Boron Neutron Capture Therapy of Anterior Chamber Melanoma With p-Boronophenylalanine

Samuel Packer, Jeffrey Coderre, Sharad Saraf, Ralph Fairchild, Julia Hansrote, and Henry Perry

Boron neutron capture therapy (BNCT) is a form of radiation therapy that requires selective uptake of boron by the tumor and irradiation with thermal neutrons. Phenylalanine is an amino acid precursor of melanin and when boronated (p-boronophenylalanine [BPA]) was found to be selectively taken up by Greene melanoma cells in the anterior chamber of rabbits. This tumor model was irradiated 24 hr after oral administration of BPA and was used for biodistribution studies that compared BPA and sodium pentaborate. Three groups were irradiated: group 1 (11 rabbits) received BPA followed by thermal neutron irradiation, group 2 (9 rabbits) received thermal neutron irradiation only, and group 3 (9 rabbits) served as unirradiated, undrugged control animals. Eight of the 11 tumors in group 1 were treated successfully; all tumors in groups 2 and 3 grew. Histopathologic examination did not reveal vascular or retina damage in group 1. These preliminary experiments confirm that newer boronated compounds, such as BPA, used in BNCT and improved neutron beams can provide selective irradiation of ocular melanomas. Invest Ophthalmol Vis Sci 33:395–403, 1992

Boron neutron capture therapy (BNCT) is a form of radiation therapy that has two components. The first is the delivery of boron to the tumor. This is accomplished by chemically boronating a tumor-seeking compound. The boronated agent must be stable in vivo and selectively deposit adequate amounts of boron in the tumor but not in normal tissues. The second is to irradiate with thermal neutrons. Only the thermal neutron will interact with the boron and give rise to an alpha and a lithium particle. These highly ionizing particles, released preferentially in the tumor, create the selective irradiation of the tumor compared with other structures in the eye (or body). The reaction products from the $^{10}$B(n,$\alpha$)$^7$Li reaction have a range of approximately 10 $\mu$m in tissue and are known to have a high relative biologic effectiveness (RBE).

Clinical trials of BNCT for the treatment of glioblastoma multiforme were done between 1953 and 1961 at Brookhaven National Laboratory and the Massachusetts Institute of Technology; the results were disappointing. Poor results were attributed to two major factors: (1) the use of boron-containing compounds without selective accumulation in the tumor and (2) the rapid attenuation in tissue of the incident thermal neutron beam. Efforts to deliver therapeutic neutron doses to deep tumors resulted in excessive damage to the normal brain and/or the skin. The high boron concentrations in the blood contributed to the damage to the vasculature of the brain. The BNCT clinical trials were discontinued in the United States in 1961.

In the intervening years since the first American clinical trial of BNCT, improvements in both areas have allowed BNCT to reemerge as a viable method for selective tumor irradiation. Epithermal neutron beams, which use tissue as a moderator to slow the neutrons into the thermal energy range at depth, provide an improved depth–dose distribution profile for thermal neutrons. Both thermal and epithermal neutron beams are now available at the Brookhaven Medical Research Reactor (BMRR). Since the end of the first American trial of BNCT, progress has been made in the development of tumor-selective boron-containing compounds. In Japan, two of the more promising boron compounds currently are being used in two separate clinical trials of BNCT: one since 1968 for treatment of patients with glioblastoma and, more recently, one for patients with cutaneous melanoma.

In theory, the full potential of BNCT should be realized when thermal and/or epithermal neutron beams can be combined with boron-containing compounds.
that selectively accumulate in the tumor and are retained, without accumulation in normal tissues.

The use of BNCT first was explored in ophthalmology 20 years ago, using a boron compound (sodium pentaborate) which had no selectivity for tumor tissue.6 We reevaluated this report and present distribution data showing an improved tumor-localizing ability with the boron-containing amino acid p-boronophenylalanine (BPA).

