Applied Radiation and Isotopes ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

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

Autoradiographic and histopathological studies of boric acid-mediated BNCT in hepatic VX2 tumor-bearing rabbits: Specific boron retention and damage in tumor and tumor vessels

C.H. Yang [a,e], Y.T. Lin [b], Y.H. Hung [c], J.W. Liao [d], J.J. Peir [b], H.M. Liu [b], Y.L. Lin [f], Y.M. Liu [g], Y.W. Chen [g], K.S. Chuang [a], F.I. Chou [b,c,*]

[a] Department of Biomedical Engineering & Environmental Sciences, National Tsing Hua University, Taiwan, ROC
[b] Nuclear Science and Technology Development Center, National Tsing Hua University, Taiwan, ROC
[c] Institute of Nuclear Engineering and Science, National Tsing Hua University, Taiwan, ROC
[d] Graduate Institute of Veterinary Pathobiology, National Chung Hsing University, Taiwan, ROC
[e] Department of Imaging Diagnosis, Taoyuan General Hospital, Ministry of Health and Welfare, Taiwan, ROC
[f] Department of Radiology, Mackay Memorial Hospital, Hsinchu, Taiwan, ROC
[g] Department of Oncology, Taipei Veterans General Hospital, Taiwan, ROC

HIGHLIGHTS

- ^{10}B-enriched boric acid (BA) was used for BNCT in hepatic tumor-bearing rabbits.
- BA was selectively targeted to tumors and tumor blood vessels.
- Radiation damage was concentrated in tumor regions during BA-mediated BNCT.
- Selective destruction to tumor regions led to the success of BA-mediated BNCT.

ARTICLE INFO

Article history:
Received 30 January 2015
Received in revised form
7 August 2015
Accepted 25 August 2015

Keywords:
Autoradiography
Histopathological observation
Boric acid-mediated BNCT
Hepatoma

ABSTRACT

Hepatoma is a malignant tumor that responds poorly to conventional therapies. Boron neutron capture therapy (BNCT) may provide a better way for hepatoma therapy. In this research, ^{10}B-enriched boric acid (BA, 99% ^{10}B) was used as the boron drug. A multifocal hepatic VX2 tumor-bearing rabbit model was used to study the mechanisms of BA-mediated BNCT. Autoradiography demonstrated that BA was selectively targeted to tumors and tumor vessels. Histopathological examination revealed the radiation damage to tumor-bearing liver was concentrated in the tumor regions during BNCT treatment. The selective killing of tumor cells and the destruction of the blood vessels in tumor masses may be responsible for the success of BA-mediated BNCT for liver tumors.

1. Introduction

Liver cancer is the second most common cause of death from cancer worldwide, estimated to be responsible for nearly 746,000 deaths in 2012 (9.1% of the total). The prognosis for liver cancer is very poor (overall ratio of mortality to incidence of 0.95) (Ferlay et al., 2015). Hepatomas can be eliminated in its early stage. However, most hepatoma patients are diagnosed at an advanced stage (Chung et al., 2006). Chemotherapy, surgical treatment and traditional radiation therapies are limited and do not eradicate the hepatoma, so a new effective therapy must be developed.

Boron Neutron Capture Therapy (BNCT) is a binary form of radiation therapy. A boron drug is administered to the patient which results in the selective uptake of ^{10}B in the tumor regions. The tumor is then irradiated with thermal neutrons or epithermal neutrons. Subsequently, the high linear energy transfer (LET) α and ^{7}Li particles are generated by the ^{10}B(n, α)^{7}Li reaction. Because the high LET particles have limited path lengths in tissue (5–9 μm), their destructive effects are limited to boron-containing cells (Barth et al., 2005). BNCT can provide an effective means of delivering curative doses to tumors while sparing normal liver

* Corresponding author at: Nuclear Science and Technology Development Center, National Tsing Hua University, Taiwan, ROC.

http://dx.doi.org/10.1016/j.apradiso.2015.08.034
0969-8043/

tissue and is therefore favored for hepatoma therapy.

BPA (boronphenylalanine) and BSH (sodium borocaptate) have been used as boron-containing drugs in clinical trials of BNCT in Japan, United States, Europe (such as Sweden and Finland), Argentina and Taiwan (Barth et al., 2005; Wang et al. .2011). BSH is mainly used in BNCT treatment for malignant glioma. However, BSH has been shown not to accumulate selectively in tumor cells either *in vitro* or *in vivo* (Obayashi et al., 2004). Furthermore, the use of BSH as a boron drug has resulted in only low tumor-to-liver boron ratios (Suzuki et al., 2000). BPA is an analog of phenylalanine, which can be transported by the L-amino acid transport (LAT) into cells because its structure is similar to that of phenylalanine. Malignant tumors and tumor cell lines express more LAT-1 than do normal cells. Therefore, BPA accumulates significantly in tumor cells, resulting in effective BPA-mediated BNCT (Wittig et al., 2000).

However, evaluations of 4-borono-2-^{18}F-fluoro-L-phenylalanine-fructose as a probe for BNCT in a glioma-bearing rat model have revealed that BPA accumulates more in the pancreas than in liver (Wang et al., 2004). Additionally, our previous *in vivo* experiments shown the accumulation of a high boron concentration in the pancreas both in SD rats that were administered BPA via intraperitoneal injection and in BALB/c nude mice that were administered BPA via intravenous injection (Chou et al., 2009; Lin et al., 2012). The pancreas is a limiting organ in the BPA-mediated BNCT of liver tumors because it accumulates a high concentration of BPA and is adjacent to the liver. When a therapeutic dose is delivered in the tumor, severe damage is very likely to be induced in the pancreas owing to the high doses that are delivered on account of the high B-10 concentration.

