

Boron Neutron Capture Therapy of Intracerebral Melanoma Using Boronophenylalanine as a Capture Agent¹

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ABSTRACT

A rat brain tumor model has been developed utilizing nude rats and the human melanoma cell line MRA 27. For pharmacokinetic and tissue distribution studies, 2 10^5 MRA 27 cells were implanted intracerebrally (i.c.), and 30 days later, 120 mg of ^{10}B -enriched L-boronophenylalanine were injected i.p. into nude rats. ^{10}B concentrations in the tumor, blood, and normal brain were 23.7, 9.4, and 8.4 $\mu\text{g/g}$, respectively, 6 h following administration. For therapy experiments, tumor bearing rats were irradiated at the Brookhaven Medical Research Reactor 30 days following implantation. The median survival time was 44 days for untreated rats, 76 days for those receiving a physical dose of 2.7 Gy, and 93 days for those receiving 3.6 Gy. Animals receiving both ^{10}B -L-boronophenylalanine and physical doses of 1.8, 2.7, or 3.6 Gy (total tumor physical doses of 5.0, 7.5, or 10.1 Gy) had median survival times of 170, 182, and 262 days, respectively. Forty % of rats that received the highest tumor dose (10.1 Gy) survived >300 days. In a replicate experiment 21% of animals that had received L-boronophenylalanine and irradiation (total tumor physical dose of 10.1 Gy) were alive 220 days after therapy. In a parallel study, animals that were irradiated with γ photons from a ^{137}Cs source with 12 Gy or 2.0 Gy 9 delivered to the head had median survival times of 86 and 79 days, respectively, compared to 47 days for untreated animals. Our results indicate that boron neutron capture therapy is effective against i.c. melanoma in a rodent model and suggest that large animal studies are warranted to further assess its efficacy.

INTRODUCTION

Malignant melanoma can metastasize to almost any organ of the body, but especially to the skin, liver, lung, and brain (1, 2). Patients with brain metastases have a very poor prognosis despite aggressive chemotherapy and radiotherapy (3, 4). In one series of 125 patients, the median survival times of patients with cerebral metastases were 9 weeks when treated with a radiation dose of 30–40 Gy to the whole brain and 26 weeks when patients had surgical excision of solitary lesions, compared to 3 weeks if they were not treated (4). Immunotherapy using interleukin 2 and low doses of cyclophosphamide or active specific immunization resulted in a prolongation of the remission phase in patients with disseminated melanoma, although many of these patients eventually succumbed to their cerebral metastases (5).

One promising therapeutic modality for the treatment of brain tumors is boron neutron capture therapy (6), which has been recently reviewed (7). BNCT⁴ is a binary system based on selective uptake of

sufficient amounts of a stable isotope, ^{10}B , by tumor cells, followed by irradiation with low energy (0.025–0.5 eV) thermal neutrons. The resulting nuclear reaction yields α particles and recoiling ^7Li nuclei, which have high linear energy transfer and path lengths of approximately one cell diameter (10–14 μm). In theory, this should minimize radiation effects to normal brain due to low uptake of ^{10}B by normal tissues but result in a significant tumoricidal effect due to selective accumulation of ^{10}B by neoplastic cells.

Mishima *et al.* (8, 9) have used the amino acid *p*-boronophenylalanine, a boron containing melanin precursor analogue, as a capture agent for the treatment of cutaneous melanomas in animals and humans. In most instances, the BPA was injected perilesionally and allowed to clear from the surrounding normal skin, and then neutron irradiation was initiated. Coderre *et al.* (10, 11) have demonstrated the efficacy of BNCT in cutaneous melanomas in mice, ocular melanoma in rabbits (12), and i.c. gliosarcomas in rats (13) using systemic injections of BPA. Furthermore, Saris *et al.* (14) recently have reported on the responsiveness of the murine glioma 261 to BNCT.

Although extensive animal studies have been carried out on the treatment of extracerebral malignant melanoma, to the best of our knowledge there is little, if anything, in the literature on the treatment of i.c. melanoma. We previously have reported that BNCT may have some potential as a modality to treat i.c. melanoma (15). The purpose of the present study was to expand upon our preliminary observations using BPA based BNCT and a human melanoma cell line implanted i.c. into nude rats.