This article describes the use of BPA as a boron delivery agent for BNCT of amelanotic Greene melanoma cells carried in the anterior chamber of the rabbit eye. The compound BPA is an analogue of the melanin precursor tyrosine. As such, it accumulates in melanoma tissue as a result of the increased metabolic demand for melanin precursors. It has been shown to enhance the killing effect of thermal neutrons on both pigmented and nonpigmented B16 melanoma in vitro.7 In addition, BPA has been used to treat superficial melanoma successfully in various animal tumor models.8,9 Our results describe the use of BNCT to irradiate a tumor selectively (melanoma in the anterior chamber) in the presence of radiosensitive tissues. The dose delivered to the tumor exceeded the dose to normal eye structures by a factor of 3.

Materials and Methods

Tumor Model

The Greene melanoma cell line has been maintained in our laboratory for 15 years. Originally a hamster melanoma, this tumor has been adapted for growth in the rabbit eye.10,11 The tumor will grow either in the anterior chamber (iris melanoma) or as a subchoroidal transplant in the posterior chamber. This neoplasm has been used to evaluate several experimental therapies, such as the use of radioactive plaques and hyperthermia.12,13 In the experiments we report, we used only the anterior chamber model. During surgical procedures, the rabbits were anesthetized with intramuscular ketamine hydrochloride and xylazine (20 mg/kg). A 2-mm incision was made in the cornea, just anterior to the limbus, with a No. 11 blade. Approximately 1 mm³ of freshly dissected tumor tissue was deposited onto the iris through the incision, which then was closed with a single suture. Antibiotic ointment was applied at the end of the procedure and daily thereafter for 5 days. When the anterior chamber tumor became visible at about 7-10 days postimplantation, photographic documentation was begun. Tumor size was measured with a handheld caliper; two dimensions, 90° apart, were recorded. The measurements were made two to three times per week by a single observer.

Biodistribution Studies

Sodium pentaborate (Na₂B₁₀O₁₆), 95% enriched in ¹⁰B, was obtained from Oak Ridge National Laboratory (Oak Ridge, TN). The sodium pentaborate was administered to tumor-bearing rabbits in two ways: (1) as an intracameral injection (0.05 ml of a 9% solution) and (2) as an intravenous injection (23 mg ¹⁰B/kg body weight). The BPA, 95.3% enriched in ¹⁰B, was synthesized according to published procedures with some minor modifications.14,15 This drug has a limited solubility at physiologic pH. Previous work in our laboratory showed that oral administration as an aqueous slurry at neutral pH (by intragastric intubation) is an effective administration route.16 The rabbits received 750 mg BPA/kg body weight (35 mg ¹⁰B/kg body weight) as a single oral dose in 15 ml of water. The boron concentration was analyzed by the prompt-gamma spectroscopic method.17 The intracocular boron distribution pattern was determined by the method of neutron capture radiography.15,16 This technique allows visualization of the boron distribution in 50-μm thick cryosections.

Neutron Irradiations

Neutron irradiations were done using the thermal neutron facility at BMRR. Dosimetry measurements for the rabbit eye tumor geometry were done using gold foils and thermoluminescent dosimeters in a plastic phantom and by positioning gold foils on an anesthetized rabbit. The dosimetric parameters of the BMRR thermal neutron irradiation facility are listed in Table 1. A RBE of 2.5 was used for the ¹⁰B reaction, 2.0 for protons from the ¹⁴N(n,p)¹⁴C reaction, and 2.0 for fast neutrons. The ¹⁰B dose was 79.4% of the total dose. The thermal neutron port (25 × 25 cm) was collimated with a 2.7-cm thick ⁶LiF-epoxy insert to a 1.55-cm aperture. The animals were anesthetized and positioned with the eye centered in the collimator aperture.

A 10-min videotape record of the eye of an anesthetized rabbit, taken from the other side of the collimator with the rabbit in place, established that the eye did not move significantly. During irradiation, online eye monitoring was not possible; therefore, the possibility of movement and a geographic miss cannot be excluded with the existing positioning apparatus. (This would not be the case with clinical irradiation.)

