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A Rat Model for Radiation-Induced Proctitis

Radiation proctitis is a frequent acute complication encountered with pelvic irradiation. This study was aimed at establishing the optimal radiation dose for radiation-induced proctitis in rats. Female Wistar rats were used. The rectal specimens were examined morphologically at 5th and 10th day following 10-30 Gy irradiation in single fraction. With increasing dose, mucosal damage became worse, and there was a prominent reaction after ≥15 Gy. We selected 17.5 Gy as an optimal dose for radiation proctitis and examined specimens at day 1-14 and at week 4, 6, 8, and 12 after 17.5 Gy. The rectal mucosa revealed characteristic histological changes with time. An edema in lamina propria started as early as 1-2 days after irradiation and progressed into acute inflammation. On day 7 and 8, regeneration was observed with or without ulcer. Four weeks later, all regeneration processes have been completed with end result of either fibrosis or normal appearing mucosa. This study showed that the radiation injury of the rectum in rat develops in dose-dependent manner as it has reported in previous studies and suggested that 17.5 Gy in single fraction is the optimum dose to evaluate the protective effect of various medications for radiation proctitis in face of the clinical situation.

Key Words: *Proctitis; Radiation; Rats*

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INTRODUCTION

Radiation treatment for pelvic malignancies frequently induces gastrointestinal side effects including enteritis and proctitis. Patients often complain of frequent bowel movements with heavy sensations like tenesmus due to swollen rectal tissues. These side effects can cause treatment interruption and result in decreased tumor control. The rectum, in particular, is frequently exposed to high dose radiation in the treatment of rectal cancer, cervical cancer or prostate cancer. Most acute side effects in rectum subside after completion of radiation therapy, but in some cases, it can be a cause of severe chronic complications. Chronic rectal complications occur in 2-5% of pelvic irradiation (1-4) and demise the patients' quality of life.

Common medical therapy for radiation-induced rectal complications in clinical practice has been the usage of nonsteroidal anti-inflammatory agents such as sulfasalazine, aspirin and ibuprofen, oral or per rectal corticosteroids and bile-acid sequestering resins (3, 5-9). However, these drugs are not sufficiently effective and have only a limited benefit.

Most studies on radiation-induced proctitis in rats evaluated the incidence of apparent or definite rectal obstruction after radiation therapy as an end point (10-16). However, complications such as intestinal or rectal obstruction are severe side effects (grade 4) which occur rarely in clinical practice after an appropriate radiation therapy for the pelvic malignancies (17).

This study was aimed at establishing the optimal radiation dose in rat model for radiation-induced proctitis in face of the clinical situation. Future studies with this rat model are to evaluate the mechanism of radiation-induced inflammatory reaction and the effective drugs to control or prevent the radiation-related rectal reactions.

MATERIALS AND METHODS

Animals

Female Wistar rats, weighing 140-200 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 the laboratory of Dong-A Pharmaceutical Company, which was maintained at 22±3℃ and a relative humidity of 55±10% with a constant 12 hr light/dark cycle. Rats were housed in standard wire cages

and fed with standard rodent chow (Cheil, Korea) and UV-sterilized tap water. After irradiation, each animal weighed and examined every 2 days until the day of sacrifice.

Irradiation

Each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (Somnopentyl, 40 mg/kg). Then, 2 to 3 rats at a time were restrained and taped by the tail on one acryl plate with supine position. Lead shielding (5 half value layer) was used to cover the rat except 3×4 cm area of low pelvis containing 2 cm length of rectum in the middle of the field. Irradiation was delivered on LINAC, 6 MV with the distance of 100 cm from the source to surface. Dose rate of the irradiation was 200-300 cGy per min. To each group of 10 rats, 9 different radiation doses in single fraction (0, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, and 30 Gy) were delivered. Five rats were sacrificed each on 5th and 10th day after irradiation for gross and histologic evaluation. Based on the dose response results, a dose of 17.5 Gy was chosen for subsequent study to evaluate the time sequential changes. Total of 90 rats were irradiated with single exposure of 17.5 Gy. Five animals were selected randomly for gross and histological evaluation on postirradiation day 1 through day 14 and on week 4, 6, 8 and 12.

