Histamine Protects Bone Marrow against Cellullar Damage induced by Ionizing Radiation

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Abstract. After surgery, radiotherapy is arguably one of the most important treatments for cancer, especially for localized disease that has not spread. However, ionizing radiation is toxic not only to tumor cells but also to healthy tissues causing serious adverse effects to patients. We have recently reported that histamine prevents ionizing radiation-induced toxicity on mouse small intestine. The aim of the present work was to determine whether histamine is able to protect bone marrow cells against ionizing radiation damage. For that purpose 56 mice were divided into 4 groups. Histamine and Histamine-10Gy groups received a daily subcutaneous histamine injection (0.1 mg/kg) starting 20 hours before irradiation and continued till the end of experimental period; untreated group received saline. Histamine-10Gy and untreated-10Gy groups were irradiated with a single dose on whole-body using Cesium-137 source (7 Gy/min) and were sacrificed 3 days after irradiation. Bone marrow was removed, fixed and stained with hematoxylin and eosin. The number of megacariocytes per 40x field, bone marrow trophism, edema, vascular damage, and other histological characteristics of bone marrow cells were evaluated. We further determined by immunohistochemistry the expression of proliferating cell nuclear antigen (PCNA) and cells in the S phase of the cell cycle were identified by immunohistochemical detection of 5-bromo-2'-deoxyuridine (BrdU) incorporation. Results indicate that histamine treatment substantially reduced the grade of aplasia, the edema and the vascular damage induced by ionizing radiation on bone marrow. Additionally, histamine preserved medullar components increasing significantly the number of megacariocytes per field (5.4 ± 0.4 vs. 2.8 ± 0.4 in Control-10 Gy, $P<0.01$). This effect was associated with an increased proliferation rate determined by the augmented PCNA expression and BrdU incorporation of bone marrow cells. On the basis of these results, we conclude that histamine radioprotects bone marrow cells. Taking into account that we have previously determined that histamine enhances radiosensitivity of breast cancer cell lines; this might be of clinical value in patients undergoing radiotherapy.

KEYWORDS: Histamine, Radioprotectant, Bone marrow, Ionizing radiation.

1. Introduction

Ionizing radiation is one of the most widely used treatments for cancer. The ratio of tumor response to normal-tissue damage is called the therapeutic index and can be manipulated by dose fractionation or by the use of drugs that preferentially either increase the tumor response (radiosensitizers) or reduce the biological effects of radiation on normal tissues (radioprotectors) [1-3].

In clinical radiotherapy, the tolerance of normal tissues for radiation depends on the ability of clonogenic cells to maintain a sufficient number of mature cells suitably structure to preserve organ function. Casarett has suggested a classification of mammalian cell radiosensitivity based on histologic observation of early cell death and has divided cells into four categories. Group I of Casarett's classification, the most sensitive group, consist of vegetative undifferentiated intermitotic cells and includes the stem cells of the self-renewing systems such as intestinal crypt cells [1,3].

We have previously reported that histamine treatment can selectively modulate cellular damage produced by ionizing radiation increasing radiosensitivity of breast cancer cells while notably preserve intestinal crypts reducing toxicity on small intestine [4,5].

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Histamine (2-(imidazol-4-yl)ethylamine) is a biogenic amine with a broad spectrum of activities in numerous physiological and pathological situations and is synthesized by the enzyme histadine decarboxylase (HDC). Histamine plays a key role in immunity and hematopoiesis and is involved in bone marrow cell physiology, enhancing differentiation and proliferation [6,7]. Histamine exerts is actions through the activation of four histamine receptors, three of which are expressed in bone marrow (H1, H2 and H4 histamine receptors) [7,8].

The bone marrow pluripotent stem cells such as erythroblast are particularly radiosensitive and after whole body irradiation an important grade of aplasia is observed increasing the possibility of hemorrhage and/or infection occurrence that could be lethal. The survival of stem cells determines the subsequent repopulation of bone marrow after irradiation [1,3].

