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Biodistribution of Boron Compounds in an Animal Model of Human Undifferentiated Thyroid Cancer for Boron Neutron Capture Therapy

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Abstract: Undifferentiated thyroid carcinoma (UTC) is a rapidly growing, highly invasive malignant tumor that currently lacks any effective treatment. Boron neutron capture therapy (BNCT) has been investigated recently for some types of tumors including glioblastoma multiforme and malignant melanoma. In previous studies we have shown the selective uptake of *p*-boronophenylalanine (BPA) by undifferentiated thyroid cancer cells in vitro and in vivo, as well as the histologic cure of 50% of the nude mice transplanted with human UTC cells when treated with BPA and an appropriate neutron beam. The present studies were performed to further optimize this treatment through the investigation of a boronated porphyrin, both alone and in combination with BPA. In vitro studies with cells in culture showed that BOPP (tetrakis-carborane carboxylate ester of 2,4-bis-(α,β-dihydroxyethyl)-deuteroporphyrin IX) is localized intracellularly, with a highest concentration in the 11500*g* (mitochondrial-enriched pellet) fraction. When BOPP was administered alone to NIH nude mice transplanted with UTC human cells, no significant tumor uptake or selectivity in our in vivo model was observed. In contrast, when BOPP was injected 5−7 days before BPA and the animals were sacrificed 60 min after administration of BPA, a significant increase in boron uptake by the tumor was found (38−45 ppm with both compounds vs 20 ppm with BPA alone). On day 5 the tissue boron selectivity ratios were tumor/blood ∼3.8 and tumor/distal skin ∼1.8. Other important ratios were tumor/thyroid ∼6.6 and tumor/lung ∼2.9. These results open the possibility of improving the efficacy of BNCT for the treatment of this so far "orphan" tumor.

Keywords: BNCT; thyroid; cancer; BOPP; BPA

Introduction

Boron neutron capture therapy (BNCT) is based on the selective uptake of boron (^{10}B) compounds by tumors. Once a significant accumulation of boron is achieved in the tumor, the area is irradiated with an appropriate neutron beam. The ^{10}B is then activated to ^{11}B, which promptly decays releasing ^{7}Li and α particles. These fission fragments have a high linear energy transfer (LET) and have mean free paths of ≤10 μm, giving rise to the possibility of extremely localized cellular destruction. Clinical trials for glioblastoma multiforme and melanomas are under development in different centers around the world, utilizing the boron compounds *p*-boronophenylalanine (BPA) and sodium borocaptate (BSH).[1] A minimum tumor boron concentration of ∼20 ppm and tumor-to-blood

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(1) Coderre, J. A.; Turcotte, J. C.; Riley K. J.; Binns P. J.; Harling, O. K.; Kiger, W. S., III; Boron Neutron Capture Therapy: Cellular Targeting of High Linear Energy Transfer Radiation. *Technol. Cancer Res. Treat.* **2003**, *2*, 1−21.

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and tumor-to-normal-tissue ratios of at least 3:1 have been established as requisites for potential success of the procedure.

Undifferentiated thyroid carcinoma (UTC) is a very aggressive tumor that is not amenable to treatment by radioiodine, chemotherapy, or radiotherapy, and therefore it has a very poor prognosis, with a mean patient survival of around 12 months.[2] In previous studies we have shown that the human UTC cell line ARO has a selective uptake of BPA with a boron concentration ratio in tumor cells to normal thyroid and in tumor cells to human follicular adenoma of around 4−5:1.[3] We have developed and characterized an in vivo model by transplanting the ARO cells into nude mice.[4] With this animal model we could also demonstrate the selective uptake of BPA by the tumor when the compound is administered at two different doses, 350 and 600 mg/kg of body weight.[3] Moreover, when mice transplanted with the ARO cells and injected with BPA were irradiated with a neutron beam, a complete halt of tumor growth was observed in 100% of the mice, and a complete cure in 50% of those animals bearing a tumor with a volume of 50 mm^3 or less.[5]

