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Histamine prevents functional and morphological alterations of submandibular glands induced by ionising radiation

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Abstract

Purpose: Xerostomia is a common, disturbing side-effect among patients treated with radiotherapy for head-and-neck cancer. The aim of the present work was to investigate whether histamine could prevent salivary gland dysfunction and histological alterations exerted by ionising radiation.

Materials and methods: Forty-eight rats were divided into four groups. Histamine and histamine-5 Gy groups received a daily subcutaneous histamine injection (0.1 mg/kg) starting 24 h before irradiation. Histamine-5 Gy and untreated-5 Gy groups were irradiated with a single dose of whole-body Cesium-137 irradiation. Control and untreated-5 Gy groups were given daily saline injections. Three days post irradiation metacholine-induced salivary secretion was measured or animals were sacrificed and submandibular gland (SMG) removed, stained and histological characteristics were evaluated. Proliferation and apoptosis markers were studied by immunohistochemistry.

Results: Radiation decreased salivary secretion by 40% in comparison to untreated rats, which was associated with loss of SMG mass, alteration of epithelial architecture, partial loss of secretor granular material, diminished proliferation and a remarkable apoptotic response. In contrast, histamine completely reversed the reduced salivation induced by radiation, conserved glandular mass with normal appearance and preserved the structural organisation of secretor granules. Radiation-induced toxicity is prevented by histamine essentially by suppressing apoptosis of ductal and acinar cells, reducing the number of apoptotic cells per field (19.0 ± 3.8 vs. 106.0 ± 12.0 in untreated animals, $P < 0.001$), and also by preventing the radiation-induced decrease in cell proliferation.

Conclusions: Histamine prevents morphological and functional radiation-induced damage on SMG, representing a potential radioprotector for treatment of patients undergoing radiotherapy for head and neck malignancies.

Keywords: *histamine, ionising radiation, radioprotectors, salivary secretion, salivary glands, apoptosis*

Introduction

Radiotherapy is a central treatment modality administered for head and neck malignancies. After irradiation, damage to the oral mucosa and particularly severe morphological and functional alterations of the salivary glands occur resulting in salivary dysfunction (Valdez et al. 1993, Burlage et al. 2001, 2008, Nagler 2002). Xerostomia is the main clinical effect, a common, disturbing side-effect among patients treated with high doses of ionising radiation that leads to considerable morbidity (Valdez et al. 1993, Nagler 2002, Vissink et al. 2003, Dirix et al. 2006).

Salivary glands consist of several cell types: Acinar cells, which are responsible for water and protein secretion, myoepithelial cells surrounding the acini and ducts, and ductal cells which mainly modulate the ionic composition of the saliva. The ductal system consists of intercalated, striated/granular convoluted tubule and excretory duct cells. Regeneration originates from putative stem cells residing in the ductal compartment from which complete recovery is induced within a week after ductal

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obstruction. Irreversible hyposalivation after irradiation-induced damage is caused not only by the death of acinar cells, but also by a marked decline in functional differentiation and proliferative capacity of all of the surviving cells, mainly by sterilisation of these primitive glandular stem cells with progenitor capability, which prevents replenishment of aged saliva producing cells, thus altering tissue homeostasis (O'Connell et al. 1999, Takahashi et al. 2004, Lombaert et al. 2008, Redman 2008). Although new radiation techniques enabled significant sparing, the amount of normal salivary gland tissue irradiated may still be substantial and results in clinically relevant and irreversible radiation-induced xerostomia. Thus, protection against radiation-induced salivary gland damage may further improve the therapeutic index of radiotherapy (Chao et al. 2001, Nagler 2002, Blanco et al. 2005, Saarilahti et al. 2005, Dirix et al. 2006, Burlage et al. 2008).

In this light, it has been previously reported that histamine significantly protects two of the most radiosensitive tissues, small intestine and bone marrow, from high doses of gamma radiation in two different species, mice and rats. Histamine prevents radiation-induced toxicity in the small intestine by increasing proliferation of damaged intestinal mucosa and also by suppressing apoptosis (Medina et al. 2007, 2010). In addition, histamine has the ability to enhance the radiosensitivity of malignant breast cells (Medina et al. 2006).

