Fractionated Radiation-Induced Acute Encephalopathy in a Young Rat Model: Cognitive Dysfunction and Histologic Findings


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BACKGROUND AND PURPOSE: Radiation-induced cognitive dysfunction is a common and serious complication after radiation therapy of brain tumor, yet knowledge of its mechanism is poorly understood. The aim of this study was to establish a young rat model for acute radiation encephalopathy, at both cognitive and pathologic levels, induced by fractionated irradiation.

MATERIALS AND METHODS: Four-week-old male rats were randomized into sham (0 Gy) and 2 experimental groups receiving fractionated irradiation of 5 Gy/day, 5 days/week, with total doses of 20 and 40 Gy, respectively. Cognition, BBB integrity, and potential astrogliosis were evaluated at 0, 4, 8, and 12 weeks’ postirradiation.

RESULTS: Twenty-Gy irradiation led to transient cognitive impairment only at 4 weeks’ postirradiation. Forty-Gy irradiation induced cognitive impairment at both 4 and 8 weeks’ postirradiation, which was more severe than that induced by 20 Gy. Cognitive impairment was accompanied by a transient increase in BWC only at 4 weeks for the 40-Gy group. Disrupted BBB permeability was detected at 4 and 8 weeks’ postirradiation for the 20-Gy group, and at 4, 8, and 12 weeks’ postirradiation for 40-Gy group, respectively. Increased astrogliosis in the hippocampus could be detected at 4 weeks’ postradiation for 40-Gy group.

CONCLUSIONS: Fractionated irradiation in this experiment could induce acute brain injury, leading to cognitive impairment in young rats. BBB disruption might be a sensitive index for acute radiation encephalopathy. In addition, reactive astrogliosis might play an important role in this process. The present model, especially the 40-Gy irradiation group, is useful for basic and therapeutic studies of acute radiation encephalopathy.

Abbreviations: 
BBB = blood-brain barrier; BWC = brain-water content; EB = Evans blue; GFAP = glial fibrillary acidic protein; IF = immunofluorescent; NPC = nasopharyngeal carcinoma; RE = radiation encephalopathy

Radiation therapy is used widely for treatment of diffuse primary and metastatic brain tumors, and its curative efficacy is well-established. With improvement of patient survival, attention is paid to its adverse effects, the radiation-induced brain injury.

In Guangdong province of southeastern China, NPC constitutes approximately 32% of all cancers. The incidence of overall NPC in Guangdong province is >20 per 100,000 people every year among men and it is the most common kind of cancer in the province. The rate in Cantonese speakers is double that in other dialect groups, such as Hakka, Hokkien, and Chiu Chau, and is, to the best of our knowledge, the highest in the world. For patients with NPC, radiation therapy is the first-choice treatment and sometimes the only effective management of the disease.

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To establish an animal model that closely resembles the most frequently used clinical protocol for cranial irradiation so as to better understand the underlying mechanisms and the clinical manifestation, we exposed young rats to fractionated irradiation treatments by using a linear accelerator and evaluated the changes in cognition, BBB permeability, and histopathology.

Materials and Methods

Animal

A total of 144 male Sprague-Dawley rats, 4 weeks of age and weighing 110 ± 20 g, were used in this study. Rats were housed socially in a temperature-controlled room with free access to food and water. Animal care and the experimental procedures in this study were approved by the Animal Care and Ethics Committee at Sun Yat-sen University, China.

Protocol of Irradiation

Rats were randomized into 3 groups (n = 48) to receive different irradiation treatments by a Linear accelerator (PRIMUS Linear Accelerator; Siemens, Erlangen, Germany) at room temperature. Animals were anesthetized with pentobarbital (30 mg/kg, peritoneal injection) and were restrained in the sternal recumbent position on a treatment table. The head was centered in the exposure field with eyes, mouth, neck, and body protected with customized lead shielding. For each experimental group of the rats, 5-Gy (dose rate, 300-cGy/min) irradiation was given per day, 5 days/week, until the total dose reached 20 Gy. For the sham control group, received 0 Gy of irradiation but underwent the same stress as did the others.

The 48 rats in each treatment group were further randomized into 4 time groups, with 12 rats in each time subgroup, for follow-up of irradiation but underwent the same stress as did the others.

