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In Vivo Evaluation of the Boronated Porphyrin TABP-1 in U-87 MG Intracerebral Human Glioblastoma Xenografts

Tomoko Ozawa,[†] Raquel A. Santos,[†] Kathleen R. Lamborn,[†] William F. Bauer,[‡] Myoung-Seo Koo,[§] Stephen B. Kahl,[§] and Dennis F. Deen*[,†,||]

Departments of Neurological Surgery, Radiation Oncology, and Pharmaceutical Chemistry, University of California, San Francisco, California 94143, and Idaho National Engineering Laboratory, Idaho Falls, Idaho 83415

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Abstract: Boron neutron capture therapy (BNCT) is an adjuvant therapy that has the potential to control local tumor growth. A selective delivery of sufficient amounts of boron to individual tumor cells, compared to surrounding normal tissues, is the key for successful BNCT. We have designed and synthesized a new highly water-soluble boronated porphyrin, TABP-1, as a possible BNCT agent. When we injected the maximum tolerated dose (MTD: 15 mg/kg) of TABP-1 systemically into the tail vein of athymic rats bearing intracerebral (ic) human glioblastoma U-87 MG xenografts, the compound accumulated preferentially in brain tumors compared to normal brain; however, the level of boron in the tumors was less than the 30 μg/g of tissue that is generally considered necessary for BNCT. We next investigated whether convection-enhanced delivery (CED) could improve the boron distribution. The compound was administered directly into ic tumors using an osmotic minipump attached to a brain-infusion cannula. TABP-1 doses from 0.25 to 1.0 mg infused locally over 24 h produced tumor boron concentrations greater than those obtained by systemic administration at the MTD. For example, CED administration of 0.5 mg of TABP-1 produced a tumor boron level of 65.4 μg/g of tumor, whereas the serum level was only 0.41 μg/g (tumor to serum ratio of approximately 160:1). CED also produced relatively high tumor to normal brain ratios of approximately 5:1 for ipsilateral brain and approximately 26:1 for contralateral brain tissues at the 0.5 mg dose. Thus, we may be able to achieve therapeutic BNCT efficacy with minimal systemic toxicity or radiation-induced damage to normal tissue by administering TABP-1 using CED.

Keywords: Boron neutron capture therapy; boronated porphyrin; U-87 MG human brain tumor xenografts; glioblastoma; convection-enhanced delivery

Introduction

The malignant gliomas—anaplastic astrocytoma and glioblastoma—are rarely curable, even by a combination of surgery, radiation therapy, and chemotherapy, and the median postoperative survival times for patients with these malignancies is 12 to 18 months.[1] Most treatment failures are due to recurrence of the tumor at the original site,[2] suggesting that more effective local therapy will be required to improve the outcome for patients with these tumors.

* Author to whom correspondence and reprint requests should be addressed: Brain Tumor Research Center, Department of Neurological Surgery, University of California, San Francisco, CA 94143-0520. Tel: (+1) 415-476-4590. FAX: (+1) 415-476-9687. E-mail address: ddeen@itsa.ucsf.edu.

[†] Department of Neurological Surgery, University of California, San Francisco.

[‡] Idaho National Engineering Laboratory.

[§] Department of Pharmaceutical Chemistry, University of California, San Francisco.

[||] Department of Radiation Oncology, University of California, San Francisco.

