Radiat Environ Biophys (2008) 47:147–155
DOI 10.1007/s00411-007-0138-8

ORIGINAL PAPER

Boron neutron capture therapy (BNCT) for the treatment of spontaneous nasal planum squamous cell carcinoma in felines

Verónica A. Trivillin · Elisa M. Heber · Monica Rao · María A. Cantarelli · Maria E. Itoiz · David W. Nigg · Osvaldo Calzetta · Herman Blaumann · Juan Longhino · Amanda E. Schwint

Received: 28 June 2007 / Accepted: 4 October 2007 / Published online: 23 October 2007

Abstract Recently, Boron neutron capture therapy (BNCT) was successfully applied to treat experimental squamous cell carcinomas (SCC) of the hamster cheek pouch mucosa, with no damage to normal tissue. It was also shown that treating spontaneous nasal planum SCC in terminal feline patients with low dose BNCT is safe and feasible. In an extension of this work, the present study aimed at evaluation of the response of tumor and dose-limiting normal tissues to potentially therapeutic BNCT doses. Biodistribution studies with ^{10}B-boronophenylalanine (BPA enriched in ^{10}B) as a ^{10}B carrier were performed on three felines that showed advanced nasal planum SCC without any standard therapeutic option. Following the biodistribution studies, BNCT mediated by ^{10}BPA was done using the thermalized epithermal neutron beam at the RA-6 Nuclear Reactor. Follow-up included clinical evaluation, assessment of macroscopic tumor and normal tissue response and biopsies for histopathological analysis. The treated animals did not show any apparent radiation-induced toxicity. All three animals exhibited partial tumor control and an improvement in clinical condition. Enhanced therapeutic efficacy was associated with a high ^{10}B content of the tumor and a small tumor size. BNCT is therefore believed to be potentially effective in the treatment of spontaneous SCC. However, improvement in targeting ^{10}B into all tumor cells and delivering a sufficient dose at a greater depth are still required for the treatment of deep-seated, large tumors. Future studies are needed to evaluate the potential efficacy of the dual mode cellular (e.g. BPA-BNCT) and vascular (e.g. GB-10-BNCT) targeting protocol in a preclinical scenario, employing combinations of ^{10}B compounds with different properties and complementary uptake mechanisms.

V. A. Trivillin · E. M. Heber · M. E. Itoiz · A. E. Schwint (✉)
Department of Radiobiology, National Atomic Energy Commission, Av. General Paz 1499, B1650KNA San Martin, Province Buenos Aires, Argentina
e-mail: schwint@cnea.gov.ar

M. Rao · M. A. Cantarelli
Veterinary Oncology Center, Avenida del Libertador 15013, 1642 Acassuso, Province Buenos Aires, Argentina

M. E. Itoiz
Department of Oral Pathology, Faculty of Dentistry, University of Buenos Aires, M.T. de Alvear 2142, 1122 Buenos Aires, Argentina

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

O. Calzetta · H. Blaumann · J. Longhino
Department of Nuclear Engineering, Bariloche Atomic Center, National Atomic Energy Commission, 8400 San Carlos de Bariloche, Province Río Negro, Argentina

Introduction

Boron neutron capture therapy (BNCT) is a binary treatment modality that involves the selective accumulation of ^{10}B carriers in tumors followed by irradiation with a thermal or epithermal neutron beam. The capture of thermal neutrons by ^{10}B nuclei gives rise to high linear energy transfer (LET) alpha particles and recoiling ^{7}Li nuclei that deposit their energy in a 9 and 5 μm range, respectively, with a high relative biological effectiveness (RBE). If ^{10}B is taken up selectively by tumor tissue, capture reactions will occur preferentially in tumor cells, and the resulting high-LET particles will travel only a very short distance (which corresponds roughly to the size of a cell/cell nucleus).

Thus, BNCT would target tumor tissue selectively, sparing normal tissue [1]. Clinical trials of BNCT for the treatment of glioblastoma multiforme and/or melanoma and, more recently, head and neck tumors using ^{10}B-boronophenylalanine (^{10}B-enriched BPA) or sodium mercaptoundecahydrododecaborane (BSH) as the ^{10}B carriers, have been performed or are underway in Argentina, Japan, the US and Europe (e.g. [2–4]). To date, results on the therapeutic advantage of this technique are encouraging but inconclusive. Translational studies have been carried out employing a variety of experimental models based on the implantation of tumor cells in normal tissue (e.g. [5]).