Histamine (2-(imidazol-4-yl)ethylamine) is a biogenic amine with a broad spectrum of activities in numerous physiological and pathological situations, and is synthesised by the histidine decarboxylase enzyme (HDC) (Dy and Schneider 2004). It was previously reported that histamine could act as a stimulator of salivary secretion in submandibular gland (SMG) of cats and dogs (Shimizu and Taira 1980, Stanovnik and Erjavec 1983). Furthermore, histamine treatment induces the translocation of aquaporin-5, which modulates water secretion to produce primary saliva, to the plasma membrane in human SMG cells (Kim et al. 2009). In addition, the histamine H1 and H2 receptors were described in rat SMG (Sim and Ang 1985, Borda et al. 2002), and histamine content was demonstrated not only in SMG but also in saliva (Erjavec and Stanovnik 1987, Kim et al. 2009).

The aim of the present study was to determine whether histamine could prevent SMG dysfunction and morphological alterations induced by ionising radiation. Furthermore, it has been suggested that the imbalance between apoptosis and proliferation induced by ionising radiation in SMG is responsible for the salivary gland function impairment that leads to a decrease in saliva secretion (Bralic et al. 2005, Muhvic-Urek et al. 2006, Avila et al. 2009). To address these issues, the histamine effect on ionising radiation-altered functional (salivary secretion and gland mass) and morphological parameters were evaluated. We also analysed proliferation and apoptosis by immunohistochemistry.

Materials and methods

Treatment and irradiation

Forty-eight 10-week-old male Sprague-Dawley rats, weighing 200–230 g were purchased from the Division of Laboratory Animal Production, School of Veterinary Sciences, University of La Plata, Buenos Aires, and were randomly separated into four groups ($n = 12$ each). Rats were maintained in our animal healthcare facility at 22–24°C and 50–60% humidity on a 12 h light/dark cycle with food and water available *ad libitum*. To determine the potential radioprotective effect of histamine on SMG, the same experimental procedures that we previously described in detail to evaluate the radioprotective effect on small intestine and bone marrow were used (Medina et al. 2007, 2010).

Histamine and Histamine-5 Gy groups received a daily subcutaneous histamine injection (0.1 mg/kg, FLUKA, Berlin, Germany) starting 24 h before irradiation and which continued until the end of the experimental period while the untreated control group and radiation only group received saline. Histamine-5 Gy group and untreated-5 Gy group were irradiated using Cesium-137 source (IBL 437C type H) of 189 TBq (dose rate: 7 Gy/min) with a single whole-body dose of 5 Gy. This dose was chosen because it is close to the dose that would be lethal to 50% of population after 30 days of exposure ($^{30}LD_{50}$) for Sprague-Dawley rat (5.8 Gy) (data not shown).

Three days post irradiation metacholine-induced salivary secretion was measured ($n = 6$ each group) or animals were sacrificed by cervical dislocation and SMG were removed, weighed, and histological and histochemical characteristics were evaluated ($n = 6$ each group).

Animal procedures were in accordance with recommendations from the Guide for the Care and Use of Laboratory Animals of the National Research Council, USA, 1996, and protocols were approved by the Ethical and Educational Committee of the Institute of Immunooncology.

Salivary secretion

Salivation was assessed in anesthetised rats (chloralose 100 mg/kg, 0.5 ml NaCl-0.9%) (FLUKA). The right femoral vein was cannulated with a polyethylene catheter (P40 catheter, Rivera & Co,

Buenos Aires, Argentina) to administer the sialagogic agonist, metacholine (FLUKA), used in the study. Through a midline incision in the neck, the trachea was intubated and the SMG ducts were exposed and cannulated with a fine glass cannula to collect saliva samples. No basal salivation was observed from the glands. Salivation was induced by the administration of different concentrations of metacholine ranging from the threshold dose to the dose that exerts the maximum stimulus (0.3, 1, 3, 10 and 30 μg/kg, in saline) sequentially injected via the right femoral vein. Salivary samples were collected for 3 min in pre-weighed aluminium foil and the quantity of saliva was determined by weighing, as previously described (Bianciotti et al. 1994). Three additional minutes were allowed until the administration of the next dose. Results were expressed as mg of saliva per gland.

Histopathological studies

Salivary glands were removed and fixed with 10% neutral buffered formalin. Tissue samples were embedded in paraffin and cut into 3 μm thick serial sections. SMG morphology and histopathological characteristics were examined on tissue sections after haematoxylin-eosin staining.

