Original Article

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

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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

Introduction

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-butyldiene 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.
Budesonide for radiation damage

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.

Materials 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.
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) and between groups 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...
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-
inherent 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 an 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

![Figure 4. A. Colon of group II taken prophylactic Budesonide. B. Colon of group IV received only radiation.](image-url)
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.

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