Applied Radiation and Isotopes 88 (2014) 59–63

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Boron neutron capture therapy as new treatment for clear cell sarcoma: Trial on different animal model

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HIGHLIGHTS

- BNCT with the use of L-BPA was applied for three human clear cell sarcoma (CCS) cell lines.
- BNCT trial was performed on a newly established intramuscularly CCS-bearing animal model.
- A significant decrease of the tumor-volume was seen by single BNCT with the use of L-BPA.
- A multiple BNCT application would be required for controlling the growth of any residual tumors.

ARTICLE INFO

Available online 17 December 2013

Keywords:
Biodistribution
p-Borono-L-phenylalanine (L-BPA)
Clear cell sarcoma (CCS)
Boron neutron capture therapy (BNCT)
Neutron irradiation

ABSTRACT

Clear cell sarcoma (CCS) is a rare malignant tumor with a poor prognosis. In our previous study, the tumor disappeared under boron neutron capture therapy (BNCT) on subcutaneously-transplanted CCS-bearing animals. In the present study, the tumor disappeared under this therapy on model mice intramuscularly implanted with three different human CCS cells. BNCT led to the suppression of tumor-growth in each of the different model mice, suggesting its potentiality as an alternative to, or integrative option for, the treatment of CCS.

1. Introduction

Clear cell sarcoma (CCS) of tendons and aponeuroses is a rare malignant tumor, called "melanoma of soft parts" because of the presence of melanin (Chung and Enzinger, 1983). It has a predilection for young adults between 20 and 40 years of age; the standard treatment is wide surgical resection, and neither common chemotherapy nor radiotherapy is effective. Since treatment other than surgical resection is lacking, a new clinical approach for the management of CCS is required.

In BNCT with the use of *p*-borono-L-phenylalanine (L-BPA), the system of L-amino acid transporter-1 (LAT-1) carries L-BPA into tumor cells and may play a major role in the efficacy of this therapy (Detta and Cruickshank, 2009). Moreover, malignant melanoma cells preferentially take up L-BPA because its chemical structure is similar to that of tyrosine requisite for melanogenesis (Mishima and Kondoh, 2000). CCS is also capable of producing melanin. As in melanogenesis, high L-BPA uptake by CCS is a promising possibility. Indeed, we have demonstrated a remarkably high uptake of ^{10}B (80 μg ^{10}B/g cells) by CCS exposed to a cell-culture medium containing L-BPA in vitro (Fujimoto et al., 2011).

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http://dx.doi.org/10.1016/j.apradiso.2013.12.007

Moreover, high-level accumulation of ^{10}B (45 μg ^{10}B/g of wet tumor tissue) has been observed after the intravenous administration of L-BPA-Fructose complex (BPA-Fr) to CCS-bearing mice in vivo (Andoh et al., 2011). This high accumulation has led to the disappearance of tumors from mice treated by BNCT (Fujimoto et al., 2013).

Thus, BNCT with the use of L-BPA could evolve into a new clinical option for the treatment of CCS, provided that a marked effect of this therapy in preclinical studies can be attained in vivo for different types of CCS-bearing animal models. With that in mind, biodistribution studies and in vivo BNCT trials with the use of L-BPA were carried out on tumor-bearing animal models established by intramuscularly implanting three human CCS cell lines (HS-MM, MP-CCS-SY and SU-CCS-1) in nude mice.

2. Materials and methods

2.1. Chemicals

L-BPA (^{10}B enriched) was kindly supplied by Stella Pharma Corporation (Osaka, Japan). Fructose, perchloric acid ($HClO_4$, 60%), hydrogen peroxide (H_2O_2, 30%) and boron standard solution (1000 μg/mL) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). L-BPA was used as a fructose complex (BPA-Fr, 4000 μg ^{10}B/mL) (Yoshino et al., 1989).

2.2. Cells

Human cell lines, HS-MM (Sonobe et al., 1993), MP-CCS-SY (Moritake et al., 2002) and SU-CCS-1 (Epstein et al., 1984) were cultured in RPMI-1640 medium containing penicillin (100 U/mL), streptomycin (100 μg/mL) and heat-inactivated fetal bovine serum (10% for HS-MM and MP-CCS-SY, 15% for SU-CCS-1), and incubated in a humidified atmosphere of 5% CO_2 in air at 37 °C.

