Int J Clin Exp Med 2014;7(4):940-946
www.ijcem.com /ISSN:1940-5901/IJCEM1402026

Original Article

Prophylactic and therapeutic effects of oral budesonide for acute radiation-induced enteritis and colitis in rats

Mehmet Odabasi[1], Suleyman Gokdemir[1], Tolga Muftuoglu[1], Ali Aktekin[1], Abdullah Saglam[1], Fugen Aker[2]

[1]Department of Surgery, Haydarpasa Education and Research Hospital, Istanbul, Turkey; [2]Department of Pathology, Haydarpasa Education and Research Hospital, Istanbul, Turkey

Received February 8, 2014; Accepted April 15, 2014; Epub April 15, 2014; Published April 30, 2014

Abstract: No satisfactory means has been found to control the symptoms of diarrhea and weight loss caused by radiation-induced enteritis and colitis. As a glucocorticoid, budesonide has multiple effects, and this study aimed to test whether it could be effective in treating these symptoms. Twenty-eight male Wistar albino rats were randomly allocated into 4 groups. Group I received 0.1 mg/kg/day budesonide at 8-h intervals for 5 days and did not undergo radiation. Group II received 0.1 mg/kg/day budesonide at 8-h intervals for 1 day before radiation treatment and 4 days after irradiation. Group III received 0.1 mg/kg/day budesonide at 8-h intervals for 4 days after irradiation. Group IV received only radiation treatment. On the fifth day after radiation treatment, the rats underwent laparotomy. The rats were weighed before irradiation and before laparotomy. Because of diarrhea, all rats lost weight except group I, which showed weight gain. Weight loss was statistically significant only in group IV. Group I rats exhibited a normal jejunum, ileum, and colon. The other groups showed varying degrees of damage. We conclude that, particularly when given before irradiation, budesonide decreased the side effects of radiation-induced enteritis and colitis both clinically and morphologically. Future pathophysiological and clinical studies will be needed to support this result.

Keywords: Budesonide, radiation, radiation-induced enteritis, radiation-induced colitis

Introductıon

Treatment of abdominal malignancies with radiation frequently results in damage to the mucosa of the small intestine and colon because of their high mitotic rates. Radiation enteritis, with symptoms of nausea, vomiting, diarrhea, pain, and weight loss, is therefore an almost inevitable consequence of therapeutic abdominal irradiation administered to patients with abdominal or gynecologic malignancy [1, 2]. No satisfactory method has been found to control the symptoms of diarrhea and weight loss, which are frequently severe enough to limit or delay further doses of treatment. In 15-20% of patients, prolongation of radiotherapy has been implicated as a factor in the reduction of the chance of cure [3, 4].

Prostaglandin (PG) release may be 1 etiologic factor in radiation-induced gastrointestinal complications [5]. There is also considerable evidence that the cytotoxic effects of ionizing radiation are mediated almost entirely by oxygen free radicals and apoptotic cell death [6].

Budesonide (16α,17α-butylidene dioxy-11β, 21-dihydroxy-1,4-pregnadiene-3,20-dione) is a non-halogenated glucocorticoid derived from 16α-hydroxyprednisolone. As a glucocorticoid, budesonide has multiple means of action. It inhibits the formation of leukotrienes and PGs, known to be mediators of inflammation. Because most cells have glucocorticoid receptors, budesonide is effective on the inflammatory cells involved in the pathogenesis of inflammatory bowel diseases [7]. The release of cytokines (TNF-α and IL-1β from monocytes) is strongly inhibited by budesonide both in healthy volunteers and in patients. The inhibitory effect of budesonide has been shown to be about 20-fold higher than that of dexamethasone. Thus, budesonide has been shown to be a strong anti-inflammatory cytokine antagonist [8].

In the treatment of inflammatory bowel diseases, the relationship between topical and systemic action is critical because it is directly correlated with the relationship between the desired therapeutic effect and adverse effects.

