RESONANCE DEPENDENCE OF SMALL INTESTINAL EPITHELIAL DAMAGE ON INTERVALS BETWEEN INJECTIONS OF THE S-PHASE-SPECIFIC AGENT, HYDROXYUREA

B. F. Dibrov, A. I. Ataullakhanov,
E. V. Gel'fand, V. S. Gorodinskaya,
A. M. Zhabotinskii, and Yu. A. Neifakh

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It is well known that antitumor compounds exhibit relatively low selectivity and damage both tumor and normal cells. Consequently, the efficacy of antitumor chemotherapy is usually limited by its toxic action on critical normal tissues [12]. For most antitumor agents the rapidly renewed hematopoietic tissue or the small intestinal epithelium are critical [12]. Reduction of the toxic action of antitumor agents on these tissues can considerably enhance their therapeutic efficacy.

It was shown by means of a mathematical model [1, 2, 4, 10] that a significant reduction of damage caused to rapidly renewed tissues (without any reduction in damage to the tumor) can be achieved by injections of a phase-specific cytotoxic agent at intervals close to the average or twice the average duration of the mitotic cycle of the cells responsible for tissue regeneration. The validity of these theoretical predictions has been confirmed in experiments on models of the regenerating small intestinal epithelium of mice [7] and on regenerating bone marrow [3].

The investigation described below is a continuation of the previous study [7] to determine how the survival rate of enterocytes of the mouse small intestine depends on the interval between injections of the S-phase-specific agent, hydroxyurea (HU). It was shown in [7] that this dependence, in principle, is of the resonance type, but the shape of the curve was estimated rather approximately. The investigations likewise were carried out on epithelium regenerating after initiating irradiation, which can change the parameters of the cell cycle of proliferating enterocytes and thus distort the type of dependence [6, 9, 11]. Accordingly, in order that the approach developed in [1, 2, 4, 10] may be used successfully in antitumor chemotherapy, it is important, first, that the type of dependence of damage to the small intestinal epithelium on the interval between repeated, periodic injections of HU be determined more precisely, and second, that this dependence be obtained, not for epithelium regenerating after irradiation, but for the intact epithelium. The investigation described below was devoted to a study of these problems. Parallel with the investigation, the rate of survival of the mice was determined (the data on survival of the mice were published previously [5]), so that the relationship between damage to a critical normal tissue and mortality among the animals can be more fully understood.

EXPERIMENTAL METHOD

Female (CBA × C57Bl)F_1 mice aged 8-10 weeks and weighing 22-24 g and HU from Serva (West Germany) were used. The animals were given eight intraperitoneal injections of HU in a dose of 5 mg/mouse (0.21-0.23 g/kg) at intervals of 6, 7, 8, 9, 10, 12, 15.5, 16.5, and 19 h (three mice at each time). The mice were killed 30 h after the last injection of HU. Segments of small intestine were separated at a distance of 3 cm from the pylorus and fixed in 10% formalin. The height of the villi and depth of the crypts were measured in the histological preparations with an ocular micrometer, and 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 section through the intestine were counted. The total number of enterocytes in the crypts per section through the intestine was calculated

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TABLE 1. Morphometric Parameters of Small Intestinal Epithelium of Mice 30 h after End of Periodic Injections of HU (M ± σ)

