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Medical Applications of Gadolinium and/or Boron-Labeled Pharmaceuticals

CRADA BNL-C-94-11

Final Report

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Abstract:

Boron neutron capture therapy (BNCT) is a binary treatment modality that can selectively irradiate tumor tissue. The key to effective BNCT is the preferential accumulation of ^{10}B in the tumor relative to the surrounding normal tissues. A screening procedure was developed under this CRADA that is an improvement over previously reported techniques. This method was used to evaluate the two compounds produced by BBI, the amino acid *p*-boronophenylalanine (BPA) and the sulfhydryl boroane $Na_2B_{12}H_{11}SH$ (BSH), for clinically useful accumulation in a panel of human tumor cell lines. BPA showed selective accumulation in: squamous cell carcinoma of the lung; small cell carcinoma of the lung; osteosarcoma; prostate carcinoma; and ovarian carcinoma. Of these it was decided to pursue application of BPA-based BNCT to lung tumors. BPA distribution in nude mice bearing subcutaneous human lung tumor xenografts showed very favorable results. At 3 hours post-injection, the tumor/blood boron concentration ratio was 5:1, the tumor/lung ratio was 6:1. The treatment planning software, already in use for the glioblastoma BNCT clinical trial underway at BNL, was used for simulation of a human lung tumor treatment using BNCT. Input data for this simulation included the nude mouse biodistribution data, human lung tumor CT geometry, and the same assumptions about relative biological effectiveness of the BNCT dose components currently in use for the human brain tumor trial. The results of this lung tumor simulation indicate significant sparing of normal lung compared to tumor. We conclude that the BBI product BPA has potential applications in BNCT of other tumor sites. BPA-based BNCT for human small cell carcinoma of the lung looks promising. Further studies into the radiation biology of the normal lung will be required prior to clinical BNCT for lung tumors.

Publications and presentations resulting from this CRADA:

Capala, J., Makar, M.S., Coderre, J.A. "Accumulation of boron in malignant and normal cells incubated *in vitro* with boronophenylalanine, mercaptoborane or boric acid." Radiation Research, **146**, 554-560, 1996.

J. A. Coderre, J. Capala, M. Makar and A. Z. Diaz, "Application of BNCT to other types of tumors." In: *Proceedings of the Seventh International Symposium on Neutron Capture Therapy for Cancer*, Zurich, Switzerland, September 4-7, 1996 (in press).

J. A. Coderre, J. Capala, and A. Z. Diaz, "BNCT for lung tumors?" Poster presented at the 45th Annual Meeting of the Radiation Research Society, May 3-7, 1997, Providence R.I.

A. Introduction

Boron neutron capture therapy (BNCT) is a binary treatment modality that can selectively irradiate tumor tissue. BNCT uses drugs containing a stable isotope of boron, ^{10}B, that are capable of preferentially accumulating in the tumor, which is then irradiated with low energy (thermal or epithermal) neutrons. The interaction of ^{10}B with a thermalized neutron (neutron capture) causes the ^{10}B nucleus to split, releasing an alpha (^{4}He) and a lithium (^{7}Li) particle. These products of the $^{10}B(n,\alpha)^{7}Li$ reaction have a combined path length in tissue of approximately 14 μm, or about the diameter of one or two cells, thus restricting most of the resulting ionizing energy to ^{10}B-loaded cells. The key to effective BNCT is the preferential accumulation of ^{10}B in the tumor relative to the surrounding normal tissues. The evaluation of new boron delivery agents, or the application of existing boron compounds to new tumor sites, would be facilitated by screening procedures capable of direct measurement of the boron concentration inside the cells relative to that in the incubation medium.

B. Objectives

1) Original Objectives: The original goals of this project were for the Industry Partner, Boron Biologicals, Inc. (BBI) to synthesize new compounds containing boron, gadolinium (Gd) or both, and for Brookhaven (BNL) to screen these compounds for the ability to accumulate in a panel of tumor cell lines. The new Gd compounds were to be evaluated as potential contrast enhancement agents in magnetic resonance imaging. The potential use of the Gd enhanced signal in MRI imaging to non-invasively quantify tissue boron concentrations was to be evaluated in compounds labeled with both Gd and boron.