MATERIALS AND METHODS

Animals and Tumor Cell Line. The human melanoma cell line MRA 27 was derived from a 60-year-old Norwegian male and has been propagated both *in vitro* and *in vivo* in nude mice and rats. MRA 27 cells were grown in McCoy's 5A medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 units/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, and 2 mM L-glutamine and were tested periodically for mycoplasma contamination by DNA-fluorochrome staining and UV microscopy (Bionique Testing Laboratory, Saranac Lake, NY). Six to 8-week-old athymic female nude rats of NIH-rnu strain were purchased from the Animal Production Branch, National Cancer Institute, Frederick, MD. The rats were maintained under specific pathogen free conditions and fed sterilized food and water.

Implantation. A stereotactic implantation procedure, previously used by us for studies on BNCT of a rat glioma (16), has been implemented. Briefly, nude rats were sedated i.p. with a 1.2/1 mixture of 120 mg/kg of ketamine/20 mg/kg of xylazine and a plastic screw was embedded in the skull. MRA 27 cells were injected through a central hole in the plastic screw into the right caudate nucleus at a concentration of 10^6 or $2 \times 10^5/10 \mu\text{l}$ of serum free McCoy's 5A medium containing 1% agarose with a gelling temperature of $<30^\circ\text{C}$. The screw hole was filled with bone wax following withdrawal of the needle, and the operative field was flushed with betadine before closing the scalp incision with a single sterilized clip. The rats were weighed 3 times/week following

neutrons; BPA, boronophenylalanine; MW-M, megawatt-minute; PE, plating efficiency; RBE, relative biological effectiveness; MeST, median survival time(s); i.c., intracerebrally; $t_{1/2}$, half-life; Gy-Eq, equivalent dose or physical dose \times RBE; n, neutrons; p, protons.

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⁴ The abbreviations used are: BNCT, boron neutron capture therapy; n_{th} , thermal

implantation and irradiation studies. The tumor volume was determined at the time of death from formalin fixed brains that had been cut coronally at 2-mm intervals. The tumor size index was defined as the cube root of the product of the largest measurements of the length, height, and width (16).

Pharmacokinetics and Tissue Distribution Studies. For injection, BPA (Callery Chemical Co., Callery, PA), as either the racemic DL mixture or the purified L enantiomer, was converted to a more soluble fructose complex. BPA and fructose were mixed in a 1:1 molar ratio to yield a final concentration of 120 mg of BPA/2 ml of water. The pH was adjusted to 8.8 with 6 N NaOH. Two ml of the complex were administered i.p. to rats 37 days following i.c. implantation of 10^6 tumor cells, unless indicated otherwise. Animals were killed 1, 3, 6, 9, 12, and 18 h later and samples of blood, brain, tumor, skin, liver, kidneys, muscle, eyes, and skull were obtained. Boron concentrations were determined by means of direct current plasma atomic emission spectroscopy, as described in detail elsewhere (17).

In Vitro Cell Irradiations and Clonogenic Assays. *In vitro* irradiations of MRA 27 cells were carried out at the Brookhaven Medical Research Reactor at 1 MW reactor power with an n_{th} flux of 2.8×10^{11} neutrons/cm² min. Prior to irradiation, cells were incubated for 24 h with BPA-fructose complex at a concentration of 10 μ g ¹⁰B/ml of growth medium. As previously described (18), the same concentration of ¹⁰B was maintained in the medium during trypsinization, harvesting, and irradiation. The cells were irradiated at a density of 2×10^5 /ml at ambient temperature but were kept on ice during transportation until plated. Following irradiation, the cells were placed in boron free medium, plated into Petri dishes, and incubated at 37°C in a humidified atmosphere containing 5% CO₂. Fourteen days later the plates were washed with phosphate buffered saline, fixed with formaldehyde, and stained with 1% crystal violet. Colonies ≥ 50 cells (0.3-mm) were enumerated visually or by means of an Artek 880 image analyzer (Artek System Co., Farmingdale, NY). The plating efficiency:

$$PE = \frac{\text{Number of MRA 27 colonies enumerated}}{\text{Total number of MRA 27 cells plated}} \times 100\%$$

ranged from 30 to 50%. The surviving fraction was determined from:

$$\frac{\text{Number of colonies enumerated}}{\text{Total number of MRA 27 cells plated} \times PE/100}$$

In vitro X-irradiations were carried out using a Stabilipan 250 kVp x-ray machine. The 250 kVp X-irradiations were performed at 15 mA with a Thoresus I filter and 50 cm SSD at a dose rate of 0.51 Gy/min. Cells were irradiated in boron free medium and boron containing medium and were assayed as described above.

