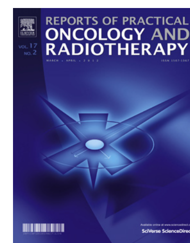
Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/rpor



Original research article

Detection of γH2AX foci in mouse normal brain and brain tumor after boron neutron capture therapy

Natsuko Kondo[a,*], Hiroyuki Michiue[c], Yoshinori Sakurai[b], Hiroki Tanaka[b], Yosuke Nakagawa[a], Tsubasa Watanabe[a], Masaru Narabayashi[a], Yuko Kinashi[a], Minoru Suzuki[a], Shin-ichiro Masunaga[a], Koji Ono[a]

[a] Particle Radiation Oncology Research Center, Research Reactor Institute, Kyoto University, Osaka, Japan
[b] Division of Medical Physics, Research Reactor Institute, Kyoto University, Osaka, Japan
[c] Department of Physiology, Okayama University, Okayama, Japan

ARTICLE INFO

Article history:
Received 9 March 2014
Received in revised form
23 July 2014
Accepted 10 October 2014
Available online 31 October 2014

Keywords:
Boron neutron capture therapy
High LET radiation
DSBs
γH2AX foci

ABSTRACT

Aim: In this study, we investigated γH2AX foci as markers of DSBs in normal brain and brain tumor tissue in mouse after BNCT.

Background: Boron neutron capture therapy (BNCT) is a particle radiation therapy in combination of thermal neutron irradiation and boron compound that specifically accumulates in the tumor. ^{10}B captures neutrons and produces an alpha (^{4}He) particle and a recoiled lithium nucleus (^{7}Li). These particles have the characteristics of extremely high linear energy transfer (LET) radiation and therefore have marked biological effects. High LET radiation causes severe DNA damage, DNA DSBs. As the high LET radiation induces complex DNA double strand breaks (DSBs), large proportions of DSBs are considered to remain unrepaired in comparison with exposure to sparsely ionizing radiation.

Materials and methods: We analyzed the number of γH2AX foci by immunohistochemistry 30 min or 24 h after neutron irradiation.

Results: In both normal brain and brain tumor, γH2AX foci induced by ^{10}B(n,α)^{7}Li reaction remained 24 h after neutron beam irradiation. In contrast, γH2AX foci produced by γ-ray irradiation at contaminated dose in BNCT disappeared 24 h after irradiation in these tissues.

Conclusion: DSBs produced by ^{10}B(n,α)^{7}Li reaction are supposed to be too complex to repair for cells in normal brain and brain tumor tissue within 24 h. These DSBs would be more difficult to repair than those by γ-ray. Excellent anti-tumor effect of BNCT may result from these unrepaired DSBs induced by ^{10}B(n,α)^{7}Li reaction.

© 2014 Greater Poland Cancer Centre. Published by Elsevier Sp. z o.o. All rights reserved.

* *Corresponding author at*: Particle Radiation Oncology Research Center, Research Reactor Institute, Kyoto University, Asashiro-Nishi, Kumatori-cho, Sennan-gun, Osaka 590-0494, Japan. Tel.: +81 72 451 2425; fax: +81 451 2627.
E-mail address: nkondo@rri.kyoto-u.ac.jp (N. Kondo).

http://dx.doi.org/10.1016/j.rpor.2014.10.005
1507-1367/© 2014 Greater Poland Cancer Centre. Published by Elsevier Sp. z o.o. All rights reserved.

1. Background

Boron neutron capture therapy (BNCT) is a modality that involves the selective uptake of boron-10 (^{10}B) compounds by tumor cells and subsequent irradiation using thermal or epithermal neutrons. ^{10}B absorbs a thermal neutron with an extremely higher probability than ^{14}N, the element with the largest cross section in the human body. The subsequent nuclear fission reaction yields an α (^{4}He) particle and a lithium (^{7}Li) nucleus, with high linear energy transfer (LET) values of 163 and 210 keV/μm, respectively. Another important characteristic of these particles is their extremely short track ranges, which are generally shorter than the diameters of tumor cells. In Kyoto University Research Reactor Institute (KUR), more than 400 cancer patients have been treated with BNCT since 1990, obtaining good results even after other conventional radiation and chemotherapies. Especially, malignant glioma patients have had great benefit from BNCT.[1–3] The neutron beam used for BNCT is generated by a nuclear reactor and is inevitably a mixed beam that contains thermal, epithermal and fast neutrons, as well as γ-rays. Understanding the biological effects of the mixed beam will help improve the efficiency of this therapy and reduce the side effects in normal tissue. The biological effects of neutrons have been studied with respect to DNA damage,[4,5] but little is known about the effects induced by the mixed beam in BNCT. Among biological endpoints, DNA double-strand breaks (DNA-DSBs) are important and have been frequently used in many recent studies. The numbers of foci that immunochemically stain for γH2AX and other DNA repair proteins have previously been shown to be related to the number of DNA-DSBs and to be efficient markers for monitoring DNA-DSB induction and repair.[6,7] As the high LET radiation induces complex DNA DSBs, large proportions of DSBs are considered to remain unrepaired in comparison with exposure to low LET radiation.[8,9]

2. Aim

In this study, we investigate γH2AX foci as markers of DSBs in normal brain and brain tumor in mouse after neutron irradiation by immunohistochemistry.

