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Protective Effect of DA-9601, an *Artemisiae Herba* Extract, on Radiation-induced Colitis in Wistar Rats

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Abstract – This study was performed to examine the effects of DA-9601, a novel antiulcer agent extracted from *Artemisiae Herba*, on radiation colitis in the rat. Female Wistar rats received a 30 Gy dose of irradiation to the 2 cm of distal colon in length using an intrarectal applicator system. 30 mg/kg or 100 mg/kg of DA-9601 was administered orally 30 min before and 4 h after radiation on day 1. And the same dose of DA-9601 was given to the animals twice a day from day 2 to 14. As a reference control, sucralfate suspension (100 or 300 mg/head) was given as an enema based on the same treatment schedule of DA-9601. Body weight change and the frequency of diarrhea were recorded during the observation period as markers of radiation-induced injury. All animals were sacrificed on day 15 for evaluation of macro- and microscopic findings and mucosal myeloperoxidase (MPO) activity. Radiated animals showed diarrhea, mucosal redness and histologic changes characterized by edema and eosinophilic infiltration of the periglandular lamina propria with loss of colonic epithelium. Radiation also significantly increased mucosal MPO activity of affected colon ($P<0.05$). However, most of these changes were completely protected by oral administration with DA-9601. DA-9601 reduced radiation-induced histologic alteration significantly in a dose-related manner ($P<0.05$). In addition, mucosal MPO activity in rats receiving high dose of DA-9601 decreased significantly when compared with that in radiated control. High dose of sucralfate (300 mg/head) alleviated radiation-induced histologic lesion, but failed to reach statistical significance. The results of this study suggest that DA-9601 can be useful for the prevention of acute clinical symptoms of radiation proctocolitis and that decrease of mucosal MPO by DA-9601 plays a role in its protective mechanism(s), at least in part.

Keywords □ DA-9601, *Artemisiae Herba*, radiation enteritis, colitis, Wistar rats

Non-selective damage to normal tissue is one of the major drawbacks to the treatment of malignancies by irradiation (Kochhar *et al.*, 1991). During treatment of pelvic malignancies with external, intracavitary, or combined irradiation, colon and rectum often receive a substantial exposure. Most of patients who undergo pelvic irradiation experience some degree of proctocolitis. The most frequent symptoms are pain, tenesmus and diarrhea, though rectal bleeding may on occasion be encountered. Stricture or fistula formation may occur months or years after cessation of therapy as an intractable sequela. Unfortunately, current treatment of radiation proctocolitis remains supportive and empirical.

DA-9601 is a quality-controlled extract of dried aerial parts of *Artemisiae Herba*, which is under clinical trials as a novel antiulcer agent (Oh *et al.*, 1996). The methods of extraction and manufacturing of DA-9601 were described in detail elsewhere (Yang, 1995). The mechanism by which DA-9601 produce its mucosal protective effect has not been fully elucidated, although stimulation of mucus and bicarbonate secretion, increase of mucosal prostaglandins and glutathione, and enhancement of mucosal blood flow are thought to play roles in producing this effect (Oh *et al.*, 1996; Oh *et al.*, 1997a; Oh *et al.*, 1997b). There is considerable evidence indicating the cytotoxic effects of ionizing radiation are mediated by ox-

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ygen free radicals (Grisham and McCord, 1986; Empey *et al.*, 1992). Rapid generation of a leukotrienes (LTs) such as LTB_4, a potent chemoattractant, by enterocytes and vascular endothelium following exposure to ionizing radiation elicits the increased accumulation of polymorphonucleated cells (PMNs), which was reported to be one of major steps in pathogenesis of radiation-induced gastrointestinal injury (Buell and Harding, 1989). Recently the authors reported that DA-9601 shows beneficial effects on experimental models of inflammatory bowel disease through decreasing oxidative stress and attenuating cytokines involved in colonic inflammation (Ahn *et al.*, 1997). The purpose of this study was to investigate the protective effect of DA-9601 on intrarectal radiation-induced colonic damage using rats.

