Utilizing the Faxitron MultiRad 225 X-ray irradiation system for the construction of mouse chronic whole brain radiation model

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ABSTRACT

Radiation-induced brain injury (RBI) is a common complication of radiotherapy for head and neck tumors while its mechanism is not fully understood. Animal whole-brain radiation (WBR) models are of key importance in experimental radiation research, and an appropriate radiation source is essential. Previous animal WBR models were administered by clinical linear accelerator to induce the pathophysiological changes of RBI. In the current study, we adopted Faxitron MultiRad 225 X-ray irradiation system to construct a mouse WBR model with a single dose of 30 Gy. In the acute phase of this mouse WBR model, brain edema and blood–brain barrier (BBB) damage were found mild. However, two months later, the results of immunofluorescence showed that astrocytes and microglia were activated continuously, and the number of immature neurons in dentate gyrus (DG) area of hippocampus was significantly reduced, in accordance with the features of chronic pathophysiological changes. Besides, data of MRI scans and behavior tests illustrated the structural changes of brain tissue and cognitive impairment in the chronic phase. To sum up, this mouse WBR model using the Faxitron MultiRad 225 irradiation system with a single dose of 30 Gy is feasible to simulate the RBI-related chronic pathophysiological changes.

Keywords: radiation-induced brain injury (RBI); animal model; neuroinflammation; microglia; animal irradiator

INTRODUCTION

Radiotherapy is an indispensable adjuvant tool for head and neck tumors, and its curative efficacy has been well-recognized [1, 2]. However, radiotherapy can also cause injury to the adjacent normal brain tissue and lead to neurological complications, known as radiation-induced brain injury (RBI) [1]. Major toxic changes of RBI include continuous activation of microglia, chronic inflammation, inhibition of neural regeneration and irreversible cognitive impairment [3–5]. However, the mechanisms of RBI still need to be further explored, and the animal model is an important research tool.

So far, models employing various animal species with different radiation dosages and fractions have been brought up to explore the mechanism of RBI [3]. An appropriate radiation source is essential in studies on tissue response in RBI animal models [6]. Previous animal whole-brain radiation (WBR) models were administered using a clinical linear accelerator with 6 MV electron beam to simulate the pathophysiological changes of RBI. Faxitron MultiRad 225 X-ray irradiation system (Wheeling, IL, USA) is a commercially available X-ray irradiator that is designed for small animals and cells [6]. Compared with traditional clinical irradiating apparatus, Faxitron MultiRad 225 X-ray irradiation system have lower radiation energy (225 kV vs 6 MV), and thus lower penetration, which helps in avoiding the damage to adjacent organs including trachea, esophagus and neck vascular tissues [6, 7]. In addition, this irradiator has a dose monitor, which can accurately measure the radiation dose applied on the experimental subjects (animals or cells) in real time, providing a more stable and uniform irradiation approach [6, 7]. Herein, we constructed a mouse WBR model by utilizing the Faxitron MultiRad 225 X-ray irradiation system, which has not been reportedly used in the construction of WBR model.

In the current study, using the Faxitron MultiRad 225 X-ray irradiation system with a single dose of 30 Gy, obvious neurological changes were observed in the brain of the mice, in accordance with the features of RBI including the chronic activation of glia cells, inhibition of neural regeneration, structural changes of brain tissue, cognitive impairment,
but mild brain swelling and destruction of blood–brain barrier (BBB) [8, 9]. To sum up, it is feasible to simulate the RBI-related chronic pathophysiological changes using the Faxitron MultiRad 225 X-ray irradiation system with a single dose of 30 Gy.