Evaluation of rectal damage

The rectums were grossly evaluated and scored as following (18): 0=normal mucosa; 1=edema, mild hyperemia or decreased vascularity; 2=diffuse hyperemia, multiple punctuated areas of hemorrhage, or confluent areas of hemorrhage; 3=presence of erosions or frank hemorrhage; and 4=ulcers. For histologic evaluation, a portion of specimen was excised, fixed in 10% neutral buffered formalin solution, and processed by routine techniques. Each specimen was then stained with hematoxylin and eosin, and was examined with a light microscope by a pathologist blinded to the study. Each specimen was graded as follows (18): 0=normal or minor alterations which cannot be correlated to radiation with certainty; 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, and/or variable degree of inflammation); and 4=severe damage (ulcer, necrosis).

Statistics

Results are presented as mean±SD. The relationship between radiation dose and grade of rectal damage, gross and microscopic, was tested by Spearman rank correlation.

RESULTS

Mortality

There were total 5 deaths after irradiation: one rat each in 10 and 20 Gy irradiation group died from anesthesia on day 1, and 1 in 22.5 Gy and 2 in 30 Gy irradiation group died from diarrhea and dehydration on day 4.

Dose response to irradiation

The scores of gross mucosal change are listed in Table 1. There was a dose dependent change at the 5th day after radiation ($p<0.05$). No mucosal damage was noted in the lower dose groups of 10 Gy to 15 Gy irradiation. With radiation doses between 17.5 Gy and 25 Gy, the minimal mucosal changes appeared in some rats. In higher dose of radiation, majority of rats showed grade 1 mucosal damage. Even on the 10th day after irradiation, there were absolutely no mucosal changes in groups that received 15 Gy or less. After 17.5 Gy radiation, grade 1 mucosal change was noted in 2 out of 5 rats. With 20 Gy and higher dose of radiation, grade 1 or 2 mucosal changes were noted in all rats. These mucosal damages were mostly observed in the distal portion of irradiated rectum, and not in the proximal end.

There was no microscopic change with 10 Gy irradiation (Table 2). However, with 12.5 Gy irradiation, some rectal specimens showed grade 1 microscopic mucosal

Table 1. Relationship between gross mucosal changes and radiation dose (mean±SD)

Dose	Gross mucosal change	
	5th day	10th day
Control	0	0
10 Gy	0	0
12.5 Gy	0	0
15 Gy	0	0
17.5 Gy	0.2±0.4	0.4±0.5
20 Gy	0	1.2±0.4
22.5 Gy	0.3±0.5	1.6±0.5
25 Gy	0.2±0.4	2.6±1.1
27.5 Gy	0.8±0.4	2.2±1.3
30 Gy	0.8±0.5	1.0±0.6

See materials and methods for grading criteria

Table 2. Relationship between histologic rectal changes and radiation dose (mean±SD)

Dose	Histologic rectal change	
	5th day	10th day
Control	0	0
10 Gy	0	0
12.5 Gy	0.2±0.4	0.4±0.5
15 Gy	2±0	2.6±0.5
17.5 Gy	2±0	2.4±1.3
20 Gy	1.8±0.4	3.8±0.4
22.5 Gy	1.4±0.5	4.0±0
25 Gy	1.2±0.4	4.0±0
27.5 Gy	1.8±0.4	4.0±0
30 Gy	1.2±1.1	3.4±1.3

See materials and methods for grading histologic changes

changes on the 5th and 10th day after irradiation (Fig. 1). The dose higher than 15 Gy caused obvious changes in all the rectal specimens. These mucosal damages were worse in the 10th day specimens as compared to the 5th day specimens. With 15 Gy and 17.5 Gy irradiation, the most common histologic findings were grade 2 and 3 changes. (Fig. 2). With 20 Gy and higher dose irradiation, grade 4 damages were most commonly observed (Fig. 3). These microscopic changes correlated well with radiation doses and proved to be statistically significant ($p<0.05$).

