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Biodistribution of GB-10 ($Na_2{}^{10}B_{10}H_{10}$) compound for boron neutron capture therapy (BNCT) in an experimental model of oral cancer in the hamster cheek pouch

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Summary *Objective:* We previously proposed the hamster cheek pouch model of oral cancer for BNCT studies. We herein present the biodistribution of a non-toxic boron compound, GB-10 ($Na_2{}^{10}B_{10}H_{10}$), in this model to assess its potential for BNCT or BNCT enhanced Fast Neutron Therapy. *Materials and methods:* We evaluated the uptake and retention of GB-10 in tumour and precancerous tissue and in potentially dose-limiting, clinically relevant normal tissues. *Results:* Mean tumour boron concentration delivered by GB-10 (50 mg B/kg) peaked to 77.7 ± 28.0 ppm at 20 min post-administration and remained at therapeutically useful values of 31.9 ± 21.4 ppm at 3 h. The clearance rate for normal tissues was faster than for tumour tissue. The consistently low brain and spinal cord values would preclude normal tissue toxicity. The uptake of GB-10 by precancerous tissue may be of potential use in the treatment of field cancerized areas. GB-10 was deposited homogeneously in different tumour areas, an asset when treating heterogenous tumours. The data suggests that the joint administration of BPA and GB-10 may improve the therapeutic efficacy of BNCT. *Conclusions:* GB-10 is a potential boron carrier for BNCT of head and neck tumours and for BNCT-FNT.

Introduction

Boron Neutron Capture Therapy (BNCT) is a binary cancer treatment modality that involves the selective accumulation of ^{10}B carriers in tumours followed by irradiation with a thermal or epithermal

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neutron beam. The high linear energy transfer alpha particles and recoiling ^{7}Li nuclei emitted during the capture of a thermal neutron by a ^{10}B nucleus[1,2] have a range of approximately 5–9 μm in tissue and are known to have a high relative biological effectiveness.[3] In this way, BNCT would potentially target neoplastic tissue selectively.[4,5] The success of BNCT is dependent on the absolute amount of boron in the tumour as well as on the ratios of boron concentration in tumour relative to blood and normal tissue. Neutron irradiations in BNCT are ideally performed at a time after administration of the boron compound when the optimum combination of high absolute boron concentration in tumour and high tumor/normal tissue and tumor/blood ratios occur.[6] As during any radiation therapy, the therapeutic tumour dose that can be administered will be limited by the tolerance of the surrounding normal tissue within the treatment volume.[7] Clinical trials of BNCT for the treatment of glioblastoma multiforme and/or melanoma using the commercially available boron compounds boronophenylalanine (BPA) or sodium mercaptoundecahydrododecarborane (BSH) have been performed at various times over the past 30 years, e.g.[5,8–10] Continued trials are currently in progress in the US, the Netherlands, Finland, Sweden, the Czech Republic and Japan. Contributory experimental studies have been carried out employing a variety of experimental models based on the implantation of tumour cells in normal tissue.[11–17] The current status of experimental and clinical BNCT warrants research on experimental models to contribute to the knowledge of BNCT and to devise strategies to improve the therapeutic advantage of this technique.

Within this context, we previously proposed and validated the hamster cheek pouch model of oral cancer for BNCT studies. This model serves to explore new applications of the technique, study the biology and radiobiology of BNCT, assess the biodistribution and pharmacokinetics of traditional and novel and/or alternative boron compounds and assess the response of tumour, precancerous tissue and potentially dose-limiting normal tissues. To date we have performed a biodistribution and pharmacokinetic study with BPA[18] and have reported the first evidence of the usefulness of BPA-mediated BNCT for the treatment of oral cancer in this experimental model.[19] Furthermore, we demonstrated that the boronated porphyrin CuTCPH, a novel boron compound, affords an advantage in terms of selectivity and absolute tumour uptake and would be potentially useful for the treatment of oral cancer in an experimental model.[20]

The hamster cheek pouch is the most widely accepted model of oral cancer.[21] The pouch anatomically resembles a pocket that is easily accessible to local tumour induction and can readily be everted for local treatment such as irradiation and macroscopic follow-up. Carcinogenesis protocols induce premalignant changes and carcinomas that closely resemble human lesions.[22] Tumours are induced by a process that mimics the spontaneous process of malignant transformation rather than by the growth of implanted tumour cells in healthy tissues as in other BNCT experimental models, e.g.[12,17] In this way, the hamster model allows for the study of precancerous tissue around the tumour, a tissue that is not available in the BNCT models used to date. Given the phenomenon of field cancerization, e.g.[23] the study of precancerous tissue is clinically relevant. Multiple primary tumours are a known phenomenon in head and neck cancer[24] and local-regional recurrence is a major concern.[25] Carcinomas would arise from multifocal areas of precancerous change involved in the process of field cancerization.[26]

There is a clinical rationale to explore the potential therapeutic advantage of BNCT in this model. Head and neck cancer patients are generally treated with surgery combined with radiotherapy and chemotherapy. Given that the survival rate for head and neck squamous cell carcinoma has remained at 52% over the past 20 years[25] and that radical tumour surgery often results in large tissue defect,[27] head and neck cancer patients may potentially benefit from alternative therapeutic strategies.[28]

Within the context of searching for alternative boron compounds that may afford a therapeutic advantage, the aim of the present study was to perform a biodistribution and pharmacokinetic study of the boron compound GB-10 ($Na_2{}^{10}B_{10}H_{10}$) provided by Neutron Therapies, LLC, USA, in the hamster cheek pouch oral cancer model. GB-10 forms the anion $(B_{10}H_{10})^{-2}$ in aqueous solution.[29] It is manufactured by oxidation of decaborane and has no special handling or storage requirements. GB-10 is a largely diffusive agent that does not traverse the intact Blood Brain Barrier. It has been shown to be non-toxic in dogs[30] and in 15 volunteer subjects with glioblastoma multiforme or non-small cell lung cancer who underwent a pharmacokinetic trial with GB-10 at the University of Washington.[31] GB-10 has been proposed as a boron agent for BNCT and BNCT enhanced Fast Neutron Therapy (BNCT/FNT).

