

Mutagenic Effect during Combined Exposure to Ionizing and Non-Ionizing Electromagnetic Radiation

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Using the method of dominant lethal mutations, we assessed the frequency of the death of *Drosophila melanogaster* embryos under combined exposure to ionizing γ -radiation and non-ionizing pulsed magnetic field at various doses and modes of exposure. Mutagenic effect of combined exposure is antagonistic in nature. The antagonism is more pronounced when the following mode of exposure was used: exposure to non-ionizing pulsed magnetic field for 5 h followed by exposure to γ -radiation at doses of 3, 10, and 60 Gy. In case of reverse sequence of exposures, the antagonistic effect was statistically significant after exposure to γ -radiation at doses of 3 and 10 Gy, whereas at a dose of 20 Gy, a synergistic interaction was noted.

Key Words: γ -radiation; pulsed magnetic field; combined effect; dominant lethal mutations; *Drosophila melanogaster*

The radiation exposure of biological systems steadily increases due to nuclear fuel cycle enterprises and application of ionizing radiation sources in medicine. At the same time, none of these factors acts separately from others, and the electromagnetic radiation of various types is not an exception. The range of electromagnetic field sources introduced into practice steadily and unavoidably increases. Higher frequencies of the electromagnetic radiation spectrum are used to maintain the work of medical and military equipment, mobile telecommunication and applications (e.g. 28, 37 and 39 GHz for 5G communication systems) [1], which surpasses our understanding of the biological regularities of the electromagnetic radiation [2,3]. As for combined effects, the scientific data are very scarce and do not provide clear understanding of the nature of interaction of ionizing and non-ionizing radiation (additive, synergetic, antagonistic, or independent) [4,5].

The objective of this study was to determine the nature of the interaction of electromagnetic radiation of ionizing and non-ionizing spectrum based on the criterion of embryonic death of *Drosophila melanogaster* using the method of dominant lethal mutations (DLM).

MATERIALS AND METHODS

The culture medium was prepared and *D. melanogaster* of Canton-S strain were cultured using the standard procedures [6]. Sexually mature males of *D. melanogaster* were irradiated, 30 males per each experimental site. Within 2-3 h after the exposure, they were crossed with intact virgin females in one-to-one ratio. Then, females were transferred to Petri dishes with agar for egg laying. In 48 h the females were taken from the Petri dishes and in another 48 h, the developed eggs and eggs with dead embryos were calculated. The percentage ratio of eggs with dead embryos to the total number of eggs was taken as DLM frequency.

The study consisted of 3 stages. At the first stage, the changes in the DLM frequency after acute exposure to γ -radiation at doses from 1 to 100 Gy (9 exper-

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imental points and one control point). Irradiation was performed on an IGUR-1M experimental γ -unit with dose rate 0.8 Gy/min and γ -field non-uniformity <5%. At the second stage we evaluated changes in the DLM frequency caused by pulsed magnetic field (PMF) with duration of exposure from 1 to 48 h (in total 8 experimental points and one control point). Shakhparonov generator intended for evaluation of the PMF effect on biological objects was used to induce PMF. The generator has the following parameters: triangular pulse with a pulse duration of 0.1 μ sec at 0.5 amplitude, pulse rise time 0.05 μ sec, pulse decay time 250 nsec, repetition frequency 1.7 MHz. The magnetic field induction of the generator at the site of biological objects location is 16 mT in the pulse. The PMF with the characteristics given above has a nonthermal mechanism of the interaction with the substance.

After measuring the effect of each factor separately and based on the obtained results, we performed the third stage of the experiment: the DLM frequency after combined exposure to PMF and γ -radiation was assessed. γ -Radiation doses of 3, 10, 20, and 60 Gy, and PMF exposure for 5 h were chosen for the experiment and combined in two variants: 1) γ -exposure at a chosen dose followed by 5-h exposure to PMF; 2) 5-h exposure to PMF followed by acute γ -irradiation at a chosen dose. Time interval between the exposures did not exceed 5 min.