Comparing the histologic changes between the 5th and 10th day specimens, the most prominent changes on the 5th day specimen were crypt dilatation with acute inflammation and crypt epithelial atrophy, and main findings on the 10th day of evaluation were regenerative epithelial changes with increased mitotic count and crypt architectural distortion.

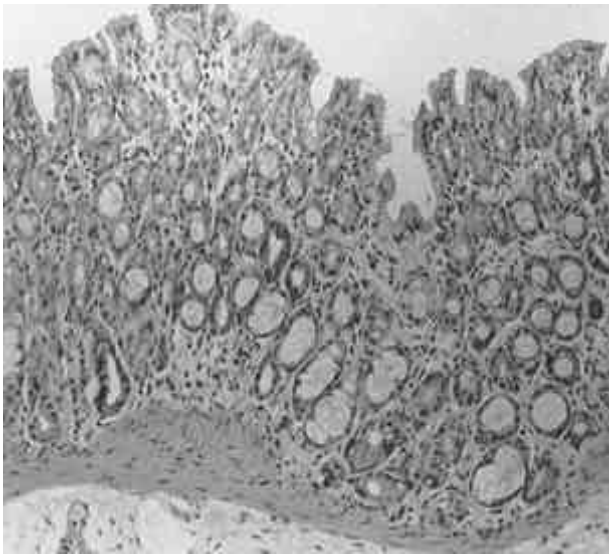

Fig. 1. Grade 1 histologic change after irradiation (H&E, ×200).

From the data shown above, we chose a single fraction of 17.5 Gy as an optimal radiation dose for a radiation proctitis model in rat.

Time-sequential changes after single dose, 17.5 Gy, irradiation

The most significant gross changes observed on given days after 17.5 Gy irradiation are as follows. A distinct mucosal damage was observed mainly on distal half of the irradiated area. Up to 4 days after irradiation, there was no obvious mucosal change. Then the irradiated rectum started to show edema on the day 5 and minute, multiple hemorrhages on the day 6 to day 8 after irradi-

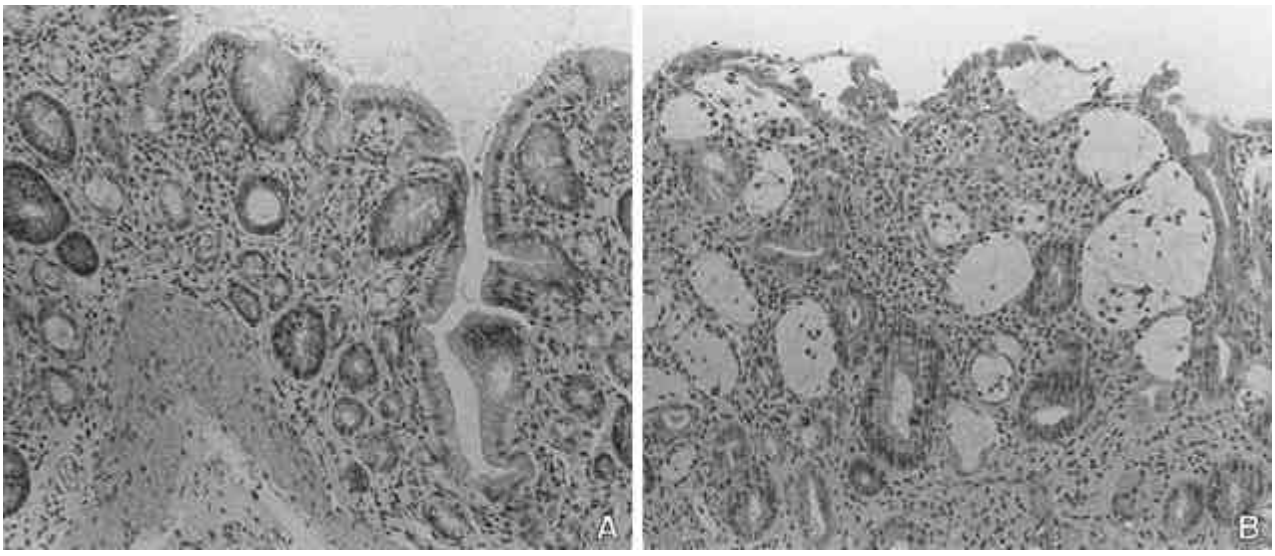

Fig. 2. A: Grade 2 histologic change after irradiation (H&E, ×200). **B:** Grade 3 histologic change after irradiation (H&E, ×200).

