Biodistribution of Boron Concentration on Melanoma-bearing Hamsters after Administration of p-, m-, o-Boronophenylalanine

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Although p-boronophenylalanine (p-BPA), a boronate analogue of tyrosine, has proven to be one of the most successful compounds for boron neutron capture therapy (BNCT) of malignant melanoma, the selective uptake mechanism of this compound into melanoma cells is not well understood. Therefore, the relationship between the structure of BPA and its specific affinity to melanoma cells appears worthy of investigation. In the present study, m- and o-boronophenylalanine (m- and o-BPA) were administered to melanoma-bearing hamsters and their uptake was measured. The time courses (0.5, 2.0, 4.0 and 48.0 h) of boron concentrations in melanoma, normal skin, and blood were determined in male Syrian (golden) hamsters bearing Greene’s melanomas following a single intraperitoneal injection of either p-, m- or o-BPA (100 mg/kg of BPA-fructose in 1.0 ml of saline). The boron concentrations in these tissues were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES). In melanoma, the order of boron uptake was p- >> m- >> o-BPA at all time points, and the boron concentrations obtained with p-BPA and m-BPA resembled each other in that they had a peak at 2 h after administration and decreased with time. The melanoma/skin boron concentration ratio of p-BPA had a peak at 4 h after administration and the ratio ranged between 7/1 and 8/1. On the other hand, m-BPA and o-BPA had a peak at 2 h and their ratios ranged between 4/1 and 5/1. The difference in the accumulations of p-BPA and m-BPA could be due to a difference in the property of p-BPA as a tyrosine analogue for melanin synthesis. The accumulation of m-BPA into melanoma might indicate the baseline level of metabolism-related amino acid transport. Our experimental findings indicate that this melanin synthesis, or the structural analogy between the boron compound and tyrosine as a precursor of melanin, is an important factor in the increased accumulation of p-BPA in melanoma cells.

Key words: BNCT — Melanoma — p-Boronophenylalanine — Structural isomers

Boron neutron capture therapy (BNCT) is based on the nuclear reaction between boron-10 (^{10}\text{B}) and thermal neutrons. Thermal neutrons are absorbed easily by ^{10}\text{B}, resulting in the emission of $\alpha$ particles and lithium atoms, which can travel distances of 10–14 $\mu$m in tissue. Therefore, when ^{10}\text{B} is selectively accumulated in tumor cells, these cells can be destroyed by thermal neutron irradiation without serious damage to the surrounding normal tissue. The successful treatment of cancer with BNCT depends mainly upon selective ^{10}\text{B} accumulation in the targeted tumor. Various drugs have been developed and used for BNCT. At present, two compounds are clinically utilized; BSH (mercaptoundecahydro-closo-dodecaborate) for brain tumors2,3 and p-BPA (para-boronophenylalanine) for malignant melanoma4,5 and brain tumors6,7. The latter is one of the most successful compounds to date from the viewpoint of tumor selectivity.8

Originally, p-BPA was developed based on the reasoning that since the biosynthesis of melanin requires tyrosine as precursor, the boronated form of this amino acid might be selectively taken up by melanoma cells.9 Since 1987 Mishima and his associates have succeeded in treating human melanoma lesions with BNCT utilizing p-BPA.4

It has been proven that p-BPA highly accumulates in melanoma cells,10 but this compound cannot be a substrate of tyrosinase. Although there have been several reports11–14 regarding the uptake of p-BPA, the mechanism involved is not well understood. Yoshino et al.14 conducted a biological uptake study using B-16 melanoma cells inoculated with ortho- and meta-boronophenylalanine (o- and m-BPA), the isomers of p-BPA. They found the order of boron uptake to be p- >> m- >> o-BPA in vitro, and considered this order to be related to the chemical properties of these isomers.

In this paper, we describe the biodistribution of boron concentration in melanoma, normal skin and blood after administration of these three structural isomers of BPA in
an in vivo study using melanoma-bearing hamsters. The uptake mechanism is discussed.

MATERIALS AND METHODS

Greene’s melanoma,\(^{15}\) which is considered to be a biological and pathological counterpart of human melanoma, was used. This melanoma was allowed to proliferate subcutaneously in male Syrian (golden) hamsters\(^{16}\) until it reached 10 mm in diameter, 14 days after implantation, at which time the experiments were performed. \(p\)-, \(m\)-, or \(o\)-BPA were synthesized from natural-abundance boron (Fig. 1).\(^{17}\) The animals received a single injection of either \(p\)-, \(m\)-, or \(o\)-BPA (100 mg/kg BPA-fructose in 1.0 ml of saline), intraperitoneally. Next, the melanoma, the surrounding normal skin and blood were collected from the hamsters under anesthesia at 0.5, 2.0, 4.0 and 48.0 h after administration of each isomer of BPA. Each group at each specified time consisted of four hamsters. Tissue and blood samples were solubilized in a mixture consisting of HClO\(_4\) (60\%, 0.3 ml) and H\(_2\)O\(_2\) (31\%, 0.6 ml). After filtration of the solubilized sample with a 0.45 \(\mu\)m filter, the boron content was measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Statistical analysis of all data was performed using a standard Student’s \(t\) test. A \(P\) value of \(<0.05\) was considered significant.

RESULTS

Boron biodistribution The boron concentration changes in melanoma, skin and blood as a function of time after administration of the three BPA isomers are shown in Fig. 2. In melanoma, the order of boron uptake was \(p\)-, \(m\)-, or \(o\)-BPA at all time points. These mean boron uptake levels at the 2 and 4 h points were statistically significant (\(P<0.05\)). The boron concentrations with \(p\)-BPA and \(m\)-BPA resembled each other in that they reached peaks at 2 h after administration and then decreased with time. In contrast, \(o\)-BPA simply decreased with time, without reaching a peak.