GB-10 was tested with encouraging results for BNCT/FNT for non-small cell lung cancer in canine patients.[30,32] The clinical utility of boron neutron capture with thermal/epithermal neutron beams has been limited to tumours located at relatively shallow depths such as in the brain and skin. Capture of the thermal neutron component within a predominantly

fast neutron beam could selectively enhance cytotoxicity in deeply located tumours, such as lung cancer. In this way, this treatment may be applicable to greater numbers of cancer patients.[30,33] Because of the steep nature of the dose—response curve for fast neutrons a selective tumour augmentation with high linear energy transfer radiation has the potential for a substantial increase in the probability of tumour control.[34]

Within this context, GB-10 would be a promising Boron compound that requires further study. The hamster cheek pouch oral cancer model allows for the examination of the properties of novel Boron compounds in tumour tissue, precancerous tissue and clinically relevant dose-limiting normal tissues. Furthermore, this type of study expands the knowledge of the biology of BNCT. Deeper insight into the different aspects of the complex BNCT technique may conceivably contribute to improve its therapeutic efficacy.

The aim of the present biodistribution study of GB-10 in the hamster cheek pouch oral cancer model was to assess the potential of GB-10 as a boron carrier for BNCT treatment of head and neck tumours, to investigate the biodistribution in tumour, precancerous and healthy tissues of a compound unable to pass through the intact Blood Brain Barrier and to examine the distribution in these tissues of a compound proposed for use in Fast Neutron Therapy.

Materials and methods

Tumor induction

Tumours were induced in the right cheek pouch of noninbred young (6 weeks old) Syrian hamsters by topical application of 0.5% dimethyl-1,2-benzanthracene (DMBA) in mineral oil three times a week for 14 weeks in keeping with a standard hamster cheek pouch carcinogenesis protocol.[35] The protocol ensures humane practices. The treated pouch was periodically everted under light intraperitoneal (i.p.) ketamine (70 mg/kg)—xylazine (10.5 mg/kg) anesthesia and examined to monitor tumour development. Once the exophytic tumours had developed and reached a diameter of approximately 3-5 mm, the animals were used for biodistribution studies.

GB-10 biodistribution studies

We employed GB-10 provided by Neutron Therapies, L.L.C, USA, isotopically enriched to over 99% in ^{10}B. $A \approx 100$ mg ^{10}B/ml stock solution was diluted tenfold with water. GB-10 was administered as a bolus injection in the surgically exposed jugular vein of animals anesthetized with an i.p. injection of ketamine (140 mg/kg) and xylazine (21 mg/kg) followed by skin suture in keeping with a technique developed previously by our laboratory.[36] Survival from the surgical procedure was 100%. We delivered a loading dose of 50 mg B/kg body weight (b.w.). Blood samples were taken from the jugular vein under i.p. ketamine (140 mg/kg)—xylazine (21 mg/kg) anesthesia at different post-administration times as indicated in the corresponding tables. The animals were sacrificed by overdose of i.p. ketamine—xylazine anesthesia 5—10, 15—20, 30—40, 60 and 90 min and 2, 3, 4 and 6 h post-administration of the compound. The following tissues were sampled: tumour, precancerous pouch tissue around tumour (pouch treated with DMBA), normal pouch tissue (nontreated pouch), cheek mucosa, cheek skin, palate mucosa, tongue, parotid gland, liver, spleen, lung, brain and spinal cord. All of the samples were weighed immediately. Tissue samples were stored at $-70\,^{\circ}C$ and blood samples were stored at $4\,^{\circ}C$ until use.

Boron analysis

Boron analysis was performed by Atomic Emission Spectroscopy with Inductively Coupled Plasma (ICP-AES). Tissue samples ($\leq$50 mg) were digested at room temperature overnight (or at $60\,^{\circ}C$ for 1 h) in 0.15 ml of a 1:1 mixture of concentrated sulphuric and nitric acids at a concentration of 50 mg tissue/ml acid solution. Addition of 0.5 ml of a 10% solution of the detergent Triton X-100 and dilution to 1 ml with water resulted in a clear solution for ICP-AES analysis.[6] Blood samples (200—300 μl) were prepared by adding 2.5% Triton X-100 at a final concentration of 0.1% in water (final volume 5 ml). Yttrium was added to the samples as an internal standard. Standard solutions of boric acid were used to prepare a calibration line during each day of operation. GB-10 biokinetics curves were obtained for blood and each of the tissues. The statistical significance of the differences between boron content of the different tissues at a given post-administration time was assessed by a one-way ANOVA. An overall level of significance was taken to be $a = 0.05$. If the residuals were not normally distributed then logarithmic transformations of the data were performed to normalize the data.

Alternative GB-10 administration protocol

Profiting from a potential difference in clearance between tumour and normal tissue at 3 h post-administration we assessed boron uptake as

previously described following administration of a single dose of GB-10 (50 mg B/kg b.w.) and a second identical dose 3 h later. The animals were sacrificed 30—60 min or 2 h after the second dose of GB-10.