Although BSH and BPA potentially provide a higher boron concentration to particular tumor cells without increased toxicity (Barth et al., 2005), these two boron drugs are not hepatoma-specific. Therefore, another boron drug for BNCT for hepatoma therapy should be developed. To achieve effective BNCT, the ideal boron drug must accumulate in a large amount in the tumor tissue (Barth et al., 2005). In our previous experiments, normal tissue toxicity and the therapeutic efficacy of BA-mediated BNCT in the hepatoma-bearing rat and the VX2 multifocal liver tumor-bearing rabbit models were evaluated. According to a color Doppler scan, BA-mediated BNCT clearly reduced the blood flow and tumor size, and eventually the tumor disappeared. The therapeutic response that was detected by ultrasound scanning was consistent with the results of the CT scan (Lin et al. .2013; Hung et al., 2014). The BA is presumed to be selectively taken up in tumor regions, and tumor therapy is associated with tumor vessel disrupture. The retention and microdistribution of BA in a tumor-bearing liver when BA-mediated BNCT is used against a hepatoma must be examined. The local concentration of boron in the various regions of tissue samples that have been treated by BNCT can be determined by evaluating the number of tracks in autoradiography images (Altieri et al., 2008). Thus, autoradiographic and histopathological assessments were conducted in preclinical studies to evaluate the microdistribution of boron and the radiobiological effects in tumor-bearing liver tissues when BA-mediated BNCT was used against a hepatoma in a VX2 multifocal liver tumor-bearing rabbit model.

2. Materials and methods

2.1. Multifocal hepatic VX2 tumor-bearing rabbit model

A multifocal hepatic VX2 tumor-bearing rabbit model was used to study the mechanisms of BA-mediated BNCT. Three- to five-month-old male New Zealand White rabbits (2.5–3.5 kg) were obtained from the Livestock Research Institute, Council of Agriculture, Executive Yuan, ROC. Before the experiment was performed, the rabbits were kept in a cage for at least one week at a temperature of 20 ± 2 °C and a humidity of 50–60%. A VX2 tumor was subcultured in the leg muscle of the rabbit (Georges et al., 1985). All surgical procedures were performed while the animals were under anesthesia by Xylazine (6 mg/kg bw) and Zoletil® 50X (5 mg/kg bw), Enrofloxacin (10 mg/kg bw) to prevent infection, and Ketoprofen (3 mg/kg bw) as an analgesic. The VX2 tumor was surgically removed from a donor rabbit, and cut into $1 \times 1 \times 2$ mm^3 pieces. VX2 tumor tissue fragments were implanted in the left lobe of the liver of the NZW rabbits using a 16G indwelling cannula. When the tumor had grown to a size of approximately 1 to 2 cm in diameter, the rabbits were ready for autoradiography and histopathological assessment to evaluate the effectiveness of the BA-mediated BNCT. The animal experiment was approved and supervised by the Institutional Animal Care and Use Committee, NTHU.

2.2. Autoradiography

The microdistribution of boron in the tumor-bearing liver was investigated by neutron capture autoradiography (Lin et al., 2012). BA powder, dissolved in a normal saline solution, was administered (50 mg ^{10}B/kg bw) from a marginal ear vein via a bolus injection. Three rabbits were sacrificed on the 5th day, and four were sacrificed on the 26th day after BNCT. The rabbit was sacrificed 35 min following the BA injection, and the tumors were removed and frozen for autoradiography analysis. The waiting time following BA injection is determined from the pharmacokinetic data obtained using the VX2 multifocal liver tumor-bearing rabbit model (Hung et al., 2014). The quantitative boron distribution was evaluated by alpha tracks analysis using an ImageJ program. The neutron autoradiograph was compared to that of a histologically prepared slice that had been cut just after the sample which had been used for quantitative analysis.

2.3. Computed tomography

When the diameter of the tumor is above 1.5 cm, BNCT treatment was delivered and its radiobiological effects subsequently evaluated. A CT scan of the tumor and neighboring organ localization was performed, and blood flow was conducted at Mackay Memorial Hospital, HsinChu on the day before BNCT. During the examination, rabbits were anesthetized with Zoletil® 50X (2 mg/kg) and Xylazine (5 mg/kg). During CT scan, the rabbits were positioned in a manner that was consistent with their position during BNCT treatment. An unenhanced baseline scan was conducted, arterial phase images and venous phase images were captured. From the CT scan images, the positions of the tumors and normal tissues were determined.

2.4. BNCT treatment

Each rabbit was intravenously injected with BA (50 mg ^{10}B /kg bw) using a 22-G catheter that was inserted into the marginal ear vein. Ten minutes following BA administration, the rabbits were injected with Xylazine (6 mg/kg) and Zoletil® 50X (3 mg/kg) to anesthetize them during neutron irradiation. The neutron irradiation was started from 35 min following BA administration. Each rabbit was placed on the holder with its tumor-bearing area close to the beam aperture of the Tsing Hua Open-pool Reactor (THOR). During BNCT irradiation, the VX2 tumor-bearing rabbits were kept lying on their right side using an L-style board on the irradiation holder. The physical dose in THOR-BNCT was calculated using Monte Carlo N-particle transport code (MCNP 4C code). The

Please cite this article as: Yang, C.H., et al., Autoradiographic and histopathological studies of boric acid-mediated BNCT in hepatic VX2 tumor-bearing rabbits: Specific boron.... Appl. Radiat. Isotopes (2015), http://dx.doi.org/10.1016/j.apradiso.2015.08.034