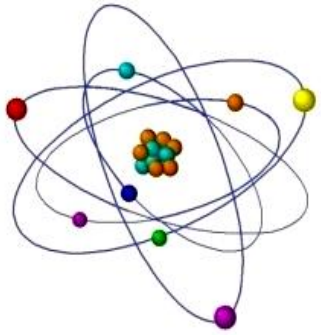
MEGAKARYOBLASTS AND ERYTHROBLASTS IN MICE BONE MARROW AFTER GAMMA-IRRADIATION WITH SUBLETHAL DOSES. EXTRACELLULAR UBIQUITIN EFFECT



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ABSTRACT**:** *Studies show that erythropoiesis and thrombopoiesis are interrelated. We provided cytological and statistical analyses of elevation-depletion picks for megakaryocytes and erythroblasts in bone marrow (BM) and the passage of mature cells into the bloodstream (PB) of irradiated mice.*
The source of radiation was ^{137}Cs with dose rate 1Gy/min., due exposure 5min. Nonlinear white mice of 23±2gr. were used for tests. Animals were divided into three groups: the first control group of intact mice; the second test group of mice irradiated with the dose of LD_{50} 5Gy; the third test group of mice irradiated with 5Gy intraperitoneally injected with ubiquitin at the 72-hour point after irradiation. PB and BM samples from the control group and the test groups of mice have been taken every 24 hours after irradiation for 7 days. Microscopy and statistical methods have been used for calculation of cell count of PB and BM.
Analysis of the most active periods of bone marrow spontaneous regeneration - 3-5th days – showed us that intraperitoneally injected ubiquitin modulates the ratio of erythroblast/megakaryocytesand descends pick sizes during proliferative activity in BM. Thrombocytosis detected in the second groupcounterbalanced by ubiquitin in the third group. Erythrocyte's count remained almost unchanged. One can assume that Platelet count increase in PB is associated with passage of both megakaryocytes and morphologically transformed erythroblasts. Further investigation with appliance of more sophisticated technics is necessary to evince feasibility of ubiquitin involvement in erythroblast/platelet transition. Probability of prevention of thrombocytosis by extracellular ubiquitin is high. More so as ubiquitins ability to moderate leukocyte regeneration picks was corroborated by our previous works.

Key words: Erythroblasts, megakaryocytes, irradiation, platelet regeneration

INTRODUCTION

Today, more than ever, the threat of radiation pollution is relevant. Radiation can be non-ionizing, that is, safe, but usually when we talk about radiation, we mean ionizing radiation that is dangerous to health. Types of ionizing radiation are high-frequency ultraviolet waves, gamma rays, X-rays, alpha and beta particle radiation [1]. The primary causes of radiation disease are radiation contamination of the environment, natural radiation sources, inadequate management of radiodiagnosis and radiotherapy. Radiation disease classically considered a blood disease, can develop when the body is irradiated with a single high dose or with a low dose for a long time [2].

Ionizing radiation can damage the human body at the molecular, cellular or organ level. The subsequent life cycle of a damaged cell can develop in three ways: the cell dies immediately, the cell can repair the damage in the DNA structure, or the cell enters a dormant state. A dormant cell, if not provoked, may remain in the body for a lifetime and not develop into a tumor.

The very first effect of irradiation is cellular depletion in bone marrow causing immunological problems during regeneration due to release of nonfunctional cells into the bloodstream.

Although high-dose chemotherapy and radiotherapy prolong survival in cancer patients, the side effects of these therapies, including pancytopenia, remain serious concerns [3,4].

Recent studies have shown that stimulation of erythroid cells with erythropoietin (Epo) leads to a decrease in the number of megakaryocytes and following thrombocytopenia. Conversely, both

endogenous and exogenous sources of thrombopoietin induce activation of thrombocytopoiesis and anemia in mice [5,6].

It should also be noted that megakaryocytes and erythrocytes have certain biochemical similarities, and various clinical conditions indicate a feedback relationship between the production of red blood cells and platelets. This hypothesis is confirmed by several studies. The discovery of agents that affect such transformation may be useful both for the regulation of malignant transformation of blood and protecting cancer patients from therapy-induced thrombosis [7].

After administration of a megakaryocyte differentiation stimulating agent phorbol 12-myristate 13-acetate (PMA)-into erythroleukemic cell culture, 4 Gy X-ray irradiation significantly increased the expression of CD41 antigens within 72 hours. Hence, radiation enhances the differentiation of erythroleukemic cells towards the megakaryocyte cell line [8,9].