To assess the type of the combined effect (antagonistic, additive, or synergistic), the expected DLM frequency was calculated as an expected additive effect of two factors [7] by the formula:

$$P\Sigma=1-(1-Pa)\times(1-Pb),$$

where P is the expected additive effect of two factors taken together; Pa and Pb are the effects of the first

and second factors. Statistically significant differences of the experimentally obtained combined effect of the factors from the calculated expected additive DLM frequency indicated the type of interaction of these two factors. The interaction was characterized as antagonistic when experimentally obtained DLM frequency was statistically significantly below the calculated expected level. The interaction was interpreted as synergism when the experimentally obtained DLM frequency surpassed the calculated expected value. In the absence of differences, the effect was considered to be additive.

The data were processed using the PAST 4.3 software (OS Windows). Experimental groups were compared by the χ^2 test. The differences were considered statistically significant at $p \leq 0.05$.

RESULTS

The evaluation of the combined effect of two factors requires assessment of the individual effect of each factor and the combinations of these factors. Thus, we first estimated the mutational effect of ionizing radiation alone and non-ionizing radiation alone, and then, their combined effect was evaluated.

The frequency of DLM in *D. melanogaster* under acute γ -irradiation increased with increasing the dose (Fig. 1). The spontaneous level of DLM was $8.4 \pm 2.4\%$. Statistically significant differences from the control group were revealed for the experimental groups subjected to γ -radiation at doses >3 Gy, and at doses >60 Gy, the mortality of embryos approximated 100%.

The frequency of DLM in *D. melanogaster* after exposure to PMF with different exposure time is presented in Figure 2. We revealed no dose-dependent changes in the DLM frequency with increasing the

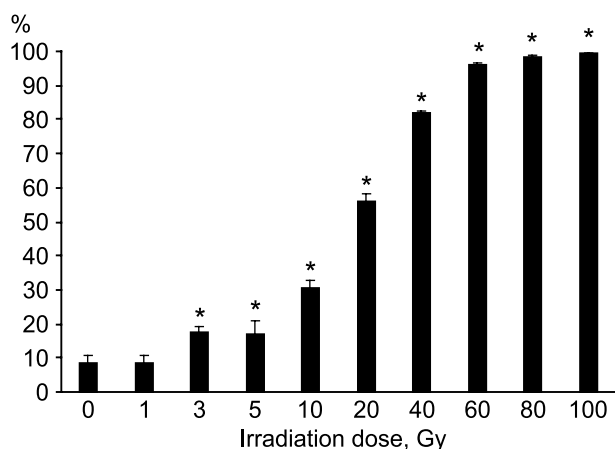


Fig. 1. Frequency of DLM in *D. melanogaster* of Canton-S strain after acute γ -irradiation. * $p < 0.05$ in comparison with the control (0 Gy).

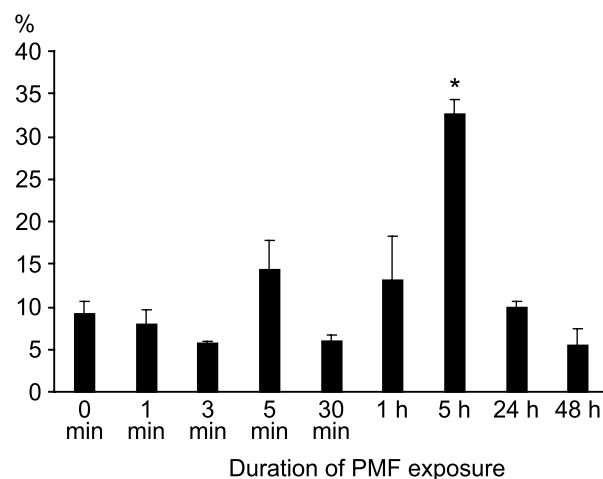


Fig. 2. Frequency of DLM in *D. melanogaster* of Canton-S strain after exposure to PMF. * $p < 0.0001$ in comparison with the control (0 min).

