## ORIGINAL ARTICLE

# Histamine reduces boron neutron capture therapy-induced mucositis in an oral precancer model

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OBJECTIVES: Searching for more effective and selective therapies for head and neck cancer, we demonstrated the therapeutic effect of boron neutron capture therapy (BNCT) to treat oral cancer and inhibit long-term tumor development from field-cancerized tissue in the hamster cheek pouch model. However, BNCT-induced mucositis in field-cancerized tissue was dose limiting. In a clinical scenario, oral mucositis affects patients' treatment and quality of life. Our aim was to evaluate different radioprotectors, seeking to reduce the incidence of BNCT-induced severe mucositis in field-cancerized tissue.

MATERIALS AND METHODS: Cancerized pouches treated with BNCT mediated by boronophenylalanine at 5 Gy were treated as follows: control: saline solution; His$_{high}$: histamine 5 mg kg$^{-1}$; His$_{low}$: histamine 1 mg kg$^{-1}$; and JNJ7777120: 10 mg kg$^{-1}$.

RESULTS: His$_{low}$ reduced the incidence of severe mucositis in field-cancerized tissue to 17% vs CONTROL: 55%; His$_{high}$: 67%; JNJ7777120: 57%. His$_{low}$ was non-toxic and did not compromise the long-term therapeutic effect of BNCT or alter gross boron concentration. Conclusion: Histamine reduces BNCT-induced mucositis in experimental oral precancer without jeopardizing therapeutic efficacy. The fact that both histamine and boronophenylalanine are approved for use in humans bridges the gap between experimental work and potential clinical application to reduce BNCT-induced radiotoxicity in patients with head and neck cancer.

## Introduction

Squamous cell carcinoma of the head and neck remains a major cause of morbidity and mortality worldwide (Jaiswal et al, 2013). The relatively poor overall 5-year survival rate for malignancies of the oral cavity (Mehrotra et al, 2011) poses the need for more effective and selective therapies. Studies in appropriate experimental models are pivotal to progress in this field.

Boron neutron capture therapy (BNCT) is a binary treatment that combines the administration of boron carriers that are taken up preferentially by neoplastic tissue and irradiation with a thermal/epithermal neutron beam. The high linear energy transfer (LET) $\alpha$ particles and recoiling lithium-7 ($^7$Li) nuclei emitted during the capture of a thermal neutron by a boron-10 ($^{10}$B) nucleus have a high relative biological effectiveness. Their short range in tissue (6–10 $\mu$m) would limit the damage largely to cells containing $^{10}$B. In this way, BNCT would target neoplastic tissue selectively, sparing normal tissue (Trivillin et al, 2006). As BNCT is based on biological rather than geometric targeting, it would be suited to treat undetectable micrometastases (Pozzi et al, 2012) and foci of malignant transformation in field-cancerized tissue (Monti Hughes et al, 2013).

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Clinical studies of BNCT for glioblastoma multiforme, melanoma, head and neck tumors, and liver metastases have been performed or are underway in the United States, Japan, Europe, Argentina, and Taiwan (González *et al*, 2004; Kankaanranta *et al*, 2012; Miyatake *et al*, 2014; Wang *et al*, 2014; Yanagie *et al*, 2014) employing nuclear reactors as the neutron source. To date, the clinical results have shown a potential therapeutic advantage, with identified opportunities for improvement. The more recent development of accelerators to be used as the neutron source for BNCT paves the way for more widespread clinical trials.

