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Radioprotective Action of Lymphokinin, a Composite Preparation of Cytokines

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Lymphokinin injected into mice in a dose of 25,000 U/kg intraperitoneally before or after their single irradiation producing acute radiation sickness is found to prolong their survival. The optimal time for administering Lymphokinin depends on the radiation dose. Its survival-prolonging effect is most marked when it is injected 1 h or 24 h before or 23 h after exposure to a dose of 6.0 Gy.

Key Words: *Lymphokinin (a composite preparation of cytokines); radiation; radiation protection; survival*

One sequela of radiation is damage to the bone marrow with subsequent impairment of hematopoiesis. As a result, various disturbances of the immune status occur and the risk of opportunistic infections and cancer rises considerably, so that the outcome for humans and animals is often fatal.

In recent years, intense efforts have been expended all over the world in the search for radioprotectants-immunostimulants, i.e., medicinal substances that can both enhance the body's resistance to radiation and strengthen the body's natural immune defenses against infection and tumor growth. Therapies relying on the use of cytokines to speed the recovery of bone marrow hematopoiesis and protect against radiation are being developed [3,7,11]. Being natural regulators of the immune and hematopoietic systems, cytokines act as powerful immunostimulants [2-5,9,10].

The purpose of the experiments described here was to evaluate the radioprotective potency of Lymphokinin, a preparation developed at the Center for Cancer Research, Russian Academy of Medical Sciences, and composed of interleukins 1, 2 (its main cytokine), and 6 (IL-1, IL-2, and IL-6) and tumor necrosis factor (TNF).

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MATERIALS AND METHODS

The experiments were carried out on random-bred mice weighing 18-20 g. After all mice had been kept under quarantine surveillance for 7-10 days in

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the vivarium on a standard diet, acute radiation sickness was produced in them by a single uniform total-body exposure to gamma quanta emitted by ^{137}Cs from an IGUR-I apparatus. Some test groups received a dose of 6.0 Gy (the dose lethal to 70% of mice, LD_{70}), 6.75 Gy (LD_{85}), or 7.5 Gy (LD_{95}) at a rate of 1.5 Gy/min. Other test groups were irradiated with 6.0 or 6.75 Gy at a rate of 0.75 Gy/min or with 7.5 or 8.75 Gy at 0.017 Gy/min (prolonged irradiation). The test animals were observed for survival over 30 days.

Lymphokinin was injected intraperitoneally in a dose of 25,000 U/kg 24 h or 1 h before irradiation to assess its preventive action or 23 h after irradiation to assess its curative potential. The control groups were injected with physiological saline intraperitoneally at the same times. Each test group consisted of 10 or 20 mice and each control group of 30 mice.

The times of Lymphokinin administration and the radiation doses were selected on the basis of a mathematical theory of experimentation [1] so as to optimize conditions for demonstrating the efficacy of this preparation. The statistical significance of the results was estimated using the coefficient of concordance χ^2.

RESULTS

In preliminary tests, a model using Lymphokinin at 25,000 U/kg in radiation-exposed mice was found to be statistically adequate and informative (data not shown), and this dose level was therefore used in the further experiments.

As seen in Table 1, the survival rate was inversely proportional to the radiation dose within the dose range used. Lymphokinin raised the survival rate significantly (up to 80%) in the groups exposed to 6 or 6.75 Gy and was less effective in those exposed to 7.5 Gy.

Among the groups administered Lymphokinin before irradiation for preventive purposes, survival rates were highest in the groups that received it 24 h before exposure to 6.0 or 6.75 Gy ($p<0.05$) and in the group given it 1 h before exposure to 7.5 Gy ($p<0.05$). Of the three groups administered Lymphokinin at 23 h postirradiation to test its curative potential, a significant effect was recorded only in the group irradiated with 6.0 Gy (80% of the mice survived vs. 40% in the corresponding control group) (Table 1). Thus, as shown by this two-factor experiment (Table 1), the best time for administering Lymphokinin is dependent on the radiation dose. Similar results came from our one-factor experiment (Table 2) staged to check the validity of the data obtained in the two-factor experiment. It can be seen in Table 2 that injecting mice with Lymphokinin 1 or 24 h before their exposure to 6.0 or 6.75 Gy resulted in a significant prolongation of their survival.