Table 1. Distribution of groups

	One day before radiation	Radiation	Four days after radiation
Group I	Budesonide 0.1 mg/kg/day by 8 hours interval	No	Budesonide 0.1 mg/kg/day by 8 hours interval
Group II	Budesonide 0.1 mg/kg/day by 8 hours interval	Yes	Budesonide 0.1 mg/kg/day by 8 hours interval
Group III	No	Yes	Budesonide 0.1 mg/kg/day by 8 hours interval
Group IV	No	Yes	No

Table 2. Animal weights

	Initial weight (g)	*Sacrifice weight (g)*
Group I	254 ± 17	265 ± 16
Group II	252 ± 11	243 ± 9
Group III	253 ± 17	235 ± 16
Group IV	255 ± 16	224 ± 10*

*p = 0.012.

Because of systemic side effects, long-term use of glucocorticoids is very limited. Therefore, budesonide, which has a low systemic bioavailability and a strong local anti-inflammatory effect, is suitable for treating inflammatory bowel disease and may also be effective in the treatment of radiation-induced enteritis.

This study aimed to determine, through histological and clinical examination, the prophylactic and therapeutic effects of oral budesonide administration in cases of experimental, acute radiation-induced enteritis and colitis in rats.

Materıals and methods

Animals

Twenty-eight male Wistar albino rats, each weighing 250 g, were randomly allocated into 4 groups of 7 rats. Budesonide (Budenofalk®) was administered as shown in **Table 1**. Group I received 0.1 mg/kg/day budesonide at 8-h intervals for 5 days and did not receive radiation treatment. Group II received 0.1 mg/kg/day budesonide at 8-h intervals for 1 day before irradiation and 4 days after irradiation. Group III received 0.1 mg/kg/day budesonide at 8-h intervals for 4 days after irradiation. Group IV received only irradiation.

The study was approved by the Animal Ethics Committee of the University of Marmara Medical School in Istanbul.

Protocol

All rats were fed standard rat chow and given free access to water. Budesonide was administered by a No. 6 feeding tube via the orogastric route. The rats were not fed for 8 h before irradiation. The rats were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and largactil (10 mg/kg), and the abdomen was subjected to a single 1000-cGy dose of radiation from an Alcyon II Co-60 apparatus (cobalt-60, 130.61 cGy/min, 80 cm source-to-skin distance). The thorax, head, and extremities were protected from radiation.

On the fifth day after irradiation, the rats were weighed again and underwent laparotomy. Under general anesthesia with ketamine and largactil, a midline incision was made, and the whole gastrointestinal system (esophagus to rectum) was resected. The lumen was washed with saline solution, and 2-cm segments of jejunum, ileum, and colon were resected. The specimens were fixed with Holland blue and alcohol, stained with hematoxylin and eosin, and examined under a light microscope.

Intestinal histology was graded using a modification of the technique described by Howarth et al [9]. For the jejunum and ileum, 15 parameters were evaluated, and damage severity scores were assigned. Each criteria was scored from 0 (normal) to 3 (maximal damage), giving a maximum score of 45. The 15 parameters were villus fusion, villus atrophy, brush border disruption, reduction in number of goblet cells, reduction in mitotic figure, changes in crypt structure, formation of crypt abscess, increase in lymphocyte and polymorph infiltration, capillary or lymphatic dilatation, thickening of the submucosa, thickening of the muscularis externa, nuclear changes, increase in eosinophils in the lamina propria, fibrinoid changes in the vessel wall, and changes in fibroblasts. For assessment of the colon, 12 parameters were evaluated (those listed above, excluding the first 3 parameters), with a maximum score of 36.