GB-10 uptake by predominantly viable and predominantly necrotic tumour areas

Given the therapeutic importance of targeting all tumour cells, we assessed the degree of homogeneity of GB-10 uptake by different tumour areas. For this purpose, during tissue sampling, when possible, we dissected the available areas of tumour that appeared to be predominantly necrotic on visual inspection and processed the tissue for boron analysis separately from the remaining tumour area that appeared to be predominantly viable on visual inspection. Tissue processing and boron analysis were performed as previously described. In each case, the ratio of GB-10 uptake between both areas was established. Given the scarce amount of necrotic tissue that could be dissected from a single tumour, a correlation between boron analysis and histology was not attempted.

Combined administration of GB-10 and BPA

To explore the possibility of profiting from the differential properties of both GB-10 and BPA to improve the potential therapeutic efficacy of BNCT, we assessed uptake of boron delivered by a combination of GB-10 and BPA as indicated in the protocols below. The doses, time points and route for BPA administration were selected based on previous work by our laboratory.[18,19]

1. GB-10 (i.v.), 50 mg B/kg b.w. + BPA (i.p.), 15.5 mg B/kg. The animals were sacrificed 3 h post-administration of GB-10 and 3.5 h post-administration of BPA. For comparative purposes the compounds were also administered separately according to the same protocol.
2. GB-10 (i.v.), 34.5 mg B/kg b.w. + BPA (i.p.), 31 mg B/kg. The animals were sacrificed 3 h post-administration of GB-10 and 3.5 h post-administration of BPA. For comparative purposes the compounds were also administered separately according to the same protocol.
3. GB-10 (i.v.), 34.5 mg B/kg b.w. + BPA, 31 mg B/kg b.w. total dose, administered as fractionated i.p. injections at 20 min intervals over a 3 h period to simulate an infusion. The animals were sacrificed 3 h post-administration of GB-10 and 1.5 h after the last i.p. injection of BPA. For comparative purposes the compounds were also administered separately according to the same protocol.

Blood and tissue sampling and processing and boron analysis were performed as previously described.

In all cases the national and institutional regulations for the care and use of laboratory animals were strictly followed.

Results

No visible signs of toxicity induced by either of the boron compounds were observed in any of the animals in terms of behaviour, appearance, and body weight changes in keeping with the studies on GB-10 by Stelzer et al.[30] and Diaz et al.[31] and the widespread literature on BPA, e.g.[18]

Following administration of GB-10 at a dose of 50 mg B/kg b.w., mean tumour boron concentration peaked at 20 min post-administration to 77.7 ± 28.0 ppm and remained at 31.9 ± 21.4 ppm at 3 h. Values for blood, precancerous tissue, normal oral tissues and skin did not differ significantly from tumour values (Table 1). Clearance from normal tissues was faster than from tumour tissue, i.e. mean boron values in normal pouch tissue fell to 28.8% of the peak value in 3 h whereas mean boron tumour values fell to only 41.1% of the peak tumour value in the same time (Table 1). There was no preferential accumulation of GB-10 in spleen and liver as compared to other normal tissues such as skin. Certain boron compounds such as boronated porphyrins do accumulate in liver and spleen.[20] This is a concern if liver and spleen are included in the treatment volume. Lung tissue took up GB-10 similarly to normal oral tissues and skin. Brain and spinal cord values remained consistently low and below tumour values at all time points. These differences reached high statistical significance between 20 min and 3 h post-administration (*P*-values ranged from 0.0000 to 0.0030 for brain versus tumour and from 0.0000 to 0.0133 for spinal cord versus tumour). At 3 h post-administration the mean tumor/brain boron ratio was 22.8/1 and the mean tumor/spinal cord ratio was 17.7/1 (Table 1).

The administration of two successive doses of GB-10 improved absolute uptake by tumour tissue but failed to afford an advantage in terms of selective incorporation of boron to tumour versus normal cheek pouch tissue (Table 2).

The analysis of boron concentration delivered by GB-10 in predominantly viable and in predominantly necrotic tumour tissue revealed that GB-10 uptake would not depend on cell viability, unlike BPA incorporation.[6] We previously reported a mean

Table 1 Boron concentration (ppm) in blood and tissue simples at different times (in min or h as indicated) after administration of GB-10 at a dose of 50 mg B/kg b.w.

