The effects of electromagnetic field and γ-radiation on cell proliferation and regeneration in planarian *Schmidtea mediterranea*

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The effects of electromagnetic field and γ-radiation on cell proliferation and regeneration in planarian *Schmidtea mediterranea*

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Abstract. We have shown that γ-irradiation at dose of 10 Gy and low-intensity electromagnetic fields (EMF, 900 MHz, 100 μW/cm^2, 180 min) significantly compromised cell proliferation of planarian *S. mediterranea* regenerating after decapitation. Using flow cytometry we have shown that 24 hours after exposure to γ-rays and EMF the index of proliferation established as the total amount of cells at S and G2/M phases was 2.8 and 1.8 lower than that in controls respectively. Our data therefore provide the first experimental evidence for the effects of medium-dose γ-irradiation on regeneration in planaria which is attributed to radiation-induced compromised proliferation activity.

1. Introduction

In recent years one of the most important problems of radiobiology and radioecology is considered to clarify radiation effects on biology of stem cells that play a key role in maintaining tissue homeostasis during adult life and tissue repair processes after damage. It is well known that neoblasts being stem cells make up 30% of the total number of cells in the multicellular organism free-living fresh-water planarian flatworms [1–3]. Planarians are characterized by a unique ability to regenerate lost or damaged body parts and provides an excellent opportunity for the study of post-traumatic recovery processes. Currently, planarians are recognized as a model for biological research in the field of regeneration, stem cell biology, study of their proliferation and differentiation, as well as the regulatory mechanisms of morphogenetic processes. The genome of the planarian *Schmidtea mediterranea* was fully sequenced which provides an excellent opportunity for the analysis of this species at the molecular biological level. It has become clear that many of the genes controlling regeneration in planarians are conserved in Metazoa [4–6], which further increases the relevance of studying the processes of posttraumatic recovery of the planarians under the impact of radiation.

The effects of low-intensity electromagnetic fields (EMF) and low and medium doses of γ-radiation on these processes are not well studied, and little is known about the response of stem cells to such exposures.

We have previously conducted a comparative analysis of preparation methods for *S. mediterranea* samples for flow-cytometric analysis of proliferative activity [7]. The aim of this study is to analyse the short- and long-term effects of radiation on the regeneration and proliferation of directly exposed *planarian*. We compared the proliferation activity of irradiated and control planarians after γ-irradiation at dose 10 Gy and low-intensity electromagnetic radiation (EMR) with mobile phone parameters. Proliferative activity was evaluated by flow cytometry with widely applied method that allows determining the proportion of cells at different phases of the cell cycle, including S- and G2/M-phases, in which there may be cycling, but not resting cells. Regeneration activity was quantified as the ratio of area of blastema to the entire body of planarian using computerised morphometry.

2. Material and methods

2.1. Design of irradiation

The planarians of 10 mm were selected, decapitated and divided into three groups. The first group of animals was irradiated for 180 min at the laboratory facility, continuously generating EMR with a frequency of 900 MHz and an energy flux density of 100 μW/cm^2. The second group was irradiated at the therapeutic installation 'Luch-1' (Latvia, ^{60}Co) at a dose of 10 Gy (dose rate 94 Gy/min). The selected dose is less than 10% of LD_{50} for this species (about 60−80 Gy).The control was in the same conditions, but without irradiation. The Index of regeneration of exposed and control planarian was evaluated at 4th day after decapitation and exposure. The analysis proliferation activity was performed at 6th, 8th and 24th hours.

2.2. Cell Cycle Analysis

For cell cycle analysis we used a well-known technique by staining cells with propidium iodide (PI) in the presence of RNase [8]. However, before this, we applied chemical disaggregation of cells in the presence of citric acid (0.1 M) for 10 min at room temperature. Then cells were slowly fixed in chilled ethanol, centrifuged and incubated with staining solution in the dark for 30 min at room temperature, filtered through 40-μm nylon filter. Finally, we used FACS Calibur flow cytometer (BDIS, USA) and CellQuestPro [9] to determine light scattering and intensity of PI fluorescence (585±42 nm wavelength).