Severe thrombocytopenia may result in spontaneous bleeding. In radiation injured patients, bleeding and thrombocytopenia are directly responsible for significant mortality.

The protein ubiquitin has been recognized for the last two decades as one of the main regulators of cellular processes.Our recent experimental results confirmed that extracellular ubiquitin has the ability to regulate the spontaneous regeneration of bone marrow cells [10,11].Hence,we speculated it probably shows effect on changes of ratio of erythroblasts and platelets and could serve as a regulator of thrombocytopenias caused by the posttherapeutic treatment of cancer patients.

MATERIAL AND METHODS

Non-linear white mice MUS MUSCULUS were used for study. Blood was obtained after decapitation of anesthetized animals. Bone marrow samples collected from femora and samples prepared immediately. Femoral marrow content was expelled by syringe containing 0.5 ml of saline. Clots of bone marrow were homogenized by agitation through the syringe. 5.000 cells per Azur-eosin-stained samples were counted. Animals were anesthetized by ether before decapitation. Statistical analyses were performed by RStudio software.

Treatment of animals performed in accordance with regulations established by the animal's ethic committee of Iv. Beritashvili Center of Experimental Biomedicine (Protocol N06/13.10.2020).

RESULTS AND DISCUSSION

We provided cytological and statistical analyses of alterations of elevation-depletion picks for megakaryocytes and erythroblasts in bone marrow (BM) and the passage of mature cells into the bloodstream (PB) of irradiated mice.

^{137}Cs was used as a source of radiation with dose rate 1Gy/min., due exposure 5min. Nonlinear white mice Mus Musculus of weight of 23±2gr. were used for tests. Animals were divided into three groups: the first control group of intact mice; the second test group of mice irradiated by the dose of LD_{50}5Gy; the third test group of mice irradiated by LD_{50} 5Gy intraperitoneally injected by 20μg/mlof ubiquitin at the 72-hour point after irradiation. PB and BM samples from the control group and the test groups of mice have been taken every 24 hours after irradiation for 7-8 days [Fig.1., Tab.1,2].

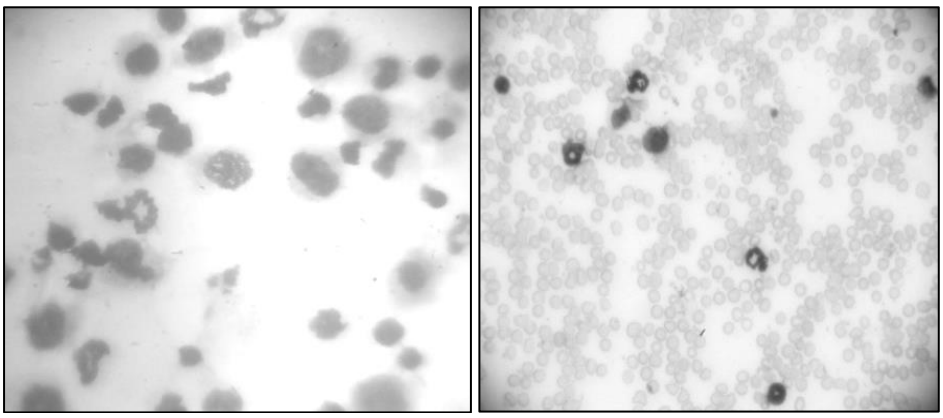

Fig. 1. Samples of microscopy figures of blood and bone marrow smears for mice of control group Azur-eosin staining. Magnification 1000X.

Count of erythroblasts and megakaryocytes decrease within 24 hours after irradiation. Elevation begins in 48 hours, followed by a depletion. The number of erythrocytes in the blood is practically unchanged. The number of platelets increases dramatically in 3-5 days, which probably occurs at the expense of both erythroid and thrombotic precursors as it is seen that both counts decline [Tab.1, Fig.2].

Tab. 1. Total amount of cells per group. The results expressed as means±SD.RStudio packages "gtsummary", "tidyverse", flextable" are used for calculation of the esults