Therefore, the aim of the present work was to determine whether histamine could be able to preserve the histological characteristics of bone marrow in whole body irradiated-mice.

2. Materials and methods

2.1 Treatment and irradiation

Fifty six nude mice (NIH nu/nu) were purchased from the Division of Laboratory Animal Production, Faculty of Veterinary Sciences, University of La Plata, Buenos Aires and were randomly separated into 4 groups (n = 14 each). Mice were maintained in our animal health care facility at 22 to 24°C and 50% to 60% humidity on a 12 h light/dark cycle with food and water available *ad libitum*.
Histamine and Histamine-10Gy groups received a daily subcutaneous histamine injection (0.1 mg/kg) starting 20 hours before irradiation and continued till the end of experimental period and untreated groups received saline. Histamine-10Gy group and untreated-10Gy group were irradiated using Cesium-137 source (IBL 437C type H) of 189 TBq (dose rate: 7 Gy/min) with a single dose of 10 Gy on whole-body and were killed 3 days after irradiation by cervical dislocation.
Four animals of each group received an intraperitoneal injection of BrdU (100 mg/kg in saline; Sigma Chemical Co., St. Louis, MO, USA) 1 hour before sacrifice.
Animal procedures were in accordance with recommendations of the Guide for the Care and Use of Laboratory Animals of the National Research Council, USA, 1996.

2.2 Histopathological studies

Bone marrows were fixed with Bouin solution and were embedded in paraffin and cut into serial sections of 3 µm thick. Tissue morphology was examined on tissue sections after hematoxylin-eosin staining.
Parameters analyzed in the bone marrow were:
a) Trophism [9,10]:
   Normal: normal appearance of bone marrow.
   Grade I Aplasia: consist of an alteration of the relationship between adipose tissue and active marrow tissue where the later is replace by adipose tissue in a different proportion according to age.
   Grade II Aplasia: hipocellular change with a clear alteration in the relationship adipose tissue/functional bone marrow tissue.
   Grade III Aplasia: adipose marrow, only lipid vacuoles and stromal cells are observed.
b) Number of megacariocytes per 40X field.
c) Type of medullar elements.
d) Stromal characteristics.

2.3 Immunohistochemical staining

After deparaffinization, specimens were placed in citrate buffer (10mM, pH 6.0) and heated in a microwave oven twice for 2 minutes for antigen retrieval. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol. After blocking, tissues were incubated with primary

mouse anti PCNA (1:40, DakoCytomation, Glostrup, Denmark), and mouse anti BrdU (1:150, Sigma Chemical Co., St. Louis, MO, USA) antibodies overnight in a humidified chamber at 4ºC. Immunoreactivity was detected by using horseradish peroxidase-conjugated anti-mouse antibody, and visualized by diamino-benzidine staining (Sigma Chemical Co., St. Louis, MO, USA). To evaluate subcellular localization of these proteins, nuclei were stained with hematoxylin. Light microscopy was performed on an Axiolab Karl Zeiss microscope (Göttingen, Germany). All photographs were taken at 630X magnification using a Canon PowerShot G5 camera (Tokyo, Japan). The immunostaining assessment was performed blind to the data in all tests by consensus agreement of 2 observers (V.M. and M.C.). To control the signal specificity, serial sections were made from five selected positive cases which were subjected to the same staining procedure, with phosphate buffered saline to replace the first antibody. This control staining did not give rise to a signal.

3. Results and Discussion

3.1 Histamine protects bone marrow cells against ionizing radiation damage

Results indicate that histamine treatment remarkably reduced the grade of medullar aplasia, edema, and vascular damage produced by ionizing radiation on bone marrow. In addition, histamine notably preserved medullar components, increasing the number of megacariocytes per field in irradiated animals (5.4 ± 0.4 vs. 2.8 ± 0.4 in untreated group, $P<0.01$) (Table 1, Figure 1).
In this line, we have previously described that histamine also protects small intestine against ionizing radiation toxicity reducing mucosal atrophy, edema, vascular damage and preserving villi, and crypts [5].