In Vivo Irradiation Studies. All irradiations were carried out at the Brookhaven Medical Research Reactor. The reactor power was maintained at 1.25 MW during the irradiation of all rats. BNCT was initiated 30 days following stereotactic implantation of 2×10^5 MRA 27 cells. Rats were divided into 6 groups of 9 or 10 animals each. Groups 1 and 2 received 6 or 8 MW-minutes of irradiation, respectively. Groups 3–5 received 4, 6, or 8 MW-M of irradiation 6 h following i.p. administration of 120 mg of 95% ¹⁰B-enriched L-BPA. Group 6 served as untreated controls. All rats were anesthetized with a 1.2/1 mixture of ketamine/xylazine and placed supine in a body shield-head stabilizer, as described elsewhere (13, 19, 20). The tumor implantation site was centered in the 1.15-cm diameter aperture of the neutron beam collimator. The adjustment of the head of the rat was established using a marked lucite plate as a template for the collimated beam.

γ -irradiations were performed 31 days following implantation of 2×10^5 MRA 27 cells. Rats were divided into 3 groups of 5 or 6 animals each. Group 1 received a single 12-Gy dose of γ photons and group 2 received 9 fractions of 2.0 Gy each, delivered over an 11-day period using a Picker ¹³⁷Cs teletherapy machine, which delivered 1.0 Gy/min at 15 cm SSD when a 2-cm (outside diameter) cone was used. Group 3 was untreated controls. The tumor bearing region of the head of the rat was irradiated directly and the body was shielded with 5 mm of lead.

Dosimetry. The mixed radiation in tissue during *in vivo* or *in vitro* BNCT includes n_{th} and fast neutrons ($>10,000$ eV), γ photons, and heavy particles that are generated from ¹⁴N(n,p)¹⁴C and ¹⁰B(n, α)⁷Li reactions. As previously described (13, 18, 20), n_{th} fluences were measured using 3-mm long gold wires implanted into the brain of a dead rat to a depth of 4–5 mm below the skull

surface, or alternatively the wires were placed inside 1.5 ml Eppendorf microfuge tubes for *in vitro* irradiations. The n_{th} flux at a depth of 4–5 mm below the skull was 3.9×10^{11} neutrons/cm²/min ($\pm 5\%$) and 2.8×10^{11} neutrons/cm²/min ($\pm 5\%$) for *in vitro* irradiations at 1 MW power. The dose contributions from the ¹⁰B(n, α)⁷Li and ¹⁴N(n,p)¹⁴C reactions were calculated using data from the measured n_{th} flux, assuming uniform boron distribution and a nitrogen content of 2.6% *in vivo* and 1.5% *in vitro* (13, 18, 20, 21). The γ photon and fast neutron components were measured using tissue equivalent plastic chambers (A-150 plastic; Far West Technology, Goleta, CA) with TE gas (Rossi gas) and graphite chambers filled with CO₂. The dosimetry of each component for both *in vitro* and *in vivo* irradiations are summarized in Table 1.

The extrinsic γ photons and fast neutron doses were measured by using paired tissue equivalent plastic chambers (A-150 plastic) with TE gas (Rossi gas) and graphite chambers filled with CO₂. The dose rates for each component are tabulated in Table 1. The γ photon dose rate for ¹³⁷Cs was measured by means of an exposure rate meter model 192X (Capintec, Montvale, NJ) with a 0.6-ml Farmer replacement ionization chamber (PR-063).

Statistical Analysis. The Wilcoxon-Gehan rank sum two sample test was applied to the survival data to test for significant differences between the treated groups and controls. All censored rats were ranked equally.

RESULTS

Pharmacokinetics and Tissue Distribution Studies. Blood, brain, and tumor concentration-time profiles of boron after a single i.p. injection of 120 mg of DL-BPA (6.3 mg of B) in nude rats carrying i.c. MRA 27 melanoma are shown in Fig. 1. Pharmacokinetic analysis was performed on the geometric mean of blood boron concentrations using a nonlinear regression program. Blood boron concentration peaked at the first sampling time (1 h) indicating rapid absorption of BPA from the peritoneal cavity. Blood boron levels exhibited biexponential decay and, consequently, were fitted to a classical two compartment open system with first order elimination from the central compartment and with the assumption of a rapid (0.1 min) zero-order input. The half-life of the rapid disposition phase, $t_{1/2\alpha}$ was 0.91 h and the half-life of the slow disposition phase, $t_{1/2\beta}$ was 5.3 h. The apparent total body blood clearance of 520 ml/min reflects a rapid elimination of boron from the body assuming the extent of bioavailability from the i.p. injection was essentially 1. The apparent volumes of distribution of the central compartment (61 ml), at the steady state (118 ml), and at equilibrium (239 ml) for rats having a mean body weight of 150 g are relatively large, indicating extensive tissue binding of BPA.

