DEPENDENCE OF SURVIVAL OF MOUSE HEMATOPOIETIC STEM CELLS ON INTERVAL BETWEEN REPEATED INJECTIONS OF HYDROXYUREA

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The effectiveness of antitumor preparations is usually restricted by their toxic action on "critical" normal tissues, especially bone marrow and intestinal epithelium. Selectivity of antitumor chemotherapy can be increased by optimizing the program of administration, making use of differences in the kinetics of tumor cell populations and stem cell populations of "critical" tissues [4].

The aim of this investigation was to determine a program of regular injection of a phase-specific cytotoxic agent, causing minimal damage to hematopoietic stem cells (CFUs). It is easy to see that during periodic administration of a phase-specific agent on a homogeneous population of dividing cells, minimal damage will take place if the interval between injections is equal to the duration of the cell cycle (T_c). In that case, during repeated injections of the agent, the blow will fall on the "empty place," and in the ideal case no additional death of cells will take place. In a real population, however, the magnitude of the effect depends on many factors: dispersion of T_c, the shape of the T_c distribution curve, the presence of an additional blocking action of the agent, and so on. Kinetic parameters of the CFUs population have not yet been determined with sufficient accuracy to enable prediction of the effect. In this investigation, a direct experimental determination was accordingly made of the proportion of surviving CFUs depending on the interval between injections of hydroxyurea (HU) into mice *in vivo*. Hydroxyurea kills cells in the S-phase of the cycle [9].

EXPERIMENTAL METHOD

Female (CBA × C57BL)F_1 mice aged 8-12 weeks and weighing about 20 g were used in the experiments.

A solution of HU (from Calbiochem, USA, and Serva, West Germany) in a concentration of 100 mg/ml was made up in physiological saline immediately before injection. Injections were given intraperitoneally in a dose of 1 g/kg body weight. A ^{137}Cs source with dose rate 24.5 rads/min was used for irradiation.

At the beginning of the experiment the animals were irradiated in a dose of 200 rads to initiate the entry of resting stem cells into the cell cycle [3, 6]. Regular injection of HU began 18-36 h after initiating irradiation. All the experimental mice received six injections of HU with intervals of 7 to 19 h. Depending on the interval between injections the animals were divided into groups with five mice in each group. Injection graphs were plotted so that animals of all experimental and control groups were killed almost simultaneously 3-4 h after the last injection.

To determine the number of stem cells Till and McCulloch's method [10] was used. Recipient mice (7-11 at each point) were irradiated beforehand in a dose of 1300 rads. Splenic colonies were counted on the 8th day. The number of endocolonies did not exceed two colonies per 10 mice.

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EXPERIMENTAL RESULTS

The results of three independent experiments are given in Table 1. They show that with injections at intervals of 12 h there was a sharp increase in the number of surviving CFUs. The data in Table 1 show that 18 and 93 h after initiating irradiation the CFUs level was about 30% of its initial value. This is in agreement with data in the literature [6, 7], according to which the number of CFUs falls during the first few hours after irradiation in a dose of 150-200 rads, then remains constant for several days. The CFUs level after initiating irradiation can thus be regarded as constant, and it is a true control. The results of two experiments are given in Fig. 1 as percentages of this control. It will be clear from Fig. 1 that injection of HU with short intervals leads to virtually total annihilation of the CFUs population. With lengthening of the interval between injections of HU a sharp increase is observed in the fraction of surviving CFUs, with a sharp peak corresponding to an interval of 12 h. With longer intervals the fraction of surviving CFUs remains relatively constant, significantly below the peak value.

