J. Vet. Sci. (2006), **7**(1), 19–23

JOURNAL OF
Veterinary
Science

Histopathological changes of testes and eyes by neutron irradiation with boron compounds in mice

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This study was performed to investigate the biological effects of boron neutron capture therapy (BNCT) on the testes and eyes in mice using HANARO Nuclear Reactor, Korea Atomic Energy Research Institute. BNCT relies on the high capacity of ^{10}B in capturing thermal neutrons. Sodium borocaptate (BSH, 75 ppm, iv) and borono-phenylalanine (BPA, 750 ppm, ip) have been used as the boron delivery agents. Mice were irradiated with neutron (flux: 1.036739E +09, Fluence 9.600200E+12) by lying flat pose for 30 (10 Gy) or 100 min (33 Gy) with or without boron carrier treatment. In 45 days of irradiation, histopathological changes of the testes and eyes were examined. Thirty-three Gy neutron irradiation for 100 min induced testicular atrophy in which some of seminiferous tubules showed complete depletion of spermatogenic germ cells. Lens epithelial cells and lens fiber were swollen and showed granular changes in an exposure time dependent manner. However, boron carrier treatment had no significant effect on the lesions. These results suggest that the examination of histopathological changes of lens and testis can be used as "biological dosimeters" for gauging radiation responses and the HANARO Nuclear Reactor has sufficient capacities for the BNCT.

Key words: boron, eye, histopathology, neutron irradiation, testes

Introduction

In cancer treatment, surgery, radiation therapy and chemotherapy are good standard procedure, but there are still many treatment failures. An ideal therapy for cancer would be destroying all tumor cells selectively without damaging normal tissues. The boron neutron capture therapy (BNCT) which had been recently developed has given great promise in cancer therapy with minimum side effects [1]. The effectiveness of BNCT depends on the relative high concentrations of ^{10}B in tumor compared with the surrounding normal tissues. It is assumed that the ^{10}B-containing compounds selectively accumulated in cancer cells will cause preferential killing of the cells and result in therapeutic effects [22]. When the boron compounds are exposed to thermal neutrons, they release two high linear energy transfer (LET) particles, an α(^{4}He) particle and a lithium (^{7}Li) recoil nucleus to the cells in which they accumulate. These particles from the ^{10}B (n, α)^{7}Li reaction have extremely short path lengths (5~10 μm) in water [16]. Capture of neutron by ^{10}B, a stable isotope, results in the formation of excited boron-11 (^{11}B). The unstable ^{11}B instantly reacts to yield the high linear energy transfer (LET) lithium-7 (^{7}Li) and energetic α-particles (^{4}He). The kinetic energy of ^{7}Li and α particles is about 2.8 million electron volts (eV) (100 million times more than what was put in). Along with high linear energy transfer (LET) makes the particles highly toxic to the cells [22]. The short range of these heavy particles (5-9 μm) and ^{10}B accumulation in target tissues provide great advantage in selective tumor destruction without significant damage to the surrounding normal tissues [7]. Two boron drugs have been used clinically, sodium borocaptate (BSH, Na(2)B(12)H(11)SH) and a dihydroxyboryl derivative of phenylalanine called borono-phenylalanine (BPA) [8,13,15].

Neutron sources for BNCT are limited to nuclear reactors that are available in a few countries, including the United States, Japan, several European countries, and Argentina. Clinical trials using the apparatus have been carried out in Japan, Europe, and the United States. The HANARO Nuclear Reactor has been recently installed in the Korea Atomic Energy Research Institute. The factors influencing beam performance, such as the neutron energy spectrum, field size and degree of collimation, are not identical in each reactor [3]. In addition, the values of the parameters to calculate a dose vary with different biological and/or medical circumstances, boron delivery agents, dose of

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neutron, and differences in beam designs. These issues should be adequately addressed in the clinical trials of BNCT. In the present study, we investigated the histopathological changes of testes and eyes of mice by neutron irradiation with or without boron treatment in mice as a first step for the investigation of biological effects of BNCT using the HANARO Nuclear Reactor.

Materials and Methods

Animals

C57BL/6 male mice (22 ± 2 g) were received at 6 weeks of age from Daehan Biolink (Eumseong, Korea). They were maintained under specific pathogen-free conditions and fed sterilized food and water *ad libitum*.

Boron compounds

Two boron compounds, BPA and BSH, were purchased from Ryscor science (USA). The aqueous solution of BPA was prepared at concentration of 750 ppm and injected intraperitoneally 3 hours before irradiation. BSH was dissolved in physiological saline at a concentration of 75 ppm and injected into caudal vein 1 hour before irradiation.

