# 101 Hz Frequency-Modulated Infrared Light Induces Cytogenetic Adaptive Response in Mouse Bone Marrow *In Vivo*

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The micronucleus test was used tu study the possibility of inducing cross-adaptive response in mouse bone marrow cells in vivo with an 850 nm infrared light modulated by a 101 Hz frequency, emitted by a light therapy device "Kurator". We demonstrated that this exposure led to a substantial reduction of cytogenetic cell damage produced by further exposure of animals to X-radiation in the dose of 1.5 Gy, *i.e.* it induced an adaptive response which did not differ by the magnitude and time course from the adaptive response to radiation.

**Key Words:** *cross-adaptive response; infrared light; X-ray radiation; micronucleus test; mice*

Investigation of the effects of low-dose radiation has not only fundamental but also practical implication, because the revelation of mechanisms of their effect on biological objects is necessary for understanding of general biological processes and for estimation and prediction of radiation effects in human body. Radiation biology possesses extensive knowledge on mechanisms and regularities of action of high doses of ionizing radiation on living objects, but this is insufficient to explain some biological effects of low-dose radiation, such as adaptive response (AR), genomic instability in generations, bystander effect and hormesis.

The phenomenon of radiation AR arouses much recent interest. The core of this phenomenon is that preliminary exposure to low dose radiation leads to increased resistance of the object to subsequent action of high deleterious doses of radiation. This phenomenon is considered as a form of cell defense from mutagenic action of oxidative stress caused by somatic diseases, ionizing radiation, and chemical agents. Comparative study of human cell protection coefficients upon antimutagen treatment and upon AR induction demonstrated that adaptive response produced the highest protection coefficient [5]. Our investigation of the time course of radiation AR development demonstrated that low doses of ionizing radiation transform the organism into a new stable state characterized by increased genomic stability and persisting throughout the life [1]. The duration of maintenance of adapted state resembles the body immune response and one may even suggest the emergence of a new phenotype [2]. The phenomenon when the adapting and the detecting exposures are of different nature is called cross-adaptation or cross adaptive response. Therefore the search for adaptogens of physical and chemical nature which are able to transform the body into adapted state similarly as low doses in an actual problem. This condition can be revealed only

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by additional exposure to high-dose radiation under laboratory conditions.

Various devices, based on the action of electromagnetic emission of the infrared subspectrum and recommended for treatment of inflammatory visceral diseases, are currently used in clinical practice. The device for light therapy "Kurator" developed by Dr. X. Muller in the Leonard Euler Institute of Investigation of Cosmic Energy (Germany), is one of them. In this device, the therapeutic effect of red and 850 nm infrared light (IRL) is optimized by 101 Hz-frequency modulation.

The aim of the study was to investigate the possibility of inducing the cross-adaptive response in the mouse bone marrow *in vivo* by exposure to IRL modulated with 101 Hz frequency produced by the light therapy device "Kurator".

## MATERIALS AND METHODS

Males of white mongrel SHK mice ($n$=293) were used in the study. The mice were housed under standard conditions of Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences (ITEB of RAS). The animals were divided into experimental and control groups, the experimental group was divided into four subgroups. The first subgroup was exposed to adapting IRL dose for 10 min on days 1, 3, 7, and 14, the second subgroup was not exposed to IRL. Then the irradiated and non-irradiated animals from the experimental group were exposed to the detecting dose of 1.5 Gy of X-radiation at a dose rate of 1 Gy/min. At the same time, the 3rd subgroup underwent irradiation imitation with the device being switched off, then it was exposed to the 1.5 Gy. The fourth group was used as positive control. These animals were exposed to radiation according to the schedule of radiation AR: to the adaptive dose of 0.1 Gy with further detecting dose of 1.5 Gy 24 h later [2]. The control group was divided into two subgroups: one group was exposed to IRL imitation using the switched off device, the other group received no radiation.

X-irradiation was conducted at the RUM apparatus at 200 kV.

In order to detect the level of cytogenetic damage, the animals were sacrificed by cervical dislocation 28 h after exposure to detecting radiation and bone marrow cytological specimen were routinely prepared [1]. No less than five mice were taken for each experimental point. No less than 1000 polychromatophilic erythrocytes (PCE) from each specimen were used for counting of cells with micronuclei (MN). To avoid cell cycle effects, the proportion of PCE and normochromatophilic erythrocytes in the bone marrow was determined. The statistical significance of differences between the groups was evaluated by the Student $t$ test.

