ORIGINAL

Experimental model for irradiating a restricted region of the rat brain using heavy-ion beams

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Abstract : Heavy-ion beams have the feature to administer a large radiation dose in the vicinity of the endpoint in the beam range, its irradiation system and biophysical characteristics are different from ordinary irradiation instruments like X-rays or gamma-rays. In order to get clarify characteristic effects of heavy-ion beams on the brain, we have developed an experimental system for irradiating a restricted region of the rat brain using heavy-ion beams. The left cerebral hemispheres of the adult rat brain were irradiated at dose of 50 Gy charged carbon particles (290 MeV/nucleon ; 5mm spread-out Bragg peak). After irradiation, the characteristics of the heavy-ion beams and the animal model were studied. Histological examination and measurement showed that extensive necrosis was observed between 2.5 mm and 7.5 mm depth from the surface of the rat head, suggesting a relatively high dose and uniform dose was delivered among designed depths and the spread-out bragg peak used here successfully and satisfactorily retained its high-dose localization in the defined region. We believe that our experimental model for irradiating a restricted region of the rat brain using heavy-ion beams is a good model for analyzing regional radiation susceptibility of the brain. J. Med. Invest. 51:103-107, February, 2004

Keywords : *cerebral hemisphere, heavy-ion beam, irradiation*

INTRODUCTION

As part of the 10-year Strategy for Cancer Control implemented by the Japanese government, the clinical trials using HIMAC (Heave Ion Medical Accelerator in Chiba) for cancer treatment have been performed more than 7 years in Research Center for Charged Particle Therapy of National Institute of Radiological Sciences. The HIMAC is the world's only heavy-ion accelerator complex dedicated to medical use in a hospital environment. Over a thousand patients have been treated using heavy-ion beams. The safety and efficacy of heavy ions have been demonstrated to a great extent (1-4). For instance, brain tumors treated by heavy-ion beams became smaller or disappearance. However, fundamental research related to such clinical phenotypes and their underlying mechanisms are little known.

HIMAC can accelerate ion particles (carbon) and it has the feature to administer a large radiation dose in the vicinity of the endpoint in the beam range (Bragg peak)(5-8). Thus, its irradiation system and biophysical characteristics are different from ordinary irradiation instruments like X-rays or gamma-rays.

In order to get clarify characteristic effects of heavy-

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ion beams on the brain, we have developed an experimental system for irradiating a restricted region of the rat brain using heavy-ion beams. The characteristics of the heavy-ion beams and the animal model were studied in the present work.

MATERIALS AND METHODS

Animals and heavy-ion beams

Twenty-eight adult male Sprague-Dawley rats, aged 12 weeks and weighing 260-340 g (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used. Animals were kept in a controlled atmosphere of $23\pm1°C$ with $55\pm5°C$ humidity under a 12-h dark/light cycle. They were given free access to commercial laboratory pellet food and tap water throughout the study. During experiment, animals were divided into two groups as follows : twenty rats were irradiated with carbon beams at 50 Gy, which is usual dose to be used in heavy ion beam therapy ; eight were sham-irradiated animals. Rats were deeply anesthetized 10-15 minutes before irradiation with ketamine (40 mg/kg) and xylazine (10 mg/kg), immobilized in a specifically designed jig, and irradiated with 290 MeV/nucleon charged carbon beams in a dorsal-to ventral direction.

The depth-dose distribution of the heavy ion beam was modified to make a spread-out bragg peak of 5 mm wide with a range modulator, and the rat left hemisphere was irradiated, that was confirmed with a left collimation (Fig.1). The area of the left brain was subjected to a single irradiation at dose of 50 Gy equivalent with a linear energy transfer of approximately 50 Ke V/µm. The position of the spread-out bragg peak was adjusted to be at 2.5-7.5 mm depth from the surface of the head with a binary filter inserted before the target.

