Recovery of Colony-Forming Capacity of Murine Stem Hemopoietic and Blood Cells under the Effect of 1-β-D-Arabinofuranosyl Cytosine

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The cytostatic 1-β-D-arabinofuranosyl cytosine (cytarabine) in a dose of 200 mg/kg decreases blood count of nuclear cells in mice to 60% and inhibits colony formation in the spleen for 8 days. The Resist repair system developed by us normalizes the total count within 3 days after cytarabine injection and repairs colony-forming activity of stem hemopoietic cells within 5 days, if Resist is injected after the cytostatic, and within 8 days, if it is injected before cytarabine. Thus, Resist restores immunity and hemopoiesis systems in cancer patients treated by radio- and chemotherapy.

Key Words: *1-β-D-arabinofuranosyl cytosine; stem hemopoietic cell; blood cells; Resist*

Effects on the genome of both malignant and normal cells of cancer patients are a drawback of radio- and chemotherapy. Like irradiation, cytostatics cause DNA rupture, chromosome aberrations, etc. [7-9], leading to leukopenia, anemia, and immune response inhibition [5,6]. Protection of stem hemopoietic cells (SHC) and, consequently, of the immune system and hemopoiesis during radio- and chemotherapy is a major priority. Interleukins, interferons, and tactivin have been used for this purpose in recent years [1,2]. These cytokines repair the parameters of blood and immunity but do not protect DNA from damage in normal cells. Therefore, these drugs exert only a maintenance effect.

This study is an attempt to develop a new approach to protection and repair of SHC and blood cells, including activation of repair processes in damaged cells. The cytostatic cytarabine (CA) was used in our experiments.

MATERIALS AND METHODS

(CBA×C57Bl/6) F_1 mice aged 3 months were used. CA was injected intraperitoneally in a dose of 200 mg/kg in 0.3 ml medium. Resist was injected intraperitoneally 24 and 3 h before CA or 3 and 24 h after CA. The total count of nuclear cells was determined in blood collected from the tail vein after 2, 3, 5, and 8 days. For estimating the count of protected or repaired functionally active SHC, the mice were irradiated with a dose of 6 Gy (0.9 Gy/min, Al filter, 3 mm thick) 3.5 or 8 days after CA injection. Endogenous colonies of hemopoietic cells in the spleen (colony-forming units, CFU) were counted on 12th day after exposure [10]. Results were processed by Wilcoxon-Mann-Whitney's nonparametrical statistical method. The data are presented as the mean of 2 experiments, each group included 10-14 animals.

RESULTS

Our approach to protection and repair of functional activity of SHC after exposure to cytostatics is based on the principle that cell injuries of similar type (caused by ionizing radiation, alkyl compounds, and nucleotide analogs) can be repaired similarly. We have developed a method for postradiation repair of SHC injuries. This method consists in restoring the capacity of SHC to form CFU in the spleen by

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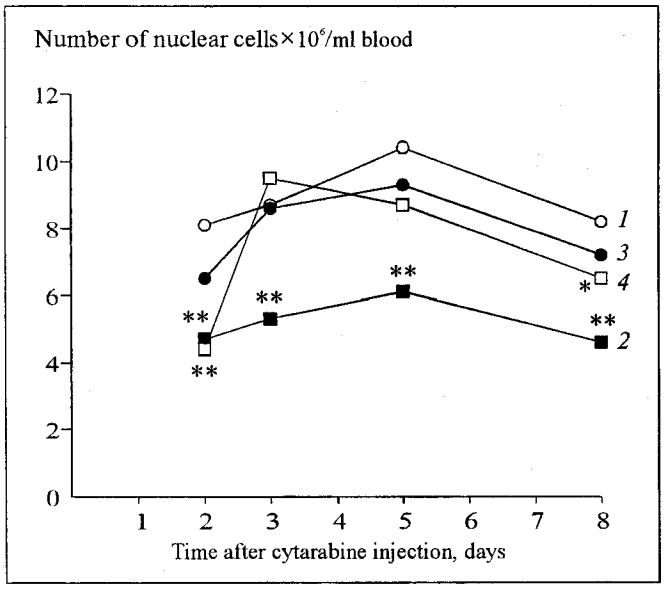