2) Modified Objectives: BBI had difficulty with the synthesis of the compounds labeled with Gd. No Gd-labeled compounds were delivered to BNL for testing. BNL focussed on the development of an improved method for evaluation of potential BNCT compounds *in vitro*. BNL applied this method to other boron compounds produced by BBI. BBI is the only U.S. supplier of the two boron compounds approved for use by the FDA for clinical BNCT: the amino acid *p*-boronophenylalanine (BPA) and $Na_2B_{12}H_{11}SH$ (BSH). The modified objective of this CRADA was to use the screening methodology to expand the clinical applications of BPA or BSH, by testing these compounds *in vitro* with a panel of human tumor cell lines. The direct benefit to the industry partner would be an increase in the potential applications (and market) for these compounds.

C. Relationship to the DOE Mission. The Department of Energy has a long-standing research commitment to all aspects of basic and clinical research in Boron Neutron Capture Therapy (BNCT). DOE funds BNCT research at a number of national laboratories and universities. DOE has supported BNCT research in the Medical Department at Brookhaven since the initial clinical trials over 35 years ago. Based on the pre-clinical BNCT studies carried out at BNL, in September of 1994 the Brookhaven BNCT group initiated a BNCT clinical trial using epithermal neutrons at the Brookhaven Medical Research Reactor (BMRR) for treatment of patients with brain tumors. Thirty three patients have been treated as of August 1997. This is a dose escalation

study, 15 patients were treated at the first dose level, 15 at the second dose level, and 3 so far at the third dose level. The initial results are encouraging. The treatment has been shown to be safe for the normal brain and the palliative effect on the tumor is roughly equivalent to that of conventional therapies. The results obtained under this CRADA will provide the direction for additional clinical trials of BNCT for other tumors in the brain or for tumor sites outside of the brain, in particular, the lung.

D. Experimental Approach

The approach to the evaluation of new boron delivery agents, or the application of existing boron compounds to new tumor sites, was to use an *in vitro* screening procedure capable of direct measurement of the boron concentration inside the cells relative to that in the incubation medium.

- Develop a method for the evaluation of compound performance.
- Evaluate new boron compounds from BBI in a panel of tumor cell lines.
- Evaluate the FDA approved boron compounds BPA and BSH, produced by BBI, for accumulation in other tumor types.
- Promising *in vitro* results are followed by distribution studies in solid tumor xenografts carried in nude mice.
- Resources available within the BNCT research group include a fully equipped tissue culture facility, extensive veterinary services facilities, boron analysis instrumentation.

E. Results:

At the outset of this project, there was a need for an improved method of screening new compounds. The screening procedure developed under this CRADA is an improvement over previously reported techniques This method has a number of advantages over the procedures that we (and others) have used in the past for the evaluation of new boron compounds.

1) This method is rapid, several days instead of more than 2 weeks for a colony forming assay method.
2) The method requires only a small amount of boron compound (10-20 mg).
3) The method does not require ^{10}B-enriched compounds.
4) The method does not require neutron irradiations.
5) The rapid quenching of the cells minimizes the possibility of washout of the intracellular boron during processing.
6) The method can be applied to other purposes: *e.g.*, rate of uptake and efflux; uptake in other cell lines, comparison of various boron compounds.

A publication resulting from this CRADA funding (Capala et al., Radiation Research, 146, 554-560, 1996) describing this technique is attached.

1) The rapid cell filtration method: The screening method developed for this project is based on rapid filtration of cells through a three-phase system. Three liquid phases were layered in a 2.0 ml centrifuge tube: the bottom layer was 750 µL of 1M trichloroacetic acid (TCA); the middle layer, 750 µL of a mixture of silicon oil (Dow-Corning 550, density = 1.06 g/ml) and light mineral oil (density = 0.84 g/ml) in the ratio of 9 to 1; the top layer, up to 250 µL of cell culture medium containing a known number of cells. The densities of these layers were such that they did not mix when centrifuged. Centrifuging this layered system (2 min, 12,000 rpm) resulted in rapid penetration of the cells though the oil layer (which separated the cells from the extracellular, boron-containing medium) and lysis of the cells in the TCA layer. The bottom of the tube was then pierced with a 27-gauge needle and a portion of the TCA layer was extracted for boron analysis. The accumulation ratio (AR) is defined as the ratio of the intracellular to the extracellular boron concentration. See attached publication: (Capala et al., Radiation Research, 146, 554-560, 1996) for details.

