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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