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Boron neutron capture therapy of brain tumors

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The Ohio State University, 1992
BORON NEUTRON CAPTURE THERAPY OF BRAIN TUMORS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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*****

The Ohio State University

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Roy F. Barth
To My Parents and My Wife
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ABBREVIATIONS

BMRR, Brookhaven Medical Research Reactor
BNCT, boron neutron capture therapy
BPA, boronophenylalanine
BSH, sodium borocaptate
cGy, centigray
cGy-Eq, effective dose
DCP-AES, direct current plasma atomic emission spectroscopy
H&E, hematoxylin and eosin
%ILS, percentage of increased life span
LET, linear energy transfer
MeST, median survival time
MCNP, Monte Carlo simulation for neutron and photon transport
MST, mean of life span
n_\text{th}, thermal neutrons
MW-M, megawatt-minute
OSURR, Ohio State Research Reactor
PE, plating efficiency
RBE, relative biological effectiveness
R_b, RBE of the \(^{10}\text{B}(n,\alpha)^7\text{Li}\) reaction
R_n, RBE of the fast neutrons
R_p, RBE of the $^{14}\text{N}(n,p)^{14}\text{C}$ reaction
SF, surviving fraction
T/Bl, tumor to blood ratio
T/Br, tumor to brain ratio
TSI, tumor size index
INTRODUCTION

The fight against cancer is continuing in order to treat all malignant tumors that affect human beings. However, the therapeutic modalities that are used, including surgery, radiotherapy, chemotherapy, immunotherapy have not succeeded in enhancing the life expectancy of patients with primary or metastatic brain tumors (1-5). Boron neutron capture therapy (BNCT) is one potential modality for treating such malignancies (6). BNCT is a binary system based on the selective accumulation of $^{10}$B-containing compounds by neoplastic cells, followed by irradiation with low energy or thermal neutrons (0.025 eV). This reaction yields alpha particles and recoiling lithium nuclei associated with 2.31 MeV energy (in 96% of the reaction) and 0.48 MeV of $\gamma$ energy (7).

$$^{10}\text{B} + ^{1}\text{n} \rightarrow ^{11}\text{B}$$

$$^{\alpha} + ^{7}\text{Li} + 2.79 \text{ MeV (6\%)}$$

$$^{\alpha} + ^{7}\text{Li} + 0.48 \text{ MeV } \gamma + 2.31 \text{ MeV (94\%)}$$

Alpha particles and lithium nuclei travel in an opposite directions with pathlength of $\sim$ 10-14 $\mu$. Since the lethal effects of these high linear energy transfer (LET) particles are confined to those cells in which this capture
agent reaction occurs. Normal cells contiguous with and adjacent to the tumor but devoid of the capture reagent will be spared from the radiation damage. In order for BNCT to be therapeutically effective, the boron compounds should have selectivity for tumor cells and achieve $^{10}$B concentration of 15-30 $\mu$g/g. Equally as important, a sufficient fluence of thermal neutrons of $10^{12}$-$10^{13}$ n.cm$^{-2}$ must be delivered to the tumor site (7). Furthermore, the $^{10}$B(n,α)$^7$Li reaction has a high relative biological effectiveness (RBE) (8), and should be effective against hypoxic tumor cells and thereby minimizing their becoming foci for tumor recurrence. In conventional low-LET radiation therapy, however, not only are the normal tissues exposed to high radiation doses equal to that of the tumor but also residual tumor cells are able to survive and proliferate.

Dr. William Sweet, a neurosurgeon at the Massachusetts General Hospital (MGH), suggested using BNCT for the treatment of brain tumors (9). With the collaboration of others, the first clinical trials of BNCT were initiated at the Brookhaven National Laboratory and at the MGH. However, these clinical trials, which were conducted between 1950-1961, failed to improve the life span of patients with brain tumors for the following reasons: poor penetration of thermal neutrons in tissues and most importantly the non-selectivity of the boron compounds used which resulted in
severe radiation damage to the brain (10).

Renewed interest in developing BNCT in the United States started following Hatanaka's work in treating patients with brain tumors (11). In addition, synthesis of more tumor selective boron compounds and optimization of neutron beams (to yield epithermal beams with better penetration in tissues) were the goals for the past 20 years.

In the following chapters, I evaluated first, the efficacy of BNCT using p-boronophenylalanine (BPA) as the capture agent and determined the increase in survival times of rats carrying an intracerebral human melanoma cell line and the F98 glioma. Secondly, I determined the radiation effect on the brain and other normal tissue following BNCT. Thirdly, I have used an intracerebral melanoma model to examine whether preloading the tumor with $^{10}$B has an enhancement on fast neutron brachytherapy using $^{252}$Cf as a neutron source. Fourthly, in vitro BNCT studies were performed to determine the RBE of the $^{10}$B(n,$\alpha$)$^7$Li reaction using two different nuclear reactors (the Brookhaven Medical Research Reactor, and the Ohio State Research Reactor). Finally, in the last chapter, a comparison review of the animal models, which have been used to evaluate different boron compounds, is presented.
REFERENCES


Chapter I

Boron Neutron Capture Therapy of Intracerebral Melanoma

Using Boronophenylalanine as a Capture Agent

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 $2 \times 10^5$ MRA 27 cells were implanted intracerebrally (i.c.), and 30 days later 120 mg of $^{10}$B-enriched L-BPA 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 $\mu$g/g respectively 6 hours following administration. For therapy experiments, tumor bearing rats were irradiated at the Brookhaven Medical Research Reactor 30 days following implantation. The median survival time (MeST) for untreated rats was 44 d, 76 d for those receiving a physical dose of 273 cGy, and 93 d for those receiving 364 cGy. Animals receiving both $^{10}$B-BPA and physical doses of 182, 273, or 364 (total tumor physical doses of 502, 754, and 1005 cGy), had MeSTs of 170, 182, and 262 d respectively. Forty percent of rats that received the highest tumor dose (1005 cGy) survived > 300 d. In a
replicate experiment 50% of animals that received BPA and irradiation (total tumor physical dose of 1005 cGy) were alive 5 months after therapy. In a parallel study, animals that were irradiated with γ photons from a ^{137}Cs source with 1200 cGy or 200 cGy X 9 delivered to the head, had MeSTs of 86 and 79 d respectively compared to 47 d for untreated animals. Our results indicate a significant therapeutic effect of BNCT and suggest that large animal studies are warranted to further assess BNCT of intracerebral melanoma.

INTRODUCTION

Malignant melanoma can metastasize to almost any organ of the body, but especially to the skin, liver, lung, and brain. The metastatic behavior of individual tumors, however, is not random and is difficult to predict (1,2). Patients with brain metastases have a very poor prognosis despite aggressive chemo- and radiotherapy (3-5). In one series of 125 patients, the median survival time of patients with cerebral metastases was 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). In another series of 26 patients with brain metastases, the median survival time did not exceed 5 months following whole brain radiotherapy and systemic chemotherapy (5).
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 (6).

One promising therapeutic modality for the treatment of brain tumors is boron neutron capture therapy. BNCT 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 eV) thermal neutrons (7). The resulting nuclear reaction yields alpha particles and recoiling $^7$Li nuclei, which have high linear energy transfer and pathlengths of approximately one cell diameter (10-14 μ). 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 have used the amino acid p-borono-phenylalanine, a boron-containing melanin precursor analog, as a capture agent for the treatment of cutaneous melanomas in animals (8) and humans (9). In most instances, the BPA was injected perilesionally, allowed to clear from the surrounding normal skin, and then BNCT was initiated. Coderre et al. (10) have demonstrated the efficacy of BNCT in cutaneous melanomas in mice and ocular melanoma in rabbits (11) using systemic injection of BPA.
We previously have reported that BNCT may have some potential as a modality to treat intracerebral melanoma (12). The purpose of the present study was to expand upon our preliminary observations using a human melanoma implanted intracerebrally into nude rats, BPA as the capture agent, followed by BNCT.

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 McCoys'SA media (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine and were tested periodically for mycoplasma contamination by DNA-fluorochrome stain and UV microscopy (Bionique Testing Laboratory, Saranac Lake, NY). Six to eight 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 (13), has been employed. Briefly, nude rats were sedated with a 1.2/1 mixture of ketamine/xylazine and a plastic screw was embedded in the skull. MRA 27 cells were injected through a central hole of the plastic screw into the right caudate nucleus at a concentration of $10^6$ or $2 \times 10^5/10 \mu l$ of serum-free McCoy's 5A medium containing 1% agarose at a gelling temperature of $<30°C$. The implantation procedure employed a relatively slow injection time, rapid filling of the screw hole with bone wax following withdrawal of the needle, and flushing of the operative field with betadiene before closing the scalp incision with a single sterilized clip.

Pharmacokinetics and Tissue Distribution Studies. A solution of either $\delta$, $\lambda$, or $\iota$ forms of BPA, (Callery Chemical Co., Callery, PA), was converted to a fructose complex by mixing BPA and fructose at a 1:1 molar ratio to yield a final concentration of 120 mg of BPA/2 ml of water at pH 8.8. Two ml of the complex were administered i.p. to rats 37 days following intracerebral implantation of $10^6$ tumor cells, unless indicated otherwise. Animals were killed 1, 3, 6, 9, 12, and 18 hours 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 (14). Briefly, 1-2 ml of concentrated sulfuric acid was added to 0.1-1 g of tissues or blood in 150 X 16 mm Pyrex culture tubes and placed in a 100°C-heated mineral oil bath under an exhaust hood for 1 hour. After the samples had cooled to ambient temperature, 1 ml of 70% hydrogen peroxide was added slowly to decolorize the digested tissue. The contents were transferred to a 15 ml graduated plastic tubes (Sarstedt, Newton, MA), the volume was adjusted to 2-3 ml by adding distilled water, and boron content was determined by DCP-AES using an ARL Spectraspan VB Spectrometer (Applied Research Laboratories, Brea, CA).

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 X 10⁵ MRA 27 cells. Rats were divided into six groups of 9-10 animals each. Groups 1 and 2 received 6 or 8 Megawatt-minutes of irradiation respectively. Groups 3, 4, and 5 received 4, 6, or 8 MW-M of irradiation 6 hrs following i.p. administration of 120 mg of 95% ¹⁰B-enriched l-BPA. Group 6 served as untreated controls. All rats were anesthetized with 1.2/1 mixture of ketamine/xylazine and placed supine in a body shield-head
stabilizer, designed by Dr. D. Slatkin, as described elsewhere (15,16). The irradiated site (tumor zone) of the rat's head was centered at 1.15 cm in diameter of the collimated neutron beam aperture. The adjustment of the rat's head was established using a marked lucite plate as a guide for centering the collimated beam.

Gamma irradiations were performed 31 days following implantation of $2 \times 10^5$ MRA 27 cells. Rats were divided into three groups of 5-6 animals each. Group 1a received a single 1200 cGy dose of gamma photons and group 2a received 9 fractions of 200 cGy each, delivered over an eleven day period using a Picker $^{137}$Cs teletherapy machine, which delivered 100 cGy/min at 15 cm SSD when a 2 cm (OD) cone was used. Group 3a was untreated controls. The tumor-bearing region of the rat's head was irradiated directly and the body was shielded with 5 mm lead.

Dosimetry. The mixed radiation in tissue during BNCT includes thermal neutrons (0.025 eV), fast neutrons (>10,000 eV), γ photons and heavy particles that were generated from $^{14}$N(n,p)$^{14}$C and $^{10}$B(n,α)$^{7}$Li reactions. As previously described (16,17), the neutron fluences were measured in a killed rat using a 3 mm long gold wire that had been implanted into the cerebrum at a depth of 4-5 mm below the skull surface. At 5 MW-M the thermal neutron fluence was $2 \times 10^{12}$ n.cm$^{-2}$. The dose contributions from the $^{10}$B(n,α)$^{7}$Li
and \(^{14}\text{N}(n,p)^{14}\) reactions were calculated using data from the measured \(n_{th}\) flux, assuming uniform boron distribution and a tissue nitrogen concentration of 2.6\% (16,17).

The extrinsic \(\gamma\) photons and fast neutron doses were measured by using paired tissue equivalent plastic chambers (A-150 plastic; Far West Technology, Goleta, CA) with TE gas (Rossi gas) and graphite chambers filled with CO\(_2\). The dose rates for each component are tabulated in Table 1. The \(\gamma\) photons dose rate for \(^{137}\text{Cs}\) 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).

Evaluation of Therapeutic Response. The therapeutic response of the treated rats was evaluated first, by the percentage of increased life span, determined from the Median Survival Time in days following implantation of the tumor. The \(\%\text{ILS}\) was determined by the following formula:

\[
\%\text{ILS} = \left( \frac{(\text{MeST of treated group} - \text{MeST of untreated group})}{\text{MeST of untreated group}} \right) \times 100.
\]

Second, the apparent percent reduction of tumor burden (cell number) was calculated for the irradiated groups from in vivo survival data of rats that had been implanted intracerebrally with logarithmically increasing numbers \((10^2 - 5 \times 10^6)\) of MRA 27 cells.

The tumor volume was determined at the time of death from formalin-fixed brains that had been cut coronally at 2
mm interval. The tumor size index was defined as the cube root of the product of the largest measurements of the length, height and width (18).

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 \( \text{d,l-} \)BPA (6.3 mg of B), in nude rats carrying i.c. MRA 27 melanoma are shown in Fig. 1. Pharmacokinetic analysis (Table 2) was performed on the geometric mean of blood boron concentrations using a nonlinear regression program (NONLIN). Blood boron concentration peaked at the first sampling time (1 hr) 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 minute) zero-order input. The half-life of the rapid disposition phase, \( t_{1/2a} \), was 0.907 hr and the half-life of
the slow disposition phase, $t_{1/2s}$, was 5.29 hrs. The apparent total body blood clearance ($Cl_t/F$) of 521.2 ml/min reflects a rapid elimination of boron from the body assuming the extent of bioavailability ($F$) from the i.p. injection is essentially 1. The apparent volumes of distribution ($V_d/F = 60.55$ ml; $V_{ds}/F = 118.26$ ml; and $V_{dg}/F = 238.62$ ml) for rats having a mean body weight of 150 gm are relatively large indicating extensive tissue binding BPA.

Tumor levels of boron, however, exhibited monoexponential decay ($t_{1/2} = 5.64$ hrs) with the terminal time points (6-18 hrs) in an apparent distribution equilibrium with boron levels observed in the blood. Tumor to blood ratios of boron within the terminal phase ranged from 2.4-3.8. Normal brain tissue reached a peak of boron concentration after 6-9 hrs of BPA administration and then declined monoexponentially, essentially superimposed on the boron blood concentrations. Tumor to normal brain ratios of boron within the terminal log-linear phase averaged 2.38.

The best composite ratio for BNCT was observed 6 hrs post administration of $\alpha$-BPA. At that time the tumor boron concentration was 13.7 µg/g of tissue and T/Bl and T/Br ratios were 2.4 and 2.5 respectively. When the same amount of $\alpha$-BPA was administered i.p. under similar conditions, the tumor boron concentration increased to 24.9 µg/g (Table 3). The T/Bl and T/Br ratios were 2.5 and 2.8 respectively. Furthermore, all other normal tissues exhibited boron
concentrations 1.5-1.7 X higher with the \( \text{L}-\text{BPA} \) than with the \( \text{D,L}-\text{BPA} \).

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

BNCT Irradiation Studies. BNCT was initiated 30 days following intracerebral implantation of \( 2 \times 10^5 \) MRA 27 cells. The TSI at the time of irradiation was approximately 5.3 mm. The calculated doses in cGy (physical doses) and cGy-Eq (effective dose or physical dose \( \times \) RBE) delivered to the tumor, blood, and brain are summarized in Table 3. The calculated physical doses represent the contributions of fast neutrons, \( \gamma \) 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 effective dose, an RBE of 2.3 was assumed for the \( ^{10}\text{B}(n,\alpha)^{7}\text{Li} \) reaction and RBE of 2 for fast neutrons and the \( ^{14}\text{N}(n,p)^{14}\text{C} \) reaction (19,20).

Kaplan-Meier plots for BNCT treated animals and the irradiated controls are shown in Fig. 2. All untreated rats
(group 6) died by 63 d following implantation and had a TSI = 8.0 ± 0.7. The MeST for group 6 was 44 d compared to 76 d for group 1 (6 MW-M) and 93 d for those animals in group 2 (8 MW-M). The %ILS for groups 1 and 2 were 73 and 111 respectively. Animals from BNCT groups 3, 4, and 5 had a MeST of 170 d, 182 d, and 262 d respectively with 286, 314, and 495 %ILS. Ten months following tumor implantation, 40% of the rats from group 5 (BPA + 8 MW-M) were still alive and clinically and neurologically appeared to be in a good condition (Table 5).

The prolongation of survival times at 300 d for all irradiated rats (groups 1-5) compared to untreated rats (group 6) were highly significant (p ≤ 0.02-0.0005). Although group 5 (BPA + 8 MW-M) received the highest irradiation dose (1005 cGy or 2115 cGy-Eq) (Table 5), the level of significance compared to group 6 (untreated) was somewhat lower (p ≤ 0.02) than the other treatment groups. This was due to the early deaths of three rats, 5, 6, and 10 days following irradiation because of rapidly expanding intracranial tumors. The most convincing evidence for therapeutic efficacy (statistically and %ILS) was seen with groups 3 (502 cGy or 1056 cGy-Eq) and 4 (754 cGy or 1587 cGy-Eq), when compared to untreated controls (group 6), p ≤ 0.0005 and %ILS = 286 and 314 respectively. The percent survival at 100, 150, and 200 days for BNCT treated rats (group 3, 4, and 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 percent survivals were significantly different at 100 and 150 days (0.05 ≥ p ≥ 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 d following stereotactic implantation of 2 x 10⁵ MRA 27 cells. The untreated controls (n=10) had a MeST of 37 d and 66 d for those (n=15) receiving irradiation dose (no BPA) of 364 cGy or 641 cGy-Eq (8 MW-M). The %ILS for the irradiated control group was 78. The MeST for the BNCT group (BPA + 8 MW-M), which received an irradiation tumor dose of 1005 cGy or 2115 cGy-Eq, could not be determined at 150 d following tumor implantation since 7 out of 14 rats were still alive (Fig. 3). The enhanced survival of the BNCT treated rats was statistically significant compared to untreated (p ≤ 0.006) and the irradiated control group (p ≤ 0.004). The younger age and lower body weight at the initiation of the experiment may provide a possible explanation for lower MeSTs of both the untreated and irradiated groups in the second compared to the first experiment (Fig. 2, Table 5). The weight factor appears to be very important in this brain tumor model, since prior to death rats usually demonstrate a dramatic weight loss of approximately 40 gm in 3-4 days.
Gamma Irradiation Studies. Kaplan-Meier plots for rats implanted intracerebrally with $2 \times 10^5$ MRA 27 and then treated 31 d later with $\gamma$ photons are shown in Fig. 4. The MeST for rats treated with 1200 cGy and 200 cGy X 9 were 86 d and 79 d respectively, compared to 47 d for the untreated animals. These differences in survival were significant at $p < 0.01$ for both irradiated groups compared to untreated animals. The %ILS of rats that had received 1200 cGy and 200 cGy X 9 were 83% and 67% respectively, and these were not significantly different from one another.

In Vivo Survival Study. The MeST of rats implanted intracerebrally with $10^2$ to $5 \times 10^5$ MRA 27 cells are shown in Fig. 5. The MeSTs were 41 d for $5 \times 10^5$ cells, 43 d for $2 \times 10^5$ cells, 60 d for $10^4$ cells, 93 d for $10^3$ cells, and 138 d for $10^2$ cells. Extrapolation from the survival curves revealed MeSTs of 185 d for 10 cells, and 264 d for 1 cell. The apparent percent reduction in tumor burden (cell number) for the BNCT groups (3, 4, and 5) and the irradiated control groups (1 and 2) was determined by extrapolation from Fig. 5. Control groups 1 and 2 had a 96.0 and 99.1% reductions of tumor burden compared to 99.99, 99.991, and 99.9991% reductions for BNCT groups (3, 4, and 5) (Table 5).
DISCUSSION

In the present study BNCT was used to treat nude rats carrying intracerebral human melanoma with $^{10}$B-BPA as the capture agent. BPA-fructose complex was administered systemically and showed selectivity for the tumor compared to the normal brain and blood. This selectivity of BPA for melanoma cells confirms other previously reported results (8-10, 21, 22). BNCT was initiated when the $^{10}$B-BPA concentration in the tumor was 23.7 $\mu$g $^{10}$B/g and T/B1 and T/Br ratio were > 2.5. 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 and %ILS. This was shown with the three BNCT treated groups and was similar to our preliminary results (12). Seventy to 80% of all BNCT treated rats showed long term survival (> 100 days) compared to 22-30% for irradiated controls. Forty percent of rats treated with BPA + 8 MW-M (2115 cGy-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 364 cGy or 641 cGy-eq survived more than 82 d. However, 50% of BNCT treated rats were still alive and in good condition > 150 d following implantation.

The tumor boron profile of human melanoma MRA 27 implanted intracerebrally into nude rats showed a
monoexponential decay while the blood boron profile exhibited a biexponential decay. This resulted in sufficient retention of boron in the tumor to sustain a $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction and concomitantly the T/B1 ratio was > 2.5. $^{10}\text{B}$ concentration in the tumor (23.7 μg/g) at the time of irradiation was within the range (15–30 μg/g) considered for BNCT to be effective (7). The 1.8 X higher uptake of the physiological L isomer compared to the D,L racemic mixture suggests that $^{10}\text{B}$-BPA accumulated in the tumor through a metabolic pathway and not by diffusion, and is similar to data reported by Coderre et al (22).