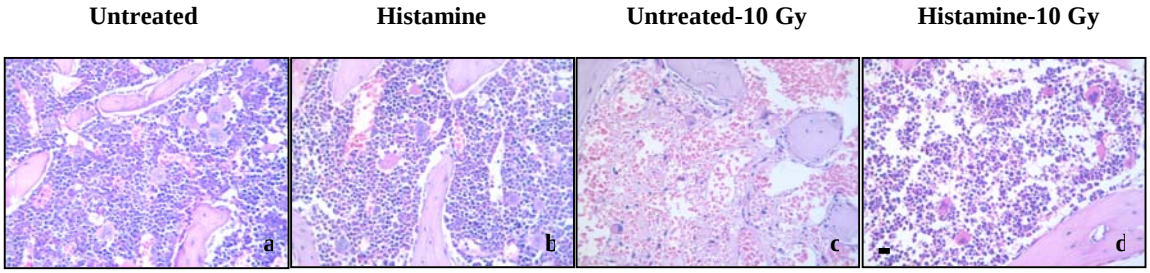
Table 1. Histopathological characteristics of bone marrow

Group	Trophism	Megacariocytes per 40x field*	Medullar elements	Stromal characteristics
Untreated[a]	Normal	7.8 ± 0.9	Erythroid cells Lymphoid cells Myeloid cells	Without alterations
Histamine[b]	Normal	6.8 ± 0.6	Erythroid cells Lymphoid cells Myeloid cells	Without alterations
Untreated-10 Gy[c]	Grade III Aplasia	2.8 ± 0.4 [e,f]	Very scarce lymphoid cells	Congestive vessels, massive hemorrhage
Histamine-10 Gy[d]	Grade I Aplasia	5.4 ± 0.4 [g]	Erythroid cells Lymphoid cells Myeloid cells	Minor congestion, slight edema

*Mean value of the experimental group calculated from the average number of megacariocytes of 10 fields examined.
[(a)]Representative of bone marrows from at least eight saline-treated mice.
[(b)]Representative of bone marrows from at least eight 0.1 mg/kg.day histamine-treated mice.
[(c)]Representative of bone marrows from at least eight saline-treated and 10 Gy-irradiated mice.
[(d)]Representative of bone marrows from at least eight histamine-treated and 10 Gy-irradiated mice.
[(e)]$P<0.01$ vs. untreated group, [(f)]$P<0.01$ vs. histamine-10 Gy group, and [(g)]$P<0.05$ vs. untreated group. (ANOVA and Newman-Keuls test).

Moreover, it was reported that intracellular HDC and histamine content in regenerating bone marrow populations in HDC+/+ mice increased in all days after total-body irradiation and a faster bone marrow repopulation was observed in wild type in comparison with HDC-/- knockout mice (completely depleted of endogenous histamine). Additionally, histamine H1 and H2 receptor expression increased while histamine H4 receptor expression was downregulated during restoration supporting the regulatory role of histamine in bone marrow regeneration [7].

Figure 1. Effect of histamine and ionizing radiation on mouse bone marrow histopathology. (a) Microphotography of bone marrow from untreated mice showing presence of medullar populations in an accurate proportion. (b) Bone marrow from histamine-treated mice displaying the same characteristics of untreated group. (c) Bone marrow from untreated and 10 Gy irradiated mice exhibiting total deprivation of all progenies that were replaced by vascular congestion and interstitial hemorrhage. Only a few megacariocytes and isolated lymphoid cells are observed. (d) Bone marrow from 10 Gy irradiated and histamine-treated mice showing marked conservation of the medullar progenies, slight interstitial edema and scarce vascular congestion. Hematoxylin-eosin staining. Pictures were taken at 400x magnification. Scale bar = 20 μm.