Three groups of animals were studied. Group 1 received BPA orally and then was irradiated. Group 2 did not receive BPA, but was irradiated. Group 3 (control group) received no BPA and was not irradiated. Table 2 gives the tumor size at time of irradiation.
Our investigations with rabbits adhered to the ARVO Resolution on the Use of Animals in Research.

Results

Boron Distribution Studies

The only previous use of BNCT for the treatment of ocular melanoma in rabbits used intracameral injections of sodium pentaborate immediately before irradiation. We did a comparative evaluation of the intraocular boron distribution produced by intracameral or intravenous injection of sodium pentaborate and the boron distribution produced by orally administered BPA.

Figure 1 is a neutron capture radiogram (NCR) that shows the boron distribution in a tumor-bearing eye after intracameral injection of sodium pentaborate. In such images, bright areas represent high boron concentrations. Aqueous humor had been removed, and 0.05 ml of sodium pentaborate 9% solution was injected into the anterior chamber. The interval between injection and death was 10 min, the same as that used in an earlier study. The large arrow in Figure 1 indicates relatively high concentrations of boron in the anterior chamber. The small arrow points to the anterior chamber tumor nodule growing on the iris. Under these conditions, the boron has not penetrated to the interior of the tumor and remains in the anterior chamber.

Figure 2 shows the boron accumulation and washout in the blood and in the anterior chamber tumor after a single oral dose of BPA (35 mg 10B/kg). At 24 hr postadministration, the boron concentration in the tumor was in the therapeutic range (>15 ppm) with a tumor/blood boron concentration ratio of approximately 4. When sodium pentaborate was administered as a single intravenous injection (23 mg 10B/kg), the boron concentrations observed in tumor and blood reached approximately 30 ppm at 30 min postinjection and decreased with a clearance half-time of 2.5 hr (data not shown). The tumor/blood boron concentration ratio after intravenous injection of sodium pentaborate never exceeded 1.

We compared the intraocular boron distribution patterns obtained after systemic administration of both BPA and sodium pentaborate in tumor-bearing rabbits. Figure 3 shows a NCR prepared from a corneal section of a tumor-bearing eye. The rabbit received intravenous BPA 7 hr before death. The large arrow points to the anterior chamber tumor. The boron concentration in the tumor shown in Figure 3 was approximately 20 ppm. Other radiosensitive ocular structures of interest in the treatment volume include the cornea, the lens, the retina, and the optic nerve (the optic nerve is not visible in this section). The estimated boron concentrations in the normal eye structures in Figure 3 are approximately 3-4 ppm. Figure 4 shows the corresponding intraocular boron distribution produced by sodium pentaborate. This NCR was obtained 7 hr after intravenous injection of sodium pentaborate. The boron distribution was non-

Table 1. Average tissue dose* using BMRR thermal neutron beam†

<table>
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<th>Dose components</th>
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<tr>
<td>1. 10B dose (20 ppm, tumor)</td>
<td>2307</td>
<td>923</td>
</tr>
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<td>312</td>
<td>156</td>
</tr>
<tr>
<td>3. Gamma dose, from reactor</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>4. 14N (n, p) (2.6% N2)</td>
<td>206</td>
<td>103</td>
</tr>
<tr>
<td>5. Gamma capture 1H(n, α) dose (10.3% H)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>6. 10B dose to normal tissue (3 ppm 10B)</td>
<td>346</td>
<td>138</td>
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* In centigray (cGy).
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Table 2. Treatment data for Group 1 (BPA + NCT)