2) Accumulation in normal tissues: Two of the normal tissues at risk during boron neutron capture therapy (BNCT) are the skin and the blood vessels. BPA, BSH and boric acid were tested in fibroblasts and in bovine aortic endothelial cells as models for skin and vasculature, respectively. BPA showed accumulation ratios significantly less than 1 in both proliferating fibroblasts and in confluent fibroblasts; BSH showed positive accumulation ratios in these cell lines. In bovine aortic endothelial cells the accumulation ratio for BPA and boric acid was equal, the accumulation ratio for BSH was approximately 1.7. This finding for BSH is consistent with other indirect evidence that the effectiveness of BSH-based BNCT may be due, at least in part, to damage to the tumor vasculature. See attached publication: (Capala et al., Radiation Research, 146, 554-560, 1996) for details.

3) Screening for preferential accumulation in other tumor types: The following human tumor lines were established in cell culture: lung tumors (both small cell carcinoma and squamous cell carcinoma), osteosarcoma, ovarian carcinoma, carcinoma of the prostate, breast carcinoma, glioblastoma. Uptake experiments were carried out after a 4- hour incubation of the cells with the boron compounds for all of these cell lines using the BBI products BPA and BSH at boron concentrations of 5, 10, 25, 50, 75 ppm in the medium. Boric acid was used as a reference compound. Comparison of the slopes of the BPA and boric acid lines is expressed as the accumulation ratio (AR).

BSH has not shown selective accumulation in any human tumor cell line tested. BPA has shown ARs significantly greater than 1 in several of these human cell lines: squamous cell carcinoma of the lung, AR = 2.2; small cell carcinoma of the lung, AR = 2.3; osteosarcoma, AR = 1.8; prostate carcinoma, AR = 2.4, ovarian carcinoma, AR = 1.3.

These results were presented at the *Seventh International Symposium on Neutron Capture Therapy for Cancer*, Zurich, Switzerland, September 4-7, 1996. A manuscript is included in the conference proceedings:

J. A. Coderre, J. Capala, M. Makar and A. Z. Diaz, Application of BNCT to other types of tumors. In: *Proceedings of the Seventh International Symposium on Neutron Capture Therapy for Cancer*, Zurich, Switzerland, September 4-7, 1996 (in press). Copy attached.

Based on these in vitro screening results, further studies with human lung tumors were pursued.

4) BPA distribution in human lung tumor xenografts: Xenografts of the human small cell lung carcinoma cell line were initiated in nude mice by subcutaneous inoculation of 4 x 10^6 cells in a volume of 0.1 ml. At 2 weeks after inoculation the tumors weighed about 100-200 mg. All mice received a single i.p. injection of a soluble BPA fructose complex (600 mg BPA/kg body weight). Animals were sacrificed at 1, 3, 5, and 7 hours post-injection for boron analysis: 6 mice per data point; 2 tumors per mouse. Figure 1 shows the accumulation of boron in tumor, lung and blood. At 3 hours post-injection, the tumor/blood boron concentration ratio is 5:1, the tumor/lung ratio is 6:1. These results are very encouraging.