Tissue	Time								
	5′–10′	15′–20′	30′–40′	60′	90′	2 h	3 h	4 h	6 h
Blood	120.5 ± 37.7 ($n = 9$)	90.0 ± 39.4 ($n = 19$)	75.0 ± 46.2 ($n = 22$)	62.9 ± 29.0 ($n = 17$)	52.9 ± 20.0 ($n = 10$)	41.2 ± 14.8 ($n = 12$)	31.8 ± 6.4 ($n = 5$)	37.3 ± 21.9 ($n = 7$)	20.7 ± 11.0 ($n = 4$)
Tumor	41.3 ± 21.3 ($n = 6$)	77.7 ± 28.0 ($n = 7$)	42.3 ± 20.7 ($n = 16$)	32.3 ± 8.3 ($n = 8$)	40.4 ± 13.5 ($n = 12$)	29.0 ± 18.5 ($n = 15$)	31.9 ± 21.4 ($n = 11$)	34.6 ± 25.9 ($n = 12$)	17.3 ± 7.9 ($n = 10$)
Tissue around tumor	44.3 ± 5.7 ($n = 3$)	83.2 ± 21.1 ($n = 3$)	64.0 ± 24.2 ($n = 6$)	42.5 ± 8.3 ($n = 6$)	41.8 ± 9.3 ($n = 5$)	25.5 ± 7.9 ($n = 6$)	34.3 ± 16.9 ($n = 4$)	38.3 ± 27.7 ($n = 3$)	17.9 ± 11.1 ($n = 4$)
Normal pouch tissue	55.4 ± 21.9 ($n = 3$)	74.9 ± 37.8 ($n = 2$)	49.3 ± 14.8 ($n = 6$)	43.8 ± 7.7 ($n = 6$)	37.7 ± 9.9 ($n = 5$)	25.1 ± 7.2 ($n = 6$)	21.6 ± 7 ($n = 5$)	34.4 ± 31.3 ($n = 3$)	14.8 ± 10.2 ($n = 4$)
Cheek mucosa	69.8 ± 9.9 ($n = 3$)	75.4 ± 21.1 ($n = 3$)	48.7 ± 15.2 ($n = 6$)	34.9 ± 9.8 ($n = 5$)	37.5 ± 10.1 ($n = 4$)	19.5 ± 2.9 ($n = 5$)	17.4 ± 8.4 ($n = 5$)	29.0 ± 18.6 ($n = 3$)	15.3 ± 10.2 ($n = 3$)
Cheek skin	58.7 ± 26.8 ($n = 3$)	70.2 ± 35.5 ($n = 3$)	51.3 ± 3.5 ($n = 6$)	38.6 ± 10.7 ($n = 6$)	41.3 ± 12.4 ($n = 5$)	27.4 ± 9.7 ($n = 5$)	26.2 ± 10.5 ($n = 5$)	36.7 ± 27.7 ($n = 3$)	14.9 ± 9.0 ($n = 4$)
Palate mucosa	99.8 ± 11.8 ($n = 3$)	97.2 ± 29.5 ($n = 3$)	55.6 ± 21.4 ($n = 5$)	58.5 ± 18.4 ($n = 6$)	45.7 ± 10.6 ($n = 5$)	31.0 ± 11.8 ($n = 6$)	27.1 ± 11.6 ($n = 5$)	37.6 ± 29.0 ($n = 3$)	19.0 ± 14.6 ($n = 4$)
Tongue	122.1 ± 72.1 ($n = 3$)	74.4 ± 10.1 ($n = 3$)	50.6 ± 23.9 ($n = 6$)	41.9 ± 19.6 ($n = 5$)	26.0 ± 21.0 ($n = 4$)	19.9 ± 5.4 ($n = 6$)	18.4 ± 11.7 ($n = 5$)	35.4 ± 14.7 ($n = 3$)	13.9 ± 10.2 ($n = 3$)
Parotid gland	43.9 ± 33.6 ($n = 3$)	30.0 ± 13.5 ($n = 3$)	19.6 ± 11.1 ($n = 6$)	15.3 ± 4.3 ($n = 6$)	17.0 ± 8.2 ($n = 3$)	10.4 ± 5.5 ($n = 6$)	9.5 ± 4.1 ($n = 5$)	14.2 ± 6.1 ($n = 3$)	8.9 ± 7.0 ($n = 4$)
Liver	95.0 ± 48.3 ($n = 3$)	90.0 ± 34.9 ($n = 3$)	87.8 ± 40.3 ($n = 6$)	72.8 ± 42.3 ($n = 6$)	42.7 ± 18.4 ($n = 5$)	44.1 ± 18.0 ($n = 6$)	27.9 ± 11 ($n = 4$)	13.8 ± 3.5 ($n = 3$)	33.0 ± 14.1 ($n = 4$)
Spleen	63.8 ± 10.2 ($n = 3$)	64.8 ± 27.5 ($n = 3$)	34.4 ± 7.0 ($n = 6$)	36.6 ± 11.3 ($n = 5$)	29.7 ± 8.8 ($n = 5$)	18.9 ± 5.7 ($n = 6$)	19.1 ± 8.3 ($n = 5$)	29.5 ± 21.5 ($n = 3$)	19.1 ± 14.9 ($n = 4$)
Lung	68.8 ± 32.9 ($n = 2$)	94.8 ± 7.3 ($n = 2$)	47.1 ± 9.6 ($n = 5$)	44.1 ± 12.1 ($n = 6$)	40.8 ± 11.8 ($n = 5$)	33.0 ± 15.8 ($n = 6$)	26.5 ± 23.1 ($n = 5$)	32.5 ± 21.8 ($n = 3$)	16.8 ± 9.6 ($n = 3$)
Brain	5.4 ± 2.7 ($n = 3$)	6.5 ± 2.1 ($n = 3$)	4.1 ± 1.2 ($n = 5$)	3.1 ± 1.6 ($n = 6$)	5.1 ± 4.2 ($n = 5$)	2.8 ± 2.3 ($n = 6$)	1.4 ± 1.1 ($n = 5$)	2.9 ± 1.8 ($n = 3$)	1.5 ± 0.7 ($n = 4$)
Spinal cord	16.2 ± 14.6 ($n = 2$)	9.2 ± 4.4 ($n = 4$)	7.9 ± 4.0 ($n = 6$)	5.4 ± 4.2 ($n = 6$)	7.4 ± 4.1 ($n = 5$)	2.9 ± 0.6 ($n = 4$)	1.8 ± 1.2 ($n = 4$)	2.2 ± 0.3 ($n = 2$)	7.7 ± 5.5 ($n = 2$)

Results are expressed as mean ± S.D. The number of samples per condition is indicated in parenthesis.

Table 2 Boron concentration (ppm) in blood and tissue samples following the administration of two successive doses of GB-10 as indicated.

