Colon Mucosal Cells after High-Dose Fractional Irradiation

(mast cells / lymphocytes / enterocytes / colon / fractional irradiation / apoptosis / morphometry / Beagle dogs)

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Abstract. The aim of this study was to investigate histological and stereological changes in cryptal enterocytes, mucosal lymphocytes and mast cells 10 days after irradiation. For experimental model, 24 Beagle dogs 1–2 years old were used. Twelve dogs were irradiated 20 days with 32 Gy over the whole pelvis and tail. Another 12 dogs represented a control group. For the detection of apoptosis, the TUNEL technique was used. Histological and stereological analyses were performed using a Wild sampling microscope M 1000. In the irradiated group, volume density ($P < 0.01$), numerical density ($P < 0.05$) and average volume of lymphocytes ($P < 0.001$) were significantly lower than in the nonirradiated group. Numerical areal density of mast cells in the irradiated group was also significantly lower ($P < 0.05$). Volume density ($P < 0.001$) and average volume of mast cells ($P < 0.001$) were significantly higher in the irradiated group. The results of our experiments show that irradiation causes injury and loss of lymphocytes and mast cells in the colon mucosa. Apoptosis was detected in enterocytes and lymphocytes in the irradiated group and in nonirradiated group in equal numbers (2.5 ± 0.3 vs. 2.3 ± 0.3; ns.), suggesting that 10 days after high-dose irradiation, the cell loss is not due to apoptosis.

Severe fibrous changes of the colon after irradiation of the gastrointestinal tract represent a difficult problem if surgical intervention is required. The purpose of this study was to investigate the histological and stereological changes in the colon occurring after experimental irradiation. The intestinal epithelium is one of the most radiosensitive structures in the body (Anderson and Scotti, 1984; Ijiri and Potten, 1984; Smith and Jerome, 1986). Individual cells in the intestinal mucosa such as enterocytes, lymphocytes and mast cells represent an important protection against noxious agents and tumor invasion (Iwanaga 1995; Thames et al., 1997). In this study we focused on lymphocytes, mast cells, cryptal enterocytes, as well as on the process of apoptosis of various cells after irradiation. There are several reports in literature concerning histological changes in the intestine after irradiation (Anderson and Scotti, 1984; Lukič et al., 1989; Potten et al., 1994; Mason et al., 1995; Langberg et al., 1996), but morphometrical data are rather scarce.

Materials and Methods

Twenty-four Beagle dogs, weighing 8–13 kg, and 1–2 years old, were included in the study. Twelve dogs were irradiated (I) with a total dose of 32 Gy γ-rays (telecobalt, Phillips, Hamburg, Germany) over the pelvic region and tail. The size of the irradiated region on the skin was 10×15 cm, the depth 5 cm. The control group consisted of twelve nonirradiated dogs (N). Dogs were irradiated for 20 days (every second day with a fractional dose of 3.2 Gy). For the detection of apoptosis, the interval of 10 days between the irradiation and the day of autopsy was used. Ten days after the last irradiation, 1-cm wide piece of colon transversum was excised from the midcolon of the anesthetized dogs. The tissue was fixed in formalin solution, embedded in paraffin and cut into step serial sections of 5 μm. The obtained sections were stained using the hematoxylin-eosin (HE), toluidine-blue and sulphate alcian blue (SAB) methods.

Detection of programmed cell death or apoptosis was performed with the terminal transferase deoxyuridine triphosphate nick end labeling (TUNEL) method (Apo Tag plus Peroxidase Kit ONCOR, Gaithersburg, MD). The slides of intestinal tissue were fixed in buffered 10% formalin and embedded in paraffin. Endogenous peroxidase was blocked by immersing sections in phosphate-buffered saline containing 0.75% H_2O_2. The nuclei of tissue sections were stripped from proteins by incubation with proteinase K (Sigma, Deisenhofem, Germany). The sections were incubated with a mixture of terminal deoxynucleotidyl transferase (TdT) and reaction buffer containing digoxigenin-labeled deoxyuridine triphosphate (dUTP). The incubation with antidigoxigenin antibody conjugated with peroxidase and staining with hematoxylin followed (Kališnik, 1985). Rat thymus sections were used as positive controls (Iwanaga, 1995).

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Abbreviations: HE – hematoxylin-eosin, V_V - volume density, N_V - numerical areal density, V - average absolute volume, SAB – sulphate alcian blue, TUNEL – terminal transferase deoxyuridine triphosphate nick end labeling.

An accurate histological analysis of the step serial sections was used to establish the changes in the mucosa of the colon crypts in each group. Lymphocyte infiltration of the mucosa as well as the distribution and morphology of mast cells were studied. Apoptosis of cryptal enterocytes and mucosal lymphocytes was evaluated. Stereological analysis (Kališnik, 1985; Kališnik et al., 1989) was performed on a Wild sampling microscope (Wild, Heerbrugg, Switzerland), using Weibel's test system. Volume density (V_v) of lymphocytes and mast cells, average absolute volume (V) of these cells and the number of apoptotic cells on 100 cryptal sections were estimated at a magnification of 400x. Numerical areal density (N_v) of lymphocytes and mast cells was estimated according to the Weibel-Gomez method. For the statistical evaluation of stereological results, Student's t-test was used.

Results

Histological analysis

In the irradiated group, the number of lymphocytes in the connective tissue and in the cryptal epithelium was lower than in the nonirradiated group of animals (Fig. 1). In the nonirradiated group, infiltration of the lamina propria was more intense than in the irradiated one (Fig. 2).

