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Radioprotective Potential of a Novel Therapeutic Formulation of Oligoelements Se, Zn, Mn Plus Lachesis Muta Venom

Ernesto CRESCENTI[1], Máximo CROCI[1], Vanina MEDINA[2], Lorena SAMBUCO[1], Rosa BERGOC[2] and Elena RIVERA[2*]

Ionizing radiation/Radioprotection/Small intestine/Bone marrow/Oligoelements.

In this study we evaluated *in vivo* the tolerance induced by the combination of Se, Zn and Mn (4 μg/ml each) plus Lachesis muta venom (4 ng/ml) (O-LM) to high doses of ionizing radiation. The protective effect of O-LM was investigated on the small-intestine and bone marrow of mice irradiated with a single whole-body dose of 10 Gy employing a ^{137}Cs source. Mice were sacrificed 3 days after irradiation. Mice receiving a subcutaneous daily O-LM injection starting 30 days before irradiation, showed a higher number of crypts, enhanced villous conservation and lack of edema or vascular damage in comparison to the untreated and irradiated group. In addition, O-LM treatment decreased vascular damage and the grade of aplasia preserving medullar progenies induced by ionizing radiation on mouse bone marrow. The protective effect of O-LM against radiation injury to the small intestine was associated with an increase in proliferation and a reduction of apoptosis in intestinal crypts and furthermore, to an enhanced intestinal immunoreactivity of MnSOD, and CuZnSOD, and also catalase. Based on the present results and taking into account that O-LM is being safely administered in phase I clinical trial as an immunomodulator, we suggest that O-LM could be an attractive candidate as a safe radioprotective agent for patients undergoing radiotherapy.

INTRODUCTION

Ionizing radiation, alone or in combination with other therapies, is one of the most commonly used treatments for cancer. Although effective, adverse effects due to radiotherapy are unavoidable, even with localized delivery techniques.[1–3] Cell death after irradiation occurs mostly as cells attempt to divide. Therefore, the early or acute effects of irradiation result from the death of a large number of cells in tissues with a rapid turnover rate. These include effects in the epidermal layer or skin, gastrointestinal epithelium, and hematopoietic system, in which the response is determined by a hierarchical cell lineage composed of stem cells and their differentiating offspring.[1)] Injury resulting from the irradiation of biological tissue is a consequence of the transfer of radiation energy to critical macromolecules. The initial chemical injury can occur directly by the absorption of radiation or indirectly through the action of free radicals. On the tissue, since water is so abundant, the contribution of free radicals to radiation damage is quite important.[4)] The effect of low linear energy transfer radiations are exerted mainly by generation of reactive oxygen species (ROS).[4–6)] Antioxidant enzymes act as the first-line defense against ROS. The most important enzymes include superoxide dismutase (SOD) that catalyzes the dismutation of superoxide into hydrogen peroxide, and catalase and glutathione peroxidase (GPx) that metabolize hydrogen peroxide.[5–11)]

The radioprotective agents are chemicals that reduce the biological effects of radiation by the scavenging of free radicals or repairing of radiation injury.[12–14)] On the other hand, radiosensitizers are chemical agents that have the capacity to increase the lethal effects of radiation.[15)]

The development of drugs that radiosensitize the malignant cell and radioprotect the normal tissues, is yet a challenge for oncologists and radiobiologists.