Immunohistochemical staining

After deparaffinisation, specimens were placed in citrate buffer (10 mM, pH 6.0) and heated at boiling temperature in a microwave oven twice for 2 min for antigen retrieval. Endogenous peroxidase activity was blocked with 3% H_2O_2 in distilled water. After blocking, tissues were incubated with primary rabbit anti histamine (1:100, Sigma Chemical Co., St Louis, MO, USA), mouse anti proliferating cell nuclear antigen (PCNA, 1:100, DakoCytomation, Glostrup, Denmark), mouse anti B-cell lymphoma 2 (Bcl-2), anti Bcl-2-associated x protein (Bax) (1:50, Santa Cruz, Santa Cruz, CA, USA) and rabbit anti HDC (1:100, Euro-Diagnostica AB, Mälmo, Sweden) antibodies overnight in a humidified chamber at 4°C. Immunoreactivity was detected by using horseradish peroxidase-conjugated anti-mouse or anti-rabbit, as appropriate, and visualised by diamino-benzidine staining (Sigma Chemical Co.). To evaluate sub-cellular localisation of these proteins, nuclei were stained with haematoxylin.

Light microscopy was performed on an Axiolab Karl Zeiss microscope (Göttingen, Germany). All photographs were taken at 630 × magnification using a Canon PowerShot G5 camera (Tokyo, Japan). To control the signal specificity, serial sections were made from five selected positive cases which were subjected to the same staining procedure, with either a normal mouse or rabbit serum or phosphate buffered saline (PBS) to replace the first antibody. No signal was detected in this control staining. Proliferation was evaluated by assessing PCNA expression and results were expressed as the number of PCNA-positive cells per field of at least 15 fields examined.

Determination of apoptosis

Apoptotic cells were determined by terminal deoxynucleotidyl transferase (TdT) mediated deoxyuridine triphosphate biotin nick end labelling (TUNEL) assay. Fragmented DNA in cells undergoing apoptosis was detected using Apoptag^{TM} plus peroxidase in situ apoptosis Detection Kit (CHEMICON International, Temecula, CA, USA) according to the manufacturer's instructions. Samples were visualised using an Axiolab Karl Zeiss microscope. All photographs were taken at 630 × magnification using a Canon PowerShot G5 camera. Negative control sections were incubated in the absence of TdT. Results were expressed as the number of TUNEL-positive cells per field of at least 15 fields examined.

Statistical analysis

Data shown are mean ± standard error of the mean (SEM). Statistical evaluations were made by analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test. P values < 0.05 were considered significant. All statistical analyses were performed with GraphPad Prism Version 5.00 software (San Diego, CA, USA).

Results

Effect of histamine on SMG function and morphology

It is well known that ionising radiation induces damage to the major salivary glands, resulting in severe alterations in their structure and function (Burlage et al. 2001, 2008, Nagler 2002, Vissink et al. 2003, Lombaert et al. 2008, Redman 2008, Avila et al. 2009).

The present results demonstrate that in non-irradiated rats, no significant effect was produced by histamine either in functional or in the histological and morphological characteristics of SMG (Table I, Figure 1C). Radiation significantly decreased salivation by approximately 35–40% at 10 and 30 μg/kg metacholine concentration in comparison to non-irradiated rats (Table I). This outcome was associated with a reduction of SMG wet weight relative to body weight (35%) and an alteration of epithelial architecture with lower cylindrical cells, vacuolisation of

Table I. Histamine preserves salivary secretion in irradiated rats.

Metacholine (μg/kg)	Untreated	Histamine	Untreated-5 Gy	Histamine-5 Gy
1	7.0 ± 1.0	5.5 ± 0.1	5.0 ± 1.5	7.8 ± 0.3
3	15.5 ± 1.5	20.5 ± 3.0	13.8 ± 3.3	21.5 ± 3.0
10	53.0 ± 0.1	45.0 ± 1.5	$32.0 \pm 5.0^{\star\star}$	$64.8 \pm 6.3^{\#\#\#}$
30	92.0 ± 1.0	81.0 ± 7.0	$60.0 \pm 0.1^{\star}$	$91.3 \pm 10.8^{\#}$

Mean metacholine-stimulated salivary secretion in irradiated and non-irradiated, untreated and histamine-treated rats ($n = 6$). Data represent the means $\pm$ SEM. $^{\#\#\#}P < 0.001$, $^{\#}P < 0.05$ vs. Untreated-5Gy.

acinar cells, and partial loss of eosinophilic secretor granular material (Table II, Figure 1C). Histamine treatment completely reversed the radiation-induced reduced salivation, significantly conserved glandular weight with normal appearance preserving the structure organisation of secretor granules and conserving the proportion between acini and ducts (Figure 1, Tables I and II).