Standard photon radiotherapy is based on the effect of low LET radiation, fractionated protocols, and geometrical targeting employing multiple beams and multileaf collimators. The fact that the treatment periods are extended (5–6 weeks) leads to anatomical changes and uncertainties in patient positioning that pose the need to adjust treatment planning to improve reproducibility. Tumor selectivity relies mainly on geometric conformation and the differential sensitivity of tumor *vs* normal tissue to radiotherapy. In the case of chemotherapy, protocols are also fractionated, involve systemic administration of the drugs, and rely on the greater sensitivity of tumor to treatment. BNCT differs from conventional radiotherapy in several ways. It is based mainly on the effect of high LET radiation with a high relative biological efficacy. The fact that it involves single or, at most, double applications contributes to the quality of life of the patient and minimizes the impact of anatomical changes. Because tumor targeting is biological rather than geometrical, relying on the preferential incorporation of boron carriers to tumor cells, BNCT is ideally suited to treat undetectable micrometastases, infiltrating malignant cells and foci of malignant transformation with minimum damage to healthy tissues in the treatment volume. Biological targeting improves the conformation of 3D dosimetry and minimizes variations due to positioning. Because BNCT is highly selective for tumor tissue, it can be employed for the treatment of recurrent lesions that have already been exposed to photon radiotherapy (Hopewell *et al*, 2011; Khan and Gerbi, 2012; Kankaanranta *et al*, 2012). A drawback of BNCT is the fact that it is not a treatment option to cure systemic disease. Also, admittedly, only a limited number of centers are currently able to offer BNCT as a treatment option.

The comparison of dose levels between standard radiotherapy and BNCT is not unequivocal due to the need to use correction factors to report estimated dose-equivalent doses (in Gy-eq), accounting for differences in biological efficacy between low and high LET radiation (Hopewell *et al*, 2011). Considering photon radiotherapy (in particular intensity-modulated radiation therapy), standard fractionation typically involves 2 Gy day$^{-1}$, 5 days a week, to reach a total of 54 Gy to the planning target volume and a boost to the clinical target volume to reach a total of 72 Gy. This treatment is administered over a 30- to 50-day period (Khan and Gerbi, 2012). An example of a successful treatment with BNCT for head and neck cancer involved a double application of BNCT at a dose of 26 Gy-eq to the planning target volume, with an interval of 41 days between applications (Kankaanranta *et al*, 2012).

The hamster cheek pouch model of oral cancer was previously proposed by our group for experimental BNCT studies (Kreimann *et al*, 2001a,b) and preceded the first clinical trial of BNCT for head and neck malignancies (Kato *et al*, 2004). It is a widely accepted model of oral cancer (Kreimann *et al*, 2001a; Vairaktaris *et al*, 2008) and oral mucositis (Bowen *et al*, 2011). Carcinogenesis protocols induce premalignant and malignant changes that closely resemble spontaneous human oral mucosa lesions (Kreimann *et al*, 2001a; Vairaktaris *et al*, 2008; Heber *et al*, 2010; Monti-Hughes *et al*, 2015). We previously demonstrated BNCT therapeutic efficacy to treat oral cancer in this experimental model employing boron compounds approved for their use in humans (Kreimann *et al*, 2001a; Trivillin *et al*, 2006) and novel boron compounds (Heber *et al*, 2014). Despite therapeutic success, the inhibition of tumor development from field-cancerized tissue remained an unresolved challenge (Heber *et al*, 2007). The relevance of field cancerization in head and neck cancer lies in the frequent occurrence of second primary tumors after treatment (Jaiswal *et al*, 2013; Monti-Hughes *et al*, 2015). In addition, the dose-limiting nature of field-cancerized tissue must be considered. In a clinical scenario, confluent oral mucositis is a frequent, dose-limiting side effect during conventional radiotherapy (Jensen and Peterson, 2014) and is a consideration in BNCT for advanced head and neck cancers (Kankaanranta *et al*, 2012; Wang *et al*, 2014). Oral mucositis can cause significant pain. The lesion can also be detrimental to diet, nutrition, oral hygiene, and quality of life. In immunosuppressed patients, secondary infection of oral mucositis lesions can lead to bacteremia, fungemia, and sepsis. In certain cases, the significant morbidity associated with oral mucositis may cause dose reductions, delays, and/or treatment interruptions in cancer therapy which in turn can jeopardize therapeutic efficacy (Jensen and Peterson, 2014). Also, oral mucositis could enhance tumorigenesis (Perez *et al*, 2005; Monti Hughes *et al*, 2013). Nowadays, it continues to represent an important unmet medical need in oncology practice, affecting patients' quality of life (Jensen and Peterson, 2014).