We previously proposed and validated [6] the use of the hamster cheek pouch oral cancer model for BNCT studies. The aim was to explore new applications of BNCT, investigate the radiobiology of BNCT, improve its therapeutic efficacy, analyze the behavior of clinically relevant, dose limiting normal tissues, and to test the potential therapeutic advantage of different ^{10}B agents. It was demonstrated that ^{10}BPA and decahydrodecaborate-^{10}B ($Na_2{}^{10}B_{10}H_{10}$ or GB-10) administered alone or jointly deliver therapeutically useful amounts of ^{10}B to squamous cell carcinomas (SCC) in the hamster cheek pouch [6–8]. ^{10}B uptake by precancerous tissue surrounding a tumor would provide a rationale to treat "field cancerized" tissue [9]. Furthermore, a remarkable therapeutic success of BNCT mediated by ^{10}BPA was reported [10]. It was also found that low-dose [11] and high-dose [12] BNCT mediated by ^{10}B-GB-10 (^{10}B-enriched GB-10) or by the combined administration of ^{10}BPA and ^{10}B-GB-10 did not induce any radiation-induced toxicity in normal tissue, and induced only slight-moderate reversible mucositis in precancerous tissue around the tumor. In a previous study, we demonstrated the feasibility and safety of treating spontaneous head and neck tumors of felines, with particular focus on SCC, with low-dose BNCT mediated by ^{10}BPA employing the thermal neutron beam of the RA-1 Nuclear Reactor (located on site) in a preclinical scenario [13]. The study of spontaneous tumors of the same histological type that we induce experimentally in the hamster cheek pouch would provide information on the clinical relevance of our experimental data.

SCC is a common tumor involving the skin and accounting for approximately 15% of cutaneous tumors in cats. SCCs are usually found in unpigmented or lightly pigmented skin. The most common cutaneous locations are sparsely haired areas of the nasal planum, eyelids and pinnae. SCCs may be locally very invasive but metastasize only rarely. Invasive SCC is usually preceded by a protracted course of disease that progresses through the following stages: crusting and erythema, superficial erosions and ulcers (carcinoma in situ or early SCC) and finally deeply invasive and erosive lesions. Many therapeutic modalities have been applied to SCC involving the facial skin in cats. Surgery and cryosurgery are the most commonly used. Radiotherapy and photodynamic therapy are also used with varying efficacy, while chemotherapy has shown little efficacy. Outcomes are generally good for most modalities if the tumors are treated early in their course. However, as carcinogenesis progresses, the degree of local invasion can be quite severe and impairs therapeutic success. Tumors of higher stages respond poorly to all therapeutic modalities [13].

It has been demonstrated that BNCT for the treatment of spontaneous nasal planum SCC in felines with low doses of irradiation employing the thermal beam at the RA-1 Nuclear Reactor is feasible and safe [13]. The aim of the present study was to evaluate the response of tumor and dose-limiting normal tissues to potentially therapeutic doses of BNCT. For this reason, three felines showing spontaneous nasal planum SCC were exposed to higher doses of irradiation employing the thermalized epithermal beam of the RA-6 Nuclear Reactor located in the Bariloche Atomic Center in the city of San Carlos de Bariloche, Argentina.

Materials and methods

Feline patients

Three felines were included in the study, with a clinical and histopathological diagnosis of advanced nasal planum SCC, and a clinical assessment indicating that standard therapy was no longer a valid option. All cats exhibited asthenia, difficulty to breathe, and lack of appetite. When possible, blood tests were performed to determine the clinical status of the animal appropriately. Tumor surfaces were evaluated by visual inspection, since it was not possible to perform Computed Tomography scans of the animals. For the same reason, degree of tumor invasion and tumor volume could not be evaluated. Consent forms were signed by the informed owners. The study was carried out in strict compliance with local and institutional regulations to protect animal subjects.

Patient 1 (Rulito)

Male, barely ate, overly thin for size (body weight: approximately 4 kg), adult (age unknown), with an extremely large, locally invasive tumor in the nose area. Tumor surface area was 17.1 cm^2 (3.8 cm × 4.5 cm). Tumor growth had obstructed the nostrils and severely impaired breathing. The histopathological diagnosis indicated semi-differentiated SCC (Fig. 1a). The tumor was actively growing, exhibited numerous mitotic figures, scarce stroma and abundant parenchyma. Laboratory tests (complete blood