Tumor levels of boron, however, exhibited monoexponential decay ($t_{1/2} = 5.6$ h) with the terminal time points (6–18 h) in an apparent distribution equilibrium with boron levels observed in the blood. Tumor to blood boron concentration ratios within the terminal phase ranged 2.4–3.8. Normal brain boron concentrations peaked at 6–9 h after BPA administration and then declined monoexponentially. These values were almost the same as the blood boron concentrations. Tumor to normal brain boron concentration ratios within the terminal log-linear phase averaged 2.4.

The best composite ratio for BNCT was observed 6 h post-administration of DL-BPA. At that time the tumor boron concentration was 13.7 μ g/g of tissue and tumor to blood boron concentration and tumor

Table 1 Dose rates for *in vivo* and *in vitro* irradiations at the Brookhaven Medical Research Reactor^a

Component	Gy/min	Gy/min
¹⁴ N (n,p) ¹⁴ C	0.076	0.031
Fast neutrons	0.27	0.13
γ photons	0.11	0.06
¹⁰ B (n, α) ⁷ Li (per μ g ¹⁰ B/g)	0.034	0.024

^a Power level, 1 MW; thermal neutron flux, 3.9×10^{11} neutrons/cm²/min at depth of 4–5 mm beneath the skull surface and 2.8×10^{11} neutrons/cm²/min inside the microfuge tubes.

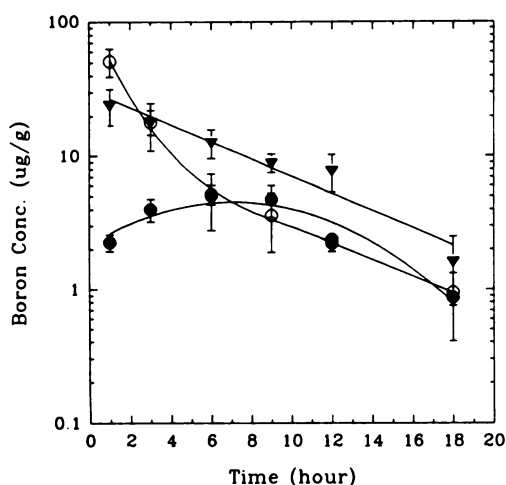


Fig. 1. Boron concentrations in blood (O), brain (●), and tumor (▼) following a single i.p. injection of 120 mg/2 ml of DL-BPA (6.3 mg of boron), as a fructose complex, in nude rats carrying 37-day-old i.c. MRA 27 human melanoma tumors. Points, (\pm SE) of four rats.

Table 2 Distribution of *p*-boronophenylalanine 6 h following administration into nude rats carrying i.c. human melanoma cell line MRA 27^a

Tissue	Boron concentration ($\mu\text{g/g} \pm \text{SD}$) ^b	
	DL-BPA	L-BPA
Brain	5.4 \pm 1.6	8.8 \pm 0.5
Blood	5.7 \pm 3.1	9.9 \pm 3.6
Tumor	13.7 \pm 5.8	24.9 \pm 2.4
Skin	7.6 \pm 2.8	10.5 \pm 4.1
Muscles	7.6 \pm 3.7	14.2 \pm 5.7
Eyes	7.4 \pm 2.3	12.2 \pm 3.9

^a Two $\times 10^5$ MRA 27 cells were implanted stereotactically into the right caudate nucleus of nude rats and 30 days later they were injected i.p. with 120 mg of either DL-BPA or L-BPA as a fructose complex.

^b Boron concentrations were determined by means of direct current plasma atomic emission spectroscopy. The mean \pm 1 SD represents 4 rats.

to normal brain boron concentration ratios were 2.4 and 2.5, respectively. When the same amount of L-BPA was administered i.p. under similar conditions, the tumor boron concentration increased to 24.9 $\mu\text{g/g}$ (Table 2). The tumor to blood boron concentration and tumor to normal brain boron concentration ratios were 2.5 and 2.8, respectively. Furthermore, all other normal tissues exhibited boron concentrations 1.5–1.7-fold higher with the L-BPA than with the DL-BPA (Table 2).

In order to evaluate the propensity of BPA to accumulate in multicentric i.c. melanomas, 5×10^4 MRA 27 cells were implanted into the right and left caudate nuclei and 37 days later rats were injected i.p. with 120 mg of L-BPA. Six h post administration animals were sacrificed and tissues were collected. The ^{10}B concentrations in tumors in the left and right cerebral hemispheres were 20 and 21 $\mu\text{g/g}$, respectively, and in the normal brain and blood they were 8.4 and 8.3 $\mu\text{g/g}$, respectively.

