Enhancement of the efficacy of x-irradiation by pentobarbital in a rodent brain-tumor model

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Radiation therapy is an important component of brain tumor treatment, but its efficacy is limited by its toxicity to the surrounding normal tissue. Pentobarbital acts as a cerebral radioprotectant, but the selectivity of its protection for the central nervous system has not been demonstrated. To determine if pentobarbital also protects tumor against ionizing radiation, five groups of Fischer 344 rats were observed after exposure to varying combinations of the presence or absence of implanted tumor, pentobarbital, and radiation treatment. The first three groups underwent cerebral implantations of a suspension of 9L gliosarcoma cells. Group 1 was left untreated and served as tumor-bearing controls. Group 2 received 30 Gy of whole-brain x-irradiation without anesthesia 8 days after tumor implantation. Group 3 received the same radiation treatment 15 minutes after pretreatment with 60 mg/kg of pentobarbital intraperitoneally. Groups 4 and 5 served as radiation controls, receiving 30 Gy of x-irradiation while awake and 30 Gy of x-irradiation after pentobarbital administration, respectively. Survival was calculated from the death of the last tumor-bearing rat.

The mean survival time in tumor-bearing control rats was 20.8 ± 2.6 days (± standard deviation). X-irradiation alone significantly enhanced the period of survival in rats implanted with the 9L tumor (29.7 ± 5.6 days, p < 0.003). Further significant prolongation of survival was seen with the addition of pentobarbital to the treatment regimen (39.9 ± 13.5 days, p < 0.01). Nontumor-bearing rats irradiated while awake (Group 4) survived 30.9 ± 2.3 days. All of their pentobarbital-anesthetized counterparts in Group 5 survived. If pentobarbital had offered radioprotection to the tumor, then Group 3 would have had a shorter survival period than Group 2. This implies that the enhancement of survival time after irradiation results from selective protection of normal brain in this model.

KEY WORDS • brain neoplasm • radiation injury • pentobarbital • rat

Materials and Methods

Animals

Male Fischer 344 rats, each weighing between 150 and 175 gm, were maintained on standard rat chow and water ad libitum. The rats were housed and cared for according to the National Institutes of Health “Guide for the Care and Use of Laboratory Animals, Revised 1985.”

Tumor Model

The 9L rat gliosarcoma was grown in tissue culture flasks in a maintenance medium of Dulbecco’s minimum essential medium (MEM) supplemented with 10% fetal calf serum, penicillin (10,000 U/ml), streptomycin (100 μg/ml), and 2 mM L-glutamine. At confluence the tumor cells were harvested with 0.4% trypsin and 5 mM ethylenediaminetetra-acetic acid (EDTA), then washed and resuspended in MEM without L-glutamine at a concentration of 10,000 cells/μl.
Rats were anesthetized with pentobarbital (45 mg/kg intraperitoneally) and placed in a stereotactic head holder. A midline sagittal incision was made and a small craniectomy was performed 1 mm anterior and 3 mm to the right of the bregma. The 9L cell suspension (4 µl) was then injected through the craniectomy with a Hamilton syringe at a depth of 3.5 mm from the outer table. Bone wax was placed over the craniectomy, the scalp was closed, and the animal was allowed to recover.

X-Irradiation

Five groups of animals were studied. Group 1 served as a tumor-bearing control and received no radiation. Group 2 animals were irradiated while awake and restrained in a cone-shaped device made of Lucite with an apical nose opening for breathing. A linear accelerator was used as 15 MeV source of x-irradiation which was delivered to the whole brain via a lateral port in a single fraction at a dose rate of 6 Gy/min and a source-to-target (midsagittal plane) distance of 86 cm. The mouth, pharynx, nose, and body were shielded with lead. Group 3 received x-irradiation with identical parameters, but the radiation was delivered 15 minutes after intraperitoneal administration of pentobarbital (60 mg/kg). Groups 4 and 5 did not undergo tumor implantation and served as irradiated controls. They received 30 Gy of x-irradiation while awake (Group 4) or after the administration of 60 mg/kg of pentobarbital (Group 5).