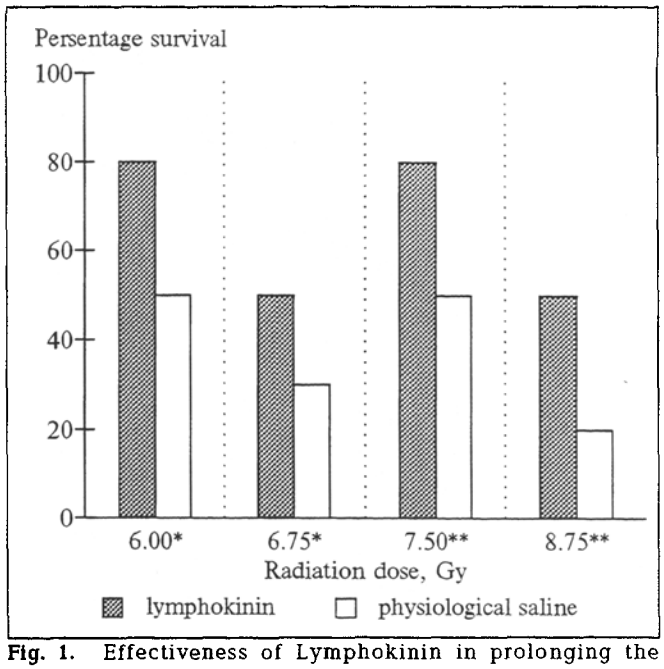

Fig. 1. Effectiveness of Lymphokinin in prolonging the survival of mice irradiated at a low dose rate. Lymphokinin or physiological saline were injected intraperitoneally 24 h prior to radiation exposure. Mice were observed for survival over 30 days. One and two asterisks denote radiation dose rates of 0.75 and 0.017 Gy/min, respectively.

In a further experiment, Lymphokinin was administered to mice 24 h before exposure to ra-

TABLE 1. Preventive and Curative Effects of Lymphokinin in Irradiated Mice (Results of the Two-Factor Experiment)

Group	Values of factors		Survival rate, %
	radiation dose, Gy	injection time, h	
Test groups			
1	6	−24	80*
2	6	−1	70*
3	6	+23	80*
4	6.75	−24	80*
5	6.75	−1	50*
6	6.75	+23	30
7	7.5	−24	20
8	7.5	−1	40*
9	7.5	+23	10
Control groups			
10	6	–	40
11	6.75	–	20
12	7.5	–	0

Note. Here and in Table 2 mice were irradiated at a dose rate of 1.5 Gy/min. The asterisk denotes a significant difference in survival from the corresponding control group at $p<0.05$.

TABLE 2. Radioprotective Efficacy of Lymphokinin Administered to Mice before Irradiation ($M \pm m$)

Group	Experimental conditions		№ of mice	Survival		Mean survival time, days
	radiation dose, Gy	time before irradiation, h		№ of surviving mice	survival rate, %	
1	6.0	1	20	13*	65	15±0.6*
2	6.75	1	20	9*	45	13±1.5*
3	7.5	1	10	4*	40	9.3±1.8
4	6.0	24	20	15*	75	13.7±0.9*
5	6.75	24	20	14*	70	16.2±1.6*
6	7.5	24	10	2	20	9.7±0.8
7	6.0	–	30	9	30	10.7±1.2
8	6.75	–	30	5	17	9.6±0.6
9	7.5	–	30	0	0	9.1±1.6

diation at low dose rates (prolonged irradiation) so as to simulate more closely the real situations which usually involve long-lasting exposure to relatively low radiation doses. This time of injection (24 h prior to irradiation) was chosen because it had been found to be optimal for securing a protective effect in mice exposed to radiation at a high dose rate (1.5 Gy/min). As shown in Fig. 1, in this experiment Lymphokinin was less effective in reducing lethality than before (e.g., the survival rate was 80% in the group exposed to 7.5 Gy at a rate of 0.017 Gy/min vs. 50% in the control group and 50% vs. 20% in the one exposed to 8.75 Gy at the same rate).

Thus, these experiments on mice with acute radiation sickness produced by various doses of ionizing radiation have demonstrated the ability of Lymphokinin to exert both prophylactic and therapeutic effects. The radioprotective activity of this preparation is possibly associated with the presence in it of such cytokines as IL-1 and TNF which, unlike IL-2 and γ-interferon, have been shown to possess radioprotective properties [6, 8].

The results of the experiments reported here permit the conclusion that Lymphokinin deserves further study as an agent that can afford protection against radiation injury.

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