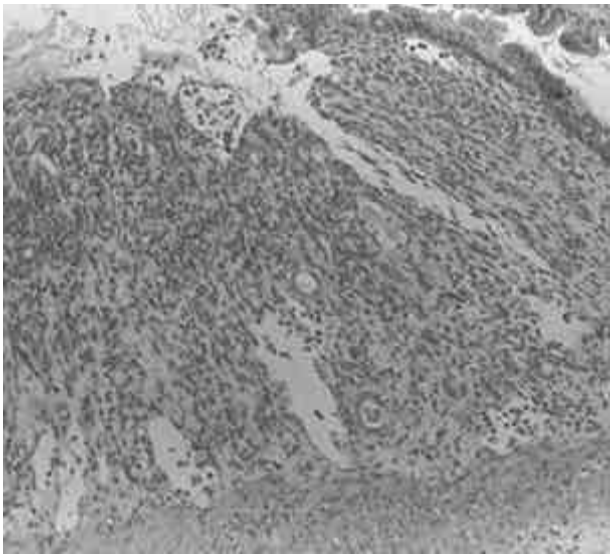

Fig. 3. Grade 4 histologic change after irradiation (H&E, ×200).

ation. Later, these changes progressed to confluent hemorrhage. Four weeks after radiation, most specimens became normal in appearances with some areas of chronic changes such as ulcer, scar or fibrosis.

The histologic changes over time are shown in Table 3 and Fig. 4A-I. There was early change of edema in the lamina propria on day 1 and 2 (Fig. 4A). Between day 4 and 6, the following changes were prominent: loss of goblet cells, infiltration of various inflammatory cells of mucosa and submucosa, some degrees of crypt dilation, formation of cryptic abscess, and atrophy of epithelium (Fig. 4B-C). On day 7-8, the distortion of crypt architecture dominated by the 'ghosts' of crypt was the main finding (Fig. 4D) and on day 9, the loss of most of the crypts and severe inflammation was observed (Fig. 4E). The erosion of surface epithelium which started on day 6 progressed into multifocal ulceration with severe inflammation until 10 day post-irradiation (Fig. 4F). On the other hand, regeneration started on day 7 and peaked at week 2 (Fig. 4G). After 4 weeks the regeneration process has been completed with features of colitis cystica profunda (Fig. 4H). With 3 months follow up after irradiation, crypt architecture was restored and fibrosis was found in some area of lamina propria (Fig. 4I).

DISCUSSION

Human rectum is relatively resistant to radiation. While chronic damages in small intestines occur in 15% to 25% of individuals receiving 50-55 Gy (19, 20), the incidence of severe proctitis is 7% to 8% after 80-95 Gy irradiation (21, 22). However, most patients undergoing pelvic irradiation experience discomfort such as hypermotility of bowel and tenesmus. These reactions correspond to the edematous, hyperemic mucosal changes throughout the rectum. Gelfand et al. described acute changes in the mucosa of large bowel in a series of 11 patients biopsied before and during therapeutic radiation of the pelvis (23). During 10 to 20 days after the initiation of radiation therapy with doses of 10 and 20 Gy, respectively, there were atypical cells in the crypts with loss of nuclear polarity, enlarged nuclei and either loss of mucus production or swellings of goblet cells. Mitosis was reduced. One month after radiation all but mild atrophy or inflammation have receded, and three of seven biopsy findings at this time were entirely normal. During the acute phase of injury the submucosa may also show edema with a few eosinophils and other leukocytes in the stroma. Fibrin will be found in the submucosa in varying amounts; its organization may contribute to the severe submucosal fibrosis which is important in the late effects.

