Advances in Brief

Hydroxylforms of p-Boronophenylalanine as Potential Boron Carriers on Boron Neutron Capture Therapy for Malignant Brain Tumors¹

Masao Takagaki, Koji Ono, Yoshifumi Oda, Haruhiko Kikuchi, Hisao Nemoto, Satoshi Iwamoto, Jianping Cai, and Yoshinori Yamamoto

Radiation Oncology Research Laboratory, Research Reactor Institute [M. T., K. O.] and Department of Neurosurgery, Graduate School of Medicine [Y. O., H. K.], Kyoto University, Kumatori-cho, Sennan-gun, Osaka 590-04; and Department of Chemistry, Graduate School of Science, Tohoku University [H. N., S. I., J. C., Y. Y.], Japan

Abstract

Hydroxylforms of boronophenylalanine (BPA) were synthesized by conjugation with a cascade of polyols to decrease the BPA uptake of normal parenchyma without affecting uptake into the tumor. We determined their tumor cell killing effect on boron neutron capture therapy (BNCT) against BPA using the human glioma cell line T98G. The thermal neutron doses yielding the D95 (dose used to inhibit 63% colony formation) values of di-p-BPA(OH)n were 1.45 x 10^12 nvt (n = 1), 3.33 x 10^12 nvt (n = 2), 3.37 x 10^12 nvt (n = 4), and 1.72 x 10^12 nvt (n = 0). The relative tumor cell killing effect on BNCT of di-p-BPA(OH)n against di-p-BPA, which was defined as the ratio of D95-BPA to D95-BPA(OH)n, was 1.18 (n = 1), 1.29 (n = 2), and 0.51 (n = 4). The tumor:normal brain ratio of di-p-BPA(OH)n in 9L rat brain tumor models was improved 1.2- (n = 1) and 1.4-fold (n = 2) against that of di-p-BPA without a decrease of its uptake into the tumor. The water solubility of BPA(OH)n increased against BPA, which was defined as the ratio of D37-BPA to D37-BPA(OH)n, was 1.18 (n = 1), 3.37 x 10^12 nvt (n = 4), and 1.72 x 10^12 nvt (n = 0). The relative neutron doses yielding the I, (dose used to inhibit 63% colony formation) against BPA using the human glioma cell line T98G. These compounds warrant further clinical study.

Materials and Methods

Synthesis of Hydroxylforms of BPA. Polyls of the cascade type (Fig. 1, 1), as a water-solubilizing element, have been developed for BNCT. Cascade polyols have no asymmetric center; therefore, no diastereoisomers are formed when they are bonded to biochemically active molecules containing boron. Furthermore, the number of hydroxyl groups can be manipulated. 1,3-bis(benzoxyl)-2-propanol (Fig. 1, 5a) was converted to the corresponding tosylate 5b upon exposure to tosyl chloride/4-(dimethylamino)pyridine in pyridine. Treatment of 5b with sodium azide in DME gave the azide derivative 5c. Reaction of 5c with LiAlH4 in ether afforded 2-amino-1,3-bis(benzoxyl)-propane (Fig. 1, 5d). The overall yield of 5d from 5a was 95%. Similarly, 1,3-bis(1,3-dibenzyloxy)-2-propanol (Fig. 1, 8a) was converted to the amine derivative 6d in 73% overall yield. BPA was converted to the Cbz-protected form 7, upon treatment with CbzCl in NaOH, in 98% yield. Treatment of 7 with 2 equivalents of N-methylthiolanamine in DME gave the boronate 8. Reduction of 8, without isolation and purification with ethanolamine in the presence of HOBr and EDC afforded 9, which was transformed to the mono-hydroxy borophenylalanine derivative 2, BPA(OH), upon hydrogenation with a Pd(OH)2-C catalyst. The overall yield of 2 from 7 was 28%. Treatment of 8 with 5d under the same conditions as above (HOBr-EDC) produced 10 in 95% yield. The direct reaction of 7 with 5d in the presence of HOBr-EDC gave 10 in a lower yield (48% yield). Removal of the Cbz and Bn groups from 10 using H2/Pd(OH)2-C afforded 3, BPA(OH)3, in 56% yield. A similar procedure was used to condense 8 with 6d. Without purification, 11 was converted to 4 upon hydrogenation. The tetrahydroxy BPA derivative 4, BPA(OH)4, was synthesized in 37% overall yield from 7. The chemical details are described by Nemoto et al. (10, 11).

