Progressive perturbations in cerebral energy metabolism after experimental whole-brain radiation in the therapeutic range

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Basic mechanisms underlying the tolerance and reaction of the central nervous system to ionizing radiation have not been fully elucidated in the literature. The authors employed the $^{[14]C}$-2-deoxy-D-glucose autoradiography method to investigate the effect of whole-brain x-irradiation on local cerebral glucose utilization in the rat brain. The animals were exposed to conventional fractionation (200 ± 4 cGy/day, 5 days/week for a total dose of 4000 cGy), and the effects of this regimen were assessed at 2 weeks and 3 months postirradiation. In rats evaluated 2 weeks after treatment, statistically significant decreases in cerebral metabolic activity were found in 13 of the 27 regions studied, compared to control animals. In rats studied 3 months after treatment, additional metabolic suppression and statistically significant decreases in cerebral metabolic activity were found in 11 of the 27 regions, compared to rats studied 2 weeks after treatment. A weighted-average rate for the brain as a whole was approximately 15% and approximately 25% below that of control animals 2 weeks and 3 months after exposure, respectively.

Although the difference in species is significant enough so that direct extrapolation to humans may not be appropriate, the data reported here may have potential clinical implications for the evaluation of the risk-benefit ratio for radiotherapy. This model can be used reproducibly for further investigations, including evaluation of therapies that may reduce irradiation-induced brain injury.

**KEY WORDS** • brain tumor • brain metabolism • quantitative autoradiography • radiation therapy

Radiation therapy plays a valuable primary or adjuvant role in all effective treatment modalities used in the management of central nervous system (CNS) tumors. Clinical regimens employing fractionated doses of whole-brain irradiation are recommended for patients with high-grade glioma and CNS lymphoma, and for those undergoing craniospinal irradiation for medulloblastoma or CNS prophylaxis for small cancers of the lung and other malignancies. There are definite clinical benefits conferred by whole-brain radiation, but its therapeutic efficacy is limited by many factors, among which the tolerance limits of the brain play a crucial role.

The same ionizing mechanism that injures the tumor also affects normal brain tissue producing both acute and chronic neurotoxic effects that, according to time of appearance, have been categorized as acute, early-delayed, and late-delayed reactions. These adverse effects range from transient functional changes to overt encephalopathy with widespread morphological changes and, occasionally, parenchymal necrosis. All these effects have well-known clinical importance, particularly in young patients in whom long-term intellectual and neuropsychological impairment with cognitive deficits and disruption of learning ability have been reported after therapeutic irradiation.

The pathophysiological mechanisms underlying the adverse effects of whole-brain irradiation have not been fully elucidated in the literature. Brain dysfunction has been ascribed either to alterations in the cerebral microvasculature, leading to brain edema and/or ischemia, or to demyelination. Recent experimental and clinical evidence suggests a possible association between metabolic alterations and whole-brain radiation-induced cerebral dysfunction.
Brain energy metabolism after whole-brain radiation

The authors have already reported on the feasibility of using a rodent model of fractionated whole-brain radiation to study irradiation-induced changes in brain energy metabolism during a period corresponding to the early-delayed phase after irradiation. Now we further examine the potentially adverse effect of whole-brain radiation in the rat model and the magnitude of changes in cerebral metabolism occurring during a period corresponding to the late-delayed phase after irradiation, that is, 3 months after the completion of the course of radiotherapy. To this purpose, we employed autoradiography with [14C]-2-deoxy-D-glucose ([14C]-2-DG), which permits measurement of local cerebral glucose utilization.47 This article describes the temporal progression of whole-brain radiation-induced changes in local cerebral glucose utilization after fractionated whole-brain irradiation of the rat brain, which are detectable as early as 2 weeks and increase in magnitude for up to 3 months postirradiation.

Materials and Methods

Experimental Design

Twenty-four male Sprague-Dawley albino rats, weighing between 250 gm and 300 gm, were used in the studies. Sixteen animals were irradiated (Groups II and III) and eight normal animals (Group I) served as controls for comparison with the other experimental groups.

To simulate the fractionated doses of clinical radiation therapy, the rats were exposed to conventional fractionation (200 ± 4 cGy/day; 5 days/week for a total dose 4000 cGy). Irradiation was performed using a linear accelerator46 delivering 6 MeV photons. Each animal was anesthetized with 5% enflurane and restrained in the prone position. Irradiation was administered by single-field dorsal-ventral exposure of the whole brain. The head was placed in a backscatter medium in the radiation field, and a tissue bolus (1.2 cm) was employed. A target-to-bolus distance of 100 cm was used with a radiation field measuring 8 cm × 6 cm. Customized lead shielding protected the body of the rat, limiting the irradiation to the head. The dose was calculated at the dose maximum on the central axis, measured at a depth of 1.5 cm. The dose rate within the radiation field, measured at a depth of 1.5 cm from the linear accelerator was 450 cGy/min. The distribution of the absorbed dose in the rat's brain ranged from 98.379 to 100% with the dorsal-ventral thickness of its brain approximated to 1 cm. The development of gross neurological symptoms, such as ataxia, paresis, or seizures, as well as changes in drinking and feeding behavior and body weight were evaluated sequentially until sacrifice.