Hubmann reported the details of dose-effect relationships using rectal obstruction as the end point in female Wistar rats which is a sigmoid curve with a ED10 of

Table 3. Histologic changes by time after 17.5 Gy of radiation

Day after irradiation	Histologic findings
D 1-2	Edema in the lamina propria
D 4	Crypt dilatation with mild inflammation
D 6	Marked crypt dilatation with epithelial atrophy Inflammation with surface epithelial erosion
D 7-8	Beginning of focal regenerative change (crypt architectural distortion with increased mitotic count)
D 9	Multifocal ulceration with severe inflammation
D 10	Ulceration with more prominent regenerative change
D 14	Healing of ulceration with extensive regenerative change
W 4-6	Complete regeneration, fibrosis with features of colitis cystica profunda
W 8-12	Minimal inflammation with fibrosis in the lamina propria

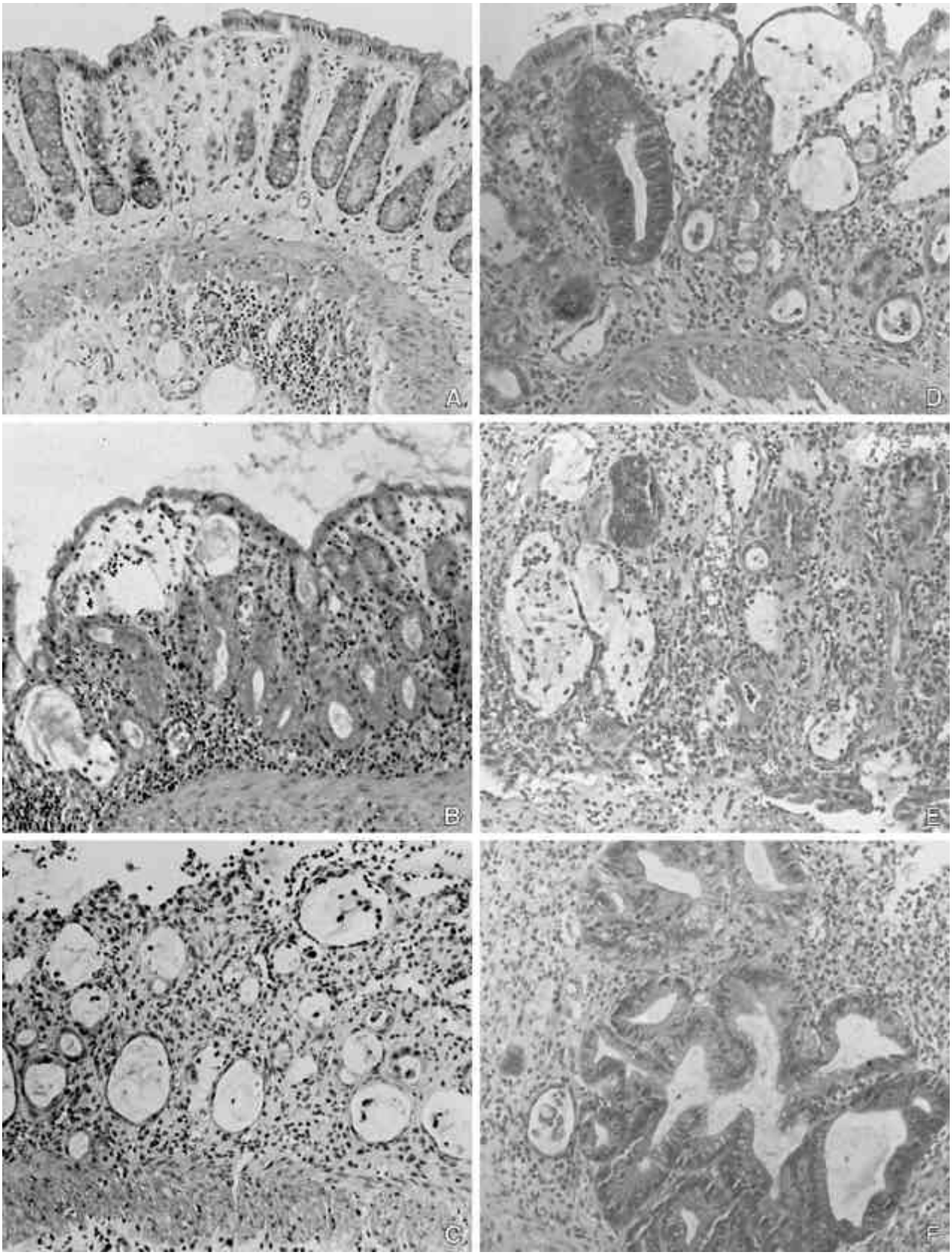

Fig. 4. The histologic changes by time sequence after 17.5 Gy irradiation in a single fraction (H&E, ×200). **A:** Day 1-2, **B:** Day 4, **C:** Day 6, **D:** Day 7-8, **E:** Day 9, **F:** Day 10. *(Fig. 4 continued next)*

