Reduction in radiation-induced brain injury by use of pentobarbital or lidocaine protection

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To determine if barbiturates would protect brain at high doses of radiation, survival rates in rats that received whole-brain x-irradiation during pentobarbital- or lidocaine-induced anesthesia were compared with those of control animals that received no medication and of animals anesthetized with ketamine. The animals were shielded so that respiratory and digestive tissues would not be damaged by the radiation. Survival rates in rats that received whole-brain irradiation as a single 7500-rad dose under pentobarbital- or lidocaine-induced anesthesia was increased from between from 0% and 20% to between 45% and 69% over the 40 days of observation compared with the other two groups (p < 0.007). Ketamine anesthesia provided no protection. There were no notable differential effects upon non-neural tissues, suggesting that pentobarbital afforded protection through modulation of ambient neural activity during radiation exposure.

Neural suppression during high-dose cranial irradiation protects brain from acute and early delayed radiation injury. Further development and application of this knowledge may reduce the incidence of radiation toxicity of the central nervous system (CNS) and may permit the safe use of otherwise "unsafe" doses of radiation in patients with CNS neoplasms.

Key Words: brain neoplasm • radiation injury • lidocaine • pentobarbital • barbiturate • rat

Although ionizing radiation therapy has the potential to eradicate most tumors, the sensitivity of surrounding normal tissues limits the tolerable dose.33,34 Therapeutic irradiation of tumors of the central nervous system (CNS) and tumors adjacent to the neural axis, such as head and neck tumors and paraspinal tumors (which require inclusion of the neural axis in the radiation field), often injures the brain and spinal cord.5,9-12,16,17,25,28,44,54 These reactions to irradiation occur either acutely (during radiation treatment), early but delayed (delayed by days or weeks after completion of radiation), or late (several months to years after treatment).5,9-12,16,17,25,28,44,54 Structural and functional evidence of radiation-induced brain and spinal cord trauma includes focal injury (demyelination, cerebral calcification, and cerebral necrosis) and widespread injury (cerebral atrophy, growth retardation and other hypothalamic-pituitary insufficiencies, vasculopathy with occlusion of large and small vessels, myelopathy, and intellectual disturbance).5,16,44 Diffuse injury is particularly prominent in children.9-11,28 Generally, the acute and early delayed types of injury limit the amount of radiation that can be safely delivered in a single fractional dose, whereas the late reactions limit the tolerable cumulative irradiation dose resulting from a multifraction course of treatment.5,16,44

Barbiturates are a commonly used class of general anesthetics in which the CNS depressant effects are derived from facilitation of a common inhibitory synaptic transmitter action involving gamma-aminobutyric acid (GABA) receptor-coupled conductance of chloride ions.3,30,42,43 Lidocaine, by blocking voltage-gated sodium channels,36 depresses activity of excitable tissues. Neural tissue is exquisitely sensitive to metabolic suppression by these substances. Thiopental and pentobarbital selectively suppress neuronal metabolism while having little influence on metabolism of normal glia or brain tumors.4,14 We sought to exploit the differential suppression of brain versus tumor for therapeutic advantage. The effects of ionizing radiation on tissue is by production of free radicals.15 Because modification of the initial chemical reactions of the ionized molecular targets of x-rays may render tissues less radiosensitive,15 we postulated that neural activity during radi-
The radiation exposure may influence tissue damage, as it does in ischemic neural injury. In ischemic injury, synaptic activity mediates death of hypoxic neurons in vitro and synaptic blockade with hypothermia, barbiturates, or lidocaine, or by antagonists of excitatory amino acids protects the brain and spinal cord against focal ischemic injury in vivo. We examined the influence of pentobarbital on the radiosensitivity of rat brains with the hypothesis that, if radiation sensitivity of brain is a function of ambient electrical activity during radiation exposure (as in neural injury by ischemia), selective suppression by barbiturates may protect brain but not tumor from radiation damage.

To determine if barbiturates would protect brain at higher radiation exposures, we compared survival rates in rats that received irradiation during pentobarbital sedation with those of animals that received no barbiturates.

