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RADIATION EFFECTS IN THE COLON

An experimental study in the rat

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In an experimental study, resembling a clinical trial of preoperative irradiation, 10 + 10 Gy was given to the pelvic and lower abdominal region of rats with a 4-day interval. The early effect on the colonic wall was evaluated by myeloperoxidase activity and hydroxyproline content of the bowel wall and correlated to histological findings. Groups of animals were followed up to eight months after irradiation for evaluation of later effects. General effects of irradiation were seen as low WBC during the first week and delayed body weight development up to two months after irradiation. Local effect in the colonic wall was noted as an increase in myeloperoxidase activity (indicating a leucocyte accumulation) in irradiated parts of colon during the first 11 days and again significantly elevated after two months in parts of colon, irradiated as well as protected. This correlated well with histological findings of inflammatory reaction, atypia and dysplasia during the first 10 days after irradiation but not at two months after irradiation. Hydroxyproline content was not affected. There were no major complications due to irradiation seen in the late course of the study period.

The high recurrence rate after resection for rectal carcinoma (1) has motivated investigations of adjuvant therapy. Preoperative radiotherapy has been suggested and tried in various doses and schemes (2). The aim of the present study was to set up an experimental model, resembling the clinical situation of preoperative radiotherapy of rectal carcinoma used in Scandinavian trials (3, 4) and to evaluate the irradiation effect in the colonic wall with special reference to early inflammatory reaction as a basis for further studies of reparative processes in areas of irradiation.

Material and Methods

One hundred and sixty male Wistar rats (Möllegaard Ltd., Denmark) with an initial weight of 249 g (range 200–300 g) were used. The animals had free access to water and standard laboratory diet. Body weight was followed throughout the experiments at regular intervals.

The animals were randomly allocated into one of two groups. The treatment group was irradiated at two separate days, four days apart. The sham group did not receive irradiation but was else handled as the treatment group. Under general anaesthesia (chloral hydrate: 0.25 g/kg intraperitoneally) the rat was placed in a plastic shell with lead shields on both the anterior and the posterior side. In both shields field-openings of 3 by 4 cm (w × l) gave access to irradiation of the pelvic and lower abdominal region (Fig. 1). The location of the radiation field was previously checked by x-ray films with barium enema and the rat positioned in the shell. Through the two openings irradiation was given using two opposing fields with an anterior–posterior technique. Irradiation was given with 240 kV x-rays, 15 mA, filter 0.2 mm Cu, total filtration HVL 1.1 mm Cu. The focus-to-skin distance was 42 cm. The dose rate was 1.135 Gy/min and the total dose given was 10 Gy at each day of irradiation. The dose was homogenous within the irradiated volume. The dosage was previ-

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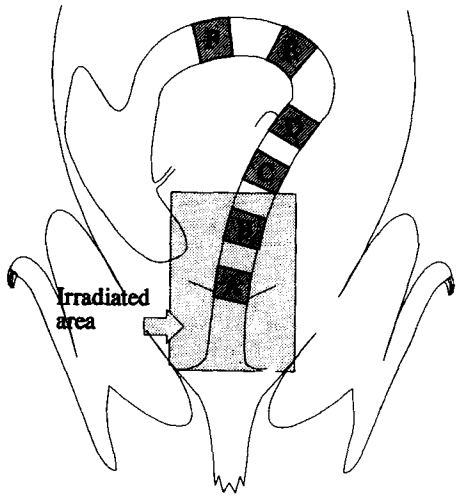

Fig. 1. Schematic view of irradiated area and segments of colon taken for analysis: A) at the peritoneal reflection. B) 1 cm, C) 2 cm and D) 3 cm above the peritoneal reflection. E) at the major flexure and F) at the transverse colon.

ously checked by a Farmer electrometer and an ion chamber placed in a rat phantom made of wax.

Blood samples were taken through a tailcut at days of irradiation and at sacrifice. Analysis of haemoglobin, albumin and white bloodcell count were made according to hospital routines.