Despite these encouraging results, it is desirable to further improve the efficacy of this form of therapy. One way of achieving this goal would be to obtain a higher boron concentration in the tumor and better selectivity of the boron carrier for tumor over blood and normal tissues. Certain compounds, such as boronated porphyrins, have been designed and synthesized to have a higher weight proportion of boron per molecule than BPA. Previous studies have shown that the tetrakis-carborane carboxylate ester of 2,4-bis-(α,β-dihydroxyethyl)-deutero-porphyrin IX (BOPP) is one such porphyrin. It contains ∼30% boron by weight and seems to be a good boron carrier in different animal models of glioma.[6−8] In contrast to both BPA and BSH, the pharmacokinetic behavior of BOPP in both small and large animals, as well as in humans, is characterized by a relatively slow rise in tumor boron levels over a period of several hours or even days.[6−9] This is accompanied by a concomitantly long terminal half-life in tumor.

In the present study, we have analyzed the biodistribution of BOPP in our UTC model, first alone and then combined with BPA, in order to determine whether the boron concentration in this tumor can be further increased above that obtained with BPA alone. The results show that the combination of BOPP and BPA gives much higher tumor boron concentrations and better tumor selectivity than either drug administered alone.

Materials and Methods

Tissue Culture. The UTC cell line ARO (kindly provided by Dr. G. Juillard, UCLA, CA) was maintained in RPMI-1640 medium containing 10% FCS, under 5% CO_2, at 37 °C. When cells reached confluence they were resuspended, counted, and diluted in PBS 1X to the appropriate concentration.

Animal Model. Male NIH nude mice (body weight ∼20 g) were implanted in the right back flank with 10^6 ARO cells. The animals were kept in a horizontal laminar flow bench in order to avoid contamination. Growth of the tumor was followed by measuring its volume twice each week with a caliper as previously described.[4] At 14 days post-transplantation, the mice were injected with the corresponding boron compound.

Preparation and Administration of BOPP. BOPP was prepared as previously described as a lyophilized powder of the sodium salt and was stored under desiccation in a vacuum at −20 °C.[10] Compound purity was determined by thin-layer chromatography and UV−visible spectroscopy. Stock solutions of 10 mg/mL in 0.9% isotonic NaCl were prepared and were stored in darkness at 4 °C for no more than 24 h before administration. Since solutions of porphyrins are known to be sensitive to visible light, all experiments were conducted under low-intensity light.

Boron Analysis. Boron measurements in tissues and blood were performed by inductively coupled plasma optical emission spectroscopy (ICP-OES) employing an axially viewed plasma using an Optima 3100 instrument (Perkin-