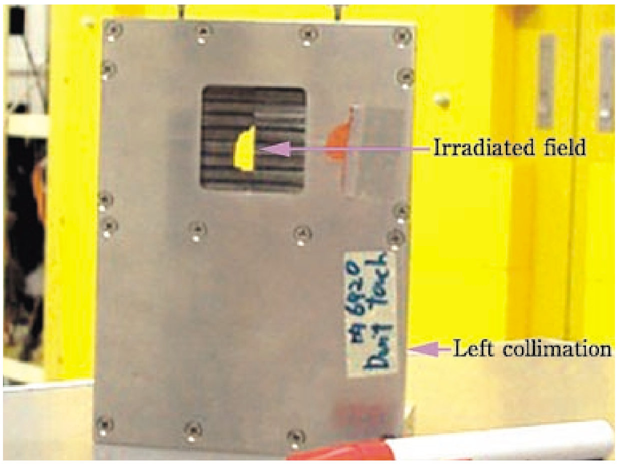

Fig. 1. The rat left hemisphere was irradiated with HIMAC (Heave Ion Medical Accelerator in Chiba), and its irradiated field (above arrow) was confirmed with this left collimation which was made of lead.

All of the animal experiments were carried out with permission and under regulation of the Institutional Committee for Animal Safety and Welfare of the National Institute of Radiological Sciences, and in accordance with Regulations on Appropriate Animal Breeding and Treatment, Ministry Office of Japan. All efforts were made to minimize the number of animals used and their suffering.

Histological treatments and examinations

Brain samples were obtained from 4, 8, 16, 32 weeks after exposure. Five rats treated with the heavy ion beam and two controls were randomly selected for study in each experimental period. Animals in either the irradiated or control groups were deeply anesthetized with ether, and perfused with Zamboni's fixitive solution (4% formaldehyde and 0.2% picric acid in 0.1 M phosphate buffer, pH7.3) with a rotary pump via the left cardiac ventricle. Following perfusion, the brain was removed and immersed in freshly made Zamboni's fixitive solution for 1 week. After a gross anatomical examination, tissue samples were embedded in paraffin and sectioned coronally at 6 µm with a microtome. Particular attention was given to the dissection and orientation of the tissue blocks in this study. The tissue blocks through the full thickness of the cerebral cortex were dissected as nearly as possible perpendicular to the longitudinal axis of the cerebral hemisphere. For each brain, every fourth section was kept from the serial sections, and then stained with hematoxylin and eosin.

Measurement of the radiation field and depth & behavioral observation

The field and depth of the heavy-ion beams were checked by an observing outline of depilation on the surface of the brain, and a measuring paraffin section of rat brain with different thickness.

Behavioral changes were observed twice a week after irradiation. Animals were removed from their cages, placed on a table, and scored for balance using a subjective scoring scale (9, 16).

RESULTS

No obvious behavioral and histological changes before 7 weeks of exposure. Loss of hairs was found in the left brain by 7 weeks after irradiation. The shape and size of depilation (Fig. 2) were almost same to the

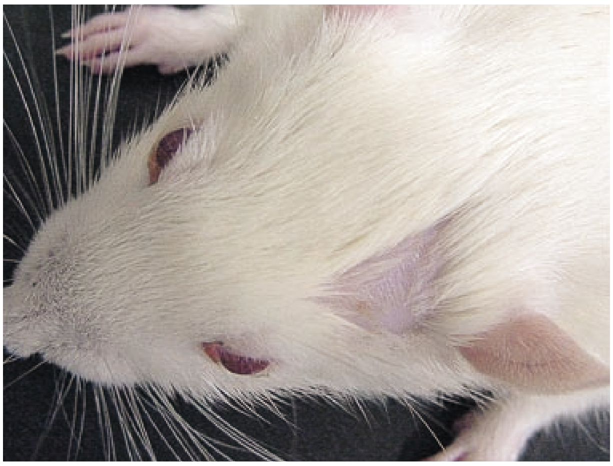

Fig. 2. Loss of hairs was observed in the rat left hemisphere by 7 weeks after irradiation of carbon ion beams. The shape and size of depilation were almost same to the left collimation (Fig.1).