Experiment 1. Eighty-four animals were used and after allocation to treatment/sham group they were again randomized to day of sacrifice: 0, 4, 7, 11, 30 and 60 days after the second irradiation/sham irradiation (at each day 8 animals for irradiation and 6 for sham irradiation). After sacrifice in a CO_2 box the abdomen was explored and the colon excised. Six standardized 5 mm colonic segments were taken (A–B within, C at the border and D–F outside the irradiation field) as marked in Fig. 1. Each segment was divided at the mesenteric and antimesenteric lines into two equal halves. One part was weighed and then dried to contant weight and subsequently analysed for hydroxyproline content (5) as a marker of collagen. The counterpart was analysed for myeloperoxidase as an indicator of neutrophil leukocyte accumulation (6).

Experiment 2. Seventy-six animals were used. Groups of animals were sacrificed in a CO_2 box 3, 10, 30, 120 and 240 days after the second irradiation. The abdomen was explored and two standardized colonic segments of 1 cm were taken from the left colon 1 cm above the peritoneal reflection (within the irradiation field) and from the transverse colon (outside the irradiation field). The specimens were fixed in formalin, stained with haematoxylin-eosin and taken for histological examination by one pathologist. Information was not given to him about treatment group, time interval from irradiation/sham or location of biopsy. Each specimen was evaluated for inflammatory reaction, atypia and dysplasia. Each parameter was judged by an arbitrary scale from 0 to 3 (normal, mild, moderate and severe reaction). Inflammatory reaction was judged according to Borgström et al (7). Atypia was judged according to degree of degenerative changes in the epithelium and gland crypts (8). Dysplasis was judged by nuclear changes of preneoplastic nature (8).

Statistical methods. The mean (m) and standard deviation (SD) were calculated. Comparisons of the means were carried out by Student's t-test for unpaired observations. Probability levels are represented by the following signs: * = $p < 0.05$ and ** = $p < 0.01$.

Results

Experiment 1. Animals receiving irradiation had a marked body weight reduction to approximately 80% on the seventh day after completion of irradiation. Body weight development was delayed in the irradiated group up to 60 days after irradiation (Fig. 2). There were no differences seen between groups in Hb and albumin (data not presented). WBC was significantly reduced in irradiated animals on the day of the second irradiation and four days after completion of irradiation (Fig. 3). During the first 11 days after irradiation myeloperoxidase activity increased in irradiated segments of the left colon with a maximum (a 20-fold increase) at 11 days after completion of irradiation. Up to the eleventh day this increase was not seen in segments outside the irradiation field (Fig. 4) but 60 days after irradiation myeloperoxidase was elevated in irradiated as well as in protected parts of colon (Fig. 5). There were no differences in hydroxyproline content between irradiated and control animals either in irradiated or in protected parts of colon irrespective of time after irradiation (data not presented).

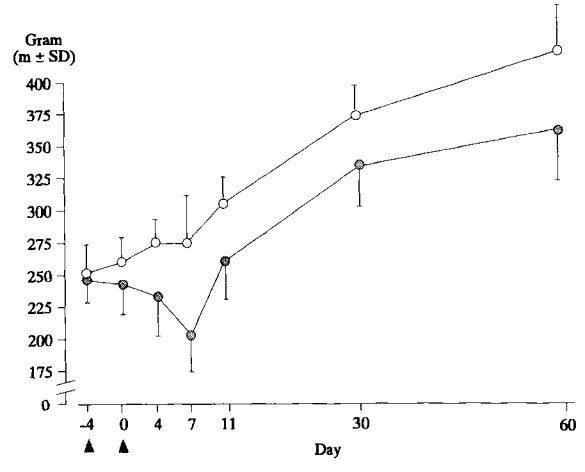

Fig. 2. Body weight development in animals in experiment 1. ▲ = days of irradiation; ● = irradiated animals; ○ = non-irradiated animals. Significant differences ($p < 0{,}01$) between groups from day 4.

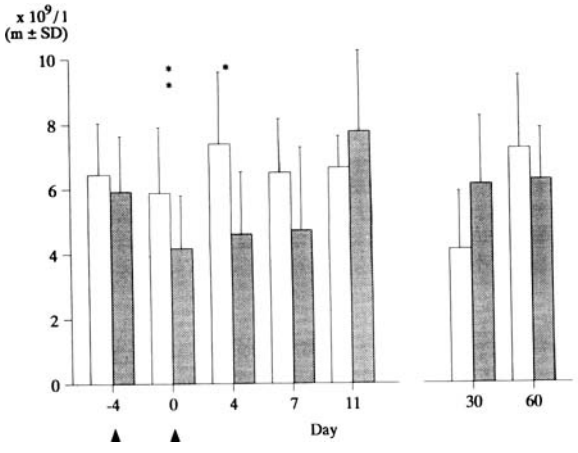

Fig. 3. White bloodcell count. ▲ = days of irradiation; ■ = irradiated animals; □ = non-irradiated animals.

