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Advances in Brief

Boron Neutron Capture Therapy for the Treatment of Oral Cancer in the Hamster Cheek Pouch Model[1]

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Abstract

We have proposed and validated the hamster cheek pouch model of oral cancer for boron neutron capture therapy (BNCT) studies and shown that boronophenylalanine delivers potentially therapeutic 36.9 ± 17.5 ppm boron to tumor tissue with tumor:normal tissue and tumor:blood ratios of 2.4:1 and 3.2:1, respectively. Here we report the first evidence of the usefulness of BNCT for the treatment of oral cancer in an experimental model. We assessed the response of hamster cheek pouch tumors, precancerous tissue, and normal oral tissue to boronophenylalanine-mediated BNCT using the thermalized epithermal beam of the RA-6 Reactor at the Bariloche Atomic Center. BNCT leads to complete remission by 15 days posttreatment in 78% of tumors and partial remission in an additional 13% of tumors with virtually no damage to normal tissue.

Introduction

BNCT[3] is a binary treatment modality that combines irradiation with a thermal or epithermal neutron beam with tumor-seeking, boron-containing drugs to produce selective irradiation of tumor tissue. The high linear energy transfer α particles and recoiling ^{7}Li nuclei emitted during the $^{10}B(n, \alpha)^{7}Li$ reaction have a range of 5–9 μm in tissue and are known to have a high relative biological effectiveness (1). In this way, BNCT would potentially target the tumor selectively, and damage to normal tissue would be scarce (2). Effective BNCT requires the uptake of sufficient ^{10}B by targeted tumor tissue and selective accumulation of ^{10}B in tumor relative to dose-limiting normal tissues and blood (3). Clinical trials of BNCT for the treatment of brain tumors and melanoma in the United States, Europe, and Japan have shown a potential, albeit nonconclusive, therapeutic advantage for this technique. Within the context of exploring new applications of BNCT, contributing to the study of BNCT biology and radiobiology to improve its therapeutic advantage, and analyzing the behavior of dose-limiting normal tissues, we have proposed and validated the use of the hamster cheek pouch oral cancer model for BNCT studies (4).

Hamster cheek pouch is the most widely accepted model of oral cancer (5). Carcinogenesis protocols induce premalignant changes and carcinomas that closely resemble human lesions (6). Tumors are induced by a process that mimics the spontaneous process of malignant transformation rather than by the growth of implanted tumor cells as seen in other BNCT experimental models (*e.g.*, Refs. 7 and 8). Thus, this model allows for the study of precancerous tissue around the tumor, an issue of clinical relevance given the phenomenon of field cancerization (*e.g.*, Ref. 9). Furthermore, the model allows us to assess the response of normal oral tissues that may potentially be dose-limiting in BNCT of brain tumors or if BNCT is eventually applied to the treatment of head and neck tumors (10). The hamster cheek pouch is easily accessible to local tumor induction and can be readily everted for local irradiation and macroscopic follow-up.

Head and neck cancer patients are generally treated with surgery combined with radiation therapy and chemotherapy. Given that the 5-year survival rate for head and neck squamous cell carcinoma has remained at 52% over the past 20 years (11) and that radical tumor surgery often results in large tissue defect (12), head and neck cancer patients may potentially benefit from alternative therapeutic strategies (13).

The boron biodistribution and pharmacokinetic study of BPA in this model showed that absolute and relative values of boron concentration in hamster cheek pouch tumor tissue would be potentially therapeutic. BPA delivered 36.9 ± 17.5 ppm boron to tumor tissue with tumor:normal tissue and tumor:blood ratios of 2.4:1 and 3.2:1, respectively (4).

The aim of the present study was to assess, for the first time, the response of hamster cheek pouch tumors, precancerous tissue, and normal tissue to BPA-mediated BNCT using the thermalized epithermal beam of the RA-6 Reactor at the Bariloche Atomic Center.

