IMPORTANCE OF INTERVALS BETWEEN HYDROXYUREA INJECTIONS FOR DAMAGE TO THE EPITHELIUM OF THE MOUSE SMALL INTESTINE

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It has been shown by the use of a mathematical model that in the course of repeated periodic injections of phase-specific agents the level of injury to rapidly renewed body tissues depends essentially on the interval between injections [1, 2, 4, 8]. This effect may be of great practical importance, in particular in connection with the problem of optimization of schedules of antitumor chemotherapy. The method of mathematical modeling led to the expectation that significantly less damage could occur in cases when the interval between injections of a phase-specific agent was close to the average or twice the average duration of the cell cycle of stem cells of rapidly renewed tissue. Direct experimental confirmation of the theoretical calculations was obtained in experiments to determine dependence of the survival rate of mice [5], and also of involvement of hematopoietic stem cells and enterocytes of the small intestine [3, 7] of mice on the interval between injections of an S-phase-specific agent, hydroxyurea (HU). The state of the small intestinal epithelium was assessed morphologically on the basis of examination of histological preparations of the small intestine obtained 2-4 h after the last injection of HU [7].

The aim of this investigation was to study the state of the epithelium of the small intestine at different times after the end of injection of HU in order to obtain a more complete idea of how damage to the epithelium of the small intestine and its ability to regenerate subsequently depend on the interval between injections of HU.

EXPERIMENTAL METHOD

Male (CBA × C57BL)F_1 mice aged 8-10 weeks and weighing 20-22 g were used. At the beginning of the experiment the mice were subjected to γ-irradiation in a dose of 200 rads from a ^{137}Cs-source (dose rate 24.5 rads/min) to activate enterocyte proliferation [6, 9]. Periodic injections of HU began 24 h after irradiation. All the experimental animals received six intraperitoneal injections, each of 5 mg HU (0.23-0.25 g/kg body weight), with intervals of 7, 9, 12, 13, 16.5, and 19 h between injections. For each interval, three mice of the corresponding group were killed by decapitation 6, 30, and 78 h after the last injection. Segments of small intestine were excised at a distance of 3 cm from the pylorus, fixed in 10% formalin, and embedded in paraffin wax. Pieces of mucous membrane from the small intestine of the mice killed after 30 h were fixed in 1% glutaraldehyde, postfixed in 2% OsO_4 solution, and embedded in Epon. Ultrathin sections were cut on an ultramicrotome, stained by Reynolds' method, and examined in the JEM-100B electron microscope. By means of an ocular micrometer, in histological preparations stained with hematoxylin and eosin the height of the villi and depth of the crypts were measured, the mitotic index (MI), the number of cell positions (NCP) in longitudinal sections through the crypts and villi, NCP in transverse sections through the crypts, and the number of crypts per transverse section through the intestine were counted. The total number of enterocytes in the crypts per section was calculated by the formula:

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TABLE 1. Morphometric Parameters of Small Intestinal Epithelium of Mice at Different Times after End of Periodic Injections of HU (M ± σ)

Time after end of injections, h	Interval between injections, h	Crypts						Villi	
		Number of crypts/section through intestine	NCP in longitudinal sections	NCP in transverse sections	Total number of enterocytes/section through intestine, ($\cdot 10^{-3}$)	Depth of crypts, μ	Mitoses (%)	NCP	Height of villi, μ
6	12	66±6	9,3±1,5	10,7±1,8	6,6±1,9	100±20	0,7±0,6	14,5±1,9	72±13
	13	85±6	15,6±1,9	11,1±1,3	14,7±1,1	84±20	1,8±0,8	21,7±4,9	152±6
	16½	161±10	31,0±3,6	25,4±5,5	126±23	123±6	0,3±0,2	68,7±4,2	398±18
	19	119±21	7,1±1,0	18,2±1,6	15,5±3,8	67±15	0±0	27,4±5	144±16
30	7	117±18	31,5±3,5	20,2±3,7	73±12	153±31	11,4±4,0	28,3±9,4	167±42
	9	166±16	30,8±1,5	26,2±4,9	134±30	187±12	30,1±6,4	40,2±6,6	230±25
	12	86±8	10,0±2,8	18,1±3,0	15,8±6,3	154±25	1,3±0,4	11,3±2,6	78±20
	16½	237±29	30,9±5,6	23,4±3,9	168±24	135±20	16,1±3,6	55,0±5,6	276±30
	19	121±22	16,5±3,0	20,0±3,5	41±16	140±18	0,77±0,6	31,1±8,5	222±73
78	7	125±17	21,2±1,1	31,8±1,6	85±13	120±6	26,8±2,9	100±8	425±11
	9	197±11	20,8±1,6	29,2±7,6	122±29	123±6	16,6±4,0	91±0	443±25
	12	97±19	22,7±3,1	28,7±4,0	63±19	112±10	22,3±4,7	85±5	425±20
	16½	203±9	22,3±1,2	26,3±4,0	118±7	121±16	34,5±5,0	98±8	405±11
	19	136±44	20,1±1,3	31,8±10,2	81±5	117±16	0,5±0,4	97±10	240±28
Control		194±14	18,8±1,2	28,6±1,4	103±6	114±8	13,3±1,3	89±3	399±17

