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Radioprotective Effects of Granulocyte-Colony Stimulating Factor in the Jejunal Mucosa of BALB/c Mice

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INTRODUCTION

Radiation therapy, intended mainly for sterilization of cancer cells, is usually accompanied by adverse effects on the normal tissues surrounding the tumor mass (1, 2). They may lead to difficulties in continuing or completing the radiation therapy and ineffective treatment results. Especially in rectal cancer or uterine cervical cancer where the radiation fields include abdomen and pelvis, it is inevitable to radiation-induced gastrointestinal mucositis.

There have been many efforts to reduce the radiation morbidity using physical methods or radiobiological methods such as radiation response modifier (2). Many of the traditional remedies for radiation injury can be discarded as they have been shown to be ineffective or even harmful (3).

Granulocyte-colony stimulating factor (G-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF) is a hematopoietic growth factor and a cytokine that has been widely used to treat neutropenia caused by chemotherapy or radiotherapy. The efficacy of recombinant human hematopoietic growth factors in improving oral mucositis after chemotherapy or radiotherapy has been demonstrated in some clinical studies recently (3, 4, 5). But, the mechanisms of the beneficial effects and the most effective route for administration of G-CSF or GM-CSF on oral mucosal ulceration are unclear.

This study was designated to determine whether G-CSF could modify the radiation injury of the jejunal mucosa in irradiated BALB/c mice.

METHODS AND MATERIALS

Male BALB/c mice aged 4 to 5 weeks and weighing an average of 20g were obtained from the Catholic University of Korea, Catholic Medical Center. The mice were kept under controlled conditions. One hundred and five BALB/c mice were divided into nine groups including G-CSF alone group (I: 10 μ g/kg or II: 100 μ g/kg), radiation alone group (750 or 1200 cGy on whole body), combination group (G-CSF I or II plus 750 cGy, G-CSF I or II plus 1200 cGy), and control group.

Radiation was administered with 6 MV linear accelerator (Mevatron MX-2, Siemens, USA) at a dose rate of 300 cGy/min without anesthesia on Day 0. RhG-CSF (Neutrogin, Korea) was injected subcutaneously for 3 days, once a day, from Day-2 to Day 0. According to the waiting period after whole body single irradiation, each group except the normal control was subdivided into Day 1, Day 3, and Day 7 groups with or without administration of G-CSF. Each group contained 5 mice.

Mice were killed by decapitation on the Day 1, Day 3, and Day 7. Proximal jejunum of 5-cm lengths were taken to prepare longitudinal and cross sectional tissue samples of bowel. All tissues were immediately rinsed with saline, pinned to cork board and immersed in 10% formalin. The intestinal specimens for crypt counts were not opened and cross sections were taken perpendicular to the long axis of the bowel, such that an intact circumferential cross section was obtained. Specimens for histologic grading were opened along the antimesenteric border and pinned with the mucosal surface up. Longitudinal axial sections were subsequently cut from these samples. Five-micron sections were prepared after paraffin embedding, and stained with hematoxylin/eosin (H & E) or with periodic acid-Schiff (PAS).

As one index of radiation injury, intestinal crypts were counted by the method of Withers and Elkind (6). Data were expressed as crypts per circumference. And microvillus lengths as another index of radiation injury were measured. For additional estimation of injury, the histologic damage scores from Mulholland's criteria (7). All results were expressed as the mean$\pm$SD.

Each group of mice was compared to the control group using Student's *t*-test for the crypt counts and microvillus lengths and the Wilcoxon rank sum test for the histologic damage scores. Significance was assessed at 95% confidence intervals. One way ANOVA (analysis of variance) and multiple comparison were also used to compare the significance of irradiation, G-CSF, and a combination of both.

RESULTS

Intestinal crypt counts, microvilli length, and histologic damage scores

The mean crypt number in the irradiated con group was 138$\pm$5.4/circumference. In both the G-CSF I and II groups, crypt number, microvilli length, and histologic damage scores were not significantly different from those in the control one ($p > 0.05$). The 750 cGy and 1200 cGy radiation alone group showed lower crypt count, shorter microvilli length, and higher histologic damage scores than the control one ($p < 0.05$). The groups

with 750 cGy radiation plus G-CSF I and II showed significantly higher crypt counts and lower histologic damage scores on the Day 3, and lower histologic damage scores on the Day 7 compared with those of the 750 cGy radiation alone one ($p < 0.05$)(Table 1, 2). The 1200 cGy radiation plus G-CSF I or II group did not show significant difference in crypt counts, microvilli length, and histologic damage scores compared with those of the 1200 cGy radiation alone one ($p > 0.05$). Most of the mice in 1200 cGy radiation with or without G-CSF group showed intestinal death within 5 days.

