RESONANCE DEPENDENCE OF MOUSE SURVIVAL ON INTERVAL BETWEEN INJECTION OF S-PHASE-SPECIFIC AGENT, HYDROXYUREA

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Antitumor agents exhibit relatively low selectivity and damage both tumor cells and normal cells. Damage to cells of "critical" normal tissues, primarily of the hematopoietic system and epithelium of the small intestime, limits the intensity and duration of antitumor therapy and, consequently, its effectiveness [11, 12].

It has been shown by the use of mathematical models [2, 4, 9] that selectivity of antitumor chemotherapy can be significantly increased by correct choice of interval between periodic doses of a phase-specific cytotoxic agent. According to results obtained, this increased selectivity is due primarily to reduction of damage to rapidly renewed "critical" normal tissues if the agent is injected at intervals similar to the average generation time of the cells responsible for regeneration of these tissues. A resonance increase in survival of polypotent hematopoietic stem cells has been found in mice receiving periodic injections of hydroxyurea (HU) at intervals of 12 h [3]. A resonance decrease in damage to the epithelium of the mouse small intestine also has been demonstrated during periodic injections of HU at intervals of 9 and 16.5 h [6].

The aim of this investigation was to study the dependence of over-all toxicity of HU on the interval between injections. Schedules of HU injections leading to significant reduction of the toxic mortality of mice were found.

EXPERIMENTAL METHOD

Female (CBA × C57BL)F_1 mice were used. A solution of HU (from Serva, West Germany) was prepared immediately before injection. HU in physiological saline was injected intraperitoneally in a dose of 5 mg per mouse (from 0.19 to 0.26 g/kg body weight). The interval between injections of HU varied for different groups of mice from 5 to 19 h and the number of injections from six to nine. All the mice remained under observation for 40 days after the end of HU injection.

TABLE 1. Survival Rate of Mice Receiving Periodic Injections of HU

Expt. No.	Number of HU injections	Interval between HU injections, h									
		5	6	7	8	9	10	12	15½	16½	19
1	6	—	0/6	—	7/7[b,c]	7/7[b,c]	—	2/8	—	7/7[b,c]	7/8[b,c]
	8	—	0/8	2/7	7/6[c,d]	5/7[c,d]	2/6	0/10	1/6	3/8	1/8
	9	—	0/6	0/6	3/6	2/6	—	0/6	—	—	—
2	8	—	—	—	7/7[c,d]	—	—	1/7	—	5/7	2/8
	8*	—	—	—	—	—	—	—	—	3/7	0/7
3	6*	—	—	—	—	7/7[c,d]	—	1/7	—	7/7[b,d]	3/7
4	6	2/7	—	5/7	—	7/7[a,c]	—	2/7	—	—	—
5	6	—	3/7	—	7/7[b]	—	—	—	—	—	—
	8	2/8	1/8	5/8	8/8[a,b]	8/8[a,b]	—	—	—	—	—
1+2	8	—	0/8	2/7	13/14[b,c,d]	5/7[b,c,d]	2/6	1/17	1/6	8/15[b,c,d]	3/16

Note. Numerator indicates number of surviving mice, denominator number of mice in group; a, b, c, d) $P < 0.05$ compared with intervals of 5, 6, 12, and 19 h, respectively. Here and in Table 2, asterisk indicates mice irradiated in a dose of 200 rads 27 h before first injection of HU.

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TABLE 2. Time of Death of Mice (in days) after Beginning of Periodic Injections of HU ($M \pm \sigma$)