The animals from each group were examined daily and survival time was recorded. The survival curves of Groups 1 through 3 began with tumor implantation, with that day being designated as Day 0. For calculation of survival time, the survival curves of Groups 4 and 5 were offset such that the day of their x-irradiation was designated as Day 8, just as in Groups 1 through 3. Survival time was calculated for Groups 4 and 5 beginning with the day of death of the last tumor-bearing animal. The data were analyzed with Student’s t-test for unpaired data.

Autopsies were performed on all tumor-implanted animals to document the success of this procedure.

**Results**

The results are summarized in Table 1 and Fig. 1. Mean survival time of Group 1 rats (the tumor control group) was 20.8 ± 2.6 days (± standard deviation). In Group 2, administration of x-irradiation while the animals were awake significantly prolonged survival to 29.7 ± 5.6 days (p < 0.003). The administration of pentobarbital 15 minutes before x-irradiation in Group 3 further extended survival to 39.9 ± 13.5 days compared to the control group (Group 1, p < 0.009) and compared to the group irradiated while awake (Group 2, p < 0.01). Survival in Group 4 (nontumor-bearing animals irradiated while awake) was 30.9 ± 2.3 days. All animals in Group 5 (those without tumors irradiated after administration of pentobarbital), survived the entire 53-day study period, clearly a better outcome than their counterparts which were irradiated while awake (Group 4, p < 0.02). At autopsy, each tumor-bearing animal had grossly visible tumor at the implantation site.

**Discussion**

The data from the investigation reported here confirms, as others have shown, that radiation therapy

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**TABLE 1**

**Survival data during 53-day observation period***

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Description</th>
<th>No. of Rats</th>
<th>Survival Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>tumor control: tumor implantation only</td>
<td>11</td>
<td>20.8 ± 2.6</td>
</tr>
<tr>
<td>2</td>
<td>tumor: XRT while awake 8 days later</td>
<td>10</td>
<td>29.7 ± 5.6†</td>
</tr>
<tr>
<td>3</td>
<td>tumor: XRT under pentobarbital 8 days later</td>
<td>8</td>
<td>39.9 ± 13.5‡</td>
</tr>
<tr>
<td>4</td>
<td>no tumor: XRT while awake</td>
<td>10</td>
<td>30.9 ± 2.3</td>
</tr>
<tr>
<td>5</td>
<td>no tumor: XRT under pentobarbital</td>
<td>10</td>
<td>53.0§</td>
</tr>
</tbody>
</table>

* Survival times are means ± standard deviation. All Group 5 rats were alive at the end of the study period. XRT = 30 Gy whole-brain x-irradiation.
† Significantly greater than Group 1 (p < 0.003).
‡ Significantly greater than Group 1 (p < 0.009) and Group 2 (p < 0.01).
§ Significantly greater than Group 4 (p < 0.02).
prolongs the survival of animals implanted with the 9L tumor. \textsuperscript{3,8,24} The addition of pentobarbital sedation before administration of the same dose of x-irradiation further enhances survival time. This demonstrates that the tumor was not protected by the pentobarbital. If the pentobarbital had protected the tumor tissue from radiation injury, the tumor would have been expected to grow more quickly than it did in the group of tumor-bearing animals that were irradiated while awake. This would have led to a more rapid death in the pentobarbital-treated group than in the awake group. This was not the case. Rather, the pentobarbital-treated group had a longer mean survival period. Although there was improvement in the survival period, all animals eventually died from tumor; thus no cure occurred with the radiation parameters used in this study.

The 30-Gy radiation control groups (Groups 4 and 5) are also reported as part of an accompanying series of experiments which investigated the mechanism and dose-response characteristics of protection against radiation-induced brain injury by pentobarbital. \textsuperscript{13} We chose 30 Gy as the radiation dose for the investigation reported here because it was the highest dose at which 100% survival occurred with the addition of pentobarbital in our radiation dose-survival response experiments. \textsuperscript{13} Because deaths occurred in nontumor-bearing animals which were irradiated while awake, the decrement in survival of the group of tumor-bearing animals that were irradiated while awake (Group 2) is due to combined radiation- and tumor-induced injury. At higher doses of radiation in the dose-survival response study, deaths occurred even with the addition of pentobarbital. \textsuperscript{13} Thus, at higher doses of radiation, deaths from radiation toxicity would have been happening simultaneously with tumor-induced deaths; the inability to separate these causes of death in the tumor-bearing group treated with pentobarbital (Group 3) would have made data interpretation difficult. Thus, limitation of the radiation dose to a level that affected tumor growth (Group 2) but at which no radiation-induced deaths occurred in control pentobarbital-treated animals (Group 5) meant that the enhanced survival time with radiation during pentobarbital treatment could be considered a result of protection of brain, and not tumor, from radiation injury.