Materials and Methods

The right cheek pouch of non-inbred young Syrian hamsters was subjected to topical administration of 0.5% dimethyl-1,2-benzanthracene in mineral oil three times a week for approximately 14 weeks, in keeping with a standard hamster cheek pouch carcinogenesis protocol (14). Once the exophytic tumors had developed and reached a diameter of more than 3 mm, the animals were taken by plane to Bariloche, a city 1200 km south of Buenos Aires, to be irradiated with the thermalized epithermal beam at the RA-6 Reactor. We were able to transport 38 animals plus an additional 5 animals to perform sham irradiations. The 38 animals (22 tumor-bearing hamsters and 16 normal hamsters) were divided up into 5 experimental groups. Fifteen animals bearing a total of 23 tumors of known sizes and locations on the pouch were irradiated under ketamine-xylazine anesthesia 3.5 h after i.p. administration of 300 mg BPA/kg b.w. Four hamsters bearing a total of four tumors were irradiated 3.5 h after i.p. administration of 600 mg BPA/kg b.w. Thirteen normal hamsters were irradiated 3.5 h after i.p. administration of 300 mg BPA/kg b.w. Three hamsters bearing a total of three tumors and three normal animals were irradiated with the beam alone. The everted pouch and, inevitably, part of the head were placed at the beam port that is 15 cm in diameter. The rest of the body was shielded by the lead and borated polyethylene that makes up the beam delimiter. Irradiations lasted 62 min, resulting in an average fluence of thermal neutrons for tumor and healthy tissue at the position of the pouch of $1.1 \pm 0.1 \times 10^{12}$ neutrons/cm^2. Boron content values for dose calculations, BPA dose, and the choice of postadministration time were based on the biodistribution and pharmacokinetic study of BPA in this model (4). Given the reported spread in hamster tumor boron values (36.9 ± 17.5 ppm), we selected a conservative rough approximation of 30 ppm boron in tumor tissue to estimate dose. Based on the boron values reported for hamster normal pouch tissue (15.6 ± 5.4 ppm) and the observation that BNCT elicited virtually no

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[3] The abbreviations used are: BNCT, boron neutron capture therapy; BPA, boronophenylalanine; b.w., body weight; CBE, compound biological effectiveness.

Table 1 *Physical absorbed doses (in Gy)*

	Fast neutrons	γ photons	Boron (30 ppm)	Boron (10 ppm)	Induced protons
Pouch	1.02 ± 0.08	1.40 ± 0.06	2.52 ± 0.25 (tumor)	0.84 ± 0.08	0.22 ± 0.02
Head	0.72 ± 0.06	0.95 ± 0.04		0.61 ± 0.06	0.15 ± 0.02
Body	0.41 ± 0.03	0.50 ± 0.02		0.31 ± 0.03	0.08 ± 0.01

damage to normal tissue, we selected a rough approximation of boron content in the lower range, *i.e.*, 10 ppm, to estimate dose to normal tissue. The γ and the fast neutron dose were measured using the paired ion chambers technique, and the thermal neutron fluence was determined by gold wire measurements (15).

The estimated physical absorbed doses in Gy from the different radiation components for the pouch (pouch tumor and normal pouch tissue), head, and body are presented in Table 1.

Assuming, as weighting factors for each dose component, a relative biological effectiveness value of 3.2 for fast neutrons and induced protons, a CBE factor of 2.5 for boron component in normal pouch tissue, a CBE factor of 3.8 for the boron component in pouch tumor, and a RBE value of 1 for photons, the total tumor dose was estimated to be 14.9 Gy equivalent, and total normal pouch dose was estimated to be 7.5 Gy eq. (3, 16). Admittedly, the CBE factor for hamster cheek pouch normal tissue remains to be determined. Some of the possibly relevant values available in the literature range from 2.4 in hamster skin (17), 2.5 in human skin (3), 3.2 in rat skin, and 2.5 in rat mucosa (16) to 4.9 in rat tongue mucosa (10). Based on the observation that normal hamster tissue treated with BNCT in the present conditions exhibited virtually no damage, we adopted one of the lower CBE values, *i.e.*, 2.5, as a reasonable estimate for our dose calculations. Here we must stress that the use of rough approximations of RBE and CBE values and uncertainties in boron content (4) result in only rough estimates of dose.

Tumor, precancerous tissue, and normal tissue response was assessed by visual inspection and a tumor volume assay at 1, 7, 14, 21, and 30 days postirradiation. Histological analysis of tumor, precancerous, and normal tissue was performed on some of the animals at the same times. Complete remission was defined as disappearance of the tumor on visual inspection and no evidence of tumor on histological analysis. Partial remission was defined as a reduction in tumor size. Continued tumor growth was interpreted as no tumor control. b.w. was monitored after all of the irradiation protocols.