(2) Ain, K. B. Anaplastic Thyroid Carcinoma: Behavior, Biology, and Therapeutic Approaches. *Thyroid* **1998**, *8*, 715−726.
(3) Dagrosa, M. A.; Viaggi, M. E.; Kreimann, E.; Farias, S.; Garavaglia, R.; Agote, M.; Cabrini, R. L.; Dadino, J. L.; Juvenal, G.; Pisarev, M. Selective Uptake of p-Borophenylalanine by Undifferentiated Thyroid Carcinoma for Boron Neutron Capture Therapy. *Thyroid* **2002**, *12*, 7−12.
(4) Viaggi, M.; Dagrosa, M. A.; Gangitano, D.; Belli, C.; Larripa, I.; Cabrini, R.; Juvenal, G.; Pisarev, M. A New Animal Model for Human Undifferentiated Thyroid Carcinoma. *Thyroid* **2003**, *13*, 529−536.
(5) Dagrosa, M. A.; Viaggi, M.; Longuino, J.; Calzetta, O.; Cabrini, R.; Edreira, M.; Juvenal, G.; Pisarev, M. Experimental Application of Boron Neutron Capture Therapy (BNCT) to Undifferentiated Thyroid Carcinoma (UTC). *Int. J. Radiat. Oncol., Biol., Phys*. **2003**, *57*, 1084−1092.
(6) Hill, J. S.; Kahl, S. B.; Kaye, A. H.: Stylli, S. S.; Koo, M. S.: Gonzales, M. F.; Vardaxis, N. J.; Johnson, C. I. Selective Tumor Uptake of a Boronated Porphyrin in an Animal Model of Cerebral Glioma. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1785−1789.
(7) Ceberg, C. P.; Brun, A.; Kahl, S. B.; Koo, M. S.; Person, B. R. R.; Salford, L. G. A Comparative Study on the Pharmacokinetics and Biodistribution of Boronated Porphyrin (BOPP) and Sulfhydryl Boron Hydride (BSH) in the RG2 Rat Glioma Model. *J. Neurosurg*. **1995**, *83*, 86−92.
(8) Tibbitts, J.; Sambol, N. C.; Fike, J. R.; Bauer, W. F.; Kahl, S. B. Plasma Pharmacokinetics and Tissue Biodistribution of Boron Following Administration of a Boronated Porphyrin in Dogs. *J. Pharm. Sci*. **2000**, *89*, 469−477.
(9) Rosenthal, M. A.; Kavar, B.; Hill, J. S.; Morgan, D. J.; Nation, R. L.; Stylli, S. S.; Basser, R. L.; Uren, S.; Geldard, H.; Green, M. D.; Kahl, S. B.; Kaye, A. H. Phase I and Pharmacokinetic Study of Photodynamic Therapy for High-Grade Gliomas Using a Novel Boronated Porphyrin. *J. Clin. Oncol*. **2001**, *19*, 519−524.
(10) Kahl, S. B.; Koo, M.-S. Synthesis of Tetrakis-Carborane Carboxylate Esters of 2,4-Di(α,β-dihydroxyethyl)-Deuteroporphyrin IX. *J. Chem. Soc., Chem. Commun*. **1990**, 1769−1771.

Table 1. Boron Biodistribution of BOPP or BPA Alone in Nude Mice with UTC[a]

		skin							
	tumor	ss[b]	ds[c]	lung	liver	spleen	kidney	thyroid	blood
BOPP									
10 mg/kg ip	2.15 ± 0.40	2.18 ± 0.24	1.27 ± 0.34	1.72 ± 0.24	5.08 ± 0.62	2.55 ± 0.15	1.58 ± 0.37	1.56 ± 0.65	1.39 ± 0.11
10 mg/kg iv	1.78 ± 0.24	1.73 ± 0.44	2.45 ± 0.17	0.92 ± 0.21	5.23 ± 0.65	1.82 ± 0.33	1.65 ± 0.34	1.57 ± 0.62	1.78 ± 0.19
100 mg/kg ip	16.59 ± 2.6	18.76 ± 2.7	6.47 ± 0.20	27.9 ± 4.56	73.71 ± 5.2	52.35 ± 4.6	22.61 ± 2.1	15.6 ± 2.15	21.4 ± 2.15
100 mg/kg iv	8.01 ± 8.1	9.35 ± 2.44	56.9 ± 15	9.8 ± 1.65	20.61 ± 2.2	10.28 ± 1.9	7.28 ± 2.28	5.94 ± 2.34	6.9 ± 0.19
BPA									
350 mg/kg ip	16.35 ± 1.91	10.62 ± 3.87	6.14 ± 0.39	6.92 ± 0.43	6.22 ± 1.19	7.50 ± 0.63	17.44 ± 1.43	4.39 ± 0.68	4.02 ± 0.75

[a] Boron concentrations (μg of B/g of tissue) were measured by ICP-OES. The BOPP treated animals were sacrificed 24 h after ip or iv administration of either 10 mg of BOPP/kg of body weight or 100 mg of BOPP/kg of body weight. The BPA treated animals were sacrificed 1 h following ip administration of 350 mg of BPA-fructose/kg of body weight. Values for BOPP treated animals are the mean ± SEM (5 animals) and for BPA treated animals values are mean ± SEM of 5−12 animals from 2 different experiments. [b] Surrounding skin. [c] Distal skin.