MATERIALS AND METHODS

Animals

Forty-eight female Wistar rats weighing 140~150 g at the age of 5 weeks were obtained from Charles River Japan (Kanagawa, Japan). All animals were acclimatized for 7 days prior to the experiment under the barrier-sustained animal facility in laboratory of Dong-A Pharmaceutical Company maintained at a temperature of 22±3 ℃, a relative humidity of 55±10 % with a constant 12 h light/dark cycle. Rats were housed in standard wire cages and fed with standard rodent chow (Cheil, Korea) and UV-sterilized tap water.

Irradiation

After overnight fasting, each rat was anesthetized with an intraperitoneal injection of sodium pentobarbitone (Somnopentyl®) 40 mg/kg, restrained in a supine position, and taped by the tail. Irradiation was done with a Ir-192 remote afterloading unit (Model: Microselectron, Nucletron Co.). The rectal applicator was designed to accommodate attachment to a Microselectron afterloading device for high dose rate. The applicator was made with a size 5 french rectal enema tubes. The length of radiation applicator is sufficient to allow a 2 cm homogenous irradiation region of colon along the length of the applicator and is similar to treatment volumes used in other studies of rectal irradiation to the rat (Zahavi *et al.*, 1989). The applicator loaded 150 cm long of 5 french nucletron catheter was inserted gently into the empty rectum. To secure the applicator in position, it was fixed to the table with surgical tape. Radiation dose was prescribed to the 0.5 cm distance from center of emission source. And the dose distribution was calculated using the attached treatment-planning computer. Exposure time was calculated from the activity of the source afterloaded into the rat considering isotope decay. All animals were irradiated using a single exposure or sham irradiation.

Treatment

To evaluate the radioprotective activity of DA-9601, rats were treated orally with DA-9601 at a dose of 30 mg/kg or 100 mg/kg, 30 min before and 4 h after irradiation. From the next day of irradiation, same dose of DA-9601 was administered orally twice a day (AM 9:00, PM 5:00) for 13 days. Control rats received vehicle (5% hydroxypropylmethylcellulose) only based on the treatment schedule. As a reference group, sucralfate suspension (Ulcermin®, Choong Wae Pharm.) 100 mg/head or 300 mg/head was administered intrarectally using a 5 cm long rubber cannula during the same treatment period. Eight rats per each group including sham-treated normal control were used.

Clinical signs and macroscopic examination

During the experimental period, clinical signs of all animals were observed and the frequency of bloody stool and diarrhea was recorded. Body weight of each rat was recorded on days 0, 4, 10 and 14 after irradiation. 14 days after irradiation, all animals were sacrificed and gross lesions of visceral organs in abdominal cavity were observed. Then, distal colon was removed and laid, mucosal side up, on a wax platform. Macroscopic mucosal damage was scored 0 (normal) to 4 (severe) as shown below: 0=normal; 1=edema, redness, decreased vascularity; 2=diffuse redness, hemorrhagic spots; 3=erosions and frank hemorrhage; 4=ulcers

Histologic observation

Samples of gross lesion were excised and fixed in 10% neutral buffered formalin solution, then processed by routine techniques. After sectioning, each specimen was stained with hematoxylin and eosin and examined with light microscope (BH-2, Olympus) by a pathologist blinded to the study. Each specimen was graded as described by Northway *et al.* (1988) with slight modification as follows: 0=normal or minor alterations which cannot be ascribed with certainty to radiation; 1=slight radiation damage (mild inflammation and/or slight crypt change); 2= mild damage (more significant inflammation, and/or crypt damage); 3=moderate damage (must have prominent loss

of epithelium, degree of inflammation variable); and 4= severe damage (frank erosion, ulcer, necrosis).

