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Boronated Unnatural Cyclic Amino Acids as Potential Delivery Agents for Neutron Capture Therapy

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Abstract

Boron delivery characteristics of *cis* and *trans* isomers of a boronated unnatural amino acid, 1-amino-3-boronocyclopentanecarboxylic acid (ABCPC) were tested in 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.

Keywords

Boron neutron capture therapy; unnatural amino acids; SIMS; imaging mass spectrometry; boronophenylalanine; boron imaging in single cells

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 et al., 2006; Li et al., 2006; Zhu et al., 2010). Kabalka et al. (2004 & 2009) 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. 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

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al., 1987), we have also employed secondary ion mass spectrometry (SIMS) to study the subcellular localization of boron atoms.

2. Materials and Methods

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 ~ 1cm, 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 hr 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 2.5 hr 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 electrical conductivity for SIMS analysis with a CAMECA IMS-3f ion microscope instrument (Chandra et al., 2000).

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 hr. 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 a 2.5 hr. 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

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.

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. Figure 1 shows B16 tumor morphology in a cryosection that was stained with hematoxylin & eosin (H&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. Quantitative observations from SIMS images of boron distribution in the tumor tissue revealed that there were no significant differences between BPA and the ABCPC compounds (not shown).

SIMS boron imaging of individual B16 melanoma cells grown in culture

The B16 cells grown *in vitro* served as a useful model for SIMS imaging studies for observing the subcellular distribution of boron in single cells delivered by *cis* or *trans* ABCPC compounds. Figure 3 shows a typical example of boron distribution imaged by SIMS in B16 cells after 2.5 hr. exposure to *cis* or *trans* ABCPC compounds. Figure 3 shows SIMS imaging analysis of the same three B16 melanoma cells reveal the subcellular distribution of ^{39}K, ^{40}Ca, and ^{11}B in *cis*-ABCPC treated cells. These cells had high-K and low-Na signatures (^{23}Na image not shown) representing viable tumor cells. In the ^{40}Ca SIMS image, the location of the cell nucleus is discernible in each cell due to its lower total calcium content in comparison to the cell cytoplasm which contains the calcium storing organelle, endoplasmic reticulum. The ^{11}B SIMS image reveals the subcellular distribution of ^{11}B atoms delivered by *cis*-ABCPC to individual cells in the field of view. The ^{11}B is distributed throughout the cell, including the nucleus. No significant differences were observed in boron delivery to the nucleus (or cytoplasm) of B16 melanoma cells between the c*is*-ABCPC and the *trans*-ABCPC compounds. The boron partitioning of approximately 4:1, between the cell interior to the nutrient medium, was observed after 2.5 hr. exposure of *cis* or *trans* ABCPC compounds.

4. Conclusions

Boronated unnatural amino acids are a class of compounds that are currently under development as potential delivery agents for BNCT. This study provides support for previously published data (Kabalka et al., 2009) suggesting that further studies with these compounds are warranted. Observations indicate that *cis* or *trans* ABCPC compounds, even when administered as racemic mixtures of their enantiomers (L and D isomers), are comparable to BPA in delivering boron to B16 melanoma cells both *in vitro* and *in vivo*. Separation of the L and D isomers of these compounds may provide even better boron targeting of tumor cells. The water solubility of these compounds is a valuable feature for their potential use as delivery agents for BNCT. Studies are underway for testing these compounds in the F98 rat and GL261 mouse glioma models.

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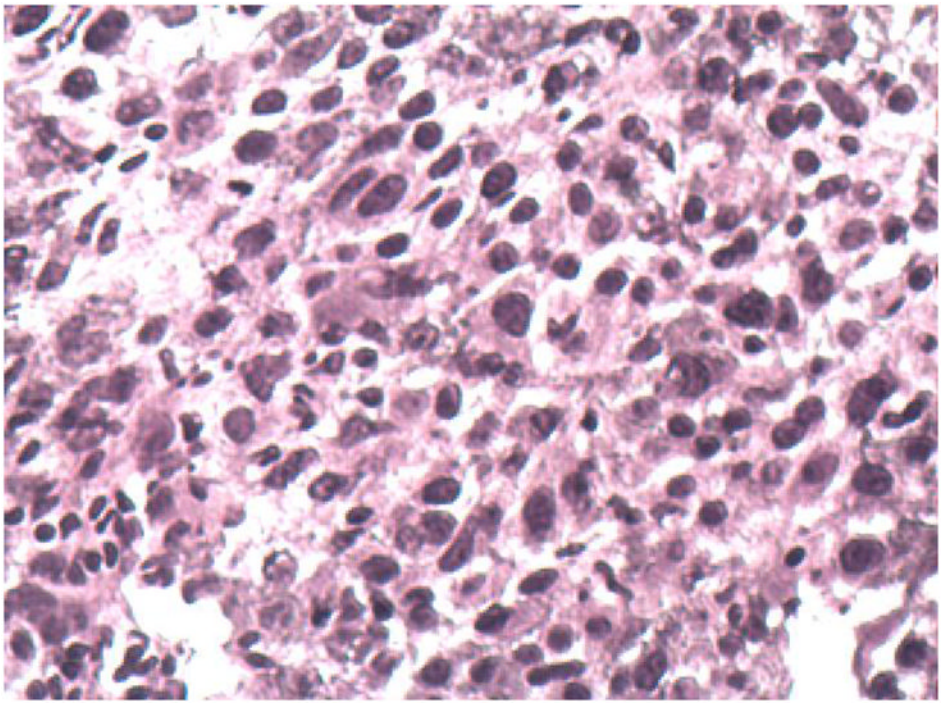