**METHODS**

**Animal and irradiation**
All animal experiments in this study were approved by our hospital. C57BL/6 mice (male, 8 ~ 10 weeks, 20 ~ 25 g) were used and randomly divided into different groups. A total of 103 mice were used in this study, 24 of which died after receiving whole-head radiation (WHR; Fig. 1). Before radiation, turn on the Faxitron MultiRad 225 X-ray irradiation system and then conduct self-examination, preheating and dose correction. Setting parameters (the distance from radioactive source to the skin: 58 cm; the thickness of aluminum filter: 2 mm; the energy voltage: 225 kV). The current was adjusted according to the dose rate measured by the dose monitor. Mice were anesthetized by intraperitoneal injection of 0.3% pentobarbital (20 ml/kg). After anesthetization, the mice were placed prone on the turntable of the radiator. In the WBR group, the mouse was placed in a 1 × 1 cm² irradiation range from the post-canthus line to the post-aurem line. The remaining area was shielded by a 1.0 cm-thick lead plate. In the WHR group, the whole head before the post-aurem line of the mouse was exposed to radiation with the rest of body covered by lead plate. In the sham group, lead plate was used to completely protect the whole body including the head from the irradiation. A single dose of 30 Gy (3 Gy/min) was used for irradiation. After irradiation, the body temperature of the mice was kept at 37°C with a heating blanket. After the animals were awake and could move freely, they were put back into the cage for further breeding.

**General observation (food, water, weight)**
Mice were randomly assigned to the sham group, the WBR group and the WHR group, with six mice in each group. They were fed in cages and each of them was labeled. Before modeling, the daily consumption weights of food and water were recorded for 3 days to calculate the mean value used as baseline. Subsequently, daily feed and potable water consumption weights were recorded every day after radiation to evaluate the change in the intake of food and water of each mouse. Daily change of body weight of each mouse were recorded.

**Cerebral water content calculation**
Mice were euthanized with pentobarbital at different time points after modeling. After removing cerebellum and olfactory bulb, unilateral cerebral hemisphere was taken to measure cerebral water content. The brain tissue was placed on the scale of 1/10000 to obtain the wet weight, and then placed in the oven at 60°C for 7 days to obtain the dry weight. The cerebral water content was calculated in line with the formula: brain water content = (wet weight – dry weight) / wet weight × 100% [10].

**Evans Blue extraction experiment**
The BBB permeability was evaluated by measuring the extravasation of Evans blue. Evans Blue was injected (2%, 4 ml/kg, Sigma, CAS:314-13-6) through tail vein at 1 h before sacrifice followed by transcardially perfused with saline to clear the intravascular dye from the vessels until the drainage was colorless [11]. After that, ipsilateral hemisphere was removed and incubated in formamide (Sigma) in 60°C water bath for 24 h. Evans Blue content was determined in supernatants at 632 nm using a spectrophotometer (BMG Multiscan Spectrum, Offenburg, Germany) and expressed in μg per gram brain.

**Evans Blue fluorescence experiment**
After perfusion following Evans Blue, ipsilateral hemisphere was fixed overnight with 4% paraformaldehyde, followed by dehydration with 15% and 30% sucrose solution with frozen with liquid nitrogen after OCT embedding, and then placed in a -80°C refrigerator for later use. Sequential 5 μm-thick coronal sections of the brain were prepared by cryotumricotomy (CM1800, Leica, Germany). After fixation with 4% paraformaldehyde, it was placed in an inverted fluorescence microscope and photographed through the red channel with the same exposure time (1s). Three regions of interest located in the cortex above
the hippocampus were selected to detect the fluorescence intensity of the EB. Image processing used ImageJ v1.8.0 to calculate the average fluorescence intensity of the cortex.