We have previously demonstrated the protective effect of a novel combination composed of three oligoelements [Selenium (Se), Zinc (Zn), Manganese (Mn)] and Lachesis muta venom (O-LM) against carcinogenic drugs and high doses of chemotherapy.[16)] In addition, O-LM inhibits malignant cell proliferation and increases survival in rodent tumor

*Corresponding author: Phone: +54-11-4964-8277,
Fax: +54-11-4962-8202,
E-mail: erivera@ffyb.uba.ar
[1]Institute of Immunooncology, Avenida Córdoba 3200, 1187 Buenos Aires, Argentina; [2]Radioisotopes Laboratory, School of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956, 1113 Buenos Aires, Argentina.
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models.[17]

The aim of the present study was to evaluate *in vivo* whether O-LM could enhance the tolerance to high doses of ionizing radiation in mice and to investigate the possible mechanisms of action. For that purpose we investigated the histological characteristics of bone marrow and small intestine in whole-body irradiated mice. Immunohistochemical studies were undertaken to define the cell type-specific detection of proliferation and apoptosis markers, and antioxidant enzymes MnSOD, Cu-ZnSOD, GPx, and catalase in small intestine.

MATERIALS AND METHODS

Irradiation source

A ^{137}Cs source of 189 TBq (7 Gy/min) calibrated by Argentine National Commission of Atomic Energy with a TLD 700 dosimeter and validated by Argentine Nuclear Regulatory Authority was employed.

O-LM treatment

In all studies the treatment employed (O-LM) was a solution composed of a combination of the oligoelements Zn, Se, and Mn (4 μg/ml each) (Merck, Argentina) plus Lachesis muta (4 ng/ml) (kindly provided by SICAE S.R.L., Santa Cruz de la Sierra, Bolivia).

Animals

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 groups. All animals were maintained in a health care facility at 22°C and 50% to 60% humidity on a 12 h light/dark cycle with food and water available *ad libitum*. To evaluate the protective capability of O-LM, nude mice were used as a suitable model to further study O-LM effect in these mice bearing xenografted human tumors. Forty animals were randomly separated into four groups: group 1, non-irradiated mice receiving daily sc saline; group 2, non-irradiated mice receiving 0.05 ml daily sc O-LM treatment starting 30 days before irradiation; group 3, 10 Gy whole-body irradiated mice receiving daily sc saline; group 4, 10 Gy whole-body irradiated mice receiving 0.05 ml daily sc O-LM treatment starting 30 days before irradiation. All animals were sacrificed 3 days after irradiation and the histopathological changes were determined on small intestines and bone marrows.

All procedures were carried out in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Research Council, USA, 1996.

Histopathological studies

The small intestine was opened along the mesenteric border and samples were pinned flat on cork board, mucosal side up, to allow immediate fixation with 10% neutral buffered formalin and comparable samples. Bone marrows were fixed with Bouin's solution. Tissue samples 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 proximal part of the small intestine were: a) mucosal trophism (Normal: normal appearance of mucosal villi and crypts; Mild atrophy: slight reduction in the number of villi and crypts only confirmable through crypt comparative counting but conserving histological normal appearance; Marked atrophy: severe reduction in the number of villi and crypts with morphological changes in shape and structure); b) number of intestinal crypts per circumference; c) mucosal ulceration; d) nuclear and cytoplasmic changes; e) villous edema; f) vascular damage.

Parameters analyzed in the bone marrow were:

a) Trophism:[18]

Normal: normal appearance of bone marrow.

Grade I Aplasia: consists of an alteration of the relationship between adipose tissue and active marrow tissue where the latter is replaced 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) Characteristics of medullar elements.

c) Stromal characteristics.

