Colon Mucosal Cells after Combined Radiotherapy and Chemotherapy

(colon mucosa / radiotherapy / chemotherapy / morphology / morphometry / apoptosis / proliferation / lymphocytes / serotonin-secreting cells / somatostatin-secreting cells / mast cells)

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Abstract. The aim of this study was to investigate early histological and stereological changes in enterocytes, lymphocytes, mast cells, serotonin- and somatostatin-secreting cells in colon mucosa the first day after the end of combined radiotherapy and chemotherapy. For experimental model 20 Beagle dogs were used. Ten dogs were given platinol every 5 days over 20 days and they were irradiated 20 days with 32 Gy (every second day with a fractional dose of 3.2 Gy) onto the whole pelvis and tail. Another 10 dogs represented a control group. For detection of apoptosis the TUNEL technique was used, whereas immunohistochemical methods were performed for detection of somatostatin- and serotonin-secreting cells, and for proliferating cell nuclear antigen in epithelial cells. The volume density of enterocytes in apoptosis was increased, and Vv of paracrine cells (mast cells, somatostatin and serotonin positive cells) was significantly increased in the treated group compared to the control group. In the treated group a significantly lower Vv of lymphocytes and PCNA-positive enterocytes was shown compared to the control group. The results of our experiments showed that combined radiotherapy and chemotherapy caused loss of enterocytes and lymphocytes early after the therapy. It was associated with an increased volume density of paracrine cells. These morphological changes in the colon mucosa might be the earliest changes leading to disruption of the mucosal barrier, malabsoption syndrome, stenosis, inflammation and other complications resulting from the radiotherapy and chemotherapy.

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Abbreviations: PBS – buffered saline solution, PCNA – proliferating cell nuclear antigen, TUNEL – terminal transferase deoxyuridine triphosphate nick-end labelling, Vv – volume density.

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Combined therapy with ionizing radiation and chemotherapy frequently causes various complications, such as increased mucosal permeability, malabsoption syndrome, ileus, inflammation, and stenosis (Muggia et al., 1978; Black et al., 1980; Trott and Kiszel, 1984; Breiter and Trott, 1986; Dewit et al., 1987; Baretton et al., 1996; Brennan et al., 1998; Bisht et al., 2000; Nejdfors et al., 2000), which require further treatment or even surgery (Fajardo and Berthrong, 1981; Fajardo, 1982; Kato et al., 2000; Kennedy et al., 2000; Anti et al., 2001). Acute radiation injuries are characterized by crypt and mucosal atrophy, and subacute or late injuries are characterized by vascular damage, fibrosis, and inflammatory cell infiltration (Hopewell et al., 1993; Langberg et al., 1994). In our previous study (Zorc-Pleskovič et al., 2000) we analysed the colon mucosa in dogs after radiotherapy alone and we found it out to be associated with lower volume density (Vv) of lymphocytes and higher Vv of mast cells, but we did not find any difference in the number of apoptotic enterocytes between the irradiated and non-irradiated dogs 10 days after irradiation. This study was undertaken to identify early morphological and morphometrical changes in colon mucosa just after combined radiotherapy and chemotherapy, and the role of these changes in predicting possible complications.

Material and Methods

Twenty Beagle dogs, aged 1–2 years and weighing 9–11 kg, were included in the study. Ten dogs were given platinol intramuscularly, 5 mg/KBW every 5 days over a period of 20 days, after previous hydration with 200 ml of saline solution. In addition, they were irradiated with γ-rays on telecobalt (Phillips, Hamburg, Germany) with 32 Gy over the pelvic region and tail. The irradiated area of the skin measured 10 × 15 cm and was 5 cm deep. The dogs were irradiated for 20 days (every second day with a fractional dose of 3.2 Gy). The control group consisted of ten non-irradiated dogs. Just

after the last irradiation (the first day after the last irradiation) and chemotherapy, 1-cm wide and long piece of tissue was excised from the middle third of colon transversum of the anaesthetized dogs for morphological and morphometrical analysis. Colon samples were also obtained from the control group of 10 sham-irradiated dogs using the same procedure.

The tissue was fixed in formalin solution, embedded in paraffin and cut into step serial sections of 5 μm. The step between two sections was 50-μm thick. The obtained sections were cut parallel with the circular muscle layer and stained with haematoxylin-eosin. For detection of mast cells, toluidine-blue (0.5% water solution in McIlvain buffer) and sulphate alcian-blue methods were used. Detection of apoptosis was done with the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) method as described previously (Zorc-Pleskovič et al., 2000). Rat thymus sections were used as positive controls.

