BIOGERONTOLOGY

Modification of Delayed Radiation-Induced Reactions of Duodenal Vessels and Mast Cells in Old Rats

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Pronounced ultrastructural changes in vessels and mast cells were observed in duodenal lamina propria of Wistar rats 1 year after single whole-body γ-irradiation in a dose of 7.5 Gy. Inhibition of adrenocortical function with methopyrone reduced structural damage and improved animal survival.

Key Words: *γ-irradiation; duodenum; vessels; mast cells*

The mechanisms underlying shortening of the life span after whole-body irradiation are directly associated with vascular disorders, tissue hypoxia, and sclerotic processes. This explains the interest of scientists to the search for means attenuating delayed radiation-induced injuries [4,5].

Gastrointestinal organs are highly sensitive to ionizing radiation. Damage to vascular and stromal elements and impairment of neurohormonal components of regulation, along with the decrease in proliferative potential of stem cells and impairment of "barrier" function of enterocytes, play an important role in the mechanisms forming radiation-induced reactions [1,5,9]. The effects of ionizing radiation on morphofunctional relationships between mast cells (MC) and vessels of the gastrointestinal mucosa are intensively studied [13-15]. The cytoplasm of MC contains bioactive substances (sulfated proteoglycanes, numerous transmitters, including peptides, histamine, serotonin (in rodents), cytokines, *etc.*), which are directly involved in the regulation of various functions in the body [10,12]. Moreover, MC participate in the development of radiation-induced fibrosis of the intestinal wall [15]. The release of bioactive substances from MC is regulated by many factors, in particular, adrenocortical hormones [10]. It was shown that inhibition of steroidogenesis after γ-irradiation in sublethal doses alleviated structural damage to vessels and MC in the early postirradiation period [8]. The possibility of modifying radiation-induced reactions in delayed periods after irradiation in lethal doses is little studied.

We investigated delayed radiation-induced reactions of the duodenal vascular and MC ultrastructure after whole-body γ-irradiation and the possibility of modifying these reactions with methopyrone, an inhibitor of adrenocortical function.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (180-200 g). The animals were divided into 3 groups. Group 1 (control) consisted of 20 intact animals, animals of groups 2 and 3 (80 animals per group) were exposed to whole-body γ-radiation in a single dose of 7.5 Gy

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on a cobalt GUB 20 000 device at a dose power of 1.8 Gy/min. Group 3 rats were injected (subcutaneously) with methopyrone (specific inhibitor of glucocorticoid hormone synthesis, Ciba) in a dose of 11 mg/100 g for 2 weeks [3,7,11]. The rats were sacrificed 1 year after irradiation at the age of 16 months. Ten rats from each group were used for morphological analysis. The duodenum was isolated under Nembutal narcosis. Fragments of the proximal portion for light microscopy were fixed for 24 h in acid Bouin's fluid, dehydrated, and embedded in paraffin. Microtome sections (7 μ) were put onto slides covered with L-polylysin films (Sigma). Specimens for electron microscopy were fixed in Karnovskii's fluid, dehydrated, and embedded in epon. Ultrathin sections (100 nm) were prepared on an LKB-7A ultramicrotome (LKB), contrasted with uranyl acetate and lead citrate, and examined under a JEM-100S electron microscope (JEOL). MC were selectively stained with 1% toluidine blue (Fluka) in 0.5 M HCl at pH 0.5.

Morphometry was carried out using a computer-assisted image analysis system (IMSTAR) equipped with Morphostar-2 and Colquant-2 software in accordance with basic principles of stereology and morphometry. Volume density of MC was evaluated on transverse sections including the whole duodenal mucosa perimeter at ×10. The test area was at least 2.5 mm^2. Quantitative data were processed statistically using nonparametric Mann—Whitney's *U* test.