In Vitro Survival Studies. The cell survival curves of MRA 27 following irradiation with 250 kVp x-rays or at Brookhaven Medical Research Reactor are shown in Fig. 2. The dose response curve for 250 kVp x-rays clearly demonstrates a shoulder, whereas none was seen following reactor irradiation alone or in the presence of 10 μg $^{10}\text{B}/\text{ml}$ (^{10}B -BPA). The PE for MRA 27 cells did not change when they were preincubated with ^{10}B -BPA. When 10 $\mu\text{g}/\text{ml}$ of ^{10}B (^{10}B -BPA) were present at ambient conditions during cell irradiations, a remarkable drop in the dose required to decrease the surviving fraction to 37% (estimated from the final slope), (0.66 to 0.14 Gy) was observed (Fig. 2). This reduction was attributed primarily to the high linear energy transfer of the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction. Furthermore, no change in the survival of MRA 27 cells was observed when irradiated with 250 kVp x-rays in the presence of 10 $\mu\text{g}/\text{ml}$ of ^{10}B (Fig. 2).

BNCT Irradiation Studies. BNCT was initiated 30 days following i.c. implantation of 2×10^5 MRA 27 cells. The tumor size index at the time of irradiation was approximately 5.3 mm. The calculated doses in Gy (physical dose) and Gy-Eq delivered to the tumor, blood, and brain are summarized in Table 3. The physical doses represent the contributions of fast neutrons, γ photons, $^{14}\text{N}(n,p)^{14}\text{C}$, and $^{10}\text{B}(n,\alpha)^7\text{Li}$ reactions. In order to convert the physical dose to the equivalent dose, an RBE of 2.3 was assumed for the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction and an RBE of 2 for fast neutrons and the $^{14}\text{N}(n,p)^{14}\text{C}$ reaction (13, 18, 20–22).

Kaplan-Meier plots for BNCT treated animals and the irradiated controls are shown in Fig. 3. All untreated rats (group 6) died by 63 days following implantation and had a tumor size index of 8.0 ± 0.7 . The MeST for group 6 was 44 days compared to 76 days for group 1 (6 MW-M) and 93 days for those animals in group 2 (8 MW-M). Animals from BNCT groups 3–5 had MeSTs of 170, 182, and 262 days, respectively. Ten months following tumor implantation, 40% of the rats from group 5 (BPA + 8 MW-M) were still alive and appeared to be in a good condition (Table 4).

The prolongation of survival times for all irradiated rats (groups 1–5) compared to untreated rats (group 6) were highly significant ($P \leq 0.04$ – 0.0006). The percentages of survival at 100, 150, and 200 days for BNCT treated rats (groups 3–5) were 70–80%, 50–70%, and 20–60% compared to 30–33%, 22–30%, and 22–30%, respectively, for the irradiated controls (groups 1 and 2). These percentages of survival were significantly different at 100 and 150 days ($0.05 \geq P \geq 0.005$) with the exception of group 3 (BPA + 4 MW-M), which was not significant at 150 days.

Another BNCT experiment was initiated 23 days following stereotactic implantation of 2×10^5 MRA 27 cells. The untreated controls ($n = 10$) had a MeST of 37 and 66 days for those ($n = 15$) receiving irradiation dose (no BPA) of 3.6 Gy or 6.4 Gy-Eq (8 MW-M). The MeST for the BNCT group (BPA + 8 MW-M), which received a tumor dose of 10.1 Gy or 21.2 Gy-Eq, was 154 days and 3 out of 14 rats were still alive 220 days following tumor implantation (Fig. 4). The enhanced survival of the BNCT treated rats was statistically significant compared to untreated ($P \leq 0.006$) and the irradiated control group ($P \leq 0.004$). The younger age and lower body weight at the initiation of the experiment may provide a possible explanation for lower MeST of both the untreated and irradiated groups in the second compared to the first experiment (Fig. 3; Table 4). The weight factor

