Published in final edited form as:

J Neurooncol. 2011 June ; 103(2): 175–185. doi:10.1007/s11060-010-0376-5.

CONVECTION ENHANCED DELIVERY OF CARBORANYLPORPHYRINS FOR NEUTRON CAPTURE THERAPY OF BRAIN TUMORS

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Abstract

Boron neutron capture therapy (BNCT) is based on the nuclear capture and fission reactions that occur when non-radioactive ^{10}B is irradiated with low energy thermal neutrons to produce α -particles ($^{10}\text{B}[\text{n},\alpha]{}^7\text{Li}$). Carboranylporphyrins are a class of substituted porphyrins containing multiple carborane clusters. Three of these have been evaluated in the present study: 5,10,15,20-tetra-(4-*nido*-carboranylphenyl)tetrabenzoporphyrin (H_2TBP), 5,10,15,20-tetra-(4-*nido*-carboranylphenyl)porphyrin (H_2TCP) and 5,15-di-[3,5-(*nido*-carboranylmethyl)phenyl]-porphyrin (H_2DCP). The goals of this study were two-fold. *First*, to determine the biodistribution of H_2TBP ,

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Conflicts of interest: There are no conflicts of interest.

H₂TCP and H₂DCP following intracerebral (i.c.) administration by means of short term (30 min) convection enhanced delivery (CED) or sustained delivery over 24 h by osmotic pumps to F98 glioma bearing rats. *Second*, to determine the efficacy of H₂TCP and H₂TBP as boron delivery agents for BNCT in F98 glioma bearing rats. Tumor boron concentrations immediately after i.c. osmotic pump delivery were high (36–88 µg/g) and they remained so at 24 h (62–103 µg/g). The corresponding normal brain concentrations were low (0.8–5.2 µg/g) and the blood and liver concentrations were all undetectable. Based on these data, therapy studies were initiated at the Massachusetts Institute of Technology (MIT) Research Reactor (MITRR) with H₂TCP and H₂TBP 24 h after CED or osmotic pump delivery. Mean survival times (MST) of untreated and irradiated control rats were 23±3 and 27±3 d, respectively, while animals that received H₂TCP or H₂TBP, followed by BNCT, had a MST of 35±4 d and 44±10 d, respectively, which were better than those obtained following i.v. administration of boronophenylalanine (37±3 d). However, since the tumor boron concentrations of the carboranylporphyrins were 3–5X > i.v. BPA (~25 µg/g), we had expected that the MSTs would have been greater. Histopathologic examination of brains of BNCT treated rats revealed that there were large numbers of porphyrin-laden macrophages, as well as extracellular accumulations of porphyrins indicating that the seemingly high tumor boron concentrations did not represent the true tumor cellular uptake. Our data are the first to show that carboranyl porphyrins are effective delivery agents for BNCT of an experimental brain tumor. Based on these results, we now are in the process of evaluating carboranylporphyrins that could have enhanced cellular uptake following administration and improved therapeutic efficacy.

Keywords

convection enhanced delivery; carboranylporphyrins; boron neutron capture therapy; F98 rat glioma

Introduction

Boron neutron capture therapy is based on the nuclear capture and fission reactions that occur when non-radioactive boron-10 is irradiated with neutrons of the appropriate energy to yield high energy alpha particles (⁴He) and recoiling lithium-7 (⁷Li) nuclei. Since these particles have pathlengths of approximately one cell diameter, their lethality primarily is limited to boron containing cells. BNCT, therefore, can be regarded as both a biologically and a physically targeted type of radiation therapy. Its success is dependent upon the selective delivery of sufficient amounts of ¹⁰B to cancer cells with only small amounts localized in the surrounding normal tissues. A wide variety of boron delivery agents have been synthesized [1], but only two of these currently are being used in clinical trials. The first, which has been used primarily in Japan, is sodium borocaptate or BSH, and the second is a dihydroxyboryl derivative of phenylalanine referred to as boronophenylalanine or BPA. The latter has been used in clinical trials in the United States, Europe, and more recently in Japan [2]. Following administration of either BPA or BSH by intravenous (i.v.) infusion, the tumor site is irradiated with neutrons, the source of which is a nuclear reactor. Interested readers are referred to the recently published proceedings of the Thirteenth International Congress on Neutron Capture Therapy for more detailed information relating to BNCT [3].

There has been a long-standing interest on the part of a number of research groups on the design, synthesis and biological evaluation of boron-containing porphyrins as delivery agents for BNCT [4–15]. For more information on this subject, interested readers are referred to comprehensive reviews on boronated porphyrins [16,17]. The carboranylporphyrins used in the present study belong to a class of *meso*-substituted porphyrins containing four *nido*-carborane clusters and 36 boron atoms per molecule

(37–31% boron by weight), linked to the porphyrin macrocycle *via* hydrolytically-stable carbon-carbon bonds. These compounds have been designated 5,10,15,20-tetra-(4-*nido*-carbo-ranylphenyl)tetrabenzoporphyrin (H₂TBP), 5,10,15,20-tetra-(4-*nido*-carboranylphenyl)porphyrin (H₂TCP) and 5,15-di-[3,5-(*nido*-methyl)phenyl]porphyrin (H₂DCP) and their structures are shown in Fig. 1. The synthesis and preliminary biological investigations of H₂TCP [18–21], H₂DCP [21,22] and H₂TBP [23,24] previously have been reported. Earlier studies [18–20,22–24] suggested that H₂TCP, H₂DCP and H₂TBP were promising boron delivery agents due to their low toxicity and tumor targeting properties.