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Boron neutron capture therapy (BNCT) is an adjuvant therapy that has the potential to improve the control of local tumor growth. BNCT is a binary therapy based on the nuclear fission reaction that occurs when stable nonradioactive boron-10 is irradiated with low-energy neutrons. The resulting boron-11 atom immediately decays into two high linear-energy transfer particles: a lithium-7 nucleus and a helium-4 nucleus (α particle). These nuclear fragments dissipate relatively high energies of 2.31 MeV (94%) and 2.79 MeV (6%) within 6−9 μm, and cells containing these fragments are destroyed.[3] Early clinical trials of BNCT on patients with malignant gliomas were conducted in the 1950s and 1960s by investigators at the Massachusetts Institute of Technology and at the Massachusetts General Hospital[4,5] using boron-10 enriched *p*-carboxyphenylboronic acid and sodium decahydrodecaborate ($Na_2B_{10}H_{10}$). Other clinical studies were carried out at the Brookhaven National Laboratory[6] using two inorganic boron-containing compounds, sodium pentaborate ($Na_2B_{10}O_{16}\cdot 10H_2O$) and borax ($Na_2B_4O_7\cdot 10H_2O$). These studies failed because the boronated compounds were not taken up selectively by tumor tissue and boron was present in the blood at concentrations equal to or greater than those found in the tumor. In addition, the available neutron beams were contaminated with γ rays and fast neutrons.[7] In the 1980s, Hatanaka and his colleagues treated more than 100 malignant glioma patients in Japan with BNCT using a tumor-selective boron compound, sodium borocaptate (BSH: $Na_2B_{12}H_{11}SH$).[8] The median survival time for patients in this study was significantly prolonged compared to conventional therapy; however, the results are difficult to interpret because patients with several different tumor types were enrolled.[9] A recent human biodistribution study in 61 brain-tumor patients by a European group found tumor to blood ratios of only 1.3 ± 1.1 at 18 h after infusion of BSH.[10] Tumor to normal brain ratios reached as high as 3 in only some instances, and maximum tumor boron concentration did not exceed 20 ppm, even for patients infused with 100 mg/kg BSH. Recently, a phase I/II trial of *p*-boronophenylalanine (BPA)-based BNCT using a reactor-based epithermal neutron beam was conducted at the Brookhaven National Laboratory.[11] Fifty-three patients with primary glioblastoma received BNCT using one, two, or three irradiation fields, yielding median survival times of 14.8, 12.1, or 11.9 months, respectively. Increasing the number of irradiation fields increased the incidence of neurological toxicity. The number of patients studied was too small to draw any definitive conclusions, but the median survival time for all BNCT-treated patients was 13.5 months. This latest study is notable because even though BPA is not an ideal BNCT drug, the average life span for the patients in this study was similar to that obtained historically using standard radiation therapy. Moreover, BNCT can be delivered to patients in 1 day, as compared to 6 weeks for standard radiation therapy, making the BNCT therapy less onerous to the patients. This suggests that a better BNCT drug might lead to a better therapy for brain-tumor patients than standard radiation therapy.

Selective delivery of sufficient amounts of boron to individual tumor cells compared to surrounding normal tissues will be required for successful BNCT for patients with malignant glioma, and a number of new delivery agents are under investigation.[12−14] We have studied porphyrins as tumor-seeking boron carriers for several reasons. Both "natural" (di-propionic acid hematoporphyrin-type) and "synthetic" (*meso*-tetraphenyl) porphyrins are known for their selective uptake and retention by a wide variety of solid tumors.[15] This property has been put to extensive clinical applications in photodynamic therapy.[16] The chemistry of

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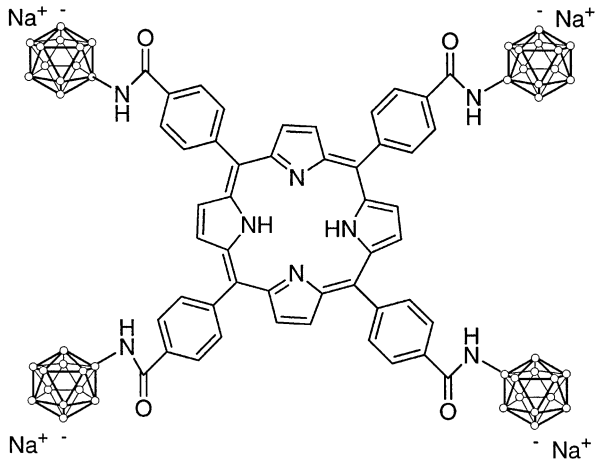

Figure 1. Chemical structure of TABP-1. Open circles in the polyhedral cages represent BH units or, in the case of the amide linkage sites, B atoms.

