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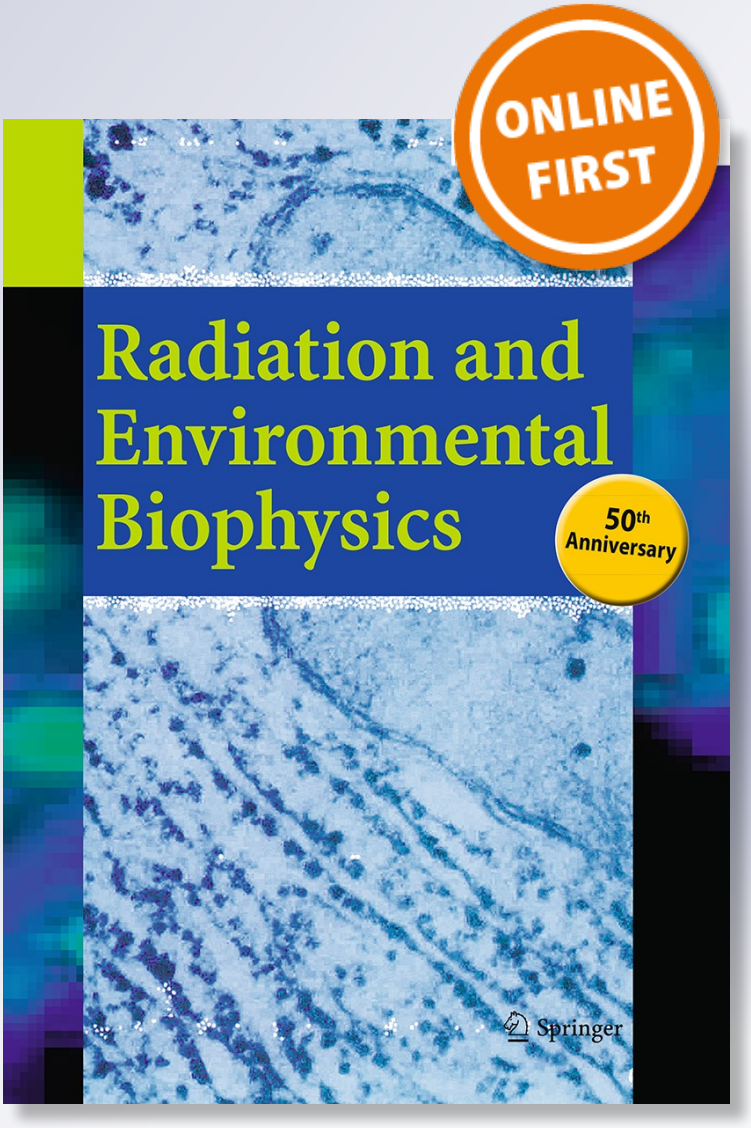

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ORIGINAL PAPER

Biodistribution of sodium borocaptate (BSH) for boron neutron capture therapy (BNCT) in an oral cancer model

Marcela A. Garabalino · Elisa M. Heber · Andrea Monti Hughes · Sara J. González · Ana J. Molinari · Emiliano C. C. Pozzi · Susana Nievas · Maria E. Itoiz · Romina F. Aromando · David W. Nigg · William Bauer · Verónica A. Trivillin · Amanda E. Schwint

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Abstract Boron neutron capture therapy (BNCT) is based on selective accumulation of ^{10}B carriers in tumor followed by neutron irradiation. We previously proved the therapeutic success of BNCT mediated by the boron compounds boronophenylalanine and sodium decahydrodecaborate (GB-10) in the hamster cheek pouch oral cancer model. Based on the clinical relevance of the boron carrier sodium borocaptate (BSH) and the knowledge that the most effective way to optimize BNCT is to improve tumor boron targeting, the specific aim of this study was to perform biodistribution studies of BSH in the hamster cheek pouch oral cancer model and evaluate the feasibility of BNCT mediated by BSH at nuclear reactor RA-3. The general aim of these studies is to contribute to the knowledge of BNCT radiobiology and optimize BNCT for head and neck cancer. Sodium borocaptate (50 mg ^{10}B/kg) was administered to tumor-bearing hamsters. Groups of 3–5 animals were killed humanely at nine time-points, 3–12 h post-administration. Samples of blood, tumor, precancerous pouch tissue, normal pouch tissue and other clinically relevant normal tissues were processed for boron measurement by optic emission spectroscopy. Tumor boron concentration peaked to therapeutically useful boron concentration values of 24–35 ppm. The boron concentration ratio tumor/normal pouch tissue ranged from 1.1 to 1.8. Pharmacokinetic curves showed that the optimum interval between BSH administration and neutron irradiation was 7–11 h. It is concluded that BNCT mediated by BSH at nuclear reactor RA-3 would be feasible.

Keywords Boron neutron capture therapy · BNCT · Oral cancer · Hamster cheek pouch · Precancerous tissue · BSH

M. A. Garabalino · E. M. Heber · A. M. Hughes · A. J. Molinari · M. E. Itoiz · R. F. Aromando · V. A. Trivillin · A. E. Schwint (✉)
Department of Radiobiology, National Atomic Energy Commission (CNEA), Avenida General Paz 1499, B1650KNA San Martin, Buenos Aires, Argentina
e-mail: schwint@cnea.gov.ar

S. J. González
Department of Instrumentation and Control, National Atomic Energy Commission (CNEA), Avenida General Paz 1499, B1650KNA San Martin, Buenos Aires, Argentina

S. J. González · V. A. Trivillin · A. E. Schwint
National Research Council (CONICET), Buenos Aires, Argentina

E. C. C. Pozzi
Department of Research and Production Reactors, National Atomic Energy Commission (CNEA), Presbítero Juan González y Aragon 15, B1802AYA Ezeiza, Buenos Aires, Argentina