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Date irradiated</th>
<th>Date enucleated</th>
<th>Initial size* (mm × mm)</th>
<th>Final size* (mm × mm)</th>
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</thead>
<tbody>
<tr>
<td>299</td>
<td>3/23/89</td>
<td>5/24/89</td>
<td>6 × 3</td>
<td>NV†</td>
</tr>
<tr>
<td>740</td>
<td>3/23/89</td>
<td>5/24/89</td>
<td>1.5 × 1.5</td>
<td>NV</td>
</tr>
<tr>
<td>323</td>
<td>3/23/89</td>
<td>6/30/89</td>
<td>1.5 × 1.5</td>
<td>1.0 × 0.5</td>
</tr>
<tr>
<td>275</td>
<td>3/23/89</td>
<td>7/23/89</td>
<td>1.5 × 1.0</td>
<td>NV</td>
</tr>
<tr>
<td>169</td>
<td>7/11/89</td>
<td>9/27/89</td>
<td>2 × 2</td>
<td>NV</td>
</tr>
<tr>
<td>167</td>
<td>7/11/89</td>
<td>7/26/89</td>
<td>6.5 × 6.5</td>
<td>12 × 8</td>
</tr>
<tr>
<td>432</td>
<td>7/11/89</td>
<td>9/11/89</td>
<td>6.5 × 5.5</td>
<td>12 × 6</td>
</tr>
<tr>
<td>503</td>
<td>7/11/89</td>
<td>7/31/89</td>
<td>3 × 4.5</td>
<td>9 × 6</td>
</tr>
<tr>
<td>097</td>
<td>7/11/89</td>
<td>11/22/89</td>
<td>6.5 × 3.5</td>
<td>NV</td>
</tr>
<tr>
<td>555</td>
<td>7/11/89</td>
<td>01/26/90</td>
<td>3.0 × 3.0</td>
<td>NV</td>
</tr>
<tr>
<td>824</td>
<td>7/11/89</td>
<td>—</td>
<td>4.5 × 4.5</td>
<td>NV</td>
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</table>

* Base dimensions 90° apart.
† NV = Not visible.
selective; the tumor/normal tissue boron concentration ratios were approximately 1.

BNCT Irradiations

A total of 29 rabbits with anterior chamber melanomas were divided into three groups. Group 1 (11 rabbits) received a single oral dose of BPA followed by thermal neutron irradiation 24 hr after the BPA administration. Table 2 gives data as to the size of the tumor at the time of treatment and the response of the tumor to the BNCT irradiation. Figure 5 shows the individual growth curves of the tumors in Group 1. Three of 11 tumors grew; the remaining tumors were monitored for various periods of time (Table 2, for the time of enucleation). Rabbits with controlled tumors were killed after increasing intervals, and the eyes

Fig. 1. Neutron autoradiograph of anterior chamber melanoma taken after sodium pentaborate was given intracamerally. The melanoma (short arrow) has no accumulation of boron (white), while the anterior chamber remains filled with boron (long arrow).

Fig. 2. Biodistribution of BPA (750 mg/kg) given orally as a single dose to rabbits containing an anterior chamber Greene melanoma.

Fig. 3. Neutron autoradiograph of anterior chamber melanoma taken 7 hr after a single dose of BPA (750 mg/kg). The melanoma (arrow) has significant accumulation of BPA compared to the rest of the eye. Whiteness reflects boron accumulation.

Fig. 4. Neutron autoradiograph of anterior chamber melanoma taken 6 hr after a single dose of sodium pentaborate (750 mg/kg). The melanoma (arrow) has accumulated amounts of sodium pentaborate similar to other ocular structures. Whiteness reflects boron accumulation.
Fig. 5. Group 1 received BPA (750 mg/kg) and was irradiated 24 hr later with thermal neutrons (see text). Three animals had tumor that continued to grow (#167, #503, and #432).

were examined histologically. At approximately 60–90 days postirradiation, cataract formation was observed in treated eyes that were otherwise free of visible signs of radiation damage. Group 2 (nine rabbits) received thermal neutron irradiation only (Table 3, for details as to tumor size and irradiation dates). Figure 6 shows that all nine melanomas grew. Group 3 (nine rabbits) served as an untreated control group and received neither BPA nor reactor irradiation. Figure 7 shows that all nine control tumors grew.