Effect of histamine on cell proliferation and apoptosis in irradiated SMG

An adequate balance between cell proliferation and programmed cell death or apoptosis is a key feature in the maintenance of the normal architecture and function of SMG (De la Cal et al. 2006, Limesand et al. 2006, Muhvic-Urek et al. 2006, Lee et al. 2006, Redman 2008, Avila et al. 2009).

PCNA is a well documented indicator of active proliferation as an essential component of the deoxyribonucleic acid replication machinery (Kelman 1997). In SMG derived from untreated rats, PCNA expression was very low and preferentially restricted to ductal cells, and no significant difference was observed after histamine treatment in non-irradiated rats. Ionising radiation markedly reduced the PCNA immunoreactivity indicating the absence of proliferation. Interestingly, histamine treatment in irradiated rats significantly prevented the decrease in PCNA-positive cells per field induced by ionising radiation (1.9 ± 0.2 vs. 0.4 ± 0.1 in untreated rats, $P < 0.001$) (Figure 2).

Enhanced apoptosis of acinar cells is suggested to be one of the major causes of salivary gland impairment after ionising radiation exposure (Limesand et al. 2006, Muhvic-Urek et al. 2006, Avila et al. 2009). In order to investigate whether the histamine radioprotective effect could be associated with a reduction of glandular cell death, we further analysed the response of SMG cells to gamma irradiation by the TUNEL assay. SMG from non-irradiated animals revealed a small number of TUNEL-positive cells primarily in ducts that were not modified by histamine treatment (Figure 3A, 3B). Irradiation drastically increased the apoptotic cells in all gland compartments including acinar cells, granular convoluted tubule cells, intercalated ductal cells and excretory ducts. A massive amount of acinar and also ductal cells were TUNEL-positive. In contrast, histamine dramatically reduced the apoptosis induced by ionising radiation that was evidenced by a diminished number of TUNEL-positive cells per field (19.0 ± 3.8 vs. 106.0 ± 12.0 in untreated animals, $P < 0.001$).

Furthermore, the reduction in ionising radiation-induced apoptosis exerted by histamine was accompanied by a decrease in the immunoreactivity levels of the pro-apoptotic protein Bax that was increased by ionising radiation, preferentially in acinar cells (Figure 3C). The anti-apoptotic protein Bcl-2 expression was weak in SMG and remained unaffected by histamine treatment or ionising radiation exposure (data not shown).

HDC expression and intracellular histamine in rat SMG

The results from the immunohistochemical analysis of histamine and histamine-synthesising enzyme, HDC, are shown in Figure 4. The expression of HDC is preferentially observed in the ductal system in the SMG, coincidently with the presence of endogenous histamine, which was principally observed in the granular convoluted and excretory ducts. Neither ionising radiation nor histamine treatment significantly modified HDC or intracellular histamine levels.

Discussion

Radiation is a major treatment modality administered for head and neck cancer. Despite the great improvement in the technology for delivering therapeutic radiation, salivary glands are inevitably irradiated (Valdez et al. 1993, Burlage et al. 2001, 2008, Nagler 2002, Vissink et al. 2003, Dirix et al. 2006). Although salivary glands should be considered to be radioresistant because of their highly differentiated cellular state, they exhibit an exquisite sensitivity to radiation, which is characterised by a reduction in salivary flow rate, irreversible and progressive loss of glandular weight and acinar cells, and morphological changes in gland structure (Nagler 2002, Dodds et al. 2005, De la Cal et al. 2006, Dirix et al. 2006).