Expt. No.	Number of HU injections	Interval between HU injections, h									
		5	6	7	8	9	10	12	15½	16½	19
1	6	—	3,5±0,3 (2,2)	—	N.D.	N.D.	—	4,7±0,7 (2,2)	—	N.D.	7,3 (3,3)
	8	—	3,2±0,4 (1,5)	3,9±0,5 (1,9)	3,4 (1,1)	5,2±1,0 (2,5)	4,3±0,5 (1,4)	5,1±0,9 (1,6)	6,2±1,4 (1,6)	6,5±0,4 (1,7)	7,2±2,3 (1,7)
	9	—	2,9±0,3 (0,9)	3,5±0,5 (1,2)	4,0±0,1 (1,3)	4,7±0,2 (1,7)	—	4,2±0,3 (0,2)	—	—	—
2	8	—	—	—	N.D.	—	—	5,1±1,2 (1,6)	—	6,4; 7,1 (1,6; 2,3)	7,5±0,7 (2,0)
	8*	—	—	—	—	—	—	—	—	6,9±0,9 (2,1)	6,8±1,5 (1,1)
3	6*	—	—	—	—	N.D.	—	3,9±1,1 (1,4)	—	N.D.	7,3±0,2 (3,3)
4	6	3,6±0,5 (2,6)	—	3,5; 5,3 (2,0; 3.8)	—	N.D.	—	4,3±0,5 (1,8)	—	—	—
5	6	—	5,1±1,5 (3,8)	—	N.D.	—	—	—	—	—	—
	8	4,5±0,8 (3,1)	5,4±2,0 (3,7)	5,6±2,4 (3,6)	N.D.	N.D.	—	—	—	—	—

Note. Average time since last injection of HU until death of mice (in h) shown in parentheses. N.D.) No mice died.

Some mice in experiment No. 2 and all the mice in experiment No. 3 were irradiated in a dose of 200 rads, 27 h before the beginning og HU injections, from a ^{137}Cs source (dose rate 24.5 rads/min) to activate regeneration of rapidly renewed tissues [5, 7, 8, 10].

The weight and age of the mice were respectively as follows: 22-24 g and 8-10 weeks (experiment No. 1), 23-25 g and 11-13 weeks (experiment No. 2), 20-22 g and 8-10 weeks (experiment No. 3), 19-20 g and 6-8 weeks (experiment No. 4), 25.27 g and 16-18 weeks (experiment No. 5).

The significance of differences in the survival rate of the mice was estimated by the chi-square test.

EXPERIMENTAL RESULTS

Table 1 shows two maxima of survival rate of mice corresponding to intervals between HU injections of 8-9 and 16.5 h. According to visual observations, repeated injections of HU cause diarrhea, emaciation of the mice, diminution of their mobility, and disturbance of the state of the fur. These external signs of toxicity were manifested particularly strongly in animals receiving HU with intervals of 5, 6, 7, 12, and 19 h, but they were significantly less marked in the case of intervals of 8, 9, and 16.5 h.

When HU was injected at intervals of 5, 6, 7, and 12 h marked worsening of the state of the mice took place during the first 36 h after the end of the injections. The state of these animals remained extremely serious for the next 6-7 days, and any subsequent improvement was slow. With intervals of 8 and 9 h between injections, manifestations of toxicity either were absent (six injections of HU in experiments Nos. 1-4 and six or eight injections of HU in experiment No. 5) or were transient, and the normal state of the mice was restored during the first 3-4 days after the end of HU injections. External signs of toxicity were rather more marked if the interval was 16.5 h. External signs of toxicity were rather more marked if the interval was 16.5 h. Increasing the interval to 19 h was followed by appreciably more prolonged and severe external signs of cytotoxicity at all times, but the state of the mice after injections at an interval of 19 h was better than after injection of HU at intervals of 5, 6, and 12 h.

Preliminary irradiation in a dose of 200 rads significantly accelerated and intensified the manifestations of cytotoxicity after injections at intervals of 12 and 19 h, but worsened the state of the mice only a little if the intervals were 9 and 16.5 h. The effect of preliminary irradiation was particularly marked if HU injections were given at intervals of 19 h. In this case sudden worsening of the state of the irradiated mice was observed as early as with the sixth injection of HU, whereas external manifestations of damage were not present in unirradiated animals, and later they were appreciably milder.