Neutron Irradiation

Fifteen mice were divided to five experimental groups. Neutron was irradiated with or without boron treatment (Table 1). Prior to each irradiation, all animals were anesthetized with 0.2 ml of 1% chloral hydrate (Fluka, Japan). They were irradiated by 10 or 33 Gy neutron (flux: 1.036739E+09, Fluence 9.600200E+12) with lying flat pose for 30 or 100 min using BNCT facility on HANARO Nuclear Reactor.

Histopathology

Mice were sacrificed at 45 days after irradiation. Testes were fixed in Bouin's fluid (picric acid, saturated aqueous sol. 75 ml, formalin 25 ml, glacial acetic acid 5 ml), and after 24 hours stored in 70% ethanol. Eyes were fixed in Davidson's fixatives (ethyl alcohol 30 ml, formalin 20 ml, glacial acetic acid 10 ml). Samples were dehydrated in 50% to 100% alcohol, and xylene was used for clearing samples.

Table 1. Experimental design

Group*	Treatment
1	Non irradiated control
2	10 Gy neutron irradiation for 30 min
3	33 Gy neutron irradiation for 100 min
4	33 Gy neutron irradiation for 100 min in combination with BPA
5	33 Gy neutron irradiation for 100 min in combination with BSH

*Three mice were in each experimental group.

Tissues were embedded in paraffin, sectioned (5 μm thick), stained with H&E, and examined with light microscope.

Results

Histopathological changes

Testes: There were no notable changes of seminiferous tubules of the mice in group 1 and 2 (Fig. 1). In groups 3, 4 and 5, moderate atrophy of seminiferous tubules was observed in the testes. Atrophic seminiferous tubules are lined with only Sertoli cells or with a few germ cells (Fig. 2). The damaged seminiferous tubules were observed mainly in the periphery of the testicles. However, other seminiferous tubules showed normal spermatogenesis with only a few germ cells degeneration. BPA and BSH had no significant effect on the lesions (Figs. 3 and 4).

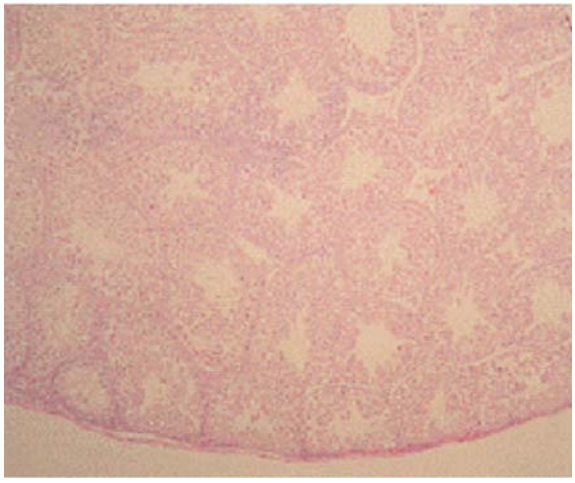

Fig. 1. Seminiferous tubules from the control mouse testis containing normal spermatogenic germ cells. H&E stain, ×200.

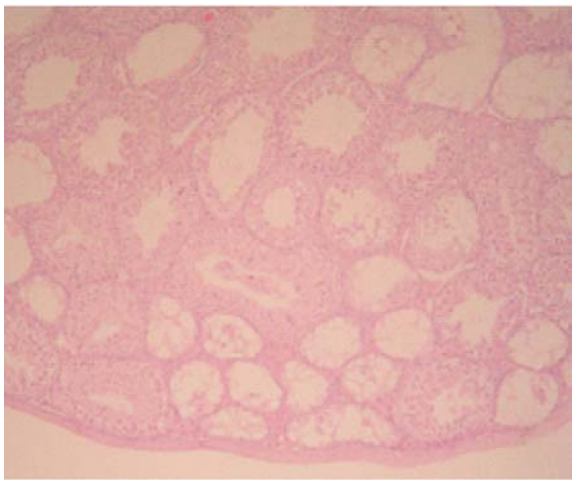

Fig. 2. Seminiferous tubules from the mouse irradiated with 33 Gy neutron for 100 min. Some tubules are lined Sertoli cells with a few germ cells. H&E stain, ×200.