2.3. Tumor bearing animals

All animal experiments were conducted according to the regulations of the Animal Care and Use Committee of Hyogo Cancer Center (Akashi, Japan), Kobe Gakuin University (Kobe, Japan) and Kyoto University Research Reactor Institute, KURRI (Osaka, Japan). Four-weeks-old female BALB/cAJcl-nu/nu nude mice (body weight of approximately 15 g) were purchased from CLEA Japan, Inc. (Tokyo, Japan). To establish CCS-bearing animal models, 0.1 mL of the culture medium containing 1×10^7 cells of HS-MM, MP-CCS-SY or SU-CCS-1 was intramuscularly implanted into the left femoral region of the mice. The three animal models were used in biodistribution studies of ^{10}B and in BNCT trials.

2.4. Biodistribution of ^{10}B

About four weeks after the implantation, the CCS tumors in the mice (body weight of approximately 20 g) grew to about 10 mm in diameter. BPA-Fr (24 mg ^{10}B/kg) was intravenously administered through the femoral vein of each mouse under anesthesia with diethyl ether. At predetermined time intervals, blood samples were collected by cardiac puncture, the residual blood in the organs was removed by saline perfusion, and the mice were euthanized with diethyl ether. Tissue samples of the kidney, the liver and the tumor were collected immediately, washed with saline and lightly blotted to remove any excess blood and water. Skin and muscles were then collected from the nates of the mice. Kidney, liver and tumor tissues were homogenized in a high-speed homogenizer.

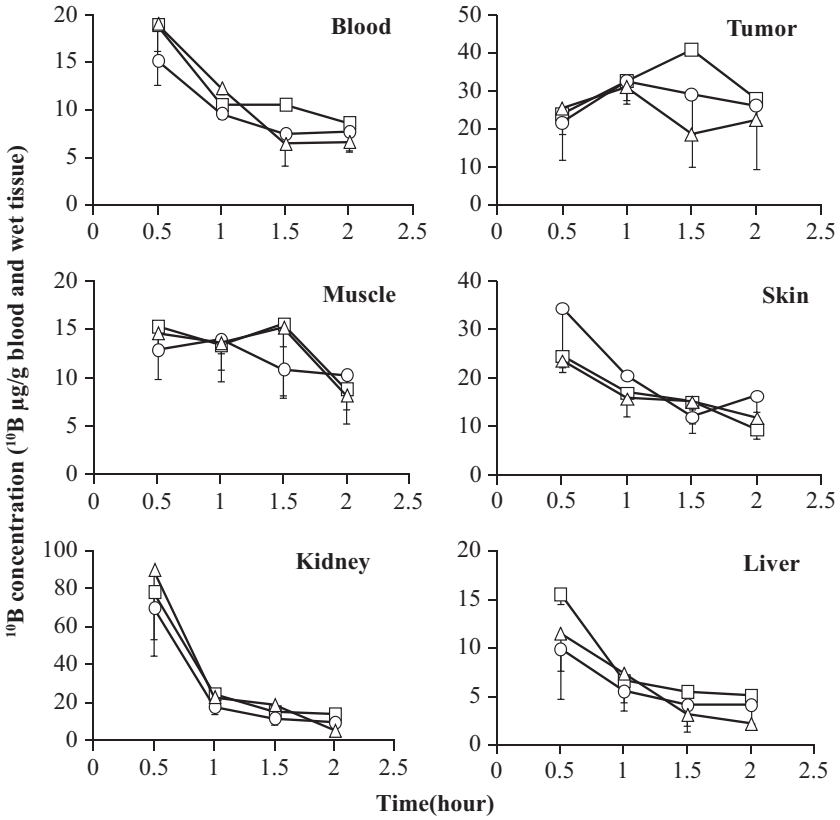

Fig. 1. Time-course changes of ^{10}B concentration in blood and tissues after i.v. administration of BPA-Fr (24 mg ^{10}B/kg) to nude mice bearing HS-MM (○), MP-CCS-SY (□) and SU-CCS-1 (Δ). Each value represents the mean ± S.D. ($n=3$).