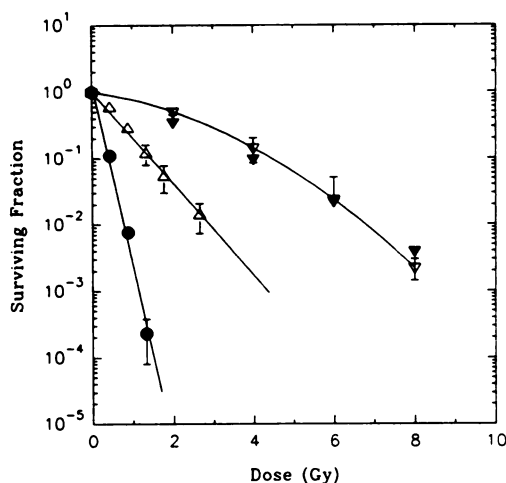


Fig. 2. Survival curves of MRA 27 cells *in vitro* after irradiation with x-rays alone (∇), x-rays + 10 μg of ^{10}B (^{10}B -BPA/ml) (\blacktriangledown), Brookhaven Medical Research Reactor (Δ), and Brookhaven Medical Research Reactor + 10 μg of ^{10}B (^{10}B -BPA) (\bullet). Data represent mean of three experiments (\pm 1 SD). Points per experiment consisted of 3–5 replicates of plated dishes.

Table 3 Radiation doses to tumor and normal tissues at different exposure times^a

Tissue	BPA ^d	Physical dose ^b			Equivalent dose ^c		
		4 MW-M	6 MW-M	8 MW-M	4 MW-M	6 MW-M	8 MW-M
Tumor ^e	-	1.8	2.7	3.6	3.2	4.8	6.4
	+	5.0	7.6	10.0	10.6	15.9	21.2
Blood	-	1.8	2.7	3.6	3.2	4.8	6.4
	+	3.1	4.6	6.2	6.1	9.2	12.3
Brain	-	1.8	2.7	3.6	3.2	4.8	6.4
	+	3.0	4.4	5.9	5.8	8.7	11.6

^a Reactor exposure times are indicated in MW-M.

^b Dose estimates were based on a tumor ¹⁰B concentration of 23.7 μg/g, a blood ¹⁰B concentration of a 9.4 μg/g, and a brain ¹⁰B concentration of 8.4 μg/g. This includes contributions from fast neutrons, γ photons, and the ¹⁴N (n,p) ¹⁴C and ¹⁰B (n,α) ⁷Li reactions.

^c For the estimation of equivalent dose the following RBE values were used: ¹⁴N (n,p) ¹⁴C reaction, 2.0; fast neutrons, 2.0; ¹⁰B (n,α) ⁷Li, 2.3; γ photons, 1.0.

^d Administered i.p. as a fructose complex 6 h prior to irradiation.

^e Two × 10⁵ MRA 27 cells were implanted stereotactically into the right caudate nucleus of nude rats and 30 days later they were irradiated at the Brookhaven Medical Research Reactor.

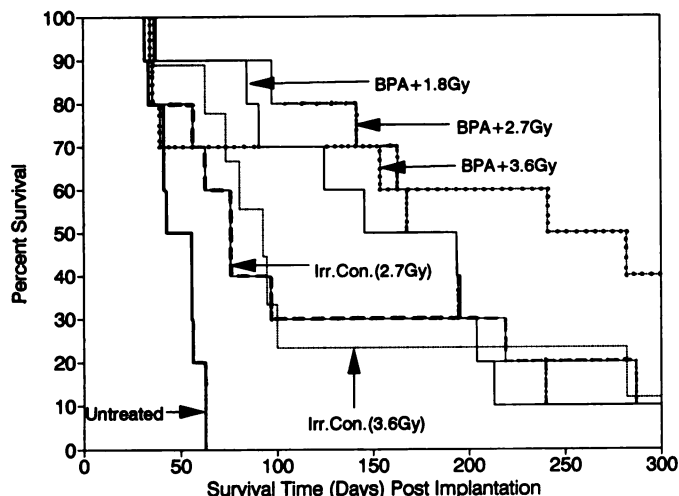


Fig. 3. Kaplan-Meier plots of rats carrying i.c. MRA 27 human melanoma tumor following BNCT or irradiation alone. One hundred twenty mg of ¹⁰B-L-BPA were injected i.p. 6 h prior to reactor irradiation. The therapy was initiated 30 days following i.c. implantation of 2 × 10⁵ of MRA 27 cells into the right caudate nucleus of nude rats.

Table 4 Survival times and percentage reduction of tumor volumes of nude rats carrying i.c. human melanoma cell line MRA 27 after irradiation^a

Group	Treatment		Tumor physical dose (Gy) ^b	MeST	P value ^c
	BPA	Reactor power (MW-M)			
1	-	6	2.7	76	0.02
2	-	8	3.6	93	0.002
3	+	4	5.0	170	0.0006
4	+	6	7.6	181	0.0006
5	+	8	10.0	262	0.04 ^d
6	-	-	0	44	-

^a Two × 10⁵ MRA 27 cells were implanted stereotactically into the right caudate nucleus of nude rats and 30 days later they were irradiated at the Brookhaven Medical Research Reactor.