Results and Discussion

Tumor response to BNCT mediated by i.p. administration of 300 mg BPA/kg b.w. as a function of time posttreatment is shown in Fig. 1, using tumor volume normalized to 100% at irradiation time as the end point. Of the 23 treated tumors, complete remission was observed in 18 tumors (78%), and partial remission was observed in an additional 3 tumors (13%) by 2 weeks postirradiation. Continued growth was seen in only one tumor. One animal was sacrificed at 1 day postirradiation for histological analysis before tumor response could be unequivocally evaluated. In some cases, an initial increase in tumor volume followed by remission was observed. This initial response was due to occasional inflammatory and edematous reactions that then subsided. Visual inspection showed that in 7 of 23 cases, the tumor had disappeared by 24 h postirradiation. This finding was confirmed by histological analysis, which showed some ulceration and basal layer lesions in the precancerous tissue surrounding the tumor site, but no evidence of tumor. The remaining 11 of 23 tumors

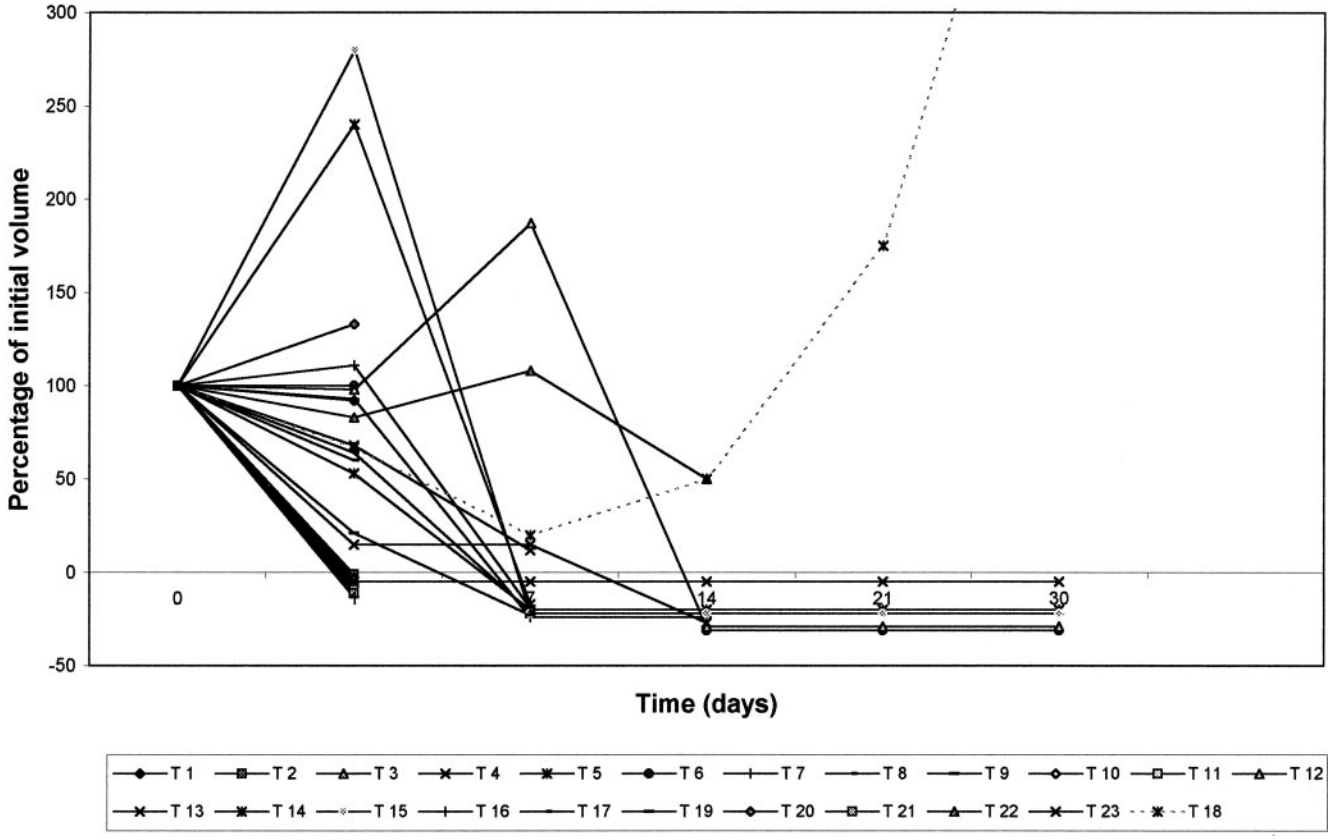

Fig. 1. Tumor response to BNCT (300 mg BPA/kg b.w.) as a function of time posttreatment using tumor volume normalized to 100% at irradiation time as the end point. The scale was chosen to optimize visualization of the response of the 21 tumors that underwent partial or complete remission. The *dotted line* indicates where tumor 18 (the only tumor that was not controlled by BNCT) fits on the existing scale. Each *line* corresponds to the evolution of the corresponding tumor listed below the plot. The negative tumor values have been artificially introduced to allow for visualization of the response of individual tumors for which volume values would otherwise be superimposed.

that underwent complete remission had disappeared by 2 weeks postirradiation. This finding was also confirmed by histological analysis, which showed an ulcerated area undergoing healing and no evidence of tumor (Fig. 2).

The animals treated with BNCT at the higher BPA dose of 600 mg/kg b.w. showed response in 75% of the tumors. One tumor had disappeared by 2 days postirradiation, and another had disappeared by 2 weeks postirradiation. One tumor underwent partial remission (76% reduction in volume) at 2 days. At this time, the animal was sacrificed for histological analysis. One tumor exhibited continued growth.

All of the tumors (three of three tumors) subjected to beam alone continued growing, exhibiting final tumor volumes at 30 days posttreatment that ranged from 110-2000% of tumor volume at irradiation time. These tumor growth values do not differ from the growth values for untreated tumors in this model.