Materials and Methods

Experimental Groups

Initially, the potential protective effect of barbiturates during whole-brain irradiation of Wistar rats, each weighing 150 to 200 gm, was examined after a single exposure of brain to irradiation. Control animals received no medication and were irradiated while awake. The treated animals received pentobarbital, 30 mg/kg intraperitoneally, and ketamine HCl, 60 mg/kg intramuscularly, 15 to 30 minutes before 5000, 7500, or 10,000 rad of x-irradiation was administered in a single dose (10 animals in each group). The animals were examined daily for 40 days and survival time was recorded. To confirm the observations in the pilot experiment, this experiment was repeated using 7500 rad only on 50 animals in a control group (no medication) and a treated group (pentobarbital, 30 mg/kg intraperitoneally, and ketamine HCl, 60 mg/kg intramuscularly).

To investigate the mechanism of the protection against CNS toxicity which was apparent from the previous experiments, and to observe the animals longer for evidence of delayed toxicity, additional animals were irradiated with 7500 rad. Treated animals received one of the following: pentobarbital, 15 mg/kg intraperitoneally (30 rats); lidocaine, 60 mg/kg intraperitoneally (23 rats); lidocaine, 8 mg/kg (30 rats); or intramuscular ketamine HCl, 60 mg/kg intramuscularly (30 rats). Control animals received no medication. The animals were observed daily for 160 days and survival time was recorded.

Irradiation

Irradiation was administered by lateral exposure of the brain to x-irradiation at 600 rad/min for sedated animals and 800 rad/min for control animals from a linear accelerator (15 mV) at a source-to-head distance of 77 cm for pentobarbital-treated rats and 87 cm for
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control and lidocaine-treated animals (the differences in distance and rate of irradiation were to accommodate differences in the animal-holding apparatus in the treated and unanesthetized groups). In sedated animals, irradiation began 15 to 30 minutes after drug administration. Unanesthetized control animals were restrained with plaster body casts to keep their heads aligned in the x-ray beam during exposure to irradiation. A multicompartemented pie-shaped Lucite device was used to position the anesthetized animals (Fig. 1). The respiratory and upper digestive tract of all animals was protected by lead shielding. All animals were observed via closed-circuit television during irradiation and those that were restless or moving during irradiation were excluded from the study. All animals were treated in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals, Revised 1985.”

Physiological Monitoring

To examine the possibility that anesthetized animals were rendered hypothermic or hypoxic during irradiation, the core temperature was monitored with a rectal probe before drug injection, for 15 minutes after drug injection, and during irradiation in control animals (20 rats) and in animals that received pentobarbital, 15 mg/kg intraperitoneally (15 rats), or ketamine HCl, 60 mg/kg intramuscularly (15 rats).

In a separate experiment, arterial oxygen pressure was measured in the femoral artery of 15 rats before and 10 to 15 and 15 to 30 minutes after administration of pentobarbital, 15 mg/kg intraperitoneally.

Pathological Examination

Two groups of rats (10 in each group) were subjected to 7500 rad of x-irradiation to the whole brain only. One group was irradiated after receiving pentobarbital, 30 mg/kg intraperitoneally, and the other group received irradiation without sedation. The animals were then monitored, perfused, and autopsied within 12 hours of death, or sacrificed, perfused, and autopsied when they had lost 25% or more of their initial body weight. At the end of 30 days all surviving animals were sacrificed, perfused, and autopsied. Autopsies included gross inspection of the brain, the oral mucosa, and the major thoracic and abdominal organs. Histological evaluation of the brain included hematoxylin and eosin, luxol fast blue, cresyl violet, Bielschowsky, and periodic acid-Schiff stains. The oral mucosa, tongue pharynx, esophagus, lungs, heart, stomach, and liver were stained with hematoxylin and eosin.

Statistical Analysis

Comparisons of survival of rats were by the two-tailed Mantel-Haenszel test.23,24

Results

Survival in Experimental Groups

In the pilot study, animals that received 5000 rad had little radiation toxicity and animals that received 10,000 rad had limited survival times, regardless of pentobarbital and ketamine administration. However, at 7500 rad of whole-brain irradiation there was a prominent difference in survival rates over the 40 days of observation between the pentobarbital-treated and control groups (Fig. 2). To confirm this observation we repeated the experiment at 7500 rad with larger numbers of animals (Fig. 3). Of the animals treated with pentobarbital, 45% to 69% were alive at 40 days in both experiments compared with 0% to 20% of the control animals.

