Protective Effect of Mesalamine Against Oxidative Injury in a Rat Model of Radiation Rectitis

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ABSTRACT

Background: Radiation rectitis is a major problem associated with high-dose irradiation used for pelvic malignancies.

Objective: The aim of this study was to investigate the possible protective effect of mesalamine against irradiation-induced oxidative tissue damage in an experimental model.

Methods: Three groups of female Sprague Dawley rats were assigned to receive treatment as follows: mesalamine enema (60 mg/mL) BID + irradiation (IR) was given to the mesalamine + IR group, and isotonic saline enema BID + irradiation to the control group. Treatments were given from the day before irradiation until euthanization (72 hours after the irradiation). Sham control rats received isotonic saline enema BID but no irradiation. On the third day of treatment, all animals were euthanized, and reduced glutathione (GSH) level, malondialdehyde (MDA) level, and myeloperoxidase (MPO) activity were measured in the rectal, intestinal, and hepatic tissue of the rats.

Results: The sham group comprised 7 rats; the control and mesalamine + IR groups, 16 rats each. The median GSH levels of rectal and intestinal specimens were lower in the control group compared with the sham group. The rectal and intestinal MDA levels were higher in the control group compared with the sham group. The rectal and intestinal MPO activities were higher in the control group compared with the sham group. All of these differences were statistically significant ($P < 0.001$) and indicated oxidative stress. With the topical application of mesalamine, the GSH and MDA levels and MPO activities were similar to those of the sham group.

Conclusion: The pelvic irradiation of rats caused oxidative rectal, intestinal, and hepatic tissue damage, which was ameliorated with the use of mesalamine. (*Curr Ther Res Clin Exp.* 2004;65:433–442) Copyright © 2004 Excerpta Medica, Inc.

Key words: pelvic irradiation, glutathione, malondialdehyde, myeloperoxidase, oxidative injury.

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INTRODUCTION

Radiation rectitis is a major problem associated with exposure to high-dose irradiation used for pelvic malignancies. The spectrum of the clinical appearance of intestine varies from mild acute effects (eg, mucositis) to late grave consequences (eg, ulceration, obstruction, hemorrhage, fistulization, infarction, and perforation).^{1,2}

The ionizing effect of radiation, responsible for the genesis of reactive oxygen species (ROS), is the starting point of the clinical disorders encountered. DNA, proteins, and lipids are the targets of the biologic and lethal effects of free radicals. Treatments for scavenging and inhibiting ROS are used to prevent the undesired effects of radiotherapy.³

The primary antioxidant defense of cells consists of reduced glutathione (GSH), melatonin, and various antioxidant enzymes (eg, superoxide dismutase, catalase, glutathione peroxidase). Lipid peroxidation is the oxidative conversion of polyunsaturated fatty acids to lipid peroxide (eg, malondialdehyde [MDA], the end-product of lipid peroxidation) and serves as an index of oxidative lipid damage.⁴

Radiation rectitis is a challenging disorder to prevent and treat. Several modalities are used to do so, including sucralfate, corticosteroids, formalin, misoprostol, and nonsteroidal anti-inflammatory drugs (eg, mesalamine).^{2,5-9}

Mesalamine is widely used in the management of inflammatory bowel diseases and radiation rectitis.¹⁰ It penetrates the colon mucosa well after rectal topical application; this has been proved using the autofluorescent properties of the molecule.¹¹ Aminosalicylates reduce the production of prostaglandins in the intestinal mucosa.¹²

The aim of this study was to investigate the possible protective effect of mesalamine against irradiation-induced oxidative tissue damage in an experimental (rat) model simulating radiation rectitis.

MATERIALS AND METHODS

This controlled study was conducted at Istanbul University, Istanbul, Turkey. The study protocol was approved by the ethics review committee of the Istanbul Faculty of Medicine at the university.