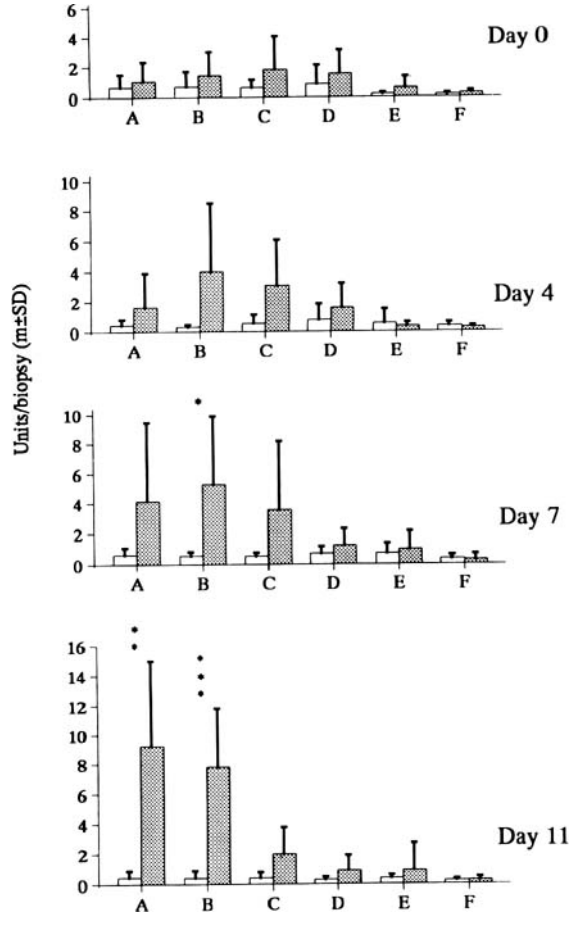

Fig. 4. Myeloperoxidase activity in different segments of colon (see Fig. 1 for details) at 0, 4, 7 and 11 days after completion of irradiation/sham. ■ = irradiated animals; □ = non-irradiated animals.

Experiment 2. In the left colon a moderate to severe inflammatory reaction and atypia was seen, 3 as well as 10 days after irradiation. In addition, dysplasia was observed in 6 out of 9 specimens 10 days after irradiation. A mild inflammatory reaction was noted in sham irradiated animals (Table). After 30 days there were no signs of inflammation, atypia or dysplasia with the exception of one animal in the irradiated group at 240 days with moderate inflammation and dysplasia. In the transverse colon no histological changes were observed during the study period.

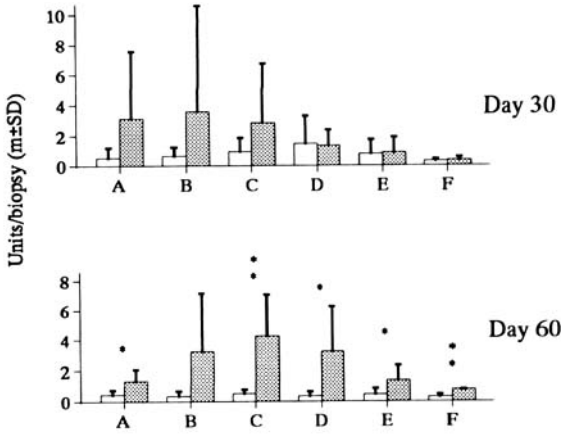

Fig. 5. Myeloperoxidase activity in different segments of colon (see Fig. 1 for details) at 30 and 60 days after completion of irradiation/sham. ■ = irradiated animals; □ = non-irradiated animals.