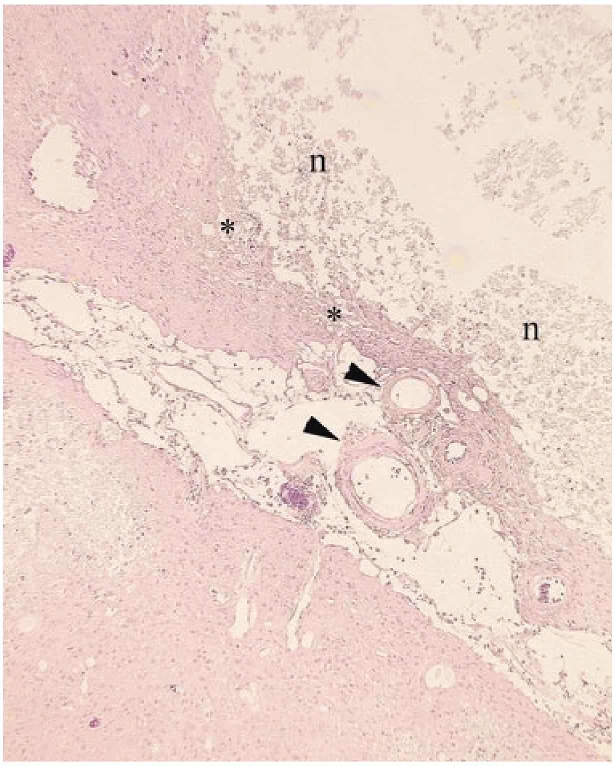

Fig. 3. High power photomicrograph of an example of histological changes since 8 weeks after irradiation of carbon ion beams. The distinctive histological changes were necrosis, vascular dilatation and tissue swelling. n : necrosis ; Arrowheads : vascular dilatation. Asterisks : tissue swelling.

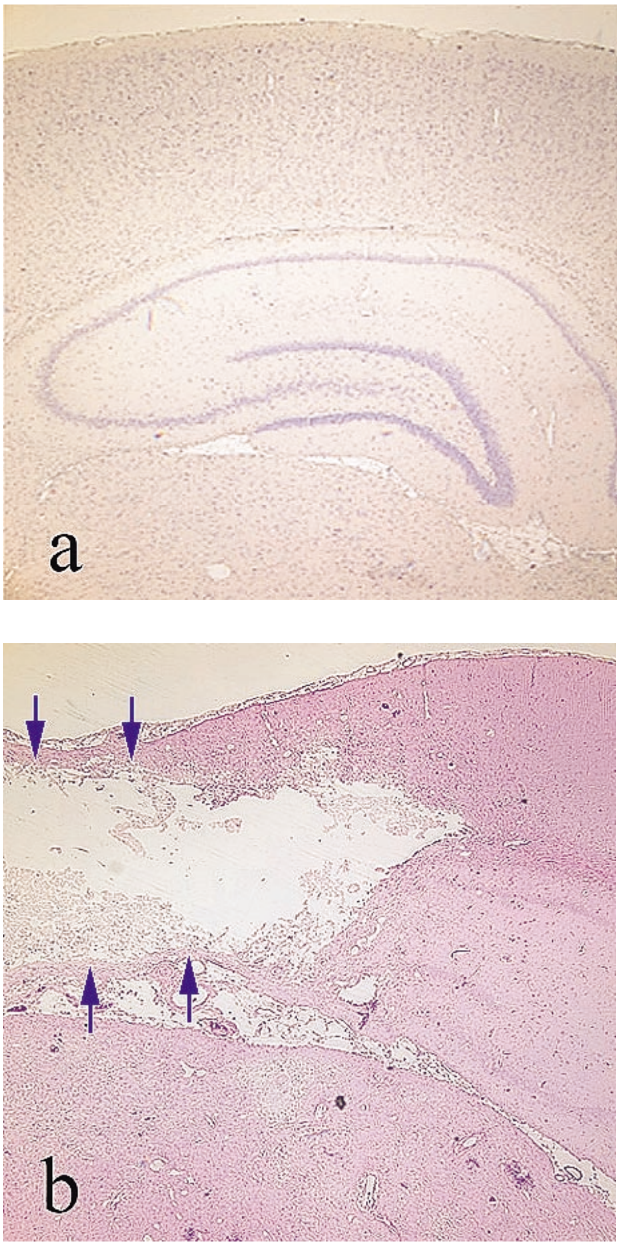

Fig. 4. Photomicrographs of examples of histological changes at 16 weeks after irradiation of carbon ion beams. b : Extensive necrosis was observed between 2.5 mm and 7.5 mm depth from the surface of the head (between arrows), suggesting a relatively high dose and uniform dose was delivered among designed depths. a : Control.