Immunohistochemical staining

After deparaffinization, specimens were placed in citrate buffer (10 mM, pH 6.0) and heated in a microwave oven twice for 2 minutes at boiling temperature for antigen retrieval. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in distillated water. After blocking, tissues were incubated with primary mouse anti catalase, (1:100, Sigma Chemical Co., St. Louis, MO, USA), sheep anti MnSOD, sheep anti Cu-ZnSOD, rabbit anti Glutathione peroxidase (1:100, Calbiochem, San Diego, CA, USA), mouse anti PCNA (1:100, DakoCytomation, Glostrup, Denmark) antibodies overnight in a humidified chamber at 4°C. Immunoreactivity was detected by using horseradish peroxidase-conjugated anti-mouse, anti-rabbit, or anti-sheep IgG, as appropriate, and developed by diamino-benzidine staining (Sigma Chemical Co., St. Louis, MO, USA). Slices were counterstained with hematoxylin and light microscopy was performed on an Axiolab Karl Zeiss microscope (Göttingen, Germany). All photographs were taken using a Canon PowerShot G5 camera (Tokyo, Japan). The immunostaining assessment was performed blind to the data in all tests. 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 IgG or phosphate buffered saline (PBS) to replace the first antibody. This control staining did not give rise to a signal. For MnSOD, CuZnSOD, Catalase, and Glutathione peroxidase a score based on the intensity of positive homogeneous staining was assigned as: - (undetectable), + (very low), ++ (low), +++ (medium), ++++ (high), +++++ (very high). For PCNA a percentage score based on the number of stained cells was assigned as: - (undetectable), + (1–20%), ++ (21–40%), +++ (41–60%), ++++ (61–80%) and +++++ (81–100%). These scoring systems were published elsewhere.[19,20] Determinations were made in cells of crypts and villi and at least 10 fields were examined.

Determination of apoptosis

Apoptotic cells were determined by terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate biotin nick end labeling (TUNEL) assay using ApoptagTM plus peroxidase in situ apoptosis Detection Kit (CHEMICON International, Temecula, CA, USA) according to the manufacturer's instructions. Tissues were visualized using Axiolab Karl Zeiss microscope (Göttingen, Germany). All photographs were taken using a Canon PowerShot G5 camera (Tokyo, Japan). Results were expressed as the number of TUNEL-positive cells per crypt. Determinations were made in cells of crypts and at least 10 fields were examined.

Cell culture

The human cell lines used were: PANC-1 (pancreatic carcinoma), WM35 (melanoma), MDA-MB-231 (estrogen receptor α negative breast carcinoma), and MCF-7 (estrogen receptor α positive breast carcinoma). Cells were obtained from the American Type Tissue Culture Collection (VA, USA) and were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 0.3 g/l glutamine, and 0.04 g/l gentamicin (Gibco BRL, NY, USA). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Clonogenic assay

Cells were seeded in 6-well plates ($1.5 – 2.0 \times 10^3$ cells/well) and incubated in the presence of O-LM treatment (20 µl of O-LM/ml of culture medium) for 8 days. Cells were then fixed with 10% formaldehyde in PBS, stained with 1% toluidine blue in 70% ethanol and the clonogenic proliferation was evaluated by counting the colonies containing 50 cells or more and was expressed as percentage values of the untreated wells.

Radiation dose-response curves

Cells were seeded in 6-well plates ($1.5 – 2 \times 10^3$ cells/well) and were treated with O-LM (20 µl of O-LM/ml of culture medium) or were left untreated. After 24 hours cells were irradiated with doses ranging from 0 to 10 Gray (Gy). Eight days post irradiation clonogenic proliferation was determined. Surviving fraction (SF) was calculated by dividing the mean colony count at each radiation dose by the mean value of non-irradiated cells from each group. Curves were adjusted to the linear-quadratic model according to the equation $SF = \exp - [\alpha D + \beta D2]$ employing the GraphPad Prism Version 5.00 software (CA, USA). The radiobiological parameter calculated from the surviving curve was SF 2Gy: fraction of surviving cells after exposure to the therapeutic dose of 2 Gy.

Statistical analysis

All statistical analyses were performed with GraphPad Prism Version 5.00 software (San Diego, CA, USA) as it is describe in each case. P values < 0.05 were considered significant.