In vitro Survival Study. A cell suspension of the human glioma cell line T98G in the logarithmic growth phase was prepared in Eagle's MEM (Nissui Co., Tokyo, Japan) containing 10% fetal bovine serum (Life Technologies Inc., Grand Island, NY) and 2 mm l-glutamine. Kanamycin was added at a concentration of 100 mg/liter. The cells were incubated in five culture dishes (100-mm tissue culture dishes; Corning Glass Works, Corning, NY) at a subconfluent concentration of 5 x 10^10/ml/dish, respectively, overnight at 36°C in a 5% carbon dioxide atmosphere. Various amounts of di-p-BPA(OH)n (n = 1, 2, and 4) and di-p-BPA-HCl (Boron Biologicals Inc., Raleigh, NC) were dissolved in MEM (FCS-), and a 10B concentration of 440 ppm was obtained by prompt γ-ray PGS (12) after balancing with MEM (FCS-). After exchanging the medium in the dishes with 10 ml MEM(FCS+), 455 μl BPA solution disinfected by membrane filtration or the same amount of boron-free MEM (FCS-), were added. The pH of the medium mixed with di-p-BPA-HCl was kept at 7.4 after balancing with a supplement with T:N ratio and the water solubility of l-p-BPA itself without significantly affecting uptake into tumors, hydroxylforms of BPA have been synthesized by Nemoto et al. (10) based on these basic concepts: (a) the permeability of the blood-brain barrier is dependent on the water solubility of a reagent and (b) the permeability of BPA through blood-brain barrier should be decreased by modifying its chemical structure, since a number of specialized mediated transport systems allow the transmission of certain amino acids (especially precursors to neurotransmitters). In this study, we investigated the effect of the hydroxylforms of BPA derivatives on BNCT using the human glioma cell line T98G. These compounds warrant further clinical study.

Footnotes

¹ The abbreviations used are: BPA, boronophenylalanine; BNCT, boron neutron capture therapy; T:B, 11B concentration ratio in tumor:brain; T:N, 11B concentration ratio in tumor:normal brain; HOBr, H-borohydronitrosaiole; EDC, ethyl-N,N'-dimeethyl-3-aminopropy)-carboxidime; PGS, prompt γ-ray spectrometry; ATA, α-tocopherol; N:B, 11B concentration ratio in normal brain: blood.

Received 2/29/96; accepted 3/25/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This study was supported by Grants-in-Aid for neurosurgical research (06544413) and cancer research (07274110) from the Ministry of Education, Science and Culture, Japan.

2 To whom requests for reprints should be addressed. Phone: 81-724-52-8194; Fax: 81-724-52-8194; E-mail: rof@ri.kyoto-u.ac.jp.

3 The abbreviations used are: BPA, boronophenylalanine; BNCT, boron neutron capture therapy; T:B, 11B concentration ratio in tumor:brain; T:N, 11B concentration ratio in tumor:normal brain; HOBr, H-borohydronitrosaiole; EDC, ethyl-N,N'-dimeethyl-3-aminopropy)-carboxidime; PGS, prompt γ-ray spectrometry; ATA, α-tocopherol; N:B, 11B concentration ratio in normal brain: blood.


5 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

6 This study was supported by Grants-in-Aid for neurosurgical research (06544413) and cancer research (07274110) from the Ministry of Education, Science and Culture, Japan.

7 To whom requests for reprints should be addressed. Phone: 81-724-52-8194; Fax: 81-724-52-8194; E-mail: rof@ri.kyoto-u.ac.jp.

8 The abbreviations used are: BPA, boronophenylalanine; BNCT, boron neutron capture therapy; T:B, 11B concentration ratio in tumor:brain; T:N, 11B concentration ratio in tumor:normal brain; HOBr, H-borohydronitrosaiole; EDC, ethyl-N,N'-dimeethyl-3-aminopropy)-carboxidime; PGS, prompt γ-ray spectrometry; ATA, α-tocopherol; N:B, 11B concentration ratio in normal brain: blood.

Boron neutron capture therapy (BNCT) involves the use of boron-10 (\(^{10}\text{B}\)) compounds to facilitate the therapeutic effects of thermal neutrons. The synthesis of BPA derivatives, \(\text{d/-p-BPA(OH)}_n\) (\(n = 1, 2, \) and 4), is shown in Fig. 1, bearing cascade polyols. Details are described by Nemoto et al. (11).