both natural and synthetic porphyrins is well understood, permitting a wide range of potential linkage chemistries between the porphyrin scaffold and the boron source. The potential for linking groups of widely variable polarity to the porphyrin backbone makes it possible to create molecules exhibiting a wide spectrum of solubility. The boronated porphyrin BOPP (tetrakis-carboxylate ester of 2,4-bis(α,β-dihydroxyethyl)deuterioporphyrin IX) has been the subject of intense study for BNCT for the past several years.[17−19] However, BOPP appears to fall short of the ideal BNCT agent for several reasons: (1) it causes toxic side effects, most notably thrombocytopenia, which limit the dose that can be tolerated by humans; (2) insufficient boron levels are reached in human tumors to permit clinically effective BNCT; and (3) the plasma pharmacokinetic behavior of BOPP in humans is characterized by a prolonged clearance phase, giving rise to potentially toxic metabolites and cutaneous photosensitivity.[20]

Recently, we have designed and synthesized a highly water soluble boronated compound, TABP-1 (Figure 1), as a possible BNCT agent. This polyboronated porphyrin was designed with the goal of increasing its water solubility, and thereby decreasing its plasma half-life relative to previously investigated boronated porphyrin compounds such as BOPP. The purpose of this study was to evaluate the biodistribution of TABP-1 in athymic rats bearing intracerebrally implanted U-87 MG human glioblastoma xenografts. We also compared tumor, normal brain, and serum boron concentrations after systemic and convection-enhanced delivery (CED) of TABP-1.

Materials and Methods

Boronated Compound. Figure 1 shows the chemical structure of TABP-1. Its synthesis and characterization will be described in detail in another publication. It was designed and synthesized to eliminate the possibility of metabolism of the borane polyhedra to toxic fragments, using the extreme stability and low toxicity of $B_{12}H_{12}^{(2-)}$ derivatives. The purity of the TABP-1 used in these experiments was determined by ultraviolet visible (UV−vis) and nuclear magnetic resonance (NMR) spectroscopy and thin-layer chromatography. TABP-1 (5 mg/mL) stock solution was prepared in phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+}, and pH was adjusted to approximately 7.4 by using sodium bicarbonate. Because TABP-1 is sensitive to visible light, the bottle containing the stock solution and all syringes containing TABP-1 were covered with aluminum foil to prevent light exposure.

Cells. U-87 MG human glioblastoma cells were obtained from the Department of Neurological Surgery Tissue Bank at the University of California, San Francisco (UCSF). Cells were maintained as exponentially growing monolayers in complete medium consisting of Eagle's minimal essential medium supplemented with 10% fetal bovine serum and nonessential amino acids. Cells were cultured at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. Cells were seeded into culture flasks 3 days before tumor implantation. For implantation, cells were harvested by trypsinization, washed once, and resuspended in Hanks balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+}.

Animals. Six-week-old male athymic rats were purchased from Harlan (Indianapolis, IN) for the biodistribution studies. Rats were housed and cared for in accordance with the United States Department of Health and Human Services Guide for the Care and Use of Laboratory Animals; all protocols were approved by the UCSF Institutional Animal Care and Use Committee.

U-87 MG Human Glioblastoma Intracerebral Tumor Model. Tumor cells were implanted into the brains of athymic rats as previously described.[21] Briefly, rats were anesthetized with intraperitoneal injections of 60 mg/kg ketamine hydrochloride and 7.5 mg/kg xylazine hydrochloride and were positioned in a stereotaxic device using ear bars. The scalp was cleaned with 2% chlorhexidine, and a skin incision approximately 15 mm in length was made electrosurgically over the middle frontal bone. The surface