S. Nievas
Department of Chemistry, National Atomic Energy Commission (CNEA), Avenida General Paz 1499, B1650KNA San Martin, Buenos Aires, Argentina

M. E. Itoiz · R. F. Aromando
Department of Oral Pathology, Faculty of Dentistry, University of Buenos Aires, Marcelo T. de Alvear 2142, 1122 Ciudad Autónoma de Buenos Aires, Argentina

D. W. Nigg · W. Bauer
Idaho National Laboratory, 2525 North Fremont Street, P.O. Box 1625, Idaho Falls, ID 83415, USA

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Introduction

Boron neutron capture therapy (BNCT) is classically described as a binary treatment modality that combines the administration of tumor-seeking boron carriers that are taken up preferentially by neoplastic tissue and irradiation with a thermal or epithermal neutron beam. The high linear energy transfer (LET) α particles and recoiling ^{7}Li nuclei emitted during the capture of a thermal neutron by a ^{10}B nucleus (Locher 1936) are known to have a high relative biological effectiveness (e.g., Gabel et al. 1984; Coderre and Morris 1999). Their short range in tissue (6–10 μm) would limit the damage largely to cells containing ^{10}B. In this way, BNCT would target neoplastic tissue selectively, sparing normal tissue. In addition, being a technique that is based on biological rather than geometrical targeting, it would be ideally suited to treat undetectable micrometastasis (Cardoso et al. 2007) and foci of malignant transformation in field-cancerized tissue (Monti Hughes et al. 2009; Monti Hughes et al. 2011).

The radiation field produced in tissue during BNCT consists of a mixture of components with different LET characteristics. In addition to the α and ^{7}Li high-LET products that give rise to the boron dose component, the interaction of the neutron beam with the nuclei of the elements in tissue (nitrogen and hydrogen) will deliver an unavoidable and non-specific background dose to both tumor and normal tissue. BNCT protocols are ideally designed to maximize the irradiation dose caused by boron neutron capture reactions and to minimize the background dose. The most effective way to do this is to maximize absolute boron content in tumor (Coderre and Morris 1999; Trivillin et al. 2006).

Clinical trials of BNCT for the treatment of glioblastoma multiforme and/or melanoma and, more recently, head and neck tumors and liver metastases have been performed or are underway in the US, Japan, Europe, Argentina and Taiwan (e.g., Chanana et al. 1999; Busse et al. 2003; Diaz 2003; Gonzalez et al. 2004; Zonta et al. 2006; Suzuki et al. 2007; Kato et al. 2009; Kankaanranta et al. 2011, 2012; Nakai et al. 2011; Wang et al. 2011; Lin and Liu 2011; Barth et al. 2012). To date, the clinical results have shown a potential therapeutic advantage for this technique. However, there is undoubtedly room for improvement in terms of therapeutic success and reduction in radiotoxicity in dose-limiting tissues. Furthermore, the search for new applications of BNCT is warranted. Within this context, contributory translational studies have been carried out employing a variety of experimental models based on the implantation of tumor cells in normal tissue (e.g., Barth et al. 2005).

Despite considerable advances in the understanding of the etiology of head and neck malignancies, the use of new treatment modalities and dramatic improvements in surgical and radiation techniques over the last decade, their management continues to be a challenge. Squamous cell carcinoma (SCC) of the head and neck region is the sixth most common cause of cancer deaths worldwide and the incidence is rising rapidly in developing countries. The relatively poor overall 5-year survival rate for malignancies of the oral cavity is estimated to range between 58.3 and 63 % (Mehrotra et al. 2011). The mortality and morbidity associated to SCC has remained relatively unaltered (Chen and Lin 2010, Tanaka and Ishigamori 2011). Within this context, there is a need for more effective and less toxic therapies which can discriminate between malignant and normal cells. Studies in appropriate experimental models are pivotal to progress in this field.

The use of the hamster cheek pouch model of oral cancer was previously proposed and subsequently validated by our group for BNCT studies to explore new applications of BNCT, study its radiobiology and improve its therapeutic efficacy (Kreimann et al. 2001a, b). Carcinogenesis protocols induce premalignant and malignant changes that closely resemble spontaneous human oral mucosa lesions (Morris 1961). This mode of tumor induction provides a tumor model surrounded by precancerous tissue. The possibility of studying precancerous tissue in addition to tumor and normal tissue is clinically relevant given the dose-limiting nature of this tissue (e.g., Monti Hughes et al. 2011) and the fact that second primary tumor locoregional recurrences that arise in field-cancerized tissue (Braakhuis et al. 2003) are a frequent cause of therapeutic failure (Smith and Haffty 1999).

Employing this model, the therapeutic efficacy of BNCT mediated by the boron carriers boronophenylalanine (BPA) and/or sodium decahydrodecaborate (GB-10) and boronated liposomes to treat oral cancer with no normal tissue radiotoxicity was previously evidenced, and without exceeding the radiotolerance of dose-limiting precancerous tissue surrounding tumors (Kreimann et al. 2001b; Trivillin et al. 2004, 2006, 2008; Pozzi et al. 2009; Aromando et al. 2010; Molinari et al. 2011; Heber et al. 2012; Molinari et al. 2012). In addition, the therapeutic potential of BNCT mediated by BPA and/or GB-10 for liver metastases was shown in a rat model (Garabalino et al. 2011; Pozzi et al. 2012). It was shown that GB-10, a diffusive boron compound, does not target hamster cheek pouch tumor cells selectively but delivers therapeutically useful amounts of boron homogeneously in heterogeneous tumors. Homogeneous tumor uptake is clearly an asset in terms of the efficacy of BNCT to target all tumor cells (Heber et al. 2004, 2006). Although GB-10 is not taken up preferentially by tumor tissue, it causes selective tumor damage while sparing normal and precancerous tissue. This selective damage to tumor tissue was ascribed to a differential effect

on aberrant tumor blood vessels (Carmeliet and Jain 2000; Trivillin et al. 2006; Folkman 2006). Furthermore, we demonstrated the inhibitory effect of BNCT mediated by BPA or the combination of BPA and GB-10 on tumor development from precancerous tissue in a model of precancer suitable for long-term follow-up (Monti Hughes et al. 2009, 2011; Heber et al. 2010).