Microscopic assessment of each specimen was performed to determine the mucosal thickness and villus height for the jejunum and ileum

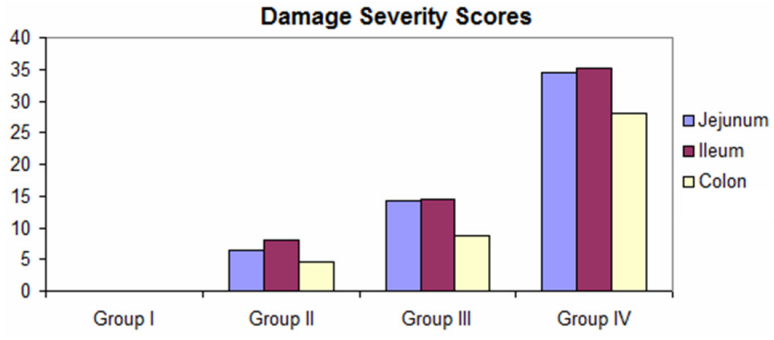

Figure 1. Damage severity scores of jejunum, ileum and colon in all groups. The damage severity score was zero in group I.

and the colonic mucosal thickness. Using a 100× eyepiece micrometer, we measured the mucosal thickness at 5 representative sites where villi were visible from base to tip.

Statistical analysis

Data analysis was performed using the Kruskal-Wallis nonparametric ANOVA test. When a significant difference was seen, a comparison was done using Dunn's multiple comparisons test (Instat-Version 2.0, Graphad Software, San Diego, CA, USA). Other data were analyzed using analysis of variance (ANOVA). Values are given as mean ± SD.

Results

Following irradiation, the animals recovered from the anesthesia without difficulty, and there was no mortality.

The rats were weighed before irradiation and before laparotomy (**Table 2**). There was weight gain in group I. All rats in groups II, III, and IV lost weight. Diarrhea was observed in these 3 groups but was worst in group IV. In group IV, weight loss was statistically significant ($p = 0.012$).

Group I (sham-operated control) had a normal jejunum, ileum, and colon. By contrast, the other groups demonstrated different degrees of injury. Damage severity scores for the jejunum, ileum, and colon were 0 in group I. Jejunum damage severity scores were 6.43 ± 2.15 in group II, 14.14 ± 3.98 in group III, and 34.43 ± 2.94 in group IV. There were significant differences between groups I and IV ($p < 0.001$), I and III ($p < 0.01$), and II and IV ($p < 0.01$). Ileum damage severity scores were 8 ± 3.27 in group II, 14.57 ± 3.46 in group III, and 35.14 ± 3.93 in group IV. There were significant differences between groups I and IV ($p < 0.005$), I and III ($p < 0.05$), and II and IV ($p < 0.05$). Colon damage severity scores were 4.57 ± 1.40 in group II, 8.71 ± 2.56 in group III, and 28 ± 2.16 in group IV. As in the ileum and jejunum, there were significant differences between groups I and IV ($p < 0.001$), I and III ($p < 0.01$), and II and IV ($p < 0.01$) (**Figure 1**).

Histological changes in the jejunum, ileum, and colon of each group are shown in **Figures 2**-**4**. There were statistical differences in villus fusion, villus atrophy, and brush border damage in the jejunum and ileum between groups I and IV ($p < 0.001$) and groups II and IV ($p < 0.05$). Group I had a normal jejunum and ileum (**Figures 2A** and **3A**).

The most prominent changes in group IV were decreased goblet cell and mitotic figure counts, changes in crypt structure, formation of crypt abscesses, lymphatic and capillary dilatation, fibrinoid changes of the vessel wall, changes in fibroblasts, thickening of the submucosa, nuclear changes, and increased lymphocyte and polymorph infiltration. There were statistical differences in the jejunum, ileum, and colon between groups I and IV ($p < 0.001$) and between groups II and IV ($p < 0.01$).

There were statistical differences in the increase in eosinophils in the lamina propria of the jejunum, ileum, and colon between groups I and IV ($p < 0.001$), II and III ($p < 0.001$), and II and IV ($p < 0.05$).

