen to compare the effects of radiosurgery and doses of fractionated radiation therapy of approximate biological equivalence for malignant tumors and to study the effects of focused (radiosurgery) versus large-field irradiation at the same 35 Gy margin dose.
Materials and Methods

All experiments were performed with the approval of the Animal Care and Use Committee of the University of Pittsburgh. Male Sprague–Dawley tumor implanted rats (average weight, 300 g) were randomized to a variety of groups: control (no irradiation), stereotactic radiosurgery (35 Gy to 50% isodose; maximum dose of 70 Gy), radiosurgery (35 Gy) plus WBRT to 20 Gy in five fractions, WBRT to 20 Gy in five fractions, large-field (hemibrain) radiotherapy to 85 Gy in 10 fractions, and single-fraction nonstereotactic hemibrain irradiation to 35 Gy. Tumors were grown as previously reported.7 We used the C6 glioma5 in this and prior experiments because of its widespread use and utility,14 its consistency in tumor histology, and because it produced a fairly well-circumscribed tumor suitable for focused radiosurgical targeting.7 We implanted C6 glioma cells into the rat frontal brain region at passage number 40 to 42. Cells were pelleted by centrifugation and resuspended to a final concentration of 10^6 cells/10^6 glioma cells into the rat frontal brain region at passage number 40 to 42, the time at which radiosurgery was performed.7 The 50% iso-
Radiation strategies tested in rat glioma

of the subjective nature of these findings. Rather we coded a characteristic as being present or absent according to the descriptions noted above. Our evaluations of these parameters proved reproducible when specimens were reexamined (no finding was changed in any animal on microscopic reassessment and all histological preparations were examined twice).

Statistical Methods
Survival data were evaluated using both a log-rank test and a two-tailed t-test for comparing independent mean values. To compare histological features we used Yates’ corrected chi-square test for column tabled data, or Fisher’s exact test for smaller samples. A probability level equivalent to 0.05 was used to determine statistical significance.

Results

Animal Survival
Table 1 details the survival results for animals in each treatment group using the log rank survival statistic. When compared to the control group (median survival, 22 days), a significant survival benefit was found after radiosurgery (median survival, 45 days; \( p < 0.0001 \)), radiosurgery plus WBRT (median, 45 days; \( p < 0.0001 \)), and WBRT alone (median survival, 40 days; \( p = 0.0002 \)). Similarly, a survival benefit was found after 10-fraction radiotherapy to 85 Gy (median survival, 49 days; \( p = 0.0001 \)) and after single fraction hemibrain irradiation to 35 Gy (median survival, 38 days; \( p = 0.005 \)). In the survival analysis, we could not identify a difference between the results after radiosurgery alone and those after radiosurgery plus WBRT (\( p = 0.72 \)), between radiosurgery alone and WBRT alone (\( p = 0.12 \)), or between radiosurgery plus WBRT and WBRT alone (\( p = 0.19 \)). There was no difference between the “biologically equivalent” groups of radiosurgery and 10-fraction radiotherapy (\( p = 0.45 \)) or between radiosurgery and single-fraction nonstereotactic irradiation at the same 35-Gy margin dose (\( p = 0.8 \)). Similar statistical results were obtained using a two-tailed t-test. Kaplan–Meier survival curves are shown in Fig. 1.

Tumor Size
When compared with the control group (mean tumor diameter, 6.8 mm \( \pm 1.3 \) mm), a significant reduction in tumor size was identified after radiosurgery alone (\( p = 0.00008 \)) and after radiosurgery plus WBRT (\( p = 0.003 \)) (Fig. 2). After radiosurgery, the mean tumor diameter was reduced to 5.2 mm \( \pm 2.6 \) mm and after radiosurgery plus WBRT, 5.3 mm \( \pm 2.3 \) mm. No significant reduction in tumor size was identified after 20-Gy WBRT alone (mean diameter 6.3 mm \( \pm 1.2 \) mm) (\( p = 0.20 \)). However, at the higher fractionated dose of 85 Gy, a significant reduction in tumor size was found (mean diameter 5.4 mm \( \pm 1.6 \) mm) (\( p = 0.004 \)), which was not different from the radiosurgery arm. Similarly, single-fraction irradiation to 35 Gy led to a reduction in tumor size compared with control (mean diameter 5.0 mm \( \pm 1.0 \) mm) (\( p = 0.001 \)).
Histological Changes

Table 2 details the individual histological changes identified among the different groups. A significant reduction in tumor cell density was identified after radiosurgery (p = 0.006) and after radiosurgery plus WBRT (p = 0.009) when compared with the control group. No significant reduction in cell density was identified after either 20-Gy WBRT alone, the 85-Gy 10-fraction regimen, or the 35-Gy single-fraction regimen. Between groups, radiosurgery plus WBRT was associated with reduced cell density when compared to any of the other nonradiosurgical groups (p = 0.02) (Fig. 3).