In all BNCT groups 3, 4, and 5 the radiation doses to the tumor were 3.3 X higher than irradiated control groups 1 and 2, and this was attributed to the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction. However, the calculated doses in cGy or cGy-Eq are based on the assumption of uniform $^{10}\text{B}$ distribution throughout the tumor and normal tissues, and this may not necessarily be the case. Utilizing a double-labelling technique with BPA and tritiated thymidine, it has been shown that $^{10}\text{B}$-BPA accumulated in proliferating regions of murine melanoma (22). Furthermore, the radiation effects of the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction are highly dependent upon the subcellular distribution of $^{10}\text{B}$ (23,24). In contrast to BPA, sodium borocaptate or BSH (Na$_2$B$_{12}$H$_{12}$SH) accumulated more in nonproliferating regions of s.c. implanted murine melanomas (25). This suggests that if both BPA and BSH 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.

In all BNCT groups 3, 4, and 5, the radiation doses (582-1163 cGy-Eq) to the brain and (612-1225 cGy) blood were 1.8 and 1.7 X less than the radiation doses to the tumor. This was due to the difference in the concentrations of $^{10}$B in the tumor versus normal brain and blood. These differences in radiation doses to the tumor and normal surrounding tissues illustrate the potential advantage of BNCT over other forms of radiation therapy. Calvo et al (26) have reported that necrosis of the cerebral white matter developed in rats 36 weeks following a single dose of $\geq 22.3$ cGy of X-ray irradiation. However, doses of less than 1200 cGy were considered to be tolerable by the brain parenchyma (27,28). 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.

The MeSTs and %ILS of animals treated with BPA and 4 MW-M of irradiation (effective dose 1056 cGy-Eq) were higher than those rats that had received 1200 cGy of $\gamma$-irradiation. This could be explained by the 48% hypoxic fraction of MRA 27 cells and their high ability to repair potential lethal
damage (Rofstad, unpublished observations). The presence or absence of oxygen highly influences the biological effectiveness of \(\gamma\)-irradiation by fixing the damage produced by free radicals. On the other hand, oxygen enhancement of tumor cell killing has no effect on the \(^{10}\text{B}(n,\alpha)^{7}\text{Li}\) and \(^{14}\text{N}(n,p)^{14}\text{C}\) reactions.

The apparent \% reduction of tumor burden in all irradiated groups was dependent upon the radiation dose and in all BNCT groups the reduction of the tumor burden was 2 to 3 logs higher than that of irradiated controls. However, after irradiation, tumor growth delay might have been due to reduction in the tumor cell number, destruction of tumor vasculature, tumor cell arrest, and tumor bed effect (29-32). However, Goodman et al have not observed a TBE when BSH was used as the capture agent, although there was a direct effect on the tumor and tumor vasculature (32).

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 intracerebral melanoma. Our model, therefore, should be useful for the evaluation of other therapeutic modalities for the treatment of intracerebral melanoma. 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.
REFERENCES


28

IAEA, Vienna, 1962.


Table 1. Dose rates for rat brain irradiations at the Brookhaven Medical Research Reactor (BMRR)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Component</th>
<th>cGy/min</th>
<th>cGy-Eq/min\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{14}\text{N}(n,p)^{14}\text{C})</td>
<td>7.56</td>
<td>15.12</td>
</tr>
<tr>
<td>Fast neutrons</td>
<td>27</td>
<td>54</td>
</tr>
<tr>
<td>Gamma photons</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>45.56</td>
<td>80.12</td>
</tr>
</tbody>
</table>

\(^{10}\text{B}(n,\alpha)^{7}\text{Li}\) | 3.38/\mu g\textsuperscript{10}\text{B} | 7.77/\mu g\textsuperscript{10}\text{B} |

\textsuperscript{a} Power level = 1 MW, thermal neutron flux = 3.9 \times 10^{11} n.cm\textsuperscript{-2}min\textsuperscript{-1} at depth of 4-5 mm beneath the skull surface

\textsuperscript{b} An RBE of 2 has been assigned to fast neutrons and the \(^{14}\text{N}(n,p)^{14}\text{C}\) reaction and 2.3 for the \(^{10}\text{B}(n,\alpha)^{7}\text{Li}\) reaction. Since the latter estimate depends upon microdosimetry it is more imprecise than the former.
Table 2. Pharmacokinetic parameters of boron after i.p. administration of \( \delta, \gamma \)-BPA in nude rats carrying i.c. melanoma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-life of rapid disposition phase</td>
<td>( t_{1/2a} )</td>
<td>hr</td>
<td>0.907</td>
</tr>
<tr>
<td>Half-life of slow disposition phase</td>
<td>( t_{1/2b} )</td>
<td>hr</td>
<td>5.29</td>
</tr>
<tr>
<td>Apparent total body clearance</td>
<td>( C_{l}/F )</td>
<td>ml/min</td>
<td>521.2</td>
</tr>
<tr>
<td>Apparent volume of distribution of the central compartment</td>
<td>( V_{i}/F )</td>
<td>ml</td>
<td>60.55</td>
</tr>
<tr>
<td>Apparent volume of distribution at steady state</td>
<td>( V_{ds}/F )</td>
<td>ml</td>
<td>118.26</td>
</tr>
<tr>
<td>Apparent volume of distribution at equilibrium</td>
<td>( V_{de}/F )</td>
<td>ml</td>
<td>238.62</td>
</tr>
</tbody>
</table>

\( a \) 10⁶ MRA.27 cells were implanted into the right caudate nucleus of nude rats

\( b \) 120 mg/2 ml of \( \delta, \gamma \)-BPA (6.3 mg of boron) was administered i.p. as a fructose complex 37 days following tumor implantation
Table 3. Distribution of $p$-boronophenylalanine (BPA) 6 hours following administration to nude rats carrying intracerebral human melanoma cell line MRA 27°

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Boron concentration (μg/g ± SD)°</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$d,l$-BPA</td>
</tr>
<tr>
<td>Brain (Br)</td>
<td>5.4 ± 1.6</td>
</tr>
<tr>
<td>Blood (Bl)</td>
<td>5.7 ± 3.1</td>
</tr>
<tr>
<td>Tumor (T)</td>
<td>13.7 ± 5.8</td>
</tr>
<tr>
<td>T/Bl ratio</td>
<td>2.4</td>
</tr>
<tr>
<td>T/Br ratio</td>
<td>2.5</td>
</tr>
</tbody>
</table>

° 2 X $10^5$ 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 $d,l$-BPA or $l$-BPA as a fructose complex.

° Boron concentrations were determined by means of direct current plasma atomic emission spectroscopy.
Table 4. Radiation doses to critical tissues at different reactor powers\textsuperscript{a}

<table>
<thead>
<tr>
<th>Tissue</th>
<th>BPA\textsuperscript{d}</th>
<th>Physical dose (cGy)\textsuperscript{b}</th>
<th>Effective dose (cGy-Eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 MW-M</td>
<td>6 MW-M</td>
<td>8 MW-M</td>
</tr>
<tr>
<td>Tumor\textsuperscript{b}</td>
<td>-</td>
<td>182</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>502</td>
<td>754</td>
</tr>
<tr>
<td>Blood</td>
<td>-</td>
<td>182</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>309</td>
<td>464</td>
</tr>
<tr>
<td>Brain</td>
<td>-</td>
<td>182</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>296</td>
<td>443</td>
</tr>
<tr>
<td>Skin</td>
<td>-</td>
<td>182</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>317</td>
<td>475</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Power levels are indicated in megawatt minutes (MW-M). This includes contributions from fast neutrons, photons, and the \textsuperscript{14}N(n,p)\textsuperscript{14}C and \textsuperscript{10}B(n,\alpha)\textsuperscript{Li} reactions.

\textsuperscript{b} 2 X 10\textsuperscript{5} MRA 27 cells were implanted stereotactically into the right caudate nucleus of nude rats and 30 days later they were irradiated at the BMRR.

\textsuperscript{c} Dose estimates were based on a tumor \textsuperscript{10}B concentration = 23.7 \mu g/g, blood = 9.4 \mu g/g, brain = 8.4 \mu g/g, and skin = 10.0 \mu g/g.

\textsuperscript{d} BPA was administered i.p. as a fructose complex 6 hours prior to irradiation.
Table 5. Survival times and percent reduction of tumor volumes of nude rats carrying intracerebral human melanoma cell line MRA 27 after irradiation

<table>
<thead>
<tr>
<th>Group</th>
<th>BPA</th>
<th>Reactor power (MW-M)</th>
<th>Physical dose (cGy)</th>
<th>MeST^c</th>
<th>%ILSd</th>
<th>Apparent % reduction of tumor volume^o</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>44</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>6</td>
<td>273</td>
<td>76</td>
<td>73</td>
<td>96.0</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>8</td>
<td>364</td>
<td>93</td>
<td>111</td>
<td>99.1</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>4</td>
<td>502</td>
<td>170</td>
<td>286</td>
<td>99.99</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>6</td>
<td>754</td>
<td>181</td>
<td>314</td>
<td>99.991</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>8</td>
<td>1005</td>
<td>262</td>
<td>495</td>
<td>99.9991</td>
</tr>
</tbody>
</table>

* 2 X 10^5 MRA 27 cells were implanted stereotactically into the right caudate nucleus of nude rats and 30 days later they were irradiated at the BMRR.

^b calculated from Table 1

^c MeST; Median Survival Time

^d %ILS; percent of increased life span

^o determined from Figure 4
Figure 1. Blood (o), brain (e), and tumor (▼) concentration-time profiles of BPA as measured by boron concentrations following a single i.p. injection of 120 mg/2 ml of d,l-BPA (6.3 mg of boron), as a fructose complex, in nude rats carrying 37 day-old i.c. MRA 27 human melanoma tumor.
Figure 1.
Figure 2. Kaplan-Meier plots of rats carrying i.c. MRA 27 human melanoma tumor following BNCT or irradiation alone. 120 mg of $^{10}\text{B-}^{-}\text{BPA}$ were injected i.p. 6 hr prior to reactor irradiation. The therapy was initiated 30 days following i.c. implantation of $2 \times 10^5$ of MRA 27 cells into the right caudate nucleus of nude rats.
Figure 2.
Figure 3. Kaplan-Meier plots of rats carrying i.c. MRA 27 human melanoma tumor following BNCT or irradiation alone. 120 mg of $^{10}\text{B}^{-}$-BPA were injected i.p. 6 hr prior to reactor irradiation. The therapy was initiated 23 days following i.c. implantation of $2 \times 10^5$ of MRA 27 cells into the right caudate nucleus of nude rats.
Figure 4. Kaplan-Meier plots of rats carrying i.c. MRA 27 human melanoma tumor following γ irradiation of 1200 cGy or 200 cGy X 9. The therapy was initiated 31 days following i.c. implantation of 2 X 10^5 of MRA 27 cells into the right caudate nucleus of nude rats.
Figure 5. *In vivo* survival study of nude rats carrying i.c. a log number of MRA 27 human melanoma cells, (●) was actual survival time, and (○) the extrapolated survival time.
Figure 5.
Chapter II

Boron Neutron Capture Therapy of F98 Glioma Bearing Rats

Using Boronophenylalanine as a Capture Agent

ABSTRACT

The purpose of the present study was to determine the efficacy of boron neutron capture therapy for the F98 glioma, using boronophenylalanine (BPA) as the capture agent. $10^5$ F98 glioma cells were implanted stereotactically into Fischer rats and 15 days later they were injected i.p. with 897 mg/kg of $\alpha,\beta$-BPA. Between 3 and 9 hours post administration, blood and tumor boron concentrations exhibited monoexponential decay with a terminal half life ($t_{1/2}$) of 4.3 and 5.3 hr respectively. When 803 mg/kg of $^10$B-1-BPA were administered, the tumor $^10$B concentration was 29.4 $\mu$g/g and tumor to blood and tumor to brain ratios were 3.5 and 3.9 respectively. Seven days following i.c. implantation of $10^6$ F98 cells BNCT was initiated at the Brookhaven Medical Research Reactor. The MeST for irradiated controls (no BPA) that had received irradiation doses of 182, 273, or 364 cGy were 27, 33, and 38 d respectively, compared to 24 d for untreated rats (p ≤
0.025-0.0001). The MeST for BNCT treated groups that had received 803 mg/kg of $^{10}$B-$\text{-}$BPA 6 hr prior to irradiation with total estimated tumor doses of 579, 889, and 1159 Gy, were 32, 37 and 59 d respectively. Although the enhanced MeSTs of two of the BNCT treated groups (889 and 1159 Gy) were significant compared to their matched, irradiated controls ($p \leq 0.0175$-$0.0277$), all BNCT treated animals died indicating that BPA may not be the ideal capture agent for treatment of the F98 glioma, and that the search for better glioma localizing boron compounds must continue.

INTRODUCTION

Fifty percent of primary malignancies of the central nervous system are anaplastic astrocytomas and glioblastoma multiforme (1). The prognosis of patients with these tumors is dismal despite aggressive surgery followed by radio- and chemotherapy, and characteristically the mean survival time of these patients still is approximately 12 months (2-4).

One potential therapeutic modality for high grade malignant brain tumors is boron neutron capture therapy (BNCT). BNCT is a binary system based on selective uptake of sufficient amounts of a stable isotope, $^{10}$B, by the tumor cells, followed by irradiation with low energy (0.025 eV) thermal neutrons ($n_{th}$) (5). The resulting nuclear reaction yields alpha particles and recoiling $^7$Li nuclei, which have
high linear energy transfer (LET) and short pathlengths of approximately one cell diameter (10-14 μm). In order for BNCT to be successful, there must be a preferential accumulation of $^{10}$B in the tumor, low levels in the blood and surrounding normal tissues, and a sufficient thermal neutron fluence delivered to the tumor site (6).

Boronophenylalanine (BPA) is boron-containing amino acid, which was synthesized as an analogue of the melanin precursor tyrosine (7,8). In Japan, Mishima has used this compound as a capture agent for BNCT of melanomas in experimental animals (9), and clinically in patients with cutaneous melanomas (10). Coderre et al have reported that BPA also was selectively taken up in vivo by non-melanotic tumors, including KHJJ murine mammary tumor, rat 9L-gliosarcoma, and human U-87 MG glioma (11). $^{10}$B-BPA has been used as a capture agent for BNCT of the 9L-gliosarcoma (12), and survival times of treated rats were significantly prolonged with an apparent cure rate of 44% compared to neutron irradiated controls. These findings have encouraged others to consider the possibility of using BPA as a capture agent for BNCT of glioblastoma in humans (13).

We previously have studied the effects of BNCT on the F98 rat glioma using sodium borocaptate or BSH ($\text{Na}_2\text{B}_12\text{H}_{12}\text{SH}$) as the capture agent (14). This compound has been used by Hatanaka et al in Japan for BNCT of patients with glioblastomas (15). Since the F98 glioma closely simulates
human glioblastoma in its biologic behavior, and has been refractory to all therapeutic agents (14,16,17), we thought it would serve as useful model to further define the efficacy of BPA as a capture agent for brain tumors. The data obtained indicate that although BPA may be useful as a capture agent for intracerebral melanoma (18,19), but it is less certain whether this is the case for high grade malignant gliomas.

MATERIALS AND METHODS

Glioma Cell Line. The F98 glioma cell line was derived from undifferentiated neoplasm induced by N-ethyl-N-nitrosourea in an inbred CD Fischer rat (20). Its morphology and growth characteristics have been described in detail elsewhere (20,21). F98 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine.

Rat Brain Tumor Model. Six-week-old male Fischer rats weighing 160-180 g were purchased from Charles River (Wilmington, MA). A previously described stereotactic implantation procedure was employed (14,22). Briefly, rats were sedated with a 1.2/1 mixture of ketamine/xylazine and a
plastic screw was embedded into the skull. F98 cells were injected into the right caudate nucleus at a concentration of $10^5$ cells/10 $\mu$l of serum-free Dulbecco’s medium containing 1% agarose at a gelling temperature of $< 30^\circ$C. The implantation procedure employed a relatively slow injection time, rapid filling of the screw hole with bone wax following withdrawal of the needle, and flushing of the operative site with betadiene before closing the scalp incision with a single sterilized clip.

Pharmacokinetics and Tissue Distribution Studies. A solution of either $\beta$- or $\alpha$- forms of BPA (Callery Chemical Co., Callery, PA), was converted to a fructose complex by mixing 1:1 molar ratio of BPA and fructose to enhance the solubility of the compound at pH 8.8. F98 tumor-bearing rats were injected i.p. with $\beta\alpha$-BPA at a dose of 897 mg/kg of body weight, which was equivalent to 47.2 mg of B/kg of body weight, 15 days following intracerebral implantation of $10^5$ tumor cells. Animals were killed 3, 6, and 9 hours later and samples of blood, brain, tumor, skin, liver, kidneys, and muscle were taken, weighed, and then frozen until boron determinations were carried out. Boron concentrations were determined by means of direct current plasma atomic emission spectroscopy (DCP-AES), as described in detail elsewhere (23). Briefly, 1-2 ml of concentrated sulfuric acid were added to 0.1-1 g of tissue or blood in
150 X 16 mm Pyrex culture tubes and placed in a 100 °C-heated mineral oil bath under an exhaust hood for 1 hour. After the samples had cooled to ambient temperature, 1 ml of 70% hydrogen peroxide was added slowly to decolorize the digested tissue. The contents were transferred to a 15 ml graduated plastic tubes (Sarstedt, Newton, MA), the volume was adjusted to 2-3 ml by adding distilled water, and boron content was determined by DCP-AES using an ARL Spectraspan VB spectrometer (Applied Research Laboratories, Brea, CA).

In Vitro Irradiation Studies and Clonogenic Assay. In vitro irradiations of F98 glioma cells were carried out at the Brookhaven Medical Research Reactor (BMRR) at 1 MW reactor power with a thermal neutron flux of 2.8 x 10^{11} n.cm^{-2}min^{-1}. Cells were incubated for 24 hours with BPA at a concentration of 25 μg ^{10}B/ml of growth medium, prior to irradiation. As previously described (24), the same concentration of ^{10}B was maintained in the medium during trypsinization, harvesting, and irradiation. The cells were irradiated at a density of 2 x 10^6/ml at ambient temperature. Following irradiation, the cells were placed in boron-free medium, plated into petri dishes and incubated at 37°C in humidified atmosphere containing 5% CO₂. Fourteen days later the cultures were terminated, the plates were washed with HBSS, fixed with absolute ethanol and stained with 10% Giemsa. Colonies ≥ 50 cells (0.3 mm) were
enumerated visually or by means of an Artek 880 image analyzer (Artek System Co., Farmingdale, New York). Plating efficiency (PE) = \[ \frac{\text{number of F98 colonies enumerated}}{\text{total number of F98 cells plated}} \] x 100%. The surviving fraction was determined from \[ \frac{\text{number of colonies enumerated}}{\left( \frac{\text{total number of F98 cells plated}}{100} \right)} \].

*In vitro* X-ray irradiations were carried out using the 250 kVp at 30 mA with 0.5 mm Cu and 1.0 mm Al filtration. The dose rate was 90 cGy/min. The cells were irradiated in boron-free media and assayed as described above.

*In Vivo* Irradiations. BNCT was initiated 7 days following i.c. implantation of \(10^6\) F98 cells. Rats were divided into seven groups of 8-10 animals each. All irradiations were carried out at the BMRR. The reactor power was maintained at 2 MW during the irradiation of all rats. Groups 1, 2, and 3 received 4, 6, or 8 Megawatt-minutes (MW-M) of irradiation respectively. Groups 4, 5, and 6 received 4, 6, or 8 MW-M of irradiation 6 hrs following i.p. administration of 803 mg/kg of body weight of \(^{10}\)B-enriched L-BPA. Animals in group 7 were untreated controls. All rats were anesthetized with 1.2/1 mixture of ketamine/xylazine and placed supine in a body shield-head stabilizer, designed by Dr. D. Slatkin (25). The irradiation site of the rat’s head (tumor zone) was centered in the 1.15 cm in diameter of the restricted neutron beam aperture. The adjustment of the
rat's head was established using a marked lucite plate as a
guide for centering the collimated beam.

Dosimetry. The mixed radiation in tissue during in vivo or
in vitro BNCT includes thermal (0.025 eV) and fast neutrons
(>10,000 eV), γ photons and heavy particles that are
generated from \(^{14}\text{N}(n,p)^{14}\text{C}\) and \(^{10}\text{B}(n,\alpha)^{7}\text{Li}\) reactions. As
described elsewhere (26,27), n\(_\text{th}\) fluences had been measured
previously 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\(_{\text{th}}\) flux at a depth of 4-5 mm below the skull was \(3.9 \times 10^{11}\)
n.cm\(^{-2}\).min\(^{-1}\) at 1 MW reactor power and \(2.8 \times 10^{11}\) n.cm\(^{-2}\).min\(^{-1}\) for
in vitro irradiations. The doses from \(^{10}\text{B}(n,\alpha)^{7}\text{Li}\) and
\(^{14}\text{N}(n,p)^{14}\text{C}\) reactions were calculated from the n\(_{\text{th}}\) fluence,
assuming uniform boron distribution and a nitrogen content
of 2.6% in vivo and 1.5% in vitro (26-28). The γ photons
and fast neutron 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 CO\(_2\). The dosimetry of each component for both
in vitro and in vivo irradiations are summarized in Table 7.

Evaluation of Therapeutic Response. The therapeutic
response of the treated rats was evaluated by the Median
(MeST) and Mean Survival Time (MST) in days following implantation of the tumor. The percentage of increased life span (%ILS) was determined by the following formula: %ILS = 
\[
\frac{(MeST or MST of treated group - MeST or MST of untreated group)}{(MeST or MST of untreated group)} \times 100.
\]

Statistical Analysis. The Wilcoxon-Gehan rank sum test was applied to test for level of significance in survival times between BNCT treated, irradiated control groups, and the untreated control group.