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Table 1. Boron Concentrations Produced by Systemic Injection of TABP-1 into Rats Bearing U-87 MG Xenografts[a]

	untreated (0 h)	2 h	6 h	24 h	48 h	72 h
			Boron Concentrations in Tissue (μg/g)[b]			
tumor	3.44 (5.72, 1.16)	7.09 (1.88)	9.34 (7.26)	7.77 (1.00)	10.01 (7.94)	9.60 (5.15)
serum	0.39 (0.38, 0.39)	50.17 (3.89)	30.49 (1.42)	4.84 (0.37)	2.01 (0.21)	1.31 (0.15)
ipsilateral brain	1.48 (1.24, 1.72)	0.77 (0.41)	0.48 (0.18)	0.28 (0.01)	1.82 (2.59)	1.82 (0.17)
contralateral brain	1.68 (1.54, 1.82)	1.24 (1.4)	0.42 (0.03)	0.30 (0.04)	0.63 (0.47)	1.06 (0.16)
			Ratio of Boron Concentrations in Tissue			
tumor:serum		0.1	0.3	1.6	5.0	7.3
tumor:ipsilateral brain		9.2	19.5	27.8	5.5	5.3
tumor:contralateral brain		5.7	22.2	25.6	15.9	9.1

[a] Athymic rats were injected with 15 mg/kg TABP-1 in the tail vein and euthanized at 2, 6, 24, 48, or 72 h postinjection. [b] For control animals (untreated), numbers in parentheses are the values from each animal. For animals euthanized at 2, 6, 24, 48, and 72 h, the numbers shown are the mean concentrations for the 4 rats euthanized at each time point and the standard deviation.

of the skull was exposed so that a small hole could be drilled with a No. 6 round-type dental burr 3.5 mm to the right of the midline and just behind the bregma. The dura was pierced with a sharp 25-gauge needle, and a screw with a hole drilled through its center was inserted into the skull hole. Animals were removed from the stereotaxic device, and a blunt 25-gauge needle attached to a Hamilton syringe (Hamilton Company, Reno, NV) was inserted into the hole in the screw. The needle had a metal sleeve that limited the depth of injection to 4.0 to 4.5 mm from the bottom of the skull. U-87 MG cells (5×10^6) in 10 μL of HBSS were injected very slowly (approximately 1 min) by free hand, and then the needle was removed. The skull and screw were swabbed with hydrogen peroxide, the screw hole was sealed with bone wax to prevent reflux, and the scalp was closed with surgical staples.

Boron Biodistribution Studies after a Bolus Systemic Injection of the Maximum Tolerated Dose of TABP-1. Eighteen days after tumor implantation, rats were randomized into groups of 4 animals in 5 TABP-1-treated groups and 2 animals in an untreated control group. Our preliminary studies showed that 15 mg/kg of this compound was the maximum tolerable dose we could administer systemically to rats (unpublished data). Rats were injected with 15 mg/kg TABP-1 into the tail vein (over approximately 5 min), and one group each was euthanized 2, 6, 24, 48, and 72 h later. A blood sample was collected by cardiac stick, and brain tumor, brain (ipsilateral and contralateral), kidney, spleen, muscle, heart, lung, liver, adrenal gland, testis, and skin were removed. All organs were weighed, quickly frozen in liquid nitrogen, and stored at −80 °C. Tissue samples were analyzed for boron levels at the Idaho National Engineering Laboratory using an inductively coupled plasma atomic emission spectrometer (ICP-AES), as described elsewhere.[22]

Boron Distribution Studies after Convection-Enhanced Delivery of TABP-1. Nineteen days after tumor implantation, 200 μL of PBS containing either 0.00025, 0.0025, 0.025, 0.25, 0.5, or 1.0 mg of TABP-1 was loaded into Alzet osmotic minipumps (Durect Corporation, Cupertino, CA). Six rats were used as an untreated control, 3 rats each received 0.00025, 0.0025, or 0.025 mg, 5 rats received 0.25 mg, and 3 rats each received 0.5 or 1.0 mg of TABP-1. A pump was implanted subcutaneously into the midscapular region of each rat, and a catheter connected the minipump to a brain-infusion cannula that was inserted into the center of the U-87 MG cell-derived tumor. The compound was infused into the tumors with a flow rate of 8.0 μL/h over a 24 h period. Twenty-four hours after completion of infusion, animals were euthanized and their blood, brains, and brain tumors were collected. All tissues were weighed, quickly frozen with liquid nitrogen, and stored at −80 °C. The tissue samples were analyzed for boron levels using ICP-AES, as above.