Theoretical curves calculated for two populations with different mean duration of the cycle (T_c^m) also are given in Fig. 1. A mathematical model, described in detail previously [1], was used for the calculations. Analysis of this model shows that the interval between injections of the phase-specific cytotoxic agent at which a peak of survival is observed is close to the mean duration of the cell cycle (T_c^m). Resonance is reduced with an increase in the coefficient of variation (V) of the distribution of T_c and is increased on account of the blocking effect possessed by certain S-phase-specific agents, including hydroxyurea. Curve 1 in Fig. 1, which is in good agreement with the experimentally obtained relationship, was calculated on a model with the following parameters: T_c^m = 12 h; duration of S-phase T_s = 0.45•

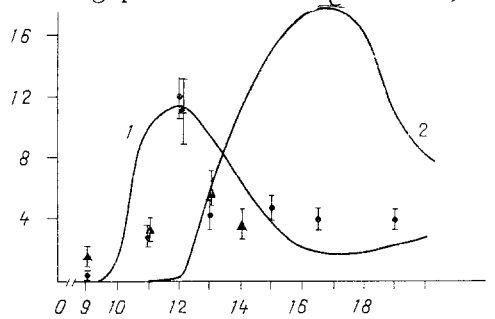

Fig. 1. Experimental and calculated dependence of number of surviving CFUs on interval between HU injections. Abscissa, interval between injections of HU (in h); ordinate, number of surviving CFUs per femur (in % of initial level, taken as number of CFUs 18 h after initiating irradiation), according to data for experiments No. 2 (circles) and No. 3 (triangles). Confidence interval with level of significance P = 0.03 indicated for each point. Calculated curves for CFUs population (curve 1) and for hypothetical population of cancer cells with longer T_c^m (curve 2) obtained with the following values of parameters:

$$1)\ T_c^m = 12\ \text{h};\ q = 0.5;\ T_s = 0.45 \cdot T_c^m;\ V = 0.39;\ T_b = 0.38 \cdot T_c^m$$

$$2)\ T_c^m = 18\ \text{h};\ q = 0.4;\ T_s = 0.45 \cdot T_c^m;\ V = 0.39;\ T_b = 0.26 \cdot T_c^m.$$

A model of the cell cycle for which the density of distribution of probability of duration of the cycle T_c, in the absence of HU, is in the form

$$F(T_c) = K^n (T_c - 0.45\, T_c^M)^{n-1} \cdot \exp[(T_c - 0.45\, T_c^M)K](n-1).$$

was used. In this case, $T_c^m = n/(0.55\ K)$; $V = 0.55/\sqrt{n}$).

TABLE 1. Survival of CFUs during Regular Injection of HU After Initiating Irradiation (M ± m)

Interval between injections of HU, h	Number of CFUs per femur, experiments		
	1	2	3
7	7,5 ± 3,5 (11)*	—	—
9	21 ± 8 (10)*	5,5 ± 1 (9)*	24 ± 5 (7)*
11	—	50 ± 8 (9)*	45 ± 6 (10)*
12	161 ± 12 (11)	171 ± 19 (8)	147 ± 25 (9)
13	—	60 ± 10 (7)*	77 ± 7 (10)*
14	—	—	51 ± 10 (9)*
15	46 ± 10 (10)*	66 ± 8 (9)*	—
16,5	—	58 ± 8 (9)*	—
19	—	56 ± 6 (8)*	—
Irradiated (200 rads) control:			
18 h after irradiation	—	1414 ± 112 (7)	1338 ± 51 (9)
93 h after irradiation	2076 ± 232 (9)	1464 ± 218 (7)	—
Unirradiated control	7068 ± 306	4500 ± 607	—

Legend. Values of M differing significantly ($P < 0.05$) from values of M for a 12 h interval are marked by an asterisk. Number of recipient mice given in parentheses. m) Error of mean.

T_c^m; coefficient of variation of duration of the cycle V = 0.39; duration of block $T_b = 0.38 \cdot T_c^m$; fraction of cells taking part in differentiation, q = 0.5. These values lie within the range cited in the literature [5, 8, 11, 12].

We know that T_c^m for most spontaneous tumors is significantly above 12 h [2]. The calculated curve 2 in Fig. 1 corresponds to a hypothetical cancer population with T_c^m = 18 h. It will be clear from Fig. 1 that injection of the agent at intervals of 12 h gives maximal gain in survival of the stem cell population relative to the cancer cell population. A similar result was obtained in all cases when T_c^m of the cancer cells differs appreciably from T_c^m of the CFUs.

Injection of a phage-specific cytotoxic agent at an interval equal to the mean duration of the stem cell cycle of normal tissue thus ensures highest selectivity of chemotherapy of spontaneous tumors.

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