RESULTS

Pharmacokinetics and Tissue Distribution Studies. Blood, brain and tumor concentration profiles following a single i.p. injection of 47 mg/kg body weight of boron (897 mg/kg of $\beta_\alpha$-BPA) are shown in Fig. 6. Pharmacokinetic analysis was performed on the geometric mean of the blood and tumor boron concentrations using a non-linear regression program (NONLIN). Between 3 and 9 hr post administration of $\beta_\alpha$-BPA, blood boron concentrations exhibited monoexponential decay with a terminal half-life ($t_{1/2}$) of 4.3 hr and a terminal rate constant of 0.16 hr$^{-1}$. Tumor boron concentrations also exhibited monoexponential decay with a $t_{1/2}\beta$ of 5.28 hr. The best tumor to blood (T/Bl) ratio was seen at 6 hr post administration of BPA, while T/Bl ratios at 3, 6, and 9 hr
ranged from 2.2-2.6. Tumor to brain (T/Br) ratios ranged from 2.9-4.9 and the best ratio was seen at 3 hr post BPA administration. The best composite ratios of T/Br and T/Bl were 3.6 and 2.6 respectively, at 6 hours post administration of BPA at which time the average tumor boron concentration was 30.6 µg/g (Table 6). Similar results were obtained when 803 mg/kg of 10B-enriched L-BPA (36.7 mg 10B/kg) was administered to tumor bearing rats, although T/Bl and T/Br ratios of 10B were somewhat higher (3.5 and 3.9 respectively).

Tumor Cell Irradiations. The survival curves of F98 cells following BNCT and X-rays are shown in Fig. 7 and clearly illustrate the difference between low LET X-rays and high LET irradiation. There was no shoulder following reactor irradiation, whereas one was evident following X-irradiation. \( D_s \) was 620 cGy and \( D_o \) was 176 cGy following X-irradiation compared to 80 cGy following reactor irradiation in the absence of 10B-BPA. \( D_o \) decreased to 20 cGy in the presence of 25 µg/ml of 10B-BPA, and this further reduction is attributable to the \( ^{10}\text{B}(n,\alpha)^7\text{Li} \) reaction.

Tumor Therapy Experiments. Reactor irradiations were carried out 7 days following i.c. tumor implantation at which time the tumor weighed ~20-30 mg. All of the irradiated animals showed a transient weight loss of 10 g at
four days following irradiation, but regained this by the 7-10th day. The calculated doses in cGy (physical dose) and cGy-Eq (effective dose) of all irradiated controls and BNCT groups to the tumor, blood, and brain are summarized in Table 8. The calculated 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 effective dose, an RBE of 2.3 was assumed for $^{10}\text{B}(n,\alpha)^{7}\text{Li}$ reaction (28,29) and 2 for fast neutrons and $^{14}\text{N}(n,p)^{14}\text{C}$ reaction.

Kaplan-Meier plots for BNCT experiments are shown in Fig. 8a, b, and c. The MeST for the irradiated control groups 1, 2, and 3 (4, 6, and 8 MW-M) were 27, 33, and 38 d respectively compared to 24 d for the untreated rats (group 7). The prolongation in survival times of groups 1, 2, and 3 compared to untreated rats were significant with p values of 0.025, 0.0001, and ≤ 0.0001 respectively (Table 9). For BNCT groups 4, 5, and 6, the MeST were 32, 37, and 59 d respectively, which, when compared to 24 d for untreated rats (group 7), were highly significant (p of 0.0093, ≤ 0.0001, and ≤ 0.0001 respectively). The enhanced MeST for BNCT treated groups 5 and 6 compared to their matched irradiated controls (groups 2 and 3) were significant with p values of 0.0175 and 0.0277 respectively. However, there was no significant difference between BNCT treated group 4 (BPA + 4 MW-M) and its matched irradiated control group 1 (4
The %ILS determined from MeST or MST of each BNCT group was higher than its matched irradiated counterpart (Table 10). The %ILS, determined from MeST, for BNCT treated groups 4, 5, and 6 were 33, 54, and 146 respectively, compared to 13, 38, and 58 for irradiated groups 1, 2, and 3. For all BNCT treated groups, the %ILS determined from MST was higher than %ILS determined from the MeST. However, for irradiated controls %ILS were the same whether calculated from either MST or MeST, suggesting that there were variations in the therapeutic response between individual rats treated with BNCT. Necropsies were performed on all rats. In all instances death was due to an expanding tumor at the site of implantation.

DISCUSSION

The present study was carried out to determine the efficacy BPA as a capture agent for BNCT of the F98 glioma for which BSH has been used as the capture agent (14). Six hours following a single i.p. injection of BPA, tumor boron concentrations were in the range of 15-30 μg B/g, and T/Bl and T/Br ratios were 3.5, and 3.9 respectively. These values should have been high enough to sustain a lethal $^{10}$B(n,$\alpha$)$^7$Li reaction within tumor cells and yet low enough to spare the normal brain and vascular endothelium of cerebral
blood vessels (30,31). Although, BNCT treated rats survived longer than their matched irradiated counterparts, only 30% and 60% of rats that received BPA and total calculated tumor physical doses of 889 and 1159 cGy respectively survived > 50 days (range 55-154 days).

Based on the experimental data (28,29), an RBE of 2.3 was assumed for the $^{10}$B(n,α)$^{7}$Li reaction and 2.0 for fast neutrons and the $^{14}$N(n,p)$^{14}$C reaction. Assuming a uniform $^{10}$B distribution throughout the tumor and normal tissues, ~ 74% of the total tumor of BNCT-treated rats was from the $^{10}$B(n,α)$^{7}$Li reaction. However, these calculations can only be regarded as approximations, since the "true" RBE can only be determined by microdosimetric methods, which require data on the intracellular boron concentration. A number of investigators have attempted to carry out such microdosimetric calculations (32,33,34), and it is very clear that the subcellular distribution of $^{10}$B is a major determinant of the RBE. Tsuji et al (8) have reported that BPA is localized primarily in the cytoplasm of melanoma cells and it is retained in the melanosomes. Furthermore, Coderre et al. (35) have provided additional data suggesting that BPA accumulation in the tumor cells depends upon the increased demand of neoplastic cells for amino acids. The problem of estimating the contribution of the $^{10}$B(n,α)$^{7}$Li reaction is illustrated by our data. Animals that received a tumor physical dose of 273 cGy (group 2), had a MeST of 33
d, while those that received a tumor physical dose of 579 cGy (group 4, BPA + 182 cGy), 397 cGy of which was attributed to the $^{10}$B(n,$\alpha$)$^7$Li reaction, had a MeST of 32 d. This discrepancy may have been due to non-uniform macro- and microdistribution of $^{10}$B within the tumor, as well as the inherent the radioresistance of the F98 glioma (16). The survival curves of BNCT treated rats were shifting to the right when a higher $n_{\text{th}}$ fluence (reactor time) was given. Due to the radioresistance of F98 cells, it can be postulated that a higher $n_{\text{th}}$ fluence (radiation time) would result in a better effect from the $^{10}$B(n,$\alpha$)$^7$Li reaction.

The F98 glioma is refractory to a wide variety of therapeutic modalities including combined treatment with cis-dichlorodiamineplatinum (cis-DPP) and X-irradiation (16) and immunotherapy using adherent LAK cells and interleukin-2 (17). In a previous study carried out by one of us (14) using BSH as the capture agent, a 52.1% increase of life span was observed in F98 glioma bearing rats. Although the rats received a physical dose of 454 cGy following the administration of BSH, the %ILS was 2.8 X less than that observed in the present study with a physical dose of 364 cGy and BPA as the capture agent. These results suggest that BPA is a more effective capture agent than BSH for the treatment of the F98 glioma, due to the lower concentration of $^{10}$B attained by the tumor following the administration of the BSH compound (14). They further suggest, however, that
if the boron concentrations of BSH and BPA in the tumor were similar, that BSH might be superior.

Significant prolongations in survival times of glioma bearing rats were observed using either BSH or BPA as the capture agent, but none of the animals were "cured". Since each of these agents target tumors by different mechanisms (36,37) it is not unreasonable to expect that a combination of them might produce a better therapeutic effect than each one individually.

The GS-9L gliosarcoma rat brain tumor models differs from the F98 glioma in that it appears to be at least partially sensitive to X-irradiation, as evidenced by limited numbers (38) of long term survivors following treatment. On the other hand, the F98 glioma more closely simulates human glioblastoma multiforme in its refractoriness to all forms of treatment. Coderre et al (12) have used BNCT and BPA as a capture agent to treat GS-9L gliosarcoma and have reported that 44% of rats survived longer than 150 d. Furthermore, we recently have reported that 40% of nude rats carrying i.c. implants of the human melanoma cell line MRA 27 survived > 300 d following BNCT and BPA as a capture agent (19).

As encouraging as Coderre et al. and our results with MRA 27 melanoma are, the present study strongly suggests that BPA might not be any better than BSH for the treatment of human glioblastomas. Furthermore, since BPA may be taken
up by critical neuroanatomic structures in the brain such as the substantia nigra, in depth normal tissue radiobiologic studies are absolutely essential before one could contemplate using BPA as a capture agent to treat any kind of brain tumor, whether primary or metastatic.
REFERENCES


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Table 6. Distribution of boron in F98 glioma bearing rats following administration of d,l-BPA

Tissue boron concentrations (μg/g ± SD) at varying times (hrs) post injection

<table>
<thead>
<tr>
<th>Tissue</th>
<th>3</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>7.5 ± 2.5</td>
<td>8.6 ± 1.7</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td>Tumor</td>
<td>37.0 ± 6.9</td>
<td>30.6 ± 8.8</td>
<td>15.7 ± 4.6</td>
</tr>
<tr>
<td>Blood</td>
<td>17.0 ± 2.8</td>
<td>11.9 ± 3.7</td>
<td>6.5 ± 1.0</td>
</tr>
<tr>
<td>Skin</td>
<td>10.8 ± 1.6</td>
<td>7.1 ± 3.1</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>Muscle</td>
<td>10.3 ± 3.4</td>
<td>10.3 ± 2.4</td>
<td>6.3 ± 0.4</td>
</tr>
</tbody>
</table>

* 10⁵ F98 cells were implanted stereotactically into the right caudate nucleus of Fischer rats and 15 days later they were injected with d,l-BPA, as fructose complex at a dose of 897 mg/kg of body weight.

* Boron concentrations were measured by DCP-AES of an average of 3-4 rats.
Table 7. Dose rates for *in vivo* and *in vitro* irradiations at the Brookhaven Medical Research reactor (BMRR)*

<table>
<thead>
<tr>
<th>Component</th>
<th><em>In vivo</em> (cGy/min)</th>
<th><em>In vitro</em> (cGy/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{14}\text{N}(n,p)^{14}\text{C}$</td>
<td>7.6</td>
<td>3.1</td>
</tr>
<tr>
<td>Fast neutrons</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>Gamma photons</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>45.6</td>
<td>22.1</td>
</tr>
<tr>
<td>$^{10}\text{B}(n,a)^{7}\text{Li}$</td>
<td>3.38/ppm$^{10}\text{B}$</td>
<td>2.4/ppm$^{10}\text{B}$</td>
</tr>
</tbody>
</table>

* At a power level of 1 MW, thermal neutron flux was $3.9 \times 10^{11} \text{ n cm}^{-2} \text{ min}^{-1}$ at a depth 4-5 mm beneath the skull surface, and $2.8 \times 10^{11} \text{ n cm}^{-2} \text{ min}^{-1}$ *in vitro.*
Table 8. Radiation doses to critical tissues during irradiations in the presence and absence of BPA at different reactor powers

<table>
<thead>
<tr>
<th>Tissue</th>
<th>BPA°</th>
<th>Physical dose (cGy)b</th>
<th>Effective dose (cGy-Eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 MW-M</td>
<td>6 MW-M</td>
</tr>
<tr>
<td>Tumor</td>
<td>-</td>
<td>182</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>579</td>
<td>869</td>
</tr>
<tr>
<td>Blood</td>
<td>-</td>
<td>182</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>294</td>
<td>441</td>
</tr>
<tr>
<td>Brain</td>
<td>-</td>
<td>182</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>284</td>
<td>427</td>
</tr>
</tbody>
</table>

a Power levels are indicated in megawatt minutes (MW-M).

b 10B-enriched BPA was administered i.p. as a fructose complex to F98 glioma bearing rats 6 hours prior to irradiation.

c Dose estimates were based on tumor 10B concentrations
= 29.4 µg/g, brain = 7.6 µg/g, and blood = 8.3 µg/g and also includes contributions from fast neutrons, photons, and the 14N(n,p)14C reaction.
Table 9. Comparison of levels of significance (p value) for prolongation in survival time of BNCT, irradiated controls, and untreated groups.

<table>
<thead>
<tr>
<th>G^b</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>4MW-M</td>
<td>6MW-M</td>
<td>8MW-M</td>
<td>4BNCT</td>
<td>6BNCT</td>
</tr>
<tr>
<td>G7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G1</td>
<td>0.0252</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G2</td>
<td>0.0001</td>
<td>0.0203</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G3</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
<td>0.0187</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G4</td>
<td>0.0093</td>
<td>0.1096</td>
<td>0.4432</td>
<td>0.1279</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G5</td>
<td>&lt;0.0001</td>
<td>0.0011</td>
<td>0.0175</td>
<td>0.4892</td>
<td>0.0793</td>
<td>-</td>
</tr>
<tr>
<td>G6</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0019</td>
<td>0.0277</td>
<td>0.0043</td>
<td>0.0882</td>
</tr>
</tbody>
</table>

^a Wilcoxon-Gehan rank sum test (one sided p value); it is assumed that the higher the dose the better the survival.

^b G; Groups 1 through 7 are as follows: G1 (4 MW-M), G2 (6 MW-M), G3 (8 MW-M), G4 (BPA + 4 MW-M), G5 (BPA + 6 MW-M), G6 (BPA + 8 MW-M), and G7 (untreated).
Table 10. Survival times of F98 glioma bearing rats following irradiation

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Physical dose (cGy) ( ^b )</th>
<th>MST (^c )</th>
<th>MeST (^d )</th>
<th>%ILS(M) (^e )</th>
<th>%ILS(Me) (^f )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>MW-M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>4</td>
<td>182</td>
<td>28</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>6</td>
<td>273</td>
<td>32</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>8</td>
<td>364</td>
<td>38</td>
<td>38</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>4</td>
<td>579</td>
<td>34</td>
<td>32</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>6</td>
<td>889</td>
<td>45</td>
<td>37</td>
<td>88</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>8</td>
<td>1159</td>
<td>64</td>
<td>59</td>
<td>167</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24</td>
<td>24</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) \(10^3\) F98 cells were implanted stereotactically into the right caudate nucleus of rats and 7 days later they were irradiated at BMRR.

\(^b\) Determined from Table 1

\(^c\) Mean and Median Survival Time were determined from groups of 9-10 rats each.

\(^e,f\) %ILS were defined relative to MST and MeST.
Figure 6. Blood (○), brain (▼), and tumor (●) concentration-time profiles of BPA as measured by boron concentrations, after a single i.p. injection of 897 mg/kg body weight of D,L-BPA into F98 glioma bearing rats.
Figure 6.
Figure 7. Survival curves of F98 glioma cell line in vitro after irradiation with X-rays (\(\bigcirc\)), and reactor (\(\blacktriangledown\)) at 0 \(\mu g/ml\) of \(^{10}\text{B}\) (\(^{10}\text{B}-\text{BPA}\)) and (\(\square\)) at 25 \(\mu g/ml\) of \(^{10}\text{B}\) (\(^{10}\text{B}-\text{BPA}\)).
Figure 7.
Figure 8. Kaplan-Meier plots of glioma bearing rats following BNCT. $^{10}$B-L-BPA (803 mg/kg) was given i.p. 6 hr prior to irradiation at three different reactor power-time (physical doses of 182, 273, 364 cGy). Each of Fig. 3a, 3b, and 3c shows 3 groups of rats. These three groups are untreated controls (heavy solid line), irradiated controls (solid line), and BNCT treated groups (dashed line).
Survival Time (Days) Post Implantation

Percent Survival

Figure 8
Figure 8 (continued)

![Graph showing survival time (days) post implantation with different treatments: Untreated, Irr.Con.(273cGy), and BNCT (BPA + 273cGy).]
Figure 8 (continued)

Survival Time (Days) Post Implantation

Percent Survival

- Untreated
- Irr. Con. (364cGy)
- BNCT (SPA + 364cGy)
Chapter III
Californium-252 Brachytherapy Enhancement of Intracerebral Melanoma in a Rat Model with Boronophenylalanine

INTRODUCTION

Californium-252 brachytherapy has been used clinically to treat bulky localized tumors such as cervical carcinomas and glioblastoma multiforme (1-4). Results from the initial therapy trials using $^{252}$Cf implants as an interstitial neutron source have been promising, and have stimulated others to consider this therapeutic modality (5). Patients with glioblastoma multiforme, who had been treated with $^{252}$Cf dose of 9-10 Gy, had a median survival time of 15.5 months compared to 10 months for patients who had surgery followed by external beam radiation therapy with a total dose of 60 Gy (4,6). The 5-year survival rate of patients with cervical carcinoma, who had been treated with an early schedule of $^{252}$Cf implants was 54% compared to 15% with patients treated with $^{137}$Cs radiotherapy (1).

$^{252}$Cf is a synthetic transuranium element, produced in the High Flux Isotope Reactor (HFIR) at the Oak Ridge National Laboratory, and purified in the Transplutonium...
Processing Plant (TRU) at Oak Ridge, TN (7). It has a half-life of 2.65 years and a 3.09% ratio of spontaneous fission. The fission of $^{252}$Cf yields neutrons and $\gamma$ photons with energies of 2.35 and 0.75 MeV respectively (8,9). The neutrons and $\gamma$ rays are emitted at a low dose rate (cGy/hr), unlike conventional photon beam therapy where the dose rate is in cGy/min. The neutron to $\gamma$ ratio is approximately 2.0 at the source, but this attenuates to 0.5 at a distance of 5-10 cm from the source.

The high linear energy transfer (LET) neutrons emitted by $^{252}$Cf are therapeutically advantageous because of their high relative biological effectiveness (RBE) and their low oxygen enhancement ratio. $^{252}$Cf brachytherapy, theoretically should be more effective against hypoxic cells thereby reducing a cell population that significantly contributes to tumor regrowth. Furthermore, the combination of neutrons and $\gamma$ photons emitted by $^{252}$Cf increases the tumoricidal activity of radiation (10).

Beach et al. (11) have suggested that the combination of $^{252}$Cf brachytherapy with boron-10 loading of the tumor could increase the tumor radiation dose due to a boost from the $^{10}$B($n$,a)$^7$Li reaction. Boron neutron capture therapy (BNCT) depends on the selective uptake of $^{10}$B by the tumor cells, followed by irradiation with thermal neutrons (0.025 eV). The resulting nuclear fission reaction yields high LET alpha particles and Li nuclei ($^{10}$B($n$,a)$^7$Li) with an energy of
2.31 MeV and in addition 96% of the fission events produce γ photons of 0.48 MeV (12). The pathlengths of the alpha particles and 7Li are approximately 10-14 μ.

We have developed a nude rat brain model utilizing a human melanoma cell line MRA 27, which when implanted intracerebrally, grows progressively and kills the host (13). This model simulates melanoma metastatic to the brain, which occurs in up to 50% of patients with disseminated melanoma (14,15). With the development of more effective chemo- and immunotherapy for extracranial melanoma (16) there is an increasing need to develop treatment modalities for intracerebral metastatic disease (16,17). Our previous experience with the implanted intracerebral melanoma in rats clearly indicated that BNCT was capable of significantly prolonging life or even eliminating implanted brain tumors in rats (13,18). In the present study, we have evaluated the effects of 10B loading of the MRA 27 melanoma by i.p. administration of boronophenylalanine (BPA) on 252Cf brachytherapy.

MATERIALS and METHODS

Animals and 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 McCoys'5A media (Gibco Grand
Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. The cell line was tested periodically for mycoplasma contamination using DNA fluorochrome staining and UV microscopy (Bionique Testing Laboratories, Saranac Lake, NY). Six to eight 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.

Tumor Implantation. A stereotactic implantation procedure, previously used by us for studies on BNCT of a rat glioma (19), has been employed. Briefly, nude rats were sedated with a 1.2/1 mixture of ketamine/xylazine and a plastic screw was embedded in the skull. MRA 27 cells were injected through a central hole of the plastic screw into the right caudate nucleus at a concentration of 2 X 10⁵/10 µl of serum-free McCoy's 5A medium containing 1% agarose at a gelling temperature of <30 °C. The implantation procedure employed a relatively slow injection time, rapid filling of the screw hole with bone wax following withdrawal of the needle, and flushing of the operative field with betadiene before closing the scalp incision with a single sterilized clip.
Tissue Distribution Studies. A solution of t-BPA (Callery Chemical Co., Callery, PA), was mixed with fructose in 1:1 molar ratio to yield a final concentration of 120 mg of BPA/2 ml of water at pH 8.8. Two ml of the complex were administered i.p. to rats 30 days following intracerebral implantation of $2 \times 10^5$ tumor cells. Animals were killed 6 hours later and samples of blood, brain, tumor, and other tissues were obtained. Boron concentrations were determined by means of direct current plasma atomic emission spectroscopy (DCP-AES), as described in detail elsewhere (20). Briefly, 1-2 ml of concentrated sulfuric acid was added to 0.1-1g of tissues or blood in 150 X 16 mm Pyrex culture tubes and placed in a 100°C-heated mineral oil bath in an exhaust hood for 1 hour. After the samples had cooled to ambient temperature, 1 ml of 70% hydrogen peroxide was added slowly to decolorize the digested tissue. The contents were transferred to a 15 ml graduated plastic tubes (Sarstedt, Newton, MA), the volume was adjusted to 2-3 ml by adding distilled water, and boron content was determined by DCP-AES using an ARL Spectraspan VB Spectrometer (Applied Research Laboratories, Brea, CA).

Irradiation Studies. All irradiation experiments were carried out in the Radiation Therapy Oncology Center, at the University of Kentucky-Lexington, KY. All animals, including untreated controls, were transported from
Columbus, Ohio on Friday and irradiated on Saturday and Sunday (in case of Exp. II), and returned back to Columbus on Sunday. Nude rats were irradiated 29 days (Exp. I) or 24 days (Exp. II) following intracerebral implantation of 2 x 10⁵ of MRA 27 cells. Animals were divided into four groups in both experiments. In Exp. I, group 1a received a radiation dose of 200 cGy of ²⁵²Cf, group 2a received the same dose as group 1a 5.5 hr following i.p. administration of 120 mg of 95% ¹⁰B-enriched β-BPA. Group 3a received a radiation dose 1200 cGy of γ photons from a ¹³⁷Cs source. Group 4a served as untreated control. In Exp. II, group 1b received a radiation dose of 341 cGy of ²⁵²Cf, group 2b received the same dose as group 1b 5.0 hr following i.p. administration of 120 mg of 95% ¹⁰B-enriched β-BPA. Group 3b received a radiation dose 600 cGy of γ photons from a ¹³⁷Cs source. Group 4b served as untreated controls. The rats were anesthetized with 1.6/1 mixture of ketamine/xylazine, placed supine with their heads attached to a Lucite plate and were then brought in contact with the irradiation source (Fig. 9).