Elmer, Norwalk, CT). Digestion of tissue samples, with masses ranging between 10 and 50 mg, was carried out for 2 h at 60 °C with 0.15 mL of a 1:1 mixture of concentrated nitric and sulfuric acids. Dilution to 1.0 mL was performed with 0.65 mL of a 5% aqueous solution of Triton X-100 (v/v) and 0.2 mL of a solution containing 25 μg/mL Sr and 0.5 μg/mL Y as internal standards. Blood samples (200−300 μL) were digested for 2 h at the same temperature with 1.0 mL of the 1:1 acid mixture and diluted to 5.0 mL with 3.0 mL of the Triton X-100 and 1.0 mL of the internal standard solution. Analytical and internal standard lines (in nm) were as follows: B 249.677 (alternative 208.880); Sr 232.235; and Y 371.029. Matrix-matched standard solutions containing the internal standard elements and boron between 0.1 and 10.0 μg/mL were employed for daily calibration.

Biodistribution Studies. (a) BOPP Alone. In the first experiment, the influence of the route of injection was compared by administering the BOPP, at a dose of 10 or 100 mg/kg body weight, either ip or iv (tail vein). Animals were sacrificed 24 h later, and the following organs were carefully dissected and weighed: tumor, surrounding skin, distal skin, lung, liver, spleen, kidney, thyroid, and blood. In previous studies we have shown that the skin surrounding the tumor is infiltrated with tumor cells.[4] Boron concentration in the tissues was determined by ICP-AOS, as described above.

(b) BPA-fructose Alone. The mice were injected ip with 350 mg/kg of body weight and sacrificed 1 h later. The same organs as noted above were dissected, weighed, and analyzed for boron.

(c) BOPP plus BPA-fructose. The nude mice were injected ip with BOPP at a dose of 60 mg/kg of body weight. After 1, 3, 4, 5, and 7 days, BPA-fructose was administered ip at a dose of 350 mg/kg of body weight. The animals were sacrificed 60 min later, and the different organs were processed as described above.

Localization of BOPP in Vitro. The ARO cells were grown to 50% confluence at 37 °C in plates of 60 cm with RPMI 1640 medium supplemented with 10% FCS. In the logarithmic phase of growth, the cells were incubated for 24 h with 20 μg/mL BOPP. The medium was then removed and the plate was washed twice with fresh RPMI medium to remove the exogenous BOPP. The cellular localization of BOPP was photographed in a Zeiss Axioplan microscope (neofluor objective 40×) with an excitation at 546 nm and the emission monitored above 590 nm.

Subcellular Distribution of Boron. ARO cells were grown in RPMI 1640 medium supplemented with 10% FCS and incubated with BOPP in a final concentration of 60 μg/mL for 24 h. At the end of the incubation, the medium was aspirated and the cells were washed twice with PBS 1X and then harvested by scraping. The cells were homogenized in a buffer containing 0.32 M sucrose, 1 mM $MgCl_2$, and 0.4 mM phosphate (pH 7.4) with a Potter−Elvehjem type tissue homogenizer, with a Teflon pestle. The homogenate was centrifuged at 900*g* for 10 min at 4 °C. The supernatant was then centrifuged at 11500*g* for 20 min at 4 °C, and the resulting supernatant was centrifuged at 105000*g* for 1 h. The pellets were resuspended and centrifuged twice with the same buffer, and the boron concentration was determined by ICP-OES as described. The corresponding values were referred to their protein content, determined after Lowry et al.[11]

Statistical Analysis. Data were tested according to analysis of variance (ANOVA) and to Bonferroni multiple comparisons test.

Results

BOPP Alone. The tissue boron results obtained by comparing the routes of administration of BOPP are shown in Table 1. With a dose of 10 mg/kg no significant differences were observed between the ip and the iv routes, while with the 100 mg/kg dose the ip boron values in the tumor at 24 h were twice as high compared to the iv route. The analysis of the boron concentration failed to show a significant uptake in the tumor when compared to the other tissues, and in all the cases the tumor/blood ratios were around 1. Liver boron concentration was higher than the boron concentration in the other tissues. No animals died after treatment with BOPP, and there was no obvious evidence of toxicity at either dose. The highest boron

(11) Lowry, OH.; Rosebrough, N. J.; Farr, A. L.; Randall, A. L. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem*. **1951**, *193*, 265.