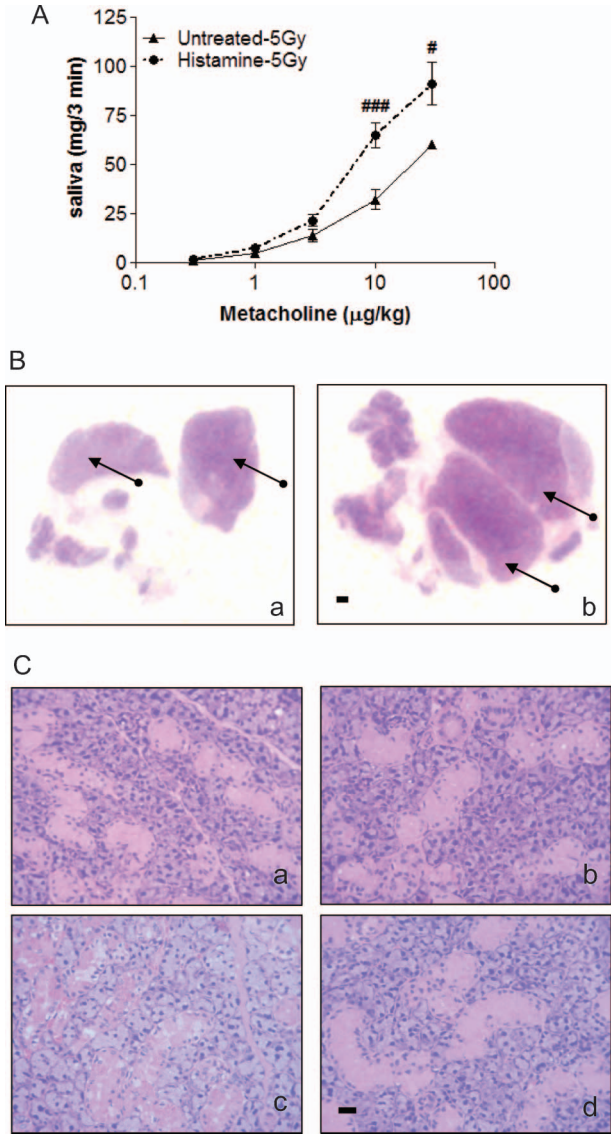

Figure 1. Histamine prevents ionising radiation damage on salivary function and morphology. (A) Mean salivary secretion in untreated and histamine treated and 5 Gy-irradiated rats. Histamine significantly preserved salivary secretion. Error bars represent the means ± SEM. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. Untreated, $^{\#\#\#}P < 0.001$, $^{\#}P < 0.05$ vs. Untreated-5 Gy. (B) Macroscopic view of hematoxylin-eosin stained histological slice showing loss of glandular surface area in irradiated SMG (a) while it was markedly conserved in histamine-treated and irradiated rats, (b) Arrows indicate SMG. Scale bar 100 μm. (C) (a) normal histological appearance of untreated SMG and (b) histamine-treated SMG. (c) SMG of irradiated rat displaying damage in the epithelial architecture of the granular convoluted ducts, and partial loss of eosinophilic secretor granular material. (d) SMG of histamine-treated and irradiated animals showing preserved structure organization of secretor granules, with homogeneity of the granule diameter and normal appearance of granular convoluted ducts. Pictures were taken at 630× magnification. Scale bar 20 μm.

The better understanding of radiation-induced damage may facilitate the development of a proper treatment or preventive strategy. For many years there has been an intense search for compounds that

Table II. Histamine conserves SMG weight in irradiated rats.

Group	SMG weight[a]
Untreated	0.36 ± 0.03
Histamine	0.33 ± 0.01
Untreated-5 Gy	0.22 ± 0.03*
Histamine-5 Gy	0.34 ± 0.01#

SMG wet weight in irradiated and non-irradiated, untreated and histamine-treated rats ($n = 6$). [a]SMG's percentage of bodyweight (SMG weights were divided by total bodyweight in grams and multiplied by 100). Data represent the means ± SEM. $^{*}P < 0.05$ vs. Untreated; $^{\#}P < 0.05$ vs. Untreated-5Gy.