Table

Histological findings in the left colon days 3 *and* 10 *after irradiation.* 0 = *normal,* 1 = *mild,* 2 = *moderate and* 3 = *severe reaction.*

Day	n	Radiation	Inflammation				Atypia				Dysplasia			
			0	1	2	3	0	1	2	3	0	1	2	3
3	10	yes	0	0	10	0	0	0	10	0	10	0	0	0
3	6	no	4	1	1	0	5	1	0	0	6	0	0	0
10	9	yes	2	0	1	6	2	5	2	0	3	1	4	1
10	5	no	1	4	0	0	5	0	0	0	5	0	0	0

Discussion

The effect of radiation in the bowel is largely dose-dependent (9, 10) and it is important to study radiation effects within a range corresponding to clinical trials. In the present experimental study we were looking for a model simulating a clinical setting of fractionated radiotherapy of 5 Gy given daily for 5 consecutive days to the pelvic region (CRE = 14.59) used in Scandinavian trials (3, 4). In a pilot study in the rat, daily anaesthesia and irradiation at the dose of 5 Gy interfered with normal food intake and was accompanied with high mortality. Local irradiation in a 2-dose scheme of 10 + 10 Gy (CRE = 14.54) was better tolerated by the animals and thus chosen for the present study. With this low number of fractions it might be somewhat inadequate to use the CRE formula but by all events it can be stated that experimental setting fairly well corresponded to the clinical setting. The used technique gave a homogeneous irradiation dose in the rectum and the lower left colon.

Previous experimental studies in the rat on the effect of irradiation in the rectum–colon have dealt with irradiation tolerance applying different fraction schemes and irradiation modalities (11–14). Endpoints in these studies have been histological evaluation of damage and macroscopic lesions, such as ulcerations and rectal stenosis. The main purpose of our study was to evaluate the prerequisites of anastomotic healing in the colon in the early phase after irradiation. Thus, in experiment 1 we were looking for the early effects of irradiation with biochemical techniques and we wanted to correlate these findings with histology in experiment 2. Moreover, we wanted to evaluate if the irradiation in our model caused later histological changes or ulcerations/stenosis as previously reported in similar studies. Thus, in experiment 2 we followed groups of animals up to 8 months but did not see any major adverse effects as judged by complications and histology.

In experiment 1, general effects from irradiation were seen as an initial loss in body weight and delayed body weight development throughout the study period in animals receiving irradiation. Further, a reduction in WBC was observed during the early period after irradiation but in spite of this reduction no obvious infections were detected. The relatively large field size employed, compared to other similar studies, could contribute to the general effects observed but we wished to include the left colon in our field to make a study of anastomotic healing after irradiation possible.

The local effect in the colonic wall, as seen in histological specimens, was observed only within irradiated area and only during the first 10 days after irradiation. There was an acute inflammatory reaction accompanied by atypia at 3 days after irradiation. This was followed by dysplasia in 6 out of 9 animals ten days after irradiation. In some sham irradiated animals a mild inflammatory reaction was seen. This could be due to intraperitoneal injections of anaesthesia.

By studying myeloperoxidase activity in biopsies it is possible to quantify the inflammatory reaction after irradiation (15). This method has not previously been used in this context but is well defined in inflammatory bowel in experimental models in the rat (16, 17). This is a technique which could be further used in small biopsies in experimental as well as in clinical studies. A gradual increase in myeloperoxidase activity was seen in the left colon of irradiated animals with a maximum on the eleventh day after irradiation, indicating a successive accumulation of leukocytes in the early phase. This increase of myeloperoxidase was confined to the irradiated part of the bowel up to 11 days. The non-irradiated parts of colon showed no signs of inflammatory reaction, neither histologically nor in myeloperoxidase activity at this time. After two months there was an overall increase in myeloperoxidase not confined to the irradiated parts but more general throughout the colon. One explanation for this increase in myeloperoxidase activity might be that it originated from monocytes as being part of a chronic reaction throughout the colon not detected by histology. Localized trauma in the left colon have in other studies caused biochemical changes throughout the colon (18).

In the early course after irradiation there was a good correlation between myeloperoxidase activity and histological findings. Thus, in this period myeloperoxidase seems well suited as a quantitative marker for inflammatory reactions caused by irradiation. Later in the postirradiation course no obvious correlation between myeloperoxidase and histological findings was observed. A small but significant increase in myeloperoxidase activity was seen in irradiated as well as in non-irradiated parts of colon two months after irradiation while in histological specimens no evidence of chronic inflammation was seen after one month. Myeloperoxidase seems to be sensitive in monitoring inflammatory reaction and elevated myeloperoxidase activity may indicate later fibrosis.