Autoradiographic Studies

The rats were conditioned to restraint in loose-fitting plaster casts. Environmental conditions were standardized. Local cerebral glucose utilization values were determined according to the method originally described by Sokoloff, et al.,47 and to standard techniques originated in our laboratory.48 Metabolic experiments were performed 2 weeks postirradiation (eight Group II rats, late-delayed phase), or 3 months postirradiation (seven Group III rats, late-delayed phase). Group I consisted of 8 nonirradiated rats.

Under light-volatile anesthetics, catheters were inserted into femoral arteries and veins to collect arterial blood samples and to inject radioactive tracers. The incision sites were sutured and infiltrated with local anesthetic. Following an intravenous pulse of 100 μCi/kg of [14C]-2-DG, timed arterial plasma samples were withdrawn to assess time courses of [14C]-2-DG concentrations by liquid scintillation counting, and glucose concentrations were assessed by enzymatic assay. Forty-five minutes after the [14C]-2-DG pulse, the rats were sacrificed with an overdose of pentobarbital, and their brains were quickly removed and immediately frozen in isopentane (−50°C).

Brain frontal plane serial sections 20 μ thick were obtained using a cryostat microtome at −20°C. The sections were mounted on glass coverslips and dried on a hotplate at 50°C. The serial sections were exposed to x-ray film in x-ray cassettes for 12 days to produce autoradiographs and the films were then developed by standard techniques. Local tissue concentrations of carbon-14 were determined by quantitative densitometric analysis using calibrated [14C]methylmethacrylate standards as reference controls, exposed together with the film and brain sections. Optical densities of single brain areas were read by a microdensitometer coupled to a computerized image-processing system. The carbon-14 content was measured in nanocuries per gram tissue. Measurements were obtained from loci in the cerebral cortices, subcortical gray matter, hypothalamus, thalamus, limbic structures, brain stem, cerebellum, and corpus callosum; these loci were standardized by referring to a stereotactic atlas of the rat brain.42

To facilitate visual inspection of the different patterns and relative amounts of glucose utilization, color-coded pictures of the autoradiographs were prepared according to the method described by Goochie, et al.49 Local cerebral glucose utilization was calculated from the operational equation developed by Sokoloff, et al.,47 which expresses the rate of glucose utilization in terms of measurable variables and defined constants. The former include the final tissue concentration of carbon-14 and the time courses of the arterial plasma glucose and carbon-14 concentrations. We used the same rate and lumped constants that had previously been derived for the brain of the anesthetized rat.47 Statistical evaluations were performed using analysis of variance to determine the difference between the values of glucose utilization for each region. When a significant variance was discovered, a two-tailed t-test was utilized to perform pairwise comparisons for each brain region. A p value of greater than 0.05 was considered to indicate significant statistical difference.

Results

Effects of Irradiation

The whole-brain irradiation course was tolerated well by the animals. No specific neurological deficits such as ataxia, paresis, or seizures were observed in the subacute or chronic phase of the experiments. Although not specifically investigated, obvious behavioral effects were not observed; motor activity within cages and exploratory behavior on the bench top were judged to be within normal limits. Fifteen irradiated animals (94%) survived the duration of their postirradiation period. One death was recorded in the late postirradiation phase. Weight loss was the most prominent feature of the irradiated rats (Table 1). At 2 weeks postirradiation, the irradiated animals displayed a 15% loss in body weight compared to the controls. At that time there was no hair loss or other notable differential effects on the exposed nonneural tissues. Three months after the completion of the irradiation course, the irradiated animals were obviously

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*Linear accelerator, Model #SL 75/S, obtained from Philips Medical System, Best, The Netherlands.
smaller and had a body weight 30% lower than the control animals. Some of them, including the rat that died before the sacrifice, appeared wasted. No significant differences were found in arterial pH, mean arterial pressure, blood gases, blood glucose, or hematocrit among the control and irradiated animals during the course of the metabolic experiments (Table 2).

Observations relevant to this model have been discussed in more detail in other publications.8,9

Measurement of Glucose Utilization

Rates of glucose utilization in 24 gray- and three white-matter structures were studied in each of the 23 rats. No right-to-left difference was found in any bilaterally represented region of the same brain. The mean metabolic activity and standard deviation for individual brain regions are presented in Table 3.