Results

Tumor Boron Distribution after a Bolus Systemic Injection of TABP-1. Table 1 lists the boron concentrations in tumors, brains, and serum that were produced by injecting 15 mg/kg TABP-1 systemically into U-87 MG tumor-bearing athymic rats. Boron concentrations in tumors were similar at all time points studied up to 72 h, ranging from 7.09 ± 1.88 (standard deviation, SD) μg/g to 10.01 ± 7.94 μg/g. However, there was significant variation among the individual brain tumors, as indicated by the large SDs at 6, 48, and 72 h. The boron concentrations in ipsilateral brain (adjacent to the tumor) and contralateral brain (in the other hemisphere) were relatively low at all times, and these concentrations were consistently less than those observed in brain tumors. Boron concentrations in serum peaked 2 h after injection with a value of 50.17 ± 3.89 μg/g and then rapidly decreased with increasing time. Tumor to ipsilateral brain ratios were relatively high, ranging from 5.3 to 27.8, while tumor to serum ratios were relatively low, ranging from 0.1 to 7.3. The maximum tumor to ipsilateral brain ratio of 27.8 was observed 24 h after administration of TABP-1, when the boron concentration in tumor was 7.77 ± 1.00 μg/g. The tumor to serum ratio at 24 h was only 1.6.

Extracranial Tissue Boron Concentrations after a Bolus Systemic Injection of TABP-1. Figure 2 shows extracranial tissue boron concentrations that were produced by systemic

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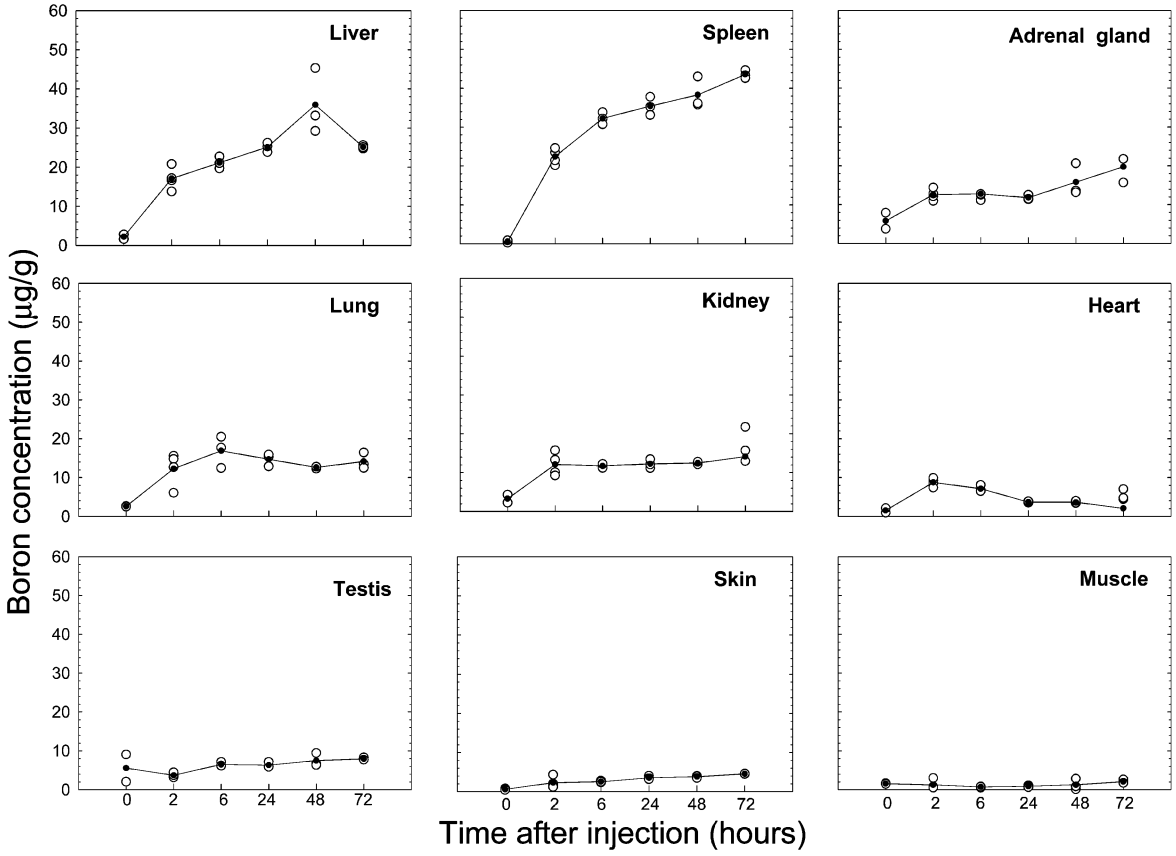