The hamster cheek pouch oral cancer model poses a unique advantage in that it allows for the study of both tumors and field-cancerized tissue (Heber *et al*, 2007). However, the aggressiveness of the model as employed in tumor control studies (Trivillin *et al*, 2006) precludes the long-term follow-up needed to evaluate the effect of BNCT on field-cancerized tissue, in terms of the development of recurrent and/or second primary tumors (Chen *et al*, 2011). Thus, we developed a model of oral precancer or field-cancerized tissue in the hamster cheek pouch that allows for long-term studies (Heber *et al*, 2010), which is essential to model (admittedly with constraints) a clinical scenario in which the risk of developing second primary tumors jeopardizes therapeutic efficacy (Heroiu *et al*, 2013). Employing this model, we demonstrated the partial inhibitory effect on the development of tumors of a *single* and *double* application of BNCT. Despite therapeutic success, BNCT-induced mucositis in field-cancerized tissue was dose limiting and favored, in some cases, tumor development (Monti Hughes *et al*, 2013).

To improve tumor control using higher doses, the dose-limiting tissue should be protected. Therefore, the role of

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radioprotective compounds is of utmost importance in clinical radiotherapy (Medina *et al*, 2011a). Histamine [2-(4-imidazolyl)-ethylamine] is an important regulator of a range of (patho)physiological conditions, acting through four histamine receptor subtypes (H1R, H2R, H3R, and H4R). In particular, H4R could be associated with inflammation and immune disorders (Medina *et al*, 2011a). Medina *et al* (2011a,b) and Martinel Lamas *et al* (2013) demonstrated that histamine and JNJ7777120 [1-[(5-Chloro-1*H*-indol-2-yl)carbonyl-]-4-methylpiperazine], another H4R ligand, prevent gamma radiation-induced toxicity in intestinal mucosa, bone marrow, and salivary glands of mice and rats. Although JNJ7777120 was initially considered as a standard H4R antagonist, recent evidence suggests that JNJ7777120 may also act as agonist to human H4R (Martinel Lamas *et al*, 2013). It has been reported that healthy oral epithelial cells are equipped with H4R, displaying a uniform staining pattern (Salem *et al*, 2015). In the digestive system, H4R expression has been evidenced in different areas and in a variety of cell types, including immune, inflammatory, neural endocrine, and epithelial cells (Medina *et al*, 2013). In particular, H2R has been reported to be overexpressed in tumors of the hamster cheek pouch (Parihar *et al*, 2013). In addition, Zampeli and Tiligada (2009) concluded that receptors H1, H2, and H4 are involved in inflammatory cell migration and H1 is specifically involved in vascular permeability and edema.

This study evaluates the ability of histamine and JNJ7777120 to reduce the incidence of BNCT-induced severe mucositis in field-cancerized tissue, without exhibiting negative local or systemic side effects, or compromising the long-term (8 months) therapeutic effect of BNCT.

## Materials and methods

### Model of oral precancer for long-term studies

Young Syrian hamsters were treated by topical application of 0.5% dimethylbenzanthracene (DMBA; Sigma Chemical Co., San Luis, MO, USA) in mineral oil in the right cheek pouch, twice a week for 6 weeks (Heber *et al*, 2010). Radiobiological and biodistribution studies were performed 1 week after the completion of the carcinogenesis protocol (T0). Animal experiments were carried out in accordance with the Guidelines laid down by the National Institute of Health in the USA regarding the care and use of animals for experimental procedures and in accordance with protocols approved by the National Atomic Energy Commission Animal Care and Use Committee (CICUAL-CNEA #17-2013).