The \(\text{d/-p-BPA(OH)}_n\) derivatives were synthesized using 10BPA. The process involves the reaction of \(\text{d/-p-BPA(OH)}_2\) with \(\text{NaOHaq}\) to form \(\text{B(OH)}_2\). The resulting \(\text{B(OH)}_2\) is then reacted with \(\text{CH}_3\text{N(CH}_2\text{CH}_2\text{OH)}_2\) to form a new compound. This process is repeated to form \(\text{d/-p-BPA(OH)}_n\) derivatives.

NaHCO\(_3\), \(^{10}\text{B}\) (20 ppm) in the medium was reconfirmed by PGS. Seven h after 20 ppm \(^{10}\text{B}\) loading, the cells were trypsinized and washed three times in boron-free MEM (FCS-), and \(5 \times 10^5\) cells/ml MEM (FCS+) were irradiated with thermal neutrons in column-shape Teflon tubes (1 x 3 cm). The cells did not adhere to the tubes, and no secondary radiation was caused by bombardment with the thermal neutrons. The thermal neutrons flux was \(7.4 \times 10^9\) cm\(^{-2}\)s\(^{-1}\), and the fluence range was 0, 0.44, 1.2, 2.2, 3.3, 4.4, 6.7, and 8.9 \(\times 10^{12}\) cm\(^{-2}\). The thermal neutron fluence was determined by averaging two gold foils symmetrically attached to the surface of the Teflon tube along the direction of incidence of thermal neutrons. The \(\gamma\)-ray dose rate, including secondary \(\gamma\)-rays, was 10.1 cSv/min, according to a thermoluminescence dosimeter attached to the surface of a Teflon tube containing 1 ml MEM. After thermal neutron exposure, 300 or 900 cells were placed in three Corning 60-mm tissue culture dishes containing 6 ml MEM to examine colony formation. Ten days later, the colonies were fixed with formaldehyde and stained with 0.1 % crystal violet for quantitative visualization by the naked eye. Values are represented as means \(\pm\) SE. Three replications of this in vitro BNCT experiments were performed. In vitro BNCT was also performed on Greene’s melanotic melanoma cells (13) that spontaneously arose in a Syrian golden hamster using \(\text{d/-p-BPA(OH)}_n\) and \(\text{d/-p-BPA(HC)}_n\) under the conditions described above.

Assessment of the T:N Ratio in the Rat Brain Tumor Model. Rats were intracranially implanted with 9L gliosarcoma cells. Fisher 344 rats (7-week-old males, approximately 170 g body weight) were anesthetized by means of an i.p. injection of 0.1 ml/kg sodium pentobarbiturate (Nembutal). A tiny burr hole was made on the right parietal region 2 mm lateral and/or caudal to the Bregma with a high-speed drill. The 9L cells (10^5/10 μl MEM (FCS+)) were slowly injected into the subcortical region to a depth of 3 mm from the dural surface using a 27-gauge Hamilton syringe. The burr hole was closed under sterile conditions using bone wax immediately after the removal of the syringe. Two weeks later, four types of BPA, \(\text{d/-p-BPA(OH)}_n\) (\(n = 1, 2, \) and 4), \(\text{d/-p-BPA \cdot HCl,}\) and \(\text{p-BPA \cdot HCl}\) were dissolved in water at a concentration of 200 mg/ml, and 0.5 ml was given by p.o. administration, using a 23-gauge catheter, to five 9L rats under Nembutal sedation (0.05 mg/kg i.p.). Seven h later, the rats were guillotined and the whole brain, liver, and blood were removed and immediately frozen in liquid nitrogen for PGS. frozen sections of the brain and the liver were prepared with a thickness of 10 μm. The sliced tissues were mounted on an \(\alpha\)-track detector (Kodak CN-85) and exposed to thermal neutrons at a flux of \(3.2 \times 10^8\) cm\(^{-2}\)s\(^{-1}\) for 45 min. The detectors were etched in 2.5 N NaOH at 60°C for 15 min, and \(\alpha\) tracks originating in \(^{10}\text{B}\) in tissue grew into the etched pits that could be quantified under light microscopic observation in a field of 0.125 \(\times\) 0.125 mm\(^2\) under a magnification of 10 \(\times\) 40 (14). The T:N ratio was calculated from the etched pit density ratio of the tumor in the right hemisphere against the left hemisphere (normal brain) on five 9L rats. The density of the etched pits is represented as the means \(\pm\) SE on 10 microscopic fields after subtracting the background counts obtained from normal brain of a rat that did not receive boron. The \(^{10}\text{B}\) concentration in the normal brain (a left hemisphere) of 9L rats was determined by PGS. We calculated the T:B ratio, therefore, by multiplying the T:N ratio determined by ATA and the N:B ratio determined by PGS, since the brain tumors were too small to obtain reliable \(^{10}\text{B}\) concentrations by our PGS.

