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APOPTOSIS AND RENEWAL OF ENTEROCYTES IN EXPERIMENTAL ATROPHY OF THE SMALL INTESTINAL MUCOSA

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UDC 616.341-018.73-007.23-092.9-07: 616.34-018.73-018.15

KEY WORDS: apoptosis; mitosis; renewal of enterocytes; hydroxyurea

The steady state of renewing tissue is maintained by equilibrium between the formation of new cells and loss of cells entering the system. Cells completing their life cycle may not only be cast off, but may also undergo self-destruction in situ. This process of "programmed cell death" [10], called apoptosis to distinguish it from necrosis, has been ascribed in recent years an important role in the regulation of the size of renewing tissues, in response to the use of cytotoxic agents and hormones [2, 5, 10]. Apoptosis has been described in normal developing tissues, in embryogenesis, metamorphosis, and endocrine-dependent tissue atrophy, in the majority of growing tumors, during irradiation with small doses, and during the action of radiomimetic agents [5, 8, 9, 11, 12]. The rapid development of atrophy when the blood supply is disturbed [13] and when stem cells are damaged by preparations disturbing DNA synthesis [4, 5], and also during involution of hyperplastic organs [7], is explained by apoptosis.

These "acute" atrophies can also undergo rapid regression after removal of the noxious agents, as a result of intensified cell division.

EXPERIMENTAL METHOD

The aim of this investigation was to assess the link between production and death of cells by apoptosis in "acute" atrophy of the mucosa caused by the S phase-specific agent hydroxyurea (HU). Female (CBA × C57Bl)F_1 mice weighing 20-22 g were used. The animals were irradiated in a dose of 200 rads 24 h before the beginning of the experiments to stimulate their stem cells [3]. Next they were given six intraperitoneal injections, each of 0.25 g/kg of HU in physiological saline. Depending on the intervals between injections the animals were divided into four groups: 1) interval 7 h; 2) 12 h; 3) 16.5 h; 4) 19 h. The mice were killed 6, 30, 78, and 126 h after the last injection of HU. The animals received an intraperitoneal injection of ^{3}H-thymidine in a dose of 1 μCi/g (specific activity 28.5 Ci/mmole) 1 h before sacrifice. Segments of the small intestine were separated at a distance of 3 cm from the pylorus and fixed in 10% formalin. The state of the architectonics of the mucosa was assessed in histological sections, the height of the villi, dimensions of the enterocytes of the crypts and villi, and the depth of the crypts were measured with an ocular micrometer, and the mitotic index (MI), labeling index (LI), and apoptosis index (AI) were calculated as percentage of the number of cells in the generative zone.

Central Research Institute of Gastroenterology. Research Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 8, pp. 238-241, August, 1987. Original article submitted December 19, 1986.

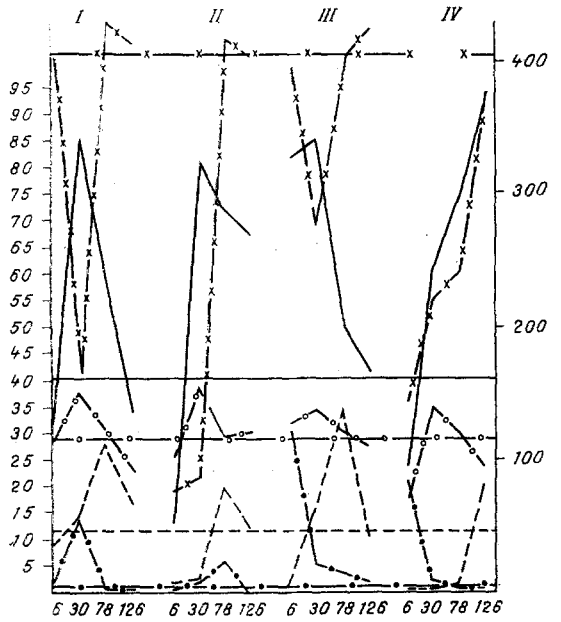

Fig. 1. Dynamics of morphological changes. Abscissa, time of sacrifice after last injection of HU (in h); ordinate: on left — Mi, Li, Ai (in %), on right — height of villi, depth of crypts (in μ). I, II, III, IV) Groups depending on intervals between injections of HU. Lines with filled circles — AI, broken lines — MI, continuous lines — LI, lines with crosses — height of villi, lines with empty circles — depth of crypts.