Boron content and distribution in tumor and normal tissues are pivotal to the efficacy of BNCT. The basic requirements for a therapeutic advantage for BNCT are classically described as selectivity for the accumulation of a non-toxic ^{10}B carrier in tumor relative to the surrounding tissues and a sufficiently high absolute concentration of ^{10}B in tumor tissue. Because successful BNCT is based on maximizing the dose to tumor tissue and minimizing the dose to normal tissue, tumor/normal tissue and tumor/blood mean boron concentration ratios greater than 1 are an asset. Furthermore, absolute boron content in tumor tissue must be high enough to allow a sufficient number of $^{10}B(n, \alpha)^{7}Li$ reactions to occur, as it is the high-LET products of this reaction that are the specific effectors of tumor damage. In addition, high, non-toxic absolute ^{10}B concentrations are an advantage in and of themselves because they allow for shorter irradiation times and a concomitant reduction in background dose (Coderre and Morris 1999).

Dose calculations in BNCT are based on boron content values in tumor and normal tissue. Because there is no non-invasive, online methodology available to date to estimate boron concentration during BNCT, dose calculations are based on values derived from boron biodistribution studies performed beforehand. In the case of experimental models, dose calculations are based on mean values obtained from biodistribution studies performed in separate sets of animals (e.g., Kreimann et al. 2001a; Heber et al. 2004). The time post-administration of the boron compound that is ideally suited for neutron irradiation, the irradiation times and the feasibility of a therapeutically useful treatment can be evaluated employing pharmacokinetic curves constructed from the measured boron concentration values. The "best" irradiation scenario maximizes absolute boron content in tumor and minimizes boron content in dose-limiting tissues while complying with pre-established radiotolerance constraints.

The degree of selectivity and absolute boron content values that are therapeutically useful will depend on the boron compound under study. Significant improvements in BNCT efficacy in terms of maximizing tumor control and minimizing radiotoxicity can be obtained by optimizing boron compound administration protocols. Boron biodistribution studies are pivotal to explore the therapeutic potential of a particular boron compound for a particular pathology and design potentially successful BNCT protocols.

The fact that sodium mercaptoundecahydro-closo-dodecaborate (sodium borocaptate or BSH) is being investigated clinically as a stand-alone boron agent for BNCT of brain tumors (e.g., Nakagawa et al. 2009) and in combination with BPA for recurrent head and neck malignancies (e.g., Kato et al. 2009) makes it a particularly interesting boron compound to explore. Sodium borocaptate is a very efficient carrier of ^{10}B, but the selectivity of tumor accumulation depends on the defective blood brain barrier (BBB) in brain tumors versus the intact BBB in normal brain (Ono et al. 2000). Within this context, its utility as a stand-alone boron agent would seem to be restricted to brain tumors. However, our findings with BNCT mediated by GB-10 in the hamster cheek pouch oral cancer model (Trivillin et al. 2006) suggested alternative mechanisms for selective tumor damage that do not rely on selective tumor boron uptake. Similarly to sodium borocaptate (BSH), GB-10 was initially proposed for the treatment of brain tumors because it does not traverse the intact BBB in normal brain and accumulates selectively in tumors surrounded by a defective BBB. In the BBB-free hamster cheek pouch model of oral cancer, GB-10 was able to deliver therapeutically useful amounts of boron to hamster tumors, albeit not selectively (Heber et al. 2004). Unlike BPA (Kreimann et al. 2001a), GB-10 was deposited homogeneously in different tumor areas, an asset when treating heterogeneous tumors (Heber et al. 2006). In contrast to the traditional BNCT paradigm that selective damage to tumor is only based on selective tumor uptake of the boron compound, previous studies demonstrated that BNCT mediated by GB-10, a chemically non-selective boron agent, would act selectively on tumors by damaging tumor blood vessels, while sparing precancerous and normal tissue blood vessels (Trivillin et al. 2006). The structure and function of angiogenic tumor blood vessels are altered (Carmeliet and Jain 2000; Folkman 2006), rendering them more sensitive to BNCT than the precancerous and normal tissue blood vessels, and thereby providing a selective mechanism of action for a chemically non-selective boron compound. Thus, even when a ^{10}B compound is not selectively taken up by tumor, it can still target tumors homogenously and produce a selective effect on tumors by preferential effects on the tumor vasculature. This is the case with GB-10 in the oral cancer model and could conceivably be the case of BSH.

Given the clinical relevance of BSH for other pathologies, the aim of the present study was to perform, for the first time, biodistribution studies of BSH as a stand-alone boron carrier in the hamster cheek pouch oral cancer model, essential for future in vivo BNCT studies that seek to optimize BNCT for oral cancer in terms of therapeutic efficacy and radiotoxicity in normal and precancerous tissue.

Materials and methods

Model of oral cancer: tumor induction

Tumors were induced in the right cheek pouch of non-inbred young (6 weeks old) Syrian hamsters by topical application of 0.5 % of the complete carcinogen dimethyl-1,2-benzanthracene (DMBA) in mineral oil twice a week for 12 weeks in keeping with a standard hamster cheek pouch carcinogenesis protocol (Shklar et al. 1979) modified as previously described (e.g., Molinari et al. 2011). The treated pouch was periodically everted under light intraperitoneal (i.p.) ketamine (70 mg/kg bw)-xylazine (10.5 mg/kg bw) anesthesia and examined to monitor tumor development. Once the exophytic tumors, that is, SCCs, developed and reached a diameter of approximately 5 mm, the animals were used for biodistribution studies. A variable number of tumors develop in each cancerized pouch. Institutional guidelines for the care and use of laboratory animals were followed throughout.