The proliferating cell nuclear antigen (PCNA) immunohistochemical method was performed for detection of the proliferative capacity of cells. The sections were washed with phosphate-buffered saline solution (PBS). Endogenous peroxidase was blocked with 3% H_2O_2 in PBS for 20 min. To block non-specific binding sites the slides were covered with 2% bovine serum albumin in PBS. After washing in PBS the primary antibodies were applied: monoclonal anti-PC-10 antibody (DAKO, A/S, Glostrup, Denmark; dilution 1 : 50, at room temperature). After washing in PBS, the primary antibodies were detected by incubation with biotinylated goat antibodies for 30 min. After washing in PBS, streptavidin-biotin complex/horseradish peroxidase was applied for 30 min at room temperature. Diaminobenzidine was used as a chromogen substrate for PCNA and TUNEL methods. As positive controls for PCNA, tonsils were used. The specificity test, performed by omission of the primary serum, produced negative results for all the antibodies used.

The primary antibody for somatostatin was polyclonal anti-somatostatin A 0566 antibody (DAKO, A/S, Denmark; dilution 1 : 200). As the primary antibody for detection of serotonin cells, monoclonal anti-serotonin M 0758 antibody (DAKO, A/S, Denmark; dilution 1 : 25) was used. The Strept ABComplex/AP (DAKO-K0391) immunohistochemical method was employed to identify enteroendocrine somatostatin-releasing and serotonin (5-hydroxytriptamine)-releasing cells with the New Fuchsines chromogen substrate system.

Changes in colon mucosa in each group were determined using accurate histological analysis of step serial sections. We evaluated the apoptotic (TUNEL-positive) cells and proliferating (PCNA-positive) cells, the lymphocyte infiltration of the mucosa, the distribution, size and shape of mast cells, somatostatin-positive and serotonin-positive cells, and the distribution of granules in paracrine cells (mast cells and somatostatin-positive and serotonin-positive cells). Degranulated mast cells, somatostatin cells and serotonin cells were identified if sparse granules were seen in the cytoplasm, and some in the vicinity of the cells.

Morphometrical analysis (Weibel, 1979; Kališnik, 1985; Kališnik et al., 1989) was performed under a Wild sampling microscope (Wild, Heerbrugg, Switzerland), using Weibel's B 100 double grid test system. The volume densities of apoptotic enterocytes, proliferative enterocytes, lymphocytes, mast cells, somatostatin-positive and serotonin-positive cells were estimated by counting points of the grid system that hit the observed cells and reference space at an objective magnification of 63x. The reference space was the epithelial layer and lamina propria. The volume density represents a quotient between hits falling on observed cells (Pf) and hits falling on the reference space (Pt) ($Vv = Pf/Pt$). For statistical evaluation Student's t-test was used.

Results

Histological analysis

Apoptotic enterocytes were localized on the top and the neck of the crypts (Fig. 1, 2), and proliferative enterocytes mostly in the neck of the crypts (Fig. 3, 4) in both groups.

Mast cells were primarily located in the intestinal lamina propria in the treated group; they were smaller, with few granules in the cytoplasm. Somatostatin-positive cells were found mainly in the surface epithelium. The treated group showed a greater number of irregularly shaped somatostatin-positive cells with sparse granules in the cytoplasm (Fig. 5). In the control group, somatostatin cells were triangular in shape, they had a central nucleus and a distinct cytoplasmic protrusion, which extended to the cryptal lumen (Fig. 6). Serotonin-positive cells resembled somatostatin cells. In the treated group, degranulation of serotonin cells with granules in the vicinity was noted.

In the treated group sparse lymphocytes were seen in the lamina propria and in the epithelial cell layer, whereas the control samples showed normal lymphocyte infiltration of the lamina propria.

Surface sloughing and mitosis of epithelial cells were seen in both groups; however, we did not quantify them. Polymorphonuclear infiltration was not seen in either group of animals.

Morphometrical analysis

The treated animals showed higher Vv of apoptotic enterocytes, higher Vv of mast cells, somatostatin-positive and serotonin-positive cells, and lower Vv of lymphocytes and PCNA-positive enterocytes compared to control animals (Table 1).