Animals and Assessments

Female Sprague-Dawley rats weighing 230 to 285 g at the age of 6 weeks were obtained from the Institute for Experimental Medical Research, University of Istanbul. All animals were acclimatized for 7 days before the experiment. The laboratory was maintained at a temperature of $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and a relative humidity of $55\% \pm 10\%$, with a constant 12-hour light/dark cycle. Rats were housed in standard wire cages and fed with standard rodent chow and ultraviolet-

sterilized tap water. After irradiation (described later), each animal was weighed and examined once daily until the day of euthanization.

Mesalamine enema (60 mg/mL) BID + irradiation (IR) was given to the mesalamine + IR group, and isotonic saline enema BID + irradiation to the control group. Treatments were given from the day before irradiation until euthanization (72 hours after the irradiation). Sham control rats received isotonic saline enema BID but no irradiation.

Irradiation

Each rat was anesthetized using sodium pentobarbital 40 mg/kg intraperitoneally. Then 3 to 4 rats at a time were restrained and taped by the tail and legs in the supine position on an acrylic plate. Lead shielding (5 half value layer corresponding to 5-cm thickness) was used to cover each rat (except for a 3×4-cm area at the low pelvis, containing a 2-cm length of rectum in the middle of the field). Irradiation was delivered on a cobalt-60 apparatus (Alcyon, General Electric, New York, New York) (gamma energy, 1.25 MeV, with a distance of 80 cm from the source to the surface). The dose rate of irradiation was 121.49 cGy/min. To each rat, 17.5 Gy in single fraction (ie, at 1 execution) was delivered to the pelvis.¹³

On the second day of irradiation, animals were euthanized by decapitation. Rectal, intestinal, and hepatic tissues were removed and stored at -80°C until the measurement of GSH and MDA levels and myeloperoxidase (MPO) activity.

Glutathione and Malondialdehyde Assays

Samples of rectal, intestinal, and hepatic tissues were homogenized in 0°C 150-mmol/L potassium chloride for measurement of GSH and MDA levels. GSH was determined by spectrophotometry using Ellman's reagent.¹⁴ Results are expressed in micrograms of GSH per gram of tissue (µg GSH/g tissue). MDA was assayed for products of lipid peroxidation.¹⁵ Results are expressed as nanomoles of MDA per gram of tissue (nmol MDA/g tissue). All samples were assayed twice, and the means of the results were used in the analysis.

Myeloperoxidase Activity

Tissue-associated MPO activity was measured using a procedure reported by Hillegass et al.¹⁶ Briefly, tissue samples were homogenized in 50-mmol/L potassium phosphate buffer (PB) (pH, 6.0) and centrifuged at 41,400g at 4°C for 10 minutes; pellets were suspended in 50-mmol/L PB containing 0.5% hexadecyltrimethylammonium bromide. After 3 freeze/thaw cycles, with sonication between cycles, the samples were centrifuged at 41,400g at 4°C for 10 minutes. Aliquots (0.3 mL) were added to 2.3 mL of reaction mixture containing 50-mmol/L PB, *o*-dianisidine, and 20-mmol/L hydrogen peroxide solution. One unit of enzyme activity was defined as the amount of the MPO present that caused a change in absorbance measured at 460 nmol for 3 minutes. MPO

activity was expressed as units per gram of tissue (U/g tissue). All samples were assayed twice, and the mean of the results was used in the analysis.

Statistical Analysis

All data are expressed as median (SE). Groups of data were compared using analysis of variance followed by Tukey's multiple comparison tests. *P* values <0.05 were considered significant. Statistical analyses were performed using SPSS version 10.0 (SPSS Inc., Chicago, Illinois).

RESULTS

The study was performed using 39 rats (the sham group comprised 7 rats; the control and mesalamine + IR groups, 16 rats each). No deaths occurred during the study.