In skin, the order of boron uptake was also \(p\)-, \(m\)-, or \(o\)-BPA at all time points, but the boron concentrations in skin did not show a peak and decreased with time.

No fixed tendency in the order of boron uptake among the three isomers was seen in the changes in boron concentration in blood. The boron concentration of \(o\)-BPA at 0.5 h was significantly high (\(P<0.05\)), after which it decreased rapidly with time.

Boron concentration ratios The melanoma/skin boron concentration ratios as a function of time after administration of the three BPA isomers are shown in Fig. 3. The concentration of \(p\)-BPA reached a peak at 4 h after administration with the ratio ranging between 7/1 and 8/1. Even at 48 h, it was still 3/1. The concentrations of \(m\)-BPA and \(o\)-BPA, on the other hand, showed a peak at 2 h, with ratios ranging between 4/1 and 5/1. There was no
cells.18) a mechanism of boron accumulation into melanoma from tyrosine or DOPA. Recently, Yoshino BPA, but they might recognize the difference between tyrosine and DOPA. Melanoma cells may not recognize the difference in the accumulation properties of these BPA isomers 2 h after administration, and then decreased with time. On the other hand, o-BPA showed no peak. The differences in the accumulation properties of these BPA isomers in the melanoma may be explained by the difference in the position of the boronic acid group. Tyrosine has one OH group at the p-position and 3,4-dihydroxyphenylalanine (DOPA) has two OH groups at the p- and m-positions. p-BPA has a boronic acid with two OH groups at the p-position. Melanoma cells may not recognize the difference between tyrosine and p-BPA, or between DOPA and p-BPA, but they might recognize m- or o-BPA as different from tyrosine or DOPA. Recently, Yoshino et al. proposed a mechanism of boron accumulation into melanoma cells.18) p-BPA enters the melanin synthesis site of melanoma cells via the tyrosine transport system, together with tyrosine. Tyrosinase promotes the oxidation of tyrosine to produce DOPA, which complexes with p-BPA. Their hypothesis is that p-BPA, which selectively accumulates in melanoma cells but cannot be a substrate of tyrosinase, may chemically bind with DOPA (including melanin monomers). In 1997, they demonstrated that BPA can form a complex with melanin monomers in chemical solution systems, as well as in malignant melanoma.19) m-BPA may not be able to enter the melanin synthesis site to the same degree as p-BPA for the above-mentioned reason.

BPA, being a tyrosine analogue and also a precursor for the synthesis of melanin, has been shown to be selectively taken up by several tumor types in animal or human models.20–22) Codre et al.11) suggested that there are other mechanisms of p-BPA uptake which are independent of melanin synthesis and that the accumulation of p-BPA in rapidly growing animal tumors (including melanoma) could be due to the metabolic demand for the amino acids needed for protein synthesis. Non-pigment tumor cells such as SCCVII, mouse squamous cell carcinoma, also showed a certain level of boron accumulation, although at a much lower level as compared to melanotic melanoma (unpublished data). The accumulation of p-BPA in SCCVII could have been due to the metabolic demand for the amino acids needed for protein synthesis.11) We also think that p-BPA passes as not only an amino acid analogue for protein synthesis, but also as a tyrosine analogue for melanin synthesis. The difference in the accumulations of p-BPA and m-BPA could be due to the effect of the latter property of p-BPA. The accumulation of m-BPA in melanoma might reflect the baseline level of metabolism-related amino acid transport. The experimental finding that the boron concentration introduced into melanoma by p-BPA is higher than that by m-BPA at any time is consistent with this assumption.

There is a possibility that o-BPA is no longer an amino acid at the physiological pH of 7.4, because a boron-nitrogen bond may be formed.13) However, o-BPA seems to retain boron delivery properties to melanoma cells, as shown in Fig. 2(a). This should be further investigated.

As shown in Fig. 3, the melanoma/skin boron concentration ratios of p-BPA were much higher than those of m- and o-BPA. This is favorable for BNCT since there are more tumor-killing effects and fewer harmful skin side effects. This is consistent with the results of our previous study, in which we investigated the tumor-killing effect of BNCT on melanoma-bearing hamsters after administration of p-, m- and o-BPA.23) Our experimental findings indicate that the structural analogy between the boron compound and tyrosine, as a precursor of melanin, is an important factor in accumulation of p-BPA in melanoma cells.

We plan to investigate further the relationship between melanin synthesis and the boron concentration introduced by these three isomers using tyrosinase-lacking mouse amelanotic melanoma cells and melanotic melanoma cells obtained by transfection of the tyrosinase gene into these cells.

![Fig. 3. Melanoma/skin boron concentration ratios against time after administration of p-, m-, and o-BPA. Each point represents the mean±SE (n=4). * P<0.05. □ p-BPA, ○ m-BPA, ○ o-BPA.](image)

DISCUSSION

The results of our biological boron uptake experiment with three structural isomers using melanoma-bearing hamsters showed the order of boron uptake in the melanoma to be p-BPA>m-BPA>o-BPA. These results are in agreement with those of a preliminary uptake experiment using cultured melanoma cells.14) There have been no other reports regarding the in vivo biodistribution of boron after administration of these three BPA isomers with which our results can be compared. As shown in Fig. 2(a), the patterns of boron uptake of p-BPA and m-BPA in the melanoma resembled each other in that both had a peak at 2 h after administration, and then decreased with time.

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