**Immunofluorescence**

Mice were euthanized after 8 weeks post-radiation and transcardially perfused with saline. Following fixation with 4% PFA overnight, brains were immersed in 15% and 30% sucrose at 4°C for cryoprotection. Sequential 6 μm-thick coronal sections of the brain were prepared by cryoutramicrotomy (CM1800, Leica, Germany). The slices were incubated at 4°C overnight with the following primary antibodies: rabbit anti-Iba-1 (1:500; Abcam, Cambridge, UK; ab178847) for microglia, rabbit anti-GFAP (1:500; Proteintech, IL, USA; 16825-1-AP) for astrocyte, and rabbit anti-Doublecortin (DCX; 1:500; Abcam; ab207175) for immature neurons. Afterwards, the slices were washed and detected with secondary antibodies (1:500; goat anti-rabbit FITC; Abcam; ab6717) preceding counterstaining with DAPI in the dark. Finally, fluorescence signal was observed under fluorescence microscope (IX73, Olympus, Japan) and analyzed using ImageJ software v1.8.0.

**Morris water maze**

Spatial learning and memory were evaluated in animals 8 weeks post-irradiation using the Morris water maze as described previously [12, 13]. Firstly, mice were trained to reach for the platform for 5 consecutive days with four trials per day. Movements of the mice were tracked by TSE VideoMot2 tracking system (Bad Homburg, Germany) to record the path and time taken to escape from four randomly assigned locations. The latency time required to locate the hidden platform was assessed among groups. After acquisition trial, the probe trial was performed on the following day, when mice were allowed 60 seconds to explore the platform which had been removed. The percentage of total time that mice spent in the target quadrant and the number of platform location crossing were recorded and analyzed.

**MRI scanning**

At certain time points before and after radiation, the mice were anesthetized with isoflurane, placed in 7.0T small animal MRI apparatus (Bruker, Germany) and fixed. T2 weighted image (T2WI) was used for coronal and horizontal scanning, with slice thickness of 0.5 mm. ADC was used for coronal scanning, with a slice thickness of 0.5 mm. The original DICOM files were imported into ImageJ, with the area of lateral ventricle measured and counted [14].

**Western Blot**

Brain tissue of the hippocampus were resolved in RIPA solution (Beyotime Biotechnology, Shanghai, China) and restored in -80°C until used. Denatured protein was separated by polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membrane (Millipore, Billerica, United States). Membrane was incubated overnight at 4°C with antibodies involving Rabbit anti-TNFα (1:5000; Abcam; ab183218). Primary antibodies were detected with anti-rabbit horseradish peroxidase–conjugated secondary antibodies. All signals were detected by enhanced chemiluminescence detection method followed by quantification with Image J and normalizing to GAPDH levels.

**Measurement of Gene Expression**

Total RNA was extracted from brain tissue in the hippocampus area. mRNA levels were measured by quantitative real-time PCR involving glyceraldehyde phosphate dehydrogenase (Gapdh), tumor necrosis factor-α (Tnf-α), interleukin (Il)-1β. Changes in the relative mRNA
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Fig. 3. Test of brain edema and BBB permeability. A. MRI scanning in acute phase between WBR group and sham group; B. Cerebral water content calculation between two groups; C. Evans blue extraction test between two groups; n = 6 in each time point; **P < 0.01.

expression were normalized to Gapdh. The mouse-specific primers were listed as followed:

Thf-α: Forward primer: 5′-CCCTCACACTCATCTTTCT; Reverse primer: 5′-GCTACGACGGGCTACAG.
Il-1β: Forward primer: 5′-GCAGCATCTCGACAAGACTT; Reverse primer: 5′-GCTCCACGGGCAAGACATAG.
Gapdh: Forward primer: 5′-AGTGCGGTGTGAACGGATTTG; Reverse primer: 5′-TGTAGACCATGTAGTTGAGGTC.

STATISTICAL ANALYSIS

The number of positive cells was counted and analyzed using the independent sample T test in immunofluorescence staining. The data of escape latency in the water maze test were analyzed with repeated-measures ANOVA comprising treatment groups, time points and groups x time interaction, followed by Tukey’s post hoc multiple comparison tests. SPSS 25.0 and GraphPad Prism 7.0 were used for data analysis. P<0.05 was considered statistically significant.

