TOXIC DEATH OF MOUSE SMALL INTESTINAL ENTEROCYTES AS A FUNCTION OF INTERVALS BETWEEN INJECTIONS OF THE S-PHASE-SPECIFIC AGENT HYDROXYUREA

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The main disadvantage of modern antitumor chemotherapy is the high toxicity of the cytotoxic agents used relative to normal, rapidly proliferating tissues, including "critical" tissues and cells such as hematopoietic tissue and intestinal enterocytes [1]. One approach to increased selectivity of antitumor agents is protection of the stem cells of normal tissues based on the use of differences in the duration of the cell cycle of cells of normal and tumor tissues [2, 6]. Previously, with the aid of a mathematical model [2, 6], and also in experiments on models of regenerating mouse bone marrow, a resonance increase in the survival rate of hematopoietic stem cells (HSC) was demonstrated in the course of repeated administration of hydroxyurea, an S-phase-specific agent with a period close to the average duration of the cell cycle of mouse HSC [3].

The aim of this investigation was an experimental study of optimal conditions for administration of hydroxyurea to mice, to ensure minimal damage to intestinal enterocytes.

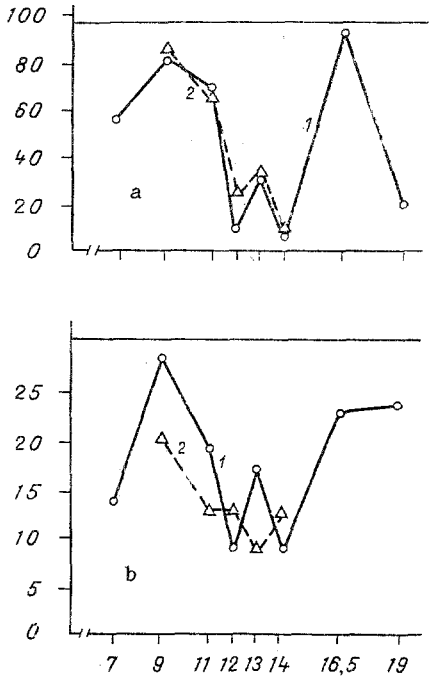

Fig. 1. Dependence of number of cell positions on the villi (a) and in the crypts (b) (average for three mice, ordinate) on interval between hydroxyurea injections (abscissa, in h). Dose of hydroxyurea: 1) 0.2 g/kg, 2) 1 g/kg. Horizontal lines indicate number of cell positions in control.

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METHODS

Experiments were carried out on 8-12-week-old female (CBA × C57BL)F_1 mice weighing 20-22 g, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. Hydroxyurea (Calbiochem, USA; Serva, West Germany) was dissolved in physiological saline immediately before use and injected intraperitoneally in a volume of 0.2 ml. The small intestine (the first 3-5 cm) was removed 2-4 h after the last injection of hydroxyurea. Fixation, section cutting, and staining followed the standard technique. For quantitative analysis of the state of the epithelium the following set of morphometric parameters was used: depth of the cryptal layer (D_c), length of the villi (D_v), height of the enterocytes in the crypts (h_c) and villi (h_v), length of the enterocytes in the crypts (d_c) and villi (d_v), and the number of cell positions (NCP). NCP in the crypts was determined as the ratio of the average depth of the cryptal layer to the average length of the cryptal enterocytes, whereas NCP on the villi was determined as the ratio of their average length to the average length of the epithelial cells of the villus. The length of the enterocytes on the villi (d_v) was measured in sections as near as possible parallel to the long axis. The length of the cryptal enterocytes (d_c) was measured in sections variously oriented, which showed that it was independent of the direction of the section: The projection of the cell on the basement membrane was virtually isoperimetric.

Before receiving an injection of hydroxyurea the mice were irradiated in a dose of 200 rads from a ^{137}Cs source (dose rate 24.5 rads/min) to activate proliferation of the epithelial cells [7]. Periodic injections of hydroxyurea began 18-36 h after irradiation. The animals, which were divided into groups (each consisting of three mice), received six injections of hydroxyurea in doses of 0.2 and 1 g/kg body weight at intervals which varied for the different groups from 7 to 19 h.