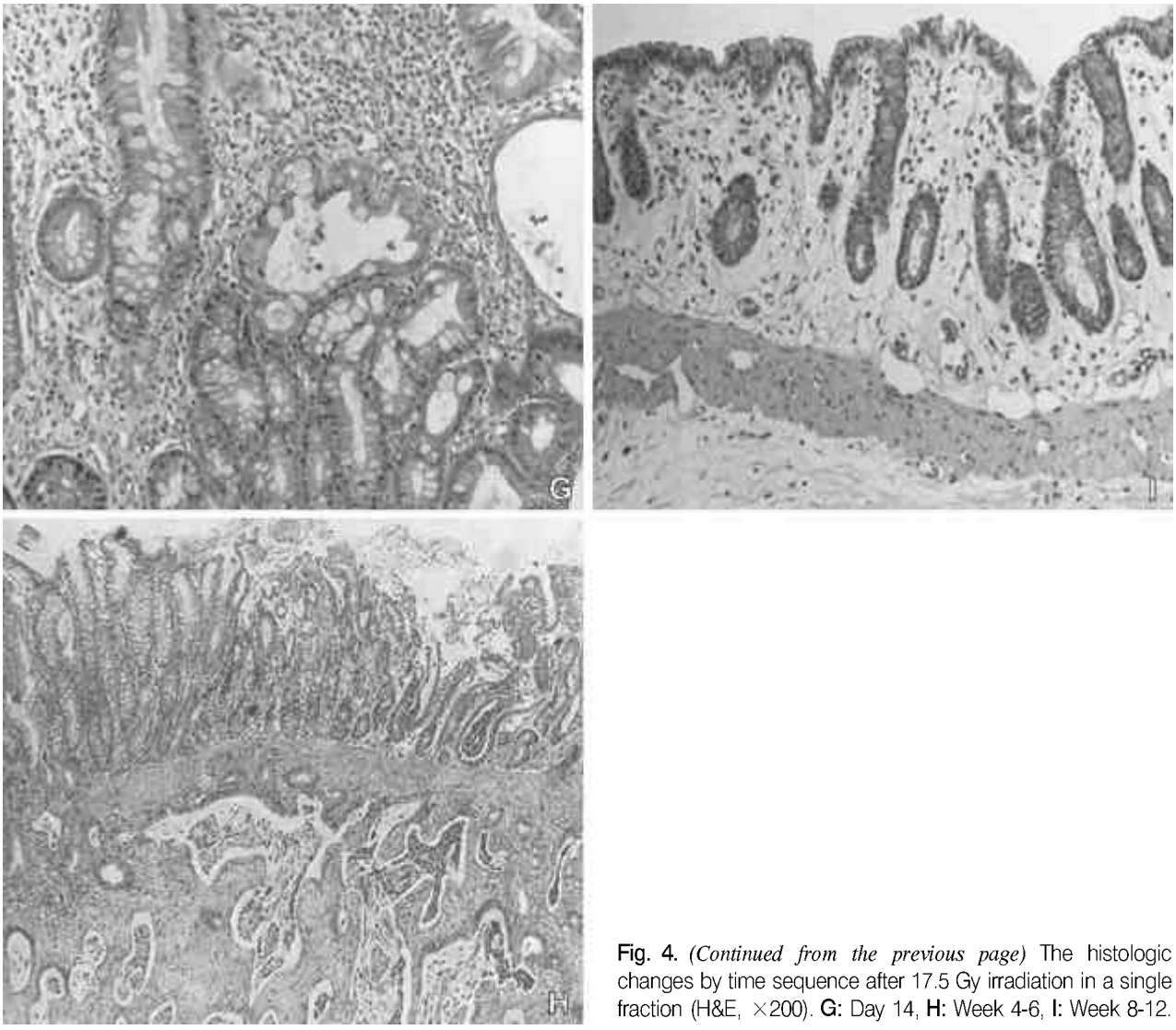

Fig. 4. *(Continued from the previous page)* The histologic changes by time sequence after 17.5 Gy irradiation in a single fraction (H&E, ×200). **G**: Day 14, **H**: Week 4-6, **I**: Week 8-12.

17.5 Gy, ED50 of 21.5 Gy and ED90 of 27.5 Gy (16). He found the latency time shortened exponentially with increasing dose and is 150 days at the ED50. He noticed a hemorrhagic radiation proctitis between the 7th and 16th day after irradiation. There were acute side effects of proctitis, which were transient, and the late reactions of fibrosis and rectal stricture with luminal obstruction and death. He suggested that rectal obstruction after irradiation was considered to be a good indicator of radiation damage and has been used as an experimental model to measure the extent of radiation injury in rats. Other studies confirmed that ED50 for acute irradiation was just over 20 Gy as shown in Hubmann studies (10, 12, 15).

Northway presented a method for sequential observations in the same animal without sacrifice and with minimal trauma to the animal (18). He found a good association between the sequences of endoscopic and histologic changes. He chose 22.5 Gy as the dose of irradiation for evaluation of effects of anti-inflammatory agents. The histologic score at the 10th day after 22.5 Gy irradiation was 2.8 ± 0.3 which meant most specimen revealed moderate damages as seen in our study. He also reported that the earliest endoscopic changes consisted of edema and loss of normal vascular pattern even at the 1st day. These changes progressed from mild erythema to punctuate hemorrhage or to confluent areas of hemorrhage. Buell also found very early increased plasma volume, indicative of an increased microvascular permeability within the tissue by 4 to 8 hr following 10 Gy irradiation in male Sprague-Dawley rat model of local abdominal irradiation (24). This finding of early edematous change could explain why patients receiving pelvic irradiation often complained abdominal discomfort with gas distention even in the first week of radiation. In our external beam radiation study, there were clear dose

