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Early effect of boron neutron capture therapy mediated by boronophenylalanine (BPA–BNCT) on mast cells in premalignant tissue and tumors of the hamster cheek pouch

Romina F. Aromando [a,b,*], Verónica A. Trivillin [b], Elisa M. Heber [b], Emiliano Pozzi [b,c], Amanda E. Schwint [b], María E. Itoiz [a,b]

[a] Department of Oral Pathology, Faculty of Dentistry, University of Buenos Aires, Argentina
[b] Department of Radiobiology, National Atomic Energy Commission, Constituyentes Atomic Center, Argentina
[c] Research and Production Reactors, National Atomic Energy Comission, Ezeiza Atomic Center, Argentina

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SUMMARY

Mast cell (MC) activation in the hamster cheek pouch cancerization model is associated with the increase in tumor cell proliferation, mediated in turn by tryptase, a protease released from mast cell granules after activation. Tryptase induces tumor cell proliferation through the activation of PAR-2 (protease activated receptor-2) on the plasma membrane of carcinoma cells. The therapeutic success of boron neutron capture therapy mediated by boronophenylalanine (BPA–BNCT) in tumor control in the hamster cheek pouch oral cancer model has been previously reported by our laboratory. Early effects of BPA–BNCT on tumors of the hamster cheek pouch include a reduction in DNA-synthesis with the concomitant decrease in the proliferation of malignant cells. The aim of the present study was to investigate the early histological changes in mast cells after BPA–BNCT in tumors and premalignant tissue of the hamster cheek pouch. Tumor-bearing pouches were treated with BPA–BNCT or beam only (neutron irradiation without prior administration of the boron compound) and sacrificed 1 day after treatment. The samples were fixed in Carnoy fixative and stained with alcian blue–safranin to identify all the populations of mast cells. Total, active and inactive mast cells (MC) were counted in the connective tissue and the adventitious tissue underlying the pouch wall and at the base of the tumors in pouches treated with BPA–BNCT, in keeping with a previously described technique. BPA–BNCT induced a marked reduction in the total number of mast cells in the pouch ($p < 0.05$). This reduction in the total number of mast cells was due to a reduction in mast cells at the base of the tumor ($p < 0.005$) and it occurred at the expense of the active mast cells ($p < 0.05$). A slight reduction that did not reach statistical significance also occurred in the amount of mast cells in the pouch wall (that corresponds to the premalignant tissue in tumor-bearing pouches), and in the adventitious tissue. In this case the reduction was seen in the inactive population. Both BPA–BNCT and beam only elicited a qualitative change in the secretion modality of the granule content. Although further studies are needed to evaluate the subcellular effect of BNCT on mast cell granule secretion, the reduction in cell proliferation induced by BPA–BNCT would be partially due to the decrease in total mast cells in the hamster check pouch.

Introduction

Boron neutron capture therapy (BNCT) is a two-component treatment modality that involves the selective accumulation of a ^{10}B compound in tumors followed by irradiation with a thermal or epithermal neutron beam. The ^{10}B accumulated in the tumor cell absorbs a thermal neutron and releases two high linear energy transfer (LET) particles, an α particle and a recoiling ^{7}Li nuclei.[1,2] These high LET particles, have a range of approximately 5–9 μm in tissue and are known to have a high relative biological effectiveness (RBE).[3] Within this context, BNCT would potentially target tumor tissue selectively, mostly sparing normal tissue.[4] The basic requirements for a therapeutic advantage for BNCT are a high degree of selectivity for the accumulation of ^{10}B in tumor relative to the surrounding normal tissues and a sufficiently high absolute concentration of ^{10}B in tumor tissue.[5] Boronophenylalanine (BPA) is one of the boron compounds actually in use for BNCT studies.[6–8] BPA is transported across the cell membrane by the L-AMINO-ACID transport system.[9] Thus, BPA uptake would depend on

* Corresponding author. Address: Department of Oral Pathology, Faculty of Dentistry, University of Buenos Aires, Argentina. Tel.: +54 11 4964 1273; fax: +54 11 6772 7188.
E-mail address: romina_aromando@yahoo.com.ar (R.F. Aromando).

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metabolic status, and preferential accumulation in tumor tissue would rely on the comparatively high metabolic activity of tumor cells compared to normal cells.[10] Clinical trials of BNCT for the treatment of glioblastoma multiforme, melanoma and tumors of the head and neck have been performed and/or are currently in progress in the US, the Netherlands, Finland, Sweden, The Czech Republic, Argentina and Japan.[6,7,11–14] Our laboratory has studied different aspects of BNCT in the treatment of experimental oral cancer.[10,15–23] These BNCT studies were performed in hamster cheek pouch tumors induced by the carcinogen dimethyl-1,2-benzanthracene (DMBA), the most widely accepted model of oral cancer.[24] This model closely mimics tumor development in human oral mucosa exposed to chemical field cancerization by tobacco and alcohol.[25–28]

Employing this model we demonstrated that certain BNCT protocols effectively control tumors, inducing partial and complete remissions and/or growth inhibition with no normal tissue radiotoxicity.[16,21,29] One of the effects of BNCT mediated by BPA (BPA–BNCT) is the early inhibition of tumor cell proliferation.[22] In previous studies carried out to further characterize the hamster cheek pouch oral cancer model, we studied mast cell (MC) kinetics in normal and cancerized pouches and found that MC activation is associated to an increase in tumor cell proliferation, in keeping with findings in a colon cancer model.[30,31] Tryptase, a protease released from mast cell granules after activation, induces tumor cell proliferation through the activation of PAR-2 receptor (protease activated receptor type 2) on the plasma membrane of carcinoma cells.[30]

The aim of the present study was to evaluate the effect of BPA–BNCT on mast cells and analyze whether this effect could contribute to the tumor growth inhibition and partial and complete tumor remission observed in our previous tumor control studies.