### Radiobiological studies

Dimethylbenzanthracene-cancerized pouches in four groups of hamsters were exposed to BNCT mediated by boronophenylalanine (BPA) at 5 Gy mean absorbed dose to field-cancerized tissue and treated 1 day before BNCT, on the day of BNCT (concomitantly with BPA injection) and daily for 14 days after BNCT with: [control group] vehicle, that is, saline solution ($n = 5$–11 depending on the endpoint); [$His_{high}$] histamine high concentration in saline solution ($n = 6$, 5 mg kg$^{-1}$, subcutaneously in the

dorsum of the neck -sc-; Sigma Chemical Co.); [$His_{low}$] histamine low concentration in saline solution ($n = 6$, 1 mg kg$^{-1}$, sc); and [JNJ7777120] JNJ7777120 in saline solution ($n = 7$; 10 mg kg$^{-1}$, sc; Johnson & Johnson Pharmaceutical Research and Development, New Brunswick, NJ, USA). A bolus injection of BPA was administered intravenously (iv) at a dose of 15.5 mg $^{10}$B kg$^{-1}$ 3 h before irradiation (Monti Hughes *et al*, 2013). The animals were irradiated at the RA-3 nuclear reactor thermal neutron facility employing lithium-6 carbonate shielding to expose the tumor-bearing pouch while protecting the body of the animal (Pozzi *et al*, 2009). Thermal neutrons are considered as well represented by a Maxwellian distribution at 38°C, corresponding to an energy of 2.7E-08 MeV. Table 1 shows the irradiation conditions, and Table 2 indicates the absorbed doses for each dose component. The 5 Gy mean absorbed dose used in each group has been proved to be therapeutically useful in previous studies, but induced a high incidence of severe mucositis in field-cancerized tissue (Monti Hughes *et al*, 2013). A pooled group of 88 untreated, cancerized animals were used to monitor tumor development and mucositis resulting from the carcinogenesis protocol.

### Follow-up

An ideal radioprotector must be minimally toxic and must reduce radiotoxicity without compromising therapeutic effect. Thus, during 8 months after irradiation (T0), we evaluated weekly

a – clinical local and systemic signs, by visual inspection and weighing.

b – potential radiotoxicity in terms of mucositis monitored in field-cancerized tissue, evaluated semiquantitatively by visual inspection according to an adaptation of oral mucositis scales (Sonis *et al*, 2000; López Castaño *et al*, 2005), that is, Grade 0 (G0): healthy appearance, no erosion, or vasodilation; G1: erythema and/or edema and/or vasodilation, no evidence of mucosal erosion; G2: severe erythema and/or edema, vasodilation and/or superficial erosion; G3: severe erythema and/or edema, vasodilation and formation of ulcers <2 mm in diameter; G4 (severe): severe erythema and/or edema, vasodilation and formation of ulcers ≥2 mm and <4 mm in diameter, and/or areas of necrosis <4 mm in diameter; G5 (severe): formation of ulcers and/or areas of necrosis ≥4 mm in diameter. Grading was based on the most severe macroscopic feature. The experiments with radioprotectors sought to minimize the incidence of severe mucositis grades 4 and 5.

c – potential development of recurrent and/or second primary tumors ('new' tumors that were not present at the time of irradiation [T0]) from field-cancerized tissue, by visual inspection and reported as percentage of animals with 'new' tumors after T0.

### Biodistribution studies

Having determined the potential of $His_{low}$ to reduce the incidence of BNCT-induced mucositis (see Results section), we performed boron biodistribution studies in two groups of DMBA-cancerized animals, 1 week after the completion of the carcinogenesis protocol (T0), to determine the effect of $His_{low}$ on BPA uptake: (i) BPA (15.5 mg$^{10}$B kg$^{-1}$) iv and (ii) BPA (15.5 mg$^{10}$B kg$^{-1}$) iv + $His_{low}$sc, administered 1 day before and concomitantly with BPA injection. Euthanasia was performed 3 h postinjection of BPA. Blood, tumor, field-cancerized, and normal tissue samples were processed for boron measurements by inductively coupled plasma atomic emission spectroscopy (Kreimann *et al*, 2001a).