Changes in crypt structure, nuclear changes, and villus fusion were the most notable changes in the jejunum and ileum in group II (**Figures 2B** and **3B**). Thickening of the muscularis propria was also seen in the ileum. Brush border disruption, capillary or lymphatic dilatation, increases in eosinophils in the lamina propria, and fibrinoid changes in the vessel wall were not seen in the jejunum (**Figure 2B**). Increases in eosinophils in the lamina propria, fibrinoid

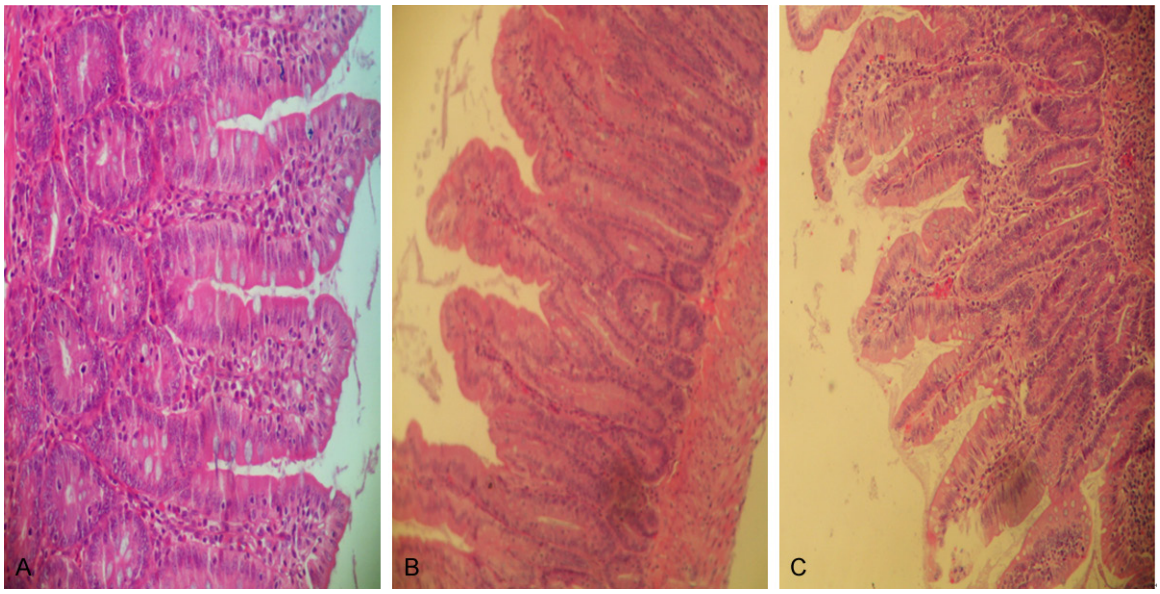

Figure 2. A. Jejunum of group I taken Budesonide without radiation. B. Jejunum of group II taken prophylactic Budesonide. C. Jejunum of group IV received only radiation.

