EFFECT OF THE S-PHASE-SPECIFIC AGENT HYDROXYUREA ON APOPTOSIS AND REGENERATION OF THE GASTRIC MUCOSA

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KEY WORDS: apoptosis; hydroxyurea; regeneration of gastric epitheliocytes.

Apoptosis, first described in 1972 [9], is widely distributed in man and animals under both normal and pathological conditions [1, 4, 5, 8, 11]. Its biological importance and its role in processes of physiological regeneration, ontogeny, and teratogenesis have been studied [8, 10, 12], and the inducing action of irradiation and of certain chemical cytotoxic compounds and hormones has been described [4, 8, 11]. The cell pool in atrophy of the liver, pancreas, and small intestine is reduced by apoptosis [1, 4, 8, 9, 13, 14].

The writers previously studied acute atrophy of the mucosa of the small intestine induced in mice by administration of the S-phase-specific agent hydroxyurea (HU), which causes death of cells by apoptosis and ultrastructural changes taking place under these circumstances in the cytoplasm of the epitheliocytes. Some general principles were discovered for relations between the parameters of atrophy and regeneration at different times after administration of HU [1, 2]. Renewal of the epithelium of the gastric mucosa is known to take place more slowly than that of the small intestine [3, 7]. The effect of S-phase-specific substances on the stomach and the role of apoptosis have not been studied.

The aim of this investigation was to study the effect of the S-phase-specific agent HU on regeneration in the gastric mucosa.

EXPERIMENTAL METHOD

Experiments were carried out on 60 female (CBA × C57B) mice weighing 20 g by the method in [1, 2]. The S-phase-specific agent HU was injected intraperitoneally in a dose of 0.25 g/kg at different intervals, as follows: group 1) 7 h, 2) 9 h, 3) 12 h, 4) 16.5 h, 5) 19 h, and 6) 21 h. The mice were killed, 6, 30, 78, and 126 h after the last injection of HU. ^{3}H-Thymidine (1 mCi/g) was injected intraperitoneally 1 h before sacrifice and pieces from the fundal part of the stomach were fixed in 10% neutral formalin. The state of the mucosa of the fundal part of the stomach was assessed in histological preparations, the thickness of the mucosa and the width of the zone of parietal cells were measured, the labeling index (LI) was calculated, the width of the zone of labeled cells was measured, and the number of apoptoses per gland counted.

EXPERIMENTAL RESULTS

Dystrophic and atrophic changes of varied severity were found in the gastric mucosa of the animals of all groups. The surface epithelium was flattened and loosened, and in some places it was separated from the basement membrane. The lumen of the pits and necks of the glands was dilated and in some places had the appearance of cysts, in which the epithelium was greatly flattened. The parietal cells were swollen, round in shape, and had pale, vacuolated cytoplasm (Fig. 1a). The chief cells were often reduced in size. Cells showing changes of apoptosis were located at the level of the necks of the glands, separately or in small groups (Fig. 1b, c), their cytoplasm was intensely stained, and small, dense basophilic nuclear fragments could be seen. The contours of these cells were smooth. Apoptotic particles were frequently found in neighboring epitheliocytes (Fig. 1d) and sometimes in the lumen of the pits. Single apoptotic cells were distributed in the depth of the glands.

