

## EFFECTS OF EARLY THERAPEUTIC ADMINISTRATION OF INTERLEUKIN-1 $\beta$ ON SURVIVAL RATE AND BONE MARROW HAEMOPOIESIS IN IRRADIATED MICE

Alexander Grebenyuk, Victor Zatsepin, Natalia Aksenova, Alexander Timoshevsky

Medical Military Academy, St. Petersburg, Russia

**Summary:** The study was aimed at the experimental assessment of the efficacy of recombinant interleukin-1 $\beta$  in the early therapy of haemopoiesis disorders caused by X-ray irradiation. The efficacy of the product was based on the 30-day survival rate and average life expectancy of dead animals. Endogenous and exogenous colony-formations were studied to reveal possible mechanisms of therapeutic effects of interleukin-1 $\beta$  on the bone marrow. The early administration (10–15 min after exposure to radiation) of recombinant interleukin-1 $\beta$  in a dose of 50  $\mu$ g/kg increased the survival rate and prevented the post-irradiation decrease in the number of endogenous and exogenous CFU-S<sub>0</sub> in irradiated mice.

---

**Key words:** Irradiation; Interleukin-1 $\beta$ ; Radiation injury; Therapeutic efficacy; Survival rate; Haemopoiesis

---

### Introduction

Early treatment of radiation injuries is of essential importance in preserving the life, health and vitality of people exposed to above-normal radiation doses (11). Some drugs of microbial origin (proteus vaccine, ribomunil, prodigiosanum etc.), polynucleotides (desoxinate) and cytokines are able to prevent the death and accelerate the recovery of the bone marrow exposed to radiation if used within the first hours/days after irradiation. Numerous investigations of domestic and foreign authors demonstrated their high radioprotective efficacy in various types of radiation exposure and their protective effects, when they were used before or after the exposure to radiation (1, 8).

One of the most promising radioprotective substances from the cytokines group is interleukin-1 (IL-1), which belongs to key mediators of body defence mechanisms (3). The most important biological properties of interleukin-1 are its ability to stimulate the proliferation, differentiation and maturation of the cells of myeloid, megakaryocytic and lymphoid lineages as well as to modulate the functional activity of mature immunity cells (2, 6).

IL-1 were shown to have a high radioprotective efficacy in acute radiation injuries caused by external irradiation at high and low dose rates, provide the radioprotective activity in local, complex and combined radiation injuries, prevent the depression of the myelopoiesis, and accelerate the recovery of peripheral blood cells after fractionated irradiation at large total doses (4, 9).

Thus, the study presented here was focused on the experimental assessment of the early therapeutic efficacy of recombinant IL-1 $\beta$  in haemopoiesis disorders caused by X-ray irradiation.

### Material and Methods

#### Study design

The therapeutic effect of the product was studied based on the 30-day survival rate and average life expectancy of dead animals. To reveal possible mechanisms of therapeutic effects of IL-1 $\beta$  on the bone marrow, endogenous and exogenous colony formations were studied by counting the number of colonies grown on the spleens of irradiated mice on the 9<sup>th</sup> day after irradiation (10).

#### Animals

The early therapeutic effect of recombinant IL-1 $\beta$  on the survival rate of irradiated animals and the number of haemopoietic early precursors were estimated in 350 linear male mice F<sub>1</sub> (CBA x C57Bl) and 210 white inbred mice.

The animals were housed in a facility for experimental animals, fed on a standard diet and were given water ad libitum until the end of the experiment. The rules of ethical treatment of experimental animals were observed during the experiments.

#### Irradiation

The animals were exposed to X-ray radiation on the RUM-17 apparatus under the following conditions: voltage

180 kV, current 15 mA, filter 0.5 mm Cu + 1.0 mm Al, source to skin distance 50 cm, dose rate 0.292 mA/kg (52.2 R/min), the direction of exposure: back-to-chest. The dosimetry was provided with the ID-11 dosimeter.

