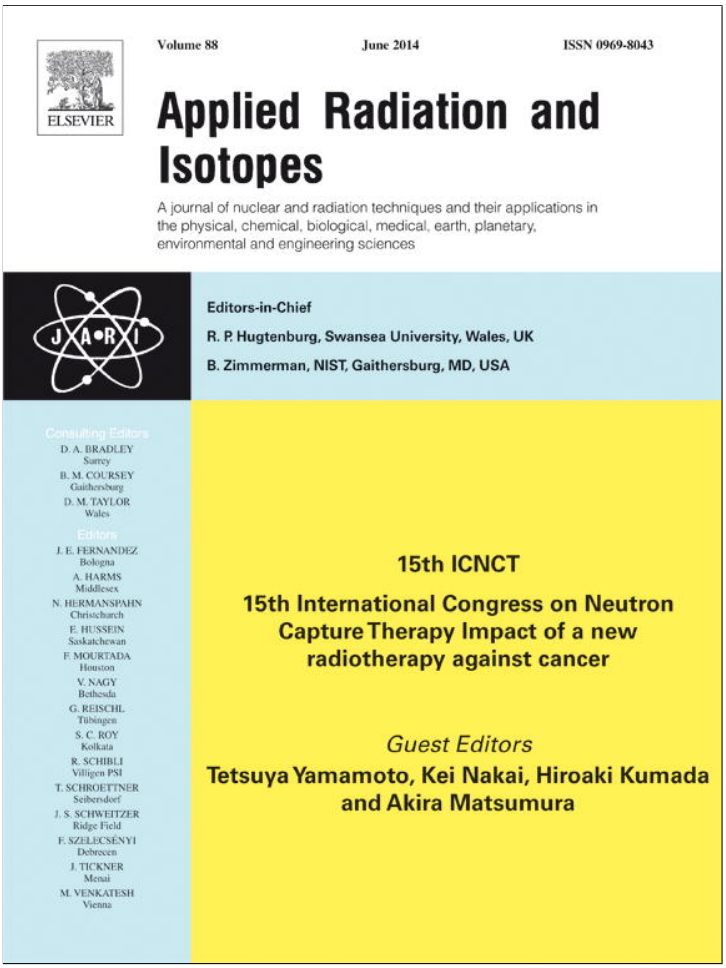
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15th International Congress on Neutron Capture Therapy Impact of a new radiotherapy against cancer

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Boron biodistribution for BNCT in the hamster cheek pouch oral cancer model: Combined administration of BSH and BPA

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HIGHLIGHTS

- We study the biodistribution of BPA+BSH for BNCT in experimental oral cancer.
- The 3 BPA+BSH protocols assayed are potentially therapeutic.
- Different proportions of B compounds with different CBE factors will affect response.

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ABSTRACT

Sodium mercaptoundecahydro-*closo*-dodecaborate (BSH) is being investigated clinically for BNCT. We examined the biodistribution of BSH and BPA administered jointly in different proportions in the hamster cheek pouch oral cancer model. The 3 assayed protocols were non-toxic, and showed preferential tumor boron uptake versus precancerous and normal tissue and therapeutic tumor boron concentration values (70–85 ppm). All 3 protocols warrant assessment in BNCT studies to contribute to the knowledge of (BSH+BPA)-BNCT radiobiology for head and neck cancer and optimize therapeutic efficacy.

1. Introduction

Boron Neutron Capture Therapy (BNCT) is a binary treatment that combines the administration of boron carriers that are taken up preferentially by neoplastic tissue and irradiation with a thermal/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 have a high relative biological effectiveness. 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. However, the interaction of the neutrons with nitrogen and hydrogen in tissue and the gamma component of the beam will deliver an unavoidable and nonspecific background dose (Coderre and Morris, 1999). As BNCT is based on biological rather than geometric targeting, it would be suited to treat undetectable micrometastases (e.g. Cardoso et al., 2007) and foci of malignant transformation in field cancerized tissue (Monti Hughes et al., 2009; Monti Hughes et al., 2011).

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). Within this context, and in view of the fact that radical surgery causes large tissue defect (Kastenbauer and Wollenberg, 1999), there is a need for more effective and selective therapies. Studies in appropriate experimental models are pivotal to progress in this field.