### Statistical analysis

The percentage of animals with 'new' tumors was analyzed using a contingency table and Fisher's exact test. The incidence of severe mucositis was

**Table 1** Irradiation conditions

| Boron concentration in field-cancerized tissue[a] | Irrad. time | Mean absorbed dose in field-cancerized tissue | Mean thermal neutron flux at the irradiation position | Gamma dose rate in air |
|---|---|---|---|---|
| 12.5 ± 2.6 ppm | 7.9 min | 5.0 ± 1.7 Gy | $(7.7 \pm 0.6) \times 10^9$ n cm$^{-2}$ s$^{-1}$ | 6.5 ± 0.6 Gy h$^{-1}$ |

[a]value used for dose prescription.

**Table 2** Absorbed doses for each dose component (Gy)

| | Fast neutrons | Gamma photons | Boron | Induced protons |
|---|---|---|---|---|
| Field-cancerized tissue, Gy | – | $0.36 \pm 0.09$ | $3.4 \pm 1$ | $0.9 \pm 0.1$ |

**Table 3** Percentage (%) of animals that reached severe mucositis (Grade 4–Grade 5). '$n$' is total number of animals in each group at the onset of the experiment

| | | Mucositis Grade 4–Grade 5 (%) |
|---|---|---|
| Control group: BPA-BNCT + saline solution | ($n = 11$) | 55 |
| BPA-BNCT + His$_{high}$ | ($n = 6$) | 67 |
| BPA-BNCT + His$_{low}$ | ($n = 6$) | 17 |
| BPA-BNCT + JNJ7777120 | ($n = 7$) | 57 |
| Cancerized, untreated | ($n = 88$) | 0 |

BNCT, boron neutron capture therapy; BPA, boronophenylalanine

**Table 4** Percentage (%) of animals with 'new' tumors after BNCT, 8 months (long-term follow-up) after treatment (T0). '$n$' is total number of animals in each group at the onset of the experiment

| | | % Animals with 'new' tumors 8 months after BNCT |
|---|---|---|
| Control group: BPA-BNCT + saline solution | ($n = 5$) | 67 |
| BPA-BNCT + His$_{high}$ | ($n = 6$) | 50 |
| BPA-BNCT + His$_{low}$ | ($n = 6$) | 40 |
| BPA-BNCT + JNJ7777120 | ($n = 7$) | 50 |
| Cancerized, untreated | ($n = 88$) | 89 |

BNCT, boron neutron capture therapy; BPA, boronophenylalanine.

**Table 5** Biodistribution studies with boronophenylalanine (BPA) and His$_{low}$ as indicated. '$n$' is number of samples.

| Tissue | BPA + saline solution[a] Mean±s.d. (ppm) | BPA + His$_{low}$[b] Mean ± s.d. (ppm) |
|---|---|---|
| Tumor | $10 \pm 3$ ($n = 3$) | $17 \pm 6$ ($n = 5$) |
| Field-cancerized pouch tissue | $16 \pm 10$ ($n = 2$) | $12 \pm 3$ ($n = 4$) |
| Normal pouch tissue | $10 \pm 3$ ($n = 2$) | $14 \pm 2$ ($n = 4$) |
| Blood | $6 \pm 1$ ($n = 2$) | $6 \pm 2$ ($n = 4$) |

[a]Two hamsters.
[b]Four hamsters.

analyzed using a contingency table and Barnard's exact test, estimating the $P$-value that will reject the null hypothesis 'The frequency of animals with severe mucositis in the control group is equal to the incidence of severe mucositis in each treatment group.' The differences in gross boron content were evaluated by Student's $t$-test. Statistical significance: $P = 0.05$.