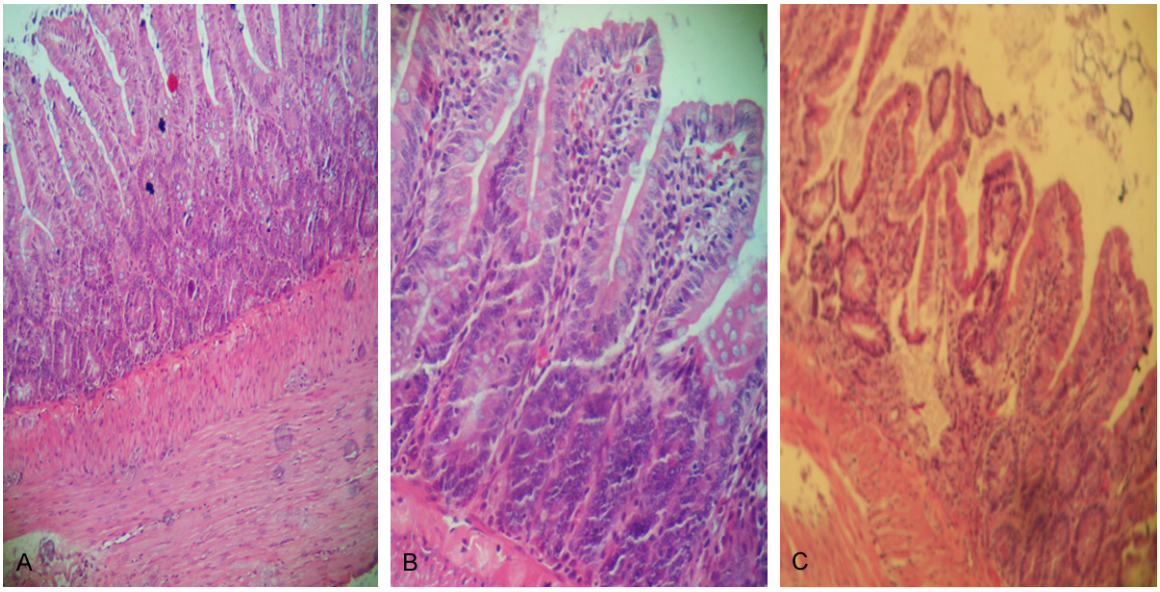

Figure 3. A. İleum of group I taken Budesonide without radiation. B. İleum of group II taken prophylactic Budesonide. C. İleum of group IV received only radiation.

changes in the vessel wall, and changes in fibroblasts were not seen in the ileum (**Figure 3B**). Nuclear changes, capillary or lymphatic dilatation, and thickening of the submucosa were the most prominent findings in the colon, where increases in lymphocyte and polymorph infiltration and thickening of the muscularis externa were not observed (**Figure 4A**).

In group III, damage was seen in all parameters for the jejunum. Nuclear changes, villus fusion, villus atrophy, changes in crypt structure, and thickening of submucosa were most prominent. By contrast, fibrinoid changes in the vessel wall and changes in fibroblasts were least prominent. Increased eosinophils in the lamina propria, fibrinoid changes in the vessel wall, and changes in fibroblasts were not observed in the ileum, where nuclear changes, villus fusion, and reduction in mitotic figures were most prominent. Capillary or lymphatic dilatation and changes in crypt structure were the most prom-

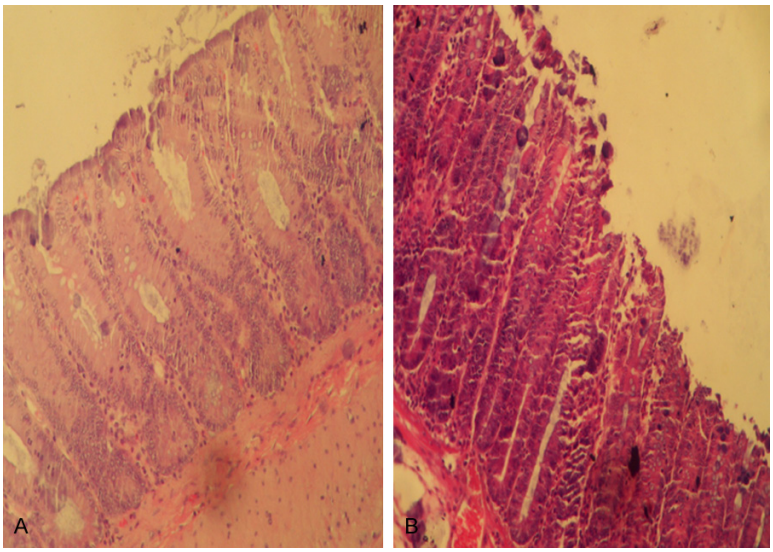

Figure 4. A. Colon of group II taken prophylactic Budesonide. B. Colon of group IV received only radiation.

inent changes in the colon, where fibrinoid changes in the vessel wall were the least common type of damage.

There was medium or heavy damage in all parameters for the jejunum, ileum, and colon in group IV. Formation of crypt abscess, villus atrophy, and increased in lymphocyte and polymorph infiltration were the most prominent types of damage in the jejunum and ileum (**Figures 2C** and **3C**). In the colon, the formation of crypt abscess and changes in crypt structure were the most prominent findings, whereas thickening of the muscularis externa was least prominent (**Figure 4B**).