Tissue	Administration protocol			
	GB-10 (50 mg B/kg) (1 h post-admin.) ($n = 5–17$)	GB-10 → 3 h → GB-10 (0.5–1 h post-admin.) ($n = 2–10$)	GB-10 (50 mg B/kg) (2 h post-admin.) ($n = 4–15$)	GB-10 → 3 h → GB-10 (2 h post-admin.) ($n = 2–7$)
Blood	62.9 ± 29.0	98.0 ± 29.5	41.2 ± 14.8	43.2 ± 12.3
Tumor	32.3 ± 8.3	79.0 ± 37.6	29.0 ± 18.5	49.1 ± 11.7
Tissue around tumor	42.5 ± 8.3	109.7 ± 20.8	25.5 ± 7.9	61.0 ± 10.2
Normal pouch	43.8 ± 7.7	76.1 ± 31.1	25.1 ± 7.2	53.5 ± 0.3
Cheek mucosa	34.9 ± 9.8	84.5 ± 34.0	19.5 ± 2.9	44.0 ± 1.3
Cheek skin	38.6 ± 10.7	74.0 ± 12.0	27.4 ± 9.7	52.0 ± 4.7
Palate mucosa	58.5 ± 18.4	130.4 ± 52.4	31.0 ± 11.8	65.8 ± 0.3
Tongue	41.9 ± 19.6	108.2 ± 45.9	19.9 ± 5.4	46.6 ± 2.5
Parotid gland	15.3 ± 4.3	36.0 ± 14.3	10.4 ± 5.5	23.3 ± 2.3
Liver	72.8 ± 42.3	268.2 ± 160.4	44.1 ± 18.0	108.7 ± 14.1
Spleen	36.6 ±11.3	74.0 ± 33.5	18.9 ± 5.7	78.6 ± 27.5
Lung	44.1 ± 12.1	62.1 ± 45.3	33.0 ± 15.8	53.3 ± 6.5
Brain	3.1 ± 1.6	7.6 ± 4.1	2.8 ± 2.3	4.1 ± 1.2
Spinal cord	5.4 ± 4.2	20.9 ± 5.0	2.9 ± 0.6	8.1 ± 1.3

The data for the corresponding single dose has been included for comparison purposes. Results are expressed as mean ± S.D. The number of samples per condition is indicated in parenthesis.

ratio of BPA uptake for predominantly viable/predominantly necrotic tissue in the present model of approximately 1.46/1 ($32.0 \pm 13.5/18.5 \pm 2.0$).[18] The ratio of GB-10 uptake for predominantly viable/predominantly necrotic tumour tissue was $0.97 \pm 0.21/1$, indicating markedly homogenous targeting of different tumour areas (Table 3).

The results of boron concentration for the different tissues following the combined administration of GB-10 and BPA according to the protocols described in the Material and Methods section are presented in Table 4. The data corresponding to the administration of the 2 compounds separately are presented for comparison purposes. In the case of protocols 1 and 2, the separate doses appeared to sum linearly to first approximation in the combined administration for all tissues except for tumour tissue, which exhibited lower than expected boron concentration values. This finding may suggest a possible antagonistic effect in tumour with this administration protocol. Protocol 3 involved the administration of BPA over a 3 h period in combination with GB-10 administration as indicated in the Materials and Methods section and resulted in a statistically significant ($P = 0.0000$) improvement in absolute mean tumour uptake in tumour as compared to protocol 2, in which BPA was administered as a bolus injection (121.7 ppm versus 59.7 ppm). Furthermore, tumor/normal pouch mean boron concentration ratios rose from 1.6/1 (59.7/38.1) to 2.0/1 (121.7/61.6) (Table 4). The administration of BPA as a simulated infusion in combination with GB-10 seemed to produce a synergistic effect in tumour tissue. More specifically, the expected mean boron concentration obtained by summing the values for the compounds administered separately, 14.5 ppm (GB-10, 34.5 mg/kg b.w.) + 59.9 ppm (BPA, 34.5 mg B/kg b.w., simulated infusion) would be 74.4 ppm. However, the corresponding actual value for the combined administration was 121.7 ± 38.6 ppm (Table 4). The administration of BPA, alone or combined with GB-10, elicited a rise in brain and spinal cord boron concentration values as compared to a comparable dose of GB-10 alone. However, in all cases, brain and spinal cord values remained well below tumour values. In particular, in the case of the administration of GB-10 combined with BPA as a simulated infusion (Protocol 3), the mean boron concentration ratios were 9.3/1 (121.7/13.1) for tumor/brain and 12.8/1 (121.7/9.5) for tumor/spinal cord (Table 4).

Discussion

The present study shows the biodistribution of GB-10 in an experimental tumour model without a Blood Brain Barrier (BBB). The fact that GB-10 does not traverse the intact BBB could be used to advantage to selectively target brain tumours surrounded by a damaged BBB. In this sense, selective uptake of GB-10 by tumour tissue versus normal tissue would

Table 3 Boron concentration (ppm) in predominantly viable and predominantly necrotic tumor tissue in the cases in which it was possible to dissect representative portions from a single tumor for separate evaluation.

Dose GB-10 mg B/kg b.w.	Time post-administ.	Predominantly viable tissue	Predominantly necrotic tissue	Ratio *V/N*
50	20′	69.4	53.6	1.3
			52.3	
50	30′	28.8	60.4	0.5
		27.6	33.7	0.8
		29.0	32.0	0.9
		55.2	47.0	1.2
50	60′	25.1	27.2	0.9
		23.9		
50	90′	39.1	45.9	0.9
		36.3		
		47.1		
		44.8		
		28.2	33.6	0.8
50	2 h	44.5	33.8	1.3
50	3 h	26.6	31.7	1.05
		24.4		
		43.6		
		32.3		
		39.8		
34.5	3 h	18.4	17.0	1.02
		21.3		
		12.6		
50	4 h	60.2	75.8	0.9
			62.5	
50	6 h	7.1	7.5	1.0
		14.6	13.0	1.1
		15.6	16.6	0.9

The cases in which several values have been recorded for predominantly viable tumor tissue correspond to cases in which the viable portion of a single tumor could be divided up into different areas for independent evaluation. The ratio of uptake of boron delivered by GB-10 was: predominantly viable tissue/predominantly necrotic tissue: 0.97 ± 0.21.