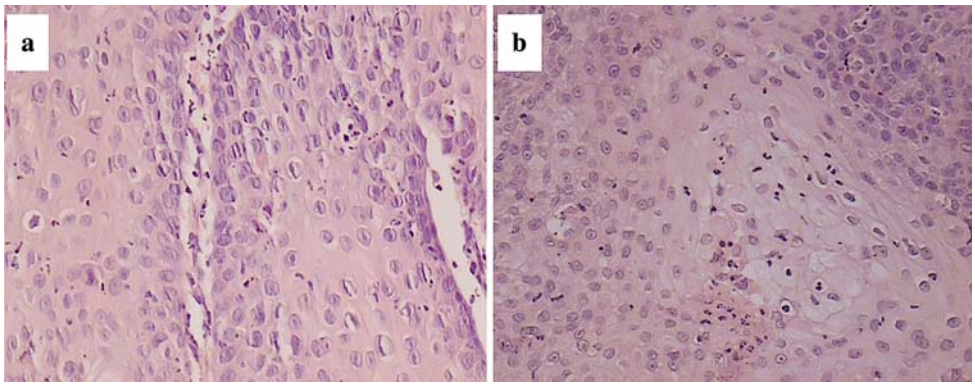

Fig. 1 Patient 1 (Rulito). **a** Light-microscope view (original magnification: 1/400) of a biopsy section stained with hematoxylin–eosin showing viable tumor cells of a semi-differentiated SCC pre-BNCT. **b** Light-microscope view (original magnification: 1/400) of a biopsy section stained with hematoxylin–eosin showing a necrotic focus in the center of a tumor cord 1 month after BNCT

count (CBC), urea, creatinine, alkaline phosphatase and alanine transaminase) showed values within the normal range (results not shown) except for a low hematocrit value of 22% (reference value 35–55%).

Patient 2 (Mario)

Male, adult (age unknown), body weight approximately 7 kg, with a medium-sized ulcerated tumor in the nose area. Tumor surface area was 3.9 cm^2 (2.3 cm × 1.7 cm). The histopathological diagnosis indicated semi-differentiated SCC (Fig. 2a). Laboratory tests (CBC, urea, creatinine, alkaline phosphatase and alanine transaminase) showed values within the normal range (results not shown).

Patient 3 (Michi)

Female, 14 years old, body weight approximately 5.5 kg, with a medium-sized tumor in the nose area. Tumor surface area was 1.6 cm^2 (1.3 cm × 1.2 cm). The histopathological diagnosis indicated undifferentiated SCC (Fig. 3a). The laboratory tests (CBC, creatinine, alkaline phosphatase and alanine transaminase) showed values within the normal range (results not shown). The blood urea nitrogen value was 55 mg/dl, slightly higher than the reference value of 14.00–40.00 mg/dl.

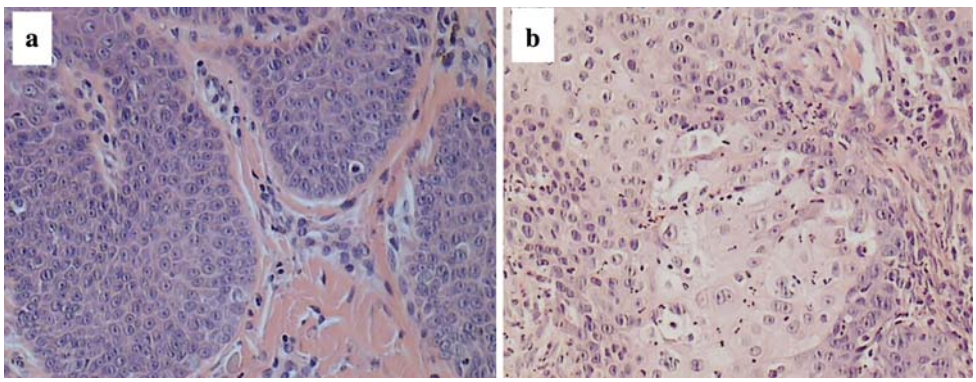

Fig. 2 Patient 2 (Mario). **a** and **b** same as Fig. 1. **b** Shows ghost images in the center of a tumor cord and signs of radiation-induced damage such as bizarre and hyperchromatic nuclei 3 months after BNCT

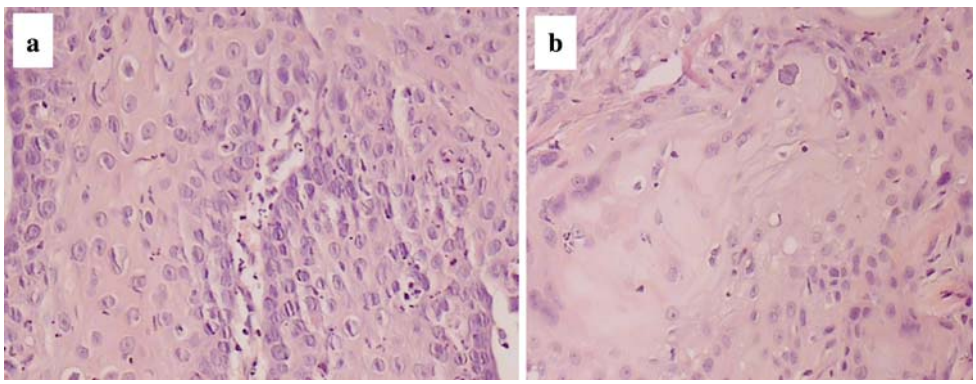

Fig. 3 Patient 3 (Michi). **a** and **b** same as Fig. 1; **b** shows a necrotic focus in the center of a tumor cord, cellular damage and cytoplasmic vacuolization 1 month after BNCT