RESULTS

No appreciable differences in histoarchitectonics of the duodenal mucosa were detected on hematoxylin-eosin stained histological preparations from animals of experimental groups. MC selectively stained with toluidine blue were detected in connective tissue of the mucosal lamina propria and were located near blood and lymph vessels (Fig. 1, *a*). Morphometry showed that the volume density of MC in the control group was 0.011±0.001. Electron microscopy of MC showed eccentric nuclei with pronounced marginal chromatin condensation. The cytoplasm usually contained numerous round or irregularly shaped large electron-dense secretory granules (Fig. 2, *a*). Fenestrated vessels in the mucosa had wide lumens filled with fine granular contents. The cytoplasm of endothelial cell contained many polyribosomes and moderate number of cell organelles (Fig. 2, *b*). The endothelium had regular structure.

In group 2 34 rats survived 1 year after irradiation. Visual examination of preparations showed high content of MC in the duodenal mucosa (Fig. 1, *b*). Quantitative analysis revealed increased volume density of MC in comparison with the control (to 0.018±0.001, $p<0.05$). Electron microscopy also revealed considerable changes in MC. Large chromatin lumps were scattered over the entire nucleoplasm. Edema of the perinuclear space and detachment of the outer karyolemma leaflet were seen. The number of secretory granules in the cytoplasm decreased. The granules greatly varied in shape and electron density (Fig. 2, *c*). Apart from dense secretory granules with typical structure, large irregularly shaped granules with finely granular contents were seen. The cytoplasm of some MC was edematous and contained mitochondria with clarified matrix. The Golgi complex was usually reduced. The endothelium was thinned. Just a narrow cytoplasmic rim with small cytoplasmatic protrusions into the capillary lumen was seen in the nucleus-containing part of the endothelium. Pronounced cytoplasmatic edema and few cytoplasmatic organelles were often seen in endothelial cells. The basal membrane was loosened; edema of the perivascular space was observed (Fig. 2, *d*).

In group 3 54 rats survived 1 year after irradiation, i.e. by 25% more than in group 2. Morphometry of the duodenum from animals treated with methopyrone showed that the volume density of MC practically did not differ from that in the control (0.009±0.001). MC ultrastructure also little differed from the normal. Chromatin was condensed along the nucleus perimeter, karyolemma was invaginated, which indirectly indicated increased functional activity of MC. The cytoplasm contained many secretory granules with typical structure and electron density (Fig. 2, *e*); there were groups of polyribosomes and short cisterns of granular endoplasmic reticulum. The cytoplasmic membrane was well preserved. Vesicular elements of the MC Golgi complex were activated. The nuclear surface of endothelial cells in the capillaries of the duodenal mucosa lamina propria of animals treated with methopyrone increased due to the formation of karyolemma invaginations. The number of polyribosomes, pynocytotic vesicles, and cytoplasm protrusions into the capillary lumen extending the area of contact with the blood were higher than in group 2. The structure of basal membrane virtually did not differ from the normal (Fig. 2, *f*).

Hence, marked structural changes in MC and vessels were detected in the duodenal mucosa one year after single whole-body γ-irradiation in a dose of 7.5 Gy. The damage caused by ionizing radiation is determined by not only its direct, but also indirect action, which is associated with rearrangement of the endocrine system and enhanced production of glucocorticoid hormones [1,2] producing genomic and membranotropic effects [8]. Experiments with methopyrone applied in a dose maintaining normal blood level of glucocorticoid hormones [3,6,7,11] demonstrated