depend a priori on the presence of an intact BBB in normal tissue and a pathologically permeable BBB in tumour tissue. BNCT relies on selective targeting of tumour tissue for therapeutic efficacy.[6] Within this context GB-10 would only be eligible as a boron carrier for BNCT of organs protected by a BBB. If we are to treat with BNCT successfully we must minimize the dose to normal tissue and maximize the dose to tumour tissue. Achieving high tumor/normal tissue and tumor/blood mean boron concentration ratios is clearly an asset. However, BNCT will not be therapeutically effective if we do not target all tumour cells, regardless of their position in the tumour, metabolic status, and degree of viability among other variables. Therefore, the microlocalization and differential accumulation of ^{10}B are critical factors for the therapeutic outcome of BNCT.[37] BPA is incorporated to tumour tissue selectively. However, uptake depends on tumour cell viability, resulting in heterogenous delivery of boron to tumour cells.[6,18,38] Thus, the combined administration of boron compounds with different properties may contribute to the therapeutic advantage of BNCT. Within this context, despite the fact that a priori we expected GB-10 uptake to be only marginally selective in organs that are not protected by a BBB, we examined the biodistribution and properties of GB-10 in the hamster cheek pouch oral cancer model to assess its potential as a boron carrier for BNCT treatment of head and neck tumours and investigate the biodistribution in tumour, precancerous and healthy tissues of a compound unable to pass through the intact Blood Brain Barrier. Furthermore, the fact that GB-10 has been

Table 4 Boron concentration (mean ± S.D.) (ppm) in blood and tissue samples for the different administration protocols as indicated.

Tissue	Adm. protocol							
	GB-10 (50 mg B/kg) + BPA (15.5 mg B/kg) GB-10: 3 h post-adm. and BPA: 3.5 h post-adm.	GB-10 (34.5 mg B/kg) + BPA (31 mg B/kg) GB-10: 3 h post-adm. and BPA: 3.5 h post-adm.	GB-10 (34.5 mg B/kg) + BPA (31 mg B/kg 3 h infusion) GB-10: 3 h post-adm. BPA: 1.5 h after end of infusion	GB-10 (50 mg B/kg) 3 h post-adm.	BPA (15.5 mg B/kg) 3.5 h post-adm.	GB-10 (34.5 mg B/kg) 3 h post-adm.	BPA (31 mg B/kg) 3.5 h post-adm.	BPA (31 mg B/kg 3 h infusion) 1.5 h after end of infusion
Blood	41.3 ± 11.1 ($n = 4$)	23.0 ± 4.6 ($n = 5$)	1.5 h infus, pre GB-10: 7.8 ± 2.9, $n = 3$ Sacr. 47.9 ± 13.3 ($n = 3$)	31.8 ± 6.4 ($n = 5$)	11.5 ± 4.1 ($n = 14$)	18.7 ± 5.6 ($n = 3$)	20.4 ± 4.2 ($n = 8$)	13.4 ± 0.3 ($n = 3$)
Tumor	42.7 ± 8.7 ($n = 9$)	59.7 ± 15.2 ($n = 21$)	121.7 ± 38.6 ($n = 12$)	31.9 ± 21.4 ($n = 11$)	33.3 ± 17.4 ($n = 26$)	14.5 ± 8.3 ($n = 25$)	64.3 ± 34.4 ($n = 12$)	59.9 ± 15.7 ($n = 12$)
Tissue around tumor	49.5 ± 9.7 ($n = 4$)	45.9 ± 15.4 ($n = 5$)	58.3 ± 19.2 ($n = 3$)	34.3 ± 16.9 ($n = 4$)	19.6 ± 5.8 ($n = 8$)	27.3 ± 20.5 ($n = 4$)	19.6 ± 5.9 ($n = 5$)	30.0 ± 8.8 ($n = 3$)
Normal pouch	41.9 ± 6.9 ($n = 4$)	38.1 ± 6.1 ($n = 5$)	61.6 ± 22.4 ($n = 3$)	21.6 ± 7 ($n = 5$)	14.4 ± 4.9 ($n = 12$)	25.2 0± 9.2 ($n = 4$)	19 ± 3.8 ($n = 8$)	33.7 ± 8.2 ($n = 3$)
Cheek mucosa	38.5 ± 12.1 ($n = 4$)	50.6 ± 17.2 ($n = 5$)	63.6 ± 10.6 ($n = 3$)	17.4 ± 8.4 ($n = 5$)	18.2 ± 8.9 ($n = 7$)	18.2 ± 10.7 ($n = 4$)	18.7 ± 5.1 ($n = 5$)	50.7 ± 35.6 ($n = 3$)
Cheek skin	37.0 ± 18.2 ($n = 4$)	29.3 ± 13.2 ($n = 4$)	59.4 ± 3.1 ($n = 3$)	26.2 ± 10.5 ($n = 5$)	14.2 ± 8.3 ($n = 7$)	20.0 ± 10.1 ($n = 4$)	13.7 ± 5.4 ($n = 5$)	26 ± 16.6 ($n = 3$)
Palate mucosa	51.1 ± 12.0 ($n = 4$)	49.8 ± 17.4 ($n = 5$)	69.2 ± 13.0 ($n = 3$)	27.1 ± 11.6 ($n = 5$)	13.0 ± 4.3 ($n = 5$)	24.0 ± 14.9 ($n = 4$)	24.2 ± 12.4 ($n = 5$)	33.8 ± 4.5 ($n = 3$)
Tongue	37.6 ± 10.9 ($n = 4$)	35.2 ± 5.5 ($n = 4$)	52.0 ± 8.0 ($n = 3$)	18.4 ± 11.7 ($n = 5$)	14.1 ± 6.9 ($n = 6$)	18.4 ± 12.2 ($n = 4$)	14.3 ± 2.8 ($n = 5$)	28.7 ± 7.5 ($n = 3$)
Parotid gland	19.3 ± 6.4 ($n = 4$)	17.7 ± 4.2 ($n = 5$)	27.1 ± 8.8 ($n = 3$)	9.5 ± 4.1 ($n = 5$)	4.3 ± 2.8 ($n = 3$)	5.1 ± 3.0 ($n = 4$)	11.0 ± 2.8 ($n = 3$)	14.3 ± 2.3 ($n = 3$)
Liver	56.4 ± 17.9 ($n = 4$)	40.9 ± 20.1 ($n = 4$)	77.2 ± 13.7 ($n = 2$)	27.9 ± 11 ($n = 4$)	11.4 ± 4.5 ($n = 5$)	39.3 ± 33.2 ($n = 4$)	12.5 ± 5.4 ($n = 5$)	17.3 ± 0.6 ($n = 3$)
Spleen	27.6 ± 7.1 ($n = 4$)	30.6 ± 6.9 ($n = 4$)	59.6 ± 29.1 ($n = 3$)	19.1 ± 8.3 ($n = 5$)	12.9 ± 6.3 ($n = 6$)	15.3 ± 9.6 ($n = 4$)	14.8 ± 5.5($n = 5$)	20.2 ± 1.9 ($n = 3$)
Lung	46.1 ± 16.9 ($n = 4$)	38.2 ± 21.4 ($n = 5$)	53.6 ± 17.4 ($n = 3$)	26.5 ± 23.1 ($n = 5$)	6.2 ± 2.6 ($n = 3$)	21.6 ± 14.9 ($n = 4$)	12.0 ± 2.4 ($n = 3$)	16.1 ± 2.7 ($n = 3$)
Brain	7.5 ± 2.3 ($n = 4$)	13.3 ± 3.4 ($n = 5$)	13.1 ± 0.97 ($n = 3$)	1.4 ± 1.1 ($n = 5$)	6.1 ± 2.0 ($n = 3$)	0.6 ± 0.6 ($n = 3$)	6.9 ± 0.4 ($n = 3$)	10.1 ± 2.3 ($n = 3$)
Spinal cord	12.6 ± 6.9 ($n = 4$)	13.4 ± 0.9 ($n = 4$)	9.5 ± 2.0 ($n = 3$)	1.8 ± 1.2 ($n = 4$)	1.6 ± 1.3 ($n = 3$)	3.9 ± 2.9 ($n = 4$)	5.7 ± 0.8 ($n = 3$)	7.3 ± 1.8 ($n = 3$)
Tumor/blood	1.0/ 1	2.6/1	2.5/1	1.0/1	4.8/1	0.8/1	2.8/1	4.5/ 1
Tumor/normal pouch	1.0/ 1	1.6/1	2.0/1	1.5/1	2.4/1	0.6/1	3.1/1	1.8/ 1