Days/N-total counted cells	**1/ N = 30[1]**	**2/ N = 30**	**3/ N = 30**	**4/ N = 30**	**5/ N = 30**	**6/N = 30**	**7/ N = 30**	**8/ N = 30**	**control, N = 60[1]**	**p-value [2]**
Erythrocytes PB	632.3 (±37.4)	610.2 (±28.4)	620.3 (±37.0)	588.0 (±40.6)	619.0 (±49.3)	617.3 (±30.1)	622.5 (±31.3)	601.8 (±35.4)	635.8 (±38.2)	<0.001
ThrombocytesPB	8.6 (±1.9)	10.3 (±2.5)	18.4 (±2.9)	20.1 (±3.0)	21.7 (±2.7)	10.7 (±2.9)	9.7 (±2.7)	10.8 (±3.0)	15.5 (±4.2)	<0.001
Megakaryocytes BM	10.9 (±2.9)	12.1 (±3.3)	10.0 (±2.3)	5.9 (±1.1)	8.2 (±2.0)	9.0 (±2.0)	9.9 (±1.8)	8.4 (±1.7)	15.7 (±5.4)	<0.001
Erythroblast BM	52.3 (±19.1)	76.1 (±19.5)	44.2 (±9.0)	35.9 (±10.6)	54.7 (±11.6)	51.6 (±12.5)	55.7 (±10.5)	57.3 (±8.2)	101.0 (±24.4)	<0.001
[1]Mean (SD)										
[2]Kruskal-Wallis rank sum test										

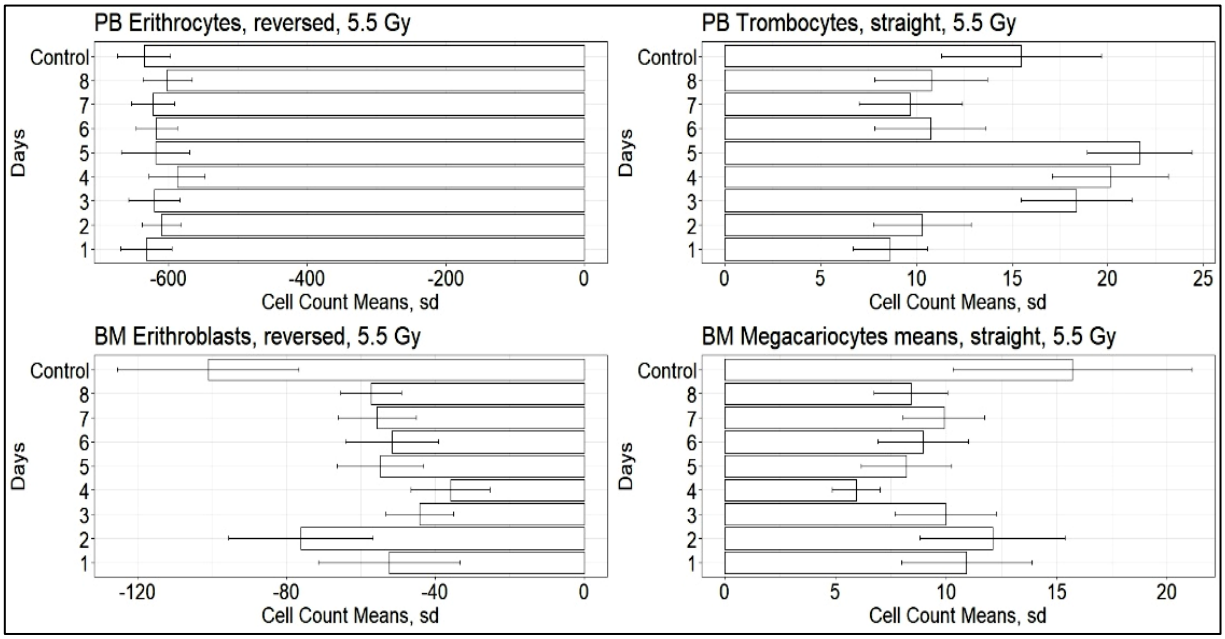

Fig. 2. Statistical analyses of PB and BM cells of irradiated mice. Source of radiation ^{137}Cs. The results expressed as means±SD. Control group presents intact animals, test group – irradiated mice 5Gy, due 1Gy/min. RStudio packages"ggplot2", "cowplot", "tidyverse" used for demonstration the results.

In ubiquitin injected groups we observed strict elevation of platelets in 6 hours due corresponding 78 hours after irradiation followed by diminish of cell number next 3 days, unlike previous irradiated groups. Erythroblast and megakaryocyte count moderated, whilst erythrocyte count remained unchanged [Tab.2, Fig.3].

Tab. 2. Total amount of cells per group. The results expressed as means±SD.RStudio packages "gtsummary", "tidyverse", flextable" are used for calculation of the results.