Fig. 1. Effect of Resist on the total number of nuclear cells in the blood of (CBA×C57Bl/6) F_1 mice after injection of 1-β-D-arabinofuranosyl cytosine. *1*) intact controls; *2*) cytarabine, 200 mg/kg; Resist before (*3*) and after (*4*) cytarabine. *$p<0.05$, **$p<0.01$ *vs.* the control.

injecting Resist and ensures a 60-70% survival of mice after lethal irradiation (8.5 Gy). The repair system Resist contains radioprotectors and substances stimulating cAMP synthesis [4]. We have assumed that stimulation of cAMP synthesis after treatment with cytostatics activates the repair of SHC damage. After a single injection of CA in a dose of 200 mg/kg the total blood count of nuclear cells decreased to 60% and remained at this level for 8 days (Fig. 1). Resist improved this count by day 3 after CA; this effect did not depend on the time of Resist injection (before or after the cytostatic). The cytological picture of the blood was virtually the same (preliminary data). A single injection of CA in a dose of 200 mg/kg inhibited endogenous colony formation in the spleen for 8 days (Table 1). Injection of Resist both before and after CA restored this process. The maximum number of endogenous CFU in the spleen was observed 8 days after CA if Resist was injected before it (216% *vs.* irradiated animals). Injection of Resist after CA restored the process of formation of endogenous CFU in the spleen with the maximum count of CFU 5 days after injection (178% *vs.* irradiated controls). Resist contains the substances stimulating cAMP synthesis in concentrations inhibiting mitotic processes and delaying the immune reactions by 24 h [3]. Resist repairs endogenous CFU in the spleen no sooner than for 5 days; therefore, it is likely that in this case SHC and blood cells are not protected but repaired. It is important that Resist repairs these cells irrespective of the time of administration (before or after CA). Presumably, Resist activates and provides better coordination of all regulatory systems due to stimulation of cAMP synthesis and activation of energy processes by radioprotectors and cAMP. As a result, DNA repair systems are activated and the injuries are repaired. Such a state is possible only in a genetically healthy cell injured for the first time by cytostatics and/or radiation. Due to autoregulation of transformed malignant clones and practically blocked cAMP system, these clones cannot respond to Resist by the recovery of coordination of intracellular regulatory systems.

Therefore, Resist selectively affects genetically healthy but not malignant cells. Our results indicate the efficacy of Resist during therapy with cytostatics and permit a conclusion that any pathological process in the organism, associated with changes in the genome structure, should be arrested with due consideration for repair of injuries under conditions of activation of the cAMP system, the key system in coordination of intracellular regulatory systems and functional activity of cells of any tissue, including the immune, and primarily SHC.

TABLE 1. Effect of Resist on the Formation of Endogenous Colonies in the Spleen of (CBA×C57Bl/6) F_1 Mice under the Effect of 1-β-D-arabinofuranosyl Cytosine (*M±m*)

Exposure	Number of CFU in spleen of mice exposed to 6 Gy, after CA					
	after 3 days		after 5 days		after 8 days	
	CFU	experiment/ control	CFU	experiment/ control	CFU	experiment/ control
Control irradiated mice	8.1±2.4	—	8.5±2.1	—	8.5±2.1	
CA	0.0	—	0.0	—	0.09±0.02	0.01
Resist:						
before CA	0.0	—	1.2±0.5*	0.14	18.4±2.6*	2.16
after CA	0.0	—	15.2±2.9*	1.78	10.5±2.5	1.24

Note. *$p<0.05$ *vs.* control.

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