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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

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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.

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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

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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.

Results

Most of the mast cells in untreated cancerized pouches and in cancerized pouches treated with BPA–BNCT and beam only exhibited the previously described morphological patterns of uniformly safranin-stained inactive mast cells and mast cells with varying degrees of activation (Fig. 1). BPA–BNCT exerted a marked, significant reduction in the total number of mast cells in the tumor-bearing pouches compared to untreated control pouches ($p < 0.001$). 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 (Tables 1 and 2).

The number of mast cells in tumor-bearing pouches treated with beam only did not differ from the control group. However, response was more heterogeneous than in the control group, as evidenced by a marked increase in standard deviation. Likewise, no differences in the ratio of active/inactive mast cells were observed between the untreated tumor-bearing pouches and those treated with beam only.

As an effect of irradiation, both tumor-bearing pouches treated with beam only or BPA–BNCT exhibited a mast cell type that has not been described to date. This particular mast cell type is characterized by inverted exocytosis compared to the traditionally described mast cell exocytosis. Thus, in this mast cell type the granules that stain with alcian blue are exocyted first, while the safranin-stained granules remain in the cytoplasm. We have called this mast cell IEMC (inverted exocytosis mast cell) (Fig. 2).

Discussion

The present study evidenced a significant reduction in the total number of mast cells following BNCT mediated by BPA. The group treated with beam only (without prior administration of the boron compound) did not exhibit a significant reduction in the number of mast cells but did show a more heterogeneous response. Mast cells in BPA–BNCT treated pouches would suffer a combination of the direct and indirect effects of the background dose (high and low linear energy transfer [LET] radiation) and of the direct effects of the boron radiation component of BNCT (high LET α and lithium particles).[4] In a previous study, we demonstrated that during carcinogenesis mast cells move to the base of tumors and activate, enhancing tumor cell proliferation via tryptase release.[31] Specific data on active transport of BPA in mast cells are not available.

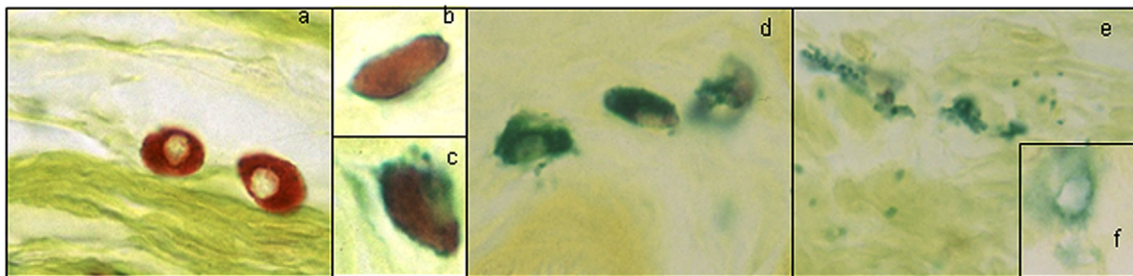

Figure 1 Morphology of mast cells at different activation stages in the hamster cheek pouch (alcian blue–safranin staining, 1000×). (a) Intact (non-activated) mast cell, (b) early activation, (c) intermediate stage, (d) advanced activation stage, (e) degranulation and (f) ghost mast cell.

Table 1
Mast cell counts in tumors and premalignant tissue pre- and post-BPA–BNCT in the hamster cheek pouch.

Experimental group	Total mast cells in the pouch (mas/mm^2)	Ratio active/inactive mast cells (AB/S)	Pouch tissue	Total mast cells in each tissue	Ratio active/inactive mast cells (AB/S) in each tissue	Inverted exocytosis mast cells (IEMC)
Normal pouch (non-cancerized, untreated)	129.3 ± 38.84 $n = 8$	1:1.64 NS	Wall	24.4 ± 4.06	1:2.15[b]	0
			Adventitious tissue	234.13 ± 34.91	1:1.60[b]	0
Cancerized, untreated pouches	107 ± 21[a] $n = 14$	1:0.95 NS	Wall	42.97 ± 20	1:0.66 NS	0
			Adventitious tissue	173.8 ± 74.5	1:0.91 NS	0
			Tumor base	162 ± 35.2[a]	1:1.05 NS	0
BPA–BNCT	63.76 ± 21.1[a] $n = 12$	1:0.65 NS	Wall	29.52 ± 25.2	1:0.3[b]	0.59 ± 1.69 $n = 6$
			Adventitious tissue	119.9 ± 76.3	1:0.29[b]	16.07 ± 34.6 $n = 6$
			Tumor base	93.48 ± 53.6[a]	1:1.64 NS	3.85 ± 9.97 $n = 6$
Beam only	108.7 ± 134.5 $n = 7$	1:1.0 NS	Wall	49.66 ± 9.48	1:0.77 NS	0.45 ± 3.79 $n = 7$
			Adventitious tissue	143.2 ± 67.76	1:0.6 NS	1.59 ± 5.69 $n = 7$
			Tumor base	196.6 ± 108.61	1:3.6 NS	0.49 ± 2.81 $n = 4$