Glutathione Levels

The GSH levels in rectum (μg GSH/g tissue) were 1.96 (0.03), 1.03 (0.02), and 1.97 (0.02) in the sham, control, and mesalamine + IR groups, respectively ($P < 0.001$ for control vs sham and mesalamine + IR groups). The GSH levels in intestine were 1.97 (0.04), 1.04 (0.02), and 1.90 (0.03) in the sham, control, and mesalamine + IR groups, respectively ($P < 0.001$ for control vs sham and mesalamine + IR groups). The GSH levels in liver were similar between all 3 groups: 1.97 (0.04), 1.84 (0.08), and 1.97 (0.02) in the sham, control, and mesalamine + IR groups, respectively.

The GSH levels in rectum and intestine were significantly reduced after irradiation (both, $P < 0.001$ for control vs sham and mesalamine + IR groups). The GSH levels in liver were not significantly different between groups. Mesalamine treatment was associated with normalization of these values. No differences in GSH levels were found between the sham and mesalamine + IR groups in any of the tissues studied (**Figure 1**).

Malondialdehyde Levels

The MDA levels in rectum (nmol MDA/g tissue) were 23.4 (0.8), 35.8 (0.6), and 21.2 (0.6) in the sham, control, and mesalamine + IR groups, respectively. The MDA levels in intestine were 7.9 (0.5), 15.1 (0.4), and 6.4 (0.4) in the sham, control, and mesalamine + IR groups, respectively. The MDA levels in liver were 34.6 (1.0), 35.8 (0.9), and 34.0 (0.5) in the sham, control, and mesalamine + IR groups, respectively.

The MDA levels in rectum and intestine obtained from the irradiated rats were significantly higher than in the sham group (both, $P < 0.001$ vs control group). The MDA levels in liver were not significantly different between groups. In the mesalamine + IR group, no significant difference in MDA levels was found compared with the sham group in any of the tissues studied (**Figure 2**).

Figure 1. The median, interquartile ranges, and extreme reduced glutathione (GSH) levels in the (A) rectum, (B) intestine, and (C) liver of rats treated with saline but no irradiation (sham group; $n = 7$), saline + irradiation (IR) (control group; $n = 16$), or mesalamine + IR ($n = 16$). The outliers, included in the statistical calculations, are shown. $*P < 0.001$ versus sham and mesalamine + IR groups.

Myeloperoxidase Activity

The MPO activities in rectum (U/g tissue) were 11.7 (0.6), 25.3 (0.3), and 15.2 (0.3) in the sham, control, and mesalamine + IR groups, respectively. The MPO activities in intestine were 47.7 (0.9), 75.4 (0.3), and 57.6 (0.5) in the sham, control, and mesalamine + IR groups, respectively. The MPO activities in liver were 10.1 (0.4), 10.9 (0.4), and 10.9 (0.2) in the sham, control, and mesalamine + IR groups, respectively.

Figure 2. The median, interquartile ranges, and extreme malondialdehyde (MDA) levels in the (A) rectum, (B) intestine, and (C) liver of rats treated with saline but no irradiation (sham group; $n = 7$), saline + irradiation (IR) (control group; $n = 16$), or mesalamine + IR ($n = 16$). The outliers, included in the statistical calculations, are shown. * $P < 0.001$ versus sham and mesalamine + IR groups.

Figure 3 shows the comparison of the median MPO activities obtained from the rectum, intestine, and liver of the rats. The MPO activities of the rectum and intestine were significantly higher in the sham group than in the control group (both, $P < 0.001$). MPO activities were similar in the mesalamine + IR and sham groups. The MPO activities in liver were statistically similar between all 3 groups.

Figure 3. The median, interquartile ranges, and extreme myeloperoxidase (MPO) activities in the (A) rectum, (B) intestine, and (C) liver of rats treated with mesalamine + irradiation (IR) ($n = 16$), saline + IR (control; $n = 16$), or saline but no irradiation (sham; $n = 7$). The outliers, included in the statistical calculations, are shown. $*P < 0.001$ versus sham and mesalamine + IR groups.