Dosimetry. The ²⁵²Cf dose rates for neutrons and γ photons were verified using a tissue equivalent (TE) proportional counter and a TE/Mg paired ionization chamber. The dosimetry were in agreement with Monte Carlo simulation for neutron and photon transport (MCNP) (21). The radiation
plaques for $^{252}$Cf emit radiation in low dose rates of 177 cGy/hr (Exp. I) at 1 cm from the source, and 171 cGy/hr (Exp. II) at 0.5 cm from the source (Table 11, Fig. 10). Using gold foil measurements positioned in a head phantom around the $^{252}$Cf sources, the thermal neutron flux in Exp. I and Exp. II at distances of 1 and 0.5 cm from the sources was estimated to be $1.6 \times 10^6$ and $2.5 \times 10^6$ n.cm$^{-2}$.sec$^{-1}$ respectively (22). At tumor $^{10}$B concentration of 23.7 µg/g, the total dose from $^{10}$B(n,α)$^7$Li reaction were calculated to be 1.3 cGy and 3.8 cGy for Exp. 1 and 2 respectively.

$^{137}$Cs plaques were calibrated in mg.rad.eq with total activity of 236.5 mg.rad.eq. Dose rates were calculated by computer program and isodose curves were generated (Fig. 11). The dose rates for $^{137}$Cs in Exp. I and II were 543 cGy/hr and 600 cGy/hr respectively at 1 cm from the source.

Evaluation of Therapeutic Response. The therapeutic response of the treated rats was evaluated by the percentage of increased life span (% ILS) determined from the Median Survival Time (MeST). %ILS was determined by the following formula: $%ILS = \left( \frac{(MeST \text{ of treated group} - MeST \text{ of untreated group})}{MeST \text{ of untreated group}} \right) \times 100%$.

Statistical Analysis. Jonckheere's statistical test (23) was applied on the survival data of the BPA + $^{252}$Cf, $^{252}$Cf, and the untreated control groups to test for a significant
trend in the enhancement of survival times between the three groups. In addition, the Wilcoxon-Gehan rank sum test was applied to test for significant differences in survival times between the treated and the untreated groups. All censored rats were ranked equally.

RESULTS

Uptake Studies. We previously have reported the blood, brain, and tumor boron profiles after administration of 120 mg of BPA in nude rats carrying intracerebral human melanoma MRA 27 cells (18). The best composite ratios were observed 6 hours following i.p. injection of either D,L or L forms of BPA. In this study, 120 mg of a fructose complex of 10B-enriched D-BPA were injected i.p. into rats 30 days following intracerebral implantation of the MRA 27 melanoma. Six hours later the tumor 10B concentration was 23.7 μg/g compared to 9.4 and 8.4 μg/g for blood and brain respectively. 10B tumor/blood and tumor/brain ratios were 2.5 and 2.8 respectively.

Irradiation Studies. In Exp. I, 252Cf and 137Cs irradiations were initiated 29 days following tumor implantation at which time the tumor weighed ~ 100 mg. Kaplan-Meier survival plots for the 252Cf, 10B + 252Cf, 137Cs treated rats, and the untreated animals are shown in Fig. 12. The MeST for the
untreated rats (group 4a) was 33 d, 43 d for animals irradiated with 200 cGy of $^{252}$Cf (group 1a), 54 d for rats that had received 120 mg of 10B-BPA 5.5 hr prior to irradiation (group 2a), and 71 d for those received 1200 cGy of $^{137}$Cs (group 3a). %ILS for groups 1a, 2a, and 3a were 30, 64, and 115% respectively. The prolongation in survival time of all irradiated groups (1a, 2a, and 3a) were statistically significant compared to untreated animals ($p \leq 0.05-0.01$) (Table 12). Although the enhancement in survival of rats treated with BPA prior to $^{252}$Cf irradiation compared to rats irradiated with $^{252}$Cf was not statistically significant, there was a statistically significant trend, as indicated by Jonckheere's test, towards increased survival times between group 1a, 2a, and 4a ($p < 0.01$).

In Exp. II, $^{252}$Cf and $^{137}$Cs irradiations were initiated 24 d following tumor implantation at which time the tumor weight was ~ 90 mg. Kaplan-Meier plots for all treated and untreated animals are shown in Fig. 13. The MeST for the untreated rats (group 4b) was 35 d, 47 d for those irradiated with 341 cGy of $^{252}$Cf (group 1b) or 600 cGy of $^{137}$Cs (group 3b), and 56 d for those receiving 120 mg of BPA 5 hr prior to irradiation with 341 cGy of $^{252}$Cf (group 2b). %ILS for groups 1b, 2b, and 3b were 34, 60, and 34 respectively (Table 13). The prolongation in survival times of all irradiated groups was statistically significant compared to the untreated rats ($p \leq 0.05 - 0.001$) (Table
This trend towards increased survival times between groups 1b, 2b, and 4b was highly significant \( p < 0.005 \). Furthermore, the difference in MeSTs between BPA treated \(^{252}\text{Cf}\) group (2b) and \(^{252}\text{Cf}\) group (1b) were significant but at a reduced level \( p \leq 0.05 \). In addition, the MeST of group 2b was significantly different \( p \leq 0.01 \) compared to MeST of group 3b which had received a \( \gamma \) photon dose of 600 cGy of \(^{137}\text{Cs}\). All rats that died were autopsied, and in every instance death was due to an expanding intracerebral melanoma originating at the implantation site.

DISCUSSION

In the present study we have evaluated the enhancement of \(^{252}\text{Cf}\) brachytherapy following the administration of \(^{10}\text{B}-\text{BPA}\) into nude rats carrying i.c. human melanoma. There was a trend towards increased survival among BPA treated rats, which had been irradiated with \(^{252}\text{Cf}\) at a dose of 200 cGy. The increased survival became statistically significant when the \(^{252}\text{Cf}\) dose increased to 341 cGy. Using the MeST of 47 d as an isoeffect endpoint, we have calculated an RBE for the neutrons \( (\text{RBE}_n) \) emitted from \(^{252}\text{Cf}\). Since the MeSTs were equal for the \(^{137}\text{Cs}\) treated group (600 cGy) and the \(^{252}\text{Cf}\) treated group (341 cGy), therefore:

\[
D_nR_n + D_R, \text{ for } ^{252}\text{Cf} = D_R, \text{ for } ^{137}\text{Cs} \quad \text{Eq. 1}
\]
\( D_n \) and \( D_y \) are the doses of neutron and \( \gamma \) rays. \( R_n \) and \( R_y \) are the RBEs of neutrons and \( \gamma \) rays. RBE of 1 was assumed for \( \gamma \) doses from either the \( ^{252}\text{Cf} \) or \( ^{137}\text{Cs} \). Solving Eq. 1, \( R_y \) was equal to 2.1, which was lower than the assigned RBE, value of 6. The value of 6 was based on several in vitro and normal tissue tolerance studies (10,24). RBE is a complex factor that depends on radiation quality (LET), dose, dose rate, and the biological system or endpoint. In our calculations, we assumed that the radiation effect of the \( \gamma \) photons from \( ^{137}\text{Cs} \) at a dose rate of 600 cGy/hr was equivalent to those of \( \gamma \) photons from \( ^{252}\text{Cf} \) at a dose rate of 111 cGy/2hr. Therefore, the actual RBE, might be somewhat higher than 2.1 because of the difference in the dose rate effects of \( \gamma \) photons delivered from \( ^{252}\text{Cf} \) or \( ^{137}\text{Cs} \). Our estimate of the RBE result is in agreement with that obtained from mouse skin reactions, calculated at a dose rate of 360 cGy/hr of \( ^{252}\text{Cf} \) (25). It was observed that the RBE of \( ^{252}\text{Cf} \) increased when smaller amounts of \( ^{252}\text{Cf} \) (i.e. decreased dose rate) were used, and reached a maximum RBE value of \( \sim 6 \) at the lowest dose rate (24,25). Furthermore, an RBE, of 2-3 has been found reported with radiosensitive target tissues such as spleen, bone marrow, and thymus (10,24).

Using MCNP calculations, Yanch et al. (21) have reported that enhancing tumor doses of \( ^{252}\text{Cf} \) from the \(^{10}\text{B}(n,\alpha)^{7}\text{Li} \) reaction depends upon the concentration of \(^{10}\text{B} \) in
the tumor and the distance of the tumor from the radiation source. The distance between source and tumor is an important factor for the moderation of fast neutrons to thermal energy range (11,21). Yanch el al. have reported a maximum dose enhancement of 18.3% when the $^{252}$Cf source was at distance of 10 cm from the target and the tumor boron concentration was 50 µg of $^{10}$B/g. A very low level of enhancement (1.3%) occurred at the same boron concentration, if the target was at a distance of 1 cm from the $^{252}$Cf source. Assuming a tumor $^{10}$B content of 60 µg/g, Beach et al. (11) have reported a dose enhancement of 8% by measuring thermal neutron flux at a distance of 1.6 cm from the $^{252}$Cf source and this increased to 12-23% at 3 cm. Neither of these two studies were based on the microdosimetry, which is essential in order to apply an accurate RBE value to the $^{10}$B(n,$\alpha$)$^{7}$Li reaction. In the present study, the macro tumor $^{10}$B concentration was 23.7 µg/g and the distances from the $^{252}$Cf sources were 1 cm, and 0.5 cm in Exp. I and Exp. II respectively. We have calculated that the doses from $^{10}$B(n,$\alpha$)$^{7}$Li reaction in Exps. I and II were 1.3 and 3.8 cGy respectively, and the total enhancement dose would have been 0.65% and 1.6% respectively. These calculations are in agreement to MCNP calculations and thermal neutron flux measurements (11,21). However, the survival data showed a 19%-25% increase in MeSTs when BPA was administered prior to irradiation with $^{252}$Cf. This might have been due to a more
favorable microdosimetric distribution of BPA in the tumor (26-28), and a higher RBE than 2.3 for the $^{10}$B(n,$\alpha$)$^{7}$Li reaction, which had been based on the assumption of uniform boron distribution within the tumor (29,30).

Using the same model as described in the present study, we have observed a very significant prolongation in survival time following BNCT (13,18). The MeSTs ranged from 170-262 days in three BNCT treated groups that had received tumor physical doses of 502, 754, or 1005 cGy (effective doses of 1056, 1587, or 2115 cGy-Eq) compared to 76-93 days of irradiated controls. Forty percent of rats that received tumor doses of 1005 cGy (2115 cGy-Eq) survived > 300 d. Of these tumor physical doses, 64% were from the $^{10}$B(n,$\alpha$)$^{7}$Li reaction. These results demonstrate the potential effectiveness of the $^{10}$B(n,$\alpha$)$^{7}$Li reaction when a high enough thermal neutron flux ($3.9 \times 10^{11} \text{ n.cm}^{-2}\text{min}^{-1}$) is delivered to the tumor site.

Data from the present study suggest that dose enhancement of $^{252}$Cf brachytherapy by means of the $^{10}$B(n,$\alpha$)$^{7}$Li reaction is feasible. Better dose enhancement could be obtained if the tumor $^{10}$B concentrations were higher > 50$\mu$g/g. This might be achievable by direct intratumoral injection of boronated compounds as has been carried out by Mishima et al. (31) using BPA, and in our laboratory using carboranyl uridine (32). In either case, the boron containing compound is allowed to clear from the surrounding normal tissues. Improved positioning and external
moderation of the $^{252}$Cf sources might further augment the dose from the $^{10}$B$(n,\alpha)^{7}$Li reaction. Further studies are required to determine whether this is indeed the case.
REFERENCES


Table 1. Dosimetry of neutrons and $\gamma$ rays of $^{252}$Cf used in Exp. I and II.

<table>
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<tr>
<th></th>
<th>Dose rate cGy/hr</th>
<th>Total physical dose cGy</th>
<th>Dn</th>
<th>Dy</th>
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<tr>
<td>Exp. I$^a$</td>
<td>177</td>
<td>200</td>
<td>134</td>
<td>66</td>
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<tr>
<td>Exp. II$^b$</td>
<td>171</td>
<td>341</td>
<td>230</td>
<td>111</td>
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</tbody>
</table>

$^a$ In Exp. I., the sources of $^{252}$Cf were estimated to be 1 cm away from the tumor.

$^b$ In Exp. II, the sources of $^{252}$Cf were estimated to be 0.5 cm away from the tumor.
Table 2. Survival times and analysis of rats carrying intracerebral human melanoma MRA 27 following $^{252}$Cf brachytherapy radiation (Exp. I)

<table>
<thead>
<tr>
<th>Group</th>
<th>BPA $^b$</th>
<th>n</th>
<th>Physical Dose (cGy) $^c$</th>
<th>MeST $^d$</th>
<th>%ILS $^e$</th>
<th>p $^f$</th>
</tr>
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<tbody>
<tr>
<td>4a</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>33</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1a</td>
<td>-</td>
<td>7</td>
<td>200</td>
<td>43</td>
<td>30</td>
<td>0.05</td>
</tr>
<tr>
<td>2a</td>
<td>+</td>
<td>6</td>
<td>200</td>
<td>54</td>
<td>64</td>
<td>0.01</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>6</td>
<td>1200</td>
<td>71</td>
<td>115</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* $2 \times 10^5$ of MRA 27 cells were implanted stereotactically into the right caudate nucleus of nude rats and 29 days later they were irradiated.

$^b$ 120 mg of $^{10}$B-enriched 1-BPA were injected i.p. as a fructose complex into rats carrying i.c. melanoma 5.5 hr prior to irradiation.

$^c$ The physical doses in groups 1 and 2 were a mixture of neutrons and $\gamma$ rays (see Table 1). The source of irradiation to groups 1a and 2a was $^{252}$Cf, and $^{137}$Cs for group 3a.

$^d$ MeST: Median Survival Time in days.

$^e$ %ILS: percentage of increased life span

$^f$ The level of significance using Wilcoxon-Gehan rank sum test.
Table 3. Survival times and analysis of rats carrying intracerebral human melanoma MRA 27 following $^{252}$Cf brachytherapy radiation (Exp. II)

<table>
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<tr>
<th>Group</th>
<th>BPA $^b$</th>
<th>n</th>
<th>Physical Dose (cGy)$^c$</th>
<th>MeST$^d$</th>
<th>%ILS$^e$</th>
<th>p$^f$</th>
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<tr>
<td>4b</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1b</td>
<td>-</td>
<td>8</td>
<td>341</td>
<td>47</td>
<td>34</td>
<td>0.05</td>
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<tr>
<td>2b</td>
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<td>8</td>
<td>341</td>
<td>56</td>
<td>60</td>
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</tr>
<tr>
<td>3b</td>
<td>-</td>
<td>8</td>
<td>600</td>
<td>47</td>
<td>34</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$^a$ As Table 2

$^b$ 120 mg of $^{10}$B-enriched $^{10}$BPA were injected i.p. as a fructose complex into rats carrying i.c. melanoma 5 hr prior to irradiation.

$^c,d,e,f$ As Table 2.
Figure 9. Rats were anesthetized, held supine, and their heads and bodies were taped to a lucite plate. The lucite plate was used as a guide for positioning of the $^{252}$Cf or $^{137}$Cs sources, and was marked to adjust the rat's head within the irradiation field.
Figure 9.
Figure 10. Isodose curves of neutrons emitted from $^{252}$Cf sources at different geometrical points (22). The 120, 100, 80, etc were the neutron doses in cGy/hr. In Exp. II, the distance between the tumor and sources estimated to be 0.5 cm. The dose rate of neutrons at 0.5 cm distance was 115 cGy/hr.
Figure 10.
Figure 11. Isodose curves of $\gamma$ rays from $^{137}$Cs sources. The dose rates of $\gamma$ rays are shown on each curve (23). The tumor was estimated to be 1 cm from the sources in both experiments. In Exp. II, the dose rate of $\gamma$ rays was 600 cGy/hr.
Figure 12. Exp. I. Kaplan Meier plots of rats carrying i.c. MRA 27 human melanoma tumor following $^{252}$Cf or $^{137}$Cs brachytherapy. Three groups were irradiated with 200 cGy of $^{252}$Cf (gp 1a), 200 cGy following 5.5 hr of i.p. administration of 120 mg of $^{10}$B-BPA (gp 2a), or 1200 cGy of $^{137}$Cs (gp 3a). Group 4a served as untreated controls.
Figure 12.
Figure 13. Exp. II. Kaplan Meier plots of rats carrying i.c. MRA 27 human melanoma tumor following $^{252}$Cf or $^{137}$Cs brachytherapy. Three groups were irradiated with 341 cGy of $^{252}$Cf (gp 1b), 341 cGy following 5.0 hr of i.p. administration of 120 mg of $^{10}$B-BPA (gp 2b), or 600 cGy of $^{137}$Cs (gp 3b). Group 4b served as untreated controls.
Figure 13.
Chapter IV
Brain, Skin, and Eye Responses Following Boron Neutron Capture Therapy

INTRODUCTION

Conventional radiation therapy is limited by the tolerance of normal tissues contiguous with and adjacent to the tumor. Following whole brain irradiation, late complications such as brain atrophy, necrosis, leukoencephalopathy, and neurological deterioration have been reported, and their severity is dose and time dependent (1-3). In boron neutron capture therapy (BNCT), these normal tissue effects theoretically can be reduced to a level less than seen following photon irradiation that since the primary effect, is at the level of individual cells. This is based on selective accumulation of $^{10}$B by the tumor cells, followed by irradiation with low energy (0.025 eV) neutrons. This reaction yields high LET alpha particles and Li nuclei, which have pathlengths of approximately 10-14 μ. The boron compounds should have selectivity for tumor cells and equally as important, a sufficient fluence of thermal neutrons must be delivered to the tumor site (4).
The first clinical trials of boron neutron capture therapy were conducted in the United States between 1951-1961 to treat patients with malignant brain tumors (5,6). The boron compounds used at that time were not selective for tumors (5), and this produced serious neuropathologic effects (6). Histological examination of the brains of 14 patients treated with BNCT revealed a variety of lesions including coagulation necrosis of the gray and white matter, necrotizing lesions with fibrin deposition and polymorphonuclear cell infiltrates in blood vessel walls, vascular thrombosis, demyelination and reactive gliosis. Furthermore, in one patient, who died 11.5 months following BNCT, histopathologic examination revealed severe thickening and fibrosis of the blood vessels. It was suggested that these pathological changes were due to the high blood boron concentration and the high radiation doses delivered to the vascular endothelium (6). In addition, non-healing moist desquamation of the skin following BNCT was observed (7).

With the advent of more tumor selective boron compounds such as sodium borocaptate (Na₂B₄H₆SH) and p-boronophenylalanine (BPA), Hatanaka (8) and Mishima (9) have initiated clinical trials for BNCT of brain tumors and (9) have treated patients with cutaneous melanomas, respectively.

We have developed a nude rat model for the treatment of intracerebral human melanoma by means of BNCT using BPA as the capture agent (10,11). The MeST ranged from 170-262
days in three BNCT groups that had received tumor physical
doses of 502, 754, 1005 cGy (effective doses of 1056, 1587,
or 2115 cGy-Eq) compared to 76-93 days for the irradiated
controls (11). In order to fully evaluate the effectiveness
of this therapy, it is essential to define the normal brain
tolerance following what otherwise might be "curative" BNCT
of intracerebral melanoma. The present study describes the
early macroscopic changes in the skin and eye, and the
histopathological changes in the brain 7 and 12 months
following BNCT using BPA as the capture agent.

MATERIALS AND METHODS

Animals. Four to six 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.

Irradiation. Forty eight (8-10 week-old) NIH-rnu nude rats
were used for this study. Rats were stratified into three
groups of 16 animals each. All groups received a single
i.p. injection of 120 mg of 95% enriched$^{10}$B-l-BPA (Callery
Chemical Co., Callery, PA) as a fructose complex, pH 8.8 in
a 2 ml of water 6 hours prior to irradiation. All
irradiations were carried out at Brookhaven Medical Research
Reactor (BMRR). The reactor power was maintained at 1.25 MW during irradiation of all rats. Groups 1, 2, and 3 received 6, 8, and 10 Megawatt-minute (MW-M) respectively. Group 4, consisting of 6 rats matched according to age and sex with the previous groups, served as controls. All irradiated animals were anesthetized with 1.2/1 mixture of ketamine/xylazine and placed supine in a body shield-head stabilizer, designed by Dr. D. Slatkin, as described elsewhere (12,13). The irradiated site of the rat's head was centered at the 1.15 cm in diameter of the restricted neutron aperture. The adjustment of the rat's head was established using a marked lucite plate as a guide for the centered collimated beam.

Perfusion Technique. At 7 and 12 month intervals following irradiation, the animals were anesthetized, as described above, and then pump perfused at a pressure of 110-120 mmHg with PBS (pH 7.2) followed by Cajal’s formol-bromide fixative (2% w/v ammonium bromide in 14% formalin). The brains were removed and kept for at least 24 hours in the same fixative. The brains were then sectioned coronally at the level of the optic chiasm, and 2 mm anterior and posterior to the chiasm. Each 2 mm thick section was allowed to fix for additional 24 hours, dehydrated, embedded in paraffin, sectioned at 6 µ and stained with either hematoxylin and eosin or luxol fast blue-PAS.
Dosimetry. The mixed radiation in tissue during BNCT includes thermal neutrons (0.025 eV), fast neutrons (>10,000 eV), γ photons and heavy particles that were generated from $^{14}\text{N}(n,p)^{14}\text{C}$ and $^{10}\text{B}(n,\alpha)^{7}\text{Li}$ reactions. As described elsewhere (13), $n_{th}$ fluences were measured using 3 mm long gold wire implanted into the cerebrum of a killed rat at a depth of 4-5 mm below the skull surface. The doses from $^{10}\text{B}(n,\alpha)^{7}\text{Li}$ and $^{14}\text{N}(n,p)^{14}\text{C}$ reactions were calculated from the $n_{th}$ flux, assuming uniform boron distribution and a tissue nitrogen concentration of 2.6% (13,14). The extrinsic γ-photons and fast neutron doses were measured by using paired tissue equivalent plastic chambers (A-150 plastic; Far West Technology, Goleta; CA) with TE gas (Rossi gas) and graphite chambers filled with CO$_2$. The dosimetry of each component is tabulated in Table 14.