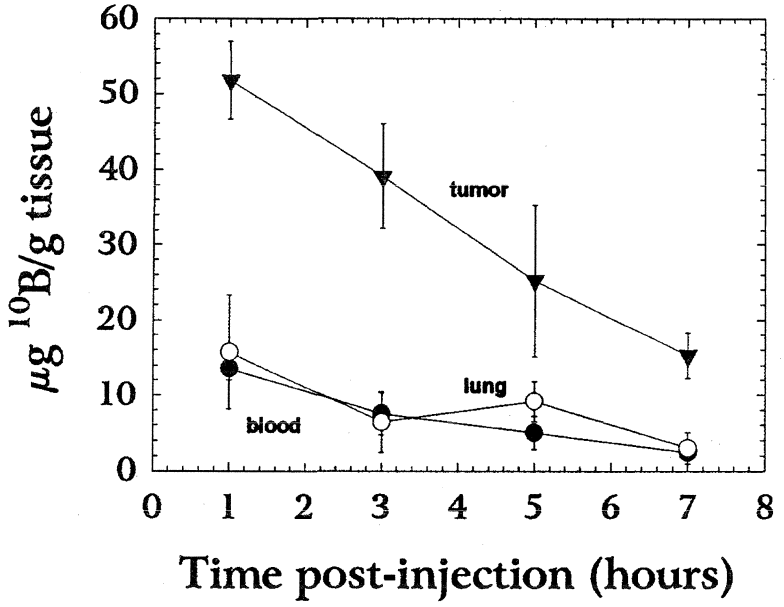

Figure 1. Accumulation of boron in human lung tumor xenografts grown subcutaneously in nude mice following a single i.p. injection of BPA.

5) BNCT treatment planning simulation: The Monte Carlo-based treatment planning software, already in use for the glioblastoma BNCT clinical trial underway at Brookhaven National Laboratory, was used for the lung tumor simulation [Nigg DW: Methods for radiation dose distribution analysis and treatment planning in boron neutron capture therapy. Int J Radiat Oncol Biol Phys 28: 1121-1134, 1994]. Human lung tumor CT data was used as input. In the absence of any experimental data on the response of lung to the high-LET BNCT dose components, the values determined for brain tumor were used for the lung tumor, and values used for skin and oral mucosa were used for the lung [assumptions: beam protons, RBE=3.2; lung tumor, CBE factor = 3.2; normal lung, CBE factor=2.5]. Figure 2 shows the isodose contours for a simulated lung tumor treatment using two fields of epithermal neutrons from the Brookhaven Medical Research Reactor (BMRR). Figure 3 shows a summary of the simulated lung tumor treatment in dose volume histogram form. The dose to the tumor is shifted significantly higher than the dose to the normal lung, indicating the potential therapeutic gain possible using BNCT for lung tumor treatments. These results in the lung are very similar to the current situation in BNCT for brain tumors: the animal biodistribution data are similar, the simulated human dose volume histograms are similar.

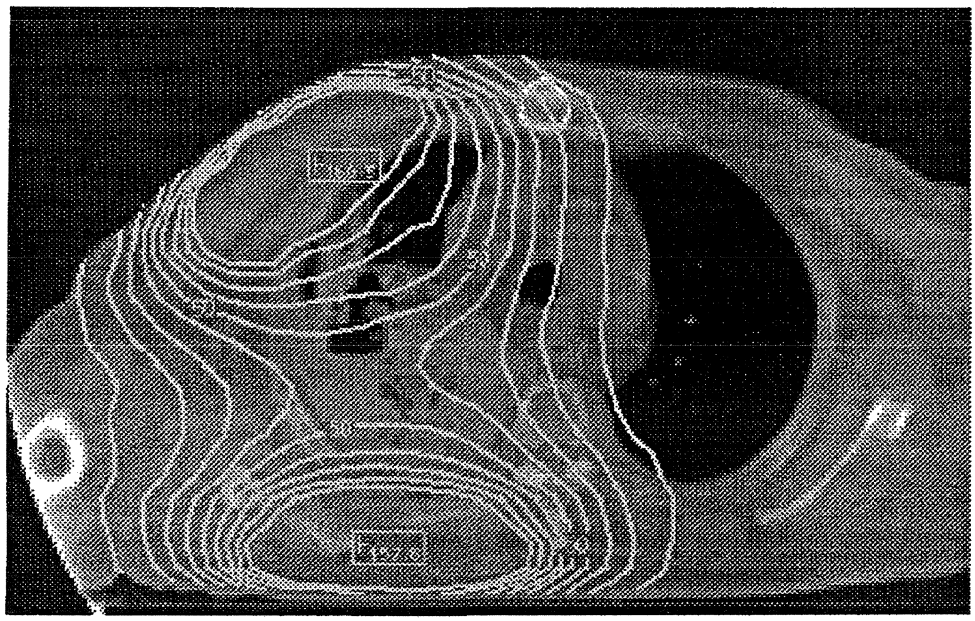

Figure 2. Isodose contours for a hypothetical BNCT treatment for a human lung tumor. Two-field irradiation with the BMRR epithermal neutron beam is shown.