Mucosal myeloperoxidase

Neutrophil infiltration into the colorectal mucosa was assessed by determination of tissue activity of myeloperoxidase (MPO). MPO activity was measured as described by Krawisz et al. (1984). MPO activity in the mucosa was determined by using *O*-dianisidine hydrochloride and hydrogen peroxide. The change in absorbance at 400 nm over 2 min was measured.

Statistical analyses

All data are expressed as mean±standard error of the mean. Body weight and mucosal MPO activity between control group and experimental group were compared by Scheffe's t-test. Rank transformation and Kruskal-Wallis test was performed to determine inter-group difference of non-parametric data and Bonferroni's test was used for multiple pair-wise comparison. P values of ≤ 0.05 were considered statistically significant.

RESULTS

Clinical signs and gross lesion

During the observation period, one animal from sucralfate treated group (100 mg/head) was found dead on day 10 after radiation (Table I). At necropsy, rectal stricture formation accompanying with severe dilatation of large intestine (Fig. 1) was observed. Intrarectal irradiation (30 Gy) did not cause body weight loss (Table I). However, the frequency of diarrhea and bloody stool (melena) was prominent in irradiated animals (Table II). With regard to consistency of stool, DA-9601 reduced the frequency of diarrhea and melena in a dose-related manner, whereas sucralfate failed to ameliorate the signs. Macroscopic examination of abdominal organs revealed that adhesion of intestine to adjacent tissue and stricture and dilation of large bowel were major features of necropsy findings (Fig. 2). Interestingly, intestinal adhesion was more frequent in terminal ileum of small bowel than

Table I. Effect of DA-9601 and sucralfate on body weight change and cumulated mortality of rats irradiated intrarectally with 30 Gy.

Radiation dose (Gy)	Treatment		Body weight (g)				Mortality
			day 0[d]	day 4	day 10	day 14	
0	-		175.6±2.7	188.4±1.5	204.0±3.1	220.4±2.3	0/8
	5% HPMC[a]		183.0±4.5	183.5±4.3	195.0±6.9	212.3±10.0	0/8
	Sucralfate[b]	100 mg/head	183.7±2.6	176.0±3.8	189.0±6.2	202.8±8.1	1/8
30		300 mg/head	184.7±6.2	182.0±6.9	203.0±7.9	215.3±7.4	0/8
	DA-9601[c]	30 mg/kg	188.7±7.3	191.7±9.0	213.0±11.2	231.3±12.0	0/8
		100 mg/kg	177.7±2.8	176.6±2.5	196.0±4.0	209.2±4.1	0/8

[a]5% suspension of hydroxypropylmethylcellulose. [b]Sucralfate was administered as an enema at a dose of 100 mg or 300 mg per head. [c]DA-9601 was administered orally based on body weight. [d]Day after irradiation.