Using ModFit 3.1 program (BDIS, USA) a region of cells was identified according to the intensity of forward and side light scattering, then the debris and conglomerates of cells (including so-called doublets) were excluded from the analysis considering the parameters FL2-H/FL2-W that characterized the maximum intensity and duration of the fluorescence signal of each event. Histograms of the cell distribution by the integrated fluorescence intensity of PI were built after gating to determine the proportion of cells at different phases of cell cycle. Proliferation activity was estimated as the total fraction of cells at the S- and G2/M phases of the cell cycle.

2.3. The analysis of regeneration

The analysis of regeneration was performed in a small sample of the planarian *S. mediterranea* (n=48 for γ-irradiation and n=57 for EMR) by computer morphometry in vivo. The Index of regeneration of exposed and control planarian was evaluated at 4th day after decapitation and exposure [10]. The blastemal growth-rate in controls and irradiated flatworms was established after decapitation by scoring the old pigmented and newly grown non-pigmented cells and quantified as the ratio of area of blastema to the entire body of planarian.

2.4. Statistical method of analysis

Three independent series of experiments were carried out for analyze of proliferation activity. In the experimental groups, 50 samples were analyzed (5 planarians per sample), in the control group − 36 samples. The number of cells in each sample was at least 200 thousand. The calculation was made in

the Goryaev's chamber. Statistical processing of the results was performed according to the Kruskal-Wallis test.

Statistical processing of the results of regeneration activity was performed according to the Mann-Whitney U-test.

3. Results and discussion

3.1. The analyses of the proliferative activity in S. mediterranea

The dynamics of changes in the proliferative activity of cells of regenerating planarians through 6, 8 and 24 h after γ-irradiation at a dose of 10 Gy compared to that after exposure to EMR (900 MHz , 100 mW/cm^2, 180 min) are presented in table 1.

It is known that mitotic activity has the first maximum in 6 h after decapitation and affects the whole body of the animal, the second maximum is observed locally at the site of damage 48–72 hours after it [11]. The first mitotic wave is determined mainly by G2 neoblasts, the second – neoblasts at G0 and G1 phases of the cell cycle at the time of damage. According to our data, the proliferative activity of planarian cells in the first six and eight hours after EMR exposure did not differ from that in control (nonradiated regenerating) animals. 24 h after EMR exposure, the number of neoblasts at the S and G2/M phases decreased by 1.5 times compared to the control. A greater drop in proliferative activity was observed in γ-irradiated planarians compared to the effect of EMR. Thus, 24 h after γ-irradiation, the fraction of S-phase cells decreased by 1.8 times, and in G2/M-phases – by 5.3 times compared to the control. At this period, the total number of (S + G2/M) cells characterizing proliferative activity after ionizing radiation exposure decreased by 2.8 times compared to the control and was lower by 1.8 times than after the action of EMR. It should also be noted that a decrease in proliferation was observed as early as 8 hours after γ-irradiation as opposed to EMR exposure.

According to the results of two-way ANOVA, there are highly significant effects of γ-irradiation ($p=1.54\cdot10^{-8}$) and time following exposure ($p=0.0009$) on the proliferation in Planarian. The contribution of time after irradiation and the interaction of these factors was significant ($p=2.93\cdot10^{-5}$). The effects of EMR ($p=0.02$) and time following exposure ($p=0.0001$) on the proliferation in Planarian were significant too.

Figure 1 shows the percentage of cells in the controls and irradiated samples of *S. mediterranea* planarian.

It can be seen that 24 hours after exposure at γ-rays and EMR the index of proliferation established as the total amount of cells at S and G2/M phases, was 2.8 and 1.8 lower than that in controls respectively.

Our data therefore provide the first experimental evidence for the effects of medium-dose γ-irradiation and low-intensity EMF on regeneration in planarians. It proves, in particular, the need for careful monitoring of anthropogenic load levels near the sources of both ionizing and non-ionizing (radio-frequency) radiation.

3.2. The analyses of the regeneration activity in S. mediterranea

Figure 2 shows the index of regeneration activity in planarians on the fourth day after decapitation and exposure at low-intensity EMF and γ-rays at dose 10 Gy.

According to our results, the regeneration activity is significantly compromised in the γ-irradiated groups. It should also be noted that the results of our study were obtained within the dose far below the semi-lethal doses for *Planaria* of 60-80 Gy. The regeneration activity after EMR exposure did not significantly differ from that in controls.

Table 1. The distribution of *S. mediterranea* cells across different phases of cell cycle at 6th, 8th and 24th hours after γ-radiation and EMR exposures.