Figure 2. Boron concentrations produced in extracranial organs by injection of 15 mg/kg TABP-1 into the tail veins of athymic rats bearing U-87 MG xenografts. Open circles represent the values from individual samples, and closed circles represent averages of the individual values.

Table 2. Boron Concentrations Produced by Convection-Enhanced Delivery of TABP-1 into U-87 MG Xenografts[a]

	untreated	1.0 mg	0.5 mg	0.25 mg	0.025 mg	0.0025 mg	0.00025 mg
			Boron Concentrations in Tissue (μg/g)[b]				
tumor	1.01 (0.42)	201.19 (92.73)	65.39 (86.16)	22.07 (16.26)	1.85 (1.48)	0.55 (0.35)	0.55 (0.24)
serum	0.40 (0.01)	0.41 (0.01)	0.41 (0.01)	0.40 (0.01)	0.39 (0.00)	0.39 (0.01)	0.39 (0.00)
ipsilateral brain	1.73 (1.20)	34.63 (23.36)	14.16 (19.28)	4.81 (3.97)	2.08 (1.94)	3.09 (0.65)	1.08 (0.90)
contralateral brain	1.70 (1.09)	4.55 (2.39)	2.51 (2.30)	1.55 (1.43)	2.90 (1.72)	1.90 (0.39)	2.80 (1.00)
			Ratio of Boron Concentrations in Tissue				
tumor:serum		490.7	159.5	55	4.7	1.4	1.4
tumor:ipsilateral brain		5.8	4.7	4.6	0.9	0.17	0.5
tumor:contralateral brain		44.2	26.1	14.2	0.6	0.3	0.2

[a] TABP-1 in 200 μL of PBS was infused into the tumors over a 24 hour period. Animals were euthanized 24 h after completion of drug infusion. [b] Numbers shown are the mean concentrations for the 3−6 rats administered each concentration of TABP-1 and the standard deviation (in parentheses).

injection of 15 mg/kg TABP-1. Boron levels were relatively high in the liver and spleen, with moderate levels occurring in the adrenal gland, lung, and kidney. Boron concentrations in the liver peaked at 48 h with a value of 35.91 ± 8.38 μg/g, and the boron concentrations in the spleen gradually increased with time after TABP-1 injection, reaching a maximum value of 43.55 ± 1.04 μg/g at 72 h. Boron concentrations in the heart, testis, skin, and muscle were relatively low at all time points. This pattern of biodistribution is similar to that observed previously for several structurally diverse boronated porphyrins.[13,14]

Convection-Enhanced Delivery of TABP-1. We chose 24 h as the postinfusion time because this is the time at which we obtained the maximum tumor to ipsilateral brain ratio of boron concentrations in the earlier experiment using systemic injections. Table 2 shows a summary of boron concentrations in tumor, brain, and serum after administration of TABP-1 at doses from 2.5×10^{-4} to 1.0 mg/200 μL directly into the U-87 MG tumors using an Alzet osmotic minipump attached to a brain-infusion cannula. Doses ≥0.25 mg produced tumor boron concentrations significantly greater than those obtained by systemic administration at the maximum tolerated dose