^b Dose estimates were as in Table 3.

^c Determined using Wilcoxon-Gehan rank sum two sample test to compare for significance between irradiated and untreated groups.

^d In this group, 3 rats died within 5–10 days following irradiation.

appears to be very important in this brain tumor model, since prior to death rats usually demonstrate a dramatic weight loss of approximately 40 g in 3–4 days. Long term surviving animals from both experiments eventually were sacrificed, and there was no evidence of residual tumor, suggesting a complete cure.⁵

γ-Irradiation Studies. Kaplan-Meier plots for rats implanted i.c. with 2 × 10⁵ MRA 27 and then treated 31 days later with γ photons are shown in Fig. 5. The MeSTs for rats treated with 12 Gy and 2.0 Gy × 9 were 86 and 79 days, respectively, compared to 47 days for the

untreated animals. These differences in survival were significant at *P* < 0.01 for both irradiated groups compared to untreated animals. However, there was no significant differences between the γ-irradiated groups.

DISCUSSION

In the present study BNCT was used to treat nude rats carrying i.c. human melanoma with ¹⁰B-BPA as the capture agent. BPA-fructose complex was administered systemically and showed selectivity for the tumor compared to the normal brain and blood, which confirms other previously reported results (8–15, 23, 24). ¹⁰B-BPA concentration in the tumor was 23.7 μg ¹⁰B/g and was within the range (15–30 μg/g) considered for BNCT to be effective (7). The 1.8× higher uptake of the physiological L isomer compared to the DL racemic mixture suggests that ¹⁰B-BPA accumulated in the tumor through a metabolic pathway and not by diffusion and is similar to data reported by Coderre *et al.* (24).

Prolongations of survival times were observed in a dose dependent relationship with all radiation doses, and the higher the calculated radiation dose, the greater the MeST. This was shown with the three BNCT treated groups and was similar to our preliminary results (15). Seventy to 80% of all BNCT treated rats showed long term survival (>100 days) compared to 22–30% for irradiated controls. Forty % of rats treated with BPA + 8 MW-M (21.2 Gy-Eq) were still alive and in good condition 300 days following tumor implantation. In the second experiment, no rats from the irradiated control that had received 3.6

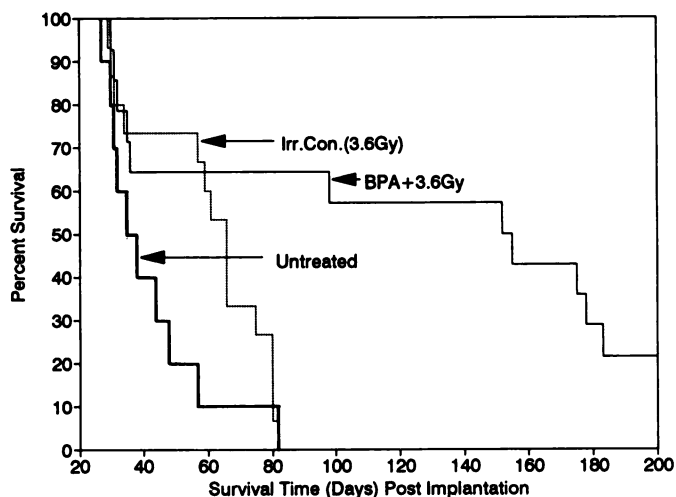


Fig. 4. Kaplan-Meier plots of rats carrying i.c. MRA 27 human melanoma tumor following BNCT or irradiation alone. One hundred twenty mg of ¹⁰B-L-BPA were injected i.p. 6 h prior to reactor irradiation. The therapy was initiated 23 days following i.c. implantation of 2 × 10⁵ MRA 27 cells into the right caudate nucleus of nude rats.

⁵ K. Z. Matalka, manuscript in preparation.