Evaluation of Skin Reactions. Early skin reactions were recorded three times a week for all the irradiated rats. The scoring system applied was: 0, no apparent change; 1, minimal; 2, mild; 3, moderate; and 4 severe moist desquamation.

RESULTS

Dosimetry. The calculated radiation doses in cGy (physical
dose) and in cGy-Eq (effective dose) of all irradiated
groups to the brain, blood, and skin are summarized in Table
15. In order to convert physical dose values to effective
doses, the relative biological effectiveness (RBE) of 2.3
was assumed for the $^{10}\text{B}(n,\alpha)^{7}\text{Li}$ reaction and 2.0 for fast
neutrons and $^{14}\text{N}(n,p)^{14}\text{C}$ reaction (15,16).

Irradiation Responses. Based on our previous
pharmacokinetic studies (11), we selected the 6 hour-time
post administration of BPA for irradiations. At that time
blood, brain, skin, and eyes $^{10}$B concentrations were 9.4,
8.4, 10.0, and 11.0 $\mu g/g$ respectively.

Weight. Following irradiation, the maximum weight
losses for groups 2 and 3 were seen at 10-15 days (Fig. 14).
Group 1, which had received BPA + 273 cGy, initially showed
a slight weight gain followed by a very mild weight loss.
In groups 2 and 3, which had received BPA + 364 cGy or 454
cGy respectively, there was a second decline in weight 40 d
following irradiation. This was due to the progressive
weight loss of some animals that culminated in their deaths.
Kaplan-Meier plots of the irradiated rats from the time of
irradiation until termination of the study are shown in Fig.
15. The cause of death could not be determined due to
cannibalization of the dead rats. The survival curves for
groups 2 and 3 were identical, although it seemed as if
there was a dose dependent relationship between the percent of deaths and radiation dose.

Skin. The evolution and time course of the moist skin response following BNCT are summarized in Fig. 16. At the time of irradiation, $^{10}$B concentration in the skin was 10.0 $\mu$g/g. Approximately fifty percent of the radiation dose was due to the $^{10}$B(n,α)$^7$Li reaction. The early erythematous reaction was seen 3-5 days following irradiation in all groups. This erythema also was seen in rats that were exposed to 481 or 641 cGy-Eq of irradiation alone (6 or 8 MW-M, and no BPA). Moist desquamation occurred only when BPA was administered prior to irradiation of the rat’s head, and was first evident between 9-12 days following irradiation. In group 1, which had received a radiation dose of 947 cGy-Eq to the skin (BPA + 6 MW-M), the reaction was less severe than those of groups 2 and 3, which had received skin doses of 1262 or 1578 cGy-Eq (BPA + 8, or 10 MW-M). Skin healing for group 1 (947 cGy-Eq) was somewhat faster (18.7 ± 1.3 d) compared to groups 2 and 3 (24.4 ± 0.7 and 26.1 ± 1.1 d). Although, the severity of the damage was dose dependent, the time course (rate) of these changes was not. Furthermore, these results were similar to those obtained in a therapy experiment conducted at the same time, indicating that 800-850 cGy-Eq was the threshold dose for moist desquamation of nude rat skin.
Ocular changes. If the neutron beam had been directed to the animals’ eyes, the irradiation doses of groups 1, 2, and 3 would have been 496 cGy (994 cGy-Eq), 661 cGy (1326 cGy-Eq), and 828 cGy (1660 cGy-Eq) respectively. Due to the geometry of the rat’s head, the eyes were close to the centerpoint of the collimated beam, and therefore could have received a significant radiation dose. Three months following BNCT, we examined the rats’ eyes in the present study as well as those of animals used in a therapy experiment, which was initiated at the same time (11). The severity of keratitis, blepharitis, conjunctivitis, and cataract formation were dose dependent. In group 3, three examined rats developed all of these changes in both eyes, except for one rat in which only the right eye was affected. At the lower irradiation doses (BPA + 6 MW or 8 MW) the severity of the eye reactions was less, and 2-3 out of 7 rats did not develop cataracts (Table 16).

Late Radiation Effects in the Brain. Major areas of interest were the fimbriae (17), choroid plexus and leptomeninges. Table 17 summarizes pathological changes seen in the choroid plexus following BNCT. Mild changes included vacuolation of epithelial cells and mild infiltrates of macrophages and polymorphonuclear cells, but these were not dose dependent (Figs. 17,18,19). Only in one rat brain, which had received brain and blood radiation dose
of 1454 and 1532 cGy-Eq, a single hemorrhagic infarct was noted (Fig. 20). A mild increase in interstitial fibrous tissue of the choroid plexus was observed 7 and 12 months following BNCT, and this increased slightly at 12 months post irradiation, especially in group 3 (Fig. 21). Although these histopathological changes were mild, they appeared to be dose and time dependent. Dilatation of small blood vessels and atrophy of the epithelial cells of the choroid plexus were observed in a few animals in groups 2 and 3 (Figs. 22, 23). Mild focal fibrosis and scattered macrophage infiltrates were noted sporadically in the leptomeninges of several animals (Fig. 24), but these were not dose or time dependent.

DISCUSSION

The skin and eye reactions at 1 and 3 months and the histopathological changes in the brains of nude rats at 7 and 12 months were determined following BNCT. A mild to moderate increase in fibrous connective tissue adjacent to the blood vessels of the choroid plexus was observed at 12 months, and this appeared to be time and dose dependent. Skin reactions were acute and healing occurred in all rats 28 days following irradiation. No second phase skin reactions were observed, and this was consistent with the calculated skin doses of ≤ 1578 cGy-Eq. In case of the
eyes, cataract formation was observed in most of the examined rats, and it was dose dependent.

Unfortunately, the cause of death of rats following BNCT irradiation could not be determined due to cannibalization of the dead animals by survivors housed in the same cage. However, deaths appeared to be dose dependent only between group 1 and the other two groups, 2 and 3 (Fig. 2). Since nude rats are more susceptible to infections especially respiratory, this could have been the primary cause, but in the absence of necropsy data, this is purely speculative. None of the animals, however, had clinical symptoms of CNS pathology. Three rats in group 3, which had received the highest dose, died 8 days following irradiation. This might be attributed to gastrointestinal radiation damage.

The radiation delivered to the brain represented an average dose distributed over the entire brain volume. In reality some parts of the brain such as the substantia nigra, might have accumulated more $^{10}$B-BPA than others. This could produce significant microdosimetric variations within the brain cellular distribution of $^{10}$B which is attributable to the $^{10}$B(n,$\alpha$)$^7$Li reaction (18). Furthermore, it has been calculated by Rydin et al. that capillary endothelial cells could received one-third to one-fifth of the blood radiation dose from the $^{10}$B(n,$\alpha$)$^7$Li reaction, depending on the capillary diameter (19). However, these
calculations were based on the reactions occurring inside the capillaries and not outside the endothelial cells or inside the blood vessel walls. Furthermore, there is a dose contribution to the vascular endothelium from $^9\text{B}(n,a)^7\text{Li}$ reaction attributable to boron that has accumulated in the brain parenchyma (15). It has been assumed that the radiation dose to the capillary endothelium was one-third of the blood dose added to two-thirds of the brain dose (13,20). Extrapolating from these assumptions, in our study the doses to the capillary endothelium would be 450, 600, 750 Gy for groups 1, 2, and 3 respectively.

Cutaneous responses in pigs following BNCT has been extensively studied because of its resemblance to human skin (7). The physical dose at which 50% of the irradiated skin fields did not heal, was estimated to be between 10-13 Gy (21). Furthermore, Morris et al. (22) reported biphasic skin reactions in rats following BNCT. In their study, skin boron concentrations were 59-65 $\mu$g/g and the total physical doses to the skin were 31-52 Gy. The acute reactions in the skin following ionizing radiation were primarily due to induced changes in the basal cell population of the epidermis (23). However, when late skin damages occurred, this indicated that the vascular endothelium of the dermis was affected. In our study, at doses of 475-793 cGy (947-1578 cGy-Eq) skin reactions were only acute and healed following 30 days of BNCT in all irradiated rats. In
addition, at higher doses, using hamsters, the maximum "safe" physical skin dose, which produced no more than moist desquamation following BNCT was 11 Gy (24). It must be pointed out that all of these studies, including our own, were based on average $^{10}$B concentration in the skin and the actual $^{10}$B distribution in the cells of the epidermis, dermis, and vascular endothelium must be known in order to accurately calculate the absorbed doses.

Rubin and Casarett (25) have proposed that damage to the blood vessel walls were responsible for development of late radiation effects. Calvo et al. (26) have shown that vascular endothelial cells of the choroid plexus were more sensitive to X-ray doses of 17.5-25 Gy than epithelial cells, and that there was a marked reduction in the number of the endothelial cells 13 weeks following X-irradiations. Recently, Reinhold et al. (27) has reported that changes within blood vessels precede radiation damage of the cerebral white matter following moderate doses (20-25 Gy) of X-rays. Necrosis of the cerebral white matter developed in rats 36 weeks following a single dose of $\geq 22$ Gy of X-rays (28), and doses of $< 1200$ cGy were considered to be threshold for injury to the brain parenchyma (29,30). In our present study, radiation doses to the brain and blood ranged 443-773 cGy (872-1532 cGy-Eq) and only group 3 had radiation doses above the 1200 cGy threshold. Although only mild vascular abnormalities were observed in the brain, this
might indicate that these changes precede any severe pathological change in the brain parenchyma. However, since the cause of death of those animals that died during first 7 months and between the 7-12 month interval was not determined, the possibility that their brains had significant histopathological changes cannot be excluded, and therefore our results should be interpreted with some caution. Using sodium borocaptate (BSH) as the capture agent Goodman et al. (31) observed minimal histopathological changes in the microvasculature of rat brains 18 months following BNCT. At a blood physical dose of 736 cGy and by means of electron microscopy, they noted collagen filaments in large capillaries, numerous dense bodies consisting of either lipid or exogenous protein, fibrous astrocytes between the capillaries, arterioles and venules, and vacuolation within membrane bound organelles in the pericytes (31).

Using BPA as the capture agent, Packer et al. have cured 8 out of 11 rabbits that had melanomas implanted in the anterior chamber of the eye. All of the rabbits developed cataracts, but no damage to the vascular structures of the eye was observed (32). In our study, although the neutron beam was not directed to the eyes, it is apparent that the eyes had received a significant radiation dose, as evidenced by cataract. The ocular pathology that we observed was due to the small size of the
rat head, and this should not present a problem in humans or lager animals, where the radiation volume is much larger, and the geometry is more favorable.

Minimal changes of normal tissues following BNCT are indicative of the strength of this therapy, at which time a regression of tumor was observed (11). However, it essential to use a large animal model for brain tumors, which in this case, higher radiation doses are delivered to the tumor and the adjacent normal tissues in order to achieve a responsive therapy. Therefore, further studies emphasizing on dose-relation effects on the brain tissue tolerance following BNCT are indispensable.
REFERENCES


28. Calvo, W., Hopewell, J. W., Reinhold, H. S., and Yeung, T. k. Time and dose related changes in the white matter of


Table 14. Dose rates for rat brain irradiations at the Brookhaven Medical Research Reactor (BMRR)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Component</th>
<th>cGy/min</th>
<th>cGy-Eq/min\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{14}\text{N}(n,p)^{14}\text{C})</td>
<td>7.56</td>
<td>15.12</td>
</tr>
<tr>
<td>Fast neutrons</td>
<td>27</td>
<td>54</td>
</tr>
<tr>
<td>Gamma photons</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>45.56</td>
<td>80.12</td>
</tr>
<tr>
<td>(^{10}\text{B}(n,\alpha)^{7}\text{Li})</td>
<td>3.38/µg(^{10}\text{B})</td>
<td>7.77/µg(^{10}\text{B})</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Power level = 1 MW, thermal neutron flux = 3.9 \(\times\) 10\(^{11}\) n.cm\(^{-2}\)min\(^{-1}\) at depth of 4-5 mm below the skull

\textsuperscript{b} An RBE of 2 has been assigned to fast neutrons and the \(^{14}\text{N}(n,p)^{14}\text{C}\) reaction and 2.3 for the \(^{10}\text{B}(n,\alpha)^{7}\text{Li}\) reaction. Since the latter estimate depends upon microdosimetry it is more imprecise than the former.
Table 15. Radiation doses to critical tissues at different reactor powers

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Physical dose (cGy)</th>
<th>Effective dose (cGy-Eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BPA° 6 MW-M 8 MW-M 10 MW-M</td>
<td>6 MW-M 8 MW-M 10 MW-M</td>
</tr>
<tr>
<td>Blood</td>
<td>- 273 364 456 481 641 805</td>
<td>481 641 805</td>
</tr>
<tr>
<td></td>
<td>+ 464 618 773 + 464 618 773</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>- 273 364 456 481 641 805</td>
<td>481 641 805</td>
</tr>
<tr>
<td></td>
<td>+ 443 591 739 + 443 591 739</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>- 273 364 456 481 641 805</td>
<td>481 641 805</td>
</tr>
<tr>
<td></td>
<td>+ 475 634 793 + 475 634 793</td>
<td></td>
</tr>
</tbody>
</table>

a Power levels are indicated in megawatt minutes (MW-M). This includes contributions from fast neutrons, photons, and the $^{14}$N(n,p)$^{14}$C and $^{10}$B(n,$\alpha$)$^7$Li reactions.

b Dose estimates were based on a blood $^{10}$B concentration = 9.4 $\mu$g/g, brain = 8.4 $\mu$g/g, and skin = 10.0 $\mu$g/g.

c BPA was administered i.p. as a fructose complex 6 hours prior to irradiation.
Table 16. Eyes reaction of rats locally brain-irradiated with thermal neutrons in the presence or absence of BPA

<table>
<thead>
<tr>
<th>Dose</th>
<th>Rat #</th>
<th>Keratitis</th>
<th>Blepharitis</th>
<th>Conjunctivitis</th>
<th>Cataract</th>
</tr>
</thead>
<tbody>
<tr>
<td>496 cGy</td>
<td>1†</td>
<td>OU</td>
<td>OU</td>
<td>OU</td>
<td></td>
</tr>
<tr>
<td>(BPA+6MW)</td>
<td>2†</td>
<td>OU</td>
<td>OU</td>
<td>OU</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>OU</td>
<td>OU</td>
<td>OU</td>
<td>early (OU)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>OU</td>
<td>OU</td>
<td>OU</td>
<td>anterior (OD)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>OU</td>
<td>OU</td>
<td>OU</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>OU</td>
<td>OS</td>
<td>OU</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>OU</td>
<td></td>
<td>OS</td>
<td>anterior capsular</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(OU)</td>
</tr>
<tr>
<td>661 cGy</td>
<td>1</td>
<td>OU</td>
<td>OU</td>
<td>OU</td>
<td>OU</td>
</tr>
<tr>
<td>(BPA+8MW)</td>
<td>2†</td>
<td>OU (severe)</td>
<td>OU</td>
<td>OU</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>OU (severe)</td>
<td>OU</td>
<td>OU</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4†</td>
<td>OU</td>
<td>OU</td>
<td>OU</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>OD</td>
<td>OD</td>
<td>OD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>OU</td>
<td></td>
<td>OS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>828 cGy</td>
<td>1</td>
<td>OU (OD worse)</td>
<td>OU (OD worse)</td>
<td>OU (OD worse)</td>
<td>OU</td>
</tr>
<tr>
<td>(BPA+10MW)</td>
<td>2†</td>
<td>OU</td>
<td>OU</td>
<td>OU</td>
<td>OU mature</td>
</tr>
<tr>
<td></td>
<td>3†</td>
<td>OD</td>
<td>OD</td>
<td>OD</td>
<td>OD mature</td>
</tr>
</tbody>
</table>

† Fundus is not visible

OU; Both eyes, OD; Right eye, and OS; Left eye

Doses of neutrons as if the eyes were irradiated ($^{10}$B in eyes = 11.0 µg/g)
Table 17. Summary of the pathological changes that occurred in the choroid plexus following BNCT.

<table>
<thead>
<tr>
<th>Pathological changes</th>
<th>Blood Dose cGy/cGy-Eq</th>
<th>Brain Dose cGy/cGy-Eq</th>
<th>Reaction score following BNCT 7 Months Score* (total no. of animals)</th>
<th>Reaction score following BNCT 12 Months Score* (total no. of animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loose connective tissue</td>
<td>464/920</td>
<td>443/872</td>
<td>2 (4/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td></td>
<td>618/1125</td>
<td>591/1163</td>
<td>1 (1/4)</td>
<td>0 (0/4)</td>
</tr>
<tr>
<td></td>
<td>773/1532</td>
<td>739/1454</td>
<td>2 (1/5)</td>
<td>0 (0/2)</td>
</tr>
<tr>
<td>Dilatation of blood vessels</td>
<td>464/920</td>
<td>443/872</td>
<td>0 (0/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td></td>
<td>618/1125</td>
<td>591/1163</td>
<td>2 (1/4)</td>
<td>2 (1/3)</td>
</tr>
<tr>
<td></td>
<td>773/1532</td>
<td>739/1454</td>
<td>2 (1/5)</td>
<td>0 (0/2)</td>
</tr>
<tr>
<td>Epithelial cell atrophy</td>
<td>464/920</td>
<td>443/872</td>
<td>0 (0/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td></td>
<td>618/1125</td>
<td>591/1163</td>
<td>0 (1/4)</td>
<td>2 (2/3)</td>
</tr>
<tr>
<td></td>
<td>773/1532</td>
<td>739/1454</td>
<td>1 (0/5)</td>
<td>0 (0/2)</td>
</tr>
<tr>
<td>Epithelial cell flattening</td>
<td>464/920</td>
<td>443/872</td>
<td>2 (2/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td></td>
<td>618/1125</td>
<td>591/1163</td>
<td>2 (1/4)</td>
<td>0 (0/3)</td>
</tr>
<tr>
<td></td>
<td>773/1532</td>
<td>739/1454</td>
<td>0 (0/5)</td>
<td>0 (0/2)</td>
</tr>
<tr>
<td>Cellular infiltrates (poly/macro.)</td>
<td>464/920</td>
<td>443/872</td>
<td>0 (0/6)</td>
<td>2 (3/6)</td>
</tr>
<tr>
<td></td>
<td>618/1125</td>
<td>591/1163</td>
<td>2 (2/4)</td>
<td>0 (0/3)</td>
</tr>
<tr>
<td></td>
<td>773/1532</td>
<td>739/1454</td>
<td>0 (0/5)</td>
<td>0 (0/2)</td>
</tr>
<tr>
<td>Vacuolation of epithelial cells</td>
<td>464/920</td>
<td>443/872</td>
<td>0 (0/6)</td>
<td>2 (2/6)</td>
</tr>
<tr>
<td></td>
<td>618/1125</td>
<td>591/1163</td>
<td>0 (0/4)</td>
<td>2 (1/3)</td>
</tr>
<tr>
<td></td>
<td>773/1532</td>
<td>739/1454</td>
<td>0 (0/5)</td>
<td>0 (0/2)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>464/920</td>
<td>443/872</td>
<td>0 (0/6)</td>
<td>2 (2/6)</td>
</tr>
<tr>
<td></td>
<td>618/1125</td>
<td>591/1163</td>
<td>1 (1/4)</td>
<td>2 (3/3)</td>
</tr>
<tr>
<td></td>
<td>773/1532</td>
<td>739/1454</td>
<td>2 (3/5)</td>
<td>3 (2/2)</td>
</tr>
</tbody>
</table>

* Changes were scored as follows: 0, no apparent change; 1, minimal; 2, mild; 3, moderate; and 4, severe.
Figure 14. The changes in rats weight following three doses of neutron irradiations (BNCT) over time.
Figure 14.
Figure 15. Kaplan Meier plots of rats following three doses of neutron irradiations BPA + 273, BPA + 364, or BPA + 454 Gy.
Figure 15.