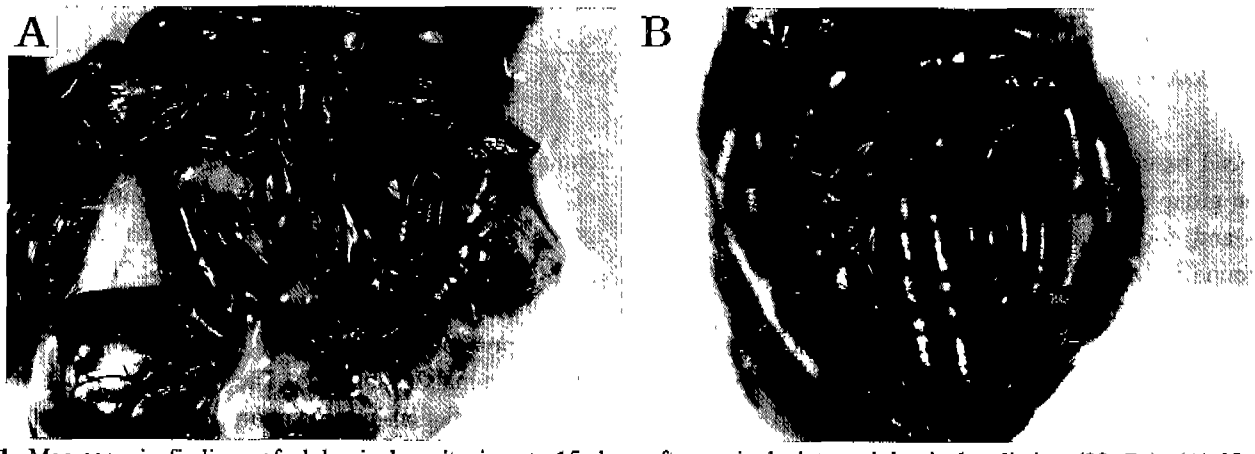

Fig. 1. Macroscopic findings of abdominal cavity in rats 15 days after a single intra- abdominal radiation (30 Gy). (A) Normal animal. (B) A rat from radiation control; dilatation of whole intestine due to rectal stricture is evident. Gross appearance of abdominal cavity is similar to the picture of megacolon.

Table II. Effect of DA-9601 and sucralfate on the frequency of diarrhea and melena in rats receiving 30 Gy of colonic irradiation

Radiation dose (Gy)	Treatment		Day after radiation													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	5% HPMC[a]		-	-	-	-	-	D3 B1[d]	D2 B1	D3	D3	D5 B1	D5	D5	D4	D4
	Sucralfate[b]	100 mg/head	-	-	-	-	-	D1	-	D2	D4	D3 B1	D3 B1	D3	D2	D2
		300 mg/head	-	-	-	-	-	D1 B1	-	D1 B1	D3	D1 B2	D3	D1 B1	D1	-
	DA-9601[c]	30 mg/kg	-	-	-	-	-	D1	-	-	-	-	D2	D1	-	-
		100 mg/kg	-	-	-	-	-	D1	-	-	-	-	-	D2	-	-

[a]5% suspension of hydroxypropylmethylcellulose. [b]Sucralfate was administered as an enema at a dose of 100 mg or 300 mg per head. [c]DA-9601 was administered orally based on body weight. [d]D3B1 represents 3 animals showing diarrhea and one animal showing melena.