γ--irradiation					
Dose (Gy)	*N*[a]	Proportion of cells at different phases (%) ± SE			
		G1/G0	*S*	*G2/M*	*S+G2/M*
6 h after irradiation					
0	9	74.3±1.0	18.6±1.3	8.0±0.6	26.5±1.0
10	9	75.0±1.1	17.2±1.0	7.8±0.4	25.0±1.1
Kruskal-Wallis test[b], df=1		0.33	1.04	0.16	1.65
P[c]		0.57	0.31	0.69	0.20
8 h after irradiation					
0	8	68.3±2.7	17.7±1.6	14.0±2.6	31.8±2.7
10	7	77.2±1.6	4.2±2.4	18.6±1.7	22.8±1.6
Kruskal-Wallis test, df=1		3.87	7.73	1.93	3.87
P		0.05	**0.01**	0.16	0.05
24 h after irradiation					
0	19	70.3±1.4	14.2±0.8	15.9±1.2	30.0±1.5
10	6	89.1±0.7	7.8±0.8	3.0±0.9	10.9±0.7
Kruskal-Wallis test, df=1		13.15	10.95	13.15	13.15
P		**0.0003**	**0.0009**	**0.0003**	**0.0003**
Two-factor analysis of variance (two-way ANOVA)					
Contribution of gamma irradiation to the effect		$2.58\cdot10^{-8}$	$5.38\cdot10^{-8}$	0.035	$\mathbf{1.54\cdot10^{-8}}$
Contribution of time after irradiation		0.0007	$1.43\cdot10^{-6}$	$6.28\cdot10^{-6}$	**0.0009**
Interaction of factors		$1.46\cdot10^{-5}$	0.0003	$3.06\cdot10^{-6}$	$\mathbf{2.93\cdot10^{-5}}$
Electromagnetic exposure					
Dose (Gy) /	*N*[*]	Proportion of cells at different phases (%) ± SE			
Exposure time (min)		*G1/G0*	*S*	*G2/M*	*S+G2/M*
6 h after irradiation					
0 (control)	9	74.3±1.0	18.6±1.3	8.0±0.6	26.5±1.0
180 min	9	75.4±1.4	15.5±1.1	9.1±0.5	24.7±1.4
Kruskal-Wallis test[b], df=1		1.22	3.30	2.14	2.69
P[c]		0.27	0.07	0.14	0.10
8 h after irradiation					
0 (control)	8	68.3±2.7	17.7±1.6	14.0±2.6	31.8±2.7
180 min	10	66.5±1.6	17.7±0.7	15.8±1.1	33.5±1.6
Kruskal-Wallis test, df=1		0.07	0.20	0.96	0.07
P		0.79	0.66	0.33	0.79
24 h after irradiation					
0 (control)	19	70.3±1.4	14.2±0.8	15.9±1.2	30.0±1.5
180 min	10	80.0±1.6	9.7±1.4	10.3±1.5	20.0±1.7
Kruskal-Wallistest, df=1		9.73	5.48	5.48	9.74
P		**0.002**	**0.019**	**0.019**	**0.002**
Two-factor analysis of variance (two-way ANOVA)					
Contribution of electromagnetic exposure		0.04	0.01	0.47	**0.02**
Contribution of time after irradiation		$3.68\cdot10^{-5}$	$1.62\cdot10^{-6}$	0.0004	**0.0001**
Interaction of factors		0.003	0.155	0.017	**0.003**