To explore new applications of BNCT and study its radiobiology to improve its therapeutic efficacy, we previously proposed and validated the use of the hamster cheek pouch model of oral cancer for BNCT studies (Kreimann 2001a, 2001b). The hamster cheek pouch model of carcinogenesis is widely accepted as a model of oral cancer (Salley, 1954). Carcinogenesis protocols induce pre-malignant and malignant changes that closely resemble spontaneous human oral mucosa lesions (Morris, 1961). In addition, the hamster cheek pouch model of oral cancer poses a unique advantage in that tumors are induced by periodic, topical application of the carcinogen dimethyl-1,2-benzanthracene (DMBA), a

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process that mimics the spontaneous process of malignant transformation. Conversely, the tumor models classically employed in BNCT small-animal studies are based on the growth of implanted cancer cells in healthy tissue (e.g. Barth et al., 2005). In the hamster cheek pouch, carcinogenesis protocols lead to the development of what has been called, globally, "precancerous tissue" (e.g. Kreimann et al., 2001a) or, more recently, "tissue with potentially malignant disorders (PMD)" (Heber et al., 2010), from which tumors arise. Thus, 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 in terms of its role as a potentially dose-limiting tissue and the fact that second primary tumor locoregional recurrences that arise in field-cancerized tissue are a frequent cause of therapeutic failure (Hoebers et al., 2011; Smith and Haffty, 1999).

Within the context of our BNCT studies in the hamster cheek pouch oral cancer model, we proved the therapeutic potential of the chemically non-selective boron compound decahydrodecaborate (GB-10) as a stand-alone boron carrier for BNCT in the hamster cheek pouch oral cancer model with no toxic effects in normal or precancerous tissue. Although GB-10 is not taken up selectively by oral tumor tissue, selective tumor lethality would result from selective aberrant tumor blood vessel damage (Trivillin et al., 2006). In addition, GB-10 contributed to homogenous boron targeting of all tumor cell populations (Heber et al., 2006). Furthermore, BNCT efficacy was enhanced when GB-10 and boronophenylalanine (BPA) were jointly administered (Pozzi et al., 2009; Trivillin et al., 2006).

The fact that sodium mercaptoundecahydro-*closo*-dodecaborate (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. BSH 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 vs 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, based on the working hypothesis that BSH might conceivably behave similarly to GB-10 in oral cancer, we previously performed biodistribution studies with BSH alone in the hamster cheek pouch oral cancer model and showed the therapeutic potential of certain administration protocols (Garabalino et al., 2010).

Based on the knowledge that targeting of all populations within a target tissue is critical to the success of BNCT, it has been postulated that the combined administration of different boron compounds with different properties and complementary uptake mechanisms may enhance the therapeutic efficacy of BNCT (e.g. Heber et al., 2007; Ono et al., 1999; Trivillin et al., 2006). Hence, our particular interest in exploring combined boron compound administration protocols.

The aim of the present study was to perform biodistribution studies of BSH+BPA administered jointly in the hamster cheek pouch oral cancer model as a starting point to contribute to the knowledge of (BSH+BPA)-BNCT radiobiology for head and neck cancer and optimize its therapeutic efficacy.

2. Materials and methods

2.1. Model of oral cancer: tumor induction

Tumors were induced in the right cheek pouch of noninbred 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 body weight)-xylazine (10.5 mg/kg body weight) anesthesia and examined to monitor tumor development. Once the exophytic tumors, i.e. Squamous Cell Carcinomas, developed and reached a diameter of approximately 5 mm, the animals were used for biodistribution studies. Institutional guidelines for the care and use of laboratory animals were followed throughout.

2.2. Biodistribution studies

Three administration protocols with different proportions of BSH and BPA were assessed: 1. BSH, 50 mg ^{10}B/kg, iv+BPA, 15.5 mg ^{10}B/kg, ip; 2. BSH, 34.5 mg ^{10}B/kg, iv+BPA, 31 mg ^{10}B/kg, ip; 3. BSH, 20 mg ^{10}B/kg, iv+BPA, 46.5 mg ^{10}B/kg, ip. For all 3 protocols the total boron dose administered was within the same range (65.5–66.5 mg ^{10}B/kg).

BSH (BBI, Cat.1921, purity 99.23%) was dissolved in saline to 0.084 M in anaerobic conditions to avoid the formation of the toxic dimers BSSB, BSSOB and BOSSOB. 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. Kreimann et al., 2001a; Trivillin et al., 2006). BPA was converted to a more soluble fructose complex by mixing BPA and fructose in water at a 1:1 M ratio. The pH was adjusted to 9.5–10 with NaOH, the mixture was stirred until all the solids dissolved and the pH was then re-adjusted to 7.4 with HCl. The concentration was then adjusted with USP water for injection to 0.14 M or 0.42 M (e.g. Garabalino et al., 2011). Based on previous studies (e.g. Kreimann et al., 2001a; Garabalino et al., 2010), groups of animals were euthanized 4 h after the administration of BSH and 3 h after the administration of BPA. Samples of blood, tumor, precancerous and normal pouch, skin, spinal cord marrow, brain, liver, kidney, and lung were processed for gross boron measurement by Atomic Emission Spectroscopy with Inductively Coupled Plasma (ICP-OES Optima 3100 XL, UV, axial, Perkin Elmer) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS, ELAN DRC2, Perkin Elmer).