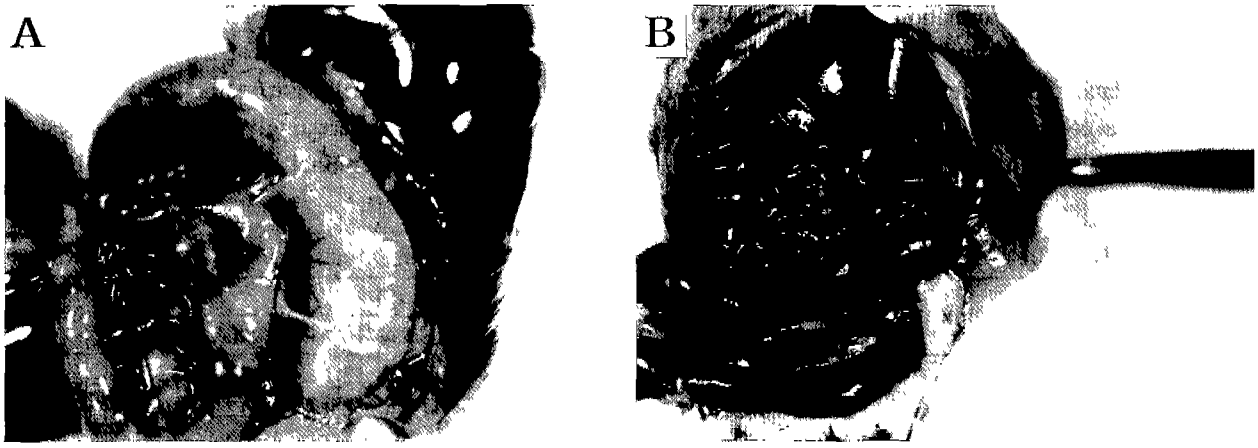

Fig. 2. Photographs of abdominal cavity of rats receiving an intrarectal radiation. (A) Radiation control. Adhesion of distal colon to adjacent small bowel is seen. Dilation of radiated colon filled with firm stool is also noted. (B) A rat received radiation and treated with 100 mg/head of sucralfate suspension twice a day for 2 weeks shows a peritoneal adhesion of affected colon to the abdominal wall.

Table III. Effect of DA-9601 and sucralfate on radiation-induced mucosal macroscopic lesion in rats

Group	Normal	Radiation control	Radiation+Sucralfate (mg/head)		Radiation+DA-9601 (mg/kg)	
			100	300	30	100
Mucosal lesion	0.0±0.0[a]	0.9±0.3[b]	1.0±0.4	0.7±0.3	0.0±0.0	0.0±0.0

[a]Mucosal lesion was scored according to the criteria described in the text. [b]Each value represents the mean±SEM of 7 to 8 rats.

colorectal tissue. Colonic mucosa of some animals in radiation group showed altered vascularity (redness or paleness) and focal hemorrhage. No mucosal abnormality was found in DA-9601 treated rats (Table III).

Histologic examination

Tissue sections of distal colonic epithelium showed normal architecture consisting of simple columnar cells, and abundant goblet cells in normal group. Animals in radiation control group exhibited statistically significant increase in histopathologic index characterized by edema, deformation and inflammatory cellular infiltration of the lamina propria, loss of epithelium, and decreased number of goblet cells when compared with animals in normal group (Fig. 3). DA-9601 reduced edema of the lamina propria, loss of colonocytes and the infiltration of polymorphonucleated cells (PMNs) to the perigladular lamina propria in a dose-related manner, when compared to control animals (Table IV). The protective effect of DA-9601 on radiation-induced morphologic alteration reached statistical significance both at low (30 mg/kg) and high dose (100 mg/kg) groups ($P<0.05$). Although there was a tendency of attenuation in the severity of edema