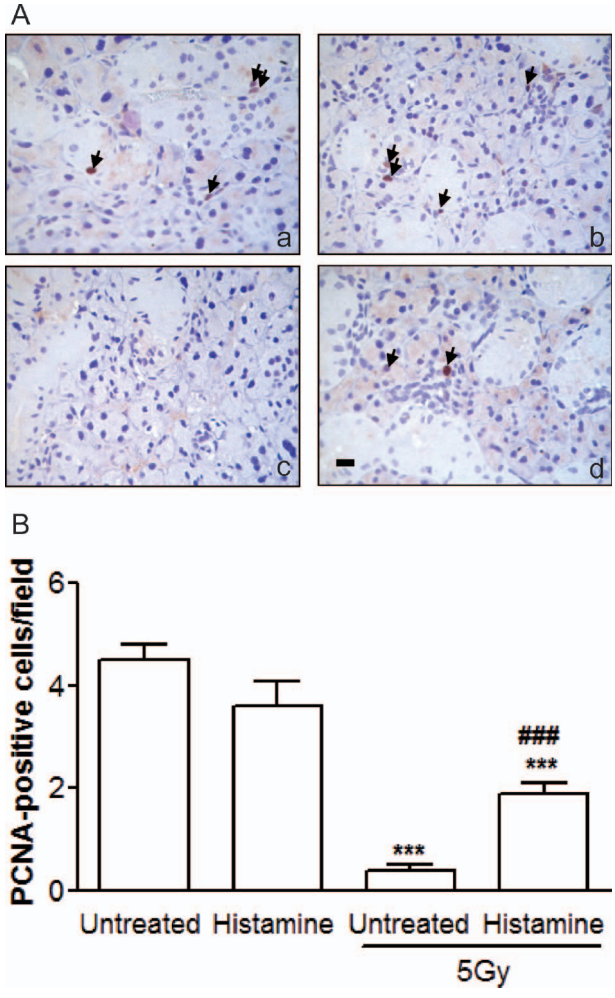

Figure 2. Histamine enhances PCNA expression in irradiated SMG. (A) Similar PCNA immunoreactivity in SMG from untreated (a) and histamine treated (b) rats. (c) Essential absence of PCNA immunoreactivity in SMG of irradiated rats. (d) Partial preservation of PCNA-positive cells in histamine treated and irradiated gland. Arrows indicate PCNA-positive cells. Pictures were taken at 630× magnification. Scale bar 20 μm. (B) Ionising radiation significantly decreased the number of PCNA-positive cells while histamine reversed in part the inhibitory effect of radiation on PCNA expression. Error bars represent the means ± SEM. $^{***}P < 0.001$ vs. Untreated, $^{\#\#\#}P < 0.001$ vs. Untreated-5 Gy.

protect normal tissues from the deleterious effects of ionising radiation. However, no ideal radio-protector has been discovered so far (Grdina et al. 2002, Weichselbaum 2005, Dziegielewski et al. 2008, Weiss and Landauer 2009).

The greatest contributor to total salivary output during the diurnal cycle is the unstimulated flow. The SMG, which produces the less serous, mucin-rich saliva, is the major source of unstimulated flow, contributing to wetting of the oral mucosa and providing lubrication and relief from desiccation. On the contrary, the parotid glands need some stimulation and its main role may be to produce copious, highly buffered, fluid to protect against extrinsic insult such as acid (Dodds et al. 2005, Burlage et al. 2008). Rat salivary glands are quite similar to human salivary glands in that salivary flow is rapidly reduced after ionising radiation (Nagler 2002).

In the present study, the potential radioprotective effect of histamine against ionising radiation-induced toxicity on rat SMG was investigated. Results demonstrate that histamine markedly prevented radiation injury on SMG ameliorating the histological and morphological alterations. Histamine treatment completely reversed the radiation-induced reduced salivation, preserving glandular mass with normal structure organisation of acini and ducts. Interestingly, the salivary secretion and SMG mass were protected to the extent that their value after irradiation was not significantly different from the value of the non-irradiated animals, therefore showing that histamine preserved the gland function. In addition, histamine was able to prevent the histological damage observed after 30 days post irradiation, reducing fibrosis and vacuolisation induced by ionising radiation while preserving the granular secretor material (data not shown), which suggests that histamine may exert a long-term protection of SMG.

Results also revealed that histamine partially prevented the radiation-induced decrease in SMG proliferation. Previously, we demonstrated that histamine prevented ionising radiation-induced damage of the small intestine and bone marrow, effects that were associated with an induction of cell proliferation (Medina et al. 2007, 2010).

A recent study showed that radiation-induced apoptosis is responsible for salivary gland dysfunction in vivo (Avila et al. 2009). Present results show