Biodistribution studies

Sodium borocaptate (BBI, Cat.1921, purity 99.23 %) was dissolved in saline at 1.8 % w/w in anaerobic conditions to avoid the formation of the toxic dimers BSSB, BSSOB and BOSSOB. Nitrogen (N_2) was used to displace oxygen and indicators of anaerobiosis (Oxoid BR0055B–sensitivity $\geq$0.1 ppm O_2) were employed to verify that the levels of oxygen were negligible during preparation of the solution. pH was adjusted to 7.0 with 0.1 M NaOH. The solution was bubbled with N_2 and stored in anaerobiosis at 4 °C in light tight conditions for a maximum of 12 h before use. The solution of BSH was injected intravenously in the surgically exposed jugular vein of tumor-bearing hamsters under ketamine—xylazine anesthesia as previously described (e.g., Trivillin et al. 2006; Kreimann et al. 2001a) at a dose of 50 mg ^{10}B/kg (88 mg BSH/kg). Blood and tissue samples were taken 3, 4, 5, 6, 7, 8, 9, 10 and 12 h post-administration. Blood samples were taken from the surgically exposed jugular vein under ketamine (140 mg/kg bw)—xylazine (21 mg/kg bw) anesthesia. The animals were then killed humanely by overdose of anesthesia immediately prior to tissue sampling. Samples of tumor, precancerous and normal pouch, cheek mucosa, palate, parotid gland, skin, tongue, spinal cord marrow, brain, liver, kidney, spleen and lung were taken for each animal. Two to three independent experiments (with 1–3 hamsters in each group) were performed for each time-point and then pooled. Precancerous pouch tissue and tumors are clearly distinguishable both macroscopically and microscopically as previously described (e.g., Kreimann et al. 2001a; Heber et al. 2007), making sampling very straightforward. Three to six samples were measured per animal for precancerous pouch tissue and normal tissues. Duplicate blood samples were measured per animal. In the case of tumor, given the characteristic spread in tumor boron concentration values (e.g., Trivillin et al. 2006), all available tissue was fractionated into 30–50 mg samples and measured. The data corresponding to the fractions of large tumors were averaged for each tumor.

Boron analysis

All of the samples were weighed immediately. Until use, tissue samples were stored at −4 °C and blood samples were stored with EDTA 5 % v/v at 4 °C. The samples were processed for boron analysis by Atomic Emission Spectroscopy with Inductively Coupled Plasma (ICP-OES Optima 3100 XL, UV, axial, Perkin Elmer). Tissue samples (30–50 mg) were digested for 1 h at 100 °C in 0.25 ml of a 1:1 mixture of concentrated sulfuric and nitric acids. Once the digestion process was complete, 0.2 ml Yttrium (0.5 ppm)-Strontium (25 ppm) were added as an internal standard, prior to the addition of 0.55 ml of a 5 % Triton X-100 solution in water. The samples were then sonicated for 90 min. Blood samples (200–300 µl) were digested at 100 °C in 1.25 ml of a 1:1 mixture of concentrated sulfuric and nitric acids. Once the digestion process was complete, 1 ml Yttrium (0.5 ppm)-Strontium (25 ppm) were added as an internal standard, prior to the addition of 2.75 ml of a 5 % Triton X-100 solution in water. Standard solutions of boric acid (enriched to 99.8 % in ^{10}B) were used to prepare a calibration line each day of operation. Boron measurements were performed using the boron 249.677 nm analytical line. In the occasional event of inadequate sample digestion, the sample was not measured. All available data were reported. When possible, boron concentration was cross-checked in random blood and tissue samples by inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC2, Perkin Elmer) as previously described (e.g., Molinari et al. 2012). In addition, the boron concentration of the BSH solution was checked in two of the independent solutions employed.

End points

Absolute boron concentration in tumor, blood and clinically relevant normal tissues were evaluated for each of the time-points. Individual tumor/blood and tumor/normal pouch boron concentration tissue ratios were calculated for each animal and then averaged for the animals in each group.

Data analysis

The average behavior of boron from BSH in blood, precancerous pouch tissue and normal pouch tissue as a

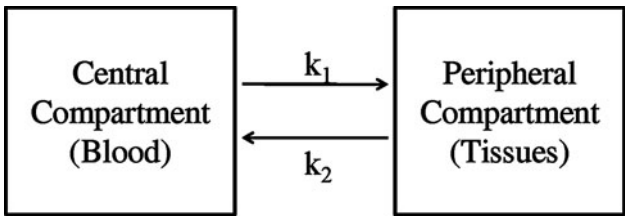

Fig. 1 Diagram of two-compartment model for BSH pharmacokinetics. The drug is administered as an intravenous bolus injection into the central compartment, which represents the blood. BSH exchange between central and peripheral compartments is governed by first order rate constants k_1 and k_2

function of time was modeled using the two-compartment model shown in Fig. 1 (Notari and DeYoung 1975). The central compartment represents the blood, and the peripheral compartment is alternatively correlated to each of the tissues considered. It was assumed that there was no interaction between the different tissues. Assuming that the blood clearance pharmacokinetic profile is adequately represented by a bi-exponential decay (Shibata et al. 2003), the model was used to determine the rate constants k_1 and k_2 which govern the exchange of BSH between both compartments. The four parameters of the blood pharmacokinetic model were first estimated by fitting a bi-exponential curve to all the collected data, using the nonlinear least square method. Based on the blood concentration profile, the values of the rate constants for tumor, precancerous pouch tissue and normal pouch tissue were obtained using all the measured data for each tissue and the same minimization method. Blood boron concentration immediately after injection (T0) was estimated based on the administered BSH dose and the blood volume of each of the animals, calculated employing body weight (Lee and Blaufox 1985).