Day/ N- total counted samples after ubiquitin injection	3/N = 30[1]	4/N = 30	5/N = 30	6/N = 30	7/N = 30	Control N = 60[1]	p-value[2]
ErythrocytesPB	611.7 (±23.6)	622.8 (±31.3)	619.3 (±30.6)	613.8 (±33.9)	619.0 (±41.2)	635.8 (±38.2)	0.057
Thrombocytes PB	24.8 (±5.7)	20.2 (±3.5)	12.4 (±2.4)	9.2 (±2.6)	8.9 (±2.2)	15.5 (±4.2)	<0.001
Megakaryocytes BM	9.2 (±1.7)	9.8 (±2.0)	7.3 (±1.9)	9.7 (±2.2)	9.2 (±1.7)	15.7 (±5.4)	<0.001
Erythroblasts BM	31.4 (±11.8)	58.8 (±16.0)	46.1 (±15.4)	50.6 (±10.1)	50.4 (±12.6)	101.0 (±24.4)	<0.001
[1]Mean (SD)							
[2]Kruskal-Wallis rank sum test							

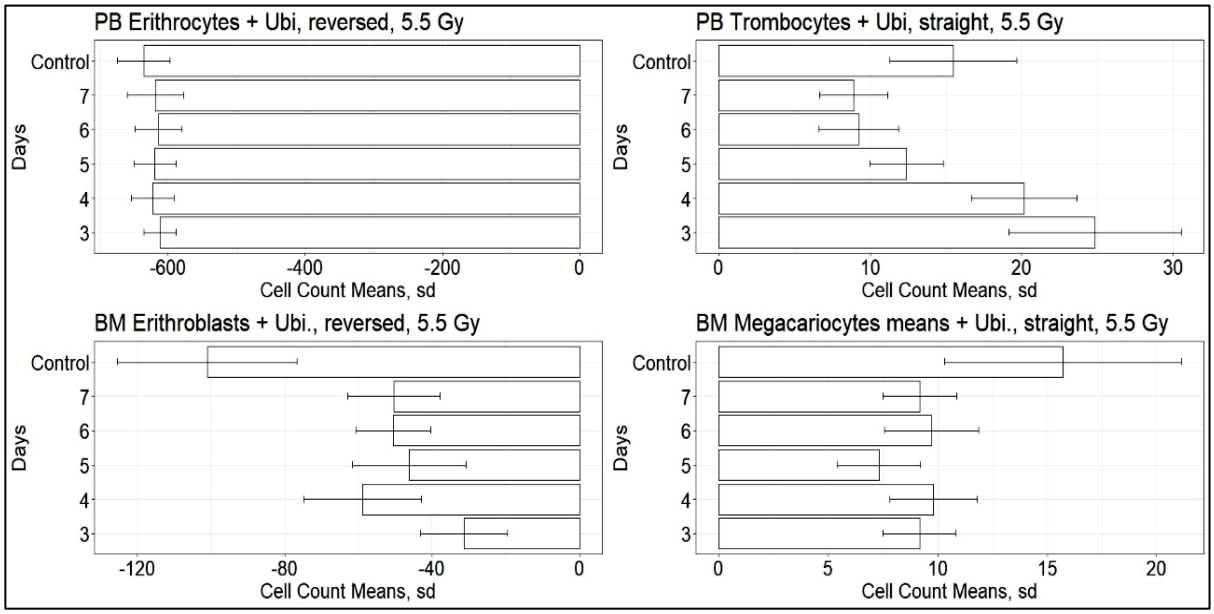

Fig. 3. Statistical analyses of PB and BM cells of irradiated mice. Source of radiation ^{137}Cs. The results expressed as means±SD. Control group presents intact animals, test group – irradiated mice 5Gy, due 1Gy/min.20µg/mlof ubiquitin at the 72-hour point after irradiation. RSudio packages "ggplot2", "cowplot", "tidyverse" are used for demonstration the results [12,13,14].

Analysis of the most active periods of bone marrow spontaneous regeneration - 3-5th days – showed us that intraperitoneally injected ubiquitin modulates the ratio of erythroblast/megakaryocytes and descends pick sizes during proliferative activity in BM. Thrombocytosis detected in the second group counterbalanced by ubiquitin in the third group. Erythrocyte's count remained almost unchanged. One can assume that Platelet count increase in PB is associated with passage of both megakaryocytes and morphologically transformed erythroblasts. Probability of prevention of thrombocytosis by extracellular ubiquitin is high. More so as ubiquitin ability to moderate leukocyte regeneration picks was corroborated by our previous works [10,11]. Ubiquitinis evidently involved in regulation of transformation of bone marrow cells and regulates platelet count, hencemay be useful for the regulation of malignant transformation of blood and protecting cancer patients from therapy-induced thrombosis. Further investigation with appliance of more sophisticated technics is necessary to evince feasibility of ubiquitin involvement in erythroblast/platelet transition.

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