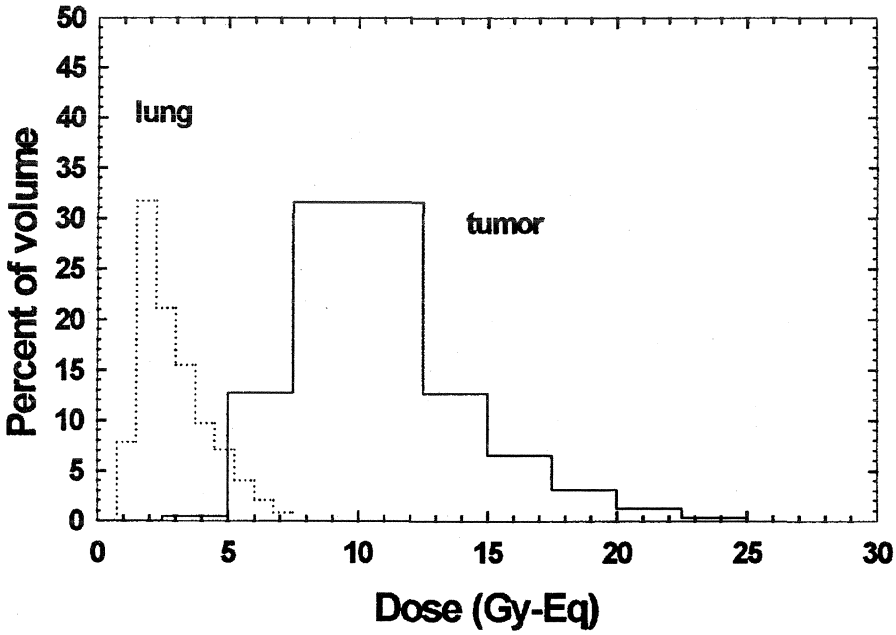

Figure 3. Dose volume histograms calculated for the hypothetical two-field BNCT treatment of a human lung tumor shown in Figure 2. The treatment planning software and assumptions about the biological effectiveness of the BNCT radiations are as used for the current brain tumor trial. Boron concentrations measured in the human lung tumor experiment shown in Figure 1 were used as input for this simulation.

We conclude that the BBI product BPA has potential applications in BNCT of other tumor sites. BPA-based BNCT for human small cell carcinoma of the lung looks promising. Further studies into the radiation biology of the normal lung will be required prior to clinical BNCT for lung tumors.

Attachments:
Publications resulting from this CRADA:

Removed for separate processing

Reprint

Capala, J., Makar, M.S., Coderre, J.A. "Accumulation of boron in malignant and normal cells incubated *in vitro* with boronophenylalanine, mercaptoborane or boric acid." Radiation Research, **146**, 554-560, 1996.

J. A. Coderre, J. Capala, M. Makar and A. Z. Diaz, "Application of BNCT to other types of tumors." In: *Proceedings of the Seventh International Symposium on Neutron Capture Therapy for Cancer*, Zurich, Switzerland, September 4-7, 1996 (in press).

Application of BNCT to Other Types of Tumors

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Introduction

The therapeutic ratio in BNCT is due primarily to the selective accumulation of the boron carrier in the tumor relative to the blood and the surrounding normal tissues. Clinical applications of BNCT have been limited to primary brain tumors and to cutaneous malignant melanoma with the two boron compounds approved for clinical use: *p*-boronophenylalanine (BPA), or the mercaptoborane $Na_2B_{12}H_{11}SH$ (BSH). Application of BNCT to other tumor sites will require the demonstration of selective accumulation of boron in tumor. If the tumor accumulation of BPA or BSH, is not sufficient, other clinical applications will only evolve as various new boron compounds are developed. The evaluation of new boron delivery agents, or the application of existing boron compounds to new tumor sites, would be facilitated by screening procedures capable of direct measurement of the boron concentration inside the cells relative to that in the incubation medium. We report here such a screening procedure and its application to the evaluation of BPA and BSH as boron carriers for a panel of human tumor cell lines.