Results and Discussion

Fig. 2 shows the surviving fraction of T98G cells after the in vitro BNCT. No significant difference in the numbers of the cells among the loaded boron compounds was evident immediately after boron loading, and there were no apparent morphological changes. The plating efficiency for the colony formation was 68 ± 5%. The surviving fraction exponentially decreased without a shoulder. The thermal neutron doses yielding the D\(_{57}\) (dose used to inhibit 63%
Melanoma

Thermal neutron fluence (n·cm⁻²)

Fig. 3. The surviving fraction of Greene's melanotic melanoma cells after in vitro BNCT. Values are means SE.
$^{10}$B atoms can be removed from melanogenesis by enzymatic activity, although BPA is initiated by tyrosinase.

The water solubility and the cytotoxicity might be irrelevant for clinical use. The water solubility of BPA(OH)$_2$ was two orders of magnitude higher than that of BPA, being $(6.6 \pm 0.1) \times 10^{-4}$ M and $(7.9 \pm 0.1) \times 10^{-3}$ M, respectively. The IC$_{50}$ value of BPA(OH)$_2$ defined as the dose that failed to inhibit tumor growth by more than 50% after a 3-day incubation, was nearly one half that of BPA on B-16 melanoma cells: $1.8 \times 10^{-2}$ M for BPA(OH)$_2$ and $8.6 \times 10^{-3}$ M for BPA (11). A fructose complex of BPA is another water-solubilizing form (17). However, it is considered that the fructose complex is immediately converted to BPA by hydrolysis in blood (11).

For BPA-based BNCT, it is desirable to reduce the dose to the normal brain, since the dose distribution ratio of the normal brain parenchyma to the capillary endothelium is nearly 1 (18), which is almost double that of BSH-based BNCT (19). The reduction rate of the absorbed dose on normal parenchyma using BPA(OH)$_2$ instead of BPA was roughly estimated using the equation

$$\frac{N_{\text{BPA(OH)$_2$}} \cdot \sigma_{c}}{N_{\text{BPA}} \cdot \sigma_{c} + N_{\text{BPA}} \cdot \sigma_{e}} \times \frac{E_{\text{B}}}{E_{\alpha}} = 0.79.$$

Supposing 20 ppm $^{11}$B is accumulating in the blood, the rate is 0.79. Therefore, the dose to the normal brain can be reduced about 21% by using BPA(OH)$_2$ instead of BPA, without a decrease in the selective tumor killing effect and radiation damage to the capillary endothelium. In our preliminary study of BNCT for patients with malignant brain tumors using a 1-p-BPA-fructose complex, intraoperative BNCT must be used to reduce the dose to the related normal brain surrounding the tumor after tumor debulking. This allowed a therapeutic dose to be absorbed even by the tumor bottom, using a large tumor cavity filled with voids as a neutron penetrator. Despite these surgical maneuvers, early necrosis and general convulsion (rarely status epilepticus) frequently occurred around 3 days after BNCT, since the protection of normal cortex from the radiation injury had to be sometimes sacrificed to fulfill the minimum requirement for the curable dose onto the bottom of the tumor. We believe that the dose to the normal brain and/or capillary endothelium must be minimized to reduce radiation injury to the surrounding normal parenchyma. The hydroxylform of BPA(OH)$_2$ might be applicable on BPA-based BNCT on malignant brain tumors, since it has lower cytotoxicity and higher water solubility.

References


Hydroxylforms of \( p \)-Boronophenylalanine as Potential Boron Carriers on Boron Neutron Capture Therapy for Malignant Brain Tumors

Masao Takagaki, Koji Ono, Yoshifumi Oda, et al.


**Updated version**  Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/56/9/2017](http://cancerres.aacrjournals.org/content/56/9/2017)