proposed as an agent for BNCT-enhanced Fast Neutron Therapy[30,32] for deep-seated tumours such as small cell lung cancer confers particular value on a biodistribution study of GB-10 in an experimental tumour model without a BBB.

For BNCT to be effective, it has been estimated that tumour cells must be loaded with a minimum of 15–30 μg ^{10}B/g tissue (ppm) to elevate the Boron Neutron Capture dose to the tumour significantly above the background radiation dose. This corresponds to a boron atomic concentration of roughly 10^9 ^{10}B atoms distributed uniformly throughout a tumour cell.[5,39] Small, fixed tumor/normal tissue and tumor/blood boron concentration ratios are not necessarily a negative factor for the use of the compound as a boron delivery agent for BNCT. The therapeutic ratio (tumour radiation dose to the normal tissue radiation dose) of BNCT improves as higher absolute ^{10}B concentrations are produced in both the tumour and the normal tissues.[5] This is because the neutron fluence can be reduced to yield the same boron dose for a lower background dose attributable to capture reactions with normal tissue ^{1}H and ^{14}N.

The present data show that absolute tumour values fell within a therapeutically useful range over the 5–10 min to 6 h observation period. In particular, at 3 h post-administration of the compound the tumor/normal pouch tissue mean boron ratio was more favourable than at other time-points, probably due to a difference in clearance rate between tumour tissue and normal tissue. This potential difference in clearance may be used to advantage in terms of contributing to selective tumour targeting. The attempt at enhancing selectivity by administering 2 successive doses of GB-10 did not prove effective in terms of preferential uptake but did increase absolute uptake. Similarly, two sequential intragastric doses of BPA have been reported to be additive in regard to loading tumours with boron in rat intracerebral gliosarcoma despite the fact that they do not increase the tumor/normal tissue and tumor/blood ^{10}B concentration ratios.[12,13] It is more effective to have higher ^{10}B levels in the tumour at a fixed tumor/normal tissue ratio than lesser amounts of ^{10}B in the tumour at very high tumor/normal tissue ratios because of the background dose as previously mentioned.[5]

The consistently low spinal cord and brain boron concentration values are a definite asset in terms of the dose limiting nature of these tissues for different tumour types. Certain compounds such as boronated porphyrins accumulate in liver in quantities that exceed by far the content of other normal tissues.[20] This is a radiotoxicity concern if the liver lies within the treatment volume. The fact that liver, spleen and lung do not accumulate GB-10 more than other normal tissues poses an advantage. This model allows us to study the behaviour of skin and oral tissues. It would be of utmost importance to evaluate, or at least estimate, actual uptake by oral mucosa and skin rather than extrapolate from concurrent blood values in patients. The study of clinically relevant, potentially dose-limiting, normal tissues in an adequate animal model such as the model described herein may contribute to elucidating and preventing previously unpredictable side-effects and establishing the conditions to eradicate tumours within the tolerance limit of the surrounding normal tissues.[18]