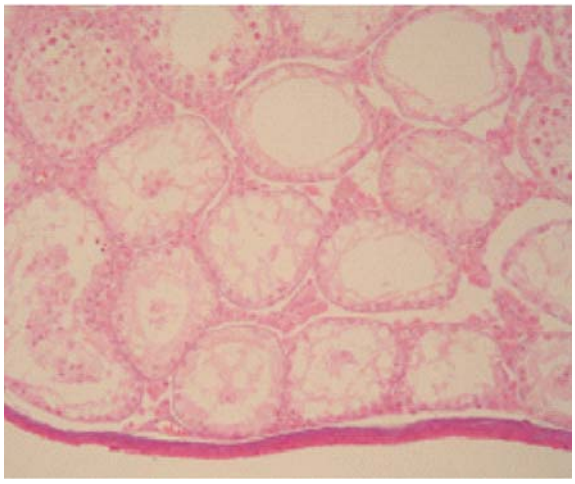

Fig. 3. Seminiferous tubules from the mouse pretreated with boronophenylalanine and irradiated with 33 Gy neutron for 100 min. Seminiferous tubules are lined Sertoli cells with a few germ cells. H&E stain, ×200.

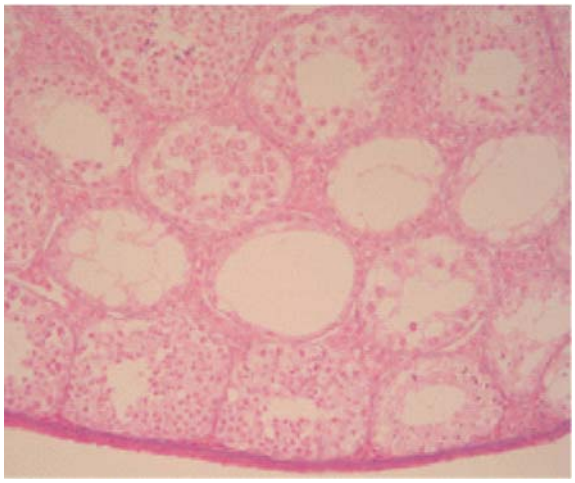

Fig. 4. Seminiferous tubules from the mouse pretreated with sodium borocaptate and irradiated with 33 Gy neutron for 100 min. Some seminiferous tubules are lined Sertoli cells with a few germ cells. H&E stain, ×200.

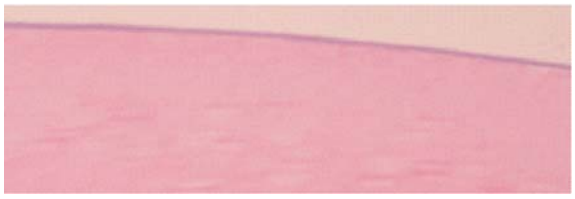

Fig. 5. Lens from the control mouse eye. Note the nucleated lens fibers below the single layer of cuboidal epithelium. H&E stain, ×400.

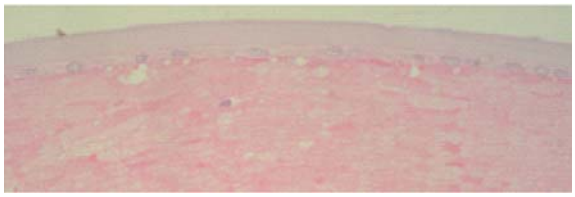

Fig. 6. Lens from the mouse irradiated with 33 Gy neutron for 100 min. Lens epithelial cells and lens fiber are swollen and showing granular changes H&E stain, ×400.

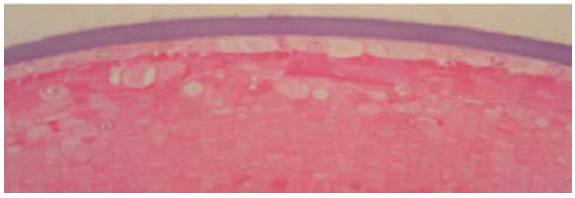

Fig. 7. Lens from the mouse pretreated with boronophenylalanine and irradiated with 33 Gy neutron for 100 min. Lens epithelial cells and lens fiber are swollen and showing granular changes and a few vacuoles are evident. H&E stain, ×400.

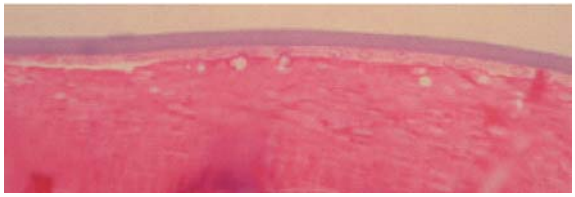

Fig. 8. Lens from the mouse pretreated with sodium borocaptate and irradiated with 33 Gy neutron for 100 min. Lens epithelial cells and lens fiber are swollen and showing granular changes and a few vacuoles are evident. H&E stain, ×400.

Eyes: The nucleated lens fibers below the single layer of cuboidal epithelium showed normal histology in the specimens obtained from the mice in groups 1 and 2 (Fig. 5). Lens epithelial cells and lens fibers were swollen and showed granular changes and a few vacuoles in groups 3, 4 and 5 (Figs. 6, 7 and 8). Only one mouse in group 5 showed more severe damages the eyes.