Based on the reports of Ozawa et al. [25,26] and our own experience with CED [27], we decided to focus on direct intracerebral (i.c.) delivery of the carboranylporphyrins rather than systemic administration. CED is an innovative method for local drug delivery to brain tumors by which a pressure gradient, or bulk flow, is used to drive an infusate through the extracellular fluid compartment [28]. It allows delivery of the infusate to the tumor and surrounding brain at much higher concentrations than could be achieved by systemic administration. As demonstrated in both animal studies [27,29] and clinical trials [30,31], CED not only increased the delivery of both low and high molecular weight agents, but also improved their therapeutic efficacy. Initially, we carried out some preliminary studies with several carboranylporphyrins using the F98 rat glioma model [32–34] and we now present a more detailed evaluation of the biodistribution and efficacy of H₂TCP, H₂DCP and H₂TBP as potential boron delivery agents for neutron capture therapy (NCT).

Material and Methods

Synthesis of carboranylporphyrins

All *nido*-carboranylporphyrins were synthesized *via* the base for H₂TCP and H₂DCP, or the fluoride ion for H₂TBP induced degradation of the corresponding *closo*-carboranylporphyrins [35]. Therefore, all of them were mixture of stereoisomers, as shown by ¹H-NMR spectra and *ab initio* calculations, and this might contribute to their therapeutic efficacy. The percent boron by weight of these was 27% for H₂TBP and 31% for H₂TCP and H₂DCP. Two of these were converted to their zinc complexes, ZnDCP and ZnTCP, for the biodistribution studies described below. These compounds were all stable for months at physiologic temperatures, but they were light sensitive and, therefore, they were shielded from light prior to their administration.

Biodistribution of ZnDCP and ZnTCP in glioma bearing mice

Biodistribution studies were carried out in female C57Bl/6 mice (Animal Production Branch, National Cancer Institute, Frederick, MD), weighing 20–25 g, bearing the syngenic murine GL261 glioma. This tumor was induced by the intracerebral (i.c.) implantation of methylcholanthrene pellets into the brain of a C57Bl/6 mouse [36]. It is composed of poorly differentiated cells histopathologically consistent with a glioblastoma. Tumor cells were implanted subcutaneously (s.c.) into the right dorsum with an inoculum of 1.8×10^6 cells. Biodistribution studies were carried out 15 d after implantation at which time the tumor volumes were $\sim 80 \text{ mm}^3$. The zinc(II) complexes of H₂DCP and H₂TCP, ZnDCP and ZnTCP, respectively, were injected intraperitoneally (i.p.) over 8 hours in 3 doses totaling 30 mg boron/kg body weight (b.w.). Since these compounds are photosensitizers, the animals were shielded from light during the course of the experiment. In order to compare their biodistribution with that of BPA, another group of mice received a single i.p. injection of BPA at a concentration of 500 mg/kg b.w. Mice were euthanized at 24 and 48 h following the first injection and tissue and blood samples were taken for boron determinations by means of direct current plasma-atomic emission spectroscopy (DCP-AES), as previously described by us [37]. All animal studies were done in accordance with the *Guide for the*

Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996) and the protocol was approved by the Institutional Laboratory Care and Use Committee of The Ohio State University.

F98 rat glioma model

The F98 rat glioma (#CRL-2397, American Type Culture Collection, Manassas, VA) was derived from an undifferentiated neoplasm transplacentally induced in the progeny of a pregnant CD Fischer rat by the i.v. administration of N-ethyl-N-nitrosourea [38]. It has been propagated *in vitro* and *in vivo* since 1971 and, as described in a recent review [39], the F98 glioma has been used in a wide variety of studies in experimental neuro-oncology. This tumor has an infiltrative pattern of growth within the brain, and an i.c. inoculum of as few as ten cells can be fatal. In the present study, F98 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT), 100 units/ml penicillin, 100 µg/mL streptomycin and 2 mM L-glutamine. Fischer rats (Animal Production Branch, National Cancer Institute, Frederick, MD), weighing 220–240 g, were used in these studies. A stereotactic implantation procedure, which has been described in detail elsewhere, was employed [40]. Briefly, rats were sedated by i.p. administration of a 1.2:1 mixture of ketamine/xylazine at a dose of 120 mg of ketamine/20 mg xylazine/kg body weight (b.w.). Following this, a small burr hole was drilled into the calvarium and a plastic screw with an entry port (Arrow Machine Manufacturing, Inc., Richmond, VA) was embedded into the skull. F98 cells at a concentration of either 10^3 for therapy studies or 10^5 cells for biodistribution studies were injected stereotactically into the right caudate nucleus over 10–15 sec through a small entry port of the plastic screw. Following this, the entry port was filled with bone wax immediately after withdrawal of the needle, and the operative field was flushed with betadine before the scalp incision was closed with a single sterilized clip. Eleven to 13 d after tumor implantation of 10^5 cells, when clinical signs of a progressively growing i.c. tumor were evident, biodistribution studies were initiated.