^{10}BPA biodistribution studies

A 0.14 M solution of L-^{10}BPA fructose (>98% enriched) was administered intravenously over approximately 15–20 min at a dose of 300 mg per kg body mass. Blood samples (0.2 ml) were taken prior to infusion, at the end of the infusion, and at 0.5, 1, 2 and 3 h after the end of the infusion under light ketamine-xylazine anesthesia. At 3 h after the end of the infusion, the animals were anesthetized with ketamine-xylazine. The choice of ^{10}BPA dose and post-administration time was based on a previous biodistribution study in the oral cancer model of the hamster cheek pouch [6]. The surgeon took one or more (if possible) samples of tumor, dorsum skin and lip mucosa. In the case of Patient 1 (Rulito), a large portion of the tumor was removed by cryosurgery to facilitate breathing. All of the samples were weighed immediately. Tissue samples were stored at −70°C and blood samples were stored at −4°C until use. Boron analysis was performed by inductively coupled plasma optical emission spectroscopy (ICP-OES). It should be noted that ICP-OES measures total boron (^{10}B and ^{11}B), and that biodistribution studies do not require the boron compound to be enriched in ^{10}B. In contrast, BNCT studies do require the boron compound to be enriched in ^{10}B (natural abundance of boron includes 20% ^{10}B and 80% ^{11}B) for a therapeutically useful number of capture reactions to take place between thermal neutrons and the ^{10}B atoms. Tissue samples (≤50 mg) were digested at room temperature overnight in 0.20 ml of a 1:1 mixture of concentrated sulfuric and nitric acids. Once the digestion process was complete, addition of 0.6 ml of a 5% solution of the detergent Triton X-100 in water and 0.2 ml of yttrium–strontium as an internal standard resulted in a clear solution for ICP-OES analysis [8, 14]. Addition of the internal standard allows for the reduction in short-term variations in signal (noise). This is accomplished by taking ratios of the measured signal (boron in this case) to a simultaneously measured signal from the added internal standard element. Furthermore, in our particular case, if sample digestion is incomplete, sample uptake by the ICP-OES nebulizer will be flawed, resulting in artificially low boron values. The operator will be alerted to this by a reduction in the expected values of internal standard and the reading is disregarded. Blood samples (200–300 µl) were digested at room temperature overnight in 1 ml of a 1:1 mixture of concentrated sulfuric and nitric acids. Once the digestion process was complete, 3 ml of a 5% solution of detergent Triton X-100 in water and 1 ml of yttrium–strontium as an internal standard was added. Finally, 1 ml samples of the resulting solutions were measured by ICP-OES. Standard solutions of boric acid were used to prepare a calibration line during each day of operation.

In vivo BNCT

Having demonstrated (see "Results") that boron values in tumor, normal tissue and blood were, from the therapeutical point of view, in an acceptable range, BNCT was performed 1–5 weeks after the biodistribution study. The animals were transported by plane to Bariloche, a city 1,600 km south–west of Buenos Aires, to be irradiated with the thermalized epithermal neutron beam at the RA-6 Nuclear Reactor [15]. This beam provides an adequate neutron spectrum for BNCT of largely superficial tumors. The beam is of the hyperthermal type, moderated to epithermal energies by aluminum oxide, and then passed through a few centimeters of hydrogenous media to partially thermalize the spectrum, creating an acceptably uniform dose-depth distribution to a depth of approximately 1 cm. Furthermore, the facility allows for exposure of the tumor area (and inevitably the head) and shielding of the rest of the body. The animals were injected intravenously with ^{10}BPA over approximately 15–20 min at a dose of 300 mg ^{10}BPA per kg body mass (same dose as that used for the biodistribution study described above). Three hours after administration of ^{10}BPA, the animals were irradiated under ketamine-xylazine anesthesia. The nasal area and, inevitably, the head, were placed at the beam port, which is 15 cm in diameter (Fig. 4). The rest of the body was shielded by the lead and borated polyethylene of the beam delimiter. The average flux of thermal neutrons at the tumor and surrounding healthy tissue was $3.4 \pm 0.3 \times 10^8$ neutrons cm^{-2} s^{-1}.