RESULTS

O-LM preserves histopathological characteristics of small intestine and bone marrow from ionizing radiation damage

Results demonstrate that O-LM treatment did not produce histological modifications in non-irradiated mice. Small intestine of untreated and 10 Gy irradiated mice showed severe degenerative changes characterized by decreased villous height, reduction of crypt number, villous edema, ulceration, vascular damage, anisokaryosis and cytoplasmic alterations. In comparison, irradiated mice receiving daily O-LM treatment significantly preserved villi, crypts and showed less nuclear and cytoplasmic alterations of small intestine cells increasing the number of crypts per circumference (87 ± 5 vs. 45 ± 3 in untreated and irradiated mice) and reduced mucosal atrophy, vascular damage, ulceration and edema after ionizing radiation exposure. In addition, O-LM decreased vascular damage and the grade of aplasia induced by ionizing radiation preserving medullar progenies on mouse bone marrow (Fig. 1, Table 1).

O-LM enhances proliferation and diminishes apoptosis in irradiated small intestine

In order to determine whether O-LM protective effect could be related to an increased proliferation rate, we evaluated the expression of the proliferation marker PCNA.[21] Results show a marked expression of PCNA in intestinal crypts and O-LM treatment did not affect significantly its expression. On the other hand, ionizing radiation completely inhibited the expression of PCNA in intestinal crypts while O-LM treatment remarkably reversed ionizing radiation effect (Fig. 2, Table 2).

In addition, apoptosis was evaluated by the TUNEL assay. In non-irradiated mice, the number of apoptotic cells in crypts in untreated or O-LM treated mice was low. Conversely, ionizing radiation significantly increased the number