In the irradiated group, the mast cells were localized mainly in the connective tissue at the basal part of the crypt. In the vicinity of irregularly shaped mast cells, numerous metachromatic granules were dispersed (Fig. 3). In the nonirradiated group, the mast cells were equally dispersed in the connective tissue, and their cytoplasm was full of metachromatically stained granules (Fig. 4). In each group of animals, some enterocytes and lymphocytes showed morphological characteristics of apoptosis such as condensation of the nuclei, appearance of apoptotic bodies and shrinkage of the cells (Fig. 1, 2).

Stereological analysis

In the irradiated group, all the measured stereological values of lymphocytes were significantly lower in comparison with the nonirradiated group: V_v (0.012 ± 0.0007 vs. 0.018 ± 0.001, $P < 0.01$, Fig. 5), V (264.2 ± 100 vs. 437.7 ± 300, $P < 0.001$, Fig. 6) and N_v (42649.5 ± 1942.7 vs. 49140.6 ± 2878.2, $P < 0.05$, Fig. 7). Numerical areal density of mast cells was also significantly lower in the irradiated in comparison with the nonirradiated group

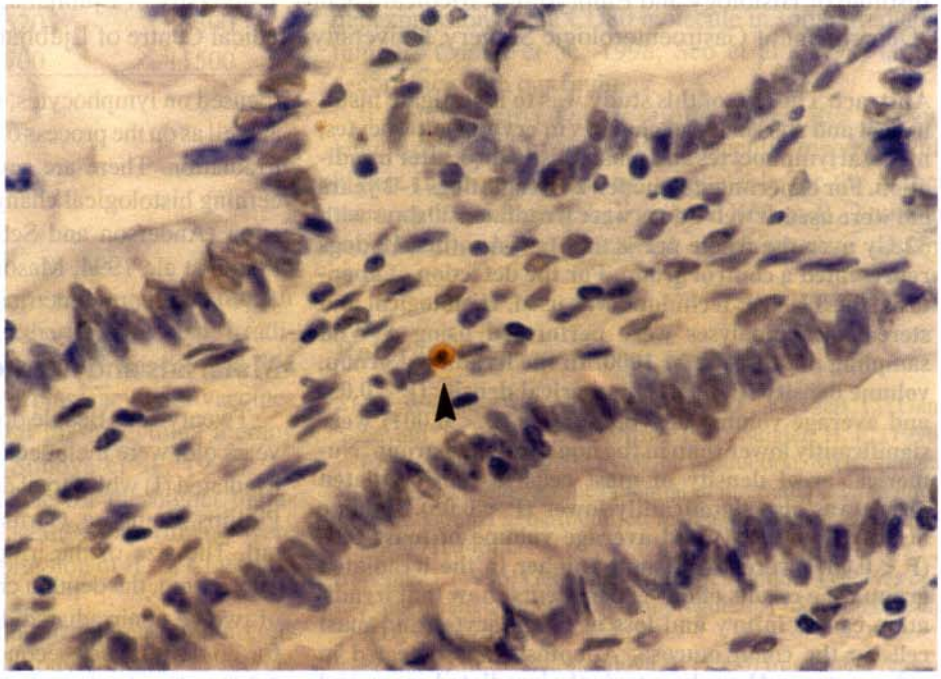

Figure 1. Rare lymphocytes and some apoptotic nuclei (arrow) in the lamina propria of an irradiated animal (TUNEL method, hematoxylin, magnification 400x).

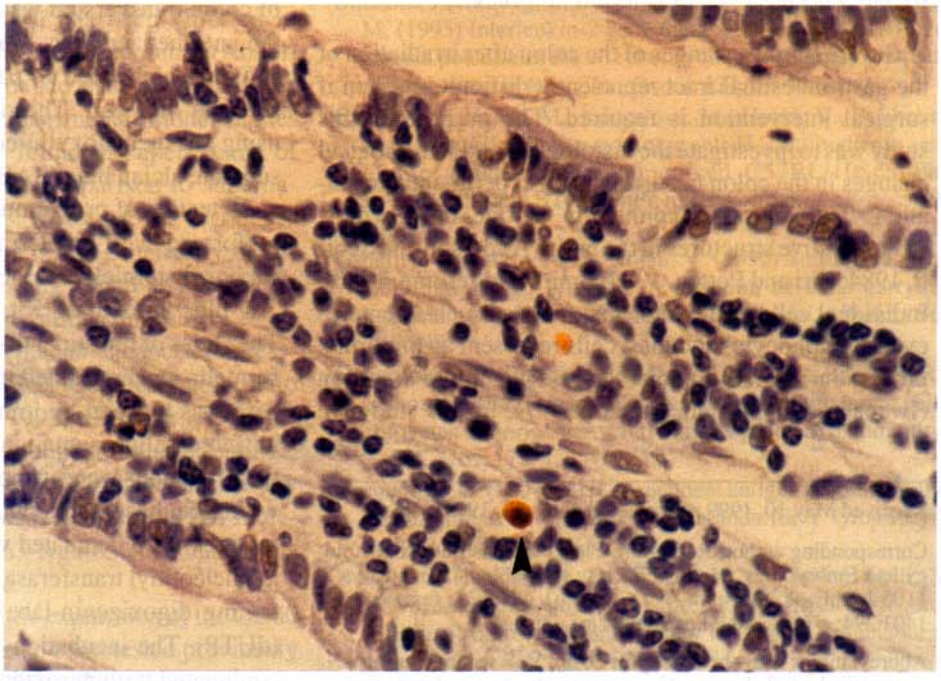

Figure 2. Lymphocytic infiltration in the lamina propria and apoptotic nuclei (arrow) of a nonirradiated animal (TUNEL method, hematoxylin, magnification 400x).