Fig. 4 Patient 1 (Rulito) positioned at the beam port for irradiation

Irradiations lasted 40 min resulting in an approximate fluence of thermal neutrons of 8.2×10^{11} neutrons cm^{-2}.

Monte Carlo neutron-photon calculations using the MCNP5 code were performed to estimate the perturbation of the RA-6 neutron beam spectrum and intensity caused by the presence of the cats' heads in the beam. The results were used to adjust previously measured in-air beam dose components as an alternative to perform detailed phantom measurements. The MCNP5 model included the detailed geometry of the beam port with a plane source entering the port that was pre-computed from the results of a calculation for the RA-6 core, coupled to the beam filtering and moderating components. An ellipsoidal representation of a typical cat head (modeled as acrylic, with semi-axis lengths similar to those of the head) was modeled at the irradiation location in the beam port with its narrower tip in the exit plane. A tally volume of $\sim$1 cm^3, representing the nasal volume, was included in that tip, and the computed neutron fluxes and doses were tallied, along with the photon-induced dose (photons generated from the neutron interactions in the material). The same tally volume was then used in a second calculation without the inclusion of the head model. The results of both calculations were combined to establish the necessary correction factors for the measured free-beam dose components.

The physical dose components were then further adjusted to take into account the results of the boron biodistribution studies (reported in "Results"). Finally, Gy-eq doses were calculated taking into account previously reported relative biological effectiveness (RBE) and compound biological effectiveness (CBE) values as indicated in the "Results" section. CBE is a derived quantity that goes beyond the concept of RBE to take into account that the boron delivery agent may not be uniformly distributed on a cellular and sub-cellular basis. It is measured in the same manner as RBE, but it implicitly includes the traditional RBE for the alpha and lithium particles as well as effects related to the micro-distribution of the boron, as noted above. For example, a compound that delivers boron directly to DNA will be expected to have a higher CBE than an agent that does not target the radiosensitive subcellular structures so precisely.

Follow-up

Follow-up of the macroscopic response of tumor and normal tissue and of the clinical status was performed periodically. A biopsy of the tumor area was taken at a representative time point after BNCT for histopathological analysis (30 days after BNCT in the case of Patient 1 (Rulito) and Patient 3 (Michi), and 3 months after BNCT in the case of Patient 2 (Mario)). Limited collaboration of the cats' owners put some constraints on the follow-up. Autopsies of two out of the three cats were performed following euthanasia as indicated in "Results". Radiation-induced toxicity was evaluated in terms of clinical signs such as presence of gastrointestinal syndrome, symptoms of neurological involvement such as convulsions, ataxia and lack of coordination, behavioral disturbances, regional loss of hair and peripheral congestion, and findings at autopsy compatible with radiation-induced toxicity such as gastric and intestinal ulcerations, ascitis, fatty deposits in liver and lung edema.

Results

^{10}BPA biodistribution studies

Table 1 shows the boron concentrations measured in the blood and tissue samples, corresponding to different times after administration of the ^{10}BPA. The number of samples taken depended on the clinical status of the cats. In all cases the pre-infusion concentrations were below the detection limit of 0.06 ppm. Absolute boron concentrations in the tumors were, particularly for patient 3 (Michi), somewhat low, but within the therapeutically useful range (Table 1). In general, ratios of boron concentrations in tumor and blood, and in tumor and normal lip mucosa were larger than one, indicating larger boron concentrations in the tumor tissue. Lip mucosa was employed to calculate the tumor/normal tissue ratio because it lay within the treatment volume and was exposed to virtually the same thermal neutron fluence as the tumor tissue. Normal dorsum skin was exposed to a lower thermal neutron fluence because it lay in the shielded area, radially outside the beam. Absolute and relative boron values warranted BNCT in all three cases.

In vivo BNCT

Table 2 presents estimated absorbed doses from the different radiation components and total dose in tumor and normal tissue. These estimates included use of a simplified Monte Carlo model to consider the modification of the initial neutron spectrum by the presence of the cat's head. The ^{10}B dose was calculated taking into account the boron content in tumor and normal tissue as obtained in the biodistribution studies (Table 1).

