Applied Radiation and Isotopes 69 (2011) 1778–1781

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

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

Boronated unnatural cyclic amino acids as potential delivery agents for neutron capture therapy

George W. Kabalka [a], Aarif L. Shaikh [a], Rolf F. Barth [b], Tianyao Huo [b], Weilian Yang [b], Pamela M. Gordnier [c], Subhash Chandra [c,*]

[a] Departments of Radiology and Chemistry, The University of Tennessee, Knoxville, TN, USA
[b] Department of Pathology, The Ohio State University, Columbus, OH, USA
[c] Department of Biomedical Engineering and Cornell SIMS Laboratory, Cornell University, Ithaca, NY, USA

ARTICLE INFO

Available online 31 March 2011

Keywords:
Boron neutron capture therapy
Unnatural amino acids
SIMS
Imaging mass spectrometry
Boronophenylalanine
Boron imaging in single cells

ABSTRACT

Boron delivery characteristics of *cis* and *trans* isomers of a boronated unnatural amino acid, 1-amino-3-boronocyclopentanecarboxylic acid (ABCPC) were tested in the B16 mouse model for human melanoma. Both ABCPC isomers delivered comparable boron to B16 melanoma tumor cells as L-*p*-boronophenylalanine (BPA). Secondary ion mass spectrometry (SIMS) analysis revealed the presence of boron throughout the tumor from these compounds, and a near homogeneous distribution between the nucleus and cytoplasm of B16 cells grown *in vitro*. These encouraging observations support further studies of these new boron carriers in BNCT.

1. Introduction

The development of new and more effective tumor-selective boron carriers than sodium borocaptate (BSH) and boronophenylalanine (BPA) would significantly improve the efficacy of boron neutron capture therapy of cancer (Barth et al., 2005; Kabalka and Yao, 2006; Li et al., 2006; Zhu et al., 2010). We previously have reported that a class of boronated unnatural cyclic amino acids had enhanced *in vitro* and *in vivo* tumor selectivity, which potentially could be far superior to BPA and BSH (Kabalka et al. 2004, 2009). One of these amino acids, 1-amino-3-boronocyclopentanecarboxylic acid (ABCPC), attained a tumor to blood ratio (T:Bl) of 8 and a tumor to normal brain ratio (T:Br) of ~21 in a murine melanoma model (Kabalka et al., 2004). ABCPC initially was synthesized and tested as a mixture of racemic diasteromers (*cis* and *trans* isomers) along with each of their enantiomers. Further separation of ABCPC into single enantiomers might result in compounds with enhanced selectivity for tumor cells. This study evaluates the biodistribution of *cis* and *trans* isomers of ABCPC (as racemic mixtures of L- and D-forms) in the B16 mouse model for human melanoma. Since localization of boron atoms within the nucleus results in more favorable radiobiologic microdosimetry for the $^{10}B(n,\alpha)^{7}Li$ capture reaction (Kobayashi and Kanda, 1982; Gabel et al., 1987), we have also employed secondary ion mass spectrometry (SIMS) to study the subcellular localization of boron atoms.

2. Materials and methods

2.1. Boron biodistribution studies in B16 mouse model for human melanoma

We have synthesized and separated the two racemic diasteriomers of ABCPC (*cis* and *trans* isomers) containing a mixture of L and D enantiomers (Kabalka et al., 2009). These compounds are water soluble and were dissolved directly in phosphate buffered saline (PBS) for studies in the B16 mouse model for human melanoma. L-*p*-boronophenylalanine (BPA) in the form of a fructose complex was used for a comparison of boron-delivery characteristics to ABCPC compounds.