The 13 normal pouches treated with BNCT (300 mg BPA/kg b.w.) were indistinguishable from untreated pouches on visual inspection and on histological analysis, except for occasional moderate congestion at 1 week posttreatment (Fig. 3). Four of the normal animals treated with BNCT were allowed to live to evaluate long-term response in normal tissue. At 5 months posttreatment, all four pouches were indistinguishable from control, untreated pouches in terms of macroscopic and microscopic features. The three normal pouches treated with beam alone failed to show macroscopic or microscopic alterations within the experimental period. Exposed (BNCT or beam alone) cheek skin damage was not observed at any of the experimental time points. None of the irradiated animals (BNCT or beam alone) showed signs of radiation toxicity. Sham irradiated animals did not show any changes.

Precancerous tissue around the tumor showed an initial, moderate inflammatory response to BNCT that had subsided by approximately 14 days posttreatment. Precancerous tissue failed to show response to beam alone. We have shown that BPA uptake in precancerous tissue tends to be greater than that in normal pouch tissue (4). The fact that precancerous tissue shows signs of response to BNCT that normal pouch tissue does not may translate to a benefit to patients with oral cancer being treated with BNCT. BNCT may have a therapeutic effect on a field cancerized area within the treatment volume, thus reducing the risk of development of multiple primary tumors and local-regional recurrence. However, the extent to which precancerous tissue may limit the dose that can be administered to tumor must be a consideration.

The light microscopy analysis of tumor-bearing pouches at different times post-BNCT shows the sequential appearance of characteristic histological features. However, the time postirradiation at which they appear is not identical for all cases. The sequence can only be inferred from histological study of tumor-bearing pouch samples of animals sacrificed at different time points after BNCT. Three hamsters bearing a total of eight tumors were sacrificed at 1 day posttreatment, four hamsters bearing a total of six tumors were sacrificed at 7 days posttreatment, three hamsters bearing a total of three tumors were sacrificed at 2 weeks posttreatment, and five animals bearing a total of six tumors were sacrificed at 30 days posttreatment. Clearly, the need to perform macroscopic follow-up precludes the possibility of sacrificing the animal to perform the histological analysis. The most evident early histological finding was vacuolization of the epithelial cells, which was much more marked in the basal layer. The connective tissue exhibited a slight inflammatory reaction. Several of the tissue sections show a separation between the epithelium and the connective tissue. Tumor remission gives way to the formation of an ulcer with fibrohyaline connective tissue. The presence of proliferating neighboring epithelium would evidence a healing process.