2.3. 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 ICP-OES or ICP-MS. In the case of ICP-OES measurements, 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) was 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 case of ICP-MS measurements, tissue samples (approximately 50 mg) and blood samples (200–300 μl) were digested in 15 ml Falcon tubes for 1 h at 100 °C in 0.25 ml of a 1:1 mixture of ultrapure concentrated sulfuric acid (J.T. Baker, Phillipsburg, USA) and sub-boiling nitric acid distilled from nitric acid 65% (p.a., Carlo Erba, Milan, Italy). Once the digestion process was complete, the mixture was allowed to cool and milli-Q water was added to bring the final volume to 10 ml. The digested samples were stored at room temperature for a maximum of 7 days prior to measurement. All the digested samples were vortexed immediately prior to preparation for actual measurement. Approximately 0.5–1 ml of the digested tissue sample or 0.2 ml of the digested blood sample (depending on estimated boron content) was placed in a new Falcon tube and mixed with 0.20 ml of a 1:1 mixture of ultrapure concentrated sulfuric acid and sub-boiling nitric acid. 0.25 ml of ^{6}Li (1 ppm) was added as an internal standard. Milli-Q water was added to bring the final volume to 10 ml. All the prepared samples were vortexed immediately prior to measurement. Different dilutions of a standard calibration solution (Multi-Element ICP-MS Calibration Standard of B, Ge, Mo, Nb, P, Re, S, Si, Ta, Ti, W, and Zr, 10 mg/l, Perkin Elmer) were used to prepare a calibration line each day of operation.

2.4. 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.

3. Results

Table 1 shows the absolute boron concentration values for blood and tissues. Considering the tissues that would be clinically most relevant in the case of head and neck cancer, mean boron concentration fell within the same range of widespread values for all 3 protocols in the case of tumor (70–85 ppm), precancerous pouch tissue (47–68 ppm), and normal pouch tissue (43–66 ppm). Spinal cord marrow and brain values were similarly low for all 3 protocols, with mean values ranging from 6 to 11 ppm. Preferential tumor uptake vs precancerous and normal pouch tissue was observed for all 3 protocols, with ratios ranging from 1.5 to 2. Differences in blood boron concentration were observed between protocols, i.e. 1. 80 ± 11 ppm; 2. 122 ± 35 ppm; 3. 43 ± 9 ppm.

4. Discussion

Absolute boron content, distribution and microdistribution in tumor and healthy tissues are central to the efficacy of BNCT. The requirements for successful BNCT are selective accumulation of a non-toxic ^{10}B carrier in tumor relative to dose-limiting healthy tissues in the treatment volume, a sufficiently high absolute boron concentration of ^{10}B in tumor tissue for sufficient ^{10}B(n,α)^{7}Li reactions to occur, and targeting of all tumor cell populations to avoid the existence of potentially refractory tumor cells that will impair tumor control (e.g. Coderre and Morris, 1999; Garabalino et al., 2011; Heber et al., 2006; Trivillin et al., 2006). 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; Pozzi et al., 2009; Trivillin et al., 2004; Trivillin et al., 2006), we defined guidelines to establish the potential therapeutic value of the administration protocols assessed, i.e. no manifest toxicity, absolute boron concentration in tumor ≥ 20 ppm, boron concentration ratio tumor/normal tissue ≥ 1, boron concentration ratio tumor/blood ≥ 1. In addition, at a given tumor/healthy tissue boron concentration ratio, high absolute ^{10}B tumor concentrations are an asset because they allow for shorter irradiation times and a concomitant reduction in background dose (Coderre and Morris, 1999). Furthermore, the microlocalization of ^{10}B also conditions the therapeutic outcome of BNCT (Santa Cruz and Zamenhof, 2004; Smith et al., 2001).

Boron biodistribution studies are essential to design and plan useful BNCT preclinical and, ultimately, clinical research protocols. In particular, they identify potentially useful boron compounds and administration protocols and enable the choice of the optimum time post-administration of the boron carrier to perform neutron irradiation, seeking to maximize tumor boron levels while minimizing healthy tissue and blood levels. To date, there is no clinically-practical online, non-invasive way to evaluate boron concentration during irradiation for BNCT. Thus, dose calculations are based on boron content values in blood, tumor and normal tissue obtained from biodistribution studies performed beforehand (e.g. Garabalino et al., 2011). At most, in the case of patient irradiation, blood samples can be taken just before and even during irradiation to infer the tissue boron concentration, assuming the tumor/blood ratios established in previously performed biodistribution studies (González et al., 2004). In the specific case of experimental models, dose calculations are based on the mean values obtained from biodistribution studies in separate sets of animals (Kreimann et al., 2001a). In this sense it is important to bear in mind that large intra-tumor, inter-tumor, intra-tissue and inter-subject variations in gross boron content values have been

Table 1
Boron concentration (mean ± standard deviation) (ppm) in blood and tissue samples for the different administration protocols (*n* denotes number of hamsters or tumors).