Table 1. The mouse jejunal crypt counts in 750 cGy irradiation and combination (irradiation + G-CSF) groups (n=5).

Day	G-CSF dose (μg/kg/day)		
	0	10	100
1	125.8±4.02	128.0±5.4	125.2±4.0
3	95.8±4.02	110.2±2.6*	111.8±4.5*
7	113.2±4.3	115.0±4.5	117.6±4.4

* : P < 0.05

Table 2. The histologic damage scores in 750 cGy irradiation and combination (irradiation + G-CSF) groups in mice (n = 5)

Day	G-CSF dose (μg/kg/day)		
	0	10	100
	median (range)	median (range)	median (range)
1	3 (2 - 4)	2 (1 - 4)	3 (1 - 3)
3	8 (7 - 10)	5*(2 - 6)	4*(2 - 5)
7	5 (3 - 7)	2*(1 - 4)	2*(1 - 3)

* : $P < 0.05$

Microscopic findings in intestine

Figure 1 shows normal jejunal villous architecture (Fig. 1A), and PAS positive mucus cells are seen in the full length of villi (Fig. 1B). On the first day after 750 cGy irradiation, mouse jejunal crypts and villi are little damaged, but individual cell necrosis in the crypt epithelium is noted. On the third day after 750 cGy irradiation alone, mouse crypts are distorted and depopulated in cells. The villi are shortened, fused, and eroded (Fig. 2A-1). Inflammatory infiltrations are noted in the lamina propria (Fig. 2A-2). On the third day after combination of 750 cGy and G-CSF I, the number of crypts are less decreased and the shapes of villi are less shortened and distorted than those of radiation alone group (Fig. 2B-1, 2). On the seventh day after 750 cGy irradiation, the evidence of regeneration in the crypts are observed but the shapes of villi are still shortened and fused (Fig. 3A). In the combination group of 750 cGy irradiation and G-CSF I, almost complete regeneration of crypts and villi are noted (Fig. 3B). On the third day after 1200 cGy irradiation with or without G-CSF, the number of crypts is markedly decreased and the villi are severely shortened and eroded. Inflammatory infiltrations and focal hemorrhage are observed.

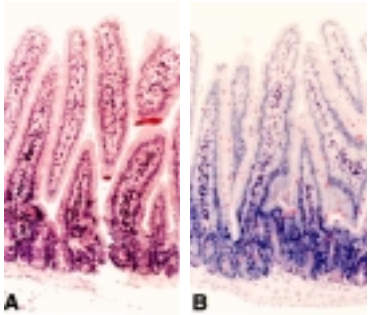

Fig. 1. Normal mouse jejunum. A, H-E stain, x100. B. PAS stain, x100.

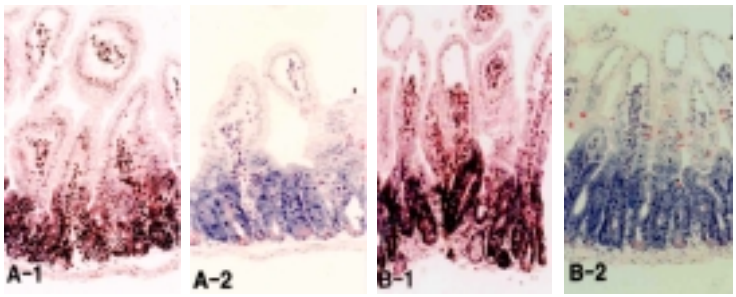

Fig. 2. Mouse jejunum 3 days after 750 cGy irradiation. A. Radiation alone, (A-1. H-E stain, x100. A-2. PAS stain x100). B. Radiation + G-CSF I. (B-1. H-E stain, x100. B-2. PAS stain x100)

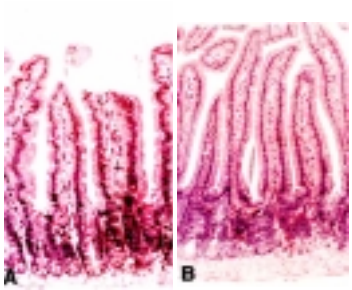

Fig. 3. Mouse jejunum 7 days after 750 cGy irradiation. A. Radiation alone (H-E stain, x100). B. Radiation + G-CSF I (H-E stain, x100)