Biodistribution studies in F98 glioma bearing rats following convection-enhanced delivery (CED) of carboranylporphyrins

Since the zinc(II) complexes of carboranylporphyrins have lower cellular uptake than the free base porphyrins, studies then were carried out with H₂DCP and H₂TCP. In order to increase tumor uptake and decrease uptake by extracranial organs and tissues, these were administered i.c. by means of CED or Alzet pump infusion. Eleven to 13 d after tumor implantation, when signs of a progressively growing i.c. tumor were evident (weight loss, lethargy, hunching, and ataxia), biodistribution studies were initiated, at which time the animals had tumors weighing ~90–180 mg. In contrast to the biodistribution studies carried out in mice that had received the test porphyrins by i.p. injection, it was not necessary to shield these rats from light. For short term CED of the boronated porphyrins, a plastic cannula was inserted into the entry port of the plastic screw and then advanced 5 mm below the dura into the tumor. The boronated porphyrins were diluted with phosphate buffered saline (PBS), pH 7.4, to yield a concentration of 0.1–0.2 mg B/10 µl. As described by us in detail elsewhere [40], these compounds were administered by CED at rate 3.3 µl/min for 30 min using a syringe pump (Harvard Apparatus Co, Cambridge, MA). Biodistribution was determined immediately after and 24 and 48 h after termination of CED. Animals were euthanized by an overdose of halothane following which tumors and normal tissues, consisting of brain, blood, liver, kidney and muscle, were removed and weighed. Uptake of boron was quantified by means of DCP-AES [37].

Biodistribution of carboranylporphyrins following i.c. infusion by osmotic pumps

Alzet osmotic pumps (model #2001 D) and brain infusion kits (rigid stainless-steel cannula, 7 mm 28 gauge) were assembled and filled with 200 μ l of the test carboranylporphyrins. The pumps were stored in the dark in a sterile solution of 0.9% saline at 37°C for 24 h prior to their use. Fourteen days after tumor implantation, the rats were anesthetized again and the scalp reopened. The pumps were implanted s.c. between the scapulae. The infusion cannula and needle were inserted stereotactically into the entry port of the plastic screw and the needle was advanced 5 mm below the dura into the tumor of glioma bearing rats. H₂DCP, H₂TCP or H₂TBP at varying doses, ranging from 0.2 – 1.0 mg, were delivered in volume of 200 μ l at a constant flow rate of 8 μ l/h over 24 h following which the animals were euthanized immediately (t=0) or 24 or 48 h later. Boron concentrations were determined in samples of tumor, brain, blood, liver, spleen, kidneys, lungs and heart by means of DCP-AES.

Therapy experiments

BNCT was performed 14 d following stereotactic implantation of 10³ F98 glioma cells. Rats were transported to the Nuclear Reactor Laboratory at the Massachusetts Institute of Technology (MIT). Based on the results of the biodistribution study, a total of either 0.2 mg of the compound was administered by CED for 30 min or 0.5 mg of the compound was infused for 24 h by Alzet pumps. The animals were then randomized on the basis of weight into experimental groups of 9–10 animals each as follows: Group 1, H₂TCP, administered by Alzet pumps and BNCT; Group 2, H₂TBP and BNCT; Group 3, H₂TBC by CED and BNCT; Group 4, CED of H₂TBP + i.v. BPA and BNCT; Group 5, i.v. BPA, followed 2.5 h later by BNCT; Group 6, CED of saline and neutron irradiation; Group 7, unirradiated controls that received H₂TCP by CED. BNCT was initiated 24 h after termination of CED or infusion and 2.5 h after i.v. administration of ¹⁰B enriched BPA at a dose of 500 mg/kg b.w. (Ryscor Science, Inc., Raleigh, NC). All irradiated rats were anesthetized with a mixture of ketamine and xylazine. BNCT was carried out at the MITR-II reactor in the M011 irradiation facility, which produces a beam of thermal neutrons of high purity and intensity with no measurable fast neutron component. Two rats at a time were positioned in a ⁶Li enriched polyethylene box that provided whole body shielding from the thermal neutrons during an irradiation. The animals' heads were aligned in the middle of a 13×2 cm² aperture, machined in the box lid, which served as the beam delimiter. Four fission counters, located at the periphery of the 15 cm circular field automatically controlled beam delivery and provided real time data on the relative neutron fluence during an irradiation.