Tissues	BSH 50 mg ^{10}B/kg iv + BPA 15.5 mg ^{10}B/kg ip	BSH 34.5 mg ^{10}B/kg iv + BPA 31 mg ^{10}B/kg ip	BSH 20 mg ^{10}B/kg iv + BPA 46.5 mg ^{10}B/kg ip
Tumor (T)	71.0 ± 38.3 (*n*=21)	85.2 ± 15.0 (*n*=14)	85.2 ± 20.4 (*n*=3)
Normal pouch (N)	41.1 ± 6.8 (*n*=5)	66.2 ± 20.2 (*n*=3)	43.3 ± 4.5 (*n*=3)
Blood (B)	80.4 ± 10.7 (*n*=5)	122.1 ± 34.5 (*n*=3)	42.7 ± 9.1 (*n*=3)
Precancerous	58.9 ± 11.6 (*n*=5)	68.2 ± 12.1 (*n*=3)	46.6 ± 7.7 (*n*=3)
Spinal cord	8.2 ± 2.0 (*n*=5)	9.2 ± 4.0 (*n*=3)	8.9 ± 2.0 (*n*=3)
Brain	5.8 ± 1.6 (*n*=5)	9.5 ± 3.3 (*n*=3)	11.3 ± 1.5 (*n*=3)
Skin	43.1 ± 12.2 (*n*=5)	60.3 ± 8.7 (*n*=3)	48.3 ± 9.4 (*n*=3)
Liver	258.7 ± 15.3 (*n*=4)	274.3 ± 33.6 (*n*=3)	55.5 ± 12.9 (*n*=3)
Kidney	116.1 ± 18.4 (*n*=4)	205.9 (*n*=2)	86.5 (*n*=2)
Lung	41.6 ± 8.2 (*n*=5)	53.0 ± 12.6 (*n*=3)	26.0 ± 9.8 (*n*=3)
T/N	1.7 ± 0.8	1.5 ± 0.5	2.0 ± 0.5
T/B	0.9 ± 0.5	0.8 ± 0.3	2.1 ± 0.8

reported (e.g. Heber et al., 2004, 2006). These variations must be accounted for in dose calculation and dose prescription, to avoid exceeding the radiotolerance of the healthy tissues within the treatment volume.

Within this context, we can conclude that the three BSH+BPA administration protocols assayed herein at a similar total boron dose, delivered similar, potentially therapeutic boron content to tumor with a large spread in values. Future studies are warranted to assess additional post-administration times. The degree of tumor selectivity was similar for all three protocols. Based on previous studies, the combined administration of BPA and GB-10 would contribute to boron targeting homogeneity (e.g. Heber et al., 2007). In addition, the use of these particular compounds has the advantage that they are both approved for use in human subjects (Molinari et al., 2011). Much effort has been expended to search for the 'ideal' ^{10}B compound that would potentially replace the three 'imperfect' compounds currently authorized for use in humans, boronophenylalanine (BPA) and sodium borocaptate (BSH), used in clinical trials and decahydrodecaborate (GB-10). Presently no other ^{10}B compounds have reached the stage for evaluation in a clinical bio-distribution study. If and when a new ^{10}B carrier is identified as promising, from cell culture studies, it still faces many hurdles: bio-distribution studies in appropriate tumor models and the evaluation of toxicity prior to experimental in vivo BNCT studies. Translation of experimental data to clinical bio-distribution studies is costly, of no direct benefit to participants, and must comply with the stringent requirements of regulatory agencies. Optimizing the delivery of the ^{10}B compounds currently authorized for use in humans is an excellent short and medium term strategy. The knowledge gained will also be applicable to potentially 'more perfect' ^{10}B compounds.

We anticipate potential differences between the three BSH+BPA protocols in terms of therapeutic efficacy and/or radiotoxicity due to differences in boron microdistribution and boron targeting homogeneity that cannot be evidenced by ICP gross boron measurements. In addition, different proportions of boron compounds with different Compound Biological Effectiveness (CBE) factors will conceivably affect response (Coderre and Morris, 1999). The radiobiological role of differences in blood boron values remains to be determined. 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). All three BSH+BPA protocols warrant assessment in in vivo BNCT studies.

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