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BORON NEUTRON CAPTURE THERAPY OF OCULAR MELANOMA AND INTRACRANIAL GLIOMA USING p-BORONOPHENYLALANINE.

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INTRODUCTION

During conventional radiation therapy, the dose that can be delivered to the tumor is limited by the tolerance of the surrounding normal tissue within the treatment volume. Boron neutron capture therapy (BNCT) represents a promising modality for selective irradiation of tumor tissue. The key to effective BNCT is the selective localization of ^{10}B in the tumor. The ratio of the ^{10}B concentration in the tumor to that in the normal tissues within the treatment volume will largely determine the therapeutic gain that can be achieved in any subsequent irradiation.

We have shown that intragastric (i.g.) administration of the synthetic amino acid p-boronophenylalanine (BPA) as an aqueous slurry at neutral pH is an effective method of drug administration (Coderre, 1988). BPA is non-toxic even at very high doses, is rapidly solubilized in the acidic environment of the stomach and is absorbed in the gastrointestinal tract. The time-course of tumor uptake and clearance of boron following i.g. doses was similar to that observed following i.p. doses of BPA. BPA is an analogue of the melanin precursor tyrosine and as such was originally intended as an agent for BNCT of melanoma. Subsequent studies with BPA have shown that this amino acid analogue is capable of selectively delivering boron to tumors other than melanoma; a murine mammary carcinoma and a rat gliosarcoma (Coderre, 1990). These results indicate that systemically administered BPA may have a general utility as a boron delivery agent for BNCT.

Our therapy experiments have progressed from irradiation of s.c. murine thigh tumors (Coderre, 1988) to the use of more sophisticated tumor models in order to demonstrate the ability of BNCT to effectively treat a tumor in the presence of radiosensitive normal tissues. Thermal neutron irradiations have been carried out at the Brookhaven Medical Research Reactor (BMRR), following i.g. administration of BPA, using the non-pigmented Greene melanoma carried in the anterior chamber of the rabbit eye and the GS-9L gliosarcoma implanted in the rat brain.

METHODS

BPA (as the free amino acid) was administered i.g. by transesophageal intubation with a feeding tube, as an aqueous slurry at neutral pH. Animals received a dose of approximately 750 mg BPA per kg body weight; rats received either 150 mg of L-BPA or 300 mg of D,L-BPA per dose in 3 ml of water, rabbits received up to 2.0 g of D,L-BPA in 15 ml of water. ^{10}B analysis was performed by the prompt gamma spectroscopic technique.

The rabbit ocular melanoma model. The Greene melanoma forms an amelanotic tumor when transplanted into the anterior or posterior chamber of the rabbit eye. During surgical procedures, rabbits were anesthetized with intramuscular ketamine hydrochloride (35mg/kg)/xylazine (5mg/kg). Iris melanomas were initiated in New Zealand White rabbits by implantation of approximately 1 mm^3 fragments of tumor tissue into the anterior chamber through an incision in the sclera at the limbus. Tumor size was measured with a hand-held caliper; 2 perpendicular dimensions were recorded. Tumors grew to about 200 mg in 2-3 weeks.

The rat glioma model. Subcutaneous (s.c.) tumors were initiated in F-344 rats by the injection of 5×10^6 cultured GS-9L gliosarcoma cells in 0.1 ml of growth medium. Tumors were palpable 4-5 days after inoculation and had a volume-doubling time of approximately two days. For initiation of brain tumors, 10^4 cultured cells in 1 μl of medium were injected, at a depth of 3-4 mm, into the left frontal lobe (Joel, 1990). This technique produced a locally expanding tumor with no evidence of blood-borne metastasis or seeding to ventricular or leptomeningeal surfaces. Rats survived an average of 22 ± 4 (SD) days after implantation.