Assuming as weighting factors, an RBE value of 3.2 for fast neutrons and induced protons, a compound biological effectiveness (CBE) factor of 3.8 for the ^{10}BPA radiation dose component in the tumor, a CBE factor of 2.5 for the ^{10}BPA radiation dose component in normal tissue and an RBE value of 1 for photons [16, 17], the total tumor dose was estimated to be 14.8 Gy-eq. for Patient 1 (Rulito), 12.2 Gy-eq. for Patient 2 (Mario) and 9.5 Gy-eq. for Patient 3

Table 1 Boron concentration (ppm) in blood and tissue samples at different times after administration of ^{10}BPA at a dose of 300 mg kg^{-1}

Tissues	Patient 1 (Rulito), tumor surface area: 17.1 cm^2	Patient 2 (Mario), tumor surface area: 3.9 cm^2	Patient 3 (Michi), tumor surface area: 1.6 cm^2
Blood			
End of infusion	44.0 ± 2.3 (n = 2)	41.6 ± 6.4 (n = 4)	45.9 ± 8.4 (n = 4)
0.5 h	19.8 ± 0.6 (n = 2)	14.3 ± 1.5 (n = 4)	17.5 ± 4.8 (n = 3)
1 h	14.9 ± 0.2 (n = 2)	8.8 ± 0.1 (n = 2)	11.2 ± 2.1 (n = 4)
2 h	10.5 ± 0.1 (n = 2)	6.6 ± 0.6 (n = 2)	7.5 ± 0.7 (n = 2)
3 h	10.3 ± 0.4 (n = 2)	6.9 ± 1.1 (n = 4)	7.7 ± 1.1 (n = 4)
Tumor, 3 h	26.6 ± 3.4 (n = 15)	19.4 (n = 1)	12.2 ± 0.9 (n = 3)
Normal dorsum skin, 3 h	9.2 (n = 1)	16.9 ± 2.9 (n = 2)	8.3 (n = 1)
Normal lip mucosa, 3 h	13.7 ± 0.4 (n = 2)	11.3 ± 0.1 (n = 2)	10.0 (n = 1)
Tumor/blood	2.6/1	2.8/1	1.6/1
Tumor/lip mucosa	1.9/1	1.7/1	1.2/1

Table 2 Absorbed doses (Gy) for the different feline patients; doses due to fast neutrons, gamma rays and induced protons apply to both tumor and normal tissue

	Fast neutrons	Gamma-ray photons	^{10}B (tumor)	^{10}B (normal tissue)	Induced protons	Total dose in tumor	Total dose in normal tissue
Patient 1 (Rulito)	0.60 ± 0.07	2.40 ± 0.12	2.54 ± 0.35	1.31 ± 0.08	0.25 ± 0.01	5.79 ± 0.39	4.56 ± 0.16
Patient 2 (Mario)	0.60 ± 0.07	2.40 ± 0.12	1.85 ± 0.09	1.08 ± 0.05	0.25 ± 0.01	5.10 ± 0.17	4.33 ± 0.15
Patient 3 (Michi)	0.60 ± 0.07	2.40 ± 0.12	1.16 ± 0.10	0.95 ± 0.05	0.25 ± 0.01	4.41 ± 0.18	4.20 ± 0.15

The combined uncertainty of the ^{10}B dose component includes the boron concentration uncertainty

(Michi), respectively. Accordingly, the total normal tissue dose was estimated to be 8.4 Gy-eq. for Patient 1 (Rulito), 7.8 Gy-eq. for Patient 2 (Mario) and 7.5 Gy-eq. for Patient 3 (Michi). It should be noted that the actual CBE factor for feline nasal planum SCC remains to be determined. Similarly, somewhat different values of the CBE factor for normal tissue than that of 2.5 used in the present work are available in the literature: 2.4 in hamster skin [18], 2.5 in human skin [16], 3.2 in rat skin and 2.5 in rat mucosa [17]. For these reasons, the doses reported in the present study should only be seen as estimates. The use of rough approximations of RBE and CBE values and the fact that the boron concentrations were extrapolated from biodistribution studies that had been performed prior to the BNCT (because to date there is no noninvasive, on-line methodology available to estimate the ^{10}B concentration during BNCT [12]), preclude accurate dose calculation.

Patient 1 (Rulito)

Rulito did not show any radiation-induced toxicity except for areas of alopecia in the treatment volume.

Over the first month after BNCT, periodic macroscopic follow-up revealed partial tumor control in terms of a halt in gross tumor growth followed by a significant reduction in tumor volume, de-obstruction of the nostrils, and development of areas of necrosis. The clinical status of the cat improved in terms of mobility, appetite and breathing. The histo-pathological analysis of a biopsy sample taken from the tumor area 1 month after BNCT showed necrotic foci in the center of the tumor cords (Fig. 1b). Images of radiation-induced damage were observed, i.e., hyperchromatic cells, cytoplasmic vacuolization, and bizarre nuclei. However, as from 1 month after BNCT, the tumor resumed growth, exhibiting a local inflammatory reaction. Two months and 5 days after BNCT, the animal was euthanized due to general decline. The autopsy did not show metastatic spread. No lesions in normal tissue attributable to radiation-induced toxicity were observed. The histopathological analysis of the necropsy tumor area revealed the presence of viable nuclei, some mitotic figures and apoptotic figures, and signs of radiation-induced damage such as bizarre and hyperchromatic nuclei.