Death of the animals took place only during the first week after the last injection of HU. In all groups maximal mortality was observed in the course of a few days after the last injection of HU (Table 2). According to data in the literature [1], death of the mice at these times was due to damage to the epithelium of the small intestime. This conclusion was confirmed by histological analysis of sections through the mouse small intestime after periodic injections of HU: The data in Table 1, showing how the survival rate of the mice depends on the interval between HU injections, correlate precisely with the corresponding relationship for survival of the cells of the small intestinal epithelium and its ability to regenerate later. The results of the histological analysis will be published separately (see also [6]). Incidentally, maxima of survival of the mice were observed when HU injections were given at intervals close to the average and twice the average duration of the cell cycle in the small intestine when regenerating after injury to the epithelium. We know [7, 13] that after exposure to factors causing damage to the small intestine the mean duration of the cell cycle of its enterocytes is 8-10 h.

It can be concluded from the facts described above and data in the literature [2, 4-6, 9] that the resonance character of dependence of survival of mice on the interval between HU injections is determined by the resonance character of dependence of survival of cells of the "critical" tissue for HM, namely the small intestinal epithelium, on the interval between injections of this agent.

The results given in Table 1 directly confirm theoretical conclusions [2, 4, 9] that toxicity of courses of antitumor chemotherapy with phase-specific cytotoxic agents can be significantly reduced by correct choice of interval between injections of the agents on the basis of data on the duration of the mitotic cycle of cells responsible for regeneration of the "critical" normal tissue.

Research into antitumor chemotherapy [11, 12] has shown that potential benefit may be obtained from reducing the interval between injections of high doses of S-phase-specific agents to the duration of the S-phase of spontaneous tumors. However, it is difficult, in principle, to apply this approach in practice because of the sharp increase in toxicity of the agent when given by repeated injections. The results show that the use of the approach developed in previous investigations [2, 4, 9] enables the interval between injections of high doses of a S-phase-specific agent to be considerably shortened (to 8-9 h) without any increase or, indeed, with a decrease in overall toxicity. The results of the present experiments may therefore lead to further progress on the road to successful cancer chemotherapy.

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LITERATURE CITED

1. N. N. Blokhin and Ch. G. Zubrod (eds.), The System for Creation of Antitumor Agents in the USSR and USA [in Russian], Moscow (1977), p. 121.
2. B. F. Dibrov, A. M. Zhabotinskii, Yu. A. Neifakh, et al., Khim.-farm. Zh., No. 12, 1415 (1983).
3. B. F. Dibrov, A. M. Zhabotinskii, A. V. Krinskaya, et al., Byull. Éksp. Biol. Med., No. 3, 345 (1984).
4. B. F. Dibrov, A. M. Zhabotinskii, Yu. A. Neifakh, et al., Biofizika, 30, 1066 (1985).
5. D. S. Sarkisov, Essays on the Structural Principles of Homeostasis [in Russian], Moscow (1977).
6. L. I. Churikova, A. V. Krinskaya, B. F. Dibrov, et al., Byull. Éksp. Biol. Med., No. 6, 746 (1986).
7. H. S. Al-Dewachi, N. A. Wright, D. R. Appleton, and A. J. Watson, Virchows Arch. B, Zellpathol., 34, 299 (1980).
8. P. A. Chervenick and D. R. Boggs, Blood, 37, 568 (1971).
9. B. F. Dibrov, A. M. Zhabotinskii (A. M. Zhabotinsky), Yu. A. Neifakh (Yu. A. Neyfakh), et al., Math. Biosci., 73, 1 (1985).
10. W. R. Hanson, D. L. Henninger, R. J. M. Fry, and A. R. Sallese, Cell Proliferation in the Gastrointestinal Tract, ed. by D. R. Appleton, J. R. Sunter, and A. J. Watson, London (1980), pp. 198-212.
11. B. T. Hill, Biochim. Biophys. Acta, 516, 217 (1978).
12. I. Tannock, Cancer Treat. Rep., 62, 1117 (1978).
13. H. R. Withers, Cancer (Philadelphia), 28, 75 (1971).