Figure 1.
H&E stained section of the B16 melanoma.

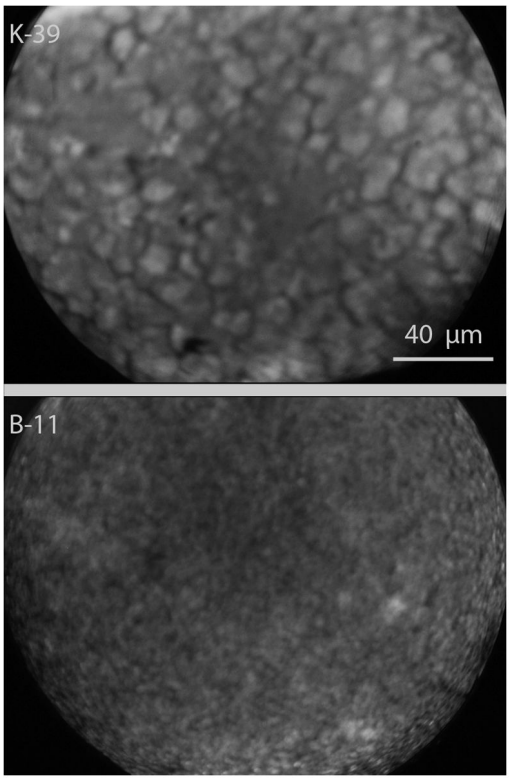

Figure 2.
SIMS images revealing the distribution of potassium-39 and boron-11 atoms in a B16 mouse melanoma tumor tissue section from *trans*-ABCPC treated animals.

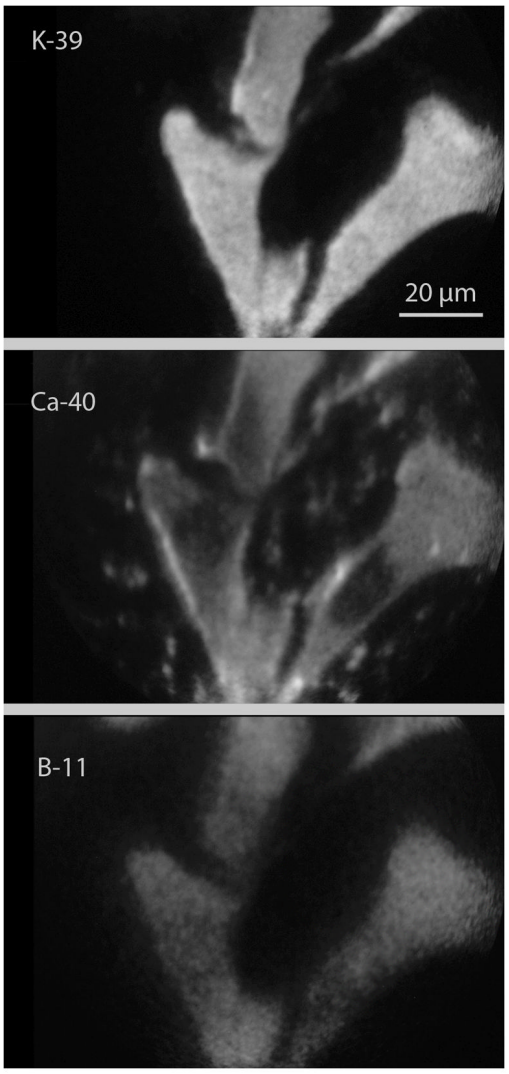

Figure 3.
SIMS images revealing the subcellular distribution of potassium, calcium, and boron atoms in B16 melanoma cells grown in cultures. The cells were treated with 50 ppm boron equivalent concentration of *cis*-ABCPC compound for 2.5 hrs.

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.

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