Results

Absolute boron concentrations in the different tissues at the different post-administration times are reported in Table 1, while tumor/normal pouch tissue and tumor/blood boron concentration ratios are reported in Table 2. Tumor/blood and tumor/normal pouch tissue boron concentration ratios were calculated for each of the tumors. Each hamster had a variable number of tumors and the number of sub-samples measured per tumor depended on tumor size. Ratios were calculated for each tumor considering the mean value of the sub-samples corresponding to that particular tumor and the mean normal pouch tissue or blood value corresponding to the hamster bearing that particular tumor. The tabulated ratio values correspond to the mean value ± standard deviation of the ratio for each of the tumors.

Based on previous radiobiological BNCT studies by our group in the hamster cheek pouch oral cancer model employing the boron compounds BPA and GB-10 (Kreimann et al. 2001b; Trivillin et al. 2004, 2006; Pozzi et al. 2009), we defined guidelines to establish the potential therapeutic value of the administration protocols assessed:

- No manifest toxicity
- Absolute boron concentration in tumor ≥20 ppm
- Boron concentration ratio tumor/normal tissue ≥1
- Boron concentration ratio tumor/blood ≥1

Earlier studies in the hamster cheek pouch oral cancer model with BNCT-mediated GB-10 showed that it was possible to achieve significant tumor control with no associated normal tissue toxicity with tumor/normal tissue and tumor/blood ratios of ≥1, provided that absolute boron content in tumor was close to or above 20 ppm (e.g., Trivillin et al. 2006). Although the actual usefulness of a specific administration protocol for BNCT can only be determined by in vivo studies in the experimental model under study, our previous studies in the hamster cheek pouch oral cancer model suggest that the administration protocols that meet the above requirements are potentially useful and worthy of radiobiological evaluation.

Tumor boron concentration peaked to approximately 24–35 ppm, 3–10 h post-administration of BSH. A large spread in tumor absolute boron concentration values was observed. The boron concentration ratio tumor/normal pouch tissue ranged from 1.1 to 1.8. Precancerous tissue absolute boron concentration values were overall in the same range as tumor and normal pouch tissue values. Blood boron values were consistently higher than tumor, precancerous and normal pouch tissue boron values. In the cases when the boron concentration values in blood and tissue samples were cross-checked by ICP-MS, no significant differences were found with ICP-OES values. Variations between the boron concentration in the BSH solutions and the expected value did not exceed 10 %. Although the characteristic spread in boron concentration values has been widely documented for different boron compounds and different tumor types (e.g., Gibson et al. 2003; Trivillin et al. 2006), the oscillations with time in *mean* boron values observed in particular for blood and also in other tissues such as liver were unexpected and counter-intuitive. The fact that it is impossible to take sequential samples over a 12-h period from the same experimental small animal precludes reliable confirmation of these oscillations. To the best of our knowledge to date, we do not have an explanation for these oscillations if, in fact, they do occur in single animals followed in time.

The average behavior of boron from BSH in blood, precancerous pouch tissue and normal pouch tissue as a function of time was obtained by fitting the data to a

Table 1 Boron concentration (mean ± standard deviation) (ppm) in blood and tissue samples at different times post-administration as indicated; *n* denotes number of animals or tumors