Dosimetry

After completion of BNCT, the animals were held at MIT for ~ 3 d to allow induced radioactivity to decay before they were returned to The Ohio State University in Columbus, OH for clinical monitoring. Dosimetric measurements were performed using bare gold foils and a graphite walled ionization chamber ($V = 0.1 \text{ cm}^3$) flushed with reagent grade CO₂ on both dead rats and phantoms made from type 6 nylon [41]. The measured dose rates in brain (2.2% nitrogen by weight), normalized to the reactor operating at a power of 5 MW, were 18.5 cGy/min for photons, 7.7 cGy/min for thermal neutrons from the nitrogen capture reaction and 3.4 cGy/min per $\mu\text{g } ^{10}\text{B}$ in tissues. For dosimetric calculations, boron concentrations were determined in tumor, normal brain, liver and blood in a separate group of animals 24 h after CED of carboranylporphyrins and 2.5 h after i.v. injection of BPA. Animal irradiations were performed with the reactor operating at a power between 4.0 and 4.8 MW, and these took approximately 7 min to deliver a thermal neutron fluence of $2.64 \times 10^{12} \text{ n.cm}^{-2}$ to complement previous dose prescriptions [42].

Monitoring of clinical status and neuropathologic evaluation

All animals were weighed three times per week and their clinical status was evaluated at the same time. Once the animals had progressively growing tumors, as evidenced by the combination of sustained weight loss, ataxia and peri-orbital hemorrhage, they were euthanized in order to minimize discomfort. Survival times were determined by adding one day to the time between tumor implantation and euthanization. The brains of all animals in the therapy studies were removed after death, fixed in 10% buffered formalin, and then cut coronally at the level of the optic chiasm and 2 mm anterior and posterior to it. Tissue sections through the tumor were embedded in paraffin, cut at 4 μ m, stained with hematoxylin and eosin (H&E), and examined microscopically to assess the histopathologic changes.

Statistical evaluation

The means and standard deviations (SD) were computed for boron concentrations in all the major organs of C57B1/6 mice bearing s.c implants of the GL261 murine glioma described in Table 1, and for the tumor, brain, and blood of the F98 glioma rats described in Table 2. Tumor: brain and tumor:blood boron concentration ratios were calculated for each group. To study the effects of BNCT on survival of F98 glioma bearing rats, the mean survival time (MST), standard error (SE), and median survival time (MeST) were calculated for each group using the Kaplan-Meier estimate [43]. Kaplan-Meier curves also were plotted for all groups. An overall log rank test was performed to test for equality of survival curves over the groups. Wald tests were used for individual comparisons between two groups, with a Bonferroni method of adjustment for multiple comparisons [44]. Since there was no censoring of the survival data, a two sample t test also was used to compare MSTs in order to increase the power of the test. The percent increased life span (% ILS) was determined from the following equation:

$$\% \text{ ILS} = \frac{\text{MeST (or MST)}_t - \text{MeST (or MST)}_u}{\text{MeST (or MST)}_u} \times 100$$

where “t” designates treated and “u” designates untreated animals

Results

Biodistribution after systemic injection of carboranylporphyrins

Following i.p. injection of either ZnDCP or ZnTCP (30 mg/kg b.w.) to GL261 tumor bearing mice, one of five animals in each group died within the first 24 h and the others demonstrated reduced activity, suggesting that these doses of the zinc complexes were toxic. In contrast, H₂DCP and H₂TCP previously were shown to have very low toxicity [22,23]. The boron uptake in tumor and normal tissues at 24 and 48 h following the third i.p. injection are summarized in Table 1. The tumor boron concentrations at 48 h were 7.6 $\pm$ 1.9 and 5.2 $\pm$ 0.3 μ g/g of tissue for ZnDCP and ZnTCP, respectively, compared to 1.2 $\pm$ 0.4 and 0.5 $\pm$ 0.1 μ g/g in normal brain. In contrast, the 48 h boron concentrations for ZnDPT and ZnTCP in the liver were 154.9 $\pm$ 28.4 and 46.3 $\pm$ 11.5 μ g/g, respectively, and the spleen concentrations were 138.5 $\pm$ 60.1 and 114.0 $\pm$ 99.1 μ g/g with marked animal to animal variability. This possibly reflected differences in the excretion of the compounds and their metabolites. The corresponding blood concentrations were 15.9 $\pm$ 3.5 and 11.3 $\pm$ 2.5 μ g/ml, respectively. Based on these data, a decision was made to administer the carboranylporphyrins i.c. either by short term (30 min) CED or by a 24 h infusion using Alzet pumps. In contrast to the high extracranial tissue uptake of ZnDCP and ZnTCP, BPA had very low concentrations in all organs except for tumor (12.5 $\pm$ 4.1 μ g/g).