Patient 2 (Mario)

Mario did not show any radiation-induced toxicity except for areas of alopecia in the treatment volume and reversible mucositis and driveling approximately 9 days after BNCT. Approximately 15 days after BNCT, the clinical symptoms associated to mucositis had resolved.

Over the first month after BNCT, periodic macroscopic follow-up revealed partial tumor control in terms of a halt in gross tumor growth. The clinical status of the animal remained constant, with a moderate increase in appetite. Twenty days after BNCT, epithelialized tumor borders were observed. The growth of normal epithelium is indicative of some degree of tumor control. The histo-pathological analysis of a biopsy sample taken from the tumor area 3 months after BNCT showed ghost images in the center of the tumor cords and signs of radiation-induced damage such as bizarre and hyperchromatic nuclei (Fig. 2b). Five months after BNCT, the tumor had resumed growth and the animal was euthanized due to generalized decline. Unfortunately, the owners declined consent to perform an autopsy.

Patient 3 (Michi)

Michi did not show any radiation-induced toxicity except for areas of alopecia in the treatment volume and reversible mucositis and driveling approximately 9 days after BNCT. Approximately 15 days after BNCT the clinical symptoms associated to mucositis had resolved.

Over the first month after BNCT, periodic macroscopic follow-up revealed partial tumor control in terms of a halt in gross tumor growth. The clinical status of the animal improved in terms of activity and appetite. Twenty-two days after BNCT, epithelialized tumor borders were observed. The growth of normal epithelium is indicative of some degree of tumor control. The histo-pathological analysis of a biopsy sample taken from the tumor area 1 month after BNCT showed necrotic foci in the center of tumor cords, cellular damage and cytoplasmic vacuolization (Fig. 3b). The lesion was partly lined by normal epithelium. Two and a half months after BNCT, the tumor exhibited some bleeding. Approximately 4.5 months after BNCT, the tumor resumed growth. Progressive tumor growth and clinical decline were observed. The animal was euthanized 7.5 months after BNCT. An autopsy did not show metastatic spread. No lesions in normal tissue attributable to radiation-induced toxicity were observed. The histo-pathological analysis of the necropsy tumor area revealed the presence of viable nuclei, necrotic foci and signs of radiation-induced tumor cell damage such as hyperchromatic and bizarre nuclei and some cytoplasmic vacuolization.

Discussion

The present study showed that ^{10}BPA might be suitable to deliver ^{10}B to squamous cells of spontaneous nasal planum carcinoma in felines in amounts sufficient to allow for BNCT. However, in one case, the absolute boron concentration in the tumor was low (Patient 3). It has been shown that BPA is transported across the cell membrane by the L amino acid active transport system [19]. Thus, ^{10}BPA incorporation depends on metabolic status and viability of the treated patient [6, 14], resulting in a heterogeneous boron biodistribution within heterogeneous tumors [8] and exhibiting considerable variations in boron content between different tumors [6]. Therefore it is not surprising that different tumors incorporate different amounts of boron delivered by BPA. Future studies will be necessary to quantify the influence of factors such as tumor differentiation on ^{10}BPA uptake.

BNCT did not cause any clinically significant radiation-induced toxicity. However, reversible mucositis was seen in the oral mucosa of Patients 2 (Mario) and 3 (Michi), but not of Patient 1 (Rulito). Morris et al. [20] described ulceration in the rat normal ventral tongue model 6–8 days following BNCT mediated by ^{10}BPA. They attributed this finding to the micro-distribution profile of ^{10}B that showed a high boron content in mucosal epithelium. In fact, mucositis is a commonly observed side effect in normal oral mucosa during conventional radiotherapy for head and neck tumors [20]. The development of reversible mucositis 1–2 weeks after BNCT mediated by ^{10}BPA was also reported previously by our group in precancerous tissue in the hamster cheek pouch oral cancer model at an estimated physical dose of 4.3 Gy (approximately 10.3 Gy-eq.). However, no mucositis was seen in normal hamster cheek pouch tissue up to 6 months after BNCT mediated by ^{10}BPA involving radiation dose ranges similar to those employed herein [10]. Probably, normal hamster pouch mucosa is less radiosensitive than normal oral tissues of felines and rats. In the present study, all three animals received similar estimated radiation doses in normal tissue (7.5–8.4 Gy-eq.), suggesting that the difference in the observed dose response was not due to a difference in the radiation dose. Thus, potential and sometimes unexpected differences in the radio-sensitivity between the animals should caution against treating to maximum normal tissue tolerance. Furthermore, to date there is no noninvasive, on-line methodology available to estimate ^{10}B concentration in tissue during BNCT. Consequently, the ^{10}B radiation dose component can only be estimated based on previous biodistribution studies, precluding accurate dosimetric calculations [12].