Anesthetic doses of pentobarbital (15 mg/kg intraperitoneally) or lidocaine (60 mg/kg intraperitoneally)
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FIG. 3. To confirm the observations illustrated in Fig. 2, the study was repeated with irradiation at only 7500 rad (50 animals in each group). Animals received pentobarbital, 15 to 30 mg/kg intraperitoneally, and ketamine HCl, 60 mg/kg intramuscularly (solid line). Sixty-nine percent of the pentobarbital-treated group versus 20% of the control group (broken line) survived at 40 days (p < 0.007). 23

provided similar protection against early radiation toxicity compared to the control group (p < 0.002 and p < 0.03, respectively). Ketamine anesthesia alone (60 mg/kg intramuscularly) did not influence survival rates (Fig. 4). The survival rate of animals that received sedative doses of lidocaine (8 mg/kg intraperitoneally) was intermediate between that of rats receiving anesthesia-inducing doses of lidocaine and control rats, although the differences were not statistically significant (p < 0.1 and p < 0.4, respectively).

Before death, animals had a decreased level of activity, but no specific neurological deficits such as ataxia, paresis, or seizures were noted. There were no notable differential effects upon the exposed non-neural tissues (the scalp, mouth, or other superficial tissues of the head).

Physiological Monitoring

There were no physiologically significant differences in body temperatures during irradiation of control animals (20 rats) and the animals treated with pentobarbital (15 rats) or ketamine (15 rats). The lowest temperature was 38.5°C ± 0.5°C, 37.5°C ± 0.5°C, and 36.5°C ± 0.5°C (mean ± standard deviation) in the control, pentobarbital, and ketamine groups, respectively. The mean nadir of the arterial oxygen pressure of animals treated with pentobarbital, 15 mg/kg intraperitoneally (15 rats), was 94.9 ± 4.9 mm Hg after receiving pentobarbital compared to 99.1 ± 3.2 mm Hg in the control period before anesthesia.

Pathological Findings

Gross external examination of animals in the experimental and control groups demonstrated that when 25% of the initial weight had been lost the fur was poorly groomed. Early conjunctivitis was noted in both groups. Gross internal examination revealed small mucosal hemorrhages of the gastric mucosa in both groups of animals.

Histological examination of the brains revealed no differences between awake and pentobarbital-treated animals. Histological examination of the remainder of the organs showed focal hepatic necrosis in one animal that was irradiated while awake. No gross or microscopic lesions were detected in the oral mucosa, tongue, pharyngeal area, or esophagus.

Discussion

Clinical Results of Radiation Therapy

Radiation therapy remains the most effective therapy for malignant primary and metastatic tumors of the brain and spinal cord. Most tumors, including the most common primary tumor of the CNS, glioblastoma multiforme, have a steep radiation dose-tumor response relationship in vitro and in vivo, even at high doses of radiation. 23,24,37,39,40,51,52 If more irradiation is administered, a greater antitumor effect is expected.

Radiation therapy is particularly effective as prophylactic treatment against secondary involvement of the brain and spinal cord in acute lymphocytic leukemia and medulloblastoma, 21 and is frequently curative against certain childhood brain tumors, such as medulloblastomas and pineal dysgerminomas. However, children are quite sensitive to the toxic effects of brain radiation. Effective doses of treatment frequently pro-
duce an unacceptable reduction in intelligence quotient, impaired coordination, dysfunction of the hypotalam-pituitary axis, and other functional disturbances which indicate brain injury. 6-11,20,28

Patients with malignant brain tumors often have limited life expectancy with medial survival times of only a few months with the best available therapy. However, when large cumulative doses are delivered, radiation treatments are limited to about 180 to 200 rad per day because acute and delayed toxicity restrict the radiation dose per treatment that can be safely administered. 3,12,16,17,25,44,52 As more rapid treatment, if tolerated, would significantly reduce the fraction of remaining meaningful survival time that is now consumed by 6 weeks of daily radiation treatments, elimination of early radiation toxicity alone after hypofractioned therapy would be beneficial.