Table 1. Histological changes in rat brain after 50 Gy irradiation with carbon ion beams

Histological changes	Time after irradiation			
	4 weeks	8 weeks	16 weeks	32 weeks
Necrosis	−	±	+	+
Vascular dilatation	−	±	+	+
Tissue swelling	±	±	+	+

Histological examination : −, no visible abnormal ; ±, sight ; +, obvious
Numbers of animals examined in each group, n=5

left collimation (Fig. 1). The skin color in the irradiated area was no difference as compared with the others in the no irradiated areas. The pathological changes, such as hemorrhage, dried scab and tissue swelling were not appearance in the cutaneous surface of the irradiated area.

At 8 weeks after exposure, three rats of fourteen exposed to the heavy ion beam exhibited slight behavioral changes, either in an abnormal walking pattern or rotation when suspended by their tail. With the process of time, these behavioral changes became more marked. The animals showed total loss of their balance both in an abnormal walking pattern and rotation from 16 weeks onwards after irradiation.

The histological examination showed obvious histological changes since 8 weeks after irradiation (Table 1). The distinctive histological changes were necrosis, vascular dilatation and tissue swelling (Fig. 3). At16 and 32 weeks after irradiation, necrotic rarefaction became dominant at the center of the irradiated region and enlarged blood vessels were present in the surrounding area (Fig. 4).

The field and depth of the heavy-ion beams were checked by a measuring paraffin section of rat brain with different thickness. A relatively high dose was delivered between 2.5 mm and 7.5 mm depth from the surface of the rat head, which was judged by cell death through the serial sections. Extensive necrosis was observed between 2.5 mm and 7.5 mm depth, but such necrosis could not found near the surface of the rat head or more than 7.5 mm depth (Fig. 4).

DISCUSSION

The shape and size of depilation found in the left cerebral hemisphere of the rat by 7 weeks after exposure were almost same to the left collimation, that was outline of the irradiation field on the surface of the brain, indicating HIMAC irradiation system and the left collimation can be satisfactory to irradiate a defined region of the brain. Histological examination and measurement showed the distinctive features of the depth-dose distribution of heavy-ion beams. Extensive necrosis was observed betweent 2.5 mm and 7.5 mm depth from the surface of the rat head, suggesting a relatively high dose and uniform dose was delivered among designed depths and the spread-out bragg peak used here successfully and satisfactorily retained its high-dose localization in the defined region. These biophysical characteristics of HIMAC irradiation system demonstrated it is good system for cancer therapy (10, 11).

Radiation therapy is a clinic way to treat brain tumors. However, damages of the normal brain induced by radiation occur several months after a treatment (13-15), so radiation damage is a limiting factor to use radiation therapy. In the present study, behavioral changes in the irradiated rats appeared both in an abnormal walking pattern and rotation from 16 weeks onwards after irradiation, indicating existence of radiation damage after heavy ion beam exposure. These facts request us to study the mechanisms of radiation damage to the brain tissue and the radiation susceptibility of each region of the brain. Therefore, it is a necessary to develop an animal model for irradiating a restricted region of the brain using heavy-ion beams.

The experimental model in this study can be expected as a novel model, it has many distinctive features which are different from others. Because ordinary irradiation instruments using X-rays or gamma-rays can not concentrate the radiation dose to a small and restricted area of the brain. The whole brain or a relatively large volume of the brain were irradiated and examined, so effects of radiation may affect with a mutual interaction with other brain regions. Heavy ion beam provides a possible to expose a small and restricted volume of animal brain to a high dose of radiation. We believe that our experimental model for irradiating a restricted region of the rat brain using heavy-ion beams is a good model for analyzing regional radiation susceptibility of the brain.

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