## RESULTS

To investigate the possibility of AR induction with light therapy device "Kurator", the mice were exposed to radiation according to the scheme of radiation AR: they were exposed to adaptive IRL and to the detecting X-radiation dose of 1.5 Gy 24h later. To make the skin contact of the device closer in order to make the experimental conditions closer to clinical conditions, one group of animals had their hair-covering depilated, the other group was not subjected to this procedure. Both groups were irradiated using "Kurator" device 10 min per day for 14 days, the exposure time was selected according to operational guideline of the device.

The level of cytogenetic damage after true and sham IRL exposure did not differ from the spontaneous level in mice with depilated hair as well as animals with non-depilated skin (Table 1). Japanese researchers also demonstrated the absence of IRL effect on the reproductive function and level of plasma components in ICR mice [10]. The absence of cytotoxic and genotoxic effects of exposure to a low-intensity red laser (660 nm) in erythroleukemic cells was also demonstrated [8]. On the other hand, long-term exposure to long-wavelength IRL both during daytime and during nighttime increased spontaneous motor activity in mice, and fractionated exposure increased this activity only during day-time [10]. Different doses of ultraviolet laser exposure (193, 223 and 248 nm) produced evident mutagenic effect on mouse cornea [6].

X-ray irradiation of mice previously exposed to IRL led to sufficient decrease of cytogenetic damage level both in animals covered with hair and in animals with removed hair as compared to mice exposed to one dose of 1.5 Gy only. This may be indicative of cross-adaptive response induction with its magnitude being equal to the magnitude of radiation AR in mice after X-ray exposure according to standard AR scheme (O.1 Gy+1.5 Gy). The protective effect was also revealed in normal human fibroblasts after IRL exposure (700-2000 nm) and subsequent ultraviolet light which is toxic for these cells [9]. Cytogenetic damage level produced by sham IRL exposure and subsequent irradiation in the dose of 1.5 Gy both in animals covered with hair and in animals with removed hair did not differ from that after the only dose of irradiation of 1.5 Gy. AR level in mice covered with hair and in animals with removed hair was comparable by mag-

**TABLE 1.** Number of PCE Containing MN in the Bone Marrow Cells of Mice Covered with Hair and in Mice with Removed Hair after Combined Exposure to IRL and X-radiation in a Dose of 1.5 Gy *in Vivo*

| Exposure | Number of mice | Number of PCE analyzed | Number of PCE containing MN | PCE with MN, % |
|---|---|---|---|---|
| Mice with removed hair | | | | |
| 0 | 5 | 7,200 | 20 | 0.31±0.08 |
| 10 min of sham IRL (14 days) | 5 | 19,000 | 99 | 0.53±0.05 |
| 10 min of IRL (14 days) | 5 | 7,000 | 30 | 0.39±0.07 |
| 10 min of IRL (14 days)+1.5 Gy 1 day later | 5 | 14,000 | 486 | 3.50±0.28* |
| 10 min of sham IRL (14 days)+ 1.5 Gy 1 day later | 5 | 20,000 | 1266 | 6.34±0.04 |
| 1.5 Gy | 5 | 14,000 | 978 | 6.98±0.07 |
| Mice with hair | | | | |
| 0 | 14 | 18,300 | 85 | 0.46±0.05 |
| 10 min of sham IRL (14 days) | 10 | 15,500 | 73 | 0.45±0.05 |
| 10 min of IRL (14 days) | 11 | 27,700 | 82 | 0.36±0.08 |
| 10 min of IRL (14 days)+1.5 Gy 1 day later | 20 | 62,000 | 2782 | 4.42±0.31* |
| 10 min of sham IRL (14 days)+ 1.5 Gy 1 day later | 19 | 52,000 | 3373 | 6.63±0.28 |
| 1.5 Gy | 30 | 54,000 | 4299 | 7.81±0.36 |
| 0.1 Gy+1.5 Gy | 11 | 26,000 | 1401 | 5.25±0.34* |