Materials and methods

Tumor induction and in vivo BNCT

The right cheek pouch of non-inbred young (6 weeks old) Syrian hamsters was subjected to topical application of 0.5% dimethyl-1,2-benzanthracene (DMBA) in mineral oil three times a week for 14 weeks in keeping with the standard hamster cheek pouch carcinogenesis protocol.[24] The treated pouch was periodically everted under light intraperitoneal (i.p.) ketamine (70 mg/kg), xylazine (10.5 mg/kg) anesthesia and examined to monitor tumor development. Once the exophytic tumors had developed, the animals were used for *in vivo* BNCT studies. Tumor-bearing hamsters were divided into three groups: (1) control group: tumor-bearing hamsters, no treatment; (2) BPA–BNCT group: tumor-bearing hamsters, treated with neutron irradiation following administration of BPA and (3) beam only group: tumor-bearing hamsters irradiated with the neutron beam without prior administration of BPA.

BNCT procedures

BPA–BNCT

BPA (L-enantiomer, >98% ^{10}B enriched; Boron Biologicals, Inc., Raleigh, NC, USA) containing 4.9% ^{10}B by weight was used as the boron delivery agent. Solutions of the BPA–fructose complex were prepared in keeping with the procedures published previously.[32] Hamsters in the BPA–BNCT group were administered BPA as an i.p. bolus injection at a dose of 15.5 mg ^{10}B/kg b.wt. (300 mg BPA/kg b.wt.). Three hours after the injection of BPA the animals were irradiated for 5.0 ± 0.1 min in the RA-3 nuclear reactor thermal neutron facility. The animals were placed in a lithium carbonate (95% enriched in lithium-6) shield to protect the body of the animal from the thermal neutron flux while exposing the cheek pouch that is everted out of the enclosure onto a protruding shelf. The thermal neutron flux at the position of the pouch was 6.5×10^9 neutrons cm^{-2} sec^{-1}. The gamma dose rate in air at the irradiation location was 4.8 ± 0.5 Gy h^{-1}. Dose calculations were based on previously reported biodistribution data for BPA in this model.[16] The total physical absorbed dose was 5.6 ± 2.5 Gy in tumor and 3.7 ± 0.9 Gy in precancerous tissue.

Beam only

Hamsters were irradiated as described above (no prior administration of BPA) with a total physical absorbed tumor dose of 1.5 ± 0.1 Gy.

Sample processing

Hamsters were killed humanely 1 day after treatment and a specimen of the right cheek containing the pouch *in situ* was obtained to preserve the integrity of the loose adventitious tissue which surrounds the pouch wall, as previously described.[31] The adventitious tissue contains most of the mast cell population of the pouch, so this procedure was employed to avoid deformations that might result from everting the cheek pouch as in standard studies of this model. Specimens were fixed in Carnoy fixative (ethanol–chloroform–acetic acid) [33,34] to guarantee the preservation of the different types of granules corresponding to the different mast cell phenotypes.

Histochemical staining

Samples were embedded in paraffin and serially sectioned at a thickness of 7 μm. To visualize heparin-sulphoconjugates, samples were stained with alcian blue 86Y–safranin[35] and counterstained with 5% methanol yellow (Holblon and Sohne, LEIPZIG, Germany) for 5 min. Alcian blue stains low sulphated-glycosaminoglycans blue while safranin stains highly sulphated-glycosaminoglycans red. The staining was followed by dehydration, clearing and mounting with synthetic balsam. Adjacent sections were stained with hematoxylin–eosin.

Mast cell counts

Mast cell counts were performed according to the categories of Dimitriadou et al.[36] as described previously.[31]

Briefly, mast cells were classified into two categories: active and inactive mast cells. Mast cells that stained uniformly red with safranin but did not stain with alcian blue were considered inactive; those that stained with alcian blue or exhibited mixed alcian blue–safranin staining were considered active. Mast cell counts were performed *in situ* by dividing the pouch into three areas: (1) tumor stroma, (2) base of the tumor (connective tissue immediately below the exophytic tumor); (3) connective and muscle tissue underlying the cancerized epithelium (pouch wall) and (4) adventitious tissue underlying the pouch wall. Mast cells were evaluated by direct counting at 200× magnification in 0.23 mm^2 fields employing a grid fitted into the light microscope eyepiece.

In the case of tumors, all the stroma and the connective tissue underlying the exophytic tumor were counted. Regarding the rest of the pouch, 20 fields were selected at random in the collagen and muscle wall and an additional set of 20 fields were selected in the loose adventitious tissue.

Statistical analysis

The data were compared by Analysis of Variance employing PRIMER® software. Differences were considered significant at $p < 0.05$. Data were expressed as mean ± SD.