**Barbiturate Protection: Radiation Biology**

Certain aspects of radiation biology indicate that barbiturates may protect the CNS against injury by x-irradiation. Therapeutic radiation is ionizing and the energy transfer by irradiation to tissue by the ionization process is immediate. The basic mechanism by which ionizing radiation produces its biological effects on cells is not clear. The critical targets are located in the nucleus of the cell and the most important target in the nucleus is deoxyribonucleic acid (DNA). In the nucleus, however, in addition to the DNA present in chromosomes, are basic proteins, histones, which comprise potential metabolic targets (other than DNA) and which, if damaged, may contribute to the biological response to x-irradiation.13 By means of low energy electron beams of limited but well defined penetration, an area of particularly high sensitivity to irradiation has been identified immediately within the nuclear membrane.15 As there is now clear evidence of attachment of chromatin to regions of the nuclear membrane, it has been proposed that sites at which DNA and membrane are complexed are peculiarly vulnerable to irradiation. It has previously been postulated that enhanced or diminished radiosensitivity may result from modification of the initial chemical reactions of ionized cellular targets.15 More recently, Cullis, et al, 7 demonstrated that when DNA is complexed to proteins, as it is in the nuclei of eukaryotes, electron transfer from the histone to DNA following irradiation leads to a significant increase in electron-gain centers on DNA. These electron centers on DNA are precursors of DNA damage.7

Since the effect of ionizing radiation on tissue is by production of free radicals12-13,34 and, as modification of the initial chemical reactions of ionized molecular targets of x-rays may render tissues less radiosensitive, we postulated that the level of ambient neural activity during radiation exposure may influence tissue damage. This action is seen in ischemic neural injury, where synaptic activity mediates death of hypoxic neurons in vitro and where synaptic blockade with hypothermia, barbiturates, lidocaine, or by antagonists of excitatory amino acids protects the brain and spinal cord against focal ischemic injury in vivo. Free radical damage to brain during radiation may depend on ambient electrical activity during the radiation exposure.

In 1957, Alvord and Brace1 reported that the acute neurological signs which arose within hours of whole-body (7500 rad) and cranial (7500-rad) x-irradiation of guinea pigs were suppressed if barbiturates were administered before irradiation. The pyknotic alterations of the cerebellar granule cells that occurred acutely after irradiation of the hindbrain only or the whole body were almost completely abolished by pretreatment with anesthetic doses of intraperitoneal pentobarbital (35 to 50 mg/kg).1 Since these alterations of cerebellar histology did not occur after similar doses of irradiation with the head shielded and since there were no pathological abnormalities in the vessels of the cerebellum, they concluded that the neurological dysfunction was related to acute brain injury and that the neural injury was diminished by pretreatment with pentobarbital. However, survival of the animals was limited and the pharmacological basis of the protective effect of barbiturates was not examined.

To determine if barbiturates would protect brain at higher doses of radiation, we compared survival rates in rats that received x-irradiation during pentobarbital sedation with those of control animals that received no barbiturates. The animals were shielded so that respiratory and digestive tissues would not be damaged by the radiation. Pentobarbital anesthesia enhanced survival in both experiments. There were no notable differential effects upon the exposed non-neural tissues (the scalp and other superficial tissues of the head) and histological evaluation of the mucous membranes of the oral cavity revealed no differences in the animals that received anesthesia with pentobarbital, lidocaine, or ketamine, or the control animals. This suggests a selective effect of protection on the CNS and that pentobarbital afforded protection through modulation of neural activity.

To eliminate the possibility that anesthetized animals were rendered hypothermic or hypoxic during anesthesia to a degree that might affect radiation sensitivity, rectal temperatures were monitored in 50 animals with a rectal probe before drug injections, for 15 minutes after drug administration, and during irradiation. There were no physiologically significant differences in the body temperatures during irradiation of control animals and of animals treated with pentobarbital or ketamine. The temperature of animals treated with ketamine, which failed to influence survival, was slightly less than that of the other two groups. The mean nadir of arterial oxygen pressure of animals treated with pentobarbital was 5 mm Hg less than that of control animals, a difference known not to affect the oxygen saturation of the blood.