RESULTS

Exploration and evaluation of mouse whole brain radiation model using the Faxitron MultiRad 225 X-ray irradiation system

Faxitron MultiRad 225 X-ray irradiation system is designed for small animals and cells irradiation (Fig. Fig. Fig.2A). The X-ray device is installed in a shielded cabinet and experimental objects are placed on a turntable below the radiation source (Fig. Fig. Fig.2B), with a monitor to measure the dose rate in real time and a beam near the radiation source to indicate the radiation range.

Previous studies on the mouse models of WBR showed that the radiation dose >25 Gy could significantly and continuously activate microglia, while the 50% lethal dose (LD50) of mice was about 32.4 Gy [3, 15, 16]. Hence, our study attempted to construct the mouse WHR model with a single dose of 30 Gy. However, we found the general state of mice (n = 10) worsened within 8–9 days after radiation and died within 2 days later (Fig. Fig. Fig.2C). Afterwards, we successively adopted different radiation dosages which covers the normal scale to
figure out the most appropriate radiation dose in our RBI model \(n = 10\) for each radiation dose: 20 Gy, 25 Gy, 40 Gy and 50 Gy \([3, 15, 16]\). We found that the majority of the mice in 20 Gy and 25 Gy radiation groups survived while those in 40 Gy and 50 Gy radiation groups died within 2 weeks.

After limiting the irradiation range to \(1 \times 1\) cm\(^2\) from the post-canthus line to the post-aurum line with a single dose of 30 Gy (Fig. 2D), all mice \((n = 10)\) showed poor general state manifested as slow reaction and decreased activity at 8–9 days after radiation and recovered gradually with successful survival to 2 months. All mice in the sham group showed no changes after modeling.

To elucidate the occurrence of lethal brain edema after radiation, another 18 mice were assigned randomly into the sham group, the WHR group and the WBR group with a \(1 \times 1\) cm\(^2\) radiation range. Via MRI scanning, four of the six mice in the WHR group showed abnormal T2WI high signal surrounding brain parenchyma on day 9 while the mice in the other two groups showed no abnormality (Fig. 2F).

Then, the changes in food and water intake and body weight in three groups \((n = 6)\) were under continuous observation within 14 days since modeling. On day 1 after modeling, all mice in the three groups had decreased food and water intake and weight loss, which was considered to be caused by anesthesia. Subsequently, the mice in two radiation groups showed significantly reduced food and water intake and weight loss on day 6. All mice in the WHR group died on day 10 after modeling. However, in the WBR group, all mice survived and gradually recovered within 2 months, while the level of food and water intake and weight were still lower than that of the sham mice \((0.839 \text{ vs } 1.066, P < 0.01, \text{Fig. 2E})\).

Hence, the WBR model using the Faxitron MultiRad 225 X-ray irradiation system was set and performed in our subsequent experiments, with the irradiation range of \(1 \times 1\) cm\(^2\) and the single dose of 30 Gy.

**No occurrence of apparent brain edema in the acute stage of the whole-brain radiation model**

To assess the changes of brain tissue in the acute phase, all mice in the modeling group and sham group \((n = 6)\) underwent sequential coronal brain MRI scanning (T2WI, ADC) before and 1, 3 and 7 days after radiation (Fig. 3A). Compared with baseline, no apparent structural change, midline shift and signal changes were found on T2WI, ADC in all animals, indicating that the current WBR method did not cause brain swelling.

To further evaluate the disruption of BBB, brain water content and Evans Blue extraction test were carried out. The disruption of BBB is a typical feature of acute RBI which usually occurs in 1–2 days and maintained for 1 month after RBI \([3]\), hence we choose a series of time points to observe the changes of BBB within 2 months. The brain water content of the irradiated mice showed no difference in several time points within 2 months after radiation compared with the sham group \((n = 6, \text{each time point})\) (Fig. 3B). Compared with the acute Evans Blue extraction induced by high radiation dose, Evans Blue extraction assay only showed mild BBB disruption in our RBI model, which peaked on days three to five \((P = 0.0093 \text{ at day } 3; P = 0.0062 \text{ at day } 5; n = 6 \text{ for each time point, Fig. 3C})\).