Clinical and Histopathologic Correlations

Figure 8 (top left) shows animal 097 (group 1) 1 day after irradiation; the tumor measured approximately 3 × 5 mm in base diameter. Figure 8 (top right) was taken 19 days after irradiation. A cataract was visible at 1 month. Histopathologic examination of animal 097 confirmed the cataract and did not reveal any residual tumor or any other evidence of radiation damage to normal ocular structures. Treated tumors, which were enucleated at periods less than 60 days (Table 2) and examined histologically, did not show cataract formation or any damage to normal ocular tissues. Figure 8 (bottom figures) shows animal 321 (Group 2) 12 and 23 days postirradiation. The tumor was measured from 2 days before irradiation to this point (Table 3). Thus, the small size of this tumor would favor a good therapeutic response. The opposite, however, occurred (Fig. 8, bottom right), as shown 23 days postirradiation. Histopathologic examination of animal 321 revealed areas of necrosis alternating with areas of viable-appearing tumor (Fig. 9).

The histopathologic examination of the BNCT group was significant because the vasculature of the eye remained normal. The major toxicity in the early BNCT trials was vascular toxicity. Thus, the lack of adverse ocular vascular events is evidence of the improved tumor/blood ratios achieved with the new boronated compounds. This is noteworthy because the thermal beam we used delivered a similar non-BNCT radiation dose to the anterior and posterior segments of the eye; thus, rabbit eyes in groups 1 and 2 received the same non-BNCT radiation exposure. Therefore, the lack of damage to the vascular structures of the eyes in group 1 indicates that they contained inadequate amounts of boron to result in radiation damage. Figure 10 shows the retina of a rabbit (321) that received BNCT with BPA. The retinal vasculature appears normal 3 months after irradiation.

Discussion

A previous study⁶ was the only other attempt to apply BNCT to the treatment of ocular melanoma to our knowledge. There were several significant differences between this earlier study and the results we report that deserve discussion. In the previous report, sodium pentaborate was administered as the boron delivery agent. Sodium pentaborate was one of the boron delivery agents used during the initial trials of BNCT in the 1950s and early 1960s and was shown to distribute rapidly in body water with no selectivity for

<table>
<thead>
<tr>
<th>Animal no.</th>
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<th>Date enucleated</th>
<th>Initial size* (mm×mm)</th>
<th>Final size* (mm×mm)</th>
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<td>5 × 4*</td>
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<td>321</td>
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<td>8/13/89</td>
<td>2 × 2</td>
<td>11 × 6.5</td>
</tr>
<tr>
<td>347</td>
<td>7/11/89</td>
<td>9/27/89</td>
<td>5 × 2</td>
<td>&quot;whole AC&quot;†</td>
</tr>
<tr>
<td>367</td>
<td>7/11/89</td>
<td>7/24/89</td>
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</tr>
<tr>
<td>621</td>
<td>7/11/89</td>
<td>7/26/89</td>
<td>4.5 × 2.5</td>
<td>12 × 7</td>
</tr>
</tbody>
</table>

* Base dimensions 90° apart. † AC = anterior chamber.
tumor tissue. A cell suspension was injected into the anterior chamber, and the eye underwent experimentation 2–5 days later when the tumor existed as a thin sheet or perhaps small nodules. In our study, we implanted tumor fragments that were allowed to grow for approximately 14 days; thus, we were treating a well-vascularized, solid tumor. The other group injected the sodium pentaborate directly into the anterior chamber and commenced reactor irradiation within 10 min. They intended to show that the intensely ionizing reaction products from BNCT were tumoricidal. Their study showed complete tumor control with minimal radiation damage in 6 of 25 treated eyes, complete tumor control with significant radiation damage in 8 of 25 treated eyes, and significant growth delay in an additional 6 treated eyes.