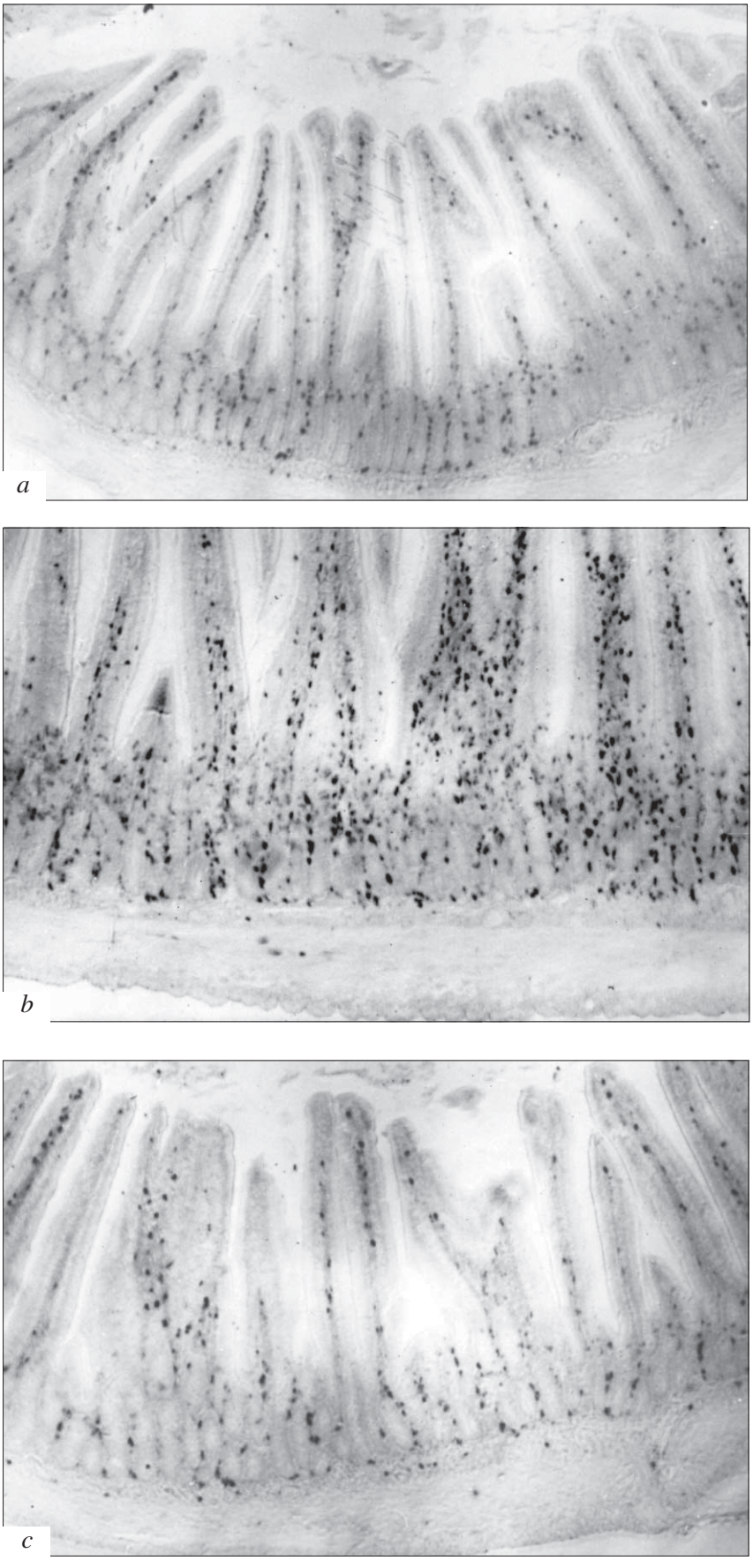

Fig. 1. Mast cells in the lamina propria of rat duodenal mucosa. Selective staining with toluidine blue. *a*) intact; *b*) 1 year after irradiation; *c*) animals treated with methopyrone, ×65.