BSH 50 mg^{10}B/kg i.v.									
	Time post-administration								
	3 h	4 h	5 h	6 h	7 h	8 h	9 h	10 h	12 h
Blood	62.5 ± 24.8 ($n = 5$)	60.8 ± 17.0 ($n = 5$)	37.1 ± 5.4 ($n = 5$)	82.6 ± 15.5 ($n = 6$)	23.4 ± 9.3 ($n = 5$)	56.3 ± 19.0 ($n = 4$)	31.1 ± 10.5 ($n = 5$)	66.7 ± 10.1 ($n = 3$)	14.4 ± 9.0 ($n = 5$)
Tumor	30.1 ± 15.0 ($n = 19$)	26.0 ± 8.6 ($n = 33$)	24.3 ± 9.3 ($n = 17$)	34.2 ± 7.1 ($n = 18$)	23.7 ± 14.0 ($n = 15$)	28.3 ± 6.4 ($n = 13$)	24.1 ± 7.9 ($n = 27$)	34.7 ± 7.2 ($n = 7$)	9.2 ± 2.4 ($n = 19$)
Normal tissues	27.2 ± 11.2 ($n = 5$)	21.4 ± 6.9 ($n = 5$)	21.3 ± 6.4 ($n = 5$)	30.9 ± 6.4 ($n = 5$)	17.1 ± 10.1 ($n = 5$)	21.7 ± 4.6 ($n = 4$)	17.5 ± 5.9 ($n = 5$)	27.2 ± 3.2 ($n = 3$)	5.3 ± 2.4 ($n = 5$)
Premalignant tissue	34.7 ± 12.4 ($n = 5$)	30.4 ± 8.5 ($n = 5$)	26.5 ± 8.3 ($n = 5$)	39.5 ± 10.8 ($n = 5$)	18.9 ± 10.5 ($n = 5$)	25.1 ± 7.2 ($n = 4$)	21.4 ± 7.7 ($n = 5$)	29.9 ± 2.9 ($n = 3$)	5.9 ± 2.7 ($n = 5$)
Cheek mucosa	23.7 ± 12.3 ($n = 5$)	21.4 ± 9.4 ($n = 5$)	16.8 ± 5.7 ($n = 5$)	37.1 ± 26.1 ($n = 5$)	13.7 ± 8.4 ($n = 5$)	16.3 ± 5.1 ($n = 4$)	22.3 ± 2.4 ($n = 5$)	19.5 ± 5.0 ($n = 3$)	4.2 ± 1.8 ($n = 5$)
Palate mucosa	40.2 ± 18.4 ($n = 4$)	33.2 ± 12.2 ($n = 5$)	24.3 ± 5.5 ($n = 5$)	49.4 ± 8.6 ($n = 3$)	18.0 ± 9.0 ($n = 5$)	22.1 ± 5.7 ($n = 4$)	28.2 ± 5.8 ($n = 5$)	44.1 ± 15.4 ($n = 3$)	8.4 ± 2.4 ($n = 5$)
Parotid gland	11.3 ± 5.1 ($n = 5$)	19.1 ± 17.8 ($n = 5$)	10.8 ± 3.6 ($n = 5$)	21.9 ± 11.6 ($n = 4$)	8.6 ± 3.3 ($n = 4$)	10.0 ± 3.9 ($n = 3$)	7.9 ± 1.6 ($n = 5$)	10.6 ± 0.8 ($n = 3$)	2.6 ± 0.9 ($n = 5$)
Tongue	29.0 ± 15.1 ($n = 4$)	19.4 ± 11.9 ($n = 5$)	20.4 ± 4.9 ($n = 5$)	33.2 ± 6.0 ($n = 5$)	17.6 ± 11.0 ($n = 5$)	21.3 ± 6.2 ($n = 4$)	15.7 ± 8.6 ($n = 5$)	21.0 ± 2.8 ($n = 3$)	4.9 ± 1.4 ($n = 5$)
Liver	143.1 ± 80.2 ($n = 5$)	171.3 ± 76.7 ($n = 5$)	94.5 ± 31.2 ($n = 5$)	214.1 ± 86.4 ($n = 5$)	51.1 ± 33.8 ($n = 5$)	77.5 ± 25.1 ($n = 4$)	76.8 ± 36.0 ($n = 5$)	93.3 ± 38.0 ($n = 3$)	13.5 ± 6.3 ($n = 5$)
Spleen	23.8 ± 10.5 ($n = 5$)	20.3 ± 10.9 ($n = 4$)	18.5 ± 8.4 ($n = 5$)	28.4 ± 6.0 ($n = 4$)	11.6 ± 5.1 ($n = 5$)	22.8 ± 3.2 ($n = 4$)	14.4 ± 5.3 ($n = 5$)	46.6 ± 2.5 ($n = 3$)	4.1 ± 0.9 ($n = 5$)
Kidney	91.0 ± 36.8 ($n = 5$)	100.7 ± 30.6 ($n = 4$)	112.8 ± 61.6 ($n = 5$)	93.7 ± 35.4 ($n = 5$)	43.7 ± 25.1 ($n = 5$)	43.7 ± 12.5 ($n = 4$)	65.0 ± 24.9 ($n = 5$)	84.5 ± 22.9 ($n = 3$)	18.6 ± 6.4 ($n = 5$)
Lung	60.9 ± 28.4 ($n = 4$)	26.8 ± 16.4 ($n = 4$)	25.5 ± 1.2 ($n = 5$)	40.7 ± 18.3 ($n = 4$)	9.2 ± 3.5 ($n = 4$)	26.6 ± 6.5 ($n = 4$)	28.1 ± 11.0 ($n = 5$)	32.6 ± 9.5 ($n = 3$)	8.4 ± 2.1 ($n = 5$)
Skin	32.8 ± 7.1 ($n = 5$)	18.6 ± 8.6 ($n = 5$)	25.9 ± 9.1 ($n = 5$)	26.2 ± 15.3 ($n = 5$)	22.6 ± 16.1 ($n = 5$)	24.4 ± 5.3 ($n = 4$)	17.4 ± 5.0 ($n = 5$)	23.8 ± 4.7 ($n = 3$)	5.7 ± 3.4 ($n = 5$)
Spinal cord marrow	1.1 ± 0.8 ($n = 4$)	5.6 ± 3.9 ($n = 5$)	4.1 ± 1.8 ($n = 5$)	5.5 ± 2.4 ($n = 5$)	2.2 ± 1.6 ($n = 5$)	1.7 ± 0.4 ($n = 4$)	4.4 ± 1.2 ($n = 5$)	2.0 ± 0.2 ($n = 3$)	0.7 ± 0.2 ($n = 5$)
Brain	1.3 ± 1.0 ($n = 4$)	2.2 ± 1.8 ($n = 4$)	1.4 ± 0.5 ($n = 5$)	4.5 ± 2.2 ($n = 5$)	0.9 ± 0.4 ($n = 4$)	1.3 ± 0.5 ($n = 4$)	2.3 ± 0.8 ($n = 5$)	0.9 ± 0.4 ($n = 3$)	0.5 ± 0.2 ($n = 5$)

Table 2 Tumor/blood and tumor/normal pouch tissue boron concentration ratios at different times post-administration as indicated; ratios were calculated for each tumor considering the mean value of the samples corresponding to that particular tumor and the mean blood or normal pouch tissue value corresponding to the animal bearing that particular tumor

BSH 50 mg^{10}B/kg i.v.									
	Time post-administration								
	3 h	4 h	5 h	6 h	7 h	8 h	9 h	10 h	12 h
Tumor/normal tissue	1.3 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	1.1 ± 0.2	1.8 ± 0.3	1.3 ± 0.2	1.4 ± 0.4	1.3 ± 0.2	1.8 ± 0.6
Tumor/blood	0.6 ± 0.2	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	1.0 ± 0.2	0.6 ± 0.2	0.8 ± 0.2	0.5 ± 0.1	0.6 ± 0.4

The tabulated ratios correspond to the mean ± standard deviation of the ratios for each of the tumors

two-compartment closed model (Fig. 2). The R-square statistic was computed for each fit shown in Fig. 2. The values were 0.997, 0.774, 0.775 and 0.744 for blood, normal, precancerous and tumor tissues, respectively, showing that the model explains at least 74 % of the total variation of the data about the average. It is noteworthy that