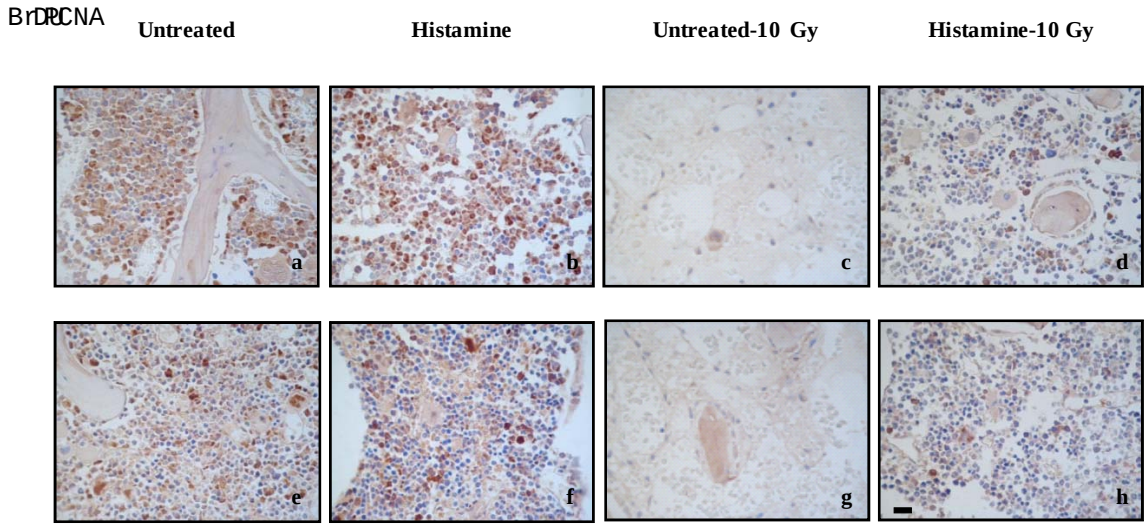


3.2 Histamine increases PCNA expression and BrdU-positive cells in irradiated bone marrow cells

In order to determine whether histamine radioprotective effect was associated with an increase in bone marrow cell proliferation, we evaluated in bone marrow cells the expression of PCNA, a well known indicator of active proliferation [11] and the BrdU incorporation, a thymidine analog.
Bone marrow from untreated mice exhibited high PCNA expression and BrdU incorporation especially in undifferentiated, immature myeloid cells; and no significant difference was observed after histamine treatment in non-irradiated mice. Complete loss of PCNA expression and a clear decrease in BrdU-positive cells was observed after irradiation indicating the absence of proliferation. Conversely, histamine treatment notably increased the level of expression of PCNA and the BrdU incorporation in irradiated-mice (Figure 2).

Present results indicate that the radioprotection exerted by histamine on bone marrow cells is mediated at least in part by an increase in the rate of proliferation as evidenced by the enhanced PCNA protein expression and BrdU incorporation. In agreement, we have recently reported that histamine prevents radiation-induced toxicity in small intestine by increasing proliferation of damaged intestinal mucosa and also suppressing apoptosis [5].

Figure 2. Effect of histamine and ionizing radiation on bone marrow cell proliferation. Representative bone marrow sections from untreated (a,e), histamine-treated (b,f), untreated and 10 Gy irradiated (c,g) and 10 Gy irradiated and histamine-treated mice (d,h). a,b,c,d illustrate PCNA immunoreactivity; e,f,g,h show BrdU immunoreactivity.



4. Conclusions

Radiation therapy is a well recognized treatment modality for cancer. During radiotherapy for intraabdominal and pelvic cancers, radiation, seriously affect radiosensitive tissues such as small intestine and bone marrow [1,12].

Present results demonstrated that histamine significantly reduced the toxicity exerted by ionizing radiation on bone marrow cells and also on small intestine, as we have previously described [5]. Considering that histamine is being safely used in clinical trials as an adjuvant for the potential treatment of different cancers [13,14] and that is as well capable of radiosensitizing breast cancer cells [4] our results suggest that histamine might increase the therapeutic index of radiation being of clinical value in patients undergoing radiotherapy.

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