Biodistribution following delivery of carboranylporphyrins by either CED or Alzet pumps

As shown in Tables 2 and 3, administration by either Alzet pumps or CED resulted in higher tumor and lower normal tissue boron concentrations, and improved tumor:brain (T:Br) and tumor: blood (T:Bl) ratios compared to those obtained following i.p. injection (Table 1). The boron concentrations in tumor and selected normal tissues in F98 glioma bearing rats following Alzet pump delivery of H₂TCP at doses of 0.2, 0.5 or 1.0 mg/200 μ l, delivered i.c. into F98 glioma bearing rats are summarized in Table 2. Tumor boron concentrations were clearly dose dependent immediately following infusion and at 24 and 48 h later. The highest tumor boron concentrations were observed at 24 h following administration of escalating doses of H₂TCP (24.6, 102.9 and 149.6 μ g/g), but these were all within 1 SD of the concentrations that were observed immediately following infusion, indicating that there was retention and possibly some accretion of boron within the tumor. In contrast, the tumor concentrations had fallen quite precipitously by 48 h to values that were one-third of their peak value, while the normal brain and blood values were low at the three time points (0.2 to 3.3 μ g/g) except for those animals that received the 1.0 mg dose. Their normal brain values were higher at 0, 25 and 48 h (6.5, 10.1 and 3.0 μ g/g, respectively). Although the highest T:Br ratio was seen with the dose of 0.5 mg at 48 h after administration, the tumor boron concentration was low (16.8 μ g/g). Therefore, the most favorable values of tumor boron (102.9 μ g/g) and T:Br ratios for H₂TCP were seen with this dose at 24 h. In contrast, the tumor boron values for H₂TBP and H₂DCP at the same time and dose were 61.9 $\pm$ 16.4 and 35.6 $\pm$ 9.0 μ g/g, respectively (Table 3). The highest tumor boron concentration was seen 24 h following short term (30 min) CED of H₂TBP (140.3 $\pm$ 70.9 μ g), but as indicated by the large SD, there was considerable animal to animal variability. It should be noted that the T:Br boron concentration ratios of the carboranylporphyrins were markedly increased over those that we have observed following either intravenous (i.v) or intracarotid (i.c.) administration of BPA and BSH [40].

Boron neutron capture therapy

The carboranylporphyrins were administered 14 days following tumor implantation and BNCT was carried out 24 h after termination of delivery. This was well tolerated and weight loss in the first week was less than 20%, following which the animals regained their weight. The estimated physical radiation doses delivered to tumor, brain and blood were calculated according to boron concentrations summarized in Table 3. In contrast, following i.c. administration of the carboranylporphyrins no boron was detected in samples of liver, spleen, kidneys, lungs and heart (data not shown). The highest physical radiation doses delivered to the tumor were 39.1 Gy for H₂TBP by CED and 29.2 Gy for H₂TCP by Alzet pump infusion. The corresponding normal brain doses were 2.3 and 2.9 Gy (Table 3). The survival data following BNCT are summarized in Table 4, and Kaplan Meier survival plots are shown in Fig 2, 3 and 4. The MSTs were 35.0 $\pm$ 3.7 d and 43.8 $\pm$ 10 d, respectively, for rats that received H₂TCP and H₂TBP by Alzet pump (Table 4). Animals that received H₂TBP had significantly longer survival times than those that received H₂DCP ($p<0.017$). Further studies were carried out using H₂TBP at a dose of 0.2 mg, administered by CED, either alone or in combination with i.v. BPA. The corresponding MSTs were 33.8 $\pm$ 3.1 d and 42.8 $\pm$ 9.0 d, respectively (Table 4 and Fig. 3). As shown in Fig. 4, there were more long term survivors among rats that received the combination of i.v. BPA and H₂TBP, compared those that received H₂TBP alone ($p<0.001$). The animals that received of H₂TBP by Alzet pump (Table 4 and Fig. 5) had longer MSTs than those that received it by CED (43.8 *versus* 33.8 d), demonstrating that Alzet pump delivery was more effective than CED ($p<0.013$). However, it must be recognized that statistical significance is not necessarily the same as biological (or clinical) significance. If the MSTs of animals that received i.v. BPA are compared to those that received H₂TBP using a two sample t test, then they were not significantly different from one another ($p=0.38$). The highest %ILS (87.2%) was observed

among those animals that received H₂TBP by osmotic pumps and this was equivalent to the %ILS of animals that received the combination of H₂TBP by CED and i.v. BPA (82.8%).

Neuropathologic evaluation

The most notable histopathologic finding was the presence of porphyrin laden macrophages and extracellular deposits of porphyrins in the tumors of many of the rats that received either H₂TCP or H₂TBP by either CED or Alzet pump infusion (Fig. 5A and B). As shown in Fig. 5B, this material was bright orange in color and was easily discernible on H&E stained sections of the tumor. In most instances, the appearance of the tumor in both treated and untreated animals was similar to that previously described by us [39]. Briefly, the tumor was composed of cells that varied in size and shape from ovoid to fusiform, sometimes displaying a whorled pattern of growth. Frequently, there were microscopic deposits of tumor cells invading the surrounding white matter and a central zone of necrosis. There were focal infiltrates of polymorphonuclear leukocytes in the tumors of several untreated and irradiate control animals, suggesting that there had been bacterial contamination at the time of their implantation. In these cases, the tumors were much smaller than those usually seen.