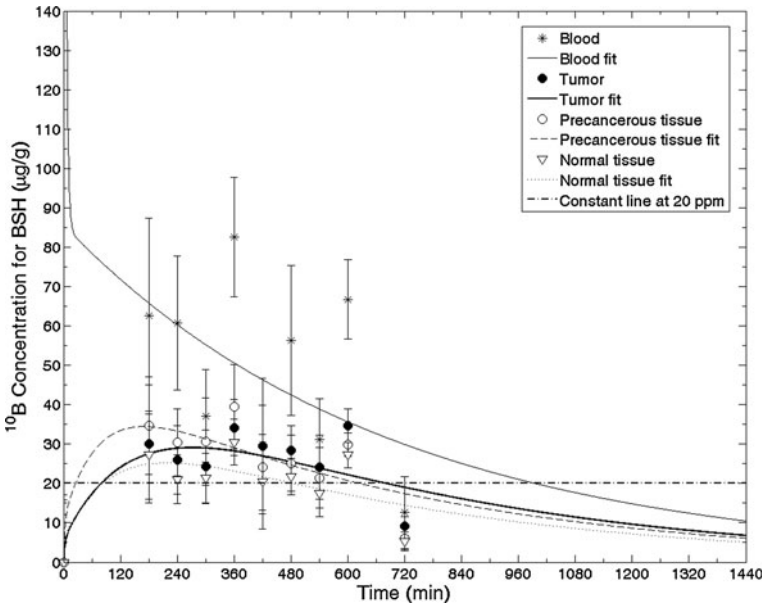

Fig. 2 Mean ^{10}B concentration values ± standard deviation for the different time-points and the corresponding fits to a two-compartment model for blood, normal, precancerous and tumor tissues. Fitting was based on all available individual data points. Boron concentration profiles are plotted as a function of time after BSH administration. A constant line is drawn at 20 ppm to indicate the minimum tumor boron concentration value considered to be therapeutically useful

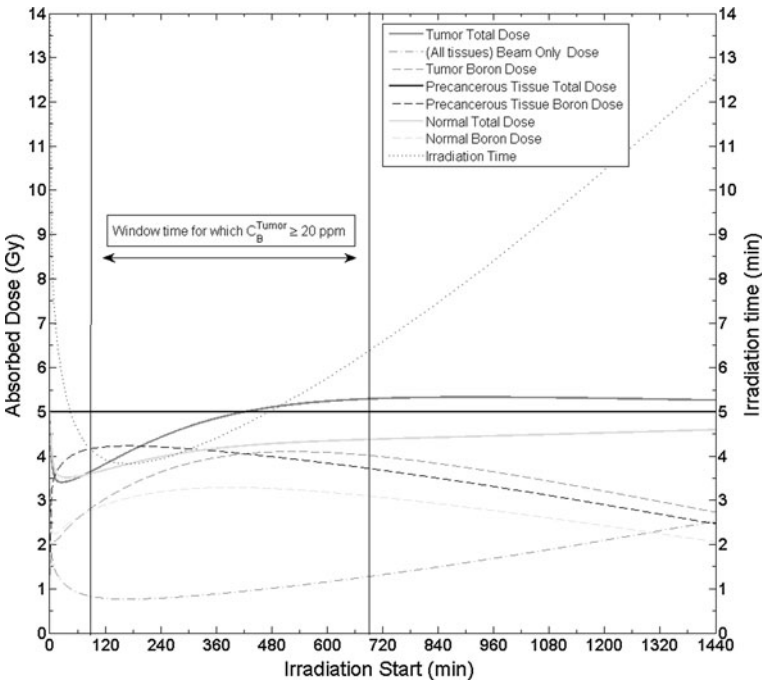

Fig. 3 Total absorbed dose in tumor (*solid dark gray line*), in precancerous tissue (*solid black line* at 5 Gy) and in normal tissue (*solid light gray line*) for different start times; contribution of the boron dose component for tumor (*dashed dark gray line*), precancerous tissue (*dashed black line*) and normal tissue (*dashed light gray line*); contribution of beam-only dose component for all tissues (*dotted-dashed dark gray line*). Irradiation time is plotted as a function of start time (*dotted black line*)

despite the previously described oscillations in mean boron concentration, the model shows how the fitted lines tend to dip down at the later time-points.

Figure 3 was constructed based on the fitted curves of Fig. 2 to determine, for nuclear reactor RA-3 (Miller et al. 2009), the "best" irradiation scenario that optimizes the interval between BSH administration and neutron irradiation. In keeping with previous studies that revealed the dose-limiting nature of mucositis in precancerous tissue (e.g., Kreimann et al. 2001b; Trivillin et al. 2006), 5 Gy was set as the limiting total absorbed dose in precancerous tissue. Figure 3 shows the total absorbed dose for different treatment start times (time interval between BSH administration and irradiation), the contribution of the boron dose component and the beam-only dose component, and the corresponding irradiation times. Considering the 5 Gy dose restriction for precancerous tissue, the requirement that tumor boron concentration should be ≥20 ppm and the choice of the interval in which tumor dose > precancerous tissue dose, the optimum interval between BSH administration and neutron irradiation would range between 7 and 11 h (420 and 660 min). Accordingly, irradiation time

would be between 5 and 6 min. The beneficial or detrimental effect of high blood boron values (Trivillin et al. 2006) remains to be determined in, in vivo BNCT studies. Due to the ostensible oscillations in blood boron values, blood boron concentration was not used as a restriction to determine the optimum interval for irradiation. However, the 7–11-h interval overlaps with the lower range of blood boron values.

High kidney and liver absolute boron concentration values suggest the need to shield these organs if they are in the treatment volume. The knowledge of boron concentration values in kidney, liver, spleen, lung, spinal cord marrow and brain are contributory to assess the potential risk of normal tissue toxicity associated to BNCT for other tumors and sites since these organs will not be in the treatment volume in the case of head and neck cancer.