EXPERIMENTAL RESULTS

The trend of the morphological changes is shown in Fig. 1. Analysis of these data reveals both general rules and also some special features due to the intervals between injections of HU. Atrophy of the villi was observed in animals of all groups, and this usually correlated with a decrease in mitotic activity and DNA synthesis in the epithelium of the crypts. At the same time round cells, lying separately or arranged in small groups, with an intensely stained cytoplasm and with smooth outlines, could be seen in the crypts (Fig. 2a). Their nuclei were pycnotic, strongly basophilic, and often consisted of small, dense basophilic fragments (Fig. 2b). These cells, undergoing apoptosis, were located on the basement membrane, but often could be seen freely lying in the lumen of the crypts (Fig. 2c). Besides extrusion, phagocytosis of the particles of these cells (apoptotic bodies) by neighboring epithelial cells also was observed (Fib. 2d). Polymorphonuclear leukocytes did not take part in phagocytosis.

In the animals of group 1, 6 h after the last injection of HU, despite a marked decrease in MI and LI the villi remained tall and thin, and their epithelium remained high and prismatic and their nuclei were elongated and located basally. The depth of the crypts was within normal limits, their epithelium was a little flattened, their nuclei large and pale, and the lumen of the crypts widened. Only single cells undergoing apoptosis were found. After 30 h the villi were very much shorter, and in some places had completely disappeared. The cytoplasma of the epithelium was swollen and vacuolated. The crypts were very deep and the cells in them were tall and basophilic, with large nuclei. Here, however, apoptotic bodies were very numerous. At the same time, LI was sharply increased (up to 85% from a normal value of 42.3%), whereas MI was reduced to 15% (normal 24.8%).

Thus at these times both hyper-regenerative atrophy of the mucosa, characterized by a combination of injury (destruction of the epithelium of the villi and apoptosis in the crystall), and regeneration (active DNA synthesis and hyperplasia of the epithelium of the crypts) developed.

After 78 h the structure of the mucosa was restored, the number of apoptotic cells sharply reduced, LI lowered, and at the same time, MI was considerably increased. After 126 h the structure of the mucosa was preserved but all parameters characterizing enterocyte proliferation (LI, MI, depth of the crypts) were a little depressed.

In the animals of group 2 a picture corresponding to hyperregenerative atrophy was observed 6 h after the last injection of HU (a sharp decrease in LI and MI, considerable shortening of the villi and crypts). Apoptosis was absent during this period, but marked degenerative changes were observed in the enterocytes of the villi. The epithelium of the crypts was flattened and elongated. Changes of this kind in the crypts are evidence of re-epithelization on account of stretching and migration of the residual cells. After 30 h the mucosa began to be restored. LI rose sharply, the crypts deepened, but the villi still remained very