Blood boron values fell progressively after a single loading dose. Diaz et al.[31] showed a similar effect in human subjects. A loading dose of approximately 30 mg ^{10}B/kg b.w. delivered intravenously to human patients resulted in blood boron values in the vicinity of 60 ppm at 2 h after administration of GB-10, whereas the present loading dose of 50 mg ^{10}B/kg b.w. in hamster resulted in blood boron values in the vicinity of 40 ppm 2 h post-administration. Within this context, it is clear that the values reported herein cannot be directly extrapolated to human subjects. Given that tissue boron concentration values for human subjects are lacking, a comprehensive comparative study cannot be attempted. However, the present study contributes a full range of biodistribution values for blood, tumour and precancerous tissue and dose-limiting normal tissues in an experimental model that may mimic potentially clinical situations. In the study by Diaz et al.[31] 5 subjects received a loading dose followed by continuous i.v. infusion of GB-10 for 2 h with the goal of achieving a steady state blood concentration of 100 ppm. High blood boron values are potentially useful to enhance capture reactions as long as the beam is circumscribed to the treatment volume. The issue of a loading dose followed by a continuous infusion has not been addressed in the present study in the oral cancer hamster model. Excessively high blood boron values may lead to unacceptable normal tissue toxicity in a BNCT scenario.

The finding that precancerous tissue surrounding tumour incorporated considerable amounts of boron would provide a rationale for the treatment of ''field cancerized'' areas surrounding tumour[26] to reduce the risk of development of additional tumours in the area. However, the dose-limiting nature of this tissue in terms of acute effects must be explored. The fact that the hamster oral cancer model can be used to study the effect of BNCT on precancerous tissue as demonstrated by our laboratory[18,19] is an advantage over the transplanted tumour models used for BNCT studies.[17]

As previously stated, BPA is known to accumulate preferentially in viable cells with uptake capacity.[6,18,38] Conversely, sodium borocaptate (BSH) which has no known uptake mechanisms and is thought to penetrate a tumour by diffusion and leakage through tumour blood cells would likely accumulate more in mostly necrotic areas.[38] Heterogenous uptake by heterogenous tumour tissues[40,41] precludes accurate treatment planning. The agreement between prescribed and actual dose depends largely on the agreement between intended and obtained ^{10}B concentration during irradiation. A large spread in boron concentrations creates additional problems in the analysis of biological responses.[42] The fact that pouch tumours occasionally undergo spontaneous partial necrosis allows us to explore, in selected pouch tumour samples with variable amounts of viable tumour tissue and small areas of necrosis, the correlation between GB-10 uptake and viability. We previously showed that mostly viable pouch tumour areas tended to incorporate more BPA than mostly necrotic pouch tumour areas, i.e. 36.9 ± 17.5 ppm versus 25.2 ± 18.5 ppm, respectively. Despite the characteristic spread in values, mean boron uptake in mostly viable tumour tissue would be approximately 50% greater than in mostly necrotic tumour tissue.[18] In the present study, we showed that GB-10 was delivered homogenously to all tumour areas, regardless of their degree of viability. This is central to targeting all areas of a heterogenous tumour and achieving tumour control. It has been suggested that the absence of long-term tumour control in patients receiving relatively high minimal tumour volume doses (40–55 Gy Eq.) may have been attributable to a nonuniform distribution of ^{10}B in tumour cells.[10] Further studies would be necessary to evaluate the effect on boron incorporation into viable tumour of other variables that have not been examined to date such as degree of differentiation of the tumour and degree of vascularization.

To explore the possibility of combining the differential properties of different boron compounds to improve tumour targeting and potential therapeutic efficacy we administered combinations of GB-10 and BPA. The rationale for the joint administration of these compounds would be to profit from the selective uptake of BPA by tumour tissue and the homogenous distribution of GB-10 in different tumour areas. The best absolute boron values and tumor/normal tissue ratios were obtained in this study by administering a loading dose of GB-10 coupled to a BPA simulated infusion. It has been reported that longer i.v. infusion times are more effective in delivering boron selectively to tumour tissue in other models.[43] The mechanisms involved in this apparent synergistic effect between GB-10 and a BPA infusion in terms of tumour boron uptake remain to be elucidated. Conceivably, the combined administration of GB-10 and an i.v. infusion of BPA may improve the therapeutic advantage of BNCT by targeting all tumour areas, reducing the probability of recurrence and improving tumor/normal tissue boron concentration ratios, allowing doses to tumour to be administered safely.

The high boron concentration values delivered by GB-10 to blood and tissues in this model and the consistently low brain and spinal cord values would suggest that GB-10 would be a useful compound to perform BNCT-enhanced FNT in certain pathologies. In particular, the tumours where fast neutrons show a therapeutic advantage appear to be those which readily repair damage from conventional low Linear Energy Transfer radiation. Normal tissue side effects preclude simply escalating the neutron dose further.[44] The radiation dose to tumour cells must be augmented without increasing the dose to normal tissue cells. A BNCT boost would be a way of accomplishing this.

We may conclude that the use of GB-10 as an adjunct to BPA-mediated BNCT may improve the potential therapeutic efficacy of BNCT. Despite the fact that tumour uptake of GB-10 is only marginally selective in organs that are not protected by the Blood Brain Barrier, the homogenous deposition of GB-10 in different tumour areas and the high absolute tumour boron values may render this compound therapeutically useful for BNCT treatment of head and neck tumours. Furthermore, the biodistribution data show that GB-10 would potentially elicit an NCT boost enhancement of FNT with no significant radiotoxicity to spinal cord, leading to a clinically-meaningful improvement in local tumour control.

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