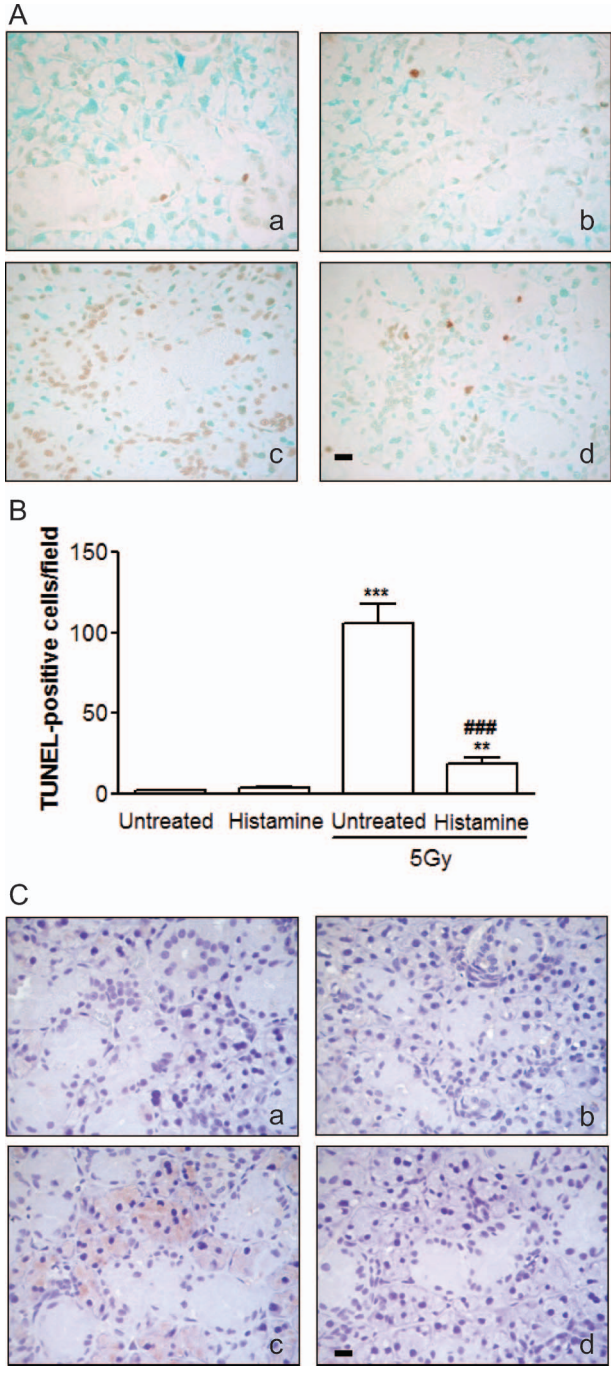

Figure 3. Histamine suppresses radiation-induced apoptosis in SMG. (A) Apoptosis was analysed by TUNEL assay. (a) Occasional TUNEL-positive cells in glandular duct cells in untreated rats. (b) Similarly, a scarce number of TUNEL-positive cells are observed in SMG of histamine-treated rat. (c) Massive presence of TUNEL-positive cells in acinar and ductal cells of the SMG of irradiated rats. (d) Drastic reduction of TUNEL-positive cells in SMG of histamine treated and irradiated rats. Pictures were taken at 630× magnification. Scale bar 20 μm. (B) Ionising radiation significantly increased the number of TUNEL-positive cells while histamine almost completely reversed the apoptotic effect of radiation. Error bars represent the means ± SEM. $^{***}P < 0.001$, $^{**}P < 0.01$ vs. Untreated, $^{###}P < 0.001$ vs. Untreated-5 Gy. (C) Immunohistochemical detection of the propapoptotic protein Bax. Minimal cytoplasmic expression of Bax in SMG from untreated (a) and histamine treated (b) rats. (c) Increased Bax protein expression especially in acini of SMG of irradiated rats. (d) Reduced immunoreactivity of Bax in histamine treated and irradiated SMG. Pictures were taken at 630 × magnification. Scale bar 20 μm.