The above results showed that the WBR model used in this experiment did not cause prominent brain edema in the acute phase, but slightly damaged BBB on days three to five.
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Increased brain atrophy in the chronic phase of the whole-brain radiation model

To assess the changes of brain tissue in the chronic phase, all sham and irradiated mice (n = 6) underwent sequential brain MRI scanning (T2, ADC) before and 1, 2 and 8 weeks after irradiation. We found that the lateral ventricles were enlarged in the coronal section (Fig. 4A). In the coronal section of the third ventricle, the area of lateral ventricles measured by ImageJ showed no difference between the two groups before radiation (1.493 vs 1.590, \( P = 0.4885 \)). One week after modeling, the lateral ventricles in the radiation group were enlarged (1.775 vs 1.550, \( P = 0.0828 \)). Two weeks and 2 months after radiation, the area of lateral ventricles in the radiation group was gradually enlarged (2.081 vs 1.550, \( P = 0.0828 \); 2.809 vs 1.625, \( P = 0.0003 \), respectively), while that in the sham group did not change significantly over time (Fig. 4B).

Irradiated mice presented decline in memory and cognitive function after 2 months

To further evaluate the impairment of cognitive function in irradiated mice, mice after receiving radiation were subjected to the Morris water maze test. Before the Morris water maze test, we made an initial evaluation on the swimming speed of the irradiated mice to screen out the final experimental mice used in water maze test to roughly exclude the effect of the basic athletic ability changes on irradiated mice. After 2 months, the mice were trained for 5 days. The latency and distance taken for mice to reach the platform were recorded. During the training, the latency and distance of mice in each group were gradually shortened over time. Compared with those of the sham group, the latency and distance in the irradiation group were longer at day 5 (\( P = 0.006 \), \( P = 0.008 \), respectively; Fig. 5A–B). In probe trial, the hidden platform below the water surface was removed, followed by recording the time staying in the target quadrant and the crossing frequency of the platform position. The crossing frequency of irradiated mice was significantly lower than that of the sham group (\( P < 0.01 \), Fig. 5C).

The results of the water maze test showed the impaired memory and cognitive function 2 months after radiation.

Activation of astrocytes and microglia with elevated pro-inflammatory cytokines and reduction of immature neurons in DG area of the hippocampus after the whole brain radiation

Continuous activation of astrocytes and microglia is one of the striking properties of radiation-induced chronic neuroinflammation [9]. In order to verify whether the present modeling method has a similar effect, we carried out the immunofluorescence for GFAP (a marker of
astrocyte) and Iba1 (a marker of microglia) of 30 Gy WBR group and sham group (n = 6) [17]. Compared with the sham group, the number of activated astrocytes were significantly increased after WBR, and the majority of GFAP+ cells were mainly located in the hippocampus, but few were found in the cortex (Fig. 6A).

Compared with the sham group, the number of Iba1-positive cells in cortex and hippocampus DG area of irradiated mice was significantly increased after 2 months (P < 0.01), suggesting that this radiation model launched the continuous microglia activation (Fig. 7A–B). Moreover, the protein expression of TNFα and mRNA level of Tnfα and Il-1β were continuously elevated in the hippocampus area on day 1, 3, 7, 30 post radiation, suggesting the existence of chronic inflammation induced by radiation (Fig. 8).

In the adult mouse brain, there are abundant neural progenitor cells in the DG area of the hippocampus, capable of differentiating to mature neurons and astrocytes. Disorder of neural progenitor cell differentiation and the decrease of newborn neurons in the DG area of the hippocampus are considered as important factors leading to RBI-related cognitive impairment [17]. In the current study, immature neurons was indicated by immunofluorescence staining with antibody against doublecortin (DCX). Results of immunofluorescence staining showed that DCX+ immature neurons were located along the inner layer of the DG region in the sham brain, while the number of DCX+ neurons was significantly decreased in the mice suffering the WBR after 2 months (P < 0.01, Fig. 6B).