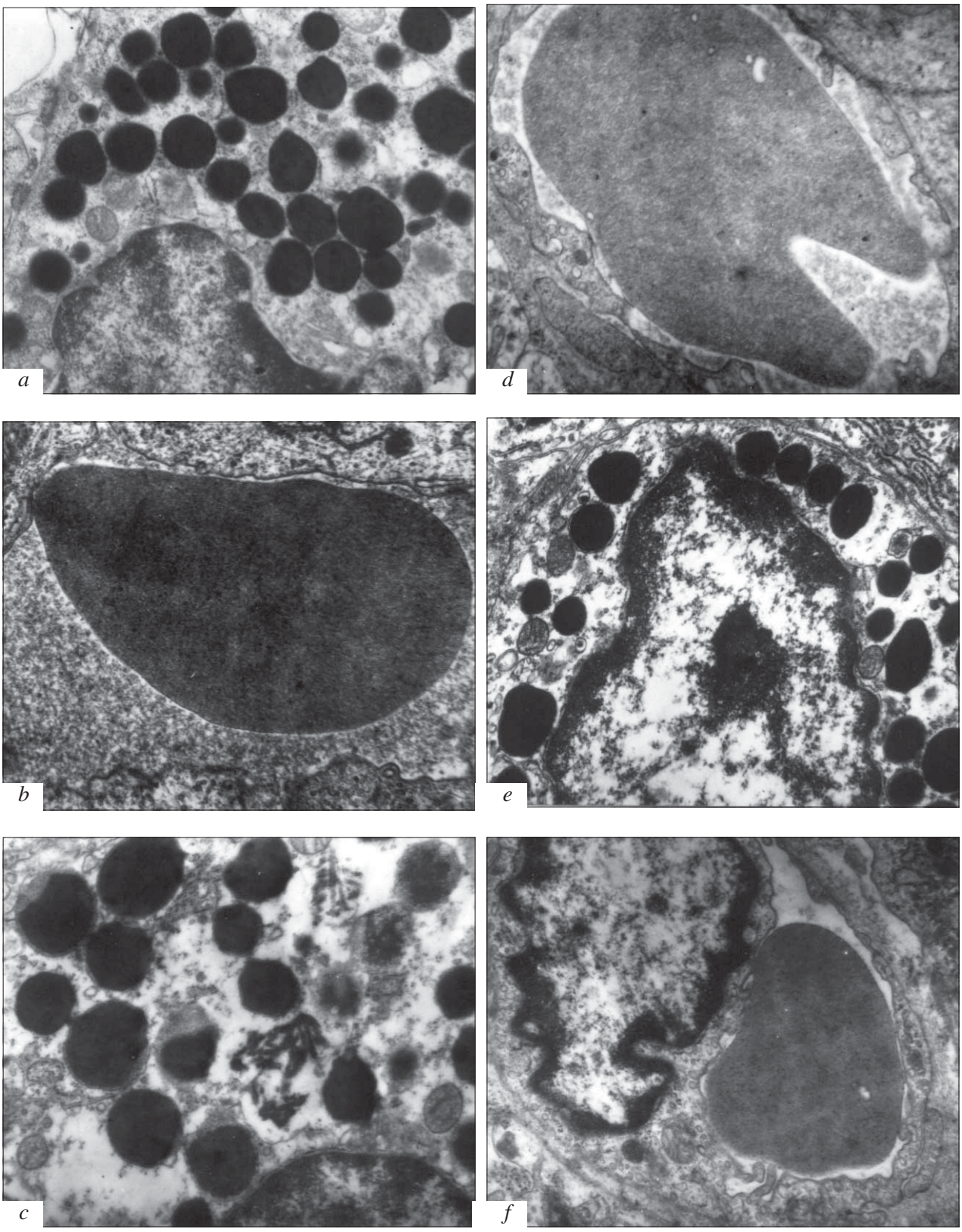

Fig. 2. Ultrastructure of mast cells and vessels in the lamina propria of the rat duodenal mucosa. *a*, *b*) intact rats; *c*, *d*) 1 year after γ-irradiation; *e*, *f*) irradiated rats treated with methopyrone, ×10,500.

that the delayed modifying effect manifested in less pronounced structural damage to duodenal vessels and mast cells and was paralleled by improvement of animal survival.

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