Time (day) Post Irradiation

Percent Survival

- BPA + 454cGy
- BPA + 364cGy
- BPA + 273cGy
Figure 16. Moist desquamation of the skin following BNCT. The scoring system applied; 0, no change; 1, minimal; 2, mild; 3, moderate; and 4, severe.
Figure 16.
Figure 17. Choroid plexus of a 9 month old rat. Note the integrity of the epithelial and the endothelial cells with little intervening connective tissue fibers (63X) (H&E stain).
Figure 17.
Figure 18. Choroid plexus of a rat, which had received brain and blood radiation dose of 1163 and 1225 cGy-Eq respectively, 12 months following BNCT. Note the vacuolation in the epithelial cells (63X) (H&E stain).
Figure 18.
Figure 19. Choroid plexus of a rat, which had received brain and blood radiation dose of 872 and 920 cGy-Eq respectively, 12 months following BNCT. Note the focal infiltration of polymorphonuclear cells (63X) (H&E stain).
Figure 20. Cerebral cortex of a rat, which had received brain and blood radiation doses of 1454 and 1532 cGy-Eq respectively, 12 months following BNCT showing a hemorrhagic infarction (63X) (H&E stain).
Figure 20.
Figure 21. Choroid plexus of a rat, which had received brain and blood radiation doses of 1454 and 1532 cGy-Eq respectively, 12 months following BNCT showing an increase in fibrous tissue (63X) (H&E stain).
Figure 21.
Figure 22. Choroid plexus of a rat, which had received brain and blood radiation dose of 1163 and 1225 cGy-Eq respectively, 6 months following BNCT showing a dilated blood vessel (63X) (H&E stain).
Figure 22.
Figure 23. Choroid plexus of a rat, which had received brain and blood radiation doses of 1454 and 1532 cGy-Eq respectively, 6 months following BNCT showing a mild atrophy in the epithelial cells (160X) (H&E stain).
Figure 24. Focal increase in fibrous tissue of the overlying meninges of a rat which had received brain and blood radiation dose of 1163 and 1225 cGy-Eq respectively, 6 months following BNCT (63X) (H&E stain).
Figure 24.
Chapter V

The Relative Biological Effectiveness of Fast Neutrons, \( ^{14}\text{N}(n,p)^{14}\text{C} \), and \( ^{10}\text{B}(n,\alpha)^{7}\text{Li} \) Reactions using Boronophenylalanine as A Capture Agent, A Human Melanoma Cell Line, and Two Different Reactors: The Ohio State Research Reactor and The Brookhaven Medical Research Reactor

INTRODUCTION

Boron neutron capture therapy (BNCT) is based on selective uptake of \( ^{10}\text{B} \) by tumor cells followed by irradiation with low energy neutrons (0.025 eV). The \( ^{10}\text{B}(n,\alpha)^{7}\text{Li} \), yields high linear energy transfer (LET) alpha particles and recoiling \(^7\text{Li} \) nuclei, which have a pathlength of approximately 10-14 \( \mu \) (1). Several reports have determined the relative biological effectiveness (RBE) of the \( ^{10}\text{B}(n,\alpha)^{7}\text{Li} \) reaction in vitro (2-4) and in vivo systems (5,6), and these were 2.3, 3.3, and 3.7 in vitro (2-4) and 2.3 and 2.0 in vivo (5,6). In the in vitro experiments, nuclear reactors were utilized as a source of neutrons, cell lines, and the calculation models were different, however, they all used boric acid as the capture agent. The
subcellular distribution of $^{10}$B within the cell is a major determinant of the lethality of the $^{10}$B(n,$\alpha$)$^{7}$Li reaction, and the RBE assigned to it will vary depending upon the boron compound used (7-9). The cellular uptake, distribution and retention will vary depending upon the chemical nature of the compound, and its ability to enter into cellular biosynthetic pathways. Furthermore, the nuclear reactors that have been used to determine the RBE of $^{10}$B(n,$\alpha$)$^{7}$Li reaction vary in the flux of thermal neutrons, and in the fast neutrons and $\gamma$ rays produced in the fission reaction within the reactor core. These variables will in part determine the doses and dose rates delivered to cells and ultimately the RBEs of each component of the beam.

Boronophenylalanine ($^{10}$B-BPA) is a boron-containing amino acid that was synthesized as an analogue of the melanin precursor tyrosine (10), and has been used as a capture agent in vitro and in vivo, as well as in BNCT of melanoma (11). BPA was utilized, as a capture agent, in the present study in order to determine the RBE of $^{10}$B(n,$\alpha$)$^{7}$Li reaction using the human melanoma cell line MRA 27 as a biological dosimeter. Irradiations were carried out at either the Brookhaven Medical Research Reactor (BMRR), which has been extensively used for BNCT experiments over a period of 30 years (12), or the Ohio State Research Reactor (OSURR), which will be used in future studies.
MATERIALS and METHODS

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 McCoys'5A media (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine and were tested periodically for mycoplasma contamination by means of DNA fluorochrome staining and UV microscopy (Bionique Testing Laboratories, Saranac Lake, NY).

Reactors. The OSURR is a pool type reactor using light water as a moderator and coolant, with a maximum power level of 0.5 MW. The design of the OSURR is based on the BULK Shielding Reactor (BSR) located at the Oak Ridge National Laboratory (ORNL). The OSURR has a relatively high flux to power ratio due to the 19.5%-enriched uranium fuel and the compact design of the core. Cell irradiations were carried out using a graphite vial holder of < 4 cm in diameter, which then was placed in a bismuth cylinder facing the reactor core. The BMRR facility has been described in detail elsewhere (13). Briefly, it is water moderated nuclear reactor with a power of 5 MW. One MW-minute generates a neutron fluence of $8 \times 10^{11} \text{ n.cm}^{-2}$ at the center.
of 254 x 254 mm² dimension of the thermal neutron port. The cell irradiations were carried out using a cell vessel rotator at a speed of 2 rpm, as described in detail by Gabel et al. (3).

In Vitro Cell Irradiations and Clonogenic Assays. In vitro irradiations of MRA 27 human melanoma cells were carried out at the BMRR and OSURR. All irradiations at the BMRR and OSURR were performed at 1 and 0.5 MW power respectively. Cells were incubated for 24 hours with BPA at different concentrations of ¹⁰B (0, 5, 10, and 25 μg/ml of growth medium) prior to irradiation. The same concentration of ¹⁰B was maintained in the medium during trypsinization, harvesting, and irradiation (ambient cells), as described previously (14) or the cells were washed thoroughly in order to remove unbound ¹⁰B-BPA, trypsinized, harvested, and irradiated in boron-free medium (washed cells). The cells were irradiated at a density of 2 x 10⁵/ml at room temperature, and were kept on ice during transportation until plated. Following irradiation, the cell concentration was adjusted to 2 x 10⁴/ml, plated into 100 mm petri dishes, and incubated at 37°C in a humidified atmosphere containing 5% CO₂. Twelve to 14 days later, the cultures were terminated, the plates were washed, fixed with formaldehyde, and stained with 1% crystal violet. Colonies ≥ 50 cells (0.3 mm) were counted by means of an Artek 880 image
The plating efficiency (PE) of the MRA 27 cells was calculated from the following formula, \( PE = \left( \frac{\text{number of MRA 27 colonies enumerated}}{\text{total number of MRA 27 cells plated}} \right) \times 100\% \). Depending upon the time of irradiation, it ranged from 25-50%. The surviving fraction was determined from \( \left( \frac{\text{number of colonies enumerated}}{\text{total number of MRA 27 cells plated} \times \text{PE/100}} \right) \).

In vitro X- or γ-irradiations were carried out using a Stabilipan 250 kVp X-ray machine (Siemens, Cleveland, OH) or a Picker \(^{137}\)Cs Teletherapy machine, respectively. The 250 kVp X-irradiations were performed at 15 mA with a Thoresus I filter and 50 cm SSD at dose rate of 51.3 cGy/min. The \(^{137}\)Cs source was used with 2 cm (OD) cone and 15 cm SSD at a dose rate of 100 cGy/min. Cells were irradiated in boron-free medium and boron containing medium and were assayed as described above.

Dosimetry. The mixed radiation from the OSURR and BMRR consisted of thermal and fast neutrons (\( > 10,000 \text{ eV} \), in case of BMRR), γ-photon and heavy particles that are generated from \(^{14}\)N(n,p)\(^{14}\)C and \(^{10}\)B(n,α)\(^{7}\)Li reactions. Thermal neutron fluences (\( n_{th} \)) were measured using 5 mm long gold wires placed inside a 1.5 ml Eppendorf microfuge tube. The \( n_{th} \) flux at 1 MW reactor power at the BMRR was \( 2.8 \times 10^{12} \text{ n. cm}^{-2} \text{min}^{-1} \) and \( 1.6 \times 10^{12} \text{ n.cm}^{-2} \text{min}^{-1} \) at the OSURR at 0.5 MW.
reactor power.

The neutron and γ doses at the OSURR were measured using paired-tissue equivalent (TE) plastic chambers with TE gas (methane, CO₂, and N₂) and Mg wall chambers filled with argon. The neutron and γ doses at the BMRR were measured by means of paired-TE plastic chambers (Shonka A-150 plastic; Far West Technology, Goleta, CA) with TE gas (Rossi gas) and graphite chambers filled with CO₂.

Radiation Dose Calculations. The absorbed doses due to the 

\(^{14}\text{N}(n,p)^{14}\text{C}\) and \(^{10}\text{B}(n,\alpha)^{7}\text{Li}\) reactions (Table 18) were calculated using the following formula:

\[
D(\text{cGy}) = (1.6 \times 10^{-8} \ C \ E \ \sigma \ \phi \ N)/A \quad \text{Eq.1}
\]

Where \(D\) is the dose in cGy, \(1.6 \times 10^{-8}\) is a constant for converting MeV/g to cGy/g/MeV, \(C\) is the concentration of the capture element (g/g), \(E\) is the energy of the emitted radiation (MeV), \(\phi\) is the fluence of thermal neutrons (n/cm²), \(\sigma\) is the thermal capture cross-section of the target element (in cm²), \(N\) is Avogadro's number (6.025 x 10²³), and \(A\) is the atomic weight (g/mol).

The nitrogen content was assumed to be 1.5% and uniformly distributed for MRA 27 cells. The cross section of nitrogen is \(1.75 \times 10^{-24}\) cm² and the energy of the emitted proton is 0.625 MeV (1). Using Eq.1, the dose rate of the
$^{14}\text{N}(n,p)^{14}\text{C}$ reaction was 3.1 for the BMRR and 1.8 cGy/min for the OSURR. The $^{10}\text{B}(n,\alpha)^{7}\text{Li}$ reaction, results in a 2.79 MeV (6% of the reaction) and 2.31 MeV with 0.48 MeV of $\gamma$ in 94% of the reaction. Using Eq.1 with $\sigma$ for $^{10}\text{B}$ of $3840 \times 10^{-24}$ cm$^2$ (1,3), the dose rate of the $^{10}\text{B}(n,\alpha)^{7}\text{Li}$ reaction was 2.4 and 1.4 cGy/min per $\mu g$ (1 ppm) of $^{10}\text{B}$ for the BMRR and OSURR.

RBE for Fast neutrons, $^{14}\text{N}(n,p)^{14}\text{C}$, and $^{10}\text{B}(n,\alpha)^{7}\text{Li}$ Reactions. At an isoeffect end point, 0.1 and 0.01 surviving fractions, the dose components resulting from the irradiation at the OSURR in the absence of $^{10}\text{B}$ (Eq.2) were equal to the doses by 250 kVp X-irradiations:

$$T_1(D_p R_p + D_\gamma R_\gamma) = \text{Dose of X-irradiation} \quad \text{Eq.2}$$

$T_1$ is the irradiation time required to reduce the surviving fraction equally in the absence of $^{10}\text{B}$-BPA. $D_p$, $D_\gamma$ are the dose rates for $^{14}\text{N}(n,p)^{14}\text{C}$ reaction and $\gamma$ respectively. $R_p$, $R_\gamma$ are the RBE values for the $^{14}\text{N}(n,p)^{14}\text{C}$ reaction, and $\gamma$ respectively. This $R_p$ value was used, then, to calculate the RBE of fast neutrons ($R_n$) for the BMRR (Eq.3).

$$T_1(D_p R_p + D_\gamma R_\gamma + D_n R_n) = \text{Dose of X-irradiation} \quad \text{Eq.3}$$
D_n is the dose rate of fast neutrons at the BMRR. 
The R_p and R_n values form Eqs. 2 and 3 were used to 
calculate the RBE of the ^10B(n,α)^7Li reaction. At an 
isoeffect end point, 0.1 and 0.01 surviving fractions, the 
dose components resulting from the irradiations at the OSURR 
or BMRR in the presence of ^10B-BPA (ambient conditions) were 
equal to the dose components in the absence of ^10B-BPA 
(equations 4 and 5).

\[ T_1(D_pR_p + D_nR_n) = T_2(D_pR_p + D_nR_n + D_0C_BRANCH) \] Eq. 4
\[ T_1(D_pR_p + D_nR_n + D_0R_n) = T_2(D_pR_p + D_nR_n + D_0C_BRANCH) \] Eq. 5

T_1 and T_2 are the irradiation times required to reduce the 
surviving fractions equally in the absence (T_1) or presence 
(T_2) of ^10B-BPA. D_n and R_n are the dose rate and the RBE 
value for ^10B(n,α)^7Li reaction respectively, and C_BRANCH is a the 
concentration of ^10B (μg/ml).

RESULTS

Survival as a Function of LET. Fig. 25 depicts the cell 
survival curves following irradiation of MRA 27 cells with 
^137Cs γ photons, 250 kVp X-rays, or neutrons at the OSURR and 
BMRR. The dose response curves for both 250 kVp X-rays and 
^137Cs derived γ photons clearly demonstrate a shoulder, 
whereas none was seen following reactor irradiation alone or
in the presence of 10 μg/ml (Table 19). The PE for MRA 27 cells did not change when they were preincubated with 10B-BPA. When 10 μg/ml of 10B (10B-BPA) where present at ambient conditions during cell irradiations at the OSURR and BMRR, a remarkable drop in the D₀ (98 to 26 cGy at the OSURR, and 66 to 14 cGy at BMRR) (Fig. 25). This reduction was attributed primarily to the high LET of the 10B(n,α)7Li 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 μg/ml of 10B (Fig. 25).

RBE of 14N(n,p)14C and fast neutrons. Solving Eq 2., the RBE of 14N(n,p)14C (Rp) was 4.9 and 3.8 at 0.1 and 0.01 surviving fractions respectively. These values were applied in Eq.3 to solve for the RBE of BMRR fast neutrons (R₀), which were 3.6 and 2.8 at 0.1 and 0.01 surviving fractions respectively (Table 20).

The survival curves of MRA 27 cells following irradiation in the absence of 10B-BPA at BMRR, OSURR, and 250 kVp X-rays are shown in Fig. 26. The doses of the survival curves were presented in cGy-Eq (effective dose), and the RBE values, obtained above, were used. The survival curves of the OSURR and BMRR are superimposable on the X-ray survival curve.
RBE of the $^{10}$B(n,$\alpha$)$^7$Li Reaction. The dose survival curves of MRA 27 cells following irradiation in the presence or absence of $^{10}$B-BPA at OSURR and BMRR are shown in Figs. 27 and 28 respectively. The survival curves clearly illustrate the cytotoxic effect of the $^{10}$B(n,$\alpha$)$^7$Li reaction compared to reactor irradiations in the absence of $^{10}$B-BPA, which was dependent upon the concentration of $^{10}$B-BPA. Solving for $R_b$, as in Eq. 4 and 5, the RBE values of the $^{10}$B(n,$\alpha$)$^7$Li reaction are summarized in Table 20. The RBE values of the $^{10}$B(n,$\alpha$)$^7$Li ($R_b$) reaction obtained following irradiation at the OSURR ranged from 1.7-2.6 and 1.8-2.0 at 0.1 and 0.01 surviving fractions respectively. However, the $R_b$ values from BMRR were higher and ranged from 5.5-6.9 and 4.4-5.4 at 0.1 and 0.01 surviving fractions respectively.

Washed cells experiment. The doses required to reduce the surviving fraction to 0.1 and 0.01 were 101 and 161 cGy for 50 $\mu$g/ml, and 60 and 103 for 100 $\mu$g/ml for the cells (Fig. 24). These were ~1.2 X less than doses required for cells incubated and irradiated in the presence of 5 and 10 $\mu$g/ml of $^{10}$B.

DISCUSSION

RBE values of 4.9 and 3.8 for the $^{14}$N(n,p)$^{14}$C and 3.6 and 2.8 for fast neutrons were observed at 0.1 and 0.01
surviving fractions respectively. Using the OSURR, an RBE of ~ 2.0 was observed for the $^{10}$B(n,$\alpha$)$^7$Li reaction, while in contrast, an RBE of ~ 5.8 was observed when the irradiations were carried out at the BMRR. The cell killing enhancement effect with the $^{10}$B(n,$\alpha$)$^7$Li reaction was dependent on the concentration of boron in the media. In the washed cell experiment, it has been observed that only 8.3% of the incubated BPA demonstrated cell killing effect, whereas the remainder (91.7%) was washed out. Furthermore, no difference in survival curves were observed with $^{10}$B and X-irradiations.

RBE is a very complex factor that depends on radiation quality (i.e. type of LET), radiation dose, dose rate, and the biological system and end point. In the case of BNCT, an additional factor, the subcellular distribution of the compounds is a major microdosimetric determinant for the RBE. Using Monte Carlo simulation, Gabel et al. (8) have calculated that the RBE of the $^{10}$B(n,$\alpha$)$^7$Li reaction changes as a function of the subcellular localization of $^{10}$B in the nucleus, cytoplasm, attached to the cell membrane, or external to the cell. Nuclear localization of boron resulted in a five fold increase in the radiation dose attributed to the $^{10}$B(n,$\alpha$)$^7$Li reaction compared to the same amount distributed uniformly throughout the cells. Furthermore, ~ 6-7 X more boron attached to the cell membrane is needed to achieve the same dose to the nucleus,
as when the boron is uniformly distributed in the nucleus (7,8). In the present study, the calculations were based on the assumption that the concentration of BPA was homogeneously distributed intra- and extracellularly. However, this is might not be the case, and therefore the RBE value for the $^{10}\text{B}(n,\alpha)^{7}\text{Li}$ reaction is not precise.

Coderre et al. have utilized the same procedure, as in the present study, to calculate the RBE values of $^{14}\text{N}(n,p)^{14}\text{C}$ reaction and the fast neutrons, using only BMRR as the irradiation source. They reported RBEs of 4.4 for both components (15). In the present study, we have utilized the fast neutron-free reactor (OSURR) to estimate the RBE of the $^{14}\text{N}(n,p)^{14}\text{C}$ reaction, and that was 3.8-4.9 depending on the end point. These estimates were consistent with those reported by Ujeno et al., who have reported an RBE of 2.3-5.5, depending upon the surviving fraction used as an end point (16). Although, the RBE of fast neutrons at the BMRR was less than the value reported by Coderre et al (15), it ranged from 2.8-3.6. This value was within the wide range of neutron RBEs as a function of energy (17,18).

The RBE values of $^{10}\text{B}(n,\alpha)^{7}\text{Li}$ reaction have been found to be 2.0-2.3 in vivo (5,6). The microdosimetric factors involved in vivo are much more complex than in vitro (9). Those factors include the homogeneous or heterogeneous distribution of $^{10}\text{B}$ in the target tissue, the blood $^{10}\text{B}$ concentration, and the accumulation of $^{10}\text{B}$ inside the cells.
In an *in vitro* culture system, there are fewer variables, and the endpoint used is simpler.

The OSURR beam is fast neutron-free and this characteristic might be useful for *in vivo* BNCT studies. Normal tissue damage from fast neutrons could be evaluated by comparing animal studies carried out in parallel at the BMRR and OSURR. The contaminating fast neutrons of the BMRR beam increases the dose rate, and therefore the time needed to reduce the surviving fraction of cells would be decreased. This might explain the differences in the RBE values of the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction between the OSURR and BMRR. Furthermore, this could prove to be a significant advantage in delineating the effects attributable to fast neutrons radiation damage from those of the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction. Utilizing the OSURR and the BMRR, we have shown a significant variation in the RBE values for the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction for the same compound, $^{10}\text{B}-\text{BPA}$, and in the same culture system. These variations were due to the mixed radiation components of the reactor beams.

In conclusion, it is unlikely that a constant RBE value can ever be assigned to the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction due to the so called "compound factor" (19,20). Therefore, new methods such as high resolution alpha track autoradiography (21), electron spectroscopic imaging/electron energy loss spectroscopy (22) and ion microscopy should provide data on subcellular localization (23), and permit more precise
microdosimetry (8,9).
REFERENCES


19. Gabel, D., Bond, V. P., Kalef-Ezra, J., and Fairchild,


Table 18. Dose rates for cell irradiations at the BMRR and the OSURR

<table>
<thead>
<tr>
<th>Component</th>
<th>BMRR (cGy/min)</th>
<th>OSURR (cGy/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{14}$N(n,p)$^{14}$C</td>
<td>3.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Fast neutrons</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Gamma photons</td>
<td>6</td>
<td>5.2</td>
</tr>
<tr>
<td>Total</td>
<td>22.1</td>
<td>7.0</td>
</tr>
</tbody>
</table>

$^{10}$B(n,α)$^7$Li 2.4/ppm$^{10}$B 1.4/ppm$^{10}$B

* At 1 MW power the thermal neutron flux = 2.8 x 10$^{11}$ n.cm$^{-2}$. min$^{-1}$ at the BMRR.
* At 0.5 MW the thermal neutron flux = 1.6 x 10$^{11}$ n.cm$^{-2}$. min$^{-1}$ at the OSURR.
Table 19. Doses (cGy) required from X-, OSURR, BMRR irradiations and in the presence of 10 μg/ml of $^{10}$B to reduce the surviving fraction 0.1 and 0.01

<table>
<thead>
<tr>
<th>Irradiation</th>
<th>Dose (cGy)$^b$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>250kVp</td>
<td>436</td>
</tr>
<tr>
<td>OSURR</td>
<td>216</td>
</tr>
<tr>
<td>BMRR</td>
<td>142</td>
</tr>
<tr>
<td>10 μg/ml of $^{10}$B at OSURR</td>
<td>69</td>
</tr>
<tr>
<td>10 μg/ml of $^{10}$B at BMRR</td>
<td>50</td>
</tr>
</tbody>
</table>

$^a$ MRA 27 cells were incubated with 10 μg/ml of $^{10}$B ($^{10}$B-BPA) for 24 hours and then were irradiated with the same 10B concentration until the clonogenic assays were performed.