Microscopic assessments of the jejunum, ileum, and colon are shown in **Table 3**. When the mean heights of the jejunal and ileal villi were compared, statistically significant differences were found between groups I and II ($p < 0.05$), I and IV ($p < 0.001$), and II and IV ($p < 0.05$). The same statistical findings were found when comparing the jejunal, ileal, and colonic mucosal thickness.

Discussion

The pathogenesis of acute radiation-induced enteritis and colitis is poorly understood, and current therapies focus on treating symptoms. The clinical sequelae of gastrointestinal exposure to radiation range from acute gastrointestinal syndrome within hours after irradiation to subacute or chronic radiation enteropathy that lasts for months to years [10, 11].

PG release might be 1 etiologic factor in radiation-induced gastrointestinal complications [5]. Other studies have investigated the use of various salicylate and other PG compounds for the control of diarrhea, based on the assumption that irradiation increases PG secretion [12, 13]. The exact mechanism of action is not known, but such compounds may act by blocking PG synthesis in the mucosa [14, 15]. Both sucralfate [16] and acetylsalicylate [5] have been reported to reduce symptoms, although these findings were not confirmed in a subsequent study [17] and the mechanisms were not assessed.

In group IV, weight loss was 31 ± 6 g, which amounted to 12% of total body weight (**Table 2**). Similarly, Howarth et al. found that rats lost 12-15% of their total body weight [9]. The weight loss can be explained by diarrhea and low food intake.

Gelfand et al. found radiation-induced histological changes in the bowels of 95% of patients [18]. In our study, radiation-induced histological changes were found in 100% of the irradiated animals. Although the pathogenesis of radiation is unclear, the mucosal damage is observed as a destruction of crypt cells, decrease in villous height, decrease in number, ulceration, and necrosis of the gastrointestinal epithelium. Submucosal edema, hyperemia, and infiltration of the lamina propria by activated inflammatory cells are also observed [19-21].

Damage severity scores were calculated to quantify radiation injury in rats. Intestinal histology was graded using 11 parameters described by Howarth et al [9]. In the jejunum and ileum, we also evaluated villus fusion and atrophy, nuclear changes, fibrinoid changes in the vessel wall, and changes in fibroblasts (a total of 15 parameters). In the colon, villus fusion and atrophy and brush border disruption were excluded (a total of 12 parameters). Kevin

Table 3. Mucosal morphometrics of jejunum, ileum and colon

	Jejunal Villus Height (mm)	*Jejunal Mucosal Thickness (mm)*	*Ileal Villus Height (mm)*	*Ileal Mucosal Thickness (mm)*	*Colonic Mukosal Thickness (mm)*
Group I	0.48 ± 0.02	0.69 ± 0.02	0.35 ± 0.01	0.52 ± 0.02	0.72 ± 0.02
Group II	0.33 ± 0.02	0.56 ± 0.03	0.26 ± 0.01	0.43 ± 0.03	0.61 ± 0.02
Group III	0.20 ± 0.02	0.33 ± 0.01	0.16 ± 0.01	0.26 ± 0.01	0.40 ± 0.02
Group IV	0.18 ± 0.01	0.28 ± 0.01	0.14 ± 0.01	0.22 ± 0.02	0.32 ± 0.04

et al. used nuclear changes as a separate parameter, as in our study [22].

Studies assessing radiation-induced histological abnormalities in the intestine have documented changes that include an interstitial accumulation of polymorphonuclear leukocytes [23, 24]. Intravital microscopic studies of radiation-induced leukocyte-endothelial cell adhesion have revealed an increased number of rolling leukocytes in the mesenteric venules at 2 h after irradiation, with a marked increase in the number of firmly adherent and immigrating leukocytes at 6 h after irradiation [25]. There was minimal lymphocyte and polymorph infiltration in the group that received prophylactic budesonide, indicating that budesonide significantly reduces radiation-induced infiltration. The mechanism of this effect is likely to involve inhibition of chemotaxis and migration. Because leukocyte-endothelial cell adhesion and infiltration occurs at 6 h after irradiation, budesonide was not totally effective in the group that received the drug at 8 h after irradiation [26].