RESULTS AND DISCUSSION

BPA distribution studies in the rabbit. Figure 1 shows the boron concentrations in the anterior chamber melanoma, blood and lens following a single i.g. dose of BPA. At 20 hrs post-administration there was ≈20 μg ^{10}B/g tumor with a tumor-to-blood concentration ratio of ≈4:1. ^{10}B concentrations in liver and muscle (not shown) were similar to those observed in the blood. Quantitative neutron capture radiography of tumor-bearing eyes following BPA administration indicated that the ^{10}B concentration in normal eye tissues was on the order of 3-5 μg/g with the exception of the lens, which showed higher levels of ^{10}B (10-15 μg/g) at the periphery. BNCT irradiations were carried out 20 hours after a single i.g. dose of BPA.

BPA distribution studies in the rat. The time course of ^{10}B accumulation and washout in the tumor as well as the maximum concentration of ^{10}B attained in the tumor were not significantly different when BPA was given as either the purified L-enantiomer (150 mg) or as the racemic mixture (300 mg). Evidently the D-enantiomer is not absorbed efficiently from the gastrointestinal tract and contributes little to the levels of ^{10}B in normal tissues.

Figure 2 shows the concentration of ^{10}B in tumor and normal tissues following i.g. administration of BPA (300 mg D,L-BPA per dose). The slow rate of accumulation of boron in the tumor following a single i.g. dose of BPA (Fig. 2) suggested that a second i.g. dose might produce an additive effect. This

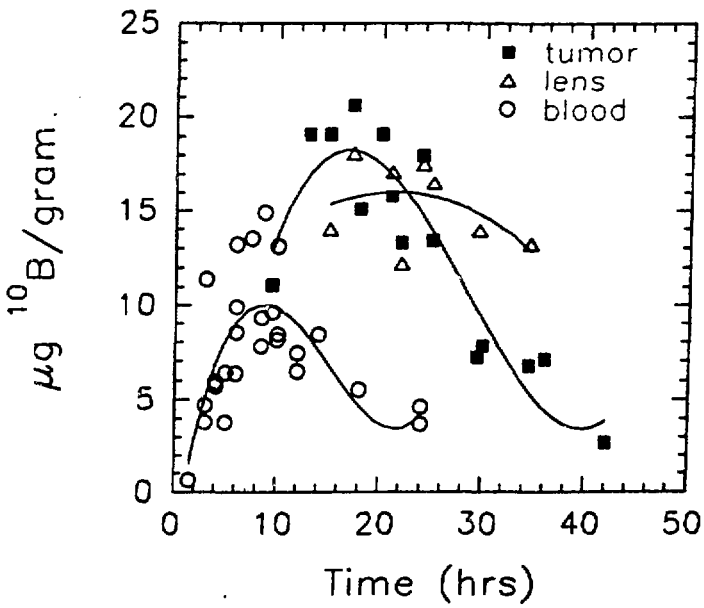

Figure 1. ^{10}B concentrations in anterior chamber Greene melanoma, blood, and lens *versus* time following one i.g. dose of BPA in the rabbit. Individual data points are shown.

was confirmed by comparison of the time courses of boron uptake and clearance from tumor, blood and normal tissues in intracerebral GS-9L rat gliomas following a single i.g. dose of BPA and following two i.g. doses of BPA given 3 hours apart (Fig. 2). The second dose in the sequence substantially increased the maximum concentration of ^{10}B in the tumor, from 24 to 39 μg $^{10}B/g$. Blood ^{10}B was also increased, but a tumor/blood ^{10}B concentration ratio of $\approx$3.3:1 was maintained. BNCT irradiations were carried out eight hours after the first of two i.g. doses given three hours apart.

Dosimetry. The dose from BNCT irradiations is expressed as gray-equivalent (Gy-Eq), calculated by summation of physical doses (Gy) of the component radiations multiplied by appropriate relative biological effectiveness (RBE) factors. RBE values of 2.3 and 2.0 have been assumed for the ionizing particles from the $^{10}B(n,\alpha)^7Li$ and $^{14}N(n,p)^{14}C$ reactions, respectively, and 2.0 has been assumed for fast neutrons.