NS, statistically not significant.
[a] Statistically significant reduction in total mast cells in pouches treated with BPA–BNCT vs. untreated cancerized pouches ($p < 0.001$).
[b] Statistically significant difference between active and inactive mast cells ($p < 0.001$).

Table 2
Effect of BPA–BNCT in the different subpopulations of mast cells.

	Mast cells in untreated tumors (AB + S)	Mast cells 1 day post-BPA–BNCT (AB + S)
Total mast cells (tumor base + wall + adventitious tissue)	108.4 ± 64.1[a] n = 14	63.76 ± 21.07[a] n = 12
Tumor base	162 ± 35.2[a] n = 9 AB = 78.8 ± 20.9[a] n = 9 S = 83.1 ± 46.8 n = 9 NS	93.48 ± 53.61[a] n = 11 AB = 34.46 ± 24.29[a] n = 11 S = 56.6 ± 62.6 n = 11 NS
Precancerous tissue		
Wall	42.97 ± 20 n = 14 NS AB = 25.93 ± 22.5 n = 14 NS S = 17.04 ± 16.7[a] n = 14	29.52 ± 25.23 n = 14 NS AB = 22.7 ± 22.9 n = 14 NS S = 6.74 ± 8.8[a] n = 14
Adventitious tissue	155.3 ± 54.5 n = 14 NS AB = 74.15 ± 35.1 n = 14 NS S = 81.19 ± 70[a] n = 14	119.9 ± 76.26 n = 14 NS AB = 80.59 ± 50.27 n = 14 NS S = 23.68 ± 36.43[a] n = 14

NS, statistically not significant; AB, alcian blue; S, safranin.
[a] Statistically significant difference between pouches pre- and post-BPA–BNCT.

However, it is known that BPA is transported across the cell membranes by the L-amino-acid transport system.[9] Thus, tumor cell uptake of BPA would depend on metabolic status and viability.[5,16] Within this context, mast cells activated during carcinogenesis would conceivably incorporate BPA and also be a target for BPA–BNCT. The BPA–BNCT-induced reduction in proliferation rate previously described in tumor and precancerous tissue[22,19] would be partly due to a direct effect on cells[21] and an indirect effect via reduction of the mast cell population. Mast cells circulate in the blood as undifferentiated precursors. When they reach the adventitious tissue (the most vascularized tissue of the pouch) from the circulation they mature and differentiate. Mast cells migrate from the adventitious tissue to the pouch wall and, during carcinogenesis, to the base of tumors. The notion that active mast cells would be more prone to BPA uptake would explain the reduction in total mast cell number at the base of the tumors where mast cells accumulate and activate during the process of carcinogenesis.[31] BPA–BNCT did not significantly affect the total number of mast cells in precancerous tissue, i.e. a slight reduction that did not reach statistical significance was observed in the pouch wall and the underlying adventitious tissue. Although there was no change in the active mast cell population (alcian blue+) there was an abrupt reduction in inactive mast cells (safranin+) both in the pouch wall and the adventitious tissue underlying precancerous tissue. The subpopulation of AB+ mast cells (potentially preferential targets for BPA) in these tissues could have been affected by BPA–BNCT to be rapidly replaced by transdifferentiation of the S+ phenotype.[37–39] It is possible to speculate that the continuous influx of mast cells from the circulation to the adventitious tissue and the phenotypic change between subpopulations would explain the finding that there was no significant variation in the total number of mast cells or in the number of active mast cells in the pouch wall and adventitious tissue. The reduction in precancerous tissue proliferation previously described in precancerous tissue 1 day post-BPA–BNCT[19] would be due to a direct effect on premalignant cells.