3. Materials and methods

3.1. *Cell line and culture conditions*

The human glioblastoma U251 cells was cultured in Dulbecco's modified Eagle's medium (Sigma–Aldrich Co. LLC, St. Louis, MO, USA) supplemented with 10% fetal bovine serum, and maintained at 37 °C in an atmosphere of 95% air and 5% CO_2.

3.2. *Animals and brain tumor model*

Female BALB/C nu-nu mice, aged 8 weeks, were purchased from Japan Animal Co., Ltd, Osaka, Japan. The mice were anesthetized with an intraperitoneal injection of Nembutal (50 mg/kg) and placed in a stereotactic frame (Narishige, Japan). A midline scalp incision was made and the bregma was identified. A 1 mm burr hole was made in the right frontal region of the skull and a Hamilton syringe was inserted 4 mm into the dura. An injection of 10^6 human glioblastoma U251 cells in 5 μl of serum free medium was administered at a rate of 1 μl/min. After the infusion, the needle was left in place for 2 min and the burr hole was then covered with bone wax.

3.3. *Drug treatment*

A stock solution of boronophenylalanine (BPA) was used for all experiments. The ^{10}B concentrations were measured by means of prompt γ-ray spectrometry using a thermal neutron guide tube installed at the KUR and the value was about 1000 ± 4.55 ppm. This stock solution consisted of sterilized water, glucose and BPA. BPA (500 mg/kg) or saline was administered subcutaneously 1 h before neutron irradiation.

3.4. *Radiation sources and neutron fluences*

The Heavy Water Neutron Irradiation Facility of the KUR was used for 1 MW neutron irradiation (1 h irradiation). The thermal neutron fluences were measured by gold foil (3 mm in diameter, 0.05 mm thick) activation analysis. The epithermal and fast neuron fluences were estimated by the normalization of the nominal values using the measured thermal neutron fluences. Contaminating gamma rays, including secondary gamma rays, were measured with thermoluminescence dosimeter (TLD). The TLD used was beryllium oxide (BeO) enclosed in a quartz glass capsule. BeO itself is sensitive to thermal neutrons. In terms of TLD sensitivity, the thermal neutron fluence of 8×10^{12} cm^{-2} is equal to approximately 1 cGy gamma-ray dose. For the neutron-sensitivity correction, gold activation foil was placed together with TLD. The average neutron fluxes were 1.0×10^9 cm^{-2} s^{-1} for the thermal neutron range (less than 0.5 eV), 1.6×10^8 cm^{-2} s^{-1} for the epithermal neutron range (0.5 eV–10 keV), and 9.4×10^6 cm^{-2} s^{-1} for the fast neutron range (more than 10 keV). The total absorbed doses were calculated as the sum of the absorbed doses attributed primarily to $^{1}H(n,n)^{1}H$, $^{14}N(n,p)^{14}C$, $^{10}B(n,\alpha)^{7}Li$ and contaminating γ-rays. The dose-converting coefficients and details of the calculation method were described previously.[10] The percentage of ^{14}N was assumed to be 2%. The cobalt-60 γ-ray source at our institute was used for the γ-ray irradiations at the dose rate of 2.7 Gy/h.

3.5. *Analysis of ^{10}B concentrations in tissue*

^{10}B concentration of normal brain was analyzed by prompt γ-ray analysis using a thermal neutron guide tube installed at KUR.

3.6. *Detection of γH2AX foci by immunohistochemistry (IHC)*

After 30 min and 24 h, the mice were sacrificed and the brains were flash-frozen. Sections of 7-μm thickness were cut on a microtome (CM 1850; Leica Microsystems, Wetzlar, Germany). Briefly, frozen tissue sections were fixed with 4% paraformaldehyde buffer solution for 30 min. The slices were then permeabilized with 0.25% Triton X-100 for 15 min,

Download English Version:

https://daneshyari.com/en/article/1855551

Download Persian Version:

https://daneshyari.com/article/1855551

Daneshyari.com