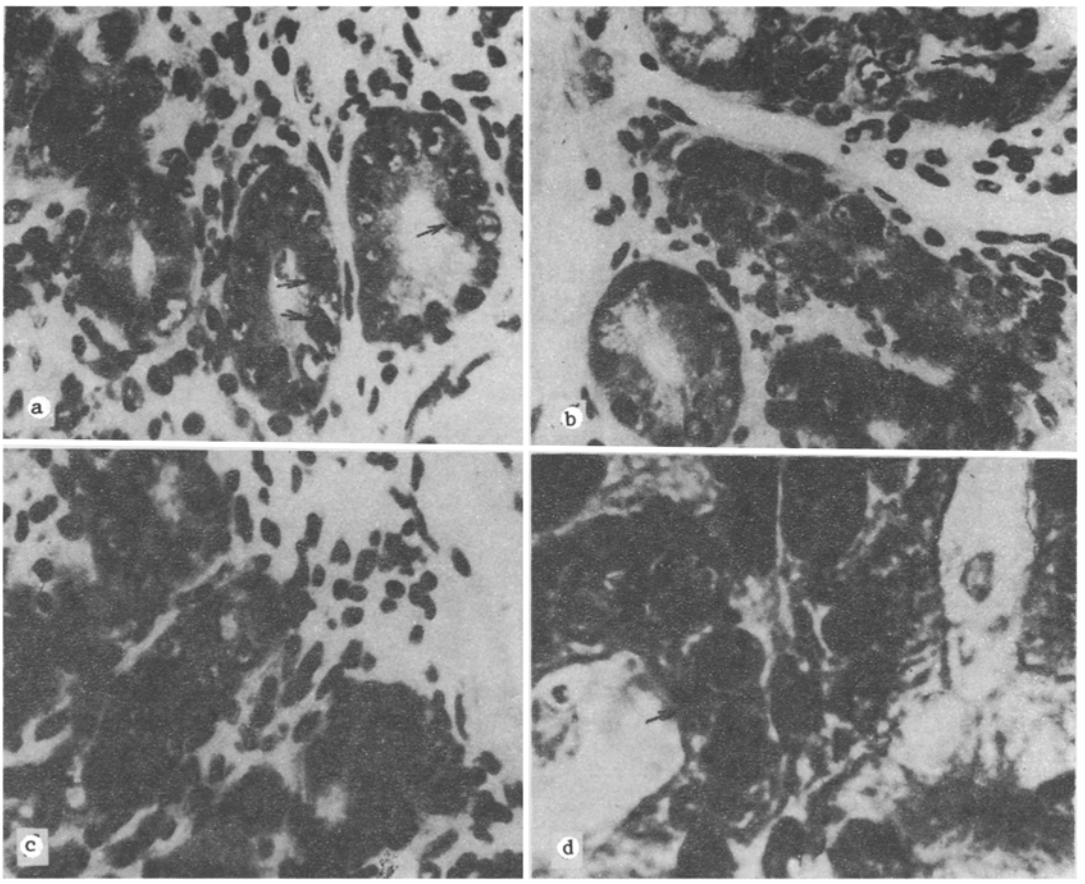

Fig. 2. Apoptosis in the small intestinal mucosa: a) apoptotic cells in crypts; b) fragmented apoptotic bodies; c) apoptotic cells in lumen of crypts; d) phagocytosed apoptotic bodies. Arrows indicate apoptotic cells and bodies. Hematoxylin and eosin. Magnification: a-c) 140; d) 630.

short, and their epithelium showed degenerative changes. After 78 h the villi were fully restored. This was accompanied by an increase in MI and a small decrease in LI. Not until this period was apoptosis present. Coincidence of the peaks of MI and AI indicates that apoptosis not only plays a complementary role opposite to that of mitosis in the regulation of the cell pool [7], but it also evidently prevents possible genetic injuries in the stem cell system [5] which could develop during restoration of the generative zone when damaged by an S phase-specific agent.

In the mice of group 3, the mucosa after 6 h appeared normal, but LI in it was high, mitoses could not be seen, but apoptoses were very numerous. This picture may predict further changes in the mucosa. The high AI in the generative zone of the intestine suggests that the development of atrophy of the mucosa can be expected within a short time, whereas a high LI, even despite the absence of mitoses, suggests that atrophy will be hyperregenerative. In fact, after 30 h the length of the villi decreased sharply, but LI and, in particular, MI increased, whereas the number of apoptoses became very small. Later, after 78 h, the length of the villi became normal, LI decreased, and MI increased correspondingly.

In the mice of group 4, changes corresponding to hyperregenerative atrophy were observed after 6 h; crossing of the curves of AI and LI, on the one hand, and between AI and MI on the other hand, were particularly conspicuous. Later these parameters changed in the opposite direction.

The 3-phase-specific agent HU thus induces "acute" atrophy of the small intestinal mucosa, the characteristics of development of which depended on the time intervals between injection of the compound (7, 12, 16.5, and 19 h). A leading role in its morphogenesis was played by apoptosis, which not only behaves in a manner complementary to mitosis, but also prevents genetic damage to stem cells. On the basis of the ratio of mitosis to apoptosis it is possible to predict both the development of atrophy and regeneration of the mucosa. Res-

toration of the mucosa in "acute" atrophy, caused by damage to cells in the S phase, of both hypo- and hyperregenerative types, unlike in "chronic" atrophy, takes place almost identically.

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ELECTRON-MICROSCOPIC AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS IN TUBULAR EPITHELIAL CELLS OF THE ALBINO RAT KIDNEY WITH NECROTOZING NEPHROSIS DUE TO MERCURIC CHLORIDE

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UDC 616.61-002.4-099-092.9-07:616.612-018.73-008.939.633.2-073.916

KEY WORDS: Mercuric chloride necrotizing nephrosis; DNA synthesis; autoradiography

Since the first study of the effect of mercury salts on animals was published, showing that the site of damage is the epithelium of the renal tubules, this model of necrotizing nephrosis has attracted the close attention of research workers hoping to discover the principles governing restoration of the structure and function of cells of the damaged epithelium. The numerous morphological investigations of the course of destructive and repair processes in the renal epithelium damaged by mercuric chloride so far undertaken at the light-optical and electron-optical levels have demonstrated that destructive changes in the epithelial cells of the proximal renal tubules are heterogeneous in character, and comprise a spectrum ranging from hardly detectable ultrastructural changes and various degrees of partial necrosis to cell death [2-5, 7, 8-11].

The question thus arises: what degree of destructive changes in the epithelial cell of the renal tubule is still compatible with its intracellular regeneration and its ability to reproduce?

To answer this question we used the technique of electron-microscopic autoradiography, whereby the ultrastructure of a damaged cell and certain of its functions, especially its ability to synthesize DNA, can be judged simultaneously.

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(Presented by Academician of the Academy of Medical Sciences of the USSR, D. S. Sarkisov.) Translated from Byulleten' Éskperimental'noi Biologii i Meditsiny, Vol. 104, No. 8, pp. 241-244, August, 1987. Original article submitted December 18, 1986.

0007-4888/87/0008-1162 $12.50