A novel finding in the hamster cheek pouch after BNCT was the presence of mast cells exhibiting an alteration in the sequence of exocytosis of their granules in the pouches treated with BPA–BNCT or beam only. In these mast cells exocytosis of alcian blue + granules occurred first, while safranin + granules persisted in the cytoplasm. Assuming that this finding is an expression of an alteration in the sequence of maturation and secretion of the granules we have called these cells *inverted exocytosis mast cells*. A similar effect was observed in the past by our laboratory[40] in odontoblasts of the tooth germs of Wistar rats irradiated with a low dose of a deuteron beam. The authors described a partial inversion of the secretion polarity of odontoblasts, with production of dentin towards the interior of the pulp chamber. A wide range of radioinduced effects on mast cells have been described, depending on the model under study and the type of radiation. Albrecht et al.[41] demonstrated that ionizing radiation induces the release of tryptase from human mast cell culture (HMC-1) *in vitro* and mast cell degranulation in *ex vivo* skin. Vasheghani et al.[42] demonstrated that low level laser therapy with helium–neon (He–Ne) in deep second-degree cutaneous burns in rats increased the number of mast cells during the inflammatory and proliferative phases of healing and decreased the total number of mast cells during the remodelling phase. The mechanism of action of BPA–BNCT that leads to a reduction in the number of mast cells in the treated pouches and, more specifically, at the tumor base, remains to be elucidated. However, the BNCT-induced reduction in the number of mast cells would suggest a colateral mechanism of action of BNCT in tumor remission. Since mast cells stimulate the proliferation of tumor cells by the release of tryptase[30], the reduction in the number of mast cells elicited by BNCT would induce a concomitant reduction in tumor proliferative capacity. These data are in keeping with previous studies by our laboratory that demonstrated a reduction in tumor cell proliferation 24 h after BPA–BNCT.[22] Although the previously described reduction in tumor proliferation 24 h after BPA–BNCT would be mostly due to the direct effect of high LET α and lithium particles on boron-loaded tumor cells, the deleterious effect of BNCT on boron-loaded mast cells would inhibit tryptase secretion and conceivably contribute to the inhibition of tumor cell proliferation.

Taking into account that tumor cells cultured with agonists of the receptor PAR-2 undergo a significant rise in proliferation rate as early as 15 min post-incubation,[43] the decrease in tryptase in the interstitial space that results from a reduction in the number of mast cells 24 h post-BPA–BNCT, would conceivably exert a

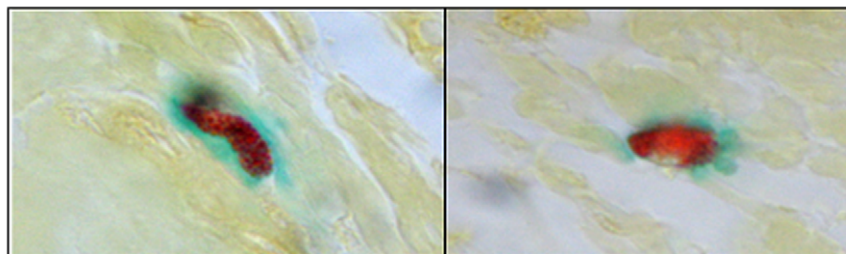

Figure 2 Inverted exocytosis mast cell (alcian blue–safranin staining, 1000×).

rapid inhibitory effect on proliferation, one of the hallmarks of cancer.[44]

Conflicts of interest statement

None declared.