Discussion

Biodistribution studies demonstrated that high tumor boron concentrations could be achieved by either short term (30 min) CED or a 24 h infusion via Alzet osmotic pumps. Based on these observations, therapy studies were carried out using H₂TCP and H₂TBP as boron delivery agents. The longest MST (43.8±10.0 d) was obtained using H₂TBP, compared to 35.0±3.7 d for H₂TCP, both delivered by Alzet pumps, and 39.8±1.6 d for i.v. BPA. Our results are in agreement with those recently reported by Jori et al. [45] who observed that the tumor boron concentrations following i.t. administration of H₂TCP to C57B1/6 mice bearing s.c. implants of the B16 melanoma were 10× greater than those observed following i.v. injection (60 *versus* 6 µg/g). However, following BNCT the tumor growth delay was practically identical for both groups and these were significantly less than those observed following i.p. administration of 200 mg/kg of BPA. Although the tumor boron concentrations for the latter animals was not determined, published data would suggest that it could have been in the range of 10–20 µg/g [46–48]. Similarly, Shibata et al. [49] have reported a very modest increase in the MST of 9L gliosarcoma bearing rats that received a BSH-porphyrin compound designated STA-BX900 (16.2 *versus* 14.8 d and 12.8 d for irradiated and untreated control animals, respectively). Therefore, our data are the first to show that carboranylporphyrins can be effective delivery agent for NCT of an experimental brain tumor.

However, since in our study the tumor boron concentrations were so much higher following either CED or infusion by Alzet pumps than those obtained with other boron compounds, it was puzzling why the survival data were similar to those obtained with BPA, which had a much lower tumor boron concentration. Histopathologic examination of brains from tumor bearing, BNCT treated rats revealed that in most animals there were large numbers of porphyrin containing macrophages (Fig. 5A and B) indicating that tumor cell uptake was much lower than would have been predicted, based on the tumor boron values, as determined by DCP-AES. This may provide a partial explanation as to why the survival data were similar to those obtained with BPA, despite the seemingly high “tumor” boron concentrations. One possible explanation for the high uptake of the carboranylporphyrins by macrophages and the relatively low uptake by tumor cells could be related to their propensity to form aggregates when high concentrations are solubilized in water. This problem could be obviated by initially dissolving them in dimethylsulfoxide (DMSO) and then diluting it down to 1% DMSO. Theoretically, bystander killing might occur if the ¹⁰B containing macrophages were adjacent to tumor cells. However, the potential lethality of the

alpha particles produced as a result of the $^{10}\text{B}(\text{n},\alpha)^7\text{Li}$ capture reaction would be much less than if they were produced within the tumor cells. Although CED has been effective in improving the distribution of a variety of agents in rats with brain tumors, its effectiveness in human has been much more problematic [30,31]. However, direct i.c. delivery of therapeutic agents, which bypass the BBB, results in much higher concentrations in the brain tumor and concomitantly lower concentrations in extracranial sites thereby reducing systemic toxicity. The major problem associated with any therapeutic study carried out using rodent brain tumor models is their relevancy to the treatment of patients with brain tumors. The rat brain weighs approximately 1.2 gms and a human brain weighs 1,200 gms, a thousand-fold difference. However, one thing that is reasonably certain, is that if it doesn't work in rats, it probably will not work in humans.

Ozawa et al. [25,26], as well as we [32–34], have observed that CED and Alzet pump infusion resulted in significantly higher tumor and lower normal brain boron concentrations than those obtained following systemic administration. They were the first to report that CED significantly increased the tumor uptake of two boronated porphyrins, designated TABP-1 and BOPP, although no therapy results were reported [29,30]. Although CED of the carboranylporphyrins has solved the problem of high extracranial tissue uptake by the liver and spleen, the seemingly high tumor boron values do not seem to accurately reflect the true intracellular uptake of the compounds, as shown for the first time by us in the present study. Direct i.c. administration appears to be the preferred route of administration for the presently available carboranylporphyrins. However, their enhanced intracellular localization and microdistribution depend on the physico-chemical properties of the carboranylporphyrins, and one of our goals is to synthesize compounds that will have enhanced tumor cell uptake. In addition, it would be highly advantageous to have tumor selective compounds that readily cross the blood-brain barrier and that could be administered systemically and obtain high tumor and low normal brain and extracranial tissue and blood concentrations. It is noteworthy that *in vitro* studies on the cellular uptake of H_2TCP studies with the murine B16 melanoma [19,45] cells and H_2TBP with human T98 glioblastoma cells [23] demonstrated intracellular fluorescence of cells that had been incubated with these compounds. Despite their tetra-anionic nature, they were able to penetrate plasma membranes to a certain extent, and may not have formed aggregates, which resulted in intense fluorescence.

In the future, we plan to carry out studies using secondary ion mass spectrometry (SIMS) [50] to obtain quantitative data on the boron concentrations of individual tumor cells in tissue sections. This method has been used by us to determine the cellular and subcellular localization of BPA [51], BSH [52] and carboranyl nucleosides [53]. Our data provide a cautionary note that high “tumor” boron concentrations do not necessarily mean that the boron delivery agent is localized *within* tumor cells. The challenge is to synthesize and evaluate non-toxic, carboranylporphyrins that attain high *in vivo* tumor cell uptake following either systemic injection or direct i.c. administration. Based on the studies of Ozawa et al., Jori, et al. and ourselves, it can be concluded that these compounds are a promising class of boron delivery agents that warrant further investigation.