Budesonide decreases vascular permeability, lymphatic and capillary dilatation, submucosal edema, and thickening of the muscularis externa. It acts as a strong inhibitor of increased microvascular permeability by inhibiting histamine release from mast cells. It decreases adhesion molecules in endothelial cells and obstruction of vessels so far protects crypt cells, villus atrophy and fusion decreases brush border damage [27].

Vascular injury is often considered to be a primary determinant of the tissue dysfunction resulting from irradiation of various organs, including the intestine [23, 26]. Many in vitro and in vivo studies of the effects of ionizing radiation on vascular endothelial cells indicate the initiation of an inflammatory response. Increases in leukocyte-endothelial cell adhesion and vascular permeability occur in microvessels exposed to γ-radiation [23]. The decreased radiation-induced damage in the groups that received budesonide prophylactically and after irradiation was probably a result of the inhibition of adhesion molecules in endothelial cells and cytokines.

Budesonide causes a marked decrease of numbers of eosinophils in the blood and tissue and reduces the chemotactic activity of eosinophils. Tissue infiltration starts with the adherence of eosinophils to endothelial cells. This process is regulated by the expression of adhesion molecules induced by IL-1. The synthesis of IL-1 is blocked by budesonide. Together, these data show that budesonide strongly impairs the survival and migration of eosinophils by inhibiting the synthesis of cytokines in lymphocytes, endothelial cells, and monocytes. In our study, the eosinophil count was minimal in the tissues of the groups that received prophylactic or therapeutic budesonide [27].

Overall, radiation-induced damage was minimal in the group that received prophylactic budesonide and mild in the group that received budesonide after irradiation. By contrast, there was medium to heavy damage in the group that received only radiation. Thus, we conclude that budesonide, especially when given before irradiation, decreases the side effects of radiation-induced enteritis and colitis both clinically and morphologically in rats. The mechanism of this effect is not clear because budesonide has multiple means of action. This study should be supported by future pathophysiological and clinical studies.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Mehmet Odabasi, Department of Surgery, Haydarpasa Numune Education and Research Hospital, Tibbiye cad. No:1 Uskudar, 34688, Istanbul, Turkey. Tel: +90 5324310630; E-mail: hmodabasi@gmail.com

References

[1] Yeoh E, Horowitz M. Radiation enteritis. Br J Hosp Med 1998; 39: 498-504.

[2] Stacey R, Green JT. Radiation induced small bowel disease: latest developments and clinical guidance. Ther Adv Chronic Dis 2014; 5: 15-29.

[3] Do N, Nagle D, Poylin V. Radiation proctitis: current strategies in management. Gastroenterol Res Practice 2011; 2011: 917941.

[4] Amdur R, Parsons J, Fitzgerald L, Million R. The effect of overall treatment time on local control in patients with adenocarcinoma of the prostate treated with radiation therapy. Int J Radiat Oncol Biol Phys 1990; 19: 1377-1382.

[5] Mennie A, Dalley V, Dinneen L, Collier H. Treatment of radiation-induced gastrointestinal distress with acetylsalicylate. Lancet 1975; 2: 942-943.

[6] Kern JC, Kehrer JP. Free radicals and apoptosis: relationships with glutathione, thioredoxin, and the BCL family of proteins. Front Biosci 2005; 10: 1727-1738.

[7] Schreiber S, Raedler A, Stenson WF, MacDermott RP. The role of the mucosal immune system in inflammatory bowel disease. Gastroenterol Clin North Am 1992; 21: 451-502.

[8] Bergstrand H, Björnsson A, Lindquist B, Nilsson A, Brattsand R. Inhibitory effect of glucocorticoides on anti-Ig E-induced histamine release from human basophilic leukocytes: evidence for a dual mechanism of action. Allergy 1984; 39: 217-230.

[9] Howarth GS, Fraser R, Frisby CL, Schirmer MB, Yeoh EK. Effects of insulin-like growth factor-I administration on radiation enteritis in rats. Scand J Gastroenterol 1997; 32: 1118-1124.