Table 2. Boron Biodistribution of BOPP and BPA Administered Sequentially[a]

	1 day	3 days	4 days	5 days	7 days
tumor	22.92 ± 1.38	25.98 ± 3.30	26.59 ± 3.40	45.19 ± 5.77	37.76 ± 2.84
blood	23.74 ± 1.38	18.25 ± 2.57	14.62 ± 1.54	12.02 ± 1.02	10.88 ± 0.88
skin					
ss[b]	26.95 ± 2.37	24.16 ± 7.97	40.58 ± 16.22	31.74 ± 3.34	33.79 ± 1.22
ds[c]	42.23 ± 6.08	20.06 ± 6.37	26.61 ± 6.80	25.59 ± 1.76	23.25 ± 0.70
liver	34.80 ± 1.67	33.50 ± 7.25	34.55 ± 2.19	21.70 ± 1.01	19.00 ± 0.79
spleen	29.14 ± 6.69	25.66 ± 5.36	21.23 ± 1.14	20.12 ± 1.21	19.14 ± 0.88
kidney	41.20 ± 0.09	43.68 ± 5.27	37.82 ± 3.93	28.05 ± 1.57	28.24 ± 2.71
lung	27.21 ± 3.25	38.86 ± 4.14	19.12 ± 3.96	15.82 ± 1.23	26.35 ± 1.75
thyroid	9.55 ± 2.83	10.02 ± 1.72	10.22 ± 2.79	6.89 ± 1.96	5.69 ± 0.82

[a] Boron concentrations (μg of B/g of tissue) were determined by ICP-OES. The animals were injected ip with 60 mg/kg of BOPP and 1, 3, 4, 5, and 7 days later with 350 mg/kg of BPA-fructose (ip). One hour later animals were sacrificed**.** Values are the mean ± SEM of 5−12 animals from 2 different experiments. [b] Surrounding skin. [c] Distal skin.

concentration in the tumor, with the dose of 100 mg/kg of body weight BOPP administered ip, was 16.59 ± 2.6 ppm. This value is below the lower limit of boron concentration established for a successful complete BNCT procedure. Moreover, the ratios of tumor/blood or tumor/normal thyroid did not exceed the desired value of 3 and in several cases were less than 1. These results indicate that BOPP alone is unlikely to be suitable for successful BNCT treatment of this tumor.

BPA-fructose Alone. When BPA was administered ip, the tumor concentration of boron was around 16 ppm, and the ratios tumor/blood and tumor/thyroid were 4.1 and 3.7, respectively (Table 1). Within experimental error, these values are identical to those we have previously found at this dose.[3]

Combined Administration of BOPP and BPA. The results of studies performed with the combined administration of BOPP and BPA are shown in Table 2. The tumor boron concentration rose from a value of 23 ppm when the compounds were given 1 day apart (day 1) to a peak of 45 ppm when the time differential was 5 days, and then declined to 38 ppm with a 1 week separation. As expected, blood boron concentrations were highest (24 ppm) with only a 1 day separation and decreased steadily throughout the observation period, reaching a value of 11 ppm on day 7. A similar behavior was observed for kidney and liver boron concentrations. At 5 days separation, the most important selectivity ratios were tumor/blood ∼3.8 and tumor/normal thyroid ∼6.6 (Figure 1; Table 2). Other important ratios were tumor/distal skin ∼1.8 and tumor/lung ∼2.9.

Subcellular Localization. The studies of cellular localization showed that BOPP localizes inside the ARO cells in agreement with previous studies of other tumor cell lines.[6,7] The subcellular fractionation studies were performed in ARO cells incubated with BOPP at a final concentration of 60 μg/mL. These studies showed that the highest boron concentration is localized in the 11500*g* pellet. (mitochondrial enriched fraction) (Table 3).