#### ***The evaluation of effects of recombinant IL-1 $\beta$ based on 30-day post-irradiation lethality of irradiated animals***

The 30-day survival rates were established in 120 white inbred male mice. The mice were divided into 8 groups (4 control groups and 4 experimental ones) and the experimental animals were exposed to radiation in doses of 6.0, 7.0, 8.0 or 9.0 Gy. The numbers of dead and surviving animals and the dynamics of their death were registered daily over 30 days after irradiation. The percentage of survivors after exposure to radiation and average life expectancy of dead animals were calculated. The dose reduction factors (DRF) of recombinant IL-1 $\beta$  for 30-days mortality are inverse values of the relative radiation effect in mice treated with the cytokine.

#### ***The evaluation of effects of recombinant IL-1 $\beta$ based on the bone marrow haemopoiesis in irradiated animals***

In the endogenous colony formation technique, mice-hybrids and white inbred mice were divided into 6 groups (3 control groups and 3 experimental ones); the experimental animals were then exposed to radiation doses of 6.5, 7.5 or 8.5 Gy. On the 9<sup>th</sup> day after irradiation, the survivors were sacrificed under ether anaesthesia, their spleens were removed and the colonies (CFU-S<sub>9</sub>) grown on them were counted.

In the exogenous colony formation technique, mice recipients F<sub>1</sub> (CBA x C57Bl) were exposed to radiation at a dose of 8.5 Gy. One day after irradiation of recipients, mice-donors of experimental (administration of IL-1 $\beta$ ) and control (administration of saline solution) groups were exposed to radiation in doses of 2 and 4 Gy, and 30 min after that, they were sacrificed under ether anaesthesia. The bone marrow was removed from the donors' femoral bones, diluted in 199 medium and then injected in the recipients' caudal vein in the amount of 10<sup>5</sup> cells. On the 9<sup>th</sup> day after irradiation the survivors were sacrificed and the colonies (CFU-S<sub>9</sub>) grown on their spleens were counted.

#### ***Chemicals***

Human recombinant IL-1 $\beta$  produced in State Research and Development Institute of Especially Pure Biological Preparations (St. Petersburg) was used as a radiomodifier. The product dissolved in 0.2 ml of the saline solution was injected intraperitoneally to mice in all the experimental groups in a dose of 50  $\mu$ g/kg (1 mg/animal) within 10–15 min after irradiation. The controls were intraperitoneally injected with the same volumes of the saline solution at the same time.

#### ***Statistical analysis***

The data obtained in experiments were used to calculate average values and standard errors. The statistical

significance of differences between particular groups was established with the use of the Student's t-test at a level of  $p < 0.05$ . The results are summarized in tables as  $M \pm m_x$ .

## **Results**

The results of the research demonstrated high effects of the recombinant IL-1 $\beta$  on the 30-days survival rate and haemopoietic precursors in irradiated mice.

The data in Table 1 show that in terms of the early therapy (10–15 min after irradiation) the recombinant IL-1 $\beta$  increased the survival rate of irradiated mice in all the radiation dose groups.

After a dose of 6.0 Gy, the early therapeutic administration of IL-1 $\beta$  increased the number of survivors by 33.3 %; after further doses, the increases were as follows: by 7 % after 7.0 Gy, by 30 % after 8.0 Gy and by 25 % after 9.0 Gy. The differences between the experimental and control groups were statistically significant after the doses of 8.0 and 9.0 Gy ( $LD_{50-70/30}$ ) only. Increases in the survival rate of mice exposed to 6.0 or 7.0 Gy ( $LD_{50-70/30}$ ) were observed, the differences between the experimental and control groups being, however, not statistically significant. The IL-1 $\beta$  dose reduction factor based on the survival criterion was of 1.13.

It is necessary to note that the early therapeutic administration of IL-1 $\beta$  increased the average life expectancy of dead animals, but a statistically significant difference from controls was only observed after a dose of 7.0 Gy.

The therapeutic effect of the product on the haemopoietic precursors was studied by endogenous and exogenous colony formation techniques.

The administration of recombinant IL-1 $\beta$  within 10–15 min after irradiation led to an increase in the proliferative activity of haemopoietic cells and to a decrease in the intensity of post-irradiation disorders of the bone marrow haemopoiesis in mice-hybrids and white inbred mice in the whole dosage range studied (see Tab. 2).