References

1. Locher GL. Biological effects and therapeutic possibilities of neutrons. *Am J Roentgenol* 1936;**36**:1–13.
2. Taylor HJ, Goldhaber M. Detection of nuclear disintegration in a photographic emulsion. *Nature (London)* 1935;**135**:341–8.
3. Gabel D, Fairchild RG, Larsson B. The relative biological effectiveness in V79 Chinese hamster cells of the neutron capture reaction in boron and nitrogen. *Radiat Res* 1984;**98**:307–16.
4. Coderre JA, Morris GM. The radiation biology of boron neutron capture therapy. *Radiat Res* 1999;**151**(1):1–18.
5. Coderre JA, Chanana AD, Joel DD, Elowitz EH, Micca PL, Nawrocky MM, et al. Biodistribution of boronophenylalanine in patients with glioblastoma multiforme: boron concentration correlates with tumor cellularity. *Radiat Res* 1998;**149**:163–70.
6. Aihara T, Hiratsuka J, Morita N, Uno M, Sakurai Y, Maruhashi A, et al. First clinical case of boron neutron capture therapy for head and neck malignancies using 18F-BPA PET. *Head Neck* 2006;**28**(9):850–5.
7. Barth RF, Coderre JA, Vicente MG, Blue TE. Boron neutron capture therapy of cancer: current status and future prospects. *Clin Cancer Res* 2005;**11**(11): 3987–4002.
8. Henriksson R, Capala J, Michanek A, Lindahl SA, Salford LG, Franzén L, et al. Boron neutron capture therapy (BNCT) for glioblastoma multiforme: a phase II study evaluating a prolonged high-dose of boronophenyalanine (BPA). *Radiother Oncol* 2008;**88**:183–91.
9. Wittig A, Sauerwein WA, Coderre JA. Mechanisms of transport of *p*-boronophenylalanine through the cell membrane in vitro. *Radiat Res* 2000;**153**: 173–80.
10. Heber EM, Trivillin VA, Nigg DW, Itoiz ME, Gonzalez BN, Rebagliati RJ, et al. Homogeneous boron targeting of heterogeneous tumors for boron neutron capture therapy (BNCT): chemical analyses in the hamster cheek pouch oral cancer model. *Arch Oral Biol* 2006;**51**:922–9.
11. Kato I, Ono K, Sakurai Y, Ohmae M, Maruhashi A, Imahori Y, et al. Effectiveness of BNCT for recurrent head and neck malignancies. *Appl Radiat Isot* 2004;**61**:1069–73.
12. Henriksson R, Capala J, Michanek A, Lindahl S, Salford L, Franzen L, et al. Boron neutron capture therapy (BNCT) for glioblastoma multiforme: a phase II study evaluating a prolonged high-dose of boronophenylalanine (BPA). *Radiother Oncol* 2008;**88**:183–91.
13. Yamamoto T, Nakai T, Matsumura A. Boron neutron capture therapy for glioblastoma. *Cancer Lett* 2008;**262**:143–52.
14. González SJ, Bonomi MR, Santa Cruz GA, Blaumann HR, Calzetta Larrieu OA, Menéndez P, et al. First BNCT treatment of a skin melanoma in Argentina: dosimetric analysis and clinical outcome. *Appl Radiat Isot* 2004;**61**:1101–5.
15. Kreimann EL, Itoiz ME, Longhino J, Blaumann H, Calzetta O, Schwint AE. Boron neutron capture therapy for the treatment of oral cancer in the hamster cheek pouch model. *Cancer Res (Adv Brief)* 2001;**61**:8638–42.
16. Kreimann EL, Itoiz ME, Dagrosa A, Garavaglia R, Farías S, Batistoni D, et al. The hamster cheek pouch model of oral cancer for boron neutron capture therapy studies: selective delivery of boron by boronophenylalanine. *Cancer Res* 2001;**61**:8775–81.
17. Kreimann EL, Miura M, Itoiz ME, Heber EM, Garavaglia RN, Batistoni D, et al. Biodistribution of a carborane-containing porphyrin as a targeting agent for boron neutron capture therapy of oral cancer in the hamster cheek pouch. *Arch Oral Biol* 2003;**48**:223–32.
18. Heber EM, Trivillin VA, Nigg DW, Kreimann E, Itoiz ME, Rebagliati RJ, et al. Biodistribution of GB-10 ($Na_2{}^{10}B_{10}H_{10}$) compound for boron neutron capture therapy (BNCT) in an experimental model of oral cancer in the hamster cheek pouch. *Arch Oral Biol* 2004;**49**:313–24.
19. Heber EM, Aromando RF, Trivillin VA, Itoiz ME, Nigg DW, Kreimann EL, et al. Therapeutic effect of boron neutron capture therapy (BNCT) on field cancerized tissue: inhibition of DNA synthesis and lag in the development of second primary tumors in precancerous tissue around treated tumors in DMBA-induced carcinogenesis in the hamster cheek pouch oral cancer model. *Arch Oral Biol* 2007;**52**:273–9.
20. Trivillin VA, Heber EM, Itoiz ME, Nigg DW, Calzetta O, Blaumann H, et al. Radiobiology of BNCT mediated by GB-10 and GB-10+BPA in experimental oral cancer. *Appl Radiat Isot* 2004;**61**:939–45.
21. Trivillin VA, Heber EM, Nigg DW, Itoiz ME, Calzetta O, Blaumann H, et al. Therapeutic success of boron neutron capture therapy (BNCT) mediated by a chemically non-selective boron agent in an experimental model of oral cancer: a new paradigm in BNCT radiobiology. *Radiat Res* 2006;**166**:387–96.