DISCUSSION

Contrary to most whole-body irradiation procedures, the experimental method chosen for the present study results in radiation rectitis.¹³ Using this method, a homogeneous radiation rectitis could be obtained with the histopathologic changes evident on days 9 to 10 after irradiation. The liver was outside the irradiated area.

Ionizing radiation has oxidative effects on tissues, which damage the macromolecules of cells, especially DNA. Ionizing radiation causes hydrolysis of the cellular water and formation of $\cdot\text{OH}$.¹⁷ The free radicals attack lipids and proteins as well.¹⁸ GSH is an important member of the cellular defense system against oxidative damage, and the tissue damage is coupled with GSH depletion.¹⁹ GSH scavenges oxygen-free radicals ($\cdot\text{O}_2^-$) and protects protein thiol groups from oxidation. GSH also has a major role in restoring other free-radical scavengers and antioxidants (vitamin E and ascorbic acid) to their reduced state. Mandel et al²⁰ reported that tissue GSH levels and the activities of glutathione reductase and glutathione peroxidase, crucial constituents of the GSH-redox cycle, were significantly reduced due to oxidative stress. In the present study, the GSH levels in the rectum and intestine, which were affected by the oxidative damage of the irradiation, significantly decreased in the irradiated group; this damage was reduced in rats receiving mesalamine. The GSH levels in liver were unchanged in all 3 groups. Our findings are in concordance with those of Sudheer Kumar et al,²¹ who reported the characteristic of mesalamine as a potent scavenger of oxygen-derived free radicals in a rat model of whole-body irradiation.

MDA level is used as an indicator of lipid peroxidation, which occurred by the oxidative effect of irradiation.⁸ In the present study, MDA levels of the rectum and intestine were elevated after irradiation. This elevation was reduced in rats receiving mesalamine. The MDA levels in liver were similar in all 3 groups because of the lead shielding.

MPO is an essential enzyme for normal neutrophil function and is released from cells when neutrophils are affected by various stimulants. MPO level is an indicator of neutrophil accumulation.²² Our findings showed an increase in MPO levels in rectum and intestine of rats after irradiation. This increase was reduced in rats receiving mesalamine. The MPO levels in liver were similar across all 3 groups.

The effect of mesalamine in this model of irradiation-induced oxidative tissue damage was similar to that reported for melatonin in a comparable experimental model.²³ The antioxidant capacity of mesalamine was reported previously in studies carried out *in vitro*.^{24,25} In the present study, this antioxidant effect was found in the rectal and intestinal tissues after topical administration, the route used to protect healthy tissues from radiation.

CONCLUSIONS

The present study demonstrated that the pelvic irradiation of rats caused oxidative organ tissue damage, which might be ameliorated with the use of mesalamine. The clinical significance and the potential effects on therapy need further study.