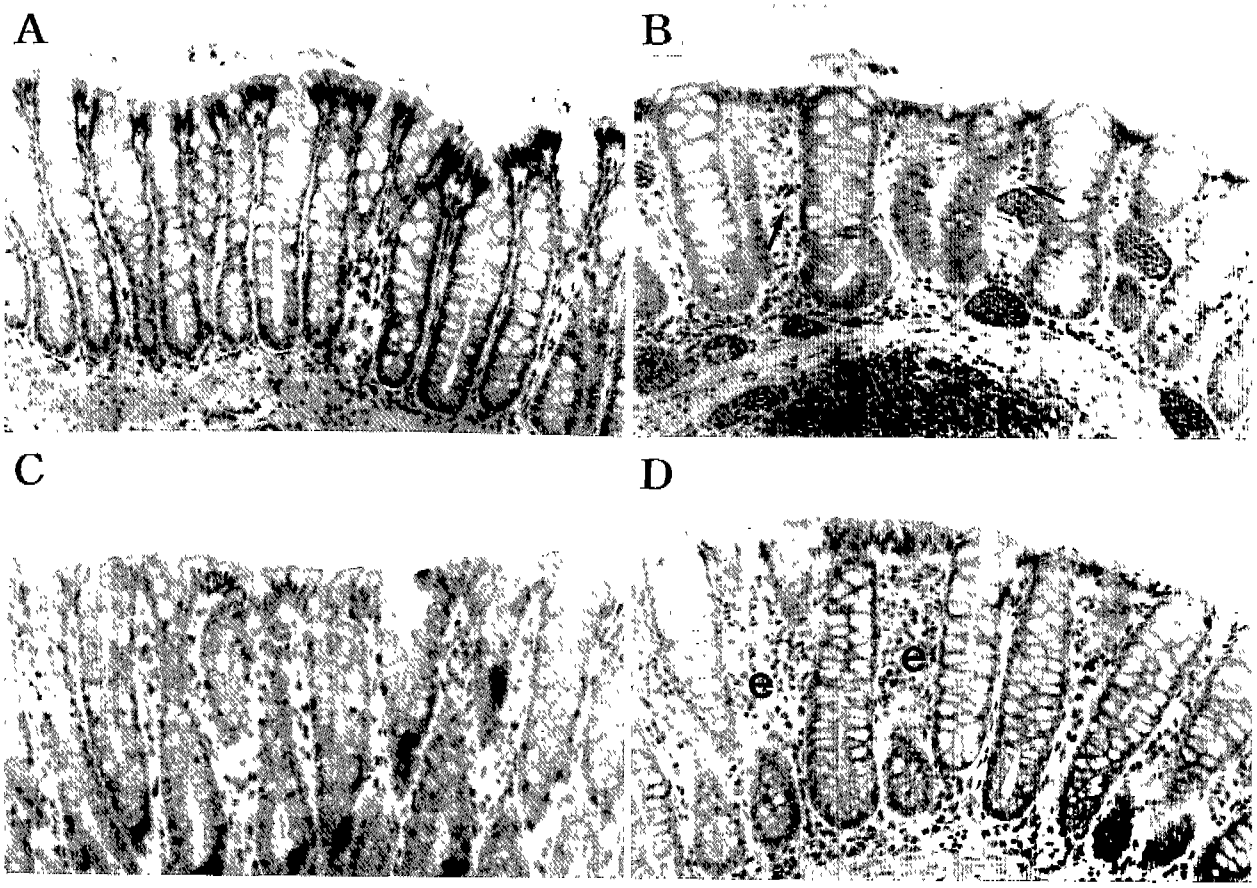

Fig. 3. Morphological changes in the colonic mucosa in the 15 days following local abdominal irradiation (30 Gy). (A) Normal (× 100). (B) Radiation control (×100); in contrast to Figure 3A, note the loss of colonocytes and swelling of periglandular lamina propria (arrows); suggestive of edema. Hyperplasia of regional lymphoid cells is also evident. (C) Colonic mucosa from a rat treated with radiation and DA-9601 (100 mg/kg); showing almost normal architecture except minimal edematous change of the lamina propria. Note intact colonic epithelium (×100). (D) Radiation and sucrafate (300 mg/kg) enema group (× 100); edema and infiltration of PMNs (mainly eosinophils) of periglandular lamina propria are observed (e).

Table IV. Effect of DA-9601 and sucralfate on radiation-induced histologic change of colon in rats

Group	Normal	Radiation control	Radiation+Sucralfate (mg/head)		Radiation+DA-9601 (mg/kg)	
			100	300	30	100
Histologic alteration index[a]	0.25±0.16[b]	2.63±0.26	1.88±0.23	1.63±0.18	1.25±0.31[b]	0.75±0.25[b]

[a]Histologic lesion index was scored according to the criteria described in the text. [b]Significantly different from radiation control ($P<0.05$). Each value represents the mean±SEM of 7 to 8 rats.

Table V. Effect of DA-9601 and sucralfate on myeloperoxidase (MPO) activity in colonic mucosa from the rat receiving a single radiation

Group	Normal	Radiation control	Radiation+Sucralfate (mg/head)		Radiation+DA-9601 (mg/kg)	
			100	300	30	100
MPO activity[a]	5.97±0.03[b]	6.30±0.05	6.32±0.09	6.11±0.03	6.07±0.10	6.02±0.02[b]

[a]Unit/mg of protein. [b]Significantly different from radiation control ($P<0.05$). Each value represents the mean±SEM of 7 to 8 rats.

and loss of colonocytes in animals treated with sucralfate (300 mg/head) after radiation, statistical significance was not obtained.