## Results

Only reversible irritation was seen at the injection site for all radioprotectors. Local scratching led to superficial wounding and scab formation, followed by healing once the series of injections had been completed. A transient weight loss of ≤10% concomitant with mucositis was seen (data not shown).

Mucositis peaked approximately at 14 days and had resolved by 28 days post-BNCT. The incidence of severe mucositis was lower for BPA-BNCT + His$_{low}$ (one of six animals) than that for the groups: control BPA-BNCT (six of 11 animals), BPA-BNCT + His$_{high}$ (four of six animals), and BPA-BNCT + JNJ7777120 (three of seven animals) (Table 3). In the case of the group BPA-BNCT + His$_{low}$, the null hypothesis is rejected with $P = 0.1$, whereas at comparable sample sizes, in the case of BPA-BNCT + His$_{high}$ and BNCT + JNJ7777120, the null hypothesis is rejected at higher $P$ values of 0.36 and 0.54, respectively. This would indicate a greater radioprotective potential for the His$_{low}$ protocol. All untreated cancerized animals (no BNCT or radioprotector) exhibited mucositis G0–G1 during 8-month follow-up. Figure 1 shows one of our most dramatic comparative examples of a cancerized pouch treated with BPA-BNCT + His$_{low}$, exhibiting only G2 mucositis, vs a control cancerized pouch treated with BPA-BNCT showing G5 mucositis, 14 days post-BNCT. None of the protocols with radioprotectors compromised the long-term therapeutic effect of BNCT (control group vs histamine and JNJ7777120, $P = 1.0000$) (Table 4). Finally, we demonstrated that His$_{low}$ did not significantly alter gross boron concentration ($P \geq 0.11$) (Table 5).

## Discussion

The present study reports the results of combining BNCT with three radioprotective treatment protocols. Mucositis in oral field-cancerized tissue affects patients' quality of life, is a dose-limiting effect (Sonis, 2009; Kankaanranta et al, 2012), and could be a tumorigenesis enhancer (Monti Hughes et al, 2013). Our results showed that treatment with His$_{low}$ reduced the incidence of severe mucositis, without affecting BNCT therapeutic effect or boron biodistribution from BPA in blood, tumor, field-cancerized, and normal tissue. Instead, His$_{high}$ and JNJ7777120 failed to exert a protective effect.

Radiation, chemotherapy, and BNCT induce DNA strand breaks and reactive oxygen species which react with DNA and other cellular molecules causing cell dysfunction and mortality (Sonis, 2009; Medina et al, 2011a; Faião-Flores et al, 2013). Free radical production is the initial stage of mucositis (Elad et al, 2006; Sonis, 2009). Previously, it was observed that histamine (0.1 mg kg$^{-1}$) prevents gamma radiation-induced toxicity on intestinal mucosa by suppressing apoptosis that was in turn associated with an enhanced antioxidant capacity in intestinal cells (Medina et al, 2007). Thus, histamine could also be enhancing the antioxidant system in oral mucosa, helping to reduce mucositis in the majority of the animals treated with BPA-BNCT + His$_{low}$.
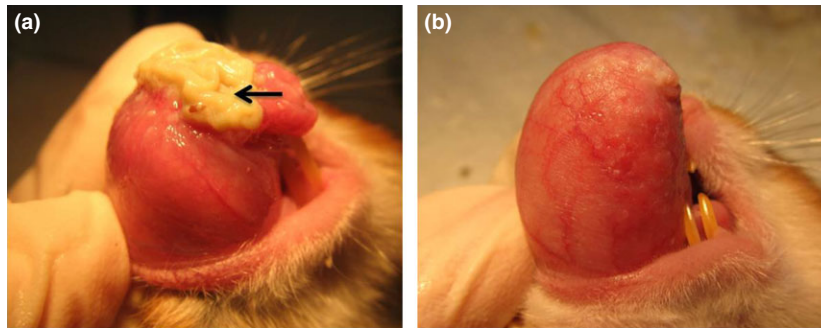
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**Figure 1** Example of radioprotective effect of His$_{low}$: (**a**) cancerized hamster cheek pouch treated with boronophenylalanine (BPA)-boron neutron capture therapy (BNCT) + saline with severe mucositis (Grade 5) *vs* (**b**) cancerized hamster cheek pouch treated with BPA-BNCT + His$_{low}$ with Grade 2 mucositis. Both pouches are shown at 14 days post-treatment (the time at which maximum mucositis was observed). The arrow indicates an area of necrosis. The pouches were everted for observation