Determination of BWC

Following cognitive tests, 12 rats in each time subgroup were further divided into 3 subgroups for histopathologic examination, BWC determination, and BBB-permeability assessment, respectively.

BBB-Permeability Assessment

Four animals from each subgroup were subjected to BBB-permeability assessment according to previously reported procedures. In brief, 2% EB dye (3 mL/kg) was injected into anesthetized rats through the femoral vein, and the brain was transcardially perfused 1 hour later. After decapsulation, the tissues were obtained and weighed and followed by incubation in formamide solution at 50°C for 72 hours. Optical attenuation of the EB formamide solution was determined by spectrophotometry at 635 nm, and BBB permeability was expressed as micrograms of EB per gram of brain tissue (microgram/gram).

IF Staining of GFAP

The death of 2 rats had reduced the sample number of planned histopathologic examinations for the subgroup of 40 Gy at 2 and 4 weeks' postirradiation, respectively. Animals were anesthetized and perfused; brains were removed and postfixed with 4% paraformaldehyde and processed for histopathology. Tissue sections (6-μm thick) were cut sequentially along the coronal profile. GFAP staining was performed as previously described, with the exception that a different primary antibody (1:100, Sigma-Aldrich, Hong Kong, China) was used in this study.

Quantitative image analysis was performed using Image-Pro Plus software, (http://www.mediacy.com/index.aspx?page=IPP) with the examiner blinded to sample identifiers. To maintain consistency across animals, we centered a rectangular box (0.72 × 0.58 mm) over the area of interest. The final results were shown as immunoreactivity per square millimeter.
All the quantitative data were expressed as mean ± SD and were analyzed with the appropriate analysis of variance, followed by post hoc mean comparisons by using the Statistical Package for the Social Sciences, Version 12.0, software (SPSS, Chicago, Illinois). P < .05 was considered statistically significant.

Results
Five rats in the 40-Gy group and 2 in the 20-Gy group displayed mild local skin reactions, including depilation, hyperemia, and edema 6–15 days after the treatment. However, these conditions were not severe enough to prevent them from undergoing the behavioral tests and subsequent analysis. Two rats in the 40-Gy group had tooth deformities and oral mucosal ulceration and died at 2 and 4 weeks’ postirradiation. All the rest of the rats were healthy. In addition, all rats showed normal daily activities, including feeding and drinking. No paralysis or convulsions were observed. Body weight slightly decreased in the first week after irradiation and returned to normal quickly. However, the changes did not reach statistical significance, and there was no difference between the groups.

Cognitive Deficits
The Morris water maze was used to assess the ability of place navigation and spatial probing. There was no difference in the swimming speeds for both parts. Place navigational function was impaired in both experimental groups. In the 20-Gy group, a transient impairment could be detected by a significantly increased latency (P < .05) and swimming distance (P < .05) compared with the sham controls only at 4 weeks’ postirradiation. The cognitive performance returned to normal at 8 weeks’ postirradiation. Compared with 20-Gy treatment, 40-Gy treatment induced more severe deficits, which became statistically significant (P < .01) by 4 weeks’ postirradiation and lasted at least to 8 weeks (P < .05); then the rats returned normal at 12 weeks’ postirradiation (Fig 1).

Effect of Irradiation on BWC and BBB Permeability
In the 20-Gy group, no significant increase of BWC could be observed during the whole experimental course. In the 40-Gy group, statistical significance (P < .05) was revealed at 4 weeks’ postirradiation. This effect was transient and gradually returned to normal by 8 weeks.

Changes in the BBB permeability caused by the irradiation were more serious than the changes in the BWC. A statistically significant increase of BBB permeability was revealed 4 weeks’ postirradiation (Fig 3) in both the 20- and 40-Gy experimental groups (P < .01 and P < .001, respectively). There was a trend toward gradual recovery. The subjects in the 20-Gy group returned to normal, but subjects remained significantly im-

Fig 1. Effects of brain irradiation on the place navigation function of rats in a water maze. Rats exposed to different doses of fractionated irradiation treatments were subjected to the Morris water maze test. The latency of target finding (A) and swimming distance (B) was plotted and shown as indicated. One asterisk indicates P < .05, double asterisks, P < .01, compared with their corresponding sham.