In control rats the distribution pattern of glucose metabolism exhibits the typical regional heterogeneity and is highly correlated to reference values.47 In rats studied 2 weeks after the completion of the radiation course (Group II), detectable decreases in glucose utilization were identified in most brain areas, and statistically significant decreases in cerebral metabolic activity, compared to control animals, were found in 13 of the 27 regions studied. When the weighted averages for rates of glucose utilization for the brain as a whole were compared, the mean values for Group II animals were approximately 15% lower than those for the control animals. In rats studied 3 months after the completion of the radiation exposure (Group III), there was an additional appreciable decrease in local cerebral glucose utilization. The magnitude of these metabolic changes was more pronounced and diffuse than that observed in Group II rats, and further statistically significant decreases in cerebral metabolic activity, in comparison to Group II animals, were found in 11 of the 27 regions studied. In general, brain areas associated with the highest baseline metabolic activity, such as the cerebral cortices and subcortical gray-matter structures involved with sensory or motor function, displayed the most impressive and consistent reductions of glucose utilization. Conversely, regions of the brain exhibiting lower basal rates of glucose utilization, such as cerebellar cortices, hypothalamic and brain-stem nuclei, or white-matter structures, showed minor variations in metabolic activity that were not statistically significant. When the weighted averages for rates of glucose utilization for the brain as a whole were compared, the mean values for Group III animals were approximately 25% lower than those for the control animals. The mean metabolic rate of glucose for cortical gray matter averaged 110 ± 10 μM, 94 ± 10 μM, and 80 ± 11 μM of glucose per 100 gm/min for control, 2 weeks postirradiation, and 3 months postirradiation animals, respectively.

Discussion

Cerebral metabolic activity can now be mapped by powerful topographical techniques that employ quantitative autoradiography to investigate in vivo energy metabolism on a regional basis within the brain tissue, both in physiological and pathological conditions. The [14C]-2-DG method developed by Sokoloff, et al.,47 is a widely accepted technique for estimating rates of cerebral metabolism, since [14C]-2-DG uptake and localization are closely linked to neuronal activity in the CNS. In our study, changes were measured in glucose utilization in specific loci of rat brains subjected to whole-brain radiation within a 90-day observation period.

The major observations derived from this study are as follows: 1) Cranial irradiation (4000 cGy) given in conventional fractionation caused both subacute and chronic significant alterations in local cerebral glucose utilization in the rat’s brain; and 2) the incidence of these metabolic perturbations was clearly time dependent, being detectable at 2 weeks postradiation, and significantly increasing in magnitude 3 months postirradiation.

Literature Review

Only a few researchers have examined the brain’s metabolic responses to radiation injury. As emphasized by Kondziolka, et al.,23 the study of regional cerebral metabolism after brain irradiation is important because metabolic changes can precede changes in cellular or tissue structure. Ito, et al.,20 who also employed the [14C]-2-DG technique, first described widespread reduction in the rate of glucose utilization...
Brain energy metabolism after whole-brain radiation

after single low-dose whole-brain radiation in the rat. Significantly lower rates of glucose utilization were found in 16 structures 4 days after irradiation and in 25 structures 4 weeks after radiation exposure. Lo and coworkers reported on rabbits irradiated with a high-dose, single-fraction charged-particle helium beam. The animals were examined in vivo by means of sequential magnetic resonance imaging and positron emission tomography (PET) evaluations of the cerebrovascular and metabolic perturbations induced by focused irradiation of a hemibrain, beginning 8 months after irradiation. The PET studies demonstrated widespread decrease in glucose uptake, indicating metabolic depression of the irradiated cortex and thalamic nuclei.

Monitoring transient effects of x-radiation on the human CNS using serial measurements of cerebral blood flow (CBF), Hylton, et al., reported a generalized metabolic derangement induced by whole-brain radiation. Positron emission tomography evidence of an early effect of radiotherapy on brain metabolic activity was identified by Mineura, et al. In other PET studies by the same group using the multiple tracers oxygen-15 and 18F-fluorodeoxyglucose (FDG), CBF and oxygen metabolism were suppressed, as was glucose metabolism at the late stage after irradiation. DiChiro, et al., reported that radiation injury was associated with depressed glucose utilization. Mineura, et al., reported a patient in whom PET scanning with FDG demonstrated that the metabolic rate of glucose was depressed markedly in several brain regions, indicative of a metabolic depression induced by radiation.