By immunofluorescence assay, we found that astrocytes and microglia were continuously activated 2 months after the whole brain irradiation, accompanied by neural regeneration disorder characterized by immature neuron reduction.

**DISCUSSION**

Radiotherapy is an effective treatment for primary head and neck tumors [20]. However, due to radiation exposure, the normal brain tissue also develops detrimental pathological changes, the so-called RBI [1]. RBI can be classified into acute, early delayed, and late delayed patterns [8, 21, 22]. Attributed to the optimization of irradiating regimen with more precise dose rate and accurate irradiation range, the incidence of acute and early delayed RBI decreases to some extent with lessened severity. However, pathological injuries during the
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Figure 7. Immunofluorescence staining with IBA-1 in cortex and in hippocampus. A. Representative pictures of immunofluorescence staining with anti-IBA-1 antibody (green) at cortex section, scale bar = 100 μm; B. Representative pictures of immunofluorescence staining with anti-IBA-1 antibody (green) at hippocampus CA1 section, scale bar = 100 μm; n = 6 in each group; **P < 0.01.

Chronic phase are irreversible and thus of significant concern, and the underlying mechanisms still need further exploration.

Animal WBR models are crucial in experimental radiation research, and an appropriate radiation source is essential [2]. Faxitron MultiRad 225 X-ray irradiation system is a 225 kV X-ray machine adapted from an X-ray imaging unit through modifications to facilitate experimental irradiation for small animals and cells [6]. This machine has been adopted to investigate breast cancer, bone imaging techniques and cell irradiation [23–25]. The X-ray device is installed in a shielded cabinet, and is adjusted to facilitate different exposure positions, adjustable radiation field, and different dose rates. There are three key characteristics of the radiation beam for the irradiation experiments: (i) dose rate of the radiation beam, which affects the time required for the experiment; (ii) field size of the beam; and (iii) the uniformity of the dose deposition [6]. Compared with traditional clinical irradiating apparatus, Faxitron MultiRad 225 X-ray irradiation system has lower radiation energy (225 kV vs 6 MV), and thus lower penetration to avoid damage to adjacent organs. In addition, this irradiator has a dose monitor and a turntable, which can accurately measure the radiation dose applied on the experimental subjects (animals or cells), providing more stable and uniform irradiation [6]. In previous study, Faxitron MultiRad 225 X-ray irradiation system was adopted to construct an RBI model with 60 Gy, mainly focusing on the brain injuries like the disruption of BBB in acute phase [26]. In contrast, we chose a lower radiation dose of 30 Gy which elicited chronic RBI as characterized by brain atrophy, memory and cognitive deficits, and neuropathological injuries 8 weeks post-whole brain irradiation. Therefore, combining our findings with Yoshida’s, Faxitron MultiRad 225 X-ray irradiation system provided a novel approach to mimic RBI in both acute and chronic phases.

So far, models employing various animal species with different dosages and fractions of the radiation have been brought up to explore the mechanisms of RBI [3]. Nevertheless, all these models have limitations, none of which is widely accepted. In animal models, RBI is related to various factors as follows: germ line of experimental animals, radiation dosage, single or fractional irradiation, irradiation range, time point after irradiation, etc. Due to the similarity of the genome and pathophysiological process between small rodents and human species, rats and mice are widely used in multiple in vivo experiments [3]. Compared with rats, the WBR model of mice has certain advantages: (i) Easy to feed; (ii) Easy to operate; and (iii) Higher genetic homology...
Fig. 8. Inflammatory cytokines expression in hippocampus region on day 1, 3, 7, 30 post radiation. A. Protein expression of TNFα in hippocampus area on day 1, 3, 7, 30 post radiation; B. mRNA expression of TNFα and IL-1β in hippocampus area on day 1, 3, 7, 30 post radiation; n = 6 in each group in each time point; **P < 0.01.