Mucosal MPO levels

MPO activities of colorectal mucosa were slightly but significantly increased after irradiation ($P<0.05$, Table V). Oral treatment with DA-9601 for 2 weeks protected the increase of the enzyme activity in a dose related manner,

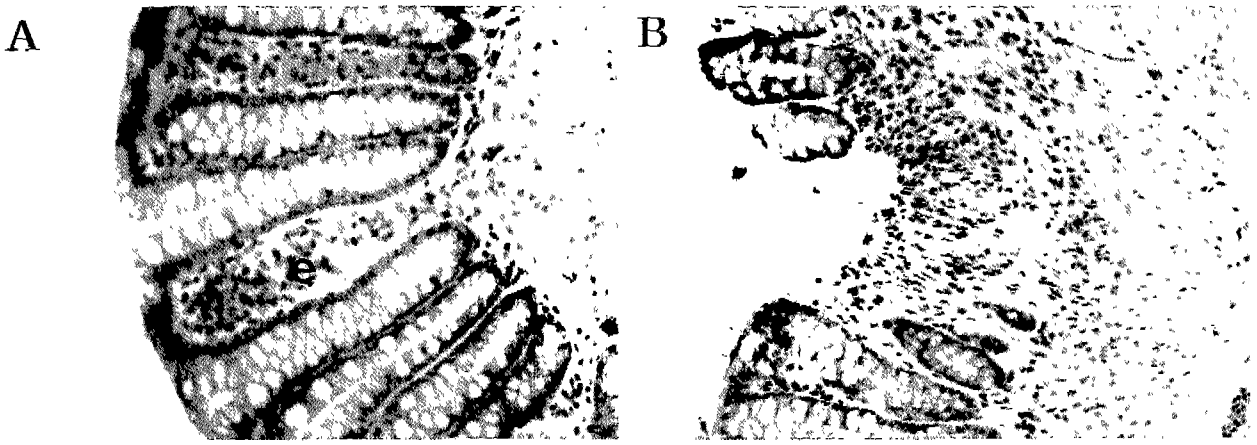

Fig. 4. (A) A photomicrograph of the colonic mucosa from a rat treated with radiation and sucralfate (100 mg/head); note eosinophils infiltrated to the edematous lamina propria (e) (×200). (B) A focal ulceration of colonic mucosa of a rat from radiation control group. Elevated ulcer bed and cellular infiltration is seen (×200).

and high dose of DA-9601 significantly prevented the increase of MPO to normal range ($P<0.05$). On the contrary, enema of sucralfate did not reduce MPO activities in colorectal tissue.

DISCUSSION

Radiation-induced enteritis is the most common complication of curative radiotherapy to the abdomen, pelvis, or rectum (Kochhar *et al*, 1991). The small and large bowel are very sensitive to ionizing radiation. Though the probability of tumor control increases as the dose of radiation increases, so does the damage to normal tissue. Acute adverse reaction to the intestines occur at approximately 10 Gy. Since curative doses for many abdominal and pelvic tumors range between 50 and 75 Gy, enteritis is likely to occur (Perez and Brady, 1987). Clinical manifestations of this disease include rectal bleeding, tenesmus, diarrhea, fistulas, and strictures (Gilinsky *et al.*, 1983). Acute radiation enteritis is a prerequisite for the development of the chronic syndrome because there has been no reported case without a prior history of acute enteritis (Donaldson *et al.*, 1975). Medical treatment of this condition has, unfortunately, not been addressed by many workers. There are a number of studies on the surgical therapy of radiation- induced bowel injury (Cooke and De Moor, 1981); however, as for other chronic radiation complications, these patients had high surgical complication rates (Buchi and Dixon, 1987). For this reason, emphasis has been placed on using nonsurgical methods, whenever possible (Buchi and Dixon, 1987). Methods to attenuate intestinal injury or to promote recovery after radiation exposure have focused on either topical or systemic administration of various agents. Steroid enema and oral sulfasalazine (Goldstein *et al.*, 1976), sucralfate (Kochhar *et al.*, 1988; Jahavi *et al.*, 1989; Henriksson *et al.*, 1990; Henriksson *et al.*, 1992), thiophosphate WR-2721 (France *et al.*, 1986), a 21-amino steroid U-74500A, vitamin E-like compound U-78518F (Delaney *et al.*, 1992), a prostaglandin E1 derivative, misoprostol (Empey *et al.*, 1992) and epidermal growth factor (McKenna *et al.*, 1994) have been used experimentally on this purpose. However, to elucidate the clinical usefulness of these compounds, a number of controlled clinical studies will be necessary.