Materials and Methods

BPA was obtained from Boron Biologicals, Inc., Raleigh NC, as the >98% ^{10}B-enriched, L-enantiomer. Boron concentrations were quantified by means of direct-current-plasma atomic emission spectroscopy (DCP-AES) [1]. Cells were maintained in humidified air at 37 °C in alpha-MEM medium supplemented with 10% fetal bovine serum (inactivated), L-glutamine (2 mM), streptomycin (100 mg/ml) and penicillin (100 IU/mL). Cells were incubated in 10 cm petri dishes for 4 hours with each boron compound at ^{10}B concentrations of 5, 10, 25, 50, 75 μg ^{10}B/ml medium, harvested by gentle scraping, concentrated by centrifugation and resuspension, and analyzed for boron content by the rapid oil filtration procedure described below. The same concentration of boron compound was maintained in the medium throughout the entire harvesting, and concentration procedure.

The screening method, which is based on rapid filtration of cells through a three-phase system, has been described [1]. Briefly, three liquid phases were layered in a 2.0 ml centrifuge tube: the bottom layer was 750 μL of 1M trichloroacetic acid (TCA); the middle layer, 750 μL of a mixture of silicon oil (Dow-Corning 550, density = 1.06 g/ml) and light mineral oil (density = 0.84 g/ml) in the ratio of 8.5 to 1; the top layer, up to 250 μL of cell culture medium containing a known number of cells. The densities of these layers were such that they did not mix when centrifuged. Centrifuging this layered system (2 min, 12,000 rpm) resulted in rapid penetration of the cells though the oil layer (which separated the cells from the extracellular, boron-containing

medium) and lysis of the cells in the TCA layer. The bottom of the tube was then pierced with a 25-gauge needle and a portion of the TCA layer was extracted for boron analysis by DCP-AES. The amount of boron in the TCA layer was linearly proportional to the number of cells applied [1].

Human lung tumor cells were grown as solid tumors in nude mice by subcutaneous injection of approximately 4 x 10^6 cells in a volume of 0.05 ml. Tumors were used for BPA uptake experiments after about 3-4 weeks of growth when they had attained a size of 100-200 mg. BPA was solubilized as the fructose complex (BPA-F) for intraperitoneal injection as previously described [2].

Results

A) Boron accumulation in tumor cell lines: The intracellular accumulation of BPA, BSH and boric acid was determined in a series of tumor cell lines. As an example, Figure 1 shows the results in human small cell carcinoma of the lung. The accumulation ratio is defined as the slope of the BPA (or BSH) line divided by the slope of the boric acid line. Table 1 summarizes the results for the entire series of tumor cell lines. All tumor cell lines were of human origin, with the exception of the rat 9L gliosarcoma. BSH showed accumulation ratios of approximately 1, indicating no preferential intracellular accumulation. The accumulation ratio for BPA in the 9L gliosarcoma was 3.2, which is in good agreement with previously published *in vivo* biodistribution data in rats bearing the intracranial 9L gliosarcoma [2].

B) BPA accumulation in lung tumor xenografts: The two human lung tumor cell lines showed accumulation ratios greater than 2 (see Table 1) after *in vitro* incubation with BPA, and were chosen for further study. Nude mice with subcutaneous human lung tumors (small cell carcinoma, n=12, or squamous cell carcinoma, n=15) received a single i.p. injection of 0.6 mg BPA/gram body weight. Tumor, blood and lung were analyzed for ^{10}B content at 1, 3, and 5 hours post-injection (Figure 2). The blood and lung data have been combined, the tumor data are graphed separately. At 3 hrs post-injection, the amount of ^{10}B in the tumor was 33 μg ^{10}B/g for the squamous cell carcinoma and 20 μg ^{10}B/g for the small cell carcinoma, the tumor/blood ^{10}B ratios were 7:1 or 4:1, and the tumor/lung ^{10}B ratios were 3:1 or 2:1, respectively.