$^b$ These are the average doses of three different experiments. The standard deviations were less than 10%.
Table 20. RBE values of fast neutrons, $^{14}$N(n,p)$^{14}$C and $^{10}$B(n,α)$^{7}$Li reactions at 0.1 and 0.01 surviving fractions obtained from the survival curves of MRA 27 human melanoma cell line after in vitro irradiations

<table>
<thead>
<tr>
<th>End point</th>
<th>$^{10}$B (μg/ml)</th>
<th>Reactor</th>
<th>Rp</th>
<th>Rn</th>
<th>Rb</th>
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</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0</td>
<td>OSURR</td>
<td>4.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>OSURR</td>
<td>2.6</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>10</td>
<td>OSURR</td>
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<td>25</td>
<td>OSURR</td>
<td>1.7</td>
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<tr>
<td>0.01</td>
<td>0</td>
<td>OSURR</td>
<td>3.8</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>5</td>
<td>OSURR</td>
<td>2.0</td>
<td></td>
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<tr>
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<td>10</td>
<td>OSURR</td>
<td>1.8</td>
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<tr>
<td></td>
<td>25</td>
<td>OSURR</td>
<td>1.8</td>
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</tr>
<tr>
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<td>0</td>
<td>BMRR</td>
<td>4.9</td>
<td>3.6</td>
<td>-</td>
</tr>
<tr>
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<td>5</td>
<td>BMRR</td>
<td>6.9</td>
<td></td>
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<td>10</td>
<td>BMRR</td>
<td>6.9</td>
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<td>25</td>
<td>BMRR</td>
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<tr>
<td>0.01</td>
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<td>BMRR</td>
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<td>2.8</td>
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<td>5</td>
<td>BMRR</td>
<td>5.4</td>
<td></td>
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<td>10</td>
<td>BMRR</td>
<td>5.5</td>
<td></td>
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<tr>
<td></td>
<td>25</td>
<td>BMRR</td>
<td>4.4</td>
<td></td>
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</tr>
</tbody>
</table>

* MRA 27 cells were incubated with different concentrations of $^{10}$B for 24 hours and then were irradiated with the same 10B concentration until the clonogenic assays were performed.

* $R_p$, $^{14}$N(n,p)$^{14}$C; $R_n$, fast neutrons; and $R_b$, $^{10}$B(n,α)$^{7}$Li reaction
Figure 25. Survival curves of MRA 27 cells following $^{137}$Cs (○), 250 kVp X-rays (▼), OSURR (□), and BMRR (△) irradiations. Cells also were irradiated with 250 kVp X-rays (▼) and neutrons at the OSURR (■) in the presence of 10 μg/ml of $^{10}$B (ambient).
Figure 25.
Figure 26. Survival curves of MRA 27 cells following irradiation at the BMRR (●), OSURR (○), and with 250 kVp X-rays (▼) machine. The cells were irradiated in the absence of boron. The survival curves were plotted versus the dose in cGy-Eq, at which the calculated RBEs for fast neutrons and $^{14}N(n,p)^{14}C$ reaction were used.
Figure 26.
Figure 27. Survival curves of MRA 27 cells following irradiation at the OSURR at different concentrations: 0 (○), 5 (▲), 10 (○), and 25 (♦) µg/ml of 10B. The cells were incubated with 10B-BPA for 24 hr and then irradiated in medium containing the same concentration (ambient).
Figure 27.
Figure 28. Survival curves of MRA 27 cells following irradiation at the BMRR at different concentrations; 0 (○), 5 (■), 10 (■), and 25 (△) μg/ml of \(^{10}\text{B}\). The cells were incubated with \(^{10}\text{B}-\text{BPA}\) for 24 hr and then irradiated in medium containing the same concentration (ambient).
Figure 28.
Figure 29. Survival curves of MRA 27 cells following irradiation at the BMRR at different concentrations; 0 (○), 25 (●), 50 (▼), and 100 (▼) μg/ml of \(^{10}\text{B}\). The cells were incubated with \(^{10}\text{B}-\text{BPA}\) for 24 hr, washed thoroughly, then irradiated in boron-free medium (washed cells).
Figure 29.
Chapter VI

Review: Animal Models for Boron Neutron Capture Therapy

INTRODUCTION

Many animal models have been used to determine the selectivity of boronated compounds and their efficacy for BNCT. This chapter will be selective in reviewing the animal models, which have been used to evaluate different generations of boronated compounds, and their possible clinical use.

Borax and Sodium Pentaborate

The first generation of compounds, which were used in early clinical trials of BNCT, were the inorganic boron compounds borax (Na\textsubscript{3}B\textsubscript{4}O\textsubscript{7}) and sodium pentaborate (Na\textsubscript{2}B\textsubscript{10}O\textsubscript{16}) (1). The rationale for using these compounds was based on their toxicity and pharmacology in animals and in man (2). In one of the first BNCT experiments in animals, borax (Na\textsubscript{3}B\textsubscript{4}O\textsubscript{7}) and boronated Red Azo dye and an Evan’s Blue analogue were evaluated for their selectivity for a murine brain tumor (3). The mice were injected i.c. with a tumor
cell suspension of a methylcholanthrene induced glioblastoma and 9-13 days later the test compounds were injected i.v. into glioma bearing mice. Using alpha autoradiography, the boron compounds were found to localize in high amounts at the periphery, none were found in the central area of the tumor or in normal brain. The accumulation of the boronated dyes within the tumor was 30 X greater than that of borax. The estimated values of tumor boron concentrations following injection of borax or Red Azo dye of Evan’s Blue analog were 60, 910, and 1750 μg/g respectively. The dyes were also taken up by the skin, muscles, kidney, liver, heart, suggesting non-selectivity for the tumor.

Locksley and Sweet (4) developed another approach to test boron compounds for their selectivity for brain tumors. Briefly, brain tumors were induced in mice by implanting pellets of the carcinogen, 20-methylcholanthrene intracerebrally. Mice developed large tumors within 200-300 days, which were then transplanted s.c. into syngeneic mice. Ten to fourteen days following s.c transplantation, sodium borate was administered i.p. and 30 minutes later all tissues, including the tumor and excluding the brain, had reached the maximum boron concentration. At that time, tumor to brain (T/Br) ratios were 3, but this dropped to 1 in one to two hours. Using the same model described above, Easterday reported a better T/Br ratio following injection of sodium pentaborate (5). During 1950s and early 1960s.
Farr et al. were the first to unequivocally demonstrate the effectiveness of BNCT in an animal model (6). They implanted a methylcholanthrene-induced glioma i.m into mice and several days later, animals were injected i.v. with $^{10}$B-enriched sodium pentaborate mixed with glucose at 2:1 molar ratio. The mixture of glucose and sodium pentaborate reduced the toxicity of sodium pentaborate (7). Neutron irradiations were carried out at the Brookhaven Medical Research Reactor (BMRR) 15, 40, 60, 85 minutes following administration of sodium pentaborate, at which time tumor boron concentrations ranged from 25-28 μg/g and tumor to blood (T/Bl) ratios were 0.7, 1.1, 1.3, and 1.6 respectively (Table 1). However with X-rays, the same s.c. tumor was also cured but with severe damage to the irradiated leg (6,8).

Boron Hydrides

The second generation of boron compounds were boron hydrides and organic compounds, including boric acid esters and aromatic monoboronic acids. The use of boron hydrides as capture agents was studied in mice carrying s.c. tumors. Sodium perhydrodecarborate (Na$_3$B$_{10}$H$_{16}$) was shown to be selective for tumor cells compared to normal brain tissue with T/Br ratio of 3.9 at 15 minutes, which increased to 7.3 at 1.8 hours following injection (9). The compound, sodium
perhydrodecaborate ($\text{Na}_2\text{B}_{10}\text{H}_{10}$), was also tested as a capture agent for BNCT in controlling malignant murine ependymoblastomas that had been implanted subcutaneously (10). BNCT was performed with tumor estimated doses of 15-20 Gy. The mean survival time (MST) for the BNCT treated group was 62 d compared to 23 d for the irradiated controls, that had received a tumor dose of 3 Gy, and 18 d for the untreated mice. It was concluded from this study that the doses were insufficient to destroy the tumor completely. It should be mentioned that 50% of mice following BNCT died early as a consequence of intestinal damage, even though the animals’ bodies were shielded with $^7\text{Li}$ and the neutron flux was reduced to 3% of the tumor dose (10). One of the boric acid esters, triisopropanolamine, which has a low rate of hydrolysis, was found to accumulate in the tumor 5-8 X more than the normal brain at 30 minutes following administration (11). However, this compound was found to be twice as toxic as boric acid.

Snyder and his colleagues synthesized aromatic boronic acids on the basis of high metabolic demand for these compounds should localize in neoplastic tissues (11). One such compound was benzeneboronic acid. Soloway et al. (11) studied a large number of substituted benzeneboronic acids and found that p-carboxybenzeneboronic acid had the best selectivity for ependymoblastomas with T/Br ratio of 5-8:1. This ratio was observed within 1 hour following
administration and retained for 3 to 4 hours. However, when p-carboxybenzeneboronic acid was used for clinical trials in patients with brain tumors, there was no evidence of therapeutic efficacy. It was realized that these compounds, including p-carboxybenzeneboronic acid, and sodium decahydrodecaborate, accumulated in high amounts in the blood, which consequently caused extensive radiation damage to the vascular endothelium (12).

Polyhedral Boranes

Studies with boron hydride compounds led to the synthesis of another series of compounds, which were developed after the discouraging clinical trials of BNCT (13). The new compounds were mercaptoundecahydrododecaborate (B_{12}S_{11}SH)^{-2} and dimercaptooctachlorodecaborate (B_{10}C_{18}(SH)_2)^{-2}. Both compounds exhibited an acceptable T/Bl ratios of boron and very low amounts of boron were found in the normal brain, although the sulfhydryl group made them toxic due to the sulfhydryl group (13). Slower infusion of the (B_{12}S_{11}SH)^{-2} into rabbits markedly decreased toxicity and that improved the retention of normal physiologic functions (13). Soloway et al. also studied the binding of Na_{11}B_{12}H_{11}SH, later referred to as BSH, to bovine serum albumin (BSA) and observed that it remained bound, even following extensive dialysis (13). They suggested that
disulfide bonds were formed between BSH and BSA. Other investigators obtained additional data that provide definitive proof for this hypothesis (14).

A series of in vivo experiments were conducted by Hatanaka in Japan, to determine the localization of BSH in tumor bearing animals (15). C57Bl mice carrying a subcutaneously implanted glioblastoma-like tumor were injected i.p. with 50 mg B/kg of BSH. Although T/Bl ratios initially were less than 1, these increased to 3 at 24 hours following injection, at which time tumor boron concentrations were 2.1 μg/g (15) and brain boron concentrations were ≤ 1.1 μg/g. BNCT experiments on mice bearing s.c. 203GL glioma were carried out following i.m. injection of 50 or 100 mg/kg of BSH (16). The survival time for one group of mice, which had received 100 mg of B/kg of BSH followed by thermal neutron (n_{th}) irradiations with a fluence of 6 x 10^{12} n.cm^{-2}, was highly significant with a MST of 576 d. All mice treated with 10-20 Gy of X-rays died with a MST of 97 d compared to 60 d for untreated controls. In another study the effectiveness of BNCT on a s.c. malignant murine ependymoblastoma was investigated (16). BSH, at a concentration of 200 mg of 10B/kg, was injected i.p. into mice bearing s.c. gliomas. Seventeen hours later, T/Bl and T/Br ratios were 9.24 and 26.4 respectively, at which time the average tumor boron concentration was 18.1 μg/g. When BNCT was performed following administration of
200 mg of $^{10}$B/kg, all mice died within one week following irradiation. Death was attributed to insufficient body shielding during irradiation because of their small body size (17). It was also found that BSH accumulated in liver and intestines (15), which increased the radiation doses attributable to the $^{10}$B(n,$\alpha$)$^7$Li reaction, and was delivered to the organs. However in the same study (16), another set of BNCT experiments was performed with i.p. injection of 100 mg of $^{10}$B/kg. Seventeen hours later, T/Bl and T/Br ratios were 1.74 and > 13.6 respectively, at which time the tumor boron concentration were 11.9 $\mu$g/g. Following $n_{th}$ irradiations of BSH treated mice, 58.3% of the animals, which had received tumor radiation doses of 10.7 Gy or 13.1 Gy, survived > 100 days. At a lower radiation dose of 7.4 Gy, 25% of the mice had an non-palpable tumors and survived 100 days (16).

A rat brain tumor model utilizing the F98 glioma was developed by Clendenon, Barth et al. in order to test the efficacy of BSH as a capture agent (18). Although, the survival times of BNCT treated rats were statistically significant when compared to unirradiated controls, there were no long-term survivors. This was due to low tumor boron concentrations, which ranged from 1-12.8 $\mu$g/g. Normal brain boron levels were very low < 0.5 $\mu$g/g, although, blood boron levels were comparable to the tumor boron levels and ranged from 0.4-10.5 $\mu$g/g. Joel et al. have evaluated the
efficacy of BNCT by utilizing the rat 9L-gliosarcoma model with the dimeric form of sodium borocaptate, BSSB as the capture agent (19). A slow infusion of the dimer at a rate of 45-50 μg of $^{10}$B/g b.w. per day for three days yielded tumor $^{10}$B concentration of 26-34 μg/g, compared to 35-45 μg/g and < 2.0 μg/g in the blood and brain respectively. Long term survivors (> 15 months) were seen in 2 out of 12 rats that had received tumor dose of 6.7 Gy (14.2 Gy-Eq), and > 10 months survivors in 6 out of 10 rats that had received 12.0 Gy (25.6 Gy-Eq) (Table 5). None of the irradiated control rats survived more than 70 days. In a similar study, gliosarcoma bearing rats were treated with X-irradiations. The median survival times (MeSTs) of rats that had received 15 or 22.5 Gy, were 26 or 31 days, and 10% and 25% of these rats survived > 10 months.

It was reported that BSH bound to albumin (13,14,20) and remained extracellular (21) and did not cross the blood brain barrier. Therefore, very low levels of boron were detected in the normal brain. Furthermore, the large amounts of the BSH and BSSB detected in the brain tumors might have been due to the localization of these boronated compounds within the tumor vasculature. The high blood values of boron following injection of BSSB could be reduced by plasmapheresis (22). Blood boron levels decreased by 65%, while the tumor boron concentrations showed little or no change over a period of 2-3 hours. Plasmapheresis, in
general, should be considered in BNCT studies for two reasons; first, it lowers blood boron concentrations, which ultimately decreases the radiation dose to the vascular endothelium of the normal tissues and second, it probably reduces the late toxic effects of the compound.

The uptake of the sulfhydryl borane monomer BSH and the disulfide dimer BSSB were both studied in mice bearing s.c. implants of the Harding-Passey melanoma (23). The compounds were injected at a dose of 200 \( \mu \text{g} \) of B/g b.w. at a very slow rate \((\sim 1 \mu \text{g} \text{ B/g b.w. per hour})\). This yielded a higher T/Bl ratio for the BSSB than the BSH at which time the tumor boron concentrations of BSSB and BSH were 14.1-30.3 and 3.8-15.1 \( \mu \text{g/g} \) respectively.

A large animal model has been used to determine the efficacy of BNCT for relatively deep seated brain tumors, using BSH as the capture agent (24). Dogs with spontaneous brain tumors were injected i.v. with 50 mg of boron (BSH)/kg b.w. Tumor boron concentrations ranged from 10-30 \( \mu \text{g/g} \), T/Bl and T/Br ratios were 0.5-1.0 and 6-14 respectively, and normal brain boron levels did not exceed 5 \( \mu \text{g/g} \). Boron concentrations of liver, spleen, lungs, and kidneys were higher than those of the blood. Nine dogs have been treated using BSH as the capture agent (25). \(^{10}\text{B}\)-enriched (95%) BSH at a dose of 50 mg B/kg was injected i.v. at an infusion rate of 1 mg B/kg per minute. The blood boron concentration was 25 \( \mu \text{g/g} \) and the physical radiation dose to the blood was
estimated to be 19 Gy. Four dogs died within 24 hours to several weeks following irradiation and 5 animals lived 3 to > 14 months. Ischemic and cerebral necrosis in the white and the gray matter were seen in one dog 4 months following BNCT with no viable tumor cells. One of the dogs that died 300 days following irradiation developed cerebral necrosis, encephalomalacia and demyelination. In summary, one animal died of CNS complications with no viable tumor, four dogs had viable tumors at the time of their death, but these were smaller than those seen at the time of diagnosis, and the last animal remained alive for > 18 months following BNCT with apparent tumor control.

Boronophenylalanine (BPA)

BPA, a boron containing aromatic amino acid, was synthesized to be selective for neoplastic tissues (26), and as which will be discussed later, this has been used as a melanafinic precursor analogue for targeting melanoma. It has been observed that BPA accumulated in amelanotic tumors (27) at 10B concentrations that was thought to be adequate (15-30 μg/g) to sustain a 10B(n,α)7Li reaction (28). Three different glioma models, 9L gliosarcoma (29), gliosarcoma 261 (30), and F98 glioma (31) were utilized in three different studies to determine uptake of BPA and its efficacy as a capture agent for BNCT (Table 5). In 9L
gliosarcoma bearing rats, $^{10}\text{B}$ concentrations in the tumor at 5 hours following one or two intragastric (i.g.) doses of BPA given 3 hours apart, were 20 or 40 µg/g respectively, at which time T/Bl and tumor to brain (T/Br) ratios of $^{10}\text{B}$ were 3.3 and 3.9. The MeSTs of BNCT treated groups, which had 2 i.g. doses of BPA and a total tumor dose of 8.9 Gy (19.3 cGy-Eq) or 13.4 cGy (29.0 cGy-Eq), were 60 and 120 days respectively. Seven out of 16 rats (8.9 Gy) and 6 out of 12 (13.4 Gy) rats survived > 12 and 5 months respectively (29). In GL261 glioma bearing mice, BPA was given orally at doses ranging from 30-100 mg of $^{10}\text{B}$/kg b.w. (30). The best T/Bl and T/Br ratios were 11 and 3 respectively at 3 hours post administration of BPA at which time the tumor $^{10}\text{B}$ concentration was 30 µg/g. The MeSTs of the BNCT treated groups, which had received tumor doses of 10.9, 24.6, 36.5, and 42.5 cGy, were 28, 25, 69, and 70 days respectively compared to 18 days for the untreated controls. However, in the same study, the animals given an equivalent dose of X-rays, had MeST of 28, 21, 17, and 15 days respectively, which were not different from untreated controls, and the animals had radiation related deaths (30). The third model has utilized F98 glioma bearing rats (31). This tumor was shown to be refractory to all other therapeutic modalities (32,33). F98 glioma bearing rats were injected with BPA-fructose complex of 47 mg of B/kg body weight. Tumor boron concentrations were 30.6 µg/g 6 hours following
administration of BPA at which time the best T/Bl and T/Br ratios were observed (2.6 and 3.6 respectively). Following BNCT, the MeSTs of rats that had received tumor doses of 5.8, 8.9, and 11.6 Gy (12.3, 16.5, and 24.8 cGy-Eq) were 32, 37, and 59 days compared to 24 for untreated animals. Twenty and 50% of the BNCT treated rats that had received tumor doses of 8.9 and 11.6 Gy (16.5 and 24.8 cGy-Eq) survived more than 2 months compared to the irradiated controls, which all had died within 42 days (Table 5).

Melanafinic Compounds

Based on the observations of Blois (34) that $^{35}$S-labeled chlorpromazine binds selectively to melanin, Mishima in 1972 proposed using boron containing derivatives of this compound as a capture agent for BNCT of melanoma (35). Malignant melanomas can be classified as either melanotic or amelanotic, based on their ability to synthesize melanin. Starting with tyrosine and 3,4-dihydroxyphenylalanine (dopa) and the enzyme tyrosinase, the biosynthetic pathway can lead to the synthesis of melanin or its biochemical precursors. Mishima postulated that $^{10}$B containing compounds that could enter into the biosynthetic pathway for melanin might be selectively incorporated into melanomas. Furthermore, the impetus for Mishima’s search for a different kind of therapy such as BNCT, was due to the inability to treat a subset of
patients with cutaneous melanoma who for one or another reason were not candidates for surgery. An even better reason is the fact that at this time there is no uniformly effective way to treat patients with metastatic disease, especially to the central nervous system (36,37).

Chlorpromazine boron hydrides were one class of the melanoma seeking compounds that were tested as capture agents for BNCT in a Greene melanoma implanted s.c. into Syrian golden hamsters (38). Intralesional injections of 12 mg (3 mg of $^{10}$B) of chlorpromazine undecahydrododecaborate ($\text{nB}_{12}-\text{CBZ}$) followed by irradiation with a thermal neutron fluence of $1.2 \times 10^{13} \text{n.cm}^{-2}$, markedly inhibited, but not completely eradicated, the growth of the malignant melanoma. $^{10}$B-dopa compound, whose incorporation is dependent upon tyrosinase activity rather than melanin synthesis, also was synthesized as a capture agent for malignant melanomas. A solution of 27.6 mg of $^{10}$B-dopa (1 mg of B) was injected s.c. into B16 melanoma bearing mice, followed by irradiation with $n_{\text{th}}$ fluence of $1.2 \times 10^{13} \text{n.cm}^{-2}$. BNCT produced a marked inhibition of tumor growth compared to irradiated control animals, but complete eradication of tumor was not achieved (38).

Mishima et al. have used BPA as an analogue of the melanin precursor phenylalanine (39). In vitro studies on B16 melanoma cells with BPA and $n_{\text{th}}$ irradiations demonstrated the efficacy of BNCT over photon irradiations
Following these, a series of BNCT experiments were initiated in Duroc pigs with spontaneous melanomas. Six grams of $^{10}$B-BPA were injected perilesionally followed by $n_{th}$ irradiation with $1.56 \times 10^{13}$ n.cm$^{-2}$. The melamomas appeared to regress following treatment and there was no evidence of residual tumor at autopsy performed 174 d later (39).