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REFERENCES

1. Ajlouni M. Radiation-induced proctitis. *Curr Treat Options Gastroenterol*. 1999;2:20–26.
2. Hovdenak N, Fajardo LF, Hauer-Jensen M. Acute radiation proctitis: A sequential clinicopathologic study during pelvic radiotherapy. *Int J Radiat Oncol Biol Phys*. 2000;48:1111–1117.
3. Chevion S, Or R, Berry EM. The antioxidant status of patients subjected to total body irradiation. *Biochem Mol Biol Int*. 1999;47:1019–1027.
4. Reiter RJ. Oxidative processes and antioxidative defense mechanisms in the aging brain. *FASEB J*. 1995;9:526–533.
5. Cavcic J, Turcic J, Martinac P, et al. Metronidazole in the treatment of chronic radiation proctitis: Clinical trial. *Croat Med J*. 2000;41:314–318.
6. Sanguineti G, Franzone P, Marcenaro M, et al. Sucralfate versus mesalazine versus hydrocortisone in the prevention of acute radiation proctitis during conformal radiotherapy for prostate carcinoma. A randomized study. *Strahlenther Onkol*. 2003;179:464–470.
7. Denton AS, Andreyev HJ, Forbes A, Maher EJ. Systematic review for non-surgical interventions for the management of late radiation proctitis. *Br J Cancer*. 2002;87:134–143.
8. Kneebone A, Mameghan H, Bolin T, et al. The effect of oral sucralfate on the acute proctitis associated with prostate radiotherapy: A double-blind, randomized trial. *Int J Radiat Oncol Biol Phys*. 2001;51:628–635.
9. Zimmermann FB, Feldmann HJ. Radiation proctitis. Clinical and pathological manifestations, therapy and prophylaxis of acute and late injurious effects of radiation on the rectal mucosa. *Strahlenther Onkol*. 1998;174(Suppl 3):85–89.
10. Cotti G, Seid V, Araujo S, et al. Conservative therapies for hemorrhagic radiation proctitis: A review. *Rev Hosp Clin Fac Med Sao Paulo*. 2003;58:284–292.
11. Schoonjans R, De Vos M, Schelfhout AM, et al. Distribution and concentrations of 5-aminosalicylic acid in rectosigmoid biopsy specimens after rectal administration. *Dis Colon Rectum*. 1996;39:788–793.
12. Stockdale AD, Biswas A. Long-term control of radiation proctitis following treatment with sucralfate enemas. *Br J Surg*. 1997;84:379.
13. Kan S, Chun M, Jin YM, et al. A rat model for radiation-induced proctitis. *J Korean Med Sci*. 2000;15:682–689.
14. Beutler E. Glutathione in red blood cell metabolism. In: *A Manual of Biochemical Methods*. 2nd ed. New York: Grune and Stratton; 1975:112–114.
15. Buege JA, Aust SD. Microsomal lipid peroxidation. *Method Enzymol*. 1978;52:302–311.
16. Hillegeass LM, Griswold DE, Brickson B, Albrightson-Winslow C. Assessment of myeloperoxidase activity in whole rat kidney. *J Pharmacol Method*. 1990;24:285–295.
17. Ward JF. DNA damage produced by ionizing radiation in mammalian cells: Identities, mechanisms of formation, and reparability. *Prog Nucleic Acid Res Mol Biol*. 1988;35:95–125.

18. Vijayalaxmi, Reiter RJ, Herman TS, Meltz ML. Melatonin reduces gamma radiation-induced primary DNA damage in human blood lymphocytes. *Mutat Res.* 1998;397:203–208.
19. Ross D. Glutathione, free radicals and chemotherapeutic agents. Mechanisms of free-radical induced toxicity and glutathione-dependent protection. *Pharmacol Ther.* 1988;37:231–249.
20. Mandel LJ, Schnellmann RG, Jacobs WR. Intracellular glutathione in the protection from anoxic injury in renal proximal tubules. *J Clin Invest.* 1990;85:316–324.
21. Sudheer Kumar M, Unnikrishnan MK, Uma Devi P. Effect of 5-aminosalicylic acid on radiation-induced micronuclei in mouse bone marrow. *Mutat Res.* 2003;527:7–14.
22. Kettle AJ, Winterbourn CC. Myeloperoxidase: A key regulator of neutrophil oxidant product. *Redox Rep.* 1997;3:3–15.
23. Şener G, Jahovic N, Tosun O, et al. Melatonin ameliorates ionizing radiation-induced oxidative organ damage in rats. *Life Sci.* 2003;74:563–572.
24. Kanski J, Lauderback C, Butterfield DA. 5-Aminosalicylic acid protection against oxidative damage to synaptosomal membranes by alkoxyl radicals in vitro. *Neurochem Res.* 2001;26:23–29.
25. Goncalves E, Almeida LM, Dinis TC. Antioxidant activity of 5-aminosalicylic acid against peroxidation of phosphatidylcholine liposomes in the presence of alpha-tocopherol: A synergistic interaction? *Free Radic Res.* 1998;29:53–66.

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