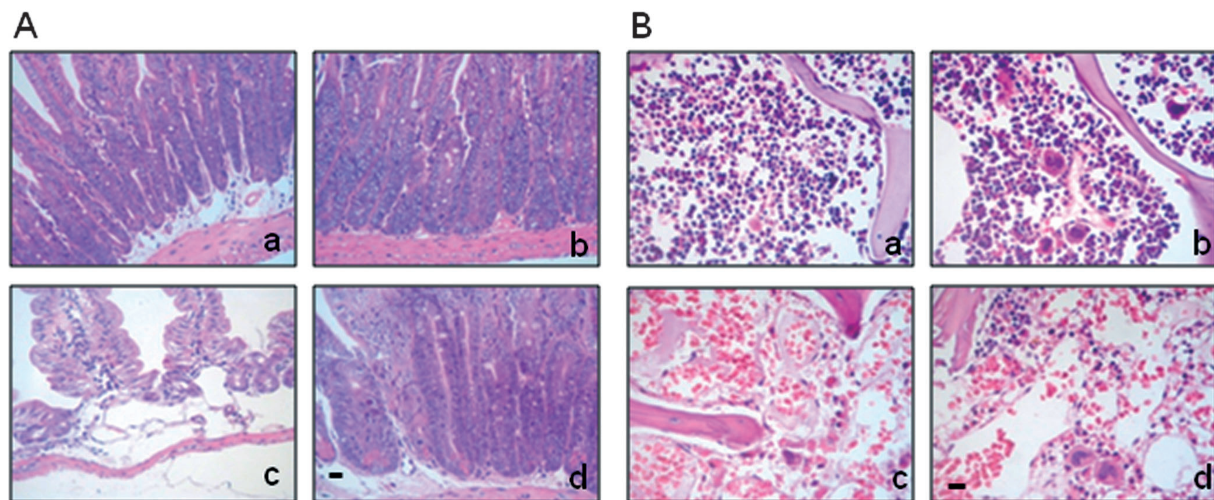

Fig. 1. Histological characteristics of mouse small intestine and bone marrow. **(A)** a) Normal appearance of intestinal mucosa of untreated and non-irradiated mice. b) Normal morphological characteristics of intestinal tissue in O-LM treated and non-irradiated mice. c) Markedly altered intestinal mucosa of 10 Gy irradiated mice, exhibiting reduced number of crypts, disorganization of the remaining villi, edema and loss of entire sectors of columnar epithelium. d) 10 Gy irradiated O-LM pretreated mice showing conservation of crypts and villi. Only slight edema and scarce epithelium loss can be observed. **(B)** a) Normal bone marrow of a non-irradiated mice b) Bone marrow of O-LM-treated mice showing normal characteristics similar to untreated bone marrow c) Grade III (total) medullar aplasia in a 10 Gy irradiated mice. d) Bone marrow of a 10 Gy irradiated O-LM pretreated mice showing grade II aplasia with partial conservation of medullar cell lines (granulocytic, megakaryocytic and erythroblastic cell lines) and also partial hemorrhagic replacement of the lost elements. Hematoxylin-eosin staining. Pictures were taken at 400x and 630x magnification. Scale bar = 20 µm.

Table 1. Histological characteristics of mouse small intestine and bone marrow

Group	Mucosal Trophism	N^0 of crypts/ intestinal circumference*	Bone marrow Trophism
Untreated[a]	Normal	110 ± 6	Normal
O-LM[b]	Normal	135 ± 7	Normal
Untreated-10 Gy[c]	Marked atrophy	45 ± 3[e,f]	Grade III Aplasia
O-LM-10 Gy[d]	Slight atrophy	87 ± 5	Grade II Aplasia

*Mean value of the experimental group calculated from the average number of crypts of 10 specimens examined.
[a]Representative of small intestines from at least six saline-treated mice.
[b]Representative of small intestines from at least six daily O-LM-treated mice.
[c]Representative of small intestines from at least six saline-treated and 10 Gy-irradiated mice.
[d]Representative of small intestines from at least six daily O-LM-treated and 10 Gy-irradiated mice.
[e]$P < 0.01$ and [f]$P < 0.01$ compared with the untreated and the O-LM-10 Gy groups, respectively. (ANOVA and Newman-Keuls Multiple Comparison Test).

of apoptotic cells per crypt, effect which was totally prevented by O-LM treatment (Fig. 2, Table 3).

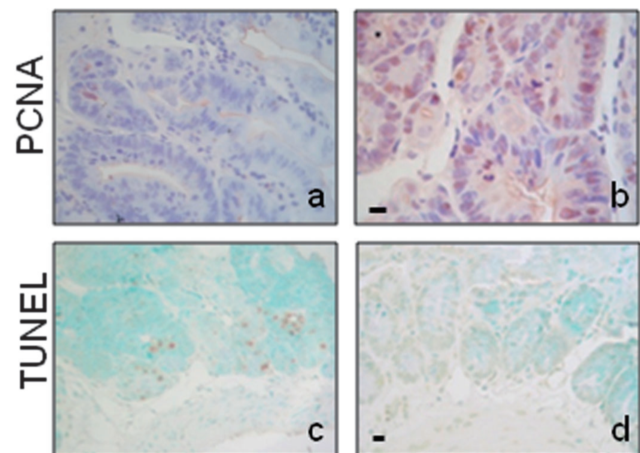

Fig. 2. Modulation of cell proliferation and apoptosis in irradiated small intestine by O-LM treatment. a) Representative intestinal mucosal sections from untreated and 10 Gy irradiated mice exhibiting absence of PCNA expression. b) 10 Gy irradiated and O-LM treated mice showing abundant PCNA positively stained cells in intestinal crypts. c) Untreated 10 Gy irradiated mice displaying numerous TUNEL positive cells in crypts. d) Absence of TUNEL positive cells in 10 Gy irradiated and O-LM treated mice. Pictures were taken at 400x and 630x magnification. Scale bar = 20 µm.

O-LM increases the expression of antioxidant enzymes in small intestine of irradiated mice

Reactive oxygen species are highly reactive and can exert

Table 2. Immunohistochemical determination of PCNA and antioxidant enzymes in small intestine. See key for groups in Table 1.