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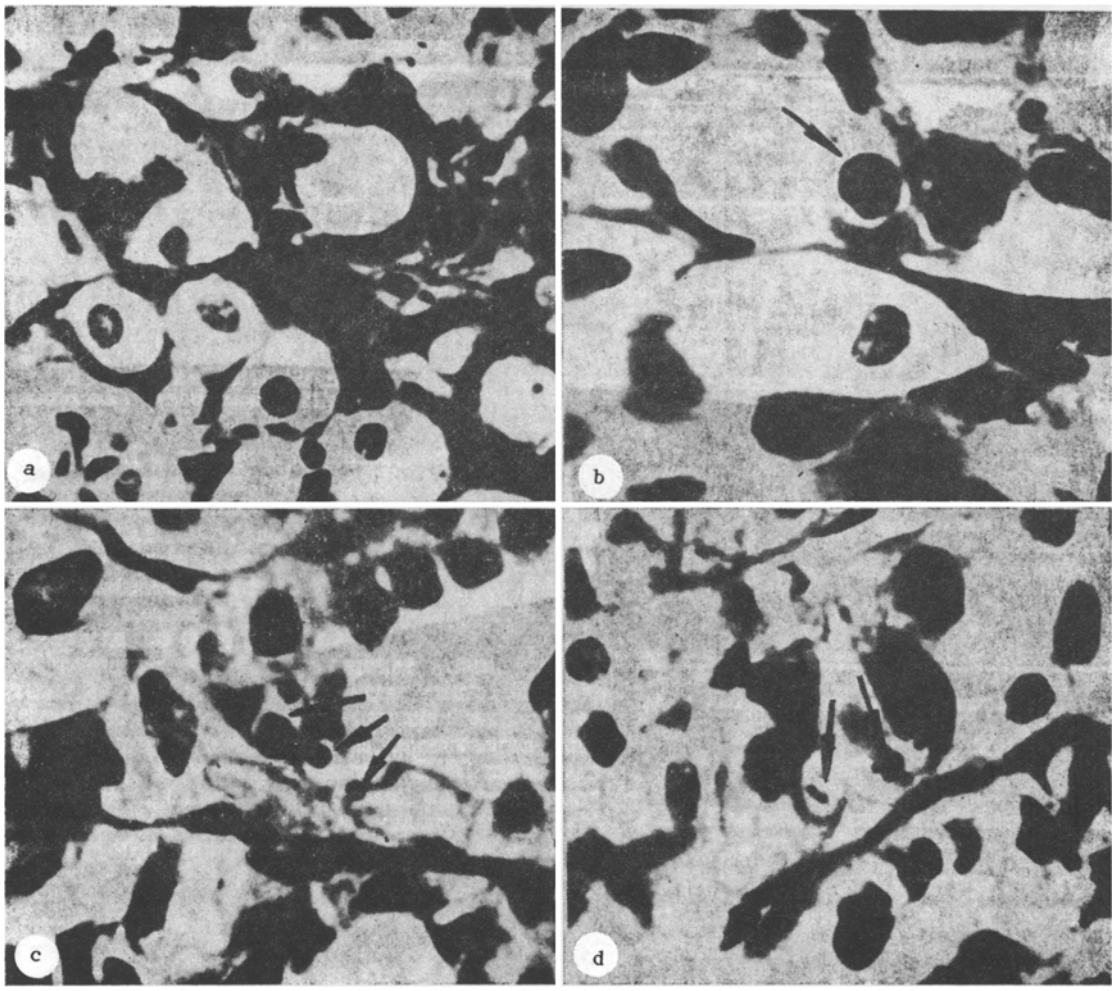

Fig. 1. Changes in gastric mucosa of mice during periodic injection of HU. Hematoxylin-eosin. 280 ×; a) Swollen parietal cells with pale, vacuolated cytoplasm in upper third of gland; b) large apoptotic body in neck of fundal gland (arrow); c) apoptoses in mouth of gland (arrows); d) apoptotic bodies, phagocytosed by epitheliocytes (arrows).

During physiological regeneration the sites of extrusion of cells in the stomach are as a rule the apices of the rugae. After desquamation of the cells, the neighboring epitheliocytes move toward these areas and the integrity of the epithelial layer is undisturbed. HU, which causes death of cells in the S-phase of the cell cycle, accelerates proliferation and migration of the cells passing through other stages of the cell cycle, and to an associated disturbance of differentiation. Immature cells, which restore the pool, migrate into the depth of the glands. The next dose of HU therefore induces apoptotic changes now in the new generation of cells, located in the deep parts of the glands.

When cell death is accelerated by HU, it would be logical to expect the development of erosions of the gastric mucosa. Their absence in our material can be explained by the mosaic pattern of cell death, the unsynchronized beginning of apoptosis in different cells, and the speed of their removal by extrusion and phagocytosis.

Some features of the regenerative changes in animals of the experimental groups are summarized in Table 1.

It can be concluded from analysis of the data that in the animals of groups 1-3 signs of dystrophy and death by apoptosis of cells of the generative zone and of the parietal cells of the apical parts of the glands, were more marked. Meanwhile in the animals of groups 1 and 2 there were signs of acceleration of regeneration, expressed as an increase in LI and widening of the zone of labeled cells. In group 3, regeneration of the gastric