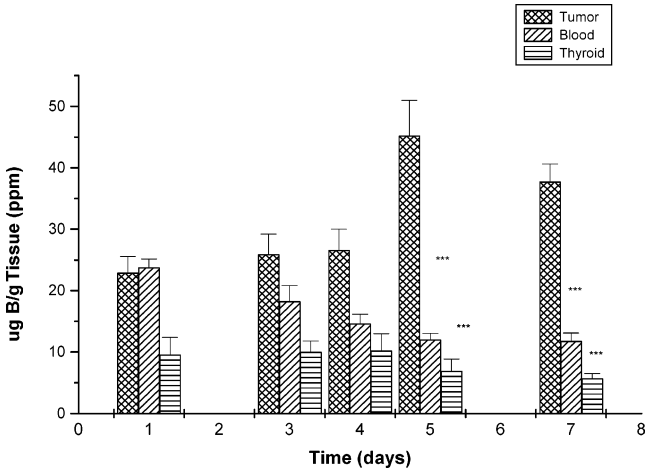

Figure 1. Boron concentrations (μg of B/g of tissue) in tumor, blood, and distal skin as a function of time between the administration of BOPP (60 mg/kg of body weight ip) and the administration of BPA-fructose (350 mg/kg of body weight ip). Each value is the mean of 5−12 samples ± SEM. (***) p (tumor vs thyroid or blood) < 0.001.

Table 3. Subcellular Distribution of BOPP in ARO Cells in Vitro[a]

subcellular fraction	boron (μg of B/μg of protein)[b]
nuclear	0.0028 ± 0.00012
mitochondria	0.0046 ± 0.00024*,**
endomembrane	0.0019 ± 0.0004

[a] Subcellular fractionation studies were performed in ARO cells incubated with BOPP at a final concentration of 60 μg/mL for 24 h. Boron concentrations (μg of B/g of tissue) were determined by ICP-OES. Values are the mean ± SEM of 3 different experiments. [b] (*) p < 0.05 vs nuclei; (**) p < 0.01 vs endosomes.

Discussion

Optimizing the therapeutic efficacy of boron neutron capture therapy is one of the long-term objectives of the many researchers and clinicians worldwide currently involved with clinical trials using the boronated amino acid *p*-boronophenylalanine. One of the potentially most effective means of accomplishing this goal is to increase the boron content of the tumor. However, simply increasing the dose of BPA does not lead to a proportionate rise in tumor boron levels and generally results in decreased tumor-to-blood and tumor-to-normal-tissue selectivity ratios. Although the mechanistic basis for BPA uptake through the cell membrane

remains somewhat unclear, there is in vitro evidence that the neutral amino acid L system is responsible.[12] Previous studies in our laboratory examining BPA in a mouse model of undifferentiated thyroid carcinoma showed a strong time dependence of peak tumor boron concentrations. At a dose of 350 mg/kg delivered ip, maximal tumor boron uptake of ~17 μg/g was observed in these earlier studies to occur at 60 min after administration with corresponding tumor-to-blood and tumor-to-normal-thyroid ratios of ~4.7 and ~3.1, respectively. Rather similar values were found in the present study at this dose. When the dose of BPA was increased to 600 mg/kg, the time of maximal tumor boron uptake was lengthened to 90 min but the absolute concentration was increased only to 23.9 μg/g and the corresponding selectivity ratios dropped to ~3.1 and ~2.8. These ratios even remained suppressed when measured at longer times (120 and 150 min). In a subsequent study, we showed that even this relatively small increase in tumor boron content could lead to dramatic differences in tumor control by BNCT.[5] With the lower dose of BPA, tumor growth was slowed but not halted, whereas at the higher dose there was an almost complete control of tumor growth in 100% of the mice until day 30 after irradiation. Thus, even incremental increases in tumor boron content can have a substantial influence on treatment outcomes in this animal model, and we sought other methods of raising tumor boron concentrations while simultaneously preserving tumor selectivity.