Experimental Design Considerations

The following features of the present experimental design should be emphasized. 1) The tissue response to whole-brain radiation was studied independently of other cerebral pathological processes, that is, in the absence of factors such as brain tumors, increased intracranial pressure, or the effects of antineoplastic drug administration. 2) Brain irradiation delivering a total dose of 4000 cGy in 20 fractions to the whole brain was performed according to conventional fractionation regimens used in clinical radiotherapy. 3) The rat model offered advantages including negligible treatment-related mortality, applicability for behavioral and pharmacological assessments, low cost, and a brain size sufficient to delineate functional anatomical organizations and to identify sites of selective response to the irradiation insult.

In this study, we utilized the lowest clinically effective dose, believed to produce no gross structural damage to the brain. Metabolic experiments were performed 2 weeks and 3 months after irradiation to reproduce the time courses of early- and late-delayed irradiation toxicity in humans, which are delayed by weeks or several months to years after completion of the therapy. This 90-day observation period was sufficient to permit investigation of the temporal progression of the pathophysiological changes.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Group I</th>
<th>Group II</th>
<th>P</th>
<th>Group III</th>
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<tr>
<td></td>
<td>124 ± 6</td>
<td>104 ± 6</td>
<td>S</td>
<td>82 ± 12</td>
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<td>90 ± 7</td>
<td>S</td>
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<td>120 ± 6</td>
<td>90 ± 6</td>
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<td>cingulate</td>
<td>103 ± 7</td>
<td>92 ± 7</td>
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<td>S</td>
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<td>53 ± 8</td>
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<td>70 ± 8</td>
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<td>56 ± 7</td>
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<td>inferior colliculi</td>
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<td>132 ± 13</td>
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<td>123 ± 12</td>
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<td>superior colliculi</td>
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<td>87 ± 5</td>
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<td>59 ± 5</td>
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<td>51 ± 6</td>
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</table>

* Cerebral metabolic activity is expressed in µM glucose/100 gm brain/min. All values are means ± standard deviation. P = probability; S = significant (p < 0.05); NS = not significant. Data were subjected to analysis of variance and Bonferroni-adjusted t-test.

Metabolic Mechanisms

The pathogenesis underlying metabolic perturbations by ionizing radiation remains speculative. The widespread distribution of regions in which the metabolic rate was reduced suggests that metabolic inhibition does not represent a specific event related to focal vascular or histological alterations. We previously reported on extensive electron microscopic investigations performed in parallel series of experiments in which we failed to identify any evidence of focal vascular or tissue damage in this rodent model at the subacute stage after irradiation. Although we did not assess ultrastructural changes at the chronic stage in the present work, their study will be incorporated into a future report.

The time course for postirradiation metabolic perturbations and their degree of recovery are unknown. However, the temporal progression of the metabolic
changes observed in the present experiments is consistent with clinical observations by Mineura, et al., who reported that in patients with glioma, oxygen metabolism as well as glucose metabolism were significantly reduced in the seemingly normal brain tissue both at the early and late stages after irradiation, in comparison with healthy volunteers. The suppressed oxygen and glucose metabolism of the irradiated brain was not relieved in the short term or even in the long term after radiotherapy.

A generalized impairment of the blood-brain barrier (BBB) related to damage of endothelial cells is a characteristic general response of brain microvessels to radiation injury. It has been speculated that, in disturbances of the BBB, an increase in vascular permeability, which produces perivascular edema and vascular collapse, may interfere with the microcirculation affecting the CBF and energy supply to the tissue. Consistent with this hypothesis is previous work from the authors’ laboratory demonstrating the occurrence of well-defined changes in the BBB in the same experimental model.

The irradiated rats weighed significantly less than controls at the 2- to 3-week and 3-month postirradiation intervals. It is unlikely, however, that undernutrition caused the observed metabolic changes, because the CNS is remarkably tolerant of the effects of starvation.

Finally, it must be taken into account that some tracer dilutional effect caused by edema may have also contributed to the observed decrease in [14C]-2-DG uptake.

Conclusions

The usefulness of PET scanning in neuro-oncology as a tool to evaluate quantitative information on subtle impairment of cerebral function has been emphasized by several investigators. In this respect, these studies may have implications for the observations made with PET scans in patients receiving x-irradiation for intracranial malignancies. Although there is a significant difference in species, and direct extrapolation to humans may not be appropriate, the association between progressive metabolic perturbations and whole-brain radiation observed in this study may also have potential clinical implications for the evaluation of the risk-benefit ratio for radiotherapy in patients suffering from neurosurgical disease or undergoing prophylactic treatment of CNS.

Finally, this model can be used reproducibly for further investigations, including evaluation of potential protective therapies that may reduce irradiation-induced brain injury.

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D. d’Avella, et al.

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