with human [3]. Besides, radiation dose is one of the most important factors determining the severity of damage and the latent period between irradiation and the occurrence of lesions. Previous studies on the mice model of WBR showed that the radiation dose > 25 Gy could significantly and continuously activate microglia, while the LD50 of mice was about 32.4 Gy [27]. In the current study, using the Faxitron MultiRad 225 X-ray irradiation system, the mice undergoing a single dose of 30 Gy exerted major pathophysiological changes in accordance with the features of chronic RBI, including continuous glial activation, inhibition of neural regeneration, cognitive dysfunction, but no obvious destruction of BBB and brain swelling [28].

In our preliminary attempt to construct RBI mouse model, mice receiving WHR died within 2 weeks. After observation of general state, we found that the mice had a significant decrease in food intake and weight loss. Hence, we speculated that whole head irradiation might cause oral mucosa injury, thus decreasing food intake and resulting in death eventually. The high signal of T2WI surrounding brain parenchyma might be caused by dehydration of brain tissue. Then, by narrowing irradiation range to 1 x 1 cm² from the post-canthus line to the post-aurem line, each mouse survived within 2 months after irradiation. Moreover, through MRI scanning and detecting BBB permeability, we found that typical acute changes of RBI were not obvious in our radiation model, which had no brain edema and slight BBB disruption [22]. It has been reported that BBB permeability increased within 24 hours after exposure to radiation at 6 Gy and that the increase was maintained for a month, which was in accordance with our findings [3].

In long-term observation of our radiation model, the chronic activation of microglia was a typical pathological change. Activated microglia have long been considered as the pro-inflammatory executioners in various central nervous system diseases including RBI, by releasing various inflammatory cytokines such as interleukin 1 beta (IL-1β), tumor necrosis factor α (TNFα), and interleukin 16 (IL-16), continuously inducing inflammatory response and neural regeneration inhibition [9, 29–31]. Our WBR model using the Faxitron MultiRad 225 X-ray irradiation system simulated such inflammatory changes mediated by activated microglia.

To sum up, the Faxitron MultiRad 225 X-ray irradiation system could be useful for simulating RBI in experiments with certain advantages: (i) easy to operate; (ii) more precise to monitor the dose rate; (iii) more uniformed radiation beam; and (iv) accurate irradiation field size.
CONCLUSION
The RBI-related chronic pathophysiological changes can be feasibly simulated by the mouse WBR model using the Faxitron MultiRad 225 X-ray irradiation system with a single dose of 30 Gy.

CONFICT OF INTEREST
The authors declare no conflict of interest.