It remains unknown whether radiation toxicities of the brain and spinal cord result primarily from neural or vascular injury, 5,12,16,17,25,44,54 and whether acute,
early delayed, and late toxicities are caused by injury to the same tissue elements and by the same cellular mechanisms. Barbiturates not only inhibit neural excitation, they bind GABA receptors on cerebral vessels and, by inhibiting the intracellular flux of ionized calcium, they block potassium- and serotonin-induced contraction of cerebral and peripheral arteries. Therefore, the protective effect against radiation toxicity reported here may derive from effects on the cerebral vasculature as well as on neurons. There was no evidence of delayed death in the control or the treated animals, which were observed for as long as 160 days, but delayed radiation toxicity of brain becomes evident in rats that receive high doses of radiation 6 to 18 months following exposure. Our studies did not determine whether protection of the brain by pentobarbital and lidocaine from the early expressions of radiation toxicity indicates similar protection against late toxicity.

In addition to suppressing activity of neural tissue by enhancing binding of GABA receptors, barbiturates scavenge free radicals. Lidocaine blocks neuronal voltage-gated sodium channels and protects against cerebral and spinal cord ischemia, but is not known to quench free radicals. To evaluate whether the mechanism of barbiturate protection against radiation damage is linked to GABA receptor binding or suppression of neural activity, or acts by free radical quenching, we compared survival rates in animals irradiated while under lidocaine anesthesia with pentobarbital-treated and control animals. Survival rates in animals anesthetized with lidocaine during irradiation were similar to those of animals treated under pentobarbital anesthesia (Fig. 4). Anesthesia per se did not influence radiation sensitivity, since survival rates of animals irradiated while under ketamine-induced anesthesia were similar to those in rats irradiated without anesthesia (Fig. 4).

**Potential Clinical Applicability**

Even if this information is to be useful for therapy of patients, it is unlikely that barbiturate coma can be used repeatedly for fractional radiation therapy over several weeks as is now employed. However, if protection is a function of neural suppression, anesthesia may not be required for the effect sought, since much of the suppression in brain metabolism can be achieved by doses of barbiturates that do not require anesthesia. Crane, et al., in their study of the relationship of pentobarbital dosage and suppression of brain glucose metabolism in rats, demonstrated that, although with increasing doses of pentobarbital the concentration of pentobarbital in the brain increases in a linear manner, most of the suppression of brain glucose metabolism occurs at preanesthetic doses (and brain concentrations) of pentobarbital. Furthermore, in humans anticonvulsant doses of phenobarbital (doses that do not alter the level of consciousness) suppress the cerebral glucose utilization rate by an average of 37%.

Potential clinical application of this protective effect requires that these agents must not also protect tumor from radiation. We have demonstrated in patients with malignant and benign brain tumors, using F-labeled 2-deoxy-D-glucose and positron emission tomography, that during thiopental-induced coma and during pentobarbital-induced sedation (in preparation) suppression of brain metabolism occurs without significantly affecting tumor metabolism. If radiation protection by barbiturates is mediated by the same mechanism that produces cerebral metabolic depression (as is protection against cerebral and spinal ischemia by barbiturates and lidocaine), then selective depression of brain metabolism by barbiturates indicates that the protection against radiation-induced brain damage by barbiturates should not also protect tumor. Previous experiments examining the sensitivity of tumor in vivo indicate that neither pentobarbital nor hypothermia significantly reduce tumor sensitivity to irradiation (see also Olson, et al.).

**Conclusions**

Our findings indicate that pentobarbital treatment during high-dose cranial irradiation protects the brain against acute and early delayed irradiation toxicity during exposure to large single fractions of irradiation. This protective effect seems to arise from general suppression of brain synaptic activity or metabolism, as ligand-gated inhibition of brain metabolism using pentobarbital and voltage-gated suppression using lidocaine, provide protection; however, anesthesia per se, shown here with ketamine anesthesia, does not provide protection.

We have demonstrated protection against acute and early delayed toxicity by suppression of brain activity during hypofractionated whole-brain irradiation in rats. However, the late delayed reactions to radiation injury that cause severe late radiation syndromes in adults and children are generally irreversible and frequently progressive. It is this late delayed toxicity that limits the cumulative dose and therefore tumor control. Whether the biological mechanisms that result in acute, early delayed, and late delayed irradiation toxicities are the same is not known. Furthermore, whether the late toxicity will be reduced in a similar manner by barbiturates remains to be determined.

**References**

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Manuscript received October 10, 1988.
Accepted in final form December 8, 1989.
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