RESULTS

The morphometric parameters of the epithelium after injection of hydroxyurea are given in Table 1. Irradiation in a dose of 200 rads had virtually no effect on the state of the enterocytes at the time the animals were sacrificed (50 h or more after irradiation). No clear dependence of the changes in the morphometric parameters of the enterocytes on the dose of hydroxyurea likewise could be discovered whatever the time interval studied. The relationship between NCP for villi and crypts and the interval between injections of hydroxyurea in doses of 0.2 and 1 g/kg is illustrated in Fig. 1. There were two peaks of survival rate of cells on the villi, corresponding to injections of hydroxyurea at intervals of 9 and 16.5 h. With these intervals, no difference was found between the epithelium in the experiment or control, with respect either to morphometric parameters (Table 1, Fig. 1) or to general morphology (Fig. 2: a, b, d). With an interval of 9 h between injections, a maximum of NCP in the crypts also was observed. With intervals of 7 and 12-14 h NCP in the crypts and on the villi was significantly reduced, and in the enterocytes definite signs of degeneration were observed.

With an interval of 10 h NCP on the villi decreased sharply, but in the crypts it did not differ significantly from NCP when the interval was 16.5 h. However, enterocytes of the crypts had virtually the normal morphology when the interval was 16.5 h, but when it was 19 h the epithelium of the crypts was considerably flattened, loose in texture, and vacuolated. With an interval of 19 h the number of viable enterocytes in the crypts capable of completing a cycle of differentiation and of reaching functional positions on the villus was much smaller than the number of cells observed.

The results thus show two peaks of survival rate of enterocytes corresponding to intervals of 9 and 16.5 h. According to data in the literature [2, 6], the first peak of survival of small intestinal cells may be due to a significant decrease in cell damage in the proliferative zone when hydroxyurea was injected with a period close to the average duration of the cell cycle (T_c) of enterocytes under conditions of regeneration. It was in fact shown experimentally previously that T_c of regenerating enterocytes of the mouse small intestine is 8-10 h [5, 8]. As the use of a mathematical model showed [2, 6], with low coefficients of variation of the duration of the cell cycle a marked decrease in the severity of damage to the cell population ought to be observed when a phase-specific cytotoxic agent is administered with a period close to $2T_c$. It was evidently with this effect that increased survival of the epithelium of the mouse small intestine when hydroxyurea was injected with a period of 16.5 h was connected.

TABLE 1. Morphometric Parameters of Small Intestinal Epithelium of Mice Receiving Periodic Hydroxyurea Injections after Inductive Irradiation (μ)

Dose of hydroxyurea, g/kg	intervals between injections, h	Villi									Crypts								
		D_V			h_V			d_V			D_C			h_C			d_C		
0,2	7	340	300	320	20	18	24	6	6	5	120	130	140	8	9	9	10	9	9
	9	500	470	490	25	28	22	5	6	7	160	180	180	17	19	15	6	6	6
	11	400	420	420	22	26	24	6	6	6	120	160	170	11	14	11	8	7	8
	12	100	140	220	16	17	16	16	16	16	160	120	140	16	15	15	15	16	15
	13	360	380	370	18	15	22	13	12	10	130	120	140	15	10	14	8	7	7
	14	100	120	100	10	15	12	17	15	12	120	120	130	10	12	13	15	12	13
	$16^1/_2$	480	500	500	24	28	27	6	5	5	180	140	160	15	13	12	7	8	6
	19	240	180	200	18	15	17	10	8	12	180	160	170	8	6	6	7	8	6
1	9	420	420	450	27	28	24	5	5	5	180	150	170	11	10	14	7	9	8
	11	380	420	400	20	22	20	6	6	7	100	80	120	8	10	14	7	8	7
	12	240	300	280	14	15	13	13	13	14	100	120	160	6	8	5	10	8	10
	13	240	280	220	15	12	17	6	7	10	100	120	110	15	15	12	10	12	14
	14	120	100	110	15	16	10	15	15	13	140	120	120	6	7	10	10	8	12
Irradiated control	—	470	480	480	27	22	25	5	5	5	160	180	170	15	17	14	5	5	5
Unirradiated control	—	480	470	480	28	26	23	5	5	5	120	180	160	18	16	17	5	5	5

Legend. Each number in a column represents the mean value of the parameter for one mouse.