Hydroxyproline content as a marker for collagen was studied primarily as a base for further studies on anastomotic healing as collagen plays an important role in the suture holding capacity of an anastomosis but it was also determined as an indicator of fibrosis. However, hydroxyproline content in the bowel wall did not differ from controls and according to this there was no sign of fibrosis development up till two month after irradiation. In the here reported part of the study we were mainly looking at the early phase after irradiation and it should be pointed out that signs of fibrosis histologically have been reported first at a later stage after irradiation.

REFERENCES

1. Eisenberg B, Decosse J, Harford F, Michalek J. Carcinoma of the colon and rectum: The natural history reviewed in 1 704 patients. Cancer 1982; 49: 1131–4.
2. Lise M, Gerard A, Nitti D, et al. Adjuvant therapy for colorectal cancer: the EORTC experience and review of literature. Dis Colon Rectum 1987; 30: 847–54.
3. Påhlman L, Glimelius B. Radiotherapy additional to surgery in the management of primary rectal carcinoma. Acta Chir Scand 1990; 156: 475–86.
4. Stockholm Rectal Cancer Study Group. Preoperative short-term irradiation therapy in operable rectal carcinoma. Cancer 1990; 66: 49–55.
5. Stegemann H, Stadler K. Determination of hydroxaproline. Clin Chem Acta 1967; 18: 267–73.
6. Bradley PP, Priebat DA, Christiansen RD, Rothstein G. Measurements of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982; 78: 206–9.
7. Borgström S, Aronsen KF, Dougan P, Jacobsson L, Lindström C, Nylander G. A study of the radioprotective effect of vasopressin induced ischemia on the small intestine of the dog and its relation to circulatory parameters. Br J Radiol 1982; 55: 568–73.
8. Jeremy RJ. The large intestine. In: Morson BC, ed. Systemic pathology/Third edition, Alimentary tract. London: Churchill Livingstone, 1987: 313–93.

9. Hubmann FH. Effect of X irradiation on the rectum of the rat. Br J Radiol 1981; 54: 250–4.
10. Black WC, Gomez LS, Yuhas JM, Kligerman MM. Quantitation of the late effects of x-irradiation on the large intestine. Cancer 1980; 45: 444–51.
11. Breiter N, Trott KR. The pathogenesis of the chronic irradiation ulcer of the large bowel in rats. Br J Cancer 1986; 53: 29–30.
12. Breiter N, Trott KR. Chronic irradiation damage in the rectum of the rat after protracted fractionated irradiation. Radiother Oncol 1986; 7: 155–63.
13. Dewit L, Oussoren Y, Bartelink H. Early and late damage in the mouse rectum after irradiation and cis-diamminedichloroplatinum (II). Radiother Oncol 1987; 8: 57–69.
14. van der Kogel AJ, Jarret KA, Paciotti MA, Raju MR. Irradiation tolerance of the rat rectum to fractionated X-rays and pi-mesons. Radiother Oncol 1988; 12: 225–32.
15. Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gastroenterology 1984; 87: 1344–50.
16. Wallace JL, Keenan CM. An orally active inhibitor of leucotriene synthesis accelerates healing in a rat model of colitis. Am J Physiol 1990; 258: 527–34.
17. Yamada Y, Marshall S, Specian RD, Grisham MB. A comparative analysis of two models of colitis in rats. Gastroenterology 1992; 102: 1524–34.
18. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses. Collagen metabolism in the colon after left colon resection. Am J Surg 1980; 139: 398–405.
19. Bubrick MP, Rolfsmeyer ES, Schauer RM, et al. Effects of high-dose and low-dose preoperative irradiation on low anterior anastomosis in dogs. Dis Col & Rect 1982; 25: 406–15.
20. Schauer RM, Bubrick MP, Feeney DA, Johnston GR, Rolfsmeyer ES, Strom RL, Hitchcock CR. Effects of low-dose preoperative irradiation on low anterior anastomosis in dogs. Dis Colon Rectum 1982; 25: 401–5.
21. Morgenstern L, Sanders G, Wahlstrom E, Yadegar J, Amodeo P. Effect of preoperative irradiation on healing of low colorectal anastomosis. Am J Surg 1984; 147: 246–9.
22. Degges RD, Cannon DJ, Lang NP. The effects of preoperative irradiation on healing of rat colonic anastomosis. Dis Colon Rectum 1983; 26: 598–600.