Legend. Total number of enterocytes per section through intestine calculated for individual mice by the formula: number of crypts per section through intestine × NCP in longitudinal sections through crypts × NCP in transverse sections through crypts. Mean results for three mice are given.

number of crypts per section × NCP in transverse sections through the crypts × NCP in longitudinal sections through the crypts.

EXPERIMENTAL RESULTS

The morphometric parameters of the small intestinal epithelium for different time intervals between injections and at different times after the end of the injections are given in Table 1. It was shown previously [7] that 2-4 h after the last injection of HU, the most severe damage to the epithelium was observed in mice receiving HU with an interval of 12 or 19 h, whereas minimal changes corresponded to intervals of 9 and 16.5 h. It will be clear from Table 1 that qualitatively the same picture was observed 30 h after the end of HU injections also. At this time, in the animals receiving HU with intervals of 12 or 19 h, the number of crypts, NCP of the crypts, and the total number of cryptal cells, and also NCP of the villi were significantly reduced compared with the control. In animals receiving HU with intervals of 9 or 16.5 h, the number of enterocytes on the villi (reflected in NCP on the villi) was reduced compared with the control, but not so much as in mice receiving HU with intervals of 12 or 19 h. In the cryptal zone, with intervals of 9 and 16.5 h, almost total regeneration or even hyperplasia of the epithelium was observed. At these times the number of crypts per section was indistinguishable from that in the control, but NCP in longitudinal sections through the crypts and the total number of enterocytes in the crypts were appreciably increased. Thus 30 h after the end of repeated injections of HU with intervals of 9 or 16.5 h, by contrast with courses with intervals of 12 or 19 h, residual damage to the epithelium of the villi was combined with complete regeneration or even hyperplasia of the epithelium of the crypts.

A somewhat unusual picture was observed if injections of HU were given with an interval of 7 h. Although 30 h after the end of HU injections NCP for the villi differed only very slightly from NCP for villi with an interval of 9 h, the ultrastructure of their epithelium was disturbed: the microvilli of the enterocytes were swollen, shortened, unequal in size and shape, chaotically arranged, and irregularly oriented. In some places small vesicles were found on the plasmalemma in the apical part of the cells. Regions of the surface of the denuded plasmalemma, free from microvilli, could be seen; the glycocalyx was absent in these regions. In the apical part of the enterocytes with disturbed, streaked brush border there were many vacuoles and various dark inclusions. In connection with swelling of the cytoplasm of the cells, the junctions between them were irregular in shape, and sometimes lacunae were formed