The histological observations suggest that rapid tumor remission

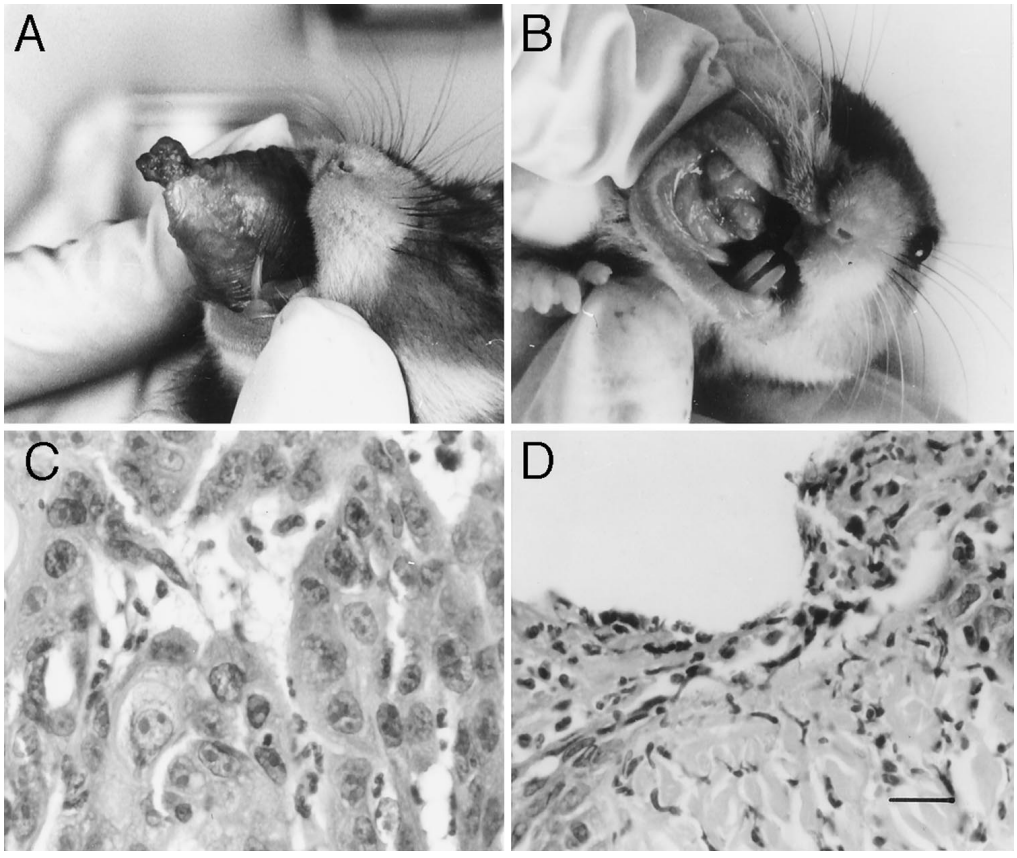

Fig. 2. *A*, macroscopic view of squamous cell carcinoma of the cheek pouch at 12 weeks of the carcinogenesis protocol. *B*, tumor site shown in *A*, 2 weeks after treatment with BNCT. A scar and fibrous bands remain. There is no evidence of tumor. *C*, characteristic light microscopy image of squamous cell carcinoma at 12 weeks of the carcinogenesis protocol. *D*, light microscopy image of the pouch area shown in *B*. Note the presence of an ulcerated area covered by a scar and the underlying fibrous tissue. A repair process following tumor remission is under way, as evidenced by the proliferation of neighboring epithelium. There is no evidence of tumor. *Bar*, 20 μm.