Tumor-bearing rabbits were irradiated for 6 minutes at 1 megawatt reactor power (6MW-min) using a collimator with a 1.55 cm diameter aperture. The thermal neutron fluence at the tumor position was 5.3 x $10^{12} n_{th} cm^{-2}$. The contributions from the beam components to the total dose within the treatment volume for the 6 MW-min eye irradiation were as follows: fast neutrons, 1.56 Gy; photons from the reactor and the collimator, 0.80 Gy; products of the $^{14}N(n,p)^{14}C$ reaction, 1.03 Gy; and particles from the $^{10}B(n,\alpha)^7Li$ reaction, 0.46 Gy per μg of ^{10}B present per gram wet tissue. In the BPA-BNCT group, the ^{10}B concentration in tumor and normal tissue at the time of irradiation was 20 and 3 $\mu g/g$, respectively. The total radiation dose was calculated to be 12.62 Gy (29.05 Gy-Eq) to the tumor and 4.77 Gy (9.44 Gy-Eq) to the normal eye tissues (cornea, retina). These data imply an average therapeutic gain of approximately 3. The ^{10}B distribution within the lens was non-uniform, as determined by neutron capture radiography; a local ^{10}B concentration of 15 μg $^{10}B/g$ would result in a dose of 10.3 Gy (21.9 Gy-Eq).

Tumor-bearing rats were irradiated for 4 minutes at a reactor power of 1.25 megawatts (5 MW-min) using a collimator with a 1.1 cm diameter aperture. The thermal neutron fluence at the tumor center was 2.0 x 10^{12} $n_{th} cm^{-2}$. The contributions from dosimetrically significant beam components to the total dose within the treatment volume for a 5 MW-min brain irradiation were as follows: fast neutrons, 1.35 Gy; photons from the reactor and the collimator, 0.55 Gy; products of the $^{14}N(n,p)^{14}C$ reaction, 0.38 Gy; and particles from the $^{10}B(n,\alpha)^7Li$ reaction, 0.17 Gy per μg of ^{10}B present per gram wet tissue. In the BPA-BNCT group, boron concentrations in the tumor, normal brain, and the blood at the time of irradiation were estimated to be 39, 12 and 10 μg $^{10}B/g$, respectively. The total radiation dose was

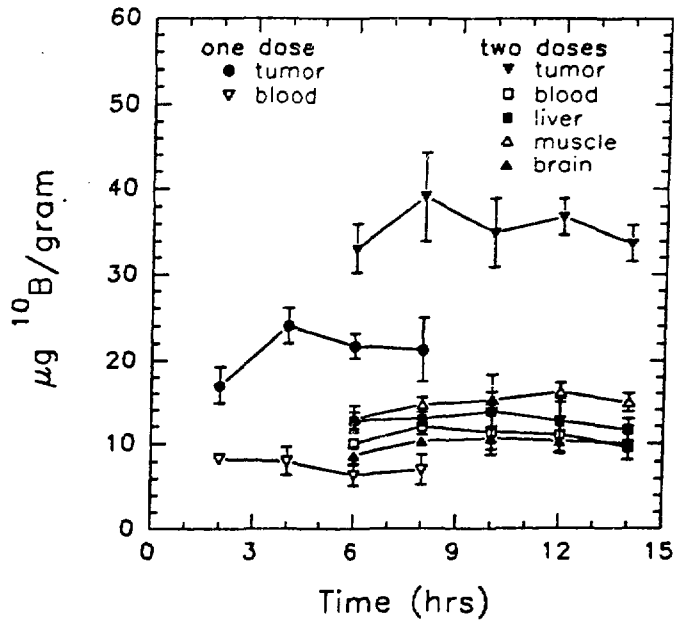

Figure 2. ^{10}B concentration in intracranial GS-9L gliosarcomas and normal tissues *versus* time following either one i.g. dose of BPA at t=0 hrs or two i.g. doses of BPA at t=0 and t=3 hours. The experiment was carried out 18 days after tumor initiation. Each point represents 4 or 5 rats, mean ± 1 SD.