We compared the presence of intratumoral hemorrhage (defined as greater than 10% of tumor volume) between groups. In the group that underwent radiosurgery plus WBRT, fewer animals experienced tumor hemorrhage than in the control group (p = 0.03). We found no other significant relations among the groups.

We compared the presence or absence of cellular edema in each group after treatment. A significant increase in the number of animals with intratumoral edema was identified after radiosurgery (p = 0.03), and after radiosurgery plus WBRT (p = 0.05) when compared with control. No significant difference in the presence of edema was identified after WBRT alone (p = 0.18), 10-fraction radiotherapy (p = 0.45), or single-fraction hemibrain irradiation (p = 0.62) when compared with control. When compared with all other groups, both radiosurgery and radiosurgery plus WBRT were associated with a significant increase in the number of animals that had tumor edema. We found no significant interrelationships among cell density, the presence of hemorrhage, or the degree of tumor edema.

Experimental Design

Previous investigators showed that single-fraction, WBRT of an in vivo rat glioma led to improved animal survival compared to that of a control group.1,4,7,16,17,19 We selected a maximum radiosurgical dose of 70 Gy (margin dose 35 Gy) because it represented a middle-range dose from our prior study, in which we had tested doses of 30, 40, 50, 70, and 100 Gy. Previously, we identified no specific dose–response relationship and thus chose a single dose for this study to provide uniformity within the radiosurgery cohort. In our prior study,1 we found no survival benefit to radiosurgery over control (p = 0.07), which perhaps was related to the small sample size examined. Schwachenwald and colleagues23 found that single-fraction irradiation using the gamma unit at doses of 25 and 45 Gy was effective in limiting growth of the D-54 Mg glioma cell line in culture. Our selection of 35 Gy for the
minimum dose received by the tumor was in accordance with their findings, although different cell lines were used.

A WBRT regimen of 20 Gy administered in five fractions was selected as a dose within cerebral tolerance for the rat brain; this scheme is a clinically relevant one used by radiation oncologists. Rogers, et al.,13 found that early radiation-induced histological changes included trigeminal and facial nerve necrosis, whereas others found central and hemispheric white matter necrosis characterized as both early5 and late effects.13 Prior studies that investigated animal survival (with 9L tumors) at different WBRT doses showed that single-fraction doses above 21 Gy led to the deaths of all animals in 8 to 12 days.4 In normal animals, a 10-fraction regimen of 21 Gy did not lead to animal death until at least Day 200. The authors suggested a conversion factor of 1.9 for whole-brain radiobiological equivalence between single-fraction and 10-fraction radiotherapy.4 Because we hoped to evaluate animals for as long as 90 days, we wanted to avoid treatment-related morbidity within that period. Our previous normal brain studies of different radiosurgery doses showed no 90-day animal morbidity with doses as high as 200 Gy.6

Radiosurgery in Addition to Radiation Therapy

Because most investigators agree that fractionated radiation is useful and beneficial in the clinical management of malignant gliomas, the possibility that radiosurgery alone might be of similar clinical benefit was not examined. However, a study of independent radiosurgical effects is important to evaluate its histological correlate and its potential clinical benefits when it might be used as an isolated treatment for recurrent tumors. At our institution, we currently use radiosurgery as a boost to fractionated radiotherapy (60 Gy) for the contrast-enhancing volume of anaplastic astrocytomas or glioblastomas multiforme less than 3 cm in diameter. Thus, the present animal study was relevant to the radiobiological evaluation of this clinical approach.