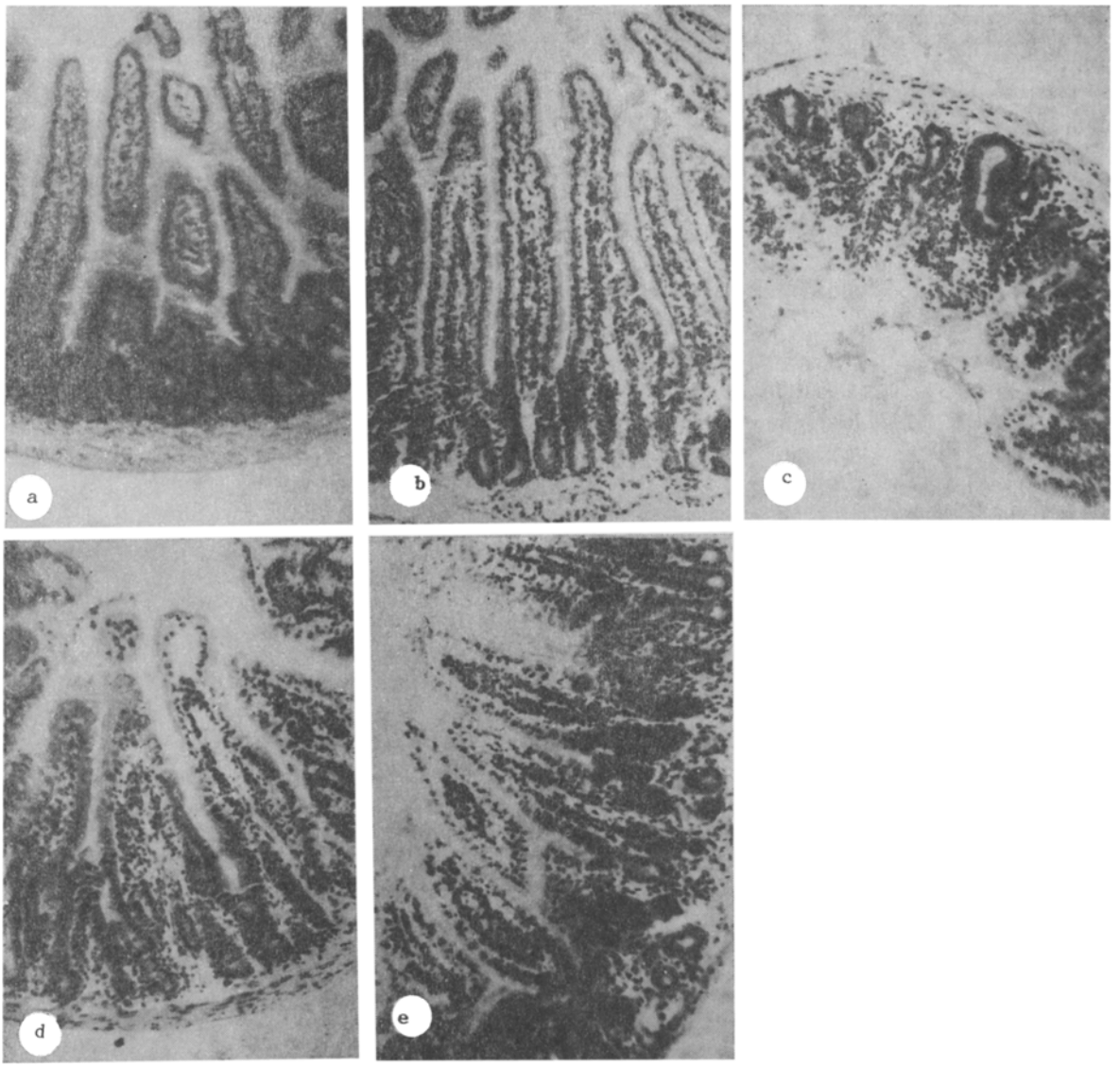

Fig. 2. Histological sections through mouse small intestine: a) intact intestine; b–e) after six injections of hydroxyurea in a dose of 0.2 g/kg, at intervals of 9, 12, 16.5, and 19 h respectively. Hematoxylin–eosin 70×.

Another possible explanation of the second peak of survival of the cells is the presence of two subpopulations (with $T_c \approx 9$ h and with T_c 16.5 h), ensuring maintenance of the epithelium, data indicative of the presence of cells with significantly different T_c values have been described by several workers [4, 5, 9].

Investigation of the sensitivity of mouse small intestinal enterocytes to the toxic action of hydroxyurea thus revealed a resonance increase in the survival rate of the enterocytes if the cytostatic was injected with a period close to the average duration, or twice the average duration, of the cell cycle of the regenerating intestinal cells.

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