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REFERENCES
1. Tofilon PJ, Fike JR. The radioreponse of the central nervous system: a dynamic process[J]. Radiat Res 2000;153:357–70.
2. Greene-Schloesser D, Robbins ME, Peiffer AM et al. Radiation-induced brain injury: a review[J]. Front Oncol 2012;2:73.
3. Yang L, Yang J, Li G et al. Pathophysiological responses in rat and mouse models of radiation-induced brain injury. Mol Neurobiol 2017;54:1022–32.
4. Abayomi OK. Pathogenesis of irradiation-induced cognitive dysfunction. Acta Oncol 1996;35:659–63.
5. Rola R, Raber J, Rizk A et al. Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. Exp Neurol 2004;188:316–30.
6. Woo M, Nordal R. Commissioning and evaluation of a new commercial small rodent x-ray irradiator. Biomed Imaging Interv J 2006;2:e10.
7. Ma CM, Coffey CW, DeWerd LA et al. AAPM protocol for 40-300 kV x-ray beam dosimetry in radiotherapy and radiobiology. Med Phys 2001;28:688–93.
8. Hopewell JW. Late radiation damage to the central nervous system: a radiobiological interpretation. Neuropathol Appl Neurobiol 1979;5:329–43.
9. Hwang SY, Jung JS, Kim TH et al. Ionizing radiation induces astrocyte gliosis through microglia activation. Neurobiol Dis 2006;21:457–67.
10. Olson JE, Mishler L, Dimlich RV. Brain water content, brain blood volume, blood chemistry, and pathology in a model of cerebral edema. Ann Emerg Med 1990;19:1113–21.
11. Goldim M, Della GA, Petronilho F. Using Evans blue dye to determine blood-brain barrier integrity in rodents. Curr Protoc Immunol 2019;126:683.
12. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984;11:47–60.
13. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006;1:848–58.
14. Chen CC, Tung YY, Chang C. A lifespan MRI evaluation of ventricular enlargement in normal aging mice. Neurobiol Aging 2011;32:2299–307.
15. Chiang CS, McBride WH, Withers HR. Myelin-associated changes in mouse brain following irradiation. Radiother Oncol 1993;27:229–36.
16. Olschowka JA, Kyrkanides S, Harvey BK et al. ICAM-1 induction in the mouse CNS following irradiation. Brain Behav Immun 1997;11:273–85.
17. Chiang CS, McBride WH, Withers HR. Radiation-induced astrocytic and microglial responses in mouse brain. Radiother Oncol 1993;29:60–8.
18. Nowak E, Etienne O, Millet P et al. Radiation-induced H2AX phosphorylation and neural precursor apoptosis in the developing brain of mice. Radiat Res 2006;165:155–64.
19. Mao XW, Favre CJ, Fike JR et al. High-LET radiation-induced response of microvessels in the Hippocampus. Radiat Res 2010;173:486–93.
20. Bondy ML, Scheurer ME, Malmer B et al. Brain tumor epidemiology: consensus from the Brain Tumor Epidemiology Consortium. Cancer 2008;113:1953–68.
21. Béhin A, Delatour JY. Complications of radiation therapy on the brain and spinal cord. Semin Neurol 2004;24:405–17.
22. Münter MW, Karger CP, Reith W et al. Delayed vascular injury after single high-dose irradiation in the rat brain: histologic immunohistochemical, and angiographic studies. Radiology 1999;212:475–82.
23. Butterfield NC, Logan JG, Waung J et al. Quantitative X-ray imaging of mouse bone by faxitron. Methods Mol Biol 2019;1914:559–69.
24. Muttalib M, Tisdall M, Scawn R et al. Intra-operative specimen analysis using faxitron microradiography for excision of mammographically suspicious, non-palpable breast lesions. Breast 2004;13:307–15.
25. Wang J, Liu Q, Yang Q. Radiosensitization effects of berberine on human breast cancer cells. Int J Mol Med 2012;30:1166–72.
26. Yoshida Y, Sejimo Y, Kurachi M et al. X-ray irradiation induces disruption of the blood-brain barrier with localized changes in claudin-5 and activation of microglia in the mouse brain. Neurochem Int 2018;119:199–206.
27. Genc M, Genc E, Genc BO et al. Significant response of radiation induced CNS toxicity to high dose steroid administration. Br J Radiol 2006;79:e196–9.
28. van der Kogel AJ. Radiation-induced damage in the central nervous system: an interpretation of target cell responses. Br J Cancer Suppl 1986;7:207–17.
29. Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. Science 2003;302:1760–5.
30. Daigle JL, Hong JH, Chiang CS et al. The role of tumor necrosis factor signaling pathways in the response of murine brain to irradiation. Cancer Res 2001;61:8859–65.
31. Bellinzona M, Gobbel GT, Shinohara C et al. Apoptosis is induced in the subependyma of young adult rats by ionizing irradiation. Neurosci Lett 1996;208:163–6.