Group	PCNA[e]	Catalase[f]	GPx[f]	CuZnSOD[f]	MnSOD[f]
Untreated[a]	+++ In crypts	+ In villi	++ In villi and crypts	+++ In villi + In crypts	+++ In villi
O-LM[b]	+++ In crypts	++ In villi	++ In villi and crypts	+++ In villi + In crypts	+++ In villi and crypts
Untreated-10 Gy[c]	–	+ In villi	–	++ In villi + In crypts	++ In villi
O-LM-10 Gy[d]	++/+++ In crypts	+++ In villi	–	+++ In villi ++ In crypts	+++ In villi and crypts

[e]Percent Positivity: – (undetectable), + (1–20%), ++ (21–40%), +++ (41–60%), ++++ (61–80%), and +++++ (81–100%).
[f]Staining Intensity: – undetectable, + very low, ++ low, +++ moderate, ++++ high, +++++ very high.

deleterious effects on cell function and viability, depending on cellular antioxidant defenses and capability to repair oxidative damage.[22] To assess whether the protective effect of O-LM could be associated with an increase in the antioxidant capacity, we studied the expression of the antioxidant enzymes responsible of reactive oxygen species metabolism. Results show that O-LM treatment in non-irradiated mice only produced a slight increase in catalase immunoreactivity. Ionizing radiation decreased the expression of GPx, CuZnSOD and MnSOD (Table 2) while O-LM treatment reversed the ionizing radiation effect on MnSOD and CuZnSOD expression, and also increased the immunoreactivity of the latter in crypts and remarkably enhanced that of catalase (Table 2, Fig. 3).

O-LM inhibits proliferation of cancer cell lines

In order to further investigate whether O-LM could behave as a valid radioprotector reducing the detrimental effects of radiation on normal tissue while maintaining tumor sensitivity, we evaluated its effect on tumor cell proliferation. As we have previously reported, O-LM significantly reduced proliferation of melanoma and pancreatic and breast cancer cells while it did not affect cell growth of a non-tumorigenic cell line.[16,17] Table 4 depicts the percentage of proliferation of cancer cell lines treated with O-LM. In addition, we studied the effect of O-LM on the SF 2Gy, a

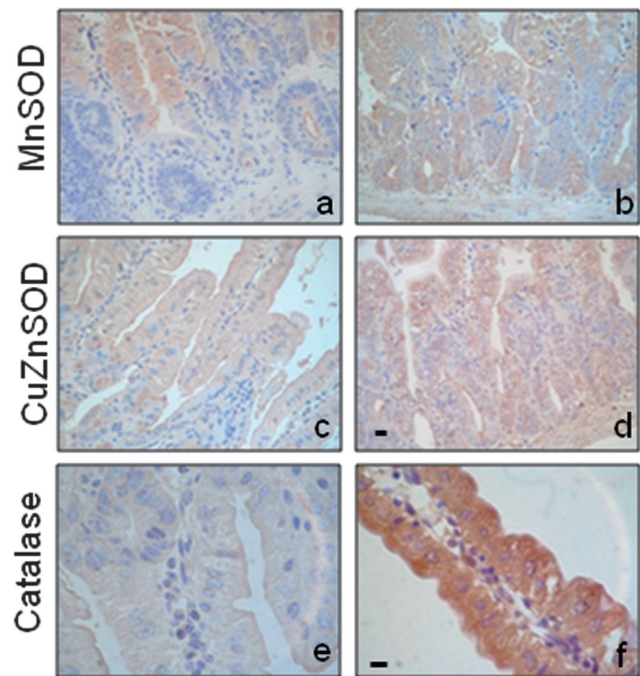

Fig. 3. Effect of ionizing radiation on antioxidant enzymes immunoreactivity of mouse small intestine, in O-LM treated and untreated mice. Representative intestinal mucosal sections from untreated and 10 Gy irradiated (a,c,e) and 10 Gy irradiated and O-LM treated mice (b,d,f). a,b illustrate MnSOD immunoreactivity; c,d display CuZnSOD immunoreactivity; e,f show catalase immunoreactivity. Pictures were taken at 400x and 630x magnification. Scale bar = 20 μm.