dependent radiation changes in rectum as was expected. Grossly and histologically, there were obvious changes with 17.5 Gy and higher irradiation but not with 15 Gy or less irradiation which caused minor changes only in part of specimens. With 20 Gy and higher dose of radiation, all the changes were severe which were more likely to be related to the late complications of fibrosis, rectal bleeding and rectal obstruction as noted in Northway and Hubmann data. Therefore, the authors suggest that a single fraction of 17.5 Gy irradiation is an ideal dose for the rat model of radiation-induced proctitis.

Several other animal studies are available for the evaluation of radiation-induced injury for the rectum of the rat. These studies used either external beam irradiation (13-16, 23, 25, 26) or brachytherapy, low-dose rate (LDR)/ high dose rate (HDR) (10-12, 27-30). One study on the incidence of late rectal stenosis of the rat reported a greater tolerance with endocavitary radiation than with external beam irradiation (30). This finding is similar to our initial observation in which there was very little rectal tissue damage even with 30 Gy, HDR (iridium-192) radiation to rectal mucosal surface using rectal tube (data not shown).

In summary, a single fraction of 17.5 Gy irradiation to the rat rectum is considered to be an optimal dose for clinically relevant proctitis where we experience frequent bowel discomfort from swollen tissue but with rare incidence of severe side effects such as death or rectal obstruction. Also this study confirms that the dose related radiation injury to the rat rectum is similar to previous data from other animal models.

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