For example, after exposure of mice-hybrids and white inbred mice to a dose of 6.5 Gy, the therapeutic administration of IL-1 $\beta$  led to an increase in "Day 9 CFU-S" by 76 % and 30 %, respectively; after further doses, the increases were as follows: after 7.5 Gy by 108 % and 115 %, respectively, and after 8.5 Gy by 196 % and 12 %, respectively. A statistically significant difference in the number of endogenous colonies on the spleens of irradiated animals in experimental and control groups was observed in mice-hybrids only, whereas the results obtained with white inbred mice showed only a stable, but statistically not significant tendency to an increase in these rates in the group of mice administered with recombinant IL-1 $\beta$  compared with the control group.

The results based on the exogenous colony formation technique also revealed pronounced therapeutic effects of the product on the haemopoietic precursors.

The data in Tab. 3 demonstrate that the early therapeutic

**Tab. 1:** Effects of recombinant interleukin-1 $\beta$  on survival rate (%) and average life expectancy (days) in irradiated white inbred mice.

Radiation dose, Gy	Survival rate		Average life expectancy of dead animals	
	irradiation	irradiation + IL-1 $\beta$	irradiation	irradiation + IL-1 $\beta$
6.0	66.7 $\pm$ 5.5 (n = 9)	100.0 (n = 9)	25.1 $\pm$ 5.3 (s = 3)	- (s = 0)
7.0	59.7 $\pm$ 15.3 (n = 17)	66.7 $\pm$ 0.05 (n = 18)	11.2 $\pm$ 0.5 (s = 7)	18.5 $\pm$ 4.6* (s = 6)
8.0	10.0 $\pm$ 0.5 (n = 20)	40.0 $\pm$ 0.0* (n = 20)	13.2 $\pm$ 1.8 (s = 18)	15.2 $\pm$ 3.2 (s = 12)
9.0	0.0 (n = 20)	25.0 $\pm$ 5.0* (n = 20)	9.6 $\pm$ 1.5 (s = 20)	10.7 $\pm$ 3.0 (s = 15)

\* - p < 0.05 in comparison with irradiation; n - the number of animals in the group; s - the number of dead animals in the group

**Tab. 2:** Effects of recombinant interleukin-1 $\beta$  on the number of “day 9 colony-forming units” grown on the spleens of irradiated mice-hybrids F<sub>1</sub> (CBA x C57Bl) and white inbred mice based on the endogenous colony formation technique.

Radiation dose, Gy	Average number of CFU-S <sub>9</sub> in the group			
	Hybrids F1 (CBA x C57Bl)		White inbred mice	
	irradiation	irradiation + IL-1 $\beta$	irradiation	irradiation + IL-1 $\beta$
6.5	7.2 $\pm$ 1.3	12.7 $\pm$ 1.2*	16.7 $\pm$ 4.0	21.7 $\pm$ 9.0
7.5	5.1 $\pm$ 0.7	10.6 $\pm$ 1.4*	9.2 $\pm$ 4.2	19.8 $\pm$ 7.8
8.5	2.6 $\pm$ 0.7	7.7 $\pm$ 1.7*	6.0 $\pm$ 0.0	6.7 $\pm$ 0.9

\* - p < 0.05 in comparison with irradiation

**Tab. 3:** Effect of recombinant interleukin-1 $\beta$  on the number of “day 9 colony-forming units” grown on the spleens of mice-hybrids F<sub>1</sub> (CBA x C57Bl) irradiated in the doses of 2 and 4 Gy based on the exogenous colony formation technique.

Terms of exposure		Radiation dose, Gy			
		2		4	
IL-1 $\beta$	irradiation	CFU in one femur		CFU/10 <sup>6</sup> myelokaryocytes	
-	-	480 $\pm$ 320	420 $\pm$ 120	20.6 $\pm$ 13.7	22.4 $\pm$ 4.9
-	+	112 $\pm$ 30	24 $\pm$ 8	5.6 $\pm$ 1.3	1.2 $\pm$ 0.4
+	+	304 $\pm$ 104*	72 $\pm$ 16*	12.7 $\pm$ 4.3*	3.5 $\pm$ 0.8*
+	-	540 $\pm$ 280	340 $\pm$ 40	22.0 $\pm$ 11.4	14.9 $\pm$ 1.8

\* - p < 0.01 in comparison with irradiation

tic administration of the recombinant IL-1 $\beta$  in a dose of 50  $\mu$ g/kg considerably prevented the post-irradiation decrease in the number of CFU-S<sub>9</sub> in irradiated mice. The therapeutic effect of the product was observed after exposures of bone marrow donors to doses of 2 Gy as well as 4 Gy. Moreover, the contents of CFU in one femur and the ratio of CFU to myelokaryocytes in animals of experimental groups was 2.5–3 times higher than that in animals of control groups (p<0.01). There is a further interesting fact that the administration of recombinant IL-1 $\beta$  itself considerably stimulates the colony growth on the spleens of intact animals.