**Note**. Here and in Tables 2, 3: *p<0.05 as compared to mice exposed to the dose of 1.5 Gy only.

**TABLE 2.** Number of PCE Containing MN in Mouse Bone Marrow Cells after Combined Exposure to IRL for 10 min per Day for 1, 3, 7 and 14 Days and X-Radiation in a Dose of 1.5 Gy One Day Later

| Exposure | Number of mice | Number of PCE analyzed | Number of PCE containing MN | PCE with MN, % |
|---|---|---|---|---|
| 0 | 14 | 18,300 | 85 | 0.46±0.05 |
| 10 min of sham IRL (14 days)+1.5 Gy | 8 | 14,000 | 1027 | 7.05±0.65 |
| 10 min of IRL (14 days)+1.5 Gy | 15 | 40,000 | 1857 | 4.67±0.24* |
| 10 min of IRL (7 days)+1.5 Gy | 15 | 40,000 | 1780 | 4.40±0.62* |
| 10 min of IRL (3 days)+1.5 Gy | 5 | 10,000 | 449 | 4.50±0.31* |
| 10 min of IRL (1 day)+1.5 Gy | 5 | 14,000 | 486 | 3.50±0.28* |
| 1.5 Gy | 15 | 27,000 | 2426 | 8.22±0.51 |

**TABLE 3.** Time Course of AR Development after Combined Exposure to IRL and X-Radiation in a Dose of 1.5 Gy

| Exposure | Number of mice | Number of analyzed PCE | Number of PCE containing MN | PCE with MN, % |
|---|---|---|---|---|
| 0 | 14 | 18,300 | 85 | 0.46±0.05 |
| 10 min of IRL | 11 | 27,700 | 82 | 0.36±0.08 |
| 10 min of IRL+1.5 Gy 20 min later | 15 | 45,000 | 2909 | 6.66±0.45 |
| 10 min of IRL+1.5 Gy 1h later | 10 | 40,000 | 2623 | 6.63±0.39 |
| 10 min of IRL+1.5 Gy 3h later | 10 | 45,000 | 2736 | 6.10±0.35 |
| 10 min of IRL+1.5 Gy 5h later | 10 | 40,000 | 1441 | 4.39±0.16* |
| 10 min of IRL+1.5 Gy 24h later | 20 | 62,000 | 2782 | 4.42±0.31* |
| 1.5 Gy | 12 | 43,000 | 2996 | 7.44±0.53 |

nitude with radiation AR after exposure of mice to standard AR scheme, therefore all subsequent experiments were done on mice covered with hair.

To determine the minimal IRL exposure time necessary for AR induction, we conducted experiments with revealed IRL exposure for 10 min for 1, 3, 7 and 14 days and subsequent X-ray irradiation (Table 2). We revealed a sufficient reduction of cytogenetic damage for all investigated terms of IRL exposure, *i.e.* AR was observed. On the basis of these data, in all subsequent experiments the mice were exposed to IRL once for 10 min in order to induce AR.

To investigate the time course of the development of AR induced by IRL, we set intervals of 1, 3, 5 and 24 h between adapting and detecting doses. These terms were specified previously in radiation AR investigation [2].

In the study of the time course of AR induction by IRL, the reduction of cytogenetic damage was observed no sooner than 5 h after the adaptive exposure (Table 3). Similar time course of AR induction was observed in different objects after exposure to low doses of ionizing radiation used as adapting exposure [1-4]. IRL irradiation of animals 20 min before X-ray radiation exposure (according to the scheme of investigation of radiation protectors effects) revealed no reduction in MN PCE number.

Thus, the radioprotective effect of "Kurator" device was revealed only 5 hours after preliminary irradiation of animals according to radiation AR mechanism and was not revealed after treatment according to the scheme of investigation of the effects of traditional radiation protectors.

In contrast to our data, the study [7] demonstrated that pretreatment of HeLa cells monolayer with HeNe laser increased the survival rate after exposure to γ-radiation in a dose of 5 Gy with intervals of 60 and 180 min between the exposures; in case of 5-min exposure, survival curves matched together. The authors hypothesized that HeNe laser exposure activates reparation processes, and this phenomenon is probably an AR.

Our study demonstrated that exposure of mice to IRL, modulated with frequency of 101 Hz, produced by "Kurator" device did not affect the level of spontaneous cytogenetic damage in bone marrow cells: combined exposure to both IRL and X-radiation in a dose of 1.5 Gy induced a cross-adaptive response, which did not differ from radiation AR by magnitude and time course. The obtained results can be indicative of the same mechanism of AR induction by IRL and X-ray exposure in mice *in vivo*.

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