Our intent was to demonstrate the ability of the boron delivery agent BPA to accumulate in tumor sites selectively after systemic administration. We chose to treat vascularized solid tumors with this approach as an approximation of the clinical situation.

The management of choroidal melanoma is still controversial. However, when we consider primarily the techniques for irradiation of choroidal melanoma, BNCT offers several worthwhile advantages. First, it is a selective form of irradiation, ie, the tumor is irradiated preferentially compared with normal ocular structures. Thus, large choroidal melanomas that currently would not be considered for radiation therapy might benefit, as would tumors located close to the macula or optic nerve. Second, the lack of manipulation (no surgery required) would obviate any need to consider the possibility that tumor manipulation might cause metastasis.

The drug BPA is absorbed by cells that synthesize melanin. Our group showed that this uptake depended more on amino acid transport than on the degree of pigmentation. In addition, we demonstrated that this uptake did not occur in metabolically active normal tissue. This study demonstrated the superiority of orally administered BPA to the intracameral technique previously used and confirmed the selectivity of BPA when given systemically. Its potential significance is the treatment of metastatic foci.

Radiation therapy has been partially effective for palliative treatment of malignant melanoma of the skin and metastases. However, BNCT delivers densely ionizing radiations that should be more effective (higher RBE) than either the x-rays or fast neutrons that have been used clinically. This, combined with the selective delivery of radiation to the tumor, the possibilities of dose fractionation, and an epithermal beam, highlights a potentially exciting new treatment of malignant melanoma not only of the eye but elsewhere in the body.

An international committee convened to advise on clinical applications of neutron capture recommended that four to six fractions be tried in such therapy. Further advantages then should be obtained for a number of reasons: (1) the usual benefits which are obtained after fractionation as represented by the "4 Rs" (repair, reassortment, repopulation, and reoxy- genation); (2) selective repair in normal tissues from the preponderance of low linear energy transfer (LET) radiation damage in the latter compared with high LET damage in the tumor; and (3) redistribution of boron compounds as a consequence of compound readministration. Fractionation also will facilitate using different boronated melanoma-seeking agents. For example, BPA may be used with a boronated monoclonal antibody.

There are 35 potential neutron sources in the United States. Therefore, this technique would be available at many locations.

Our results are encouraging, but further studies are necessary.
needed. The problems remaining with BNCT are related to optimization of boron delivery and neutron beam characteristics. As highlighted by others, an adequate tumor concentration of boron is required (> 35 μg/g 10B/g tumor), and higher uptake by the tumor compared with normal tissue is desirable. This will require pharmacokinetic and biodistribution studies because the absolute amount of 10B in the tumor will influence the tumor/normal tissue ratio required to assure a therapeutic gain. The alpha particle has a range of one cell diameter; therefore, cells without boron may not be treated successfully. Thus, the uni-

Fig. 8. Comparison of results, Group 1 vs. Group 2. Top left, #097 1 day after irradiation. Top right, #097 19 days after therapy, tumor (arrow) smaller than at 1 day. Bottom left, #321 12 days after receiving irradiation and no BPA. Bottom right, #321 23 days after irradiation, tumor (arrow) significantly larger.
form cellular distribution of boron in a tumor is critical. This problem is similar to that faced in radiation therapy where it is generally considered that treatment requires reduction of the surviving cell fraction to $10^{-9}$. Given the heterogeneity of malignant melanoma, this is a formidable task and may require manipulation of boron delivery (perhaps with multiple agents, eg, melanin precursors or monoclonal antibodies), multiple-dose schedules including fractionation, and the judicious use of thermal and epithermal neutron beams. The latter will decrease the radiation dose to the anterior structures of the eye further and
increase the therapeutic gain, ie, allow a tumoricidal dose to be delivered with less radiation to the normal anterior segment of the eye.

Key words: boron neutron capture, melanoma, phenylalanine, rabbit, radiation therapy

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