Wheeler and Kaufman19 identified an improvement in animal survival using a 26-Gy fractionated regimen in their 9L rat model. Barker and colleagues1 found a survival benefit after 20-Gy single-fraction WBRT (administered Day 16 after implantation) in a similar model. Although those authors categorized animals without any evidence of tumor cells as “cured,” we excluded all such animals from our analysis in the belief that failure of initial tumor grafting was a serious, potentially confounding variable in such studies.

Radiobiological Interpretation

We detected no significant difference in median survival after radiosurgery, fractionated radiotherapy (whole-brain or hemibrain), or a combination of both. Thus, we did not define a dose–response relationship for survival. This may be related to continued growth of persistently viable cells, and thus an inability to attain a mean survival longer than 50 days despite any treatment regimen. In addition, irradiating a larger volume, as used in all the nonradiosurgery groups, did not impact survival benefit. However, the cytotoxic effects between groups were different, as reflected by changes in tumor size, cellularity and edema. The exception to this was that radiosurgery and 85-Gy radiation therapy showed similar reductions in tumor size. It seems clear that the observed effects within this early 90-day period are primarily “direct” cellular responses, and not “indirect” vascular responses (most of which occur later), due to the absence of vessel thrombosis or infarction. Thus, the degree of decreased tumor cellularity was proportional to the observed edema (or expansion of extracellular spaces); these changes were noted only in the radiosurgery groups. This result was consistent with our prior work.5

FIG. 3. Photomicrographs showing the tumor–brain interface. a: Control; tumor is densely hypercellular. A similar appearance was found in rats that had fractionated radiation therapy. b: Radiosurgery; the entire tumor is small, necrotic, and appears to contain nonviable cells. c: Radiosurgery plus whole-brain radiotherapy; marked necrosis and a hypocellular appearance at the tumor margin is shown. H & E, original magnification × 25.
Although we found more animals with tumor edema in the control group than in the WBRT group, this was not statistically significant. One could hypothesize that WBRT led to a reduction in cytotoxic edema compared with control; however, further studies using brain weight measurements would be necessary to confirm this.

Animals that underwent radiosurgery showed a decrease in tumor size that was not observed after the 5-fraction regimen or after the 35-Gy single-fraction irradiation. It is possible that a nonedematous tumor in the radiosurgery arms might have been smaller, indicating an even greater cytotoxic effect (a conclusion not reached according to the numbers of animals in this experiment). It would be logical to expect a greater decrease in tumor size or even less tumor cellularity after combined radiosurgery/radiotherapy than after the other therapies because more radiation was administered. However, in the “biologically equivalent” groups of 35-Gy radiosurgery and 10-fraction 85-Gy radiotherapy, even when compared to the single-fraction 35-Gy arm, radiosurgery led to greater cytotoxic effects, as noted by a greater reduction in cellular density. This was most likely due to the spectrum of dose delivered across the tumor in radiosurgery (35 Gy at the margin, increasing to 70 Gy at the center) versus a much more uniform dose delivered across the tumor in the other regimens.

This finding is an example of the potential benefit of dose heterogeneity within solid neoplasms and may indicate not only the importance of the minimum dose, but also of isodose and the corresponding maximum dose. Such higher central doses, as well as a delayed vascular response from vessels irradiated at the tumor periphery, are probably responsible for the marked loss of central contrast enhancement often found after human acoustic tumor radiosurgery.10,12 Fractionated radiotherapy to the 100% isodose line did not appear to be as efficacious as radiosurgery to the 50% isodose line. This finding might be clinically relevant to those now using stereotactic fractionated techniques, in which both the benefits of precise targeting as well as the use of less homogeneous doses (at or below the 80% isodose) are being explored.

Future Experiments

Because the goal of radiosurgery is tumor control rather than tumor elimination, we next plan to investigate the in vitro behavior of irradiated cells and the molecular correlates of tumor dormancy. First, irradiated tumor cells within the rat brain will be removed and regrown in culture. This will allow quantification of their in vitro growth rate (in comparison to control) as well as an analysis of tumor-regulated growth factor production after radiosurgery. Similar experiments are planned for our acoustic tumor xenograft model to study such responses after benign tumor radiosurgery.

Acknowledgments

The authors would like to thank Wendy Fellows-Mayle for excellent technical support and Kim Tonet for manuscript preparation.

References


Manuscript received September 14, 1995. Accepted in final form January 15, 1996.

Dr. Kondziolka is supported by Grant No. 1 K08 NS01723 from the National Institutes of Health.

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