(MTD) (Table 1). CED of 0.5 mg of TABP-1 produced a tumor boron level of 65.39 ± 86.16 (SD) μg/g, and a serum level of only 0.41 ± 0.01 μg/g, resulting in a tumor to serum ratio of 160:1. Even at the highest TABP-1 dose tested,1.0 mg, serum boron levels equaled untreated control levels. At this dose, the tumor boron level was 201.19 ± 92.73 μg/g, yielding a tumor to serum ratio of 491:1. Doses of 0.25, 0.5, and 1.0 mg produced siginificant variation in boron levels among the individual brain tumors, as indicated by the high SD values. This mode of local delivery also produced relatively high tumor to normal brain ratios. Tumor to ipsilateral brain ratios were 4.7:1 and 5.8:1 after the 0.5 and 1.0 mg doses, respectively. Tumor to contralateral brain ratios were 26.1:1 and 44.2:1 after the 0.5 and 1.0 mg doses, respectively. Doses of 0.5 and 1.0 mg produced relatively high boron concentrations in the surrounding brain of approximately 14 and 35 μg/g, respectively; however, none of the animals displayed any neurological or systemic symptoms following TABP-1 infusion.

Discussion

The delivery of therapeutic agents to intracerebral tumors poses a particularly difficult challenge. Intravascular delivery of most high molecular weight (HMW) drugs, such as targeted proteins, and even low molecular weight (LMW) compounds is severely retarded by the blood-brain barrier (BBB) and the increased interstitial pressure within the tumor. Systemic treatment also requires the administration of high concentrations of drugs that are almost universally associated with systemic toxicities, yet which achieve relatively ineffective concentrations of drug within the tumor. Intra-arterial delivery techniques and specific chemical disruption of the BBB have provided modest improvements at best. Avoiding the BBB altogether through the use of direct interstitial, intrathecal, or intratumoral injection or implantable biodegradable polymers inevitably relies on diffusion processes. Diffusive methods are inherently limited because diffusion rates are dependent on concentration gradients and result in high peak concentrations with a rapid decrease in dose from the point source. This restricts the delivery of most therapeutic agents to within a few millimeters of the source, while malignant tumor cell populations existing beyond the tumor border escape exposure to the drug.

Recently, CED has been used to surmount these deficiencies. In contrast to diffusion, CED uses a pressure gradient established at the tip of an infusion catheter to establish bulk flow and distribute drug and solvent throughout the extracellular space. CED has the potential to homogeneously distribute even large therapeutic molecules up to several centimeters from the point of infusion. Convection enhances the distribution of HMW molecules in the normal rat, cat, and primate brain by an order of magnitude relative to diffusion. Using flow rates <10 μL/min, the apparent volume of distribution has exceeded the volume of infusion by 5-fold to 10-fold in these models.[23,24] Thus, CED should allow the infusion of therapeutic molecules to the infiltrative margins of the tumor/normal brain. Also, in CED, 100% of the drug is delivered directly to target tissue. Therefore, the brain tumor sees the highest concentrations of the drug and the dose to normal brain and other normal tissues is significantly smaller. Since the systemic circulation is exposed to very small doses of drug, systemic toxicity should be limited. As a result, drugs with low MTDs when given systemically should produce few toxic effects when delivered by CED.

In the present study, we investigated the newly synthesized, highly water-soluble boronated compound TABP-1 as a possible BNCT agent in vivo. A successful BNCT compound must localize preferentially in tumor tissue, the amount of compound in tumors must be sufficient to ensure the BNCT reaction, and the compound must be minimally toxic to the normal tissues of the host.

In these experiments, injecting the MTD of TABP-1 intravenously did not produce satisfactory boron accumulation in the experimental brain tumors. Average tumor boron concentrations ranged from approximately 7 to 10 μg/g for time points up to 72 h after drug injection (Table 1), far below the generally accepted therapeutic range of ≥30 μg/g. A maximum tumor to normal brain ratio of 27.8 occurred at 24 h, indicating preferential accumulation in tumors, and the tumor to serum ratio at 24 h was 1.6, a relatively low ratio for BNCT agents. We also observed relatively high boron levels of approximately 40 μg/g in both the liver and spleen in rats (Figure 2) injected at the same dose. The accumulation of boron in the liver and spleen after injection of boronated compounds has also been reported by others.[13,14,25] However, nontoxic boron accumulation in extracranial organs may not pose substantial problems in the treatment of brain tumors, owing to the localized neutron irradiation.