Table 3. Number of apoptotic cells in small intestinal crypts. See key for groups in Table 1.

Untreated[a]	O-LM[b]	Untreated-10 Gy[c]	O-LM-10 Gy[d]
0.36 ± 0.20	0.33 ± 0.21	2.30 ± 0.49*	0.33 ± 0.16[#]

Apoptotic cells were determined using TUNEL assay. The number of TUNEL positive cells per crypt are expressed as mean ± SEM. *P < 001 compared with the untreated group; [#]P < 0.01 compared with the untreated and 10 Gy irradiated group. (ANOVA and Newman-Keuls Multiple Comparison Test).

Table 4. Effect of O-LM on the clonogenic proliferation of human cell lines.

Cell line	Proliferation (% of Control)
WM35	45 ± 9*
PANC-1	34 ± 8*
MCF-7	60 ± 8*
MDA-MB-231	55 ± 7*

Human cells were cultured in presence or absence of O-LM and proliferation was determined by the clonogenic assay. Values are means and SEM from three independent experiments. *P < 0.01 versus Control (ANOVA and Newman-Keuls Multiple Comparison Test).

Table 5. Effect of O-LM on the SF 2Gy of human breast cell lines.

Cell line	SF 2Gy	SF 2Gy (O-LM)
MCF-7	0.23 ± 0.06	0.16 ± 0.02
MDA-MB-231	0.25 ± 0.06	0.14 ± 0.03*

Human breast cells were cultured in presence or absence of O-LM and clonogenic survival was determined after single radiation exposures between 0 and 10 Gy. Values are means and SEM from three independent experiments. *$P < 0.05$ versus Control (ANOVA and Newman-Keuls Multiple Comparison Test).

radiobiological parameter that correlates best with clinical responsiveness,[23] of breast cell lines. Results show that O-LM significantly reduced the SF 2Gy in MDA-MB-231 breast cancer cells. Although not significant, we observed a decrease of this radiobiological parameter in MCF-7 breast cancer cells (Table 5).

DISCUSSION

Most of the side effects of radiation on normal tissues result from the depletion of a cell population by cell killing and are inevitable, even with localized radiotherapy.[3] During the radiotherapy for many intraabdominal and pelvic cancers ionizing radiation affects principally the most radiosensitive organs which are the small intestine and bone marrow.[3,24,25] Ionizing radiation causes mucosal damage in the gastrointestinal epithelium that comprises destruction of crypt cells, decrease in villous height and number, ulceration and necrosis.[25–28] Therefore, protection of the crypts that house multipotent stem cells is essential for long-term maintenance of the intestinal epithelium.[29,30] In addition, 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]

Despite many years of research, no appropriate radioprotective chemical agents have yet been introduced for routine clinical use in the treatment of cancer due to their systemic toxicity.[12,13,31]

In the present study, we examined *in vivo* the radioprotective effect of O-LM on irradiation induced bone marrow and small intestine damage.

Our results demonstrated that O-LM treatment effectively mitigated the ionizing radiation induced toxicity on small intestine and bone marrow. O-LM preserved intestinal crypts and villi, increasing the trophism of the enterocytes and preventing the histological and vascular damage. In addition, O-LM reduced the grade of bone marrow aplasia produced by whole-body irradiation increasing the medullar components. In agreement with this, we have previously reported that O-LM treatment enhanced the tolerance of normal tissues to high cytostatic doses *in vivo* and *in vitro*.[16] Furthermore, we determined that O-LM protected BALB/c mice from irradiation by recovering the immune function, improving T lymphocyte activity and modulating the production of key cytokines as IFNγ and TNFα (manuscript in preparation).