DISCUSSION

Radiation repair, a classical mechanism of radiomodification, has in recent times been defined broadly as any processes for replacing damaged base pairs, absorbing or detoxifying free radicals, altering the cell cycle, or inhibiting apoptosis and other cellular phenomena that influence the fixation of DNA damage. Typically, improved repair is manifest on a radiation dose-response curve by differences in the shoulder and/or slope. Several cytokines are known to augment the shoulder of the dose-response curve of hematopoietic progenitor cells in vitro including interleukin 1 (IL-1), granulocyte-macrophage colony- stimulating factor (GM-CSF) and G-CSF (8). G-CSF and GM-CSF belong to a family of glycoprotein growth factor, which promote the proliferating and differentiation of neutrophil and monocyte/ macrophage precursors and enhance the effector functions of mature neutrophils in vitro and in vivo. Recently, many of the major hematopoietic growth factors that have been produced from recombinant DNA technology in highly purified form have entered clinical trials. The action of these glycoprotein molecules was thought to be restricted to cells of hematopoietic cells only, but laboratory studies have shown that both G-CSF and GM-CSF influence the migration and proliferation of human endothelial cells, suggesting that these molecules may act as regulatory signals outside the hematopoietic system (9). Clinical experience has also shown that these regulating proteins could stimulate the cells of the mucous membranes of the oropharynx. Chi et al who conducted a prospective clinical controlled study, observed that GM-CSF had a beneficial effect in the reduction of the severity and duration of chemotherapy-induced oral mucositis (10). Kannan et al, who evaluated the subcutaneous administration of GM-CSF on the severity of radiation mucositis, observed a mucosal protection by GM-CSF during radiotherapy in patients with head and neck carcinoma (11). Nicolatou et al observed that local administration of GM-CSF significantly reduced and almost healed radiation-induced oral mucositis in 14 of 17 patients during radiotherapy (12). Karthaus showed that topical oral G-CSF mouthrinses could be beneficial to reduce oral mucositis in randomized placebo-controlled trial (13). However, in vivo radiomodifying effects of G-CSF or GM-CSF have not been tested for normal tissues of gastrointestinal tract except oral mucosa.

The lining of jejunum is a classic example of a self-renewal system with rapid turnover of mucosal cells. Acute effects of radiation on the small intestine are related to the rapid turnover rate. Within 24 hours of a single dose of 5 to 10 Gy, the mitotically active cells of the crypts show maximum destruction. Reepithelization is extensive within 96 hours (14). At the dose more than 10 Gy, a significant proportion of the dividing cells in the crypts is sterilized and unable to regenerate (15). Our data indicate that G-CSF significantly protected against acute radiation reactions in mouse intestine. The radioprotective effect on the jejunal mucosa by G-CSF was significant in the 750 cGy radiation groups but not in the 1200 cGy ones. The latter dose may have been too high, since most of the mice died within 5 days after 1200 cGy whole-body irradiation. We used recombinant human G-CSF (rhG-CSF), which has been shown to be species-crossreactive (16). The timing, duration and dosing of G-CSF for radioprotection against hematopoietic syndrome have been variable in many studies (17, 18) and the use of G-CSF for radioprotection against gut mucosa has not been tried. Uckun et al observed that in vivo

administration of rhG-CSF before radiation protected mice from the lethal effects of TBI due to G-CSF-induced increase in the ability of bone marrow progenitor cells to repair sublethal radiation damage (17). In the present study, we administrated G-CSF (10 μ g/kg or 100 μ g/kg) to the BALB/c mice during consecutive 3 days before TBI so that the constant blood level of G-CSF was maintained .

The mechanism of the beneficial effect of G-CSF or GM-CSF on oral mucosal ulceration in recent clinical studies is unclear. Spickermann et al suggested that the protective effect of G-CSF is possibly due to local activation of neutrophils (19), since it is well known that G-CSF stimulates migration, phagocytosis, and cytotoxicity of neutrophils, the precise mechanism, however, being unknown and possibly multimodal. So, much research work is needed and the optimal administration schedules of G-CSF for radiation injury under varying conditions still need to determine. Also, further studies are required to evaluate the effect of G-CSF on the normal tissues as well as the tumor tissues. Future studies focusing on radioprotection of the bowel during fractionated irradiation will be important for determining the clinical utility of G-CSF.

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