Fig 2. Effects of brain irradiation on the space probe function of rats in a water maze. Rats exposed to different doses of fractionated irradiation treatments were subjected to the Morris water maze test. The journey distance of target to total (A) and target quadrant (B) staying time was plotted and shown as indicated. The asterisk indicates P < .05 compared with the corresponding sham.
paired in the 40-Gy group at 12 weeks’ postirradiation. Furthermore, the deleterious effects of the 40-Gy treatment were significantly stronger than those of the 20-Gy treatment.

Pathologic Changes
To better understand the potential changes of astrogliosis in animals after irradiation, we quantified the expression of GFAP, an astrocyte-specific intermediate filament protein, in the hippocampus. Our quantitative image analysis showed that in the 40-Gy group, significantly more GFAP+ astrocytes in the hippocampus could be observed at 4 weeks’ postirradiation compared with the sham control group (P < .01). This change returned to normal by 8 weeks, while in the 20-Gy group, there was no change at any examined time points (Fig 4).

Discussion
Radiation therapy–associated neuroinjury of the central nervous system can be detected within hours after exposure to a dose higher than 15-Gy radiation, and fatality may occur within approximately 2 days.16 Acute RE, the radiation-induced brain injury, occurs in months after exposure to radiation and is a major health problem for patients. Cognitive dysfunction, with a dose-dependent impairment in working memory, is perhaps the most common sequela of acute RE.8,17,18 There is growing concern regarding the cognitive consequences of whole-brain irradiation for long-term cancer survivors.19

We have established an acute RE model in young rats undergoing single-dose whole-brain irradiation by using a linear accelerator before this study.20 However, most patients with cancer are treated with fractionated therapy currently, and there might be differences in the injury patterns between single-dose exposure and multiple fractioned exposures. To mimic the clinical protocol as closely as possible, young rats received a course of conventional whole-brain irradiation according to a schedule commonly used in the clinical setting in
this study; and during the 3 months after irradiation, sequen-
tial behavioral and immunohistologic studies were observed
in irradiation and sham groups.

Previous studies mainly focused on late-onset RE. Hodges
et al reported radiation-induced deficits in T-maze forced-
choices alternation and subsequent dose-dependent water
maze deficits during a period of 44 weeks. They indicated that
local cranial irradiation with low dose (20-Gy) of x-rays could
produce cognitive deficits in adult rats without evidence of
pathologic changes. Yoneoka et al found that fractionated
irradiation at the 40-Gy level could cause memory deficits at
12 months after irradiation, but not at 6 or 9 months after
irradiation in adult rats. Shi et al reported that radiation-
induced spatial learning and memory deficits could be de-
tected 12 months after irradiation in 12-month-old rats. How-
ever, the response to the radiation observed in young rats
differed from that observed in old rats. Lamproglou et al studied
the influence of age on learning and memory dysfunction
induced by cranial radiation. They found that the radia-
tion-induced (30-Gy) memory deficits could be detected at 1
month postirradiation in 4-month-old Wistar rats. Young rats
showed an earlier decrease in learning and memory than older
rats, and this deficit was followed by partial recovery.

In our study, we used young rats (4 weeks) for the tests, and
our results suggest that progressive learning and memory dys-
function could be induced early after fractionated irradiation
in young rats. This cognitive defect in the 20-Gy group was
transient and could only be detected at 4 weeks’ postirradi-
ation, while in the 40-Gy group, this decline was more obvious
with a longer duration compared with the 20-Gy group. The
significant defects in cognitive function could be observed at 4
weeks and lasted at least until 8 weeks’ postirradiation. In
comparison with large single-dose irradiation reported previ-
ously, the cognitive impairment caused by the fractionated
irradiation was less severe and started later and did not last as
long.

Mechanisms of radiation encephalopathy remain to be elu-
cidated. Shi et al indicated that an altered glutamate neu-
rotransmission and/or excitatory neurotoxicity in the hip-
ocampal CA1 region might be involved in the radiation-
induced cognitive impairments. Other mechanisms, such as
the death of vascular cells, changes in cytokines, reduction in
regional glucose metabolism, and inhibition of the forma-
tion of new neurons in the hippocampus might also be in-
volved. Considering that many proposed molecular mecha-

References