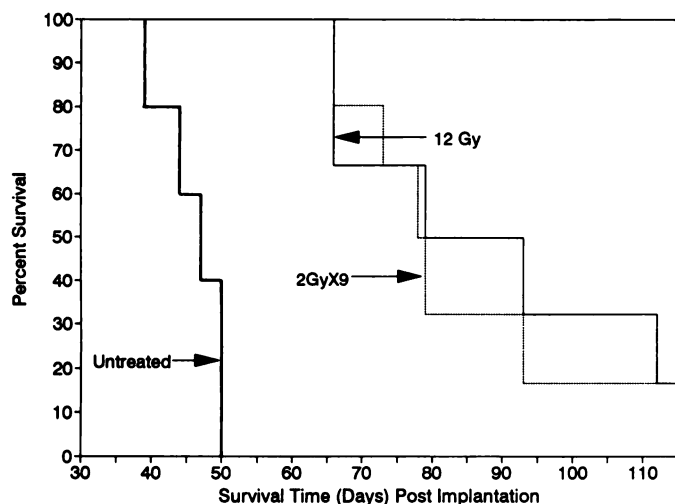


Fig. 5. Kaplan-Meier plots of rats carrying i.c. MRA 27 human melanoma tumor following a single dose of γ -irradiation (12 Gy) or fractionated γ -irradiation (2.0 Gy \times 9). The therapy was initiated 31 days following i.c. implantation of 2×10^5 of MRA 27 cells into the right caudate nucleus of nude rats.

Gy or 6.4 Gy-Eq survived more than 82 days. However, 21% of BNCT treated rats were still alive and in good condition >220 days following implantation.

The MeST of animals treated with BPA and 4 MW-M of irradiation (effective dose, 10.6 Gy-Eq) was higher than that of those rats that had received 12 Gy of γ -irradiation. This could be explained by the 48% hypoxic fraction of MRA 27 cells and their high ability to repair potentially lethal damage.⁶ The presence or absence of oxygen highly influences the biological effectiveness of γ -irradiation by repairing the damage produced by free radicals. On the other hand, oxygen enhancement of tumor cell killing should have no effect on the $^{10}\text{B}(n,\alpha)^7\text{Li}$ and $^{14}\text{N}(n,p)^{14}\text{C}$ reactions.

The toxicological studies of BPA injected into mice and rats at neutral pH have shown no significant systemic toxicity for a dose of 5 and 3 g/kg, respectively (25, 26). In addition, the clonogenic assays of MRA 27 cells with or without BPA showed no change in the plating efficiency. Saris *et al.* (14) have shown that the MeST of glioma bearing mice with or without the administration of BPA (no irradiation) were 18.7 and 18.3 days, respectively. These studies indicate that ^{10}B -BPA is neither toxic nor tumoricidal unless it is subjected to neutron irradiations.

In BNCT groups, the radiation doses to the tumor were 3.3-fold higher than irradiated control groups, and this was attributed to the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction. The radiation effects of the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction are highly dependent upon the subcellular distribution of ^{10}B (27, 28) and since the subcellular localization of ^{10}B -BPA is unknown, the calculated doses in Gy or Gy-Eq are imprecise because they are based on uniform ^{10}B distribution throughout the tumor and normal tissues. Utilizing a double-labeling technique with BPA and tritiated thymidine, it has been shown that ^{10}B -BPA accumulated in proliferating regions of murine melanoma (24). In contrast to BPA, sodium borocaptate or $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$ accumulated more in nonproliferating regions of s.c. implanted murine melanomas (29). This suggests that if both BPA and sodium borocaptate $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$ were used in combination as capture agents, even more favorable tumor boron uptake might be achieved, and the results of BNCT might be better than those obtained with BPA alone.

The first clinical trials of BNCT of patients with brain tumors revealed very serious neurological lesions including coagulation ne-

crisis of both the white and gray matter, vascular thrombosis, fibrin deposition and polymorphonuclear cell infiltrates in the wall of blood vessels, demyelination, and reactive gliosis (30). This was primarily due to the boron compounds used, which were not selectively accumulated by tumors. In the present study, however, the difference in the concentrations of ^{10}B -BPA in the tumor *versus* normal brain and blood resulted in radiation doses to the brain (5.8–11.6 Gy-Eq) and blood (6.1–12.3 Gy-Eq) that were 1.8 and 1.7 times less than those delivered to the tumor. This illustrates the potential advantage of BPA based BNCT over other forms of radiation therapy. Calvo *et al.* (31) have reported that necrosis of the cerebral white matter developed in rats 36 weeks following a single dose of ≥ 22.3 Gy of x-ray irradiation. However, doses of less than 12 Gy, as in the present study, were considered to be tolerable by the brain parenchyma (32, 33). At the present time we are in the process of studying the late radiation effects produced in the rat brain by BNCT following administration of BPA. This should provide information on the normal tissue tolerance of the brain parenchyma and cerebral vasculature following BNCT.

Our data suggest that BPA has promise as a capture agent for BNCT of melanoma metastatic to the brain, but prior to the initiation of any clinical studies, it is essential that the therapeutic efficacy should be determined in a large animal model. Furthermore, it is essential that the long term radiation effects, which may be produced in normal brain following BNCT, be clearly defined.

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⁶ E. K. Rofstad, unpublished observations.

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