TABLE 1. Frequency of DLM in *D. melanogaster* of Canton-S Strain under the Influence of the Factors

Experimental conditions	Dose of γ -radiation, Gy			
	3	10	20	60
Control (spontaneous DLM)	8.8			
5-h exposure to PMF	32.5			
γ -Irradiation at specified doses	17.6	30.3	56.1	95.8
Combined effect of radiation (expected frequency of DLM)	44.4	53.0	70.4	97.2
γ -Irradiation followed by PMF exposure (experimental frequency of DLM)	31.2* $\chi^2=3.6$; $p=0.049$	38.8* $\chi^2=4.3$; $p=0.038$	92.6* $\chi^2=16.4$; $p<0.001$	98.7
PMF exposure followed by γ -irradiation (experimental frequency of DLM)	22.3* $\chi^2=11$; $p<0.001$	32.8* $\chi^2=8.7$; $p=0.003$	63.5	80.9* $\chi^2=15.4$; $p<0.001$

Note. *Significant differences from the expected DLM frequency, $p \leq 0.05$.

duration of PMF exposure in the range from 1 min to 48 h. However, PMF exposure for 5 h produced the greatest damaging effect, the frequency of DLM (32.5%) was higher than in the control group by 3.7 times ($\chi^2=16.5$; $p<0.0001$).

Thus, on the basis of the above results, we have chosen the γ -radiation doses of 3, 10, 20, and 60 Gy and PMF exposure for 5 h. For the first variant of combined exposure, i.e. γ -irradiation at a chosen dose followed by 5-h exposure to PMF, the DLM frequency was significantly lower than the expected effect of γ -radiation alone in doses 3 and 10 Gy (Table 1), exceeded the expected effect after γ -irradiation at a dose of 20 Gy, and did not significantly differ from the expected value in case of γ -irradiation at a dose 60 Gy.

For the second variant of combined exposure, i.e. 5-h exposure to PMF followed by acute γ -irradiation at a chosen dose, a decrease in DLM frequency was noted at all doses: by 50% at a dose 3 Gy and by 17% at a dose of 60 Gy.

Special attention should be paid to the group exposed to γ -radiation at a dose of 60 Gy followed by 5-h exposure to PMF. The frequency of DLM in *D. melanogaster* approximated 100% after acute γ -irradiation alone at a dose ≥ 60 Gy (Fig. 1), but after combined exposure, the mortality of embryos considerably decreased, and therefore, antagonistic effect was observed.

Thus, mutagenic effect under combined exposure to ionizing and non-ionizing electromagnetic radiation is antagonistic in nature. The antagonism was most expressed when the objects were first exposed to PMF for 5 h and then to γ -radiation. In case of the inverse sequence of exposures, the statistically significant antagonistic effect was observed for γ -irradiation at doses of 3 and 10 Gy, while at 20 Gy, a synergetic type of interaction was observed.

Biological mechanisms of the genotoxic effect of non-ionizing electromagnetic radiation could be rela-

ted to increased activity of free radicals and reduced activity of antioxidants [2,8]. These two processes result in increased level of DNA molecule damage. At the physical level, the influence of electric and magnetic fields leads to the polarization processes in biological macromolecules and the environment. As a result, conformation modifications become probable, for example, changes in bond angle in a molecule and changes in dipole–dipole and ion–dipole interaction affecting functional activity of the biomolecules [9,10]. We could assume that our findings are a result of some of these. It can be hypothesized that polarization of the environment or biological macromolecules, including the repair enzymes, by magnetic field, prior to or after acute γ -irradiation leads to increased activity of the reparation system of a cell.

The results show the need for further research to better understand the molecular mechanisms behind this interaction of electromagnetic radiations. Further research can help to develop strategies for managing and protecting against exposure to electromagnetic radiation, as well as to expand knowledge about the interaction of different types of radiation to achieve maximum benefit in practical applications.

Conflict of interest. The authors have no conflicts of interest to declare.

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