As with other drugs, the biologic effect of the drug may be dependent on its dosage (Elad *et al*, 2006). In particular, Medina and Rivera (2010) reported that histamine would modulate proliferation in a dose-dependent manner in normal and malignant cells, depending also on the biological system assayed. Both His$_{high}$ and JNJ7777120 failed to reduce severe mucositis. This could be partially due to a different effect on cell proliferation produced by these compounds at higher concentrations that could enhance radiosensitivity (Hashibe *et al*, 2005). However, this effect would apply mainly to the low LET component of the BNCT dose (Hopewell *et al*, 2011). Furthermore, Murphy *et al* (2008) demonstrated that extensive removal of superoxide anions during the specific time window of mucositis may interfere with the healing process of mucositis. Within this context, high doses of histamine and JNJ7777120 over a relatively long period might interfere with the healing process and paradoxically increase the severity of mucositis.

In the case of BNCT, where the main components of the dose are high LET (Hopewell *et al*, 2011), a signifi-cant proportion of the damage occurs directly via DNA double-strand breaks, inducing cell death and inhibiting the proliferation of surviving cells (Aromando *et al*, 2009). The fact that mucositis is initiated by mucosal injury in particular in the basal layer (Mais, 2006), that BPA would accumulate preferentially in basal cells (Kreimann *et al*, 2001b), and that BNCT would inhibit DNA synthesis and proliferation (Aromando *et al*, 2009) would partially explain why BPA-BNCT induces a high incidence of severe mucositis. While scarce and controversial data are available on the effect of BNCT on apoptosis (Aromando *et al*, 2009; Faião-Flores *et al*, 2013), Kamida *et al* (2008) described apoptosis associated with BNCT in a model of human oral squamous cell carcinoma cells. Within this context, the capacity of histamine to act as a growth factor, favoring proliferation and repair and inhibiting apoptosis (Medina *et al*, 2007; Medina and Rivera, 2010) and other properties such as its ability to reduce the levels of ROS, suppress proinflammatory cytokines, and increase blood flow which may favor healing (Treede *et al*, 1990; Hellstrand *et al*, 1998; Agarwala and Sabbagh, 2001; Azuma *et al*, 2001;
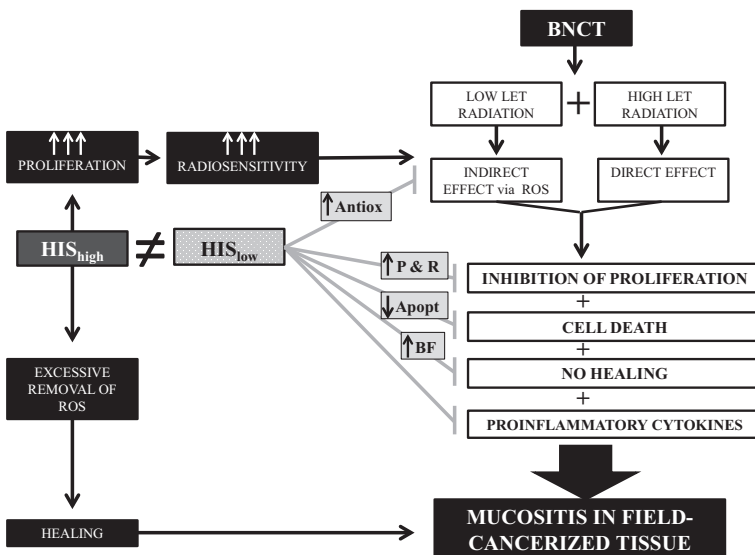