The protective effect of O-LM against radiation injury to the small intestine was associated with an increase in proliferation as evidenced by the enhanced PCNA protein expression in crypts. In rapidly proliferating tissues, such as the small intestine epithelium, the stringent control of cell proliferation and cell death by apoptosis is central to the maintenance of tissue homeostasis.[29,30] It is well known that radiation produced small intestine apoptosis.[1,3] In our study we clearly show that the radiation-induced apoptosis was also diminished by O-LM administration.

Radiation is a recognized producer of ROS originating a pro-oxidant state which contributes to cell radiation injury and can activate apoptosis.[32,33] The mechanisms involved in the control of the initiation and propagation of free-radical chain reactions are compartmentation, antioxidant defenses such as chain-breaking antioxidant compounds capable of forming stable free radicals (e.g. ascorbate, alpha-tocopherol) and the enzyme systems that diminish the intracellular concentration of the ROS.[34] Thus, the net intracellular concentration of ROS is the result of their production and the ability of antioxidants to remove them. The antioxidant enzymes superoxide dismutases (both copper zinc, CuZn-SOD and manganese containing, MnSOD), catalase and GPx (containing selenocysteine at the catalytic center) are key intracellular antioxidants in the metabolism of ROS.[7–11] Therefore and considering that the O-LM combination contains the cofactors of these enzymes we determined the expression of these antioxidant enzymes in the small intestine.

Present results demonstrated that ionizing radiation reduced the expression of CuZnSOD, MnSOD and GPx in untreated animals. This is in agreement with previous reports that show that whole-body irradiation of mice caused a dose-dependent depletion for these enzymes in both liver as well as small intestine.[6] Conversely, an enhanced immunoreactivity of MnSOD, and CuZnSOD, and also catalase was observed in the irradiated and O-LM treated mice compared to the untreated ones. One of the important radiation-induced free-radical species is the hydroxyl radical which indiscriminately attacks neighboring molecules often at near diffusion-controlled rates. Hydroxyl radicals are generated by ionizing radiation either directly by the dissociation/ionization of water, or indirectly by the formation of secondary partially ROS. This type of free radical can be formed from superoxide anion and hydrogen peroxide via the Harber-Weiss reaction. The interaction of copper or iron and hydro-

gen peroxide also produce hydroxyl radical as first observed by Fenton. Radiation injury is therefore influenced by the cellular antioxidant status and the amount and availability of activating mechanisms.[7,8,34] Our results suggest that O-LM modulated oxidant/antioxidant balance toward a more reduced state could contribute to a less radiation-induced damage. In this light, previous studies described a higher SOD and catalase activities in radiation-resistant than in radiation-sensitive mice suggesting a role for these antioxidant enzymes in the process of radiation sensitivity.[35] In addition, it was suggested that MnSOD may play a central role in protecting cells against ROS injury during ionizing radiation exposure among MnSOD, CuZnSOD, and GPx.[5]

The clinical use of radioprotectors in radiation therapy continues to be plagued by issues relating the possible tumor protection and diminution of therapeutic gain.[12] Therefore, we further investigated the effect of O-LM on the radiobiological response of cancer cells and we showed that O-LM slightly decreased the SF 2Gy of MCF-7 breast cancer cells while significantly radiosensitized MDA-MB-231 breast cancer cells.

Nowadays O-LM is being administered in a completely safe way to patients in phase I clinical trial as an immunomodulator (Reference: National Administration of Food, Drugs and Medical Technology of Argentina–ANMAT- 1-47-6840-08-4). Previously, the preclinical phase was successfully completed demonstrating a total absence of toxicological or undesirable side effects in two rodent and one non-rodent species, both in acute and in chronic studies. On the other hand, O-LM treatment did not modify the response to ionizing radiation or was even capable of radiosensitizing breast malignant cells. Based on these results and in the fact that no side effects have been reported so far, we conclude that O-LM could be an attractive candidate as a safe radioprotective agent for patients undergoing radiotherapy.

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