Female BALB/c mice were injected subcutaneously with 10^6 B16 melanoma cells. After 8–10 days, when the tumors reached a diameter of ~1 cm, biodistribution studies were initiated. Compounds were administered intraperitoneally (i.p.) to the tumor bearing mice. The dose of each compound was equivalent to 24 mg boron/kg body weight (b.w). Mice were euthanized 2.5 h post-injection by exposure to isofluorane following which they were bled. The tumor, liver and kidneys were collected for boron determination by means of inductively coupled plasma-optical emission spectroscopy (ICP-OES). The selection of the 2.5 h time interval between administration and euthanization was based on BPA's optimal localization in tumor and blood concentrations in another melanoma model (Matalka et al., 1993). For SIMS studies of boron imaging, the tumor and adjacent muscle tissues were frozen and cryo-sectioned at 4 μm. The sections were attached to silicon wafers, freeze-dried and sputter coated with a 10 Å layer of Au/Pd for enhancing their

* Corresponding author. Tel.: +607 255 3884; fax: +607 254 4780.
E-mail address: sc40@cornell.edu (S. Chandra).

0969-8043/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.apradiso.2011.03.035

electrical conductivity for SIMS analysis with a CAMECA IMS-3f ion microscope instrument (Chandra et al., 2000).

2.2. In vitro boron imaging studies of cis and trans isomers of ABCPC with SIMS

B16 melanoma cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum, supplemented with L-glutamine and antibiotics. When the cells reached approximately 70% confluency, they were exposed to the nutrient medium containing 50 ppm boron equivalent of the racemic mixture (both L- and D-forms) of either *cis* or *trans* isomers of ABCPC for 2.5 h. Both *cis* and *trans* isomers were soluble in the nutrient medium but a slight adjustment of the pH was required to restore the pH to 7.4. After 2.5 h exposure to the test compound, the cells were cryogenically prepared with a freeze-fracture method and freeze-dried for quantitative SIMS imaging (Chandra et al., 1986; Chandra, 2010).

3. Results and discussion

3.1. Boron biodistribution studies in B16 melanoma mouse model

ICP-OES data shown in Table 1 reveal that both ABCPC compounds delivered boron concentrations to tumor cells that were equivalent to that of BPA. Although the mean boron concentrations in the blood of animals were higher for ABCPC compounds than BPA, these differences were not statistically significant ($p < 0.05$). Hepatic uptake in animals that received BPA had significantly less boron ($p = 0.01$) than those that received *cis*-ABCPC. No significant differences between the compounds were observed for boron concentrations in the kidney. In general, these observations indicate that the ABCPC compounds seem to be comparable to BPA in delivering boron to tumor cells but their blood clearance (or metabolism in the liver) may be somewhat longer than that of BPA.

3.2. SIMS imaging of subcellular boron distribution in B16 melanoma mouse model

To determine the microdistribution of boron within the tumor, we analyzed tumor tissues prepared for SIMS imaging. The CAMECA IMS-3f SIMS instrument used in this study is capable of imaging the distribution of any elements from H to U (via isotopic detection) at 500 nm spatial resolution with ppm to ppb sensitivity. Fig. 1 shows B16 tumor morphology in a cryosection that was stained with hematoxylin and eosin (H and E). The tumor was composed of a monomorphic population of cells with large, hyperchromatic nuclei and cytoplasmic melanin. SIMS analysis of an adjacent cryosection shows typical observations of boron distribution for both *cis* and *trans* ABCPC compounds in B16 tumor cells (Fig. 2). The positive secondary ion images of ^{39}K and ^{11}B, show the potassium and boron distributions in tumor cells. In ^{39}K SIMS image, some tumor cell nuclei are discernible. The boron from ABCPC compounds is distributed throughout the tumor with some degree of heterogeneity.

Table 1
Biodistribution of boron from BPA and ABCPC compounds in B16 melanoma mouse model.

Compound	*n*	Boron concentration (μg/g tissue) (mean ± SD)			
		Blood	Tumor	Liver	Kidney
BPA	4	5.1 ± 2.7	19.6 ± 5.5	5.5 ± 3.7[a]	15.1 ± 12.4
trans-ABCPC	6	9.3 ± 4.1	21.3 ± 8.9	15.1 ± 11.7	17.1 ± 9.3
cis-ABCPC	4	9.9 ± 3.3	26.5 ± 4.9	18.9 ± 8.1[b]	30.3 ± 20.2

Different superscript letters "a" and "b" indicate statistically significant difference in liver boron concentrations between the compounds as determined by means of Student's t-test.