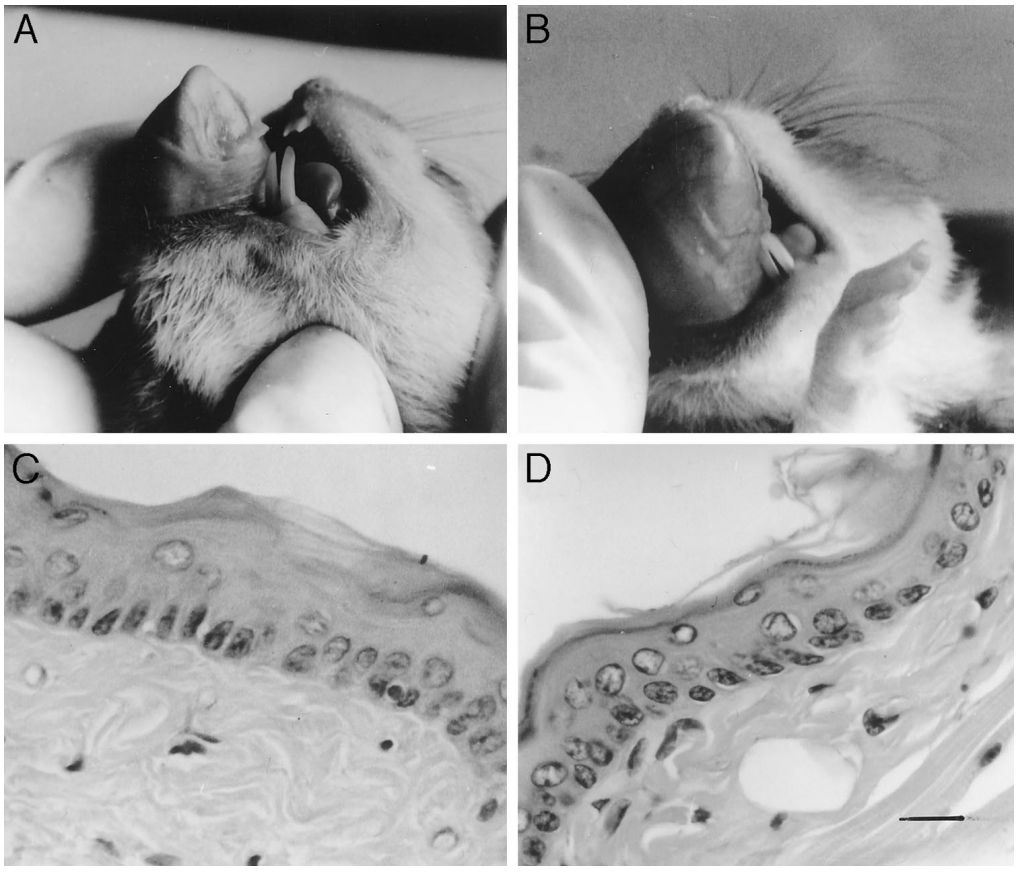

Fig. 3. *A*, everted normal pouch. *B*, pouch shown in *A*, 2 weeks after treatment with BNCT. An occasional slight congestion is the only observable change. *C*, characteristic light microscopy view of a normal pouch. *D*, light microscopy view of the pouch shown in *B*. No unusual microscopic features are apparent. *Bar*, 20 μm.

was due to basal layer damage induced by BNCT, followed by loss of the upper strata. Preferential accumulation of BPA in basal cells could partially explain this effect. Additional studies in this sense are warranted.

To the best of our knowledge, there is a single report in the literature on the therapeutic effect of X-irradiation (20 Gy, single dose) on fully developed hamster cheek pouch tumors. This study showed an initial (14 days) therapeutic effect followed by regrowth (18). All irradiated cheek pouches showed extensive necrosis and inflammation. The present *in vivo* BNCT study would suggest a therapeutic advantage for BNCT in this model, given that the therapeutic total tumor doses with negligible tissue toxicity were estimated to be 5.2 Gy physical dose, *i.e.*, 14.9 Gy eq. (assuming a CBE factor of 3.8 for the boron component in pouch tumor), as compared with the reportedly ineffective 20-Gy single dose of X-rays. Here we must stress that the CBE factor for pouch tumors remains to be determined and that the value used is only a rough approximation.

b.w. showed a slight, albeit not statistically significant, reduction at 1 week posttreatment in animals with BNCT-treated tumor, perhaps due to inflammation in the precancerous tissue at this time. Normal animals treated with BNCT or beam alone failed to exhibit variations in b.w. Tumor-bearing hamsters treated with beam alone showed a reduction in b.w. at 4 weeks postirradiation, compatible with continued tumor growth (results not shown).

The results of BPA-mediated BNCT reported herein suggest that the hamster cheek pouch model would be useful for testing other boron compounds (19) and BNCT protocols and examining the mechanisms underlying BNCT-based tumor remission.

BNCT leads to a dramatic and very rapid tumor response with virtually no damage to normal tissue, the first demonstration *in vivo* of successful BNCT in a tumor model that imitates the process of spontaneous malignant transformation. The present data are the first evidence of the usefulness of BNCT for the treatment of oral cancers in an experimental model.

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