[10] Earnest DL, Trier JS. Radiation enteritis and colitis. In: Sleisenger MH, Fordtran JS, eds. Gastrointestinal disease: pathophysiology, diagnosis and management. 4th ed. Philadelphia: Saunders, 1989. pp: 1369-1382.

[11] Galland RB, Spencer J. The natural history of clinically established radiation enteritis. Lancet 1985; 1: 1257-1258.

[12] Henriksson R, Franzen L, Littbrand B. Prevention of irradiation-induced bowel discomfort by sucralfate: a double-blind, placebo-controlled study when treating localized pelvic cancer. Am J Med 1991; 91: 151-157.

[13] Rotman M, McDonald AE, Wasserman TH. Supportive care and quality of life in radiation oncology. In: Perez CA, Brady LW, eds. Principles and practice of radiation oncology. Philadelphia: Lippincott; 1998, pp: 2243-56.

[14] Rao SSC, Dundas SAC, Holdsworth CD, Cann PA, Palmer KR, Corbett CL. Olsalazine or sulphasalazine in first attacks of ulcerative colitis. A double blind study. Gut 1989; 30: 675-679.

[15] Watkinson G. Sulphasalazine: a review of 40 years' experience. Drugs 1986; 32: 1-11.

[16] Henriksson R, Franzen L, Littbrand B. Effects of sucralfate on acute and late bowel discomfort following radiotherapy of pelvic cancer. J Clin Oncol 1992; 10: 969-975.

[17] Gibson RJ, Keefe DM, Lalla RV, Bateman E, Blijlevens N, Fijlstra M, King EE, Stringer AM, van der Velden WJ, Yazbeck R, Elad S, Bowen JM; Mucositis Study Group of the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO). Systematic review of agents fort he management of gastrointestinal mucositis in cancer patients. Supportive Care Cancer 2013; 21: 313-326.

[18] Gelfand MD, Tepper M, Katz LA, Binder HJ, Yesner R, Flock MH. Acute radiation proctotitis in man. Gastroenterology 1968; 54: 401-411.

[19] Giriş M, Erbil Y, Oztezcan S, Olgaç V, Barbaros U, Deveci U, Kirgiz B, Uysal M, Toker GA. The effect of heme oxygenase-1 induction by glutamine on radiation-induced intestinal damage: the effect of heme oxygenase-1 on radiation enteritis. Am J Surg 2006; 191: 503-509.

[20] Olgaç V, Erbil Y, Barbaros U, Oztezcan S, Giriş M, Kaya H, Bilge H, Güler S, Toker G. The efficacy of octreotide in pancreatic and intestinal changes: radiation-induced enteritis in animals. Dig Dis Sci 2006; 51: 227-232.

[21] Hepgül G, Tanrikulu S, Unalp HR, Akguner T, Erbil Y, Olgaç V, Ademoğlu E. Preventive effect of pentoxifylline on acute radiation damage via antioxidant and anti-inflammatory pathways. Dig Dis Sci 2010; 55: 617-625.

[22] McKenna KJ, Ligato S, Kauffman GL Jr, Abt AB, Stryker JA, Conter RL. Epidermal growth factor enhances intestinal mitotic activity and DNA content after acute abdominal radiation. Surgery 1994; 5: 626-630.

[23] Buell MG, Harding RK. Proinflammatory effects of local abdominal irradiation on rat gastrointestinal tract. Dig Dis Sci 1989; 34: 390-399.

[24] Narayan K, Cliff WJ. Morphology of irradiated microvasculature: a combined in vivo and electron microscopic study. Am J Pathol 1982; 106: 47-62.

[25] Panes J, Anderson DC, Miyasaka M, Granger DN. Role of leukocyte-endothelial cell adhesion in radiation-induced microvascular dysfunction in rats. Gastroenterology 1995; 108: 1761-1769.

[26] Law MP. Radiation induced vascular injury and its relation to late effects in normal tissues. Adv Radiat Biol 1981; 9: 37-73.

[27] Targan SR, Shanahan F. Inflammatory bowel disease: From bench to bedside. Baltimore: Williams & Wilkins, 1994. pp: 487-502.