In contrast, the results of the CED study clearly demonstrate that this mode of local delivery of TABP-1 significantly enhances the tumor uptake of boron compared to an intravenous injection and results in no apparent toxicity. CED of 1.0 and 0.5 mg of TABP-1 produced tumor boron concentrations of 201.19 and 65.39 μg/g, respectively, and a serum level of only 0.4 μg/g at both doses, resulting in tumor to serum ratios of 491:1 and 160:1, respectively (Table 2). In addition, high absolute boron levels in tumor would permit a reduction in exposure time to the neutron beam, resulting in a reduction in background radiation dose. This could be a significant advantage of CED of TABP-1. Moreover, CED of TABP-1 produced relatively high tumor

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to brain ratios of approximately 5:1 for ipsilateral brain and approximately 26:1 for contralateral brain at the 0.5 mg dose. In comparison, human biodistribution studies in patients with malignant gliomas treated intravenously with BPA showed tumor to normal brain ratios of only approximately 3.5.[26] The fact that TABP-1 doses $\geq$0.5 mg produced relatively high boron levels of approximately 14−35 μg/g in ipsilateral brain suggests that there was a leakage of boron from the tumor into the surrounding healthy brain tissue. Although animals that received 0.5 or 1.0 mg of TABP-1 did not display any neurotoxicities of the compound itself at 24 h after completion of CED, the potential radiotoxic effects in surrounding brain at these boron levels induced by BNCT should be considered. We also note that we achieved higher concentrations of boron in tumor than needed for BNCT. Therefore it should be possible to lower the amount of boron injected via CED into the tumor, and this will also lower the relatively high boron concentrations in surrounding brain. Thus it should be possible to find dosing conditions that are not toxic to surrounding brain. A limited amount of boron leakage into surrounding brain may be desirable if the boronated compound is nontoxic, because it may permit the boron to reach tumor cells that have invaded or migrated into brain that immediately surrounds the tumor. More than 90% of malignant gliomas recur within 2 cm of the original margin of resection, and local control of gliomas could potentially prevent or delay tumor recurrence.[27]

Heterogeneity is a hallmark of brain tumors, and cells within the tumors may reside in various microenvironments. Whether boronated compound will be equally distributed to cells in these microenvironments has not yet been investigated. Tumor heterogeneity also might explain the significant variation in boron levels found in the individual brain tumors, as indicated by the large SD values.

To our knowledge, the present report is the first to demonstrate significantly increased tumor boron concentrations of an LMW agent (approximately 1.3 kDa) in a human brain tumor model through the use of CED. Yang and colleagues[28] have reported using CED to deliver a boronated epidermal growth factor (28−39 kDa) to normal rats bearing intracerebral F98 gliomas, but these authors did not measure the resulting boron distribution in tumor and normal brain. Instead, they determined the volume of distribution (V_d) of an ^{125}I-labeled construct and compared it to the volume of infusion (V_I). They reported a relatively constant V_d/V_I of approximately 6.5 and a nearly linear dependence of V_d on V_I. Perhaps most importantly, they found that the amount of injected dose per gram of tumor tissue was almost 50% greater using CED than by direct intratumoral injection and almost 4 times as great compared with systemic intravenous injection of the agent. They concluded on the basis of these observations that CED is more effective than either intratumoral or intravenous injection for delivery of boronated EGF to EGFR(+) gliomas for BNCT.

Our study demonstrates that the newly synthesized boronated compound TABP-1 may be useful for BNCT for malignant brain tumors. Changing drug delivery from intravenous to CED significantly enhances boron concentrations in tumors and produces desirable tumor to serum and tumor to normal brain ratios. Using CED of TABP-1, we may be able to achieve therapeutic BNCT efficacy with minimal systemic toxicity or radiation-induced damage to normal brain tissue. We will next investigate this possibility in brain tumor xenograft models using 0.5 and 1.0 mg doses of TABP-1.

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