Interval between injections of HU, h	Crypts					Villi	
	number of crypts per intestinal section	NCP in transverse sections	NCP in longitudinal sections	depth, μ	total number of enterocytes in crypts (× 10^{-3})	NCP	length
6	3±1	8,7±3,2	7,5±2,2	50±14	0,2	7,8±2,5	88±21
	10±4	6,7±2,5	3,8±0,4	97±25	0,3	4,8±1,0	136±15
	7±3	7,5±2,8	5,1±1,3	78±32	0,3	5,1±1,7	109±31
7	115±9	29,8±3,5	15,7±0,6	113±30	53,8	13,7±3,3	140±34
	79±2	19,7±6,4	14,8±1,9	97±21	23,0	9,2±3,5	131±18
	108±10	30,2±11,0	20,2±2,1	90±26	65,9	11,0±1,8	82±25
8	134±19	27,3±7,1	21,5±4,1	107±12	78,7	24,2±6,4	207±31
	150±18	26,9±5,3	20,8±5,2	137±25	83,9	22,3±2,7	247±61
	185±16	23,2±4,6	28,9±2,3	187±21	124,0	31,3±4,5	367±9
9	141±8	14,1±3,6	16,8±5,0	94±6	33,4	10,2±3,3	133±32
	127±17	20,1±2,7	16,5±1,3	101±10	42,1	11,8±2,5	67±12
	149±14	15,7±1,5	20,8±2,8	74±15	48,7	13,2±4,3	77±20
10	104±5	23,3±3,8	19,2±3,4	70±10	46,5	11,8±3,7	140±26
	94±14	15,9±9,6	15,3±3,6	63±12	22,9	13,2±1,9	113±15
	85±10	18,8±2,0	10,8±1,5	60±10	17,3	6,0±3,7	123±25
12	31±3	9,7±2,0	8,8±1,0	93±31	2,6	3,8±0,3	67±12
	41±10	10,1±4,4	12,0±3,0	83±24	5,0	6,5±2,3	90±36
	24±7	9,6±3,1	9,1±1,3	60±20	2,1	4,1±1,9	60±22
15,5	165±9	16,7±4,5	20,5±2,3	170±10	56,5	41,5±5,2	194±12
	132±25	19,7±2,1	18,5±3,1	130±10	48,1	44,3±1,0	280±40
	167±7	21,3±2,5	23,5±3,3	153±12	83,6	45,7±2,2	247±12
16,5	190±13	31,8±4,5	21,2±2,1	153±13	128,1	38,3±2,1	240±15
	187±21	26,9±2,0	28,7±1,5	173±15	144,4	44,8±3,5	237±25
	198±20	28,1±4,1	23,4±1,8	170±18	130,2	43,4±4,1	242±18
19	123±16	17,1±3,6	18,7±1,8	167±15	39,3	18,0±2,0	230±26
	120±15	17,3±5,5	15,2±1,6	103±15	31,6	8,2±2,8	80±20
	86±20	20,6±2,5	17,2±2,6	150±10	30,5	18,3±2,3	196±15
Control	221±27	26,9±6,0	20,1±2,1	96±19	119,5	108±13	427±15
	219±30	23,3±4,1	18,3±4,0	103±15	93,4	111±15	401±25
	179±4	22,7±4,0	18,3±2,5	111±17	74,4	100±5	426±24

Legend. Total number of enterocytes in crypts per intestinal section calculated by the formula: number of crypts per section × NCP in transverse sections through crypts × NCP in longitudinal sections through crypts. Data for one mouse given on each line.

by the formula: number of crypts per section × NCP in transverse sections of crypts × NCP in longitudinal sections of crypts.

EXPERIMENTAL RESULTS

Morphometric parameters of the epithelium in the control and after courses of periodic injections of HU are given in Table 1. Two minima of damage to the small intestinal epithelium were found, corresponding to injections of HU at intervals of close to 8 or 16.5 h, together with three maxima of damage, corresponding to injections of HU at intervals of 6, 12, or 19 h. It must be emphasized, in particular, that this dependence of damage on the interval between injections was apparent in all parameters used to characterize the state of the small intestinal epithelium.

The severest damage to the epithelium was observed after injections of HU at intervals of 6 h. In this case the enterocyte populations of the crypts and villi were virtually completely annihilated. The number of crypts also was close to zero, an indication of severe and, evidently, irreversible damage to the stem cell population. Rather less marked signs of damage were observed at the second minimum of survival of the epithelium (interval of 12 h), and weaker signs still at the third (interval 19 h). However, even when the interval was 19 h a marked decrease in the number of crypts and in the size of the enterocyte populations in the crypts and villi was observed.

With intervals of close to 8 or 16.5 h the number of enterocytes of the villi was less than in the control, although the decrease was less than with intervals of 6, 12, or 19 h. Meanwhile parameters characterizing the state of the epithelium of the crypts did not differ significantly from the control. With an interval of 8 h, for instance, damage to the crypt epithelium was reflected in a very small decrease in the number of crypts (Table 1), whereas