22. Aromando RF, Heber EM, Trivillin VA, Nigg DW, Schwint AE, Itoiz ME. Insight into the mechanisms underlying tumor response to boron neutron capture therapy in the hamster cheek pouch oral cancer model. *J Oral Pathol Med* 2009;**38**(5):448–54.
23. Heber EM, Monti Hugges A, Pozzi ECC, Itoiz ME, Aromando RF, Molinari AJ, et al. Development of a model of tissue with potentially malignant disorders (PMD) in the hamster cheek pouch to explore the long-term potential therapeutic and/or toxic effects of different therapeutic modalities. *Arch Oral Biol* 2010;**55**(1): 46–51.
24. Salley JJ. Experimental carcinogenesis in the cheek pouch of the Syrian hamster. *J Dent Res* 1954;**33**:253–62.
25. Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, et al. Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Res* 1988;**48**(11):3282–7.
26. Cogliano V, Straif K, Baan R, Grosse Y, Secretan B, El Ghissassi F. Smokeless tobacco and tobacco-related nitrosamines. *Lancet Oncol* 2004;**5**(12):708.
27. Petersen PE. Tobacco and oral health. The role of the world health organization. *Oral Health Prev Dent* 2003;**1**(4):309–15.
28. Petersen PE. Oral cancer prevention and control – the approach of the World Health Organization. *Oral Oncol* 2009;**45**(4–5):454–60.
29. Pozzi E, Nigg DW, Miller M, Thorp SI, Heber EM, Zarza L, et al. Dosimetry and radiobiology at the RA-3 reactor boron neutron capture therapy (BNCT) facility: application to the treatment of experimental oral cancer. *Appl Radiat Isot* 2009;**61**:939–45.
30. Yoshii M, Jikuhara A, Mori S, Iwagaki H, Takahashi H, Nishibori M, et al. Mast cell tryptase stimulates DLD-1 carcinoma through prostaglandin and MAP kinase-dependent manners. *J Pharmacol Sci* 2005;**98**:450–8.
31. Aromando RF, Pérez MA, Heber EM, Trivillin VA, Tomasi VH, Schwint AE, et al. Potential role of mast cells in hamster cheek pouch carcinogenesis. *Oral Oncol* 2008;**44**:1080–7.
32. LaHann TR, Lu DR, Daniell G, Kraft SL, Gavin PR, Bauer WF. Bioavailability of intravenous formulations of *p*-boronophenylalanine in dog and rat. In: Soloway R, Barth R, Carpenter D, editors. *Advances in neutron capture therapy*. New York: Plenum Press; 1993. p. 585–9.
33. Enerbäck L. Mast cells in rat gastrointestinal mucosa. 2. Dye-binding and metachromatic properties. *Acta Pathol Microbiol Scand* 1966;**66**(3):303–12.
34. Enerbäck L. Mast cells in rat gastrointestinal mucosa. I. Effects of fixation. *Acta Pathol Microbiol Scand* 1966;**66**(3):289–302.
35. Mayrhofer G. Fixation and staining of granules in mucosal mast cells and intraepithelial lymphocytes in the rat jejunum with specimen reference to the relationship between the acid glycosaminoglycans in the two cell types. *Histochem J* 1989;**12**:513–26.
36. Dimitriadou V, Buzzi MG, Moskowitz MA, Theoharides TC. Trigeminal sensory fiber stimulation induces morphological changes reflecting secretion in rat dura matter mast cells. *Neuroscience* 1991;**44**:97–112.
37. Kanakura Y, Thompson H, Yamamura T, Asai H, Kitamura Y, Metcalfe DD, et al. Multiple bi-directional alterations of phenotype and changes in proliferative potential during the in vitro and in vivo passage of clonal mast cell populations derived from mouse peritoneal mast cells. *Blood* 1988;**72**(3):877–85.
38. Kitamura Y. Heterogeneity of mast cells and phenotypic change between subpopulations. *Annu Rev Immunol* 1989;**7**:59–76.
39. Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CMM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol* 2005;**23**:749–86.
40. Cabrini RL, Itoiz ME, Carranza FA, Mayo J, Smolko EE. Histological and histochemical analysis of the effect on oral tissues and tooth germs of irradiation with deuteron beams. *Helv Odont Acta* 1967;**11**:124–30.
41. Albrecht M, Müller K, Köhn FM, Meineke V, Mayerhofer A. Ionizing radiation induces degranulation of human mast cells and release of tryptase. *Int J Radiat Biol* 2007;**83**(8):535–41.
42. Vasheghani MM, Bayat M, Rezaei A, Karimipour M. Effect of low level laser therapy on mast cells in second-degree burns in rats. *Photomed Laser Surg* 2008;**26**(1):1–5.
43. Jikuhara A, Yoshii M, Iwagaki H, Mori S, Nishibori M, Tanaka N. MAP kinase-mediated proliferation of DLD-1 carcinoma by the stimulation of protease-activated receptor 2. *Life Sci* 2003;**73**(22):2817–29.
44. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;**100**(1):57–70.