Acknowledgments

This paper is dedicated to Professor Otto Harling in recognition of his outstanding contributions to the field of BNCT research, and more specifically to his vision and foresight to make the Massachusetts Institute of Technology Research Reactor one of the leading facilities in the world to carry out BNCT studies. We thank Ms. Michelle Van Fossen for expert secretarial assistance in the preparation of this manuscript and Dr. Michael Pennell, Division of Biostatistics, OSU, College of Public Health, for his helpful comments relating to statistical evaluation of the data. The studies described in this report were supported by N.I.H. grants R01 CA098902 (M.G.H.V.) and R01 CA098945 (R.F.B.), and the United States Department of Energy through the program of Innovations in Nuclear Infrastructure and Education, Office of Nuclear Energy, Science and Technology (contract no. DE-

FG07-02ID14420DE-FG07-02, K14420), and the Office of Environmental and Biological Research (contract no. DE-FG02-02ER63358) (K.J.R. and P.J.B.).

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Figure 1.

Chemical structures of the carboranylporphyrins used in this study: A. di[3,5-(*nido*-carboranylmethyl)phenyl]porphyrin (H₂DCP), and its zinc(II) complex (ZnDCP), B. tetra(4-*nido*-carboranylphenyl)porphyrin (H₂TCP), and C. tetra(4-*nido*-carboranylphenyl)tetra-benzo-porphyrin, H₂TBP (C).

Figure 2. Kaplan-Meier survival curves for F98 glioma bearing rats following Alzet pump delivery of H₂TCP or H₂TBP followed by BNCT. Survival times in days after implantation have been plotted for untreated animals (●), radiation controls (○), H₂TCP (▲) or H₂TBP (□).

Figure 3.

Kaplan –Meier survival curves for F98 glioma bearing following CED of H₂TBP followed by BNCT. Survival times in days after implantation have been plotted for untreated animals (●), irradiation controls (○), H₂TBP (■), i.v. BPA (◇) and H₂TBP plus BPA (◆).

Figure 4.

Kaplan-Meier survival curves for F98 glioma bearing rats following either CED or Alzet pump delivery of H₂TBP followed by BNCT. Survival times in days after implantation have been plotted for untreated animals (●), radiation controls (○), CED delivery of H₂TBP (■), Alzet pump delivery of H₂TBP (□).

Figure 5.

Low power photomicrograph from the brain of a BNCT treated, F98 glioma bearing rat following administration of H₂TBP by CED. There are large numbers of porphyrin laden macrophages (arrows). B. High power photomicrograph showing porphyrin laden macrophages. These photos were taken at the time of death of the animal at which time they had progressively growing brain tumors.

Table 1

Boron biodistributions in tumor and normal tissue following i.p. injection of either ZnDCP or ZnTCP in mice bearing s.c. implants of the GL261 murine glioma*

Agent ^a	Time (hrs)	Boron concentrations $\pm$ SD ($\mu\text{g/g}$) ^b						Ratios ^c	
		Tumor	Brain	Blood	Liver	Spleen	Muscle	T/Br	T/Bl
ZnDCP	24	8.3 $\pm$ 2.7	1.7 $\pm$ 0.8	44.5 $\pm$ 12.6	94.7 $\pm$ 7.3	109.4 $\pm$ 10.0	10.9 $\pm$ 6.6	4.8	0.2
	48	7.6 $\pm$ 1.9	1.2 $\pm$ 0.4	15.9 $\pm$ 3.5	154.9 $\pm$ 28.4	138.5 $\pm$ 60.1	6.7 $\pm$ 3.2	6.3	0.5
ZnTCP	24	2.0 $\pm$ 0.1	0.7 $\pm$ 0.6	25.5 $\pm$ 31.1	43.1 $\pm$ 4.8	115.9 $\pm$ 73.6	1.5 $\pm$ 0.2	2.6	0.1
	48	5.2 $\pm$ 0.3	0.5 $\pm$ 0.1	11.3 $\pm$ 2.5	46.3 $\pm$ 11.5	114.0 $\pm$ 99.1	3.0 $\pm$ 0.1	10.4	0.5
BPA	2.5	12.5 $\pm$ 4.1	1.0 $\pm$ 0.2	2.6 $\pm$ 0.8	1.1 $\pm$ 0.2	3.2 $\pm$ 1.9	1.1 $\pm$ 0.6	12.5	4.8

^aZnDCP or ZnTCP was administered by 3 i.p. injections, administered over 8 hours to C57BL/6 mice bearing s.c. implants of the GL261 murine glioma. Animals were euthanized at 24 and 48 hrs following the third injection and tissues and blood samples were removed for boron determinations. BPA was administered i.p. at a dose of 25 mg b/kg b.w. and mice were euthanized 2.5 h later.

^bBoron concentrations were determined by DCP-AES. The mean boron values ($\mu\text{g/g}$ wt of tissue) $\pm$ the standard deviation are shown for groups of 4 animals.

^cT:Br indicates the tumor to normal brain ratio and T:Bl indicates the tumor to blood ratio.