Discussion

The oil filtration technique offers a simple *in vitro* method for direct measurement of the intracellular boron concentration following incubation with any boronated compound. This approach is well suited for the first level of screening in a compound evaluation protocol. The method has several advantages over other reported techniques that require manipulations of the cells such as washing, or indirect approaches to estimate the intracellular accumulation of boronated compounds. For example, cell survival experiments have been reported in which cells preincubated with boronated compounds were irradiated with thermal neutrons and the cell survival curves following BNCT compared to the survival curves obtained with boric acid to produce an indirect estimate of the intracellular boron concentration of the test compound [3,4]. BNCT irradiations of cells that, following preincubation with a boronated test compound, have been placed in boron-free medium can provide, at best, a crude estimate of the rate of compound

washout. Irradiation of cells with boron present in the extracellular medium [3,4] requires a correction for the dose component coming from outside the cells before the results can be interpreted. The oil filtration method described here is suitable for detailed pharmacokinetic studies, such as the kinetics of uptake into the cell, or the kinetics of efflux from the cell after removal of the boronated compound from the extracellular medium. The oil filtration method allows direct determination of the ratio of intracellular to extracellular boron concentrations without the need for washing the cells or the use of corrections for the extracellular boron trapped in cell pellets. The technique is rapid, does not require a colony-forming assay, does not require ^{10}B-enriched compounds, does not require a reactor (or a thermal neutron source), and can be carried out with small amounts (< 10 mg) of test substances. The rapid quenching of the cells minimizes the possibility of washout of the intracellular boron during processing.

These screening experiments have identified human lung tumor as a potential candidate for BPA-based BNCT. Further studies are planned to investigate the feasibility of BNCT for lung tumors using BPA. Radiobiological studies in human tumors grown in nude mice and the effects of BNCT on normal rat lung will provide the input data for computer simulations of BNCT for human lung tumors.

Acknowledgements

P. Micca, C. Fisher, and A. LoMonte provided technical assistance in the DCP-AES measurements of boron concentrations. This work was supported by the Office of Health and Environmental Research of the U.S. Department of Energy under Contract DE-AC02-76CH00016. The U.S. government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or allow others to do so, for U.S. government purposes.

References

1. Capala J, Makar MS, Coderre JA. Accumulation of boron in malignant and normal cells incubated *in vitro* with boronophenylalanine, mercaptoborane or boric acid. Radiat Res 1996; 146:554-560.

2. Coderre JA, Button TM, Micca PL, Fisher CD, Nawrocky MM, Liu HB. Neutron capture therapy of the 9L rat gliosarcoma using the *p*-boronophenylalanine-fructose complex. Int J Radiat Oncol Biol Phys 1994; 30:643-652.

3. Bond VP, Laster BH, Wielopolski L. The equal effectiveness ratio: A quantitative approach to the evaluation of compounds for boron neutron capture therapy. Radiat Res 1995; 141:287-293.

4. Fairchild RG, Kahl SB, Laster BH, Kalefezra J, Popenoe EA. *In vitro* determination of uptake, retention, distribution, biological efficacy, and toxicity of boronated compounds for neutron capture therapy - A comparison of porphyrins with sulfhydryl boron hydrides. Cancer Res 1990; 50:4860-4865.

Table 1. Intracellular accumulation of BPA or BSH relative to boric acid in a panel of human tumor cell lines (plus the rat 9L gliosarcoma).

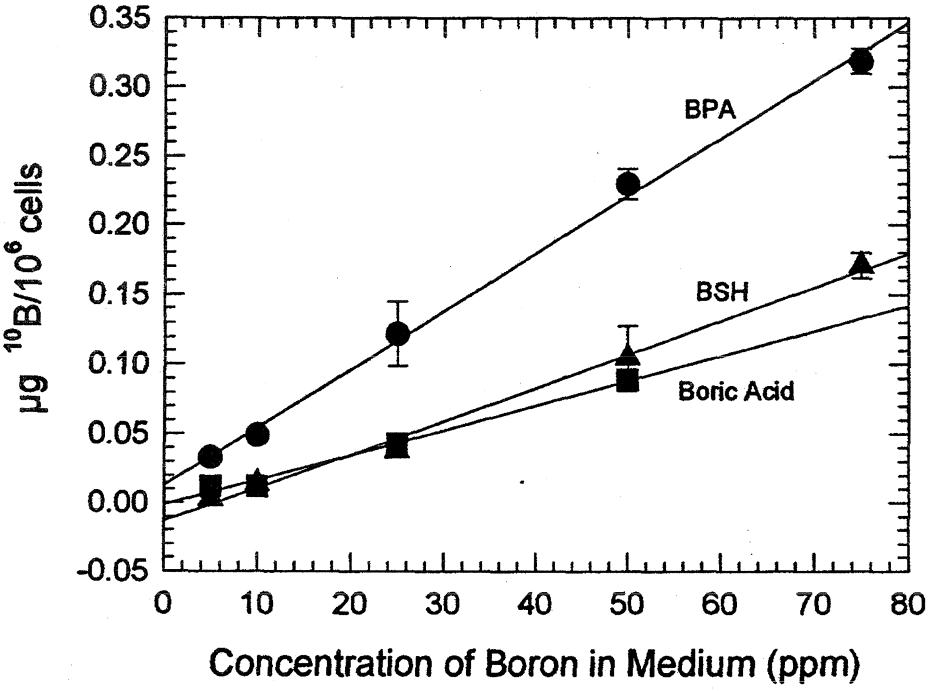
Tumor cell line	Accumulation Ratio	
	BPA	BSH
9L gliosarcoma, rat	3.2	0.9
osteosarcoma	1.8	nd*
glioblastoma	2.6	nd
breast carcinoma	0.6	0.4
prostate carcinoma	2.4	1.2
ovarian carcinoma	1.3	1.1
lung carcinoma, small cell	2.3	1.3
lung carcinoma, squamous cell	2.2	nd