In this study, we examined the protective effect of an *Artemisiae Herba* extract, DA-9601, against intrarectal radiation-induced colonic damage. In the results, oral administration of DA-9601 effectively alleviated acute clinical signs including diarrhea and bloody stool as well as macroscopic lesion index. Because these signs are major clinical manifestations in acute radiation-induced enteritis in human, it will be possible to use DA-9601 in order to control acute signs associated with abdominal radiation. In the previous study, it was found that DA-9601 reduces the frequency and severity of diarrhea in animal models of inflammatory bowel disease (Ahn *et al.*, 1997), which shares clinical signs with radiation proctocolitis in many respects. It is thought that this action of DA-9601 is from its mucoprotective effect, because DA-9601 lacks the effect on functional motility of intestine (Ahn *et al.*, 1997). And increased mucus secretion following DA-9601 adminstration may be helpful for mucosal re-

generation (Oh *et al.*, 1996).

A single intrarectal radiation of 30 Gy dose did not affect the body weight of rats in this study, though most of animals from radiation control showed emaciation of the face and limbs. Most of these rats showed pot belly-like abdominal distension due to intestinal dilatation filled with feces, which compensated for weight loss.

Histologically, the morphologic changes observed in the present study following colonic radiation were generally consistent with those reported previously by others (Guzman-Stein *et al.*, 1988; McKenna *et al.*, 1994). Eosinophilic infiltration to the affected mucosa was one of major histological findings in radiated animals. Hasleton *et al.* (1985) reported that excessive eosinophilic infiltrate was noted in both acute and chronic phase reaction. In most cases, this infiltrate was confined to the periglandular lamina propria. Possibly, this mucosal reaction is due to a long-lasting alteration of the antigenic character of epithelial cells induced by early radiation damage.

In the present study, radiation induced significant increase of mucosal myeloperoxidase (MPO) activity. The reduced MPO activity by oral administration with DA-9601 may explain, in part, the protective effect of DA-9601 against radiation-induced colonic injury, because PMN-mediated oxidative stress was reported to play an important role in many gastrointestinal disorders (Sanchez *et al*, 1996; Yamada *et al.*, 1993) and the reduction of MPO activation was reported to be beneficial to attenuate the injury in these diseases (Park, 1989; Tepperman *et al.*, 1993).

Interestingly, the small bowel was shown to be more sensitive to radiation damage than large bowel in this study. The frequency of intestinal adhesion, ulceration and transmural inflammation was more common in jejunum and ileum than colorectum (data not shown), although we adopted intrarectal brachytherapy system which gives higher dose to colorectal tissue than small intestine.

In summary, data from this study showed that in animals exposed to acute colorectal radiation, repeated oral treatment with DA-9601 decreased the frequency of diarrhea and histologic alteration as well as mucosal MPO level, a marker of neutrophil infiltration. These results illustrate that there might be a role for DA-9601 in the prevention or treatment of radiation-induced colorectal symptoms, and that the underlying mechanism of radioprotective effect of DA-9601 might be associated with its inhibition of neutrophil infiltration to affected mucosa, at least in part.

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