The work of Mishima and his collaborators have stimulated worldwide interest in BNCT of melanoma. Several investigators (40-43) have demonstrated the selectivity of BPA for Harding Passey, B16 melanoma, SK-MEL-3, and Green melanomas when they were implanted s.c. compared to normal tissues. T/Bl ratios of 4, 4, 4, 5, and 2.1 were reported at which time the average tumor concentrations were 30, 15, 20, 40, and 14.5 $\mu$g/g respectively (Table 2). These variations in tumor boron concentrations were due to the melanoma cell lines used, dose, and route of administration. However, these data suggest that BPA has selectively localized in various subcutaneously implanted melanomas. Furthermore, Coderre et al (42) have reported that BPA selectively accumulated in amelanotic clone of B16 melanoma (B16-013) and in human melanoma cell line LOX at T/Bl ratio of 3 at which time the tumor boron concentration was 15 $\mu$g/g. The accumulation of BPA in the melanotic and amelanotic melamomas appeared to be dependent upon the increased demand for amino acid of fast growing tumors, and to a lesser degree upon the amount of melanin
Several investigators have conducted BNCT experiments with s.c. melanomas in mice. Coderre et al. (44) have treated BALB/c mice carrying s.c. implants of the Harding-Passey melanoma. Tumor $^{10}\text{B}$ concentrations at the time of irradiation, were 40 $\mu$g/g, which increased the tumor irradiation dose from 3 Gy to 17.8 Gy (38.7 cGy-Eq) of which 14.8 Gy were due to the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction. Eleven out of 19 mice showed long-term tumor growth control compared to 4 out of 22 mice of the irradiated control group, which had received a radiation dose of 3 Gy (Table 3). In a similar study (45), s.c. B16 melanoma bearing mice were subjected to different treatment regimens of BNCT or with 250 kVp X-irradiations. The mice, which had received 30 or 45 cGy of X-rays, had severe radiation damage to the skin, as evidenced by severe moist desquamation, edema and muscular atrophy of the foot. However, BNCT treated mice, which received a total tumor dose of 38.7 cGy-Eq, only had moist desquamation of the skin and pedal edema. These were primarily due to the differential accumulation of $^{10}\text{B}$ in tumor versus blood, skin, and muscles. In a xenograft model, nude mice carrying s.c. Harding-Passey melanoma were utilized to demonstrate the equivalence of the nude mice model with the syngeneic mice (44,46). Nude mice carrying s.c. implants of the Harding-Passey melanoma were treated with BNCT to determine tumor growth rather than survival.
BPA was injected i.p. followed by irradiation with thermal neutrons, which demonstrated a complete tumor regression for 200 days in 7 out of 8 mice compared to 21 days when mice were treated with neutron irradiation only (Table 3) (46).

We have developed a model for melanoma metastatic to the brain using nude rats. When human melanoma MRA 27 cells were implanted into the right caudate nucleus of a rat brain, they grew progressively and ultimately killing the animal (47). The tumor boron concentrations 6 hours following i.p. administration of 120 mg of $^7$-BPA in fructose complex mixture were 24.9 at which time T/Bl and T/Br ratios were 2.5 and 2.8 respectively. Thirty days following i.c. injection of $2 \times 10^5$ MRA 27 cells, BNCT was carried out (48). The MeSTs of BNCT treated rats that had received tumor doses of 5.0, 7.5, and 10.0 Gy (10.6, 15.9, and 21.1 cGy-Eq) were 170, 181, and 262 days respectively, compared to 44 days for untreated animals. In addition, the MeST for the irradiated controls that had not received BPA but only radiation doses of 2.7 or 3.6 Gy (4.8, 6.4 cGy-Eq) were 76 and 93 days respectively. Four out of 10 rats that had received tumor dose of 10.0 Gy (21.1 Gy-Eq) survived more than 300 days. Another BNCT experiment was performed with a larger numbers (n=15) of rats. Eight out of 14 rats that had received 10.0 Gy (21.1 Gy-Eq), survived > 5 months. The MeSTs of the irradiated, which had not receive BPA and untreated contols were 66 and 38 days respectively.
A large animal model for spontaneous canine oral melanoma has been used by Gavin et al. to study the biodistribution of BPA (49). \(d,l\)-BPA was administered either orally or s.c. at various dose levels of boron to 9 dogs. Three dogs had tumor boron concentrations > 20 \(\mu g/g\) at variable times following injections of BPA. In 8 dogs that had complete tissue boron determinations, T/B1 ranged from 3-32.

An ocular melanoma model of the rabbit was used by Packer et al. (50) to determine the efficacy of BNCT with BPA as a capture agent. Greene melanoma cells were deposited in the iris of rabbits and 7 days later BPA was administered orally. The average tumor \(^{10}\text{B}\) concentration was 20 \(\mu g/g\), whereas, \(^{10}\text{B}\) levels in the cornea, retina, and lens were 3-4 \(\mu g/g\). When BNCT was performed on the BPA treated rabbits, the tumor dose was 12.6 Gy (29.1 Gy-Eq), compared to a normal tissue dose of 4.8 Gy (9.4 Gy-Eq). Histological examination revealed that the tumors in 8 out of 11 BNCT treated rabbits were eradicated and in 7 of them the tumors were not visible. Although cataracts were seen in all BNCT treated rabbits, no retinal or vascular damage was seen observed.
Boronated Porphyrins.

A new group of boron compounds that appeared to have affinity for tumors is the boronated porphyrins. Boronated porphyrins such as the nido-carboranyl porphyrin, boron tetraphenyl porphyrin (BTPP), and the closo-carboranyl protoporphyrin (BOPP) have been shown to have high uptake and retention in all types of tumors tested either in vitro or in vivo. The in vivo uptake studies of BTPP in glioma and carcinomas, which had been implanted subcutaneously in mice, were 18 and 45 μg of B/g seven days following infusion of 29.2 mg/kg b.w. of BTPP. The boron concentration in the glioma increased to 22 μg/g when the amount of the injected BTPP increased to 65 mg/kg b.w. (51) (Table 4). Normal organs such as liver, spleen, and kidney also accumulated high amounts of 10B-BTPP. The selectivity of the boronated porphyrin, BOPP has been tested in mice carrying i.c. C6 glioma (52). Hundred mg/kg of BOPP were injected i.v. into C6 glioma bearing mice and 24 hours later, the maximum tumor boron concentration was 65 μg/g and persisted for up to 3 days.

A boronated protoporphyrin compound, 2,4-bis-vinyl-ono nidocarboranyl-deuteroporphyrin IX (VCDP), was tested for its selectivity in s.c. B16 (G3.12 subclone) melanoma and KHJJ carcinoma mouse models (53). KHJJ tumor boron concentrations ranged from 25–60 μg/g between 18 hours and 4
days following 275 μg VCDP/g b.w. of multiple injections (3/day for 4 days). Blood boron levels dropped from 30 to 10 μg/g between 18 hours and 6 days following administration of VCDP (Table 4). In B16 melanoma (G3.12 subclone) model, the tumor boron concentration was 30 μg/g at 4 days following administration of VCDP, compared to 15 μg/g in the blood. BNCT was carried out on the s.c. B16 melanoma bearing mice. Tumor growth delay of BNCT treated mice was found to be 50 days compared to 20 days for the irradiated controls (no VCDP). Normal organs such as liver, spleen, and kidney also accumulated large amounts of VCDP. In addition, significant but reversible thrombocytopenia was observed in the VCDP treated mice (53).

Macromolecular Agents.

Shelly et al. (54) have investigated the potential use of liposomes containing boron hydrides such as Na₂B₁₀H₁₀ or BSH as a delivery agents. The highest tumor boron concentration in BALB/c mice carrying s.c. EMT6 tumor was < 10 μg/g following i.v. administration of liposomes containing 110 μg of B as Na₂B₁₀H₁₀. Blood boron concentration was 40 μg/g, 5 hours following administration. However, when BSH containing liposomes were injected i.v., tumor boron concentrations ranged from 8.8-12 μg/g between 5 and 30 hours following administration. Blood boron
concentrations were higher than tumor, but at 24 hours T/Bl ratio was > 1. At all times following injections, the liver boron values were higher than those of the tumor. When the dimeric form of the sodium hydrides, Na₂B₂₀H₁₈, were encapsulated in liposomes and injected into BALB/c mice carrying s.c. EMT6 tumor, tumor boron concentrations were in the ≤ 20 μg/g between 5 and 30 hours following administration of the compound. Forty-eight hours post administration the tumor boron concentration was 13.6 μg/g, at which time T/Bl ratio was 3.3. Other experiments using boronated thiouracils in nude mice bearing human or murine melanoma showed T/Bl and tumor to liver ratios ranged from 2.5-4.9 and 2.0-3.2 respectively (55). These initial trials were encouraging and it seems that boron retention in the tumor was dependent upon the boron compound used. Another macromolecule are the boronated Loe density lipoproteins (LDLs), which were shown to be promising following in vitro BNCT (56), but no in vivo data have been reported to date.

DISCUSSION

The purpose of the present review was to summarize the animal models and the different boronated compounds that have been used in vivo. It seemed that BPA has potential as a capture agent for BNCT to treat s.c. melanomas. In melanotic and amelanotic melanomas BPA was found to be
selective, and was incorporated in the tumors in high concentrations (15-40 μg of B/g). There were variations of BPA tumor uptake in the animal models described. We related those variations to the different cell lines, and route of administration, and the dose. As proposed by Glass (40), it has been observed by us and Coderre et al., that the physiological L isomer of BPA accumulated 1.5-2X more than the D,L racemic mixture, suggesting that BPA accumulated in the tumor through a metabolic pathway rather than diffusion. The BNCT experiments conducted on s.c. melanomas revealed long term tumor control or even "cure". These encouraging results support the potential usefulness of BNCT for s.c. melanomas. In cutaneous melanoma, the application of BNCT is simple. The injection of the compound could be systemic or intralesional or both, to assure high $^{10}$B concentration in the tumor, and high ratios of T/B1 and tumor to muscle. Once the compound has cleared from the surrounding normal tissues neutron irradiations is carried out (39).

In glioma bearing rats, BNCT with BPA and BSSB as capture agents, showed very encouraging results with 9L-gliosarcoma cell line. However, in F98 glioma bearing rats, the results were not encouraging, even though, the tumor $^{10}$B concentration was 29 μg/g. This might be due to the radioresistance of the F98 glioma cells. However, if higher radiation doses (thermal fluences) was delivered to the tumor, better results might be seen obtainable (31). In
addition, BNCT results of the GL261 glioma model in mice, were better than the with X-irradiations. The challenge for BNCT appears when BNCT is unable to enhance the survival or "cure" small percentage of tumor bearing animals, despite having tumor $^{10}\text{B}$ concentration of $\geq 25 \mu\text{g/g}$, and T/B1 and T/Br ratios were $> 2-3$. In this case, the distribution of $^{10}\text{B}$ in the tumor, and microdistribution of $^{10}\text{B}$ in the cells are important factors to determine the actual effect of $^{10}\text{B}(n,\alpha)^{7}\text{Li}$ reaction on the tumor. Techniques such as high resolution alpha track autoradiography (65), ion microscopy and electron spectroscopic imaging combined with transmission electron microscopic microscopy are needed to determine the subcellular localization of boronated compounds in tumor cells (57). This would permit more accurate calculations of the radiation doses delivered to the tumor those obtained using an average of tumor boron concentration (58-60). The best available information on BPA and BSH indicat that they target tumors by different mechanisms (41,61), and it is not unreasonable to expect that a combination of BPA and BSH might produce a better therapeutic effect than each one alone.

To date, only one the MRA 27 cerebral melanoma model has been described (48). In this model three BNCT experiments were carried out and the results were very encouraging. However, other cerebral melanoma models are needed to fully investigate the usefulness of BNCT.
Although small animal models are important for evaluating BNCT and its capture agents, they have their own limitations. The uptake of boron compounds in spontaneous tumors compared to transplantable tumors, are more relevant to clinical situations. In transplantable tumors the vasculature is artificially induced whereas in spontaneous tumors the vasculature is not. Furthermore, spontaneous tumors have slow growth rates, and most are non-immunogenic. Therefore, it would be advantageous to study BNCT in spontaneous tumor models. Second, large animal models are important to evaluate BNCT in treating deep seated tumors, including brain tumors. In the large animal models such as dogs with spontaneous brain tumors epithermal beam is needed because epithermal neutrons have higher energy and enable them to penetrate deeper in tissues than thermal neutrons. However, using heavy water (D₂O) reduces the attenuation of thermal neutrons in deep tumors and also reduces the capture gamma dose form the 'H(n,γ)⁷H reaction (62,63). Therefore, this might overcome the expensive epithermal neutron facilities. Slatkin et al (62) have also shown that deuteration did not decrease the cytotoxicity of ¹⁰B(n,α)⁷Li reaction as in the case of γ photon irradiation. However, D₂O replacement should not exceed 30% of body water replacement, since larger amounts are toxic (64).

Successful BNCTs of cancer in animals suggest that the chance for a clinical application is high. The above
mentioned studies with BPA, BSSB, and BSH were in the right direction in increasing that chance. However, other capture agents are also needed to assure better homogeneous distribution of boron throughout the tumor which will facilitate to deliver an equal radiation doses to all tumor cells. This depends on the development of the $^{10}\text{B}$-microlocalization techniques that should facilitate such distribution studies of all boronated compounds. It should be emphasized, however, that combining BNCT with other therapeutic modalities including surgery, chemotherapy, and immunotherapy in order to eradicate all tumor cells is essential. Further studies of such combined therapy experiments are required to determine whether this is indeed the case.
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Table 21. BNCT of mice bearing glioma implanted intramuscularly using $^{10}$B-sodium pentaborate as a capture agent.$^{a,b}$

<table>
<thead>
<tr>
<th>$^{10}$B concentration (µg/g)</th>
<th>Percent of permanent regression in relation to tumor diameter (mm)$^{c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Blood</td>
<td>8-9</td>
</tr>
<tr>
<td>27</td>
<td>25</td>
</tr>
</tbody>
</table>

$^{a}$ Farr and Konikowski experiments during 1950-1960s at Brookhaven National Laboratory.

$^{b}$ 35 µg $^{10}$B/g of sodium pentaborate mixed with $\alpha$-glucose at 2:1 molar ratio were injected i.v. into mice and then were irradiated with $n_{th}$ fluence of $1.41-1.67 \times 10^{12}$ n.cm$^{-2}$

$^{c}$ In each BNCT treated group 285-542 mice were used.
Table 22. *In vivo* uptake of BPA in animals carrying s.c. melanoma

<table>
<thead>
<tr>
<th>Tumor</th>
<th>BPA injected (mg)/isomer</th>
<th>Route</th>
<th>(^{10}\text{B} \text{ Concentration (µg/g)}</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Blood</td>
<td></td>
</tr>
<tr>
<td>Harding Passey</td>
<td>12 (D,L) i.p.</td>
<td>30</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td>Harding Passey</td>
<td>6 (L) i.p.</td>
<td>15</td>
<td>3</td>
<td>41</td>
</tr>
<tr>
<td>B16 melanoma</td>
<td>15 (L) i.g.</td>
<td>20</td>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td>SK-MEL-3</td>
<td>15 (L) i.g.</td>
<td>40</td>
<td>8</td>
<td>42</td>
</tr>
<tr>
<td>Greene melanoma</td>
<td>30 (D,L) i.v.</td>
<td>14</td>
<td>7</td>
<td>43</td>
</tr>
</tbody>
</table>

* In all the above experiments mice were the animals of choice except in Green melanoma cells which were transplanted in hamsters.
Table 23. BNCT of mice carrying s.c. melanoma following administration of BPA

<table>
<thead>
<tr>
<th>Tumor</th>
<th>$^{10}$B concentration (μg/g)</th>
<th>Tumor dose (cGy)</th>
<th>Result*</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harding Passey</td>
<td>15</td>
<td>8.7</td>
<td>0%</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>17.8</td>
<td>50%</td>
<td>44</td>
</tr>
<tr>
<td>B16</td>
<td>20</td>
<td>10.2</td>
<td>0%</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>17.5</td>
<td>20%</td>
<td>45</td>
</tr>
<tr>
<td>Harding Passey</td>
<td>29</td>
<td>NR</td>
<td>88%</td>
<td>46</td>
</tr>
</tbody>
</table>

* Percent long term growth control

b NR; not recorded
Table 24. Distribution studies of boronated porphyrins in different tumor models

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Compound</th>
<th>Amount (mg/kg)</th>
<th>Route</th>
<th>Concentration (μg B/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tumor</td>
</tr>
<tr>
<td>U-87 MG</td>
<td>BTPP</td>
<td>65</td>
<td>i.p.(i)</td>
<td>22</td>
</tr>
<tr>
<td>C6</td>
<td>BOPP</td>
<td>100</td>
<td>i.v.</td>
<td>65</td>
</tr>
<tr>
<td>KHJJ</td>
<td>VCDP</td>
<td>275</td>
<td>i.p.(m)</td>
<td>45</td>
</tr>
<tr>
<td>B16 (G3.12)</td>
<td>VCDP</td>
<td>232</td>
<td>i.p.(m)</td>
<td>30</td>
</tr>
</tbody>
</table>

a All tumors were transplanted s.c. except C6 glioma cells which were transplanted intracerebrally into mice.

b BTPP, boron tetraphenyl porphyrin; BOPP, closo-carboranyl protoporphyrin; and VCDP, 2,4-bis-vinyl-o-nidocarboranyl-deuteroporphyrin IX

c i.p.(i); intraperitoneal infusion for 3 days, i.p.(m); multiple doses of intraperitoneal injections 3/day for 4 days

d The best composite ratio of tumor to blood at which time tumor boron concentration was > 20 μg/g.
Table 25. BNCT experiments performed at the BMRR on two different glioma cell lines bearing rats

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Compound</th>
<th>$^{10}$B Concentration (µg/g)</th>
<th>Dose Gy/cGy-Eqa</th>
<th>Percent dose of $^{10}$B(n,$\alpha$)'Li $^b$</th>
<th>MeST T/U $^c$</th>
<th>Percent of long term survivors $^a$</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>F98</td>
<td>BSH</td>
<td>12.8</td>
<td>8.2/16.5</td>
<td>54%</td>
<td>30/28</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td>F98</td>
<td>BSH</td>
<td>1.1</td>
<td>4.6/8.2</td>
<td>9%</td>
<td>37/24</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>F98</td>
<td>BPA</td>
<td>29.4</td>
<td>8.9/16.5</td>
<td>74%</td>
<td>37/24</td>
<td>-</td>
<td>31</td>
</tr>
<tr>
<td>F98</td>
<td>BPA</td>
<td>29.4</td>
<td>11.6/24.8</td>
<td>74%</td>
<td>59/24</td>
<td>10%</td>
<td>31</td>
</tr>
<tr>
<td>9LGL</td>
<td>BSSB</td>
<td>26.2</td>
<td>6.7/14.2</td>
<td>75%</td>
<td>60/21</td>
<td>13%</td>
<td>19</td>
</tr>
<tr>
<td>9LGL</td>
<td>BSSB</td>
<td>33.7</td>
<td>12.0/25.6</td>
<td>75%</td>
<td>&gt;305/21</td>
<td>60%</td>
<td>19</td>
</tr>
<tr>
<td>9LGL</td>
<td>BPA</td>
<td>40.0</td>
<td>8.9/19.3</td>
<td>75%</td>
<td>60/20</td>
<td>44%</td>
<td>29</td>
</tr>
<tr>
<td>9LGL</td>
<td>BPA</td>
<td>40.0</td>
<td>13.4/29.0</td>
<td>75%</td>
<td>120/20</td>
<td>50%</td>
<td>29</td>
</tr>
</tbody>
</table>

$^a$ The calculated absorbed (cGy) doses at the Brookhaven Medical Research Reactor to the tumor which includes the contributions of $^{10}$B(n,$\alpha$)'Li, $^6$N(n,p)$^13$C, fast neutrons, and photons. The effective doses (cGy-Eq) are the physical doses multiplied by the assigned relative biological effectiveness (RBE) factor of each component. A RBE of 2.3 was assumed for the $^{10}$B(n,$\alpha$)'Li reaction, 2 for $^6$N(n,p)$^13$C, fast neutrons, and 1 for photons.

$^b$ The percent radiation dose of the tumor due to the $^{10}$B(n,$\alpha$)'Li reaction.

$^c$ T: BNCT treated group, U: untreated controls

$^a$ The percent of long term survivors is defined by rats that survived > 5 months after implantation of tumor (number of animals ≥ 10).
APPENDIX A

Thermal neutron capture cross-section values of potential nuclides for neutron capture therapy

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Cross-section (σ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^3\text{He})</td>
<td>5,500</td>
</tr>
<tr>
<td>(^6\text{Li})</td>
<td>953</td>
</tr>
<tr>
<td>(^{10}\text{B})</td>
<td>3,837</td>
</tr>
<tr>
<td>(^{113}\text{Cd})</td>
<td>20,000</td>
</tr>
<tr>
<td>(^{135}\text{Xe})*</td>
<td>2,720,000</td>
</tr>
<tr>
<td>(^{149}\text{Sm})</td>
<td>41,000</td>
</tr>
<tr>
<td>(^{151}\text{Eu})</td>
<td>5,900</td>
</tr>
<tr>
<td>(^{152}\text{Gd})</td>
<td>58,000</td>
</tr>
<tr>
<td>(^{157}\text{Gd})</td>
<td>240,000</td>
</tr>
<tr>
<td>(^{199}\text{Hg})</td>
<td>2,000</td>
</tr>
<tr>
<td>(^{238}\text{U})*</td>
<td>678</td>
</tr>
<tr>
<td>(^{241}\text{Pu})*</td>
<td>1.375</td>
</tr>
<tr>
<td>(^{242}\text{Am})*</td>
<td>8,000</td>
</tr>
</tbody>
</table>

*Radioactive nuclide capture cross-section (σ) are given in barns (1 barn = 10^{-24} cm²)
APPENDIX B

Thermal neutron capture cross-section values of normal tissue element

<table>
<thead>
<tr>
<th>Element</th>
<th>Cross-section ($\sigma$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>0.332</td>
</tr>
<tr>
<td>Na</td>
<td>0.536</td>
</tr>
<tr>
<td>K</td>
<td>2.07</td>
</tr>
<tr>
<td>Mg</td>
<td>0.069</td>
</tr>
<tr>
<td>Ca</td>
<td>0.44</td>
</tr>
<tr>
<td>C</td>
<td>0.0037</td>
</tr>
<tr>
<td>N</td>
<td>1.75</td>
</tr>
<tr>
<td>P</td>
<td>0.19</td>
</tr>
<tr>
<td>O</td>
<td>&lt; 0.0002</td>
</tr>
<tr>
<td>S</td>
<td>0.52</td>
</tr>
<tr>
<td>Cl</td>
<td>33.8</td>
</tr>
<tr>
<td>Fe</td>
<td>2.62</td>
</tr>
</tbody>
</table>
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