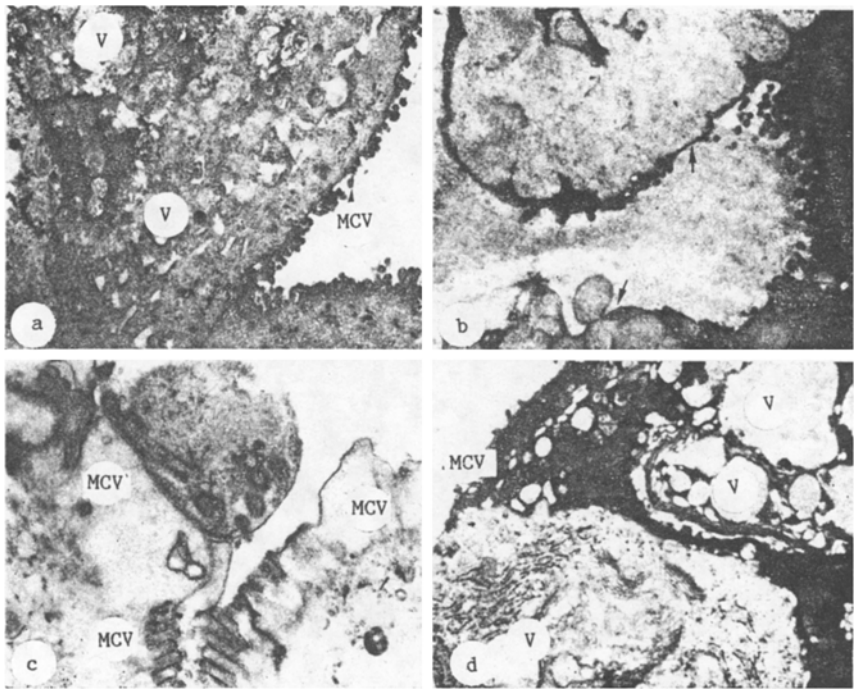

Fig. 1. Enterocytes of villus of mouse small intestine 30 h after end of periodic injections of HU. a) Destruction of microvilli (MCV), increase in number of vacuoles (V) and accumulation of dark granular masses in the cytoplasm after injections of HU with an interval of 7 h, magnification 12,000 ×; b) evagination of goblet cell into intestinal lumen and disturbance of plasma membranes (arrows) after injections of HU with interval of 7 h, magnification 12,000 ×; c) swelling of plasmalemma of enterocytes as a result of fusion of microvilli (MCV) after injections of HU with interval of 12 h. d) Destruction of microvilli (MCV) with denudation of plasmalemma, formation of vacuoles (V), and regions of condensation of cytoplasm in enterocytes after injections of HU with interval of 19 h, magnification 12,000 ×.

in the region of the terminal laminae and desmosomes. There were very few intact mitochondria and the number of cytogranules was sharply reduced. The number of double membranes of tubules of the endoplasmic reticulum also was reduced. The Golgi complex was invisible in many cells (Fig. 1a). Goblet cells were packed with optically empty vacuoles, evidence of reduced production of mucinogen and disturbance of mucus formation. These swollen goblet cells appeared to project from a row of enterocytes, with disturbance of the plasmalemma. Under these circumstances the contents of the cytoplasm could be seen to be extruded. Together with destructively changed enterocytes, there were others which were almost intact, and the ratio between altered and unaltered was 3:2 (Fig. 1b). In this group the number of crypts per section also was appreciably reduced (Table 1), indicating involvement of the stem cell population.

Processes of regeneration accompanied in some cases by hyperplasia of the epithelium were clearly observed 78 h after the end of injections of HU irrespective of the intervals between them.

On electron-microscopic investigation most of the changed cells were found in the epithelium of the small intestine of mice receiving HU with intervals of 7, 12, or 19 h. These changes were manifested mainly as destruction of the microvilli, with in some cases denudation of the plasmalemma and its swelling in these regions, in the form of projections into the intestinal lumen. Lysis of membranes of the endoplasmic reticulum and in increase in the number of dark inclusions and vacuoles were observed in the cytoplasm. Destructive changes also were found in the nuclei. In most cells these destructive changes were the morphological reflection of cell death after repeated injections of HU. An increase in the number of translucent vacuoles was observed in the cytoplasm of the goblet cells, as a result of which the

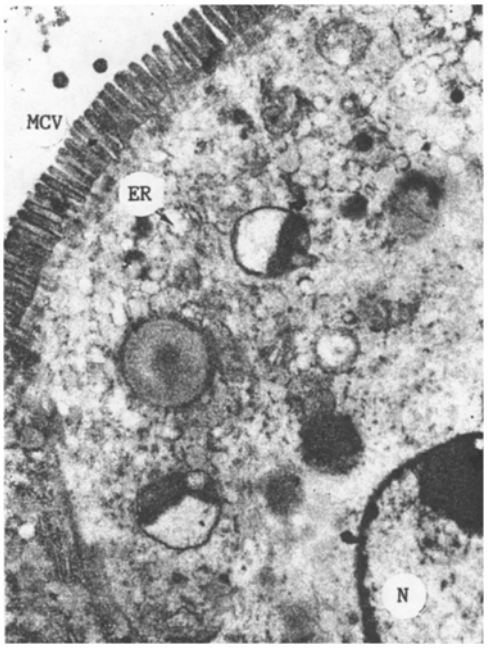

Fig. 2. Enterocyte on villus 30 h after end of periodic injections of HU with interval of 9 h. Microvilli (MCV) intact, nuclei (N) oval in shape, tubules of endoplasmic reticulum (ER) moderately dilated. Magnification 18,000 ×.

cells swelled and protruded in the form of pear-shaped formations, with disturbance of the integrity of the outer plasmalemma, into the intestinal lumen (Fig. 1c, d).

The destructive changes observed in the epithelium of the small intestine are not specific and are found in other pathological conditions. They were found not in all cells, and they varied in severity. A distinctive mosaic distribution was observed at both cellular and ultrastructural levels. On electron-microscopic examination of the small intestinal enterocytes of mice receiving HU with intervals of 9 or 16.5 h, mainly the typical structure of the microvilli was observed on the apical part of the enterocytes on the villi. The cytoplasm contained numerous cytogranules, tiny vacuoles, short membranes of the endoplasmic reticulum, and lipid inclusions. The nuclei were oval in shape and had no visible disturbances (Fig. 2).

The results thus show that two maxima of survival of small intestinal enterocytes occur in mice, corresponding to injections of HU with intervals of about 9 and 16.5 h. This conclusion is in full agreement with the results of an investigation [7] and provides direct experimental confirmation of the theoretical calculations [1, 2, 4, 8].

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