TABLE 1. Parameters of Regeneration of Gastric Mucosa after Administration of HU

Group of animals	Time of investigation, h	Thickness of mucosa, μ	Width of zone of parietal cells, μ	Width of zone of labeled cells, μ	Number of apoptoses (per gland)	LI
1	6	380±11,3	190±5,9	196±3,2	1—2	32,8
	30	360±8,8	170±3,7	120±0,9	0	10,8
	78	400±7,4	190±9,1	152±1,8	0	25,4
	126	380±12,1	190±2,2	93±0,6	0	12,7
2	6	360±6,5	213±8,8	125±0,6	0—1	33,4
	30	340±4,7	224±11,2	89±0,9	0	24,7
	78	360±9,8	209±3,5	92±1,2	0	24
	126	400±11,2	251±12,7	61±0,3	0	5
3	6	360±5,5	183±7,1	50±1,1	18—20	5,1
	30	380±6,8	202±8,4	45±0,6	2—4	1,3
	78	370±11,4	175±3,2	41±0,9	0	5
	126	410±10,7	215±5,6	38±2,8	0	9
4	6	300±6,9	153±3,2	34±0,5	5—7	22,3
	30	330±10,5	170±7,7	37±0,3	0—2	15,2
	78	350±7,2	188±4,8	30±0,5	0	11
	126	370±12,6	163±9,1	32±1,2	0	10,3
5	6	330±4,8	126±7,6	36±3,1	17—19	11,4
	30	300±9,1	115±3,5	41±2,4	8—10	9,9
	78	330±7,3	150±9,8	39±1,8	5—7	5
	126	340±8,8	147±5,2	37±0,2	0	14,3
6	6	300±2,4	133±3,9	55±0,8	12—15	10,2
	30	280±7,7	127±8,8	38±1,1	9—12	2,8
	78	320±9,4	142±1,7	44±2,3	1—2	12,3
	126	360±6,7	149±6,4	40±1,7	0	15,5
Control (physiological saline)	6	420±7,5	240±9,3	42±3,1	0	12
	30	390±8,2	196±11,5	40±1,8	0	8,3
	78	380±3,1	200±3,4	37±0,0	0	9,6
n = 12	126	400±5,6	218±7,2	41±1,3	0	11,2

Legend. Each group except control contained 12 animals.

mucosa was delayed: LI was depressed and the width of the zone of labeled cells was close to the control value. Parallel with these changes the mucosa was restored: in the mice of groups 1 and 2 the architectonics was restored as early as after 30 h, whereas in group 3 it was not restored until 126 h.

In groups 4-6 the most conspicuous feature was the appearance of atrophy of the gastric mucosa and slowing of repair processes: the thickness of the mucosa and the diameter of the parietal cells were reduced, LI was depressed, and the zone of labeled cells narrower. Apoptotic bodies were located not only in the pits, but also in the glands.

Renewal of thd surface epithelium and of the mucous cells of the neck of the gastric glands is known to take 5 days, compared with 10 days for the fundal glands. The duration of the S-phase of the epitheliocytes of the stomach is 8-10 h [6]. Because of the asynchronous nature of regenerative processes in the cells it can be postulated that the cytotoxic action of HU on them is manifested differently. Some cells die by apoptosis, the genetic apparatus of others is injured, and this leads to dystrophic changes in the parietal cells.

In experiments under 4 days in duration, complete renewal of the fundal glands did not take place in the animals and phenomena of dystrophy in the upper layers of the mucosa were compensated by more rapid regeneration. In experiments lasting over 4 days, when the injured cells migrated into the deeper parts of the mucosa, signs of atrophy of the gastric mucosa became more marked, and with the delayed regeneration, its restoration was less complete.

The character and severity of changes in the gastric mucosa during periodic administration of the S-phase-specific agent HU, by contrast with rapidly renewed systems, are associated not so much with the interval between injections of HU as with the total duration of the experiment. Apoptosis plays an important role in the genesis of these changes.

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CHANGES IN THE HEMOSTASIS AND FIBRINOLYSIS SYSTEM IN RATS WITH EXPERIMENTAL RENAL HYPERTENSION

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In arterial hypertension resistance to the blood flow is increased [1, 8], due not only to anatomical changes in the microcirculatory bed (thickening of the walls, diminution of the lumen of the vessels), but also to changes in the rheologic properties of the blood. Some workers consider that an increase in viscosity of the blood plays an essential role not only in stable hypertension, but also in the initial stages of development of the disease [7]. The viscosity of the blood is significantly affected by the concentration and properties of its macromolecular proteins, namely fibrinogen, which can form aggregates with a molecular weight of up to 1000 kD, and fibronectin. An important role also is undoubtedly played by the fibrinolytic system, responsible for the hydrolysis of fibrinogen and of its high-molecular-weight fragments [10].

The aim of this investigation was to study concentrations of some plasma components of hemostasis and the role of the fibrinolytic system in experimental renal hypertension.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats. Renal hypertension was induced by Goldblatt's method: after removal of the left kidney, a nichrome coil with a lumen of 0.25 mm was applied to the right renal artery. Animals undergoing unilateral nephrectomy alone (group 1, mock operation) and intact animals (group 2) served as the controls. Twice a week the systolic blood pressure of all animals was measured by an indirect method [6] in the caudal artery. Blood for analysis was taken from the jugular vein. The following parameters were determined: fibrinogen concentration by Lazar's method, fibrinolytic activity (FA) by Niewierowski's method and on fibrin plates by the method of Astrup and Mullertz, the level of soluble fibrin monomer complexes (SFMC) by Stakhurskaya's

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