[a] sample size

[b] Kruskal-Wallis test values (df=1) for comparison with the control group

[c] the probability of differences adjusted for multiple comparison Bonferroni

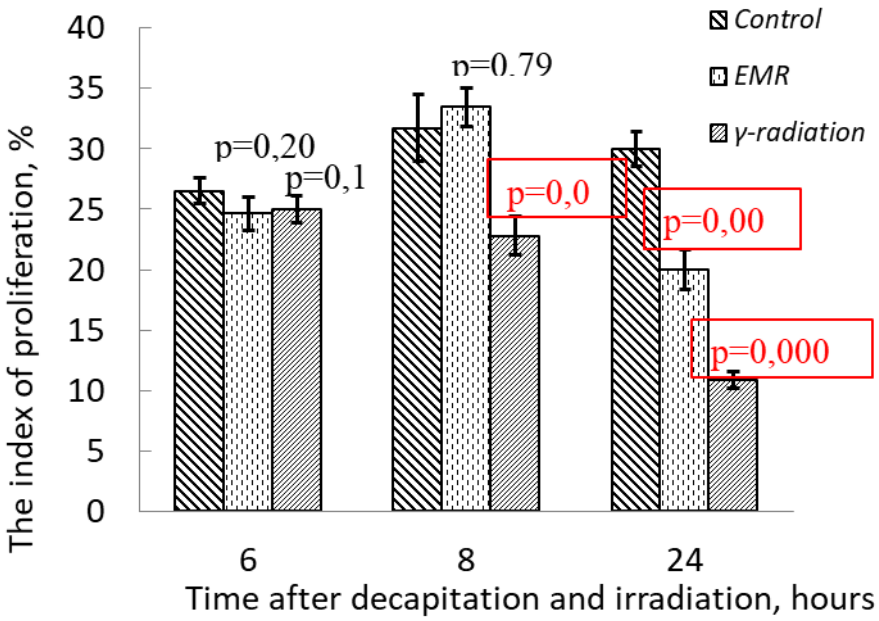

Figure 1. The percentage of cells at the S- and G2/M-phases of cell cycle in the samples of *S. mediterranea* regenerating planarians in control and exposed groups.

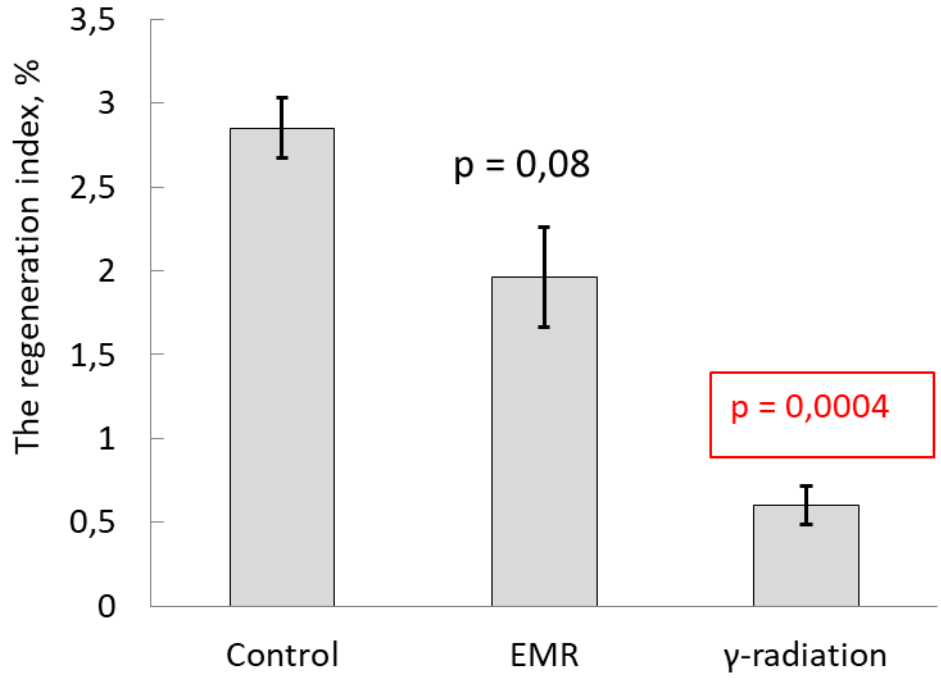

Figure 2. The index of regeneration in the controls and samples of *S. mediterranea* planarians exposed to EMR (900 MHz, 100 μW/cm^2, 180 min) and γ-radiation at dose of 10 Gy.

4. Conclusions

The decrease in the proliferative activity of the *S. mediterranea* planarian cells after EMR exposure was found to be less pronounced than that after γ-irradiation and not be reflected in the regeneration. This indicates the leveling of the EMR effect in the more distant periods, possibly due to the subsequent stimulation of the neoblast proliferation, the increase in migration activity or the intensification of the differentiation processes.

Using of planarians as the object of study allowed us to analyze the effects of ionizing and non-ionizing radiation on both cellular and organismic levels.

Our data therefore provide the first experimental evidence for the effects of medium-dose γ-irradiation on regeneration in planaria which is attributed to radiation-induced compromised proliferation activity.

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