BNCT improved the clinical condition of the animals. The present study also demonstrated that BNCT mediated by ^{10}BPA is capable of inducing partial tumor control in nasal planum SCC in terms of a halt in gross tumor growth, a significant reduction in tumor volume [as in the case of Patient 1 (Rulito)], the development of necrotic foci and the induction of cell damage in tumor tissue with no significant radiation-induced toxicity in normal tissue. Tumor control was clearly more pronounced in Patient 1 (Rulito) than in the other two cats. This difference is probably due to the

higher tumor boron content in Patient 1 (Rulito), who undoubtedly showed the most advanced tumor of the three. If future studies confirm that less advanced tumors incorporate low amounts of ^{10}B delivered by ^{10}BPA, BNCT mediated by ^{10}BPA may not be an adequate choice for early stage SCC.

Patient 1 (Rulito) exhibited a marked reduction in tumor volume followed by eventual re-growth rather than by complete tumor remission. This finding could be attributed to the fact that at-depth radiation dose was insufficient in this very large tumor. A similar observation was made by Kato et al. [21] who performed several (two to three) BNCT applications in recurrent head and neck malignancies due to insufficient at-depth dose in large tumors.

It should be noted that, given the sensitivity of SCC to high-LET neutrons alone [22], the 13–20% contribution of the fast neutron weighted dose component to total weighted dose might have played a role in tumor growth delay.

Undoubtedly, there is room for increasing the therapeutic efficacy of BNCT. The present study clearly demonstrates the need to increase at-depth radiation dose in large tumors and improve ^{10}B targeting into tumors. At-depth radiation dose could be probably improved by applying more than one BNCT treatment [21] or by increasing thermal neutron flux [23]. Previous studies by our group already suggested potential strategies to improve ^{10}B targeting to tumor cells. Successful BNCT is based on minimizing the dose to normal tissue and maximizing the dose to tumor tissue. However, radiation dose must be delivered to all tumor cell populations within a heterogeneous tumor to a therapeutically optimal level. Thus, it has been postulated (see e.g. [24]) that the combined administration of different ^{10}B compounds with different properties and complementary uptake mechanisms may enhance the therapeutic effect of BNCT. In fact, a remarkable tumor control without radiation-induced toxicity to normal tissue was reported in the hamster cheek pouch oral cancer model using ^{10}BPA and ^{10}B-GB-10 ($Na_2{}^{10}B_{10}H_{10}$, a diffusive non tumor-selective compound) combined [11, 12]. Future studies are warranted to evaluate the potential efficacy in a preclinical scenario of this dual mode cellular (^{10}BPA ^{10}B radiation dose component) and vascular (^{10}B-GB-10 ^{10}B radiation dose component) targeting protocol, which was proved highly effective in an experimental model of SCC [7, 9, 11, 12].

Conclusion

From the present study it can be concluded that BNCT mediated by ^{10}BPA is capable of inducing partial tumor control in SCC without any significant radiation-induced toxicity in normal tissue. Because BNCT targets tumor tissue preferentially, the present findings might have relevance to human therapy in terms of avoiding the known severe side effects of photon radiotherapy in the head and neck such as osteoradionecrosis [25].

It was shown that BNCT mediated by ^{10}BPA involves tumor-weighted doses that are about a factor 2 higher than the doses to normal tissue. The results show that potential and sometimes, unexpected differences in radio-sensitivity between the animals should caution against treating to maximum normal tissue tolerance. Further work is required to focus on at-depth dose distributions for large tumors, and to evaluate the potential efficacy in a preclinical scenario of the dual mode cellular (^{10}BPA ^{10}B radiation dose component) and vascular (^{10}B-GB-10^{10}B radiation dose component) targeting protocol.

Acknowledgments This work was partially supported by the United States Department of Energy, Office of Nonproliferation and International Security under the DOE/CNEA Sister Laboratory Program, and by the US Department of Energy, Office of Science through the Idaho National Laboratory under DOE Idaho Operations Office Contract DE-AC07-05ID14517.

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