calculated to be 8.9 Gy (19.3 Gy-Eq) to the tumor, 4.3 Gy (8.7 Gy-Eq) to the blood, 4.0 Gy (7.9 Gy-Eq) to the brain, and 4.1 Gy (8.2 Gy-Eq) to the capillary endothelium. The dose to the capillary endothelial cell was calculated as 1/3 of the dose to the blood plus 2/3 of the dose to the normal brain. These data imply average therapeutic gains of 2.2 - 2.4.

BNCT of ocular melanoma. A total of 31 rabbits with anterior chamber melanomas were divided into three groups. Figure 3 shows the tumor growth *versus* time in the groups that received no treatment (Fig.3A), reactor irradiation only (Fig.3B), or BPA-based BNCT (Fig.3C). Three of 13 BNCT-treated tumors grew (Fig.3C); the remaining tumors were monitored for various periods of time. Rabbits with controlled tumors were euthanized at increasing time intervals and the eyes examined histologically. At approximately 3 to 6 months post-irradiation cataract formation was observed in treated eyes as well as a mild to moderate keratitis; the vasculature in the treated eyes appeared normal. All 9 tumors in the reactor-irradiation-only group (Fig.3B) as well as all 9 control tumors (Fig.3A) grew at roughly the same rate.

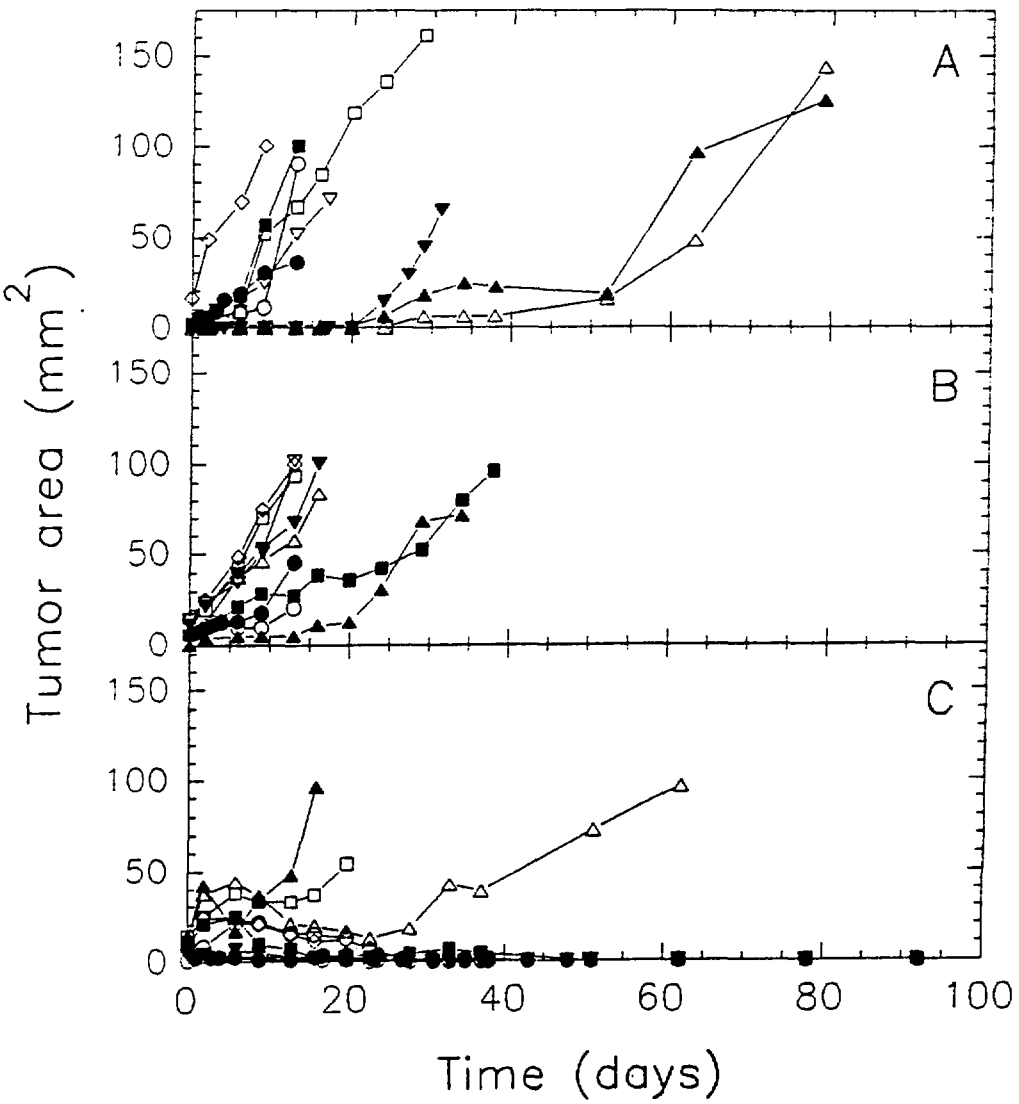

Figure 3. Growth of the Greene melanoma in the anterior chamber of the rabbit eye (tumor area *versus* time) following (A) no treatment (n=9); (B) reactor irradiation only (n=9) and (C) BPA-based BNCT (n=13).

BNCT of intracranial glioma. A total of 44 rats bearing intracerebral tumors were divided into three treatment groups; 1) no treatment (n = 13); 2) reactor irradiation alone (n = 15); and 3) reactor irradiation after two i.g. doses of BPA (n = 16). Reactor irradiations alone caused a transient weight loss. There was no evidence of radiation-induced nasopharyngitis. No post-irradiation supportive therapy was given. Figure 4 shows