

The Changes of the Cellular Indicators of Leucopoiesis in Irradiated Animals under Hypomotile Conditions

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Abstract It is studied the nature of the changes of the morpho-functional indicators of leukopoiesis in hypokinesia dynamics on the background of multiple impact of the millimeter range electromagnetic waves. It is shown that the preliminary radiation increases the potential capacity of the regulatory systems of leukopoiesis, activates the proliferative and maturation processes of the stem cells, and boosts the adaptive compensatory mechanisms thus resulting in preventing of the negative impact of hypokinesia. The obtained data allow us to assume that the low-power microwaves with the frequency of 42.2 GHz are means of non-medication treatment under stress rectifying the changes of the morpho-functional indicators. The changes in the sympathoadrenal system, the increase of the immunoreactivity and non-specific resistance, the activation of endogenous immunomodulators have important role in the mechanism of the physiological impact of non-ionizing millimeter electromagnetic waves.

Keywords: hypokinesia, millimeter electromagnetic waves, leukopoiesis, non-ionizing radiation

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1. Introduction

In the past few decades, due to the technological progress, the level of exposure of biological systems to unfavorable factors of the external environment, such as vibration, noise and radiation, has been increased. At the same time, a dramatic change in the human life style has led to restriction of motive activity and to a sedentary lifestyle. Along with the development of technology, many processes have been automatized; the majority passing under intense and almost no motile conditions. Today, the computer technology is evolving into different fields of human activity and is becoming indispensable for work and the learning process. Relative non-motile stages create a stress reaction, the tension of regulatory mechanisms, the movements in the immune system and the reduction of the reserve capacity of the organisms. These processes are responsible for the development of pathologic processes in the organisms [1,2].

The results of experimental investigations show that in the case of hypokinesia, the concentration of lysozyme, the amount of complimentary lymphocytes and immunoglobulins are decreased. In addition, it was observed that the functional activity of neutrophils is decreased as well. As a consequence of this the resistance of the organism against various infections and diseases decreases [3,4]. Therefore, it is important to find means that under the hypomotile conditions will help both to prevent and correct such deviations.

The clinical and experimental data of numerous investigations have shown that the electromagnetic waves are considered to be a new, highly efficient method for the treatment of various diseases. They have anti-stress effect on neuroendocrine and immune systems, as well as on the peripheral regulatory structures [5-16].

The anti-stress effect of the millimeter wave electromagnetic radiation (EMR) has been studied in [17,18,19] by Temuryants et al. and Kirichuk et al. It has been shown that the millimeter waves increase