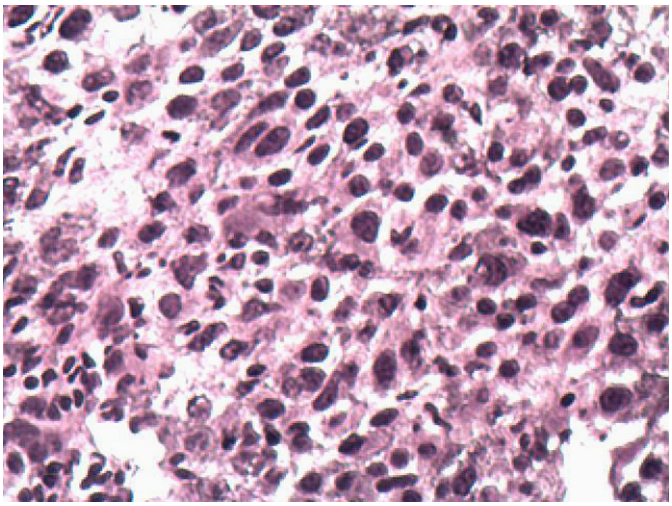

Fig. 1. H and E stained section of B16 melanoma.

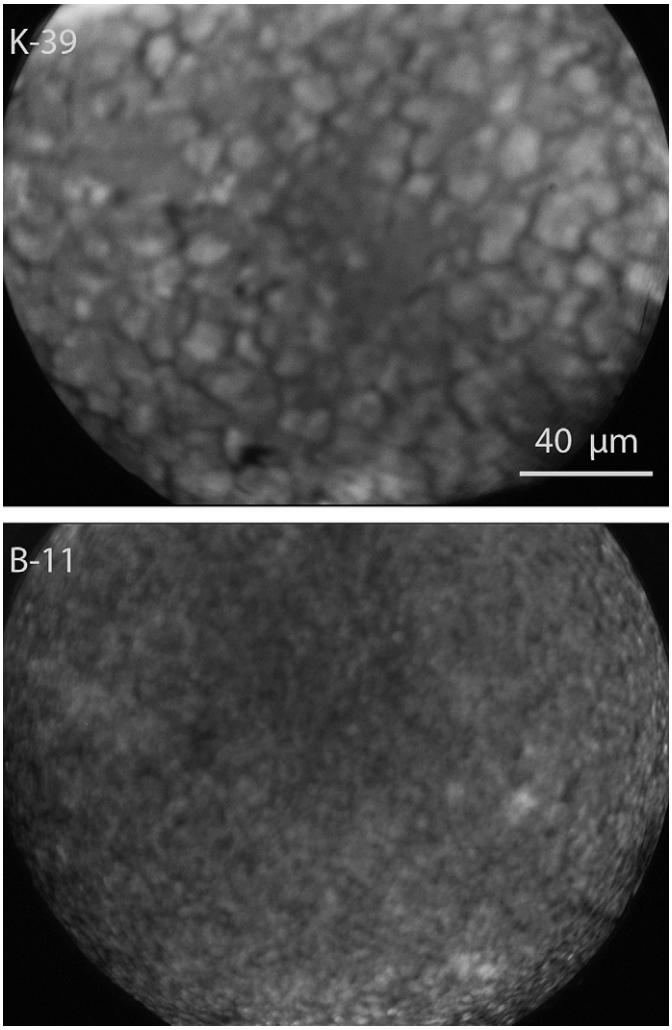

Fig. 2. SIMS images revealing the distribution of potassium-39 and boron-11 atoms in a B16 mouse melanoma tumor tissue section from *trans*-ABCPC treated animals.