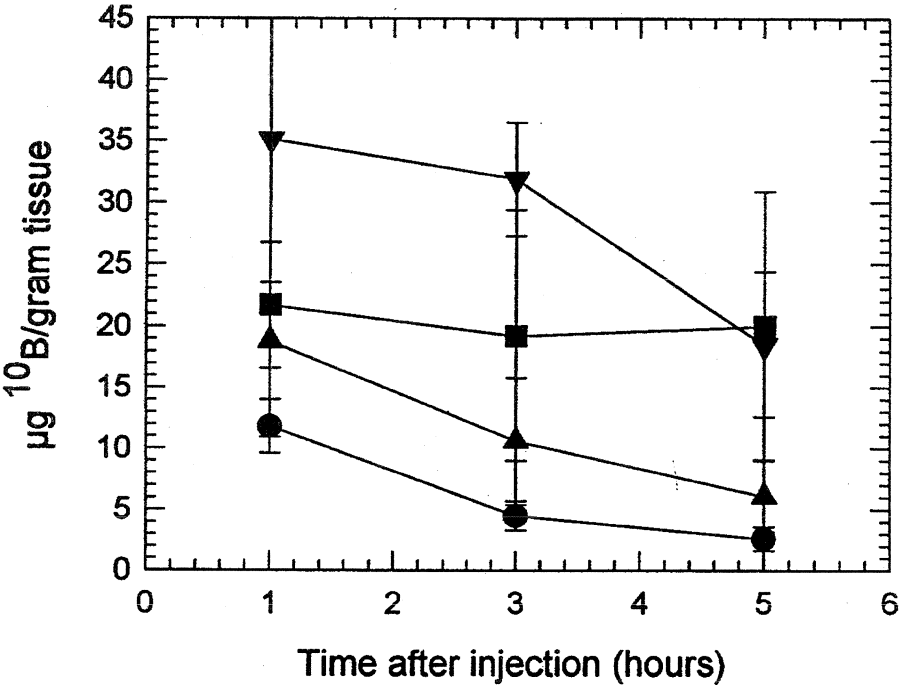
* not determined

Figure Legends

Figure 1. Accumulation of BPA, BSH and boric acid *in vitro* in human small cell carcinoma of the lung. Data points represent the mean ± sd of three independent measurements. The straight lines were fit to the data using a least squares fitting model. The accumulation ratio is defined as the ratio of the slope of the boron compound to that of boric acid.

Figure 2. Boron concentrations in squamous cell carcinoma (▼), small cell carcinoma(■), lung (▲) and blood (●) as a function of time after a single i.p. injection of BPA. Each tumor data point is the mean ± sd of 4-5 animals; lung and blood data have been combined and represent the mean of 8-10 animals.


0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
μg ^{10}B/10^{6} cells
BPA
BSH
Boric Acid
0
10
20
30
40
50
60
70
80
Concentration of Boron in Medium (ppm)


45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
µg ^{10}B/gram tissue
Time after injection (hours)