To examine the influence of pentobarbital on radiation-induced tumor response required a tumor-bearing model in which tumor response could be measured. We chose to deliver radiation 8 days following tumor implantation because, at 7 days after implantation, tumor is visible to the naked eye and treatment of a small tumor was more likely to demonstrate any possible benefits. Alternative hypotheses to explain the improved survival of animals pretreated with pentobarbital are that this manipulation increased the radiosensitivity of the tumor or that pentobarbital as used here had some direct tumor-inhibiting effect. Prior work addressing the alteration of radiosensitivity of tumors or normal tissue by pentobarbital has been performed with many different experimental models and has produced conflicting conclusions. Investigators utilizing rodent models with subcutaneously implanted tumors have reported that pentobarbital is capable of increasing, decreasing, or having no effect on tumor radiosensitivity and have demonstrated no direct effect of pentobarbital on these tumors. \textsuperscript{6,17,18} In 1957, Alvord and Brace \textsuperscript{1} described alteration of radiation injury to cerebellar granule cells in a guinea pig; however, no specific work on the alteration of radiosensitivity using intracranial tumor models has been reported since. As all tumor-bearing animals in this study eventually died of their tumor at the dose of radiation used, whether treated with pentobarbital or not, the alteration of radiosensitivity and tumor growth which might be significant in terms of final outcome requires investigation of increasing doses of radiation.

This investigation addresses the issue of preferential radioprotection of normal brain over tumor based on implanted tumor model. The prospect of application of cerebral radioprotection by pentobarbital is most easily projected in reference to tumor treatment. It may offer a mechanism by which survival time as well as quality of survival can be enhanced. Although the data presented here are of potential relevance, we caution that 1) there is a significant species difference, 2) the tumor is sarcomatous in nature, and 3) the most important dose-limiting toxicity with radiation therapy of brain-tumor patients is late delayed toxicity. This study investigated the differential protection of tumor and normal brain against acute and early delayed radiation damage. Direct extrapolation to humans with malignant brain tumors is not appropriate. Further investigation of this phenomenon is warranted prior to unqualified recommendations about its potential utility in the clinic.

References

11. Marks JE, Baglan RJ, Prassad SC, et al: Cerebral radione-
crosis: incidence and risk in relation to dose, time, frac-
tionation and volume. Int J Radiat Oncol Biol Phys 7:
243–252, 1981
radiation-induced brain injury by use of pentobarbital or
13. Olson JJ, Friedman R, Orr K, et al: Cerebral radioprotec-
tion by pentobarbital: dose-response characteristics and
association with GABA agonist activity. J Neurosurg 72:
749–758, 1990
14. Prosnitz LR, Kapp DS, Weissberg JB: Radiotherapy (first
15. Prosnitz LR, Kapp DS, Weissberg JB: Radiotherapy (sec-
16. Scholz W, Hsu YK: Late damage from roentgen irradia-
tion of the human brain. Arch Neurol Psychiatry 40:
928–936, 1938
17. Sheldon PW, Hill SA, Moulder JE: Radiation protection
by pentobarbitone sodium of a murine tumour in vivo.
18. Sheline GE, Wara WM, Smith V: Therapeutic irradiation
and brain injury. Int J Radiat Oncol Biol Phys 6:
1215–1228, 1980
anesthesia and the response of tumor and normal tissue
in the C3Hf/Sed mouse to radiation. Radiat Res 104:
47–65, 1985
20. Teicher BA, Rose CM: Perfluorochemical emulsions can
increase tumor radiosensitivity. Science 223:934–936,
1984
I study of hypoxic cell radiosensitizer RO-07-0582, a 2-
high-dose metronidazole in supratentorial glioblastomas.
23. Walker MD, Strike TA, Sheline GE: Analysis of dose-
effect relationship in the radiotherapy of malignant glo-
schedules on the response of a rat brain tumor to therapy
with BCNU and radiation. Int J Radiat Oncol Biol Phys
6:845–849, 1980

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