Boronated porphyrins bearing polyhedral carboranes or borane anions have been reported by us and others to have significant potential for binary therapies such as BNCT and photodynamic therapy (PDT).[6−8,13−15] In particular, the carborane-bearing protoporphyrin derivative BOPP has been shown to have strong tumor localizing and retention properties in other solid tumors, and a significantly different pharmacokinetic and pharmacodynamic profile from BPA. In mice with intracerebrally implanted brain tumors, ip or iv treatment with BOPP produced maximal tumor boron uptake at ~24 h with very high selectivity for tumor over normal brain (up to 400:1) and good tumor/blood ratios.[6] Also in contrast to BPA, approximately 80% of the maximum tumor boron is retained in the tumor for at least 3 days. There is also substantial evidence that BOPP is taken up by cells through its association with plasma lipoproteins. Callahan et al. have shown in vitro that the uptake of BOPP in human glioma cells and fibroblasts depends directly on the amount of lipoproteins in the medium and that the compound is internalized through the LDL receptor.[16] Like most human tumor cell lines, the LDL receptor status of ARO cells has not been measured, but the aggressive, rapid growth of this tumor suggests the potential for high levels of LDLR expression. Since its pharmacokinetic behavior and mechanism of tumor cell uptake are quite different from those of BPA, we hypothesized that BOPP might be a suitable companion drug to use with BPA to obtain increased tumor boron.

The biodistribution results obtained with BOPP alone in our UTC tumor in nude mice failed to demonstrate a significant selective tumor uptake. At both 10 mg/kg and 100 mg/kg tumor boron levels at 24 h were significantly below the levels generally considered the minimum for successful therapy. Highest levels of boron were found in liver and spleen, the probable sites of metabolism. In fact, the organ uptake profile for BOPP in this tumor model was generally similar to that seen previously for BOPP and other boronated porphyrins in other models. It has previously been shown in tumor bearing mice that the pharmacokinetic behavior of BOPP following either ip or iv administration is characterized by a peak tumor concentration at 1 day after administration,[6] identical to its behavior in the present study. In the prior mouse study, this 24 h peak was followed by a steady decline in tumor boron levels over time out to 3 days. It thus seems highly probable that the tumor pharmacokinetic behavior of BOPP alone is similar in our nude mouse model. The biodistribution of BPA alone confirmed previous data showing that there is selective uptake by the tumor and by the surrounding skin, which is usually infiltrated with tumor cells.

However, combined administration of BOPP and BPA, assayed at different time intervals between administrations, clearly produces a significant improvement in the tumor boron concentration, which reached 45 ppm 5 days after BOPP−BPA injection. This amount of boron greatly exceeds that established as a requirement for a successful neutron capture therapy and confirms our hypothesis that the combination of drugs might be better than either drug alone. At earlier times (1−4 days), there seems to be an additive accumulation of boron due to both BOPP and BPA uptake. These studies were performed at different time intervals between the administration of BOPP and BPA in order to find the optimum time at which the concentration of boron in the nontumor tissues decreases, thus allowing us to obtain the best ratios, as already shown by other studies.[6−8] The

(12) Wittig, A.; Sauerwein, W. A.; Coderre, J. A. Mechanisms of Transport of *p*-Borono-Phenylalanine Through the Cell Membrane *In Vitro*. *Radiat. Res*. **2000**, *153*, 173−180.

(13) Miura, M.; Micca, P. L.; Fisher, C. D.; Gordon, C. R.; Heinrichs, J. C.; Slatkin, D. N. Evaluation of Carborane-Containing Porphyrins as Tumour Targeting Agents for Boron Neutron Capture Therapy. *Br. J. Radiol*. **1998**, *71*, 773−781.

(14) Matsumura, A.; Shibata, Y.; Yamamoto, T.; Yoshida, F.; Isobe, T.; Nakai, K.; Hayakawa, Y.; Kiriya, M.; Shimojo, N.; Ono, K.; Sakata, I.; Nakajima, S.; Okumura, M.; Nose, T. A. New Boronated Porphyrin (STABX909) for Neutron Capture Therapy: An *In Vitro* Survival Assay and *In Vivo* Tissue Uptake Study. *Cancer Lett*. **1999**, *141*, 203−209.