survival versus time following tumor initiation. The median survival of untreated rats with implanted gliosarcomas was 22 ± 4 (SD) days. Reactor irradiation was performed on day 14 after tumor initiation. Rats receiving reactor irradiations alone had a median survival of 25 days. There were no survivors in group 1 or group 2. The median survival of rats in group 3 was 80 days. Seven of the 16 animals in group 3 are long-term survivors; 4 are alive 13 months post-irradiation and 3 are alive 10 months post-irradiation. All of the seven long-term surviving rats developed cataracts in the left eye 3 to 6 months after BNCT; 4 developed cataracts in the right eye 6-9 months after irradiation.

Joel et al. (1990) have recently reported the results of successful BNCT trials in the GS-9L rat gliosarcoma using the dimer form of the sulfhydryl borane ($Na_4B_{24}H_{22}S_2$). There are differences in the distributions of ^{10}B in blood and normal brain between BPA and dimer which may have radiobiological implications *in vivo*. Table 1 shows, for BPA and the two dimer experiments reported by Joel *et al.* (1990), radiation doses to critical tissues based on comparable tumor ^{10}B concentrations and the corresponding fractions of long-term survivors. These data suggest a dose-response relationship with a 50% tumor control dose of roughly 23 Gy-Eq. Barker et al. (1979) reported a long-term survival of only 1 of 20 rats bearing the intracerebral GS-9L gliosarcoma following 20 Gy of x-rays to the whole head. Joel et al. (1990) also reported long-term survival fractions of 1/10 and 4/16 following x-ray doses of 15.0 and 22.5 Gy, respectively. X-ray doses in the 20 Gy range may be effective in controlling tumor growth in a small percentage of animals but have also been reported to cause delayed brain damage up to two years later (van der Kogel, 1983).