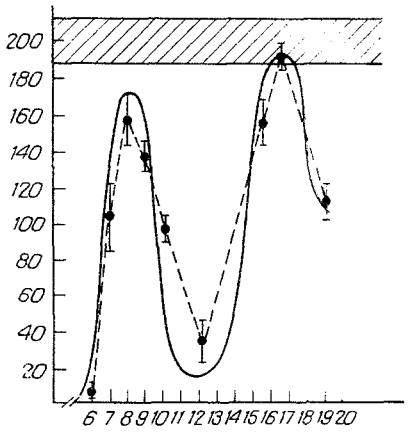

Fig. 1. Experimental and theoretical dependences of number of crypts on interval between injections of HU. Abscissa, interval between injections of HU (in h); ordinate, number of crypts after 8 injections of HU. Each experimental point obtained by averaging data for 3 mice. Shaded region corresponds to M ± m for control animals. It was assumed during the calculation that normally the number of crypts per small intestinal section was 206, and that the mean number of stem cells was 17. The calculated curve (continuous line) was obtained with the following values of the parameters of the cell cycle of stem cells of the small intestinal epithelium: mean duration of the cell cycle (T_c) 8.3 h; coefficient of variation of duration of the cell cycle 0.25; relative duration of determined phase of cycle 0.5 T_c; duration of phase of sensitivity to the cytotoxic action of HU 0.5 T_c; duration of blocking action for a single dose of HU 0; effective multiplication factor per cycle 1.3.

the total number of enterocytes of the crypts was not different from the control. With an interval of 16.5 h the number of crypts was close to the control value, but the total number of enterocytes in the crypts was actually greater than in the control animals.

Comparison of the data given in this paper with those on survival of mice after repeated periodic injections of HU [5] reveals clear correlation between damage to the small intestinal epithelium and the survival rate of the mice. For instance, maxima of survival of the mice and minima of damage to the intestine correspond to injections of HU with intervals of close to 8 or 16.5 h, whereas minima of survival of the mice and maxima of epithelial damage were observed after injections of HU at intervals of 6, 12, or 19 h. It can thus be concluded that the resonance character of dependence of the survival of mice on the interval between repeated injections of HU is determined by the resonance character of the corresponding dependence for the small intestinal epithelium.

The theoretical approach developed in [1, 2, 4, 10] not only predicts the resonance character of damage to a self-renewing tissue on the interval between injections of the agent, but it also enables the parameters of the cell cycle responsible for regeneration of that tissue to be estimated on the basis of this dependence. For the intestinal epithelium the cells concerned are: the self-maintaining stem cells, occupying some of the first positions in the column of epithelium of the intact crypt, and the transient cells occupying the next 10-12 positions. Investigations by the labeled mitosis method show that the duration of the stem cell cycle in intact epithelium is much longer than that of the transient cells, and according to some estimates, measures 30 h, but in damaged epithelium it is much shorter [8, 9]. In the investigations cited only mild lesions of the epithelium were examined, when the stem cells could still be identified by their positions. It is interesting, however, to estimate the limit to which the stem cell cycle can be shortened when more severe damage is present. Dependence of the number of crypts on the period of HU injections is shown in Fig. 1. This dependence characterizes injury to the stem cell population, for death of the crypt evidently corresponds to annihilation of all its stem cells. Analysis of the experimental dependence by means of the model developed in [1] gives an estimate of 8.3 h for the

mean duration of the stem cell cycle. As will be clear from Fig. 1, the experimental points fall accurately on the theoretical curve calculated for this value of the mean duration of the cycle.

As was pointed out above (Table 1), all the parameters characterizing epithelial damage which were investigated, namely the number of crypts and the number of enterocytes in the epithelium of the villi and crypts, changed in phase. This fact indicates that regeneration of all parts of the epithelium is effected by a cell population that is homogeneous with respect to the average duration of its cycle. It can thus be tentatively suggested that if the damage is severe enough, the difference in duration of the cycle between stem cells and transient cells disappears. This disappearance of the difference in the durations of the cycle may perhaps reflect the disappearance of functional differences between stem and transient cells, in agreement with data in the literature [13, 14] to the effect that under conditions of regeneration most or even all proliferating crypt cells form microcolonies just as do stem cells. It is stated in [15] that the population of microcolony-forming cells doubles itself in 8 h. This is in agreement with our estimate of the duration of the cycle of cells responsible for regeneration of the small intestinal epithelium.

The results described above thus directly confirm the theoretical predictions [1, 2, 4, 10] that a resonance decrease in the severity of damage to rapidly renewing tissues can be achieved by correct choice of the interval between injections of phase-specific cytotoxic agents. This offers new prospects for the successful chemotherapy of tumors.

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