Table 2

Boron biodistribution in F98 glioma bearing rats following administration of escalating doses of H₂TCP by osmotic pumps*

Dose (mg/200µl)	Time (hrs)	Boron concentration ±SD (µg B/g) ^a			Ratios ^b	
		Tumor	Brain	Blood	T:Br	T:Bl
0.2	0	18.7 ± 8.7	1.3 ± 1.0	0.3 ± 0.3	14.0	64.7
	24	24.6 ± 5.1	2.9 ± 2.7	0.5 ± 0.7	8.5	54.1
	48	9.0 ± 2.7	0.5 ± 0.5	0.3 ± 0.1	18.0	31.9
0.5	0	88.4 ± 20.2	2.0 ± 0.9	0.4 ± 0.2	45.2	207.6
	24	102.9 ± 26.3	3.3 ± 4.1	0.5 ± 0.1	31.3	196.0
	48	16.8 ± 8.4	0.3 ± 0.1	0.3 ± 0.1	49.9	50.5
1.0	0	123.3 ± 61.1	6.5 ± 3.8	0.2 ± 0.1	18.9	593.3
	24	149.6 ± 55.5	10.1 ± 12.6	0.2 ± 0.1	14.8	708.8
	48	34.8 ± 10.8	3.0 ± 1.9	0.3 ± 0.1	11.5	105.5

^aH₂TCP (0.2 – 1.0 mg) was administered by means of Alzet osmotic pumps (#2001D) over 24 hours to Fischer rats bearing i.c. implants of the F98 glioma. Animals were euthanized at different time point (0, 24, 48 hrs after termination) and tissues and blood samples were removed for boron determinations by DCP-AES. The values indicated are means ± SD for groups of 4–5 rats.

^bT:Br indicates the tumor to normal brain ratio of the ipsilateral cerebral hemisphere and T:Bl indicates the tumor to blood ratio.

Table 3

Boron concentrations and physical radiation doses delivered in F98 glioma bearing rats 24 hrs following administration of carboranylporphyrins by osmotic pumps or CED*

Agent/Route ^a	Boron concentrations (μg/g) ^b			Physical radiation dose ^c		
	Tumor	Brain [†]	Blood	Tumor	Brain	Blood
H ₂ TCP/pump	102.9 ± 26.3	3.3 ± 4.1	0.7 ± 0.9	25.4	2.5	1.9
H ₂ TBP/pump	61.9 ± 16.4	0.8 ± 0.9	0.2 ± 0.4	16.0	1.9	1.8
H ₂ DCP/pump ^d	35.6 ± 9.0	5.2 ± 6.8	0.5 ± 0.5	9.9	2.9	1.9
H ₂ TBP/CED	140.3 ± 70.9	0.8 ± 0.3	0.2 ± 0.1	34.0	1.9	1.8

^a H₂DCP, H₂TCD and H₂TBP at doses of 0.5 mg were administered to Fischer rats bearing i.c. implants of the F98 glioma by Alzet osmotic pumps or at a dose of 0.2 mg by CED. The animals were euthanized 24 h after administration and tissues and blood samples were taken for boron determinations.

^b Boron concentrations were determined in samples of tumor, brain, blood, liver, spleen, kidneys, lung and heart, by means of direct current plasma-atomic emission spectroscopy. Except as indicated above, boron concentrations were at the undetectable level (<0.5 μg/g) in all other tissues. The values indicated are means ± standard deviation for groups of 4–5 rats.

^c Physical dose estimates include contributions from gamma photons, ¹⁴N (n,p), ¹⁴C and ¹⁰B (n,α) ⁷Li reactions.

^d Based on the low tumor boron values and the calculated physical radiation doses, BNCT was not carried out using H₂DCP.

Table 4

Survival times of F98 glioma bearing rats following i.c. delivery of H₂TCP and H₂TBP by CED or osmotic pumps

Agent/Route		Survival Time			%ILS ^b	
Group	N ^a	Range	Mean ± SD	Median	Mean	Median
H ₂ TCP/pump	18	30 – 41	35.0±3.7	36	49.6	60.0
H ₂ TBP/pump	10	32 – 59	43.8±10.0	40.5	87.2	80.0
H ₂ TBP/CED	10	30 – 39	33.8±3.1	33.5	44.4	48.9
H ₂ TBP/CED+ i.v. BPA	9	33 – 61	42.8±9.0	41	70.0	75.5
BPA/i.v.	10	33–48	39.8±1.6	39.5	59	58
Irradiated controls	9	24 – 31	27.4±2.7	27	17.3	20.0
Untreated Controls	10	21 – 29	23.4±2.5	22.5	-	-

^a A total of either 0.2 mg of the compound was administered by CED for 30 min or 0.5 mg by Alzet osmotic pumps for 24 hrs. BNCT was initiated 24 hrs after termination of either CED for Alzet osmotic pump infusion or 2.5 hrs after i.v. administration of BPA.

^b N is the number of animals per group.

^c Percent increased life span (%ILS) was defined relative to the mean and median survival times of untreated controls.