(15) Hill, J. S.; Kahl, S. B.; Stylli, S. S.; Nakamura, Y.; Koo, M.-S.; Kaye, A. H. Selective Tumor Kill of Cerebral Glioma by Photodynamic Therapy Using a Boronated Porphyrin Sensitizer. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12126−12130.

(16) Callahan, D. E.; Forte, T. M.; Afzal, J.; Deen D. F.; Kahl, S. B.; Bjornstad, K. A.; Bauer, W. F.; Blakely, E. A. Boronated Protoporphyrin (BOPP): Localization in Lysosomes of the Human Glioma Cell Line SF-767 with Uptake Modulated by Lipoprotein Levels. *Int. J. Radiat. Oncol., Biol., Phys.* **1999**, *45*, 761−777.

tumor/blood and tumor/thyroid ratios showed improved values of around 3.8 and 6.6, respectively, over BOPP alone and were equivalent to or better than similar values for BPA alone. Moreover, it is known that one of the main organs where this tumor produces metastasis is the lung, and the tumor/lung ratio was around 2.9, again better than the ratio for either drug alone. The underlying explanation for these results appears to reside in the quite different pharmacokinetic behaviors of BOPP and BPA. Boron from BPA rises in all organs relatively quickly compared with BOPP and also decreases in most tissues except tumor at a relatively rapid rate. In particular, liver boron levels in animals treated with BPA never become very substantial since this organ is not primarily responsible for BPA metabolism. BPA appears to be metabolized primarily by the kidney, perhaps in the form of boric acid resulting from cleavage of the boron−phenyl ring bond. For example, in our previous study in nude mice, kidney boron levels maximized within the first 30 min after injection and remained higher than in any other tissue except tumor at all subsequent time points.[3] The metabolism of BOPP follows a very different pattern and appears to be largely hepatic. Tibbitts et al. observed in dogs treated with BOPP that the boron concentration in the bile after 10−28 days is rather low and suggested that BOPP might have a low enterohepatic circulation.[8] In the biodistribution studies of BOPP performed in a variety of small and large animals, a high concentration of boron was always found in the liver.[6−8] It was proposed that this organ might accumulate the compound at early times, acting as a reservoir, and releasing it or a metabolite into the circulation at later times (5−7 days). This proposition is at least consistent with our present results.

The present data also clearly show the intracellular localization of BOPP in UTC ARO cells, similar to the observations made in both rat and human glioma cells.[6,16] Fluorescence photomicrography of UTC cells treated with BOPP showed the intense characteristic red fluorescence of BOPP clearly localized within the cytoplasm (not shown). The subcellular distribution studies of BOPP showed that most of the boron is localized in the 11500*g* pellet. This pellet is enriched with mitochondria, and such localization agrees with previous results obtained by Hill et al. in the C6 rat glioma cell line.[6] However, these results contrast with those of Callahan and co-workers, who found BOPP primarily localized in lysosomes of SF 767 human glioma cells.[16]

Conclusions

To our knowledge, these results represent the first reported example of the successful tumor-selective delivery of therapeutic amounts of boron through use of a “cocktail” of two BNCT drugs in a human tumor model in vivo. Taken together, these data support an encouraging view of the possible application of the complete BNCT procedure in an experimental model of human UTC by the combined administration of BOPP and BPA. In vivo irradiation studies are currently being performed toward this goal. Preliminary results from such a study may be found in ref 17.

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(17) Viaggi, M.; Dagrosa, M. A.; Longhino, J.; Blaumann, H.; Calzetta, O.; Kahl, S. B.; Juvenal, G. J.; Oisarev, M. A. Boron Neutron Capture Therapy for Undifferentiated Thyroid Carcinoma: Preliminary Results with the Combined Use of BPA and BOPP. *Appl. Radiat. Isot.* **2004**, *61*, 905−909.