## Discussion

Our findings agree with those found by the previous research dealing with the estimation of the therapeutic efficacy of IL-1 $\beta$ , supplement them and enhance knowledge of the mechanism of the therapeutic action of this recombinant cytokine.

Among other things, it was formerly shown (7) that the biological effect of IL-1 aims mainly at the stimulation of the proliferation activity of haemopoietic stem cells at their early development stages. In endogenous and exogenous colony formation tests it was also established that due the action of IL-1, the number of CFU-S<sub>12</sub> increased and the number of CFU-S<sub>5</sub> remained practically unchanged (5). There was also an increase in the number of late precursor cells in the bone marrow due to the action of IL-1 but this was mainly caused by their recruitment from early haemopoietic stem cells (7). The results of our study obviously suggest that the early therapeutic administration of IL-1 $\beta$  to irradiated animals exerts therapeutic effects, the mechanisms of which being mediated through the activation of the bone marrow haemopoiesis.

## Conclusions

The early therapeutic administration of recombinant IL-1 $\beta$  in a dose of 50  $\mu$ g/kg increases the survival rate in ir-

radiated mice. The IL-1 $\beta$  dose reduction factor based on the survival criterion was of 1.13.

The administration of recombinant IL-1 $\beta$  within 10–15 min after the exposure to radiation prevents the post-irradiation decrease in the number of endogenous and exogenous CFU-S<sub>9</sub> in irradiated mice.

### References

1. Aksenova NV, Grebenyuk AN, Ketlinsky CA, Simbirtsev AS, Sidorov DA, Timoshevsky AA. Radioprotective effect of recombinant IL-1 $\beta$  on hematopoietic early precursors. *Med Immunol* 2003; 5(5–6):621–5.
2. Dinarello CA. The biological properties of interleukin-1. *Eur Cytokine Netw* 1994; 5(3):517–31.
3. Grebenyuk AN. Prospects of the use of recombinant cytokines in radiobiology and toxicology. *Cytokines & Inflammation* 2002; 1(2): 86–7.
4. Grebenyuk AN, Sarkisyan KG, Timoshevsky AA. Antiradiation properties of interleukin-1. *Vestnik of Russian Medical Military Academy* 2005; 1(13): 44–53.
5. Gromykhina NY, Orlovskaya IA, Dubinina LV. Participation of hematopoietic stem cells in the mechanisms of immunostimulating effect of interleukin-1 in mice. *Immunol* 1995; 2: 29–33.
6. Ketlinsky SA, Simbirtsev AS. *Cytokines*. St. Petersburg: Foliant, 2008: 552.
7. Lebedev VG, Moroz BB, Deshevoi YuB. Study of interleukin-1 $\beta$  radioprotective mechanisms on the model of bone marrow long-term cultures. *Rad Biol Radioecol* 2002; 42(1): 60–4.
8. Neta R. Modulation of radiation damage by cytokines. *Stem Cells* 1997; 15 (suppl. 2): 87–94.
9. Rozhdestvenskiy LM. Interleukin-1 – a central proinflammatory cytokine of pleiotropic action from the angle of treatment of radiation damages in the experiment and clinic. *Med Radiol & Rad Safety* 2001; 46(4): 5–11.
10. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Rad Res* 1961; 14(2): 213–22.
11. Waselenko JK, MacViltie TJ, Blekely WF et al. Medical management of the acute radiation syndrome: Recommendations of the strategic national stockpile radiation working group. *Ann Internal Med* 2005; 140: 1037–51.

Received: 04/05/2010.

Accepted: 27/07/2010.

### Corresponding author:

---

Prof. Alexander Grebenyuk, DrSc., Lebedeva street 6, 194044 Saint-Petersburg, Russian Federation;  
e-mail: grebenyuk\_an@mail.ru

---