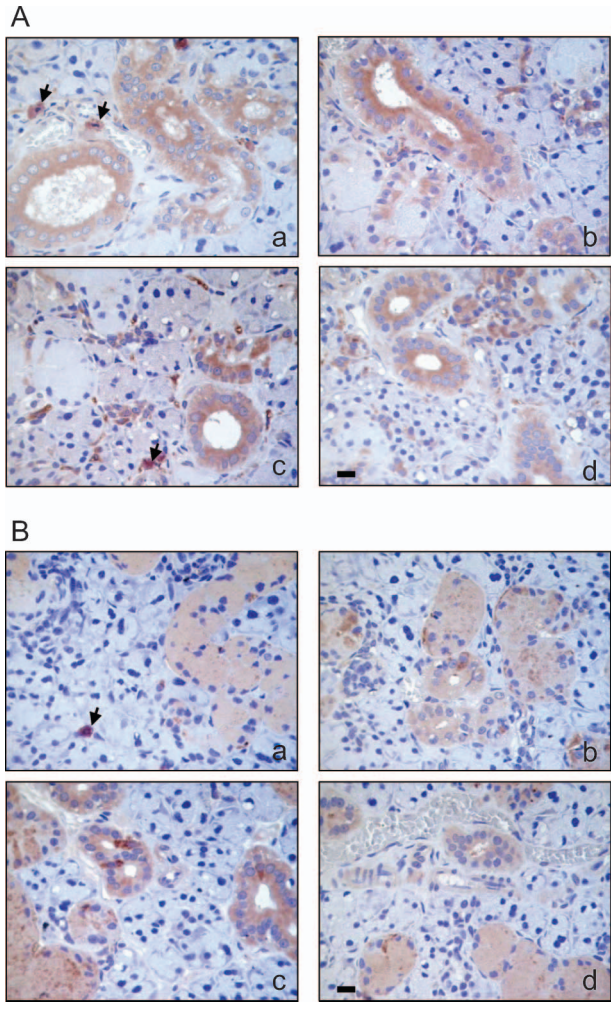

Figure 4. HDC immunoreactivity and intracellular histamine content in rat SMG. (A) High HDC expression principally in excretory ductal cells of untreated (a), histamine treated (b), irradiated (c) and irradiated and histamine treated (d) SMG. Pictures were taken at 630× magnification. Scale bar 20 μm. Arrows indicate mast cells. (B) Intense and uniform histamine intracellular content in ductal cells of untreated (a), histamine treated (b), irradiated (c) and irradiated and histamine treated (d) SMG. Pictures were taken at 630× magnification. Scale bar 20 μm. Arrows indicate mast cells.

that radiation produced a remarkable increase in the number of TUNEL-positive cells, indicating an enhancement of apoptotic cell death, and also an increase in Bax immunoreactivity in acini. Accordingly, it was reported that during atrophy of SMG Bax promotes apoptosis of acinar cells (Takahashi et al. 2008). SMG of histamine-treated rats demonstrated a considerable decrease in apoptosis in both acinar and also ductal cells that might represent the major effect of histamine on the SMG radioprotection. This outcome was associated with a reduction of Bax-induced immunoreactivity produced by ionising radiation. In agreement with this result, we previously described an anti-apoptotic action of histamine in irradiated small intestine (Medina et al. 2007).

Histamine has been reported to modulate salivary flow and has been postulated as a possible secretory signal. In addition, it was described that human SMG cells express HDC (Stanovnik and Erjavec 1983, Sim and Ang 1985, Kim et al. 2009). In this study, HDC expression and histamine content and their localisation were also evaluated. We observed that neither histamine treatment nor ionising radiation produce any changes in the immunoreactivity for histamine or HDC, preferentially detected in the ductal system. In fact, the histamine effect seemed to be mediated through histamine receptor subtypes expressed in SMG. Preliminary studies indicate that the histamine protective effect on SMG morphology was partially prevented by the combined treatment with a histamine H2 receptor antagonist (data not shown).

Although no radioprotective drug currently available has all the requisite qualities of an ideal radioprotector, sulfhydryl radioprotectors such as cysteine, N-acetylcysteine, and the phosphorothioate amifostine used to reduce xerostomia, are the best known radioprotectors today. However, their use encounters two great difficulties: their dose-limiting toxicity and the short period during which they are active, which limited their clinical administration (Grdina et al. 2002, Dziegielewski et al. 2008, Weiss and Landauer 2009). In addition, the clinical use of radioprotectors in radiation therapy continues to be plagued by issues related to possible tumour protection interfering with the irradiation anticancer effect and therefore diminution of therapeutic gain. Amifostine is the only U.S. Food and Drug Administration-approved radioprotector; however, it has many adverse effects and recent reports indicate induced elevation of superoxide dismutase 2 activity in tumours that could have an unanticipated deleterious effect on tumour responses to fractionated radiation therapy (Weiss and Landauer 2009, Grdina et al. 2009). Recently, preclinical studies demonstrated a potential usage of Tempol as a selective protector against radiation-induced salivary gland damage; however, human clinical studies are still missing (Cotrim et al. 2007).

On the other hand, histamine dihydrochloride is currently safely used in clinical trials as an adjuvant for the potential treatment of different cancers exhibiting no unexpected or irreversible side effects (Romero et al. 2009). Furthermore, in our study no local or systemic side-effects were observed upon histamine administration (data not shown).

Conclusion

Based on the presented evidence, we conclude that histamine is a potential candidate as a safe radioprotective agent that could prevent salivary gland damage during radiotherapy for head and neck

cancer, and might enhance patient quality of life by protecting normal tissue from radiation injury. However, the efficacy of histamine needs to be carefully investigated in prospective clinical trials.

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