Discussion

In the present study, histopathological changes of eyes and testis were assessed in 45 days of neutron irradiation with or without pretreatment of boron compounds in mice. It has been reported that irradiation induces several histopathological consequences in the organs and tissues, including the increase of loose fibrous tissues, infiltration of macrophages and polymorphonuclear leukocytes, and vacuolation of epithelial cells in the choroid plexus, desquamation of the skin and ocular changes consisting of keratitis, blepharitis, conjunctivitis, cataract formation, and morphological changes in retina [11,14,21]. Our study demonstrates that 33 Gy neutron irradiation for 100 min induced cataract in eyes of the mouse, although it was

predicted the threshold dose that may cause damage to retinal structures is 250 cGy [11]. Thirty three Gy neutron did not induce other ocular lesions such as retinal changes and keratitis. Due to the geometry of the mouse's head, the eyes were close to the center of the collimated beam, and would have received a significant radiation dose [14]. Other parameters, such as examination methods, exposure time, dose of neutron, and differences in beam designs, may be related to the results. Since the lens have been recognized as a "biological dosimeter" for gauging radiation responses, a standardized methodology of appraisal of eye lesions has to be established.

The testis is also a main target organ of irradiation damage. In the this study, a severe tubular atrophy with complete loss of germ cells was noted in some seminiferous tubules in the mice irradiated with neutron for 100 min. However, other tubules showed normal spermatogenesis with degenerating germ cells within a normal range. This result indicates the spermatogonial damages by neutron could induce permanent impairment of spermatogenesis. The most neutron-sensitive spermatogenic germ cells are spermatogonia when the majority of these cells are in G0 phase. However, the cells are most resistant when they are stimulated for proliferation, and exhibit intermediate sensitivities during active proliferation [20]. Spermatogonial depletion by testicular toxicants results in seminiferous tubules atrophy by impairment of spermatogenesis [18]. In addition, 30 min neutron irradiation did not induce histopathological changes in the eyes and testes. Further studies on time-cause observation of spermatogenesis and ocular changes immediately after irradiation are required in order to investigate this.

BNCT is a binary system for treatment of cancers which is based on absorption of low-energy neutrons by nonradioactive boron-10 (^{10}B) atoms delivered to neoplastic cells in the form of a boron carrying drug [2,6,10,22]. Over the past 20 years, many classes of boron-containing compounds have been designed and synthesized. Those compounds include boron-containing amino acids, biochemical precursors of nucleic acids, DNA-binding molecules, porphyrin derivatives, high molecular weight delivery agents such as monoclonal antibodies and their fragments that recognize a tumor-associated epitopes, such as epidermal growth factor, and liposomes. Two ^{10}B delivery agents, amino acid p-boronophenylalanine (BPA) and sulfhydryl borane (BSH), are being used in current clinical protocols for the treatment of cancers [7]. BSH, which was developed by Soloway [17], has been applied in malignant glioma patients in Japan [9]. Subsequently, BPA, a melanin precursor, was developed for BNTC of melanomas and is in clinical trials as a neutron capture agent in glioma patients [4,12].

This study shows that neutron irradiation induced testis atrophy and lens degeneration in a time and dose-dependent manner. However, boron carriers, BPA and BSH had no significant effect on the lesions, except one case of mouse showing more severe lesions in the lens by BPA. Studies have been carried out in both normal and neoplastic tissues to characterize the relative biological effectiveness of each radiation component. The distribution patterns and radiobiological characteristics of the two ^{10}B delivery agents in current clinical use have been evaluated in a range of normal tissues and tumor types [5]. Wide range of differences in the distribution of the ^{10}B-labeled compound in tissues have been reported [19]. In addition, BPA or BSH are mutagenic and the retention of these boron compounds in the cells causes accurate assaults on the cell and lessens the chance of misrepair after neutron irradiation [13]. These previous reports have been warranted further studies on toxicological effects in other organs under various experimental conditions.

In conclusion, 33Gy (flux: 1.036739E+09, Fluence 9.600200E+12) neutron with lying flat pose in mice, induced atrophy of some seminiferous tubules and swelling, vacuolation and granular changes of lens epithelial cells and lens fiber in an exposure time dependent manner in mice. In addition, Pretreatment of boron carriers, BSH or BPA had no significant effect on the lesions. These data demonstrated that histopathological changes of lens and testis could be used as "biological dosimeters" for gauging radiation responses and the HANARO Nuclear Reactor has sufficient capacity for the BNCT. Further investigations regarding the precise dose distribution and a wide range of preclinical studies should be conducted for human clinical trials.

Acknowledgments

This work was supported by grant No. 2003-6819 from the Office of Academic Promotions, Chungnam National University, Korea

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