**Figure 2** Schematic representation of the effects that might contribute to the protective action of histamine. Promotes ⟶; Inhibits ⊣; Antiox, antioxidant capacity; P & R, proliferation and repair; Apopt, apoptosis; BF, blood flow; ≠, The biological effect of the drug may be dependent on its dosage

Elad *et al*, 2006) would conceivably contribute to prevent severe mucositis. Figure 2 shows a schematic representation of the effects that might contribute to the protective action of histamine.

In the present study we explored, based on the effect of histamine in other models (Medina *et al*, 2011a,b), the radioprotective effect of histamine in the hamster cheek pouch oral cancer model. Admittedly, this study was not designed to determine the mechanisms involved in this effect. H2R has been reported to be overexpressed in tumors of the hamster cheek pouch (Parihar *et al*, 2013) and preliminary studies by our group in the hamster cheek pouch model showed negative immunoreactivity for H4R in epithelial cells and positive staining for $H_4R$ in immunocompetent cells. Future studies will be necessary to elucidate the role of the histamine receptors in this effect.

There are two assets in considering the use of histamine as a radioprotector in BNCT mediated by BPA for head and neck tumors: (i) a synthetic derivative of histamine, histamine dihydrochloride, is currently safely used in clinical trials (Romero *et al*, 2009) and (ii) histamine also prevents radiation-induced alterations in submandibular glands and completely reversed radiation-induced reduced salivation in rats (Medina *et al*, 2011b). It has been shown that BNCT is also a potential curative therapy for patients with salivary gland carcinoma (Aihara *et al*, 2014). Apart from mucositis, xerostomia is also a negative side effect in BNCT (Kankaanranta *et al*, 2012; Wang *et al*, 2014). Overall, histamine reduces oral mucositis and could preserve submandibular salivary glands, preventing xerostomia during BNCT treatment.

None of the protocols compromised the long-term therapeutic effect of BPA-BNCT. The fact that $His_{low}$ did not affect boron biodistribution in tumor or field-cancerized tissue is an asset in terms of preserving therapeutic efficacy.

This study suggests the potential use of $His_{low}$ treatment to minimize BPA-BNCT-induced severe mucositis in field-cancerized tissue at a therapeutic absorbed dose of 5 Gy, in an oral precancer model. These results may be of clinical value in reducing radiation-induced toxicity without compromising therapeutic efficacy in patients with head and neck cancer undergoing BNCT treatment. The fact that both histamine and BPA are approved for use in humans narrows the gap between research and clinical application.

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## Author contributions

Study concepts: A. Monti Hughes, V.A. Trivillin, A.E. Schwint. Study design: A. Monti Hughes, V.A. Trivillin, A.E. Schwint, V.A. Medina, D.J. Martinel Lamas, E.S. Rivera. Data acquisition: A. Monti Hughes, M.A. Garabalino, E.C.C. Pozzi, S.I. Thorp, P. Curotto, S.J. Gonzalez, R.O. Farías. Quality control of data: A. Monti Hughes, V.A. Trivillin, A.E. Schwint, E.C.C. Pozzi, S.I. Thorp. Data analysis and interpretation: A. Monti Hughes, V.A. Trivillin, A.E. Schwint, M.E. Itoiz, R.F. Aromando, V.A. Medina, D.W. Nigg, E.M. Heber. Manuscript preparation: A. Monti Hughes, A.E. Schwint. Manuscript editing: A. Monti Hughes. Manuscript review: E.C.C. Pozzi, S.I. Thorp, P. Curotto, V.A. Trivillin, M.E. Itoiz, R.F. Aromando, V.A. Medina, D.J. Martinel Lamas, M.A. Garabalino, R.O. Farías, S.J. Gonzalez, D.W. Nigg, E.M. Heber.

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