Discussion

Sodium borocaptate employed as a stand-alone boron carrier in the hamster cheek pouch oral cancer model at 50 mg ^{10}B/kg (88 mg BSH/kg), a dose that lies within the range employed in clinical studies (Bendel et al. 2010), yields absolute boron values in tumor 3–10 h post-administration that fall within a therapeutically useful range for in vivo BNCT studies at RA-3 nuclear reactor (Pozzi et al. 2009). Absolute tumor boron values resemble those previously reported for GB-10 and BPA in this model but retention times were longer (Heber et al. 2004). As previously described for other boron compounds in different experimental models and in different tumor types in patients (e.g., Heber et al. 2004; Cardoso et al. 2009), the spread in tumor boron values among individual animals was large. The fact that no preferential tumor uptake was observed at any of the time-points does not necessarily imply that the compound is not eligible as a stand-alone boron carrier for BNCT. Similarly to GB-10, it could conceivably act selectively on tumor via selective blood vessel damage (Trivillin et al. 2006). Although high blood boron values as observed herein involve a potential risk of vascular damage (Wittig et al. 2009), they could potentiate the selective damage of structurally and functionally altered tumor blood vessels while preserving the blood vessels of normal and precancerous pouch tissue as previously described for GB-10-BNCT (Trivillin et al. 2006).

As previously established, precancerous tissue is the dose-limiting tissue in the hamster cheek pouch oral cancer model. Dose escalation is limited by mucositis in this tissue (e.g., Molinari et al. 2012). In a clinical scenario, confluent oral mucositis is a frequent, dose-limiting side effect during conventional radiotherapy for advanced head and neck tumors (Coderre and Morris 1999; Sonis 2004). Within this context, boron content in precancerous tissue is of particular importance and must be evaluated in terms of radiotoxicity in actual BNCT studies. Additionally, the fact that precancerous tissue boron values are, overall, somewhat higher than normal pouch tissue values would make it potentially possible to achieve a therapeutic effect in precancerous tissue in terms of inhibition of tumor development, without significant damage to normal pouch tissue (Heber et al. 2007; Monti Hughes et al. 2009). Even when precancerous tissue and normal pouch tissue do not differ in terms of gross boron values, preferential microlocalization (undetectable by ICP-OES measurements) of boron in precancerous foci at a higher risk of malignant transformation would conceivably favor a preferential effect in these areas (Monti Hughes et al. 2009, 2011).

No preferential uptake of BSH has been observed in peripheral tumors as described herein in keeping with previous studies (e.g., Obayashi et al. 2004). However, very importantly, BSH would contribute to boron targeting homogeneity in tumor (Ono et al. 1999; Obayashi et al. 2004), an issue that is pivotal to the therapeutic efficacy of BNCT. Furthermore, BSH would be particularly suited to target quiescent cells that are characteristically refractory to BPA targeting (Ono et al. 1996). It is known that BPA is taken up selectively, albeit heterogeneously, in the hamster cheek pouch oral cancer model (Kreimann et al. 2001a; Heber et al. 2006). Within this context, co-administration of BPA and BSH would conceivably improve selective tumor boron uptake and boron targeting homogeneity (Ono et al. 1996; Obayashi et al. 2004; Wittig et al. 2009). The similarities between boron concentration values for BSH (this study) and GB-10 (Heber et al. 2004) in this model in terms of tumor incorporation levels and lack of preferential tumor uptake could be ascribed to the passive diffusion uptake mechanism described for both boron compounds (Heber et al. 2004; Yoshida et al. 2002), although alternative uptake mechanisms cannot be ruled out (Neumann et al. 2002). Given the proven success of GB-10 as a stand-alone boron carrier for BNCT and the previously demonstrated therapeutic efficacy of BNCT mediated by GB-10 and BPA administered jointly in this model (Trivillin et al. 2006) with no normal tissue radiotoxicity, the present biodistribution data for BSH warrant in vivo BNCT studies to analyze the radiobiology of BNCT mediated by BSH and evaluate the therapeutic potential of BNCT mediated by BSH or BSH and BPA administered jointly. Due to the high boron content in kidney and liver, particular attention must be paid to protecting these organs during neutron irradiation. However, assuming that these levels of the compound are not chemically toxic in themselves, moderately high kidney and liver boron levels would not be a concern for head and neck cancer, where the kidney and liver would not be in the treatment volume. In particular, in

the case of the hamster cheek pouch oral cancer model, the body of the animal is shielded while the tumor-bearing pouch is exposed to the neutron beam as previously described (e.g., Molinari et al. 2011).

Sodium borocaptate boron concentration values in blood, tumor and normal tissues observed in the present study tend to be higher and retention times tend to be longer than those reported for other experimental models at similar dose levels (e.g., Wittig et al. 2009; Obayashi et al. 2004; Ichikawa et al. 2009; Yoshida et al. 2008) and more similar to BSH boron values found in patients with glioma after i.v. infusion of BSH (50 mg ^{10}B/kg) (Goodman et al. 2000). Certain differences in boron concentration values can be attributed to the experimental model employed and the BSH administration route. Variations could also be due to the fact that the assay used by here and by others to determine boron concentrations cannot distinguish between the parent drug (BSH) and any boronated metabolites and/or oxidation products that may have been formed in vivo (Gibson et al. 2003) and to the fact that retention of the dimer BSSB is significantly longer than that of the monomer BSH (Elhanati et al. 2001). However, other sources of variation cannot be ruled out.

Conclusion

Sodium borocaptate (BSH) delivered potentially therapeutically useful amounts of boron in the oral cancer model. The present biodistribution data suggest the potential value of BSH as a stand-alone boron carrier for the treatment of head and neck cancer with BNCT and allowed to establish the feasibility of BNCT mediated by BSH at RA-3 nuclear reactor for experimental oral cancer.

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