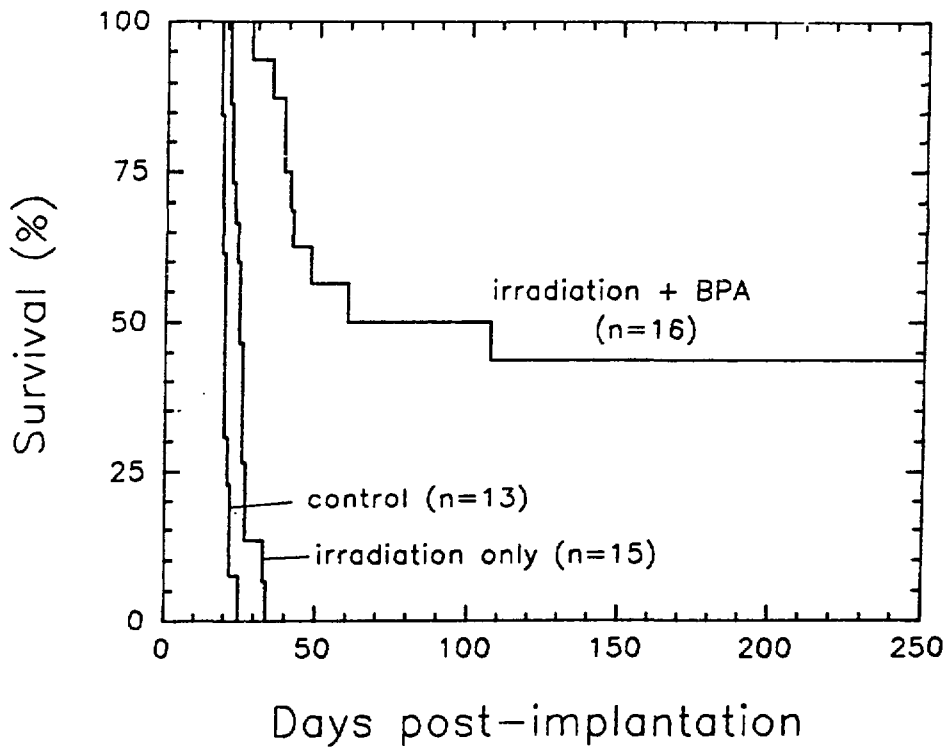

Figure 4. Survival of control and BNCT-treated rats bearing intracerebral GS-9L gliosarcomas *versus* time. Control rats (n = 13) received no treatment. All reactor irradiations were performed 14 days following tumor initiation. Fifteen rats received reactor irradiation only. Sixteen tumor-bearing rats received 2 i.g. doses of BPA (300 mg D,L-BPA each) given 3 hours apart; the rats were irradiated 5 hours after the second dose.

Table 1. BNCT of the Intracerebral GS-9L Gliosarcoma: Comparison of BPA with the Sulfhydryl Borane Dimer.

	BNCT Treatment Conditions		
	Dimer 5MW-min[1]	BPA 5MW-min[2]	Dimer 7.5 MW-min[3]
Tumor Dose (Gy-Eq)	14.2	19.3	25.6
Endothelial Cell Dose (Gy-Eq)	8.8	8.2	15.2
Long-Term Survivors	2/12	7/16	6/10

[1] At the time of irradiation, ^{10}B concentrations in tumor, blood and brain were 26, 35, and 1.4 μg ^{10}B/gram, respectively (Joel, 1990).

[2] At the time of irradiation, ^{10}B concentrations in tumor, blood and brain were 39, 12, and 10 μg ^{10}B/gram, respectively.

[3] At the time of irradiation, ^{10}B concentrations in tumor, blood and brain were 35, 45, and 1.4 μg ^{10}B/gram, respectively (Joel, 1990).

The radiation dose to the vascular endothelium in the brain is more significant, in terms of delayed damage, than similar doses to brain parenchyma (Hopewell, 1989). The lower ^{10}B concentrations in blood following BPA administration compared to the concentrations in blood following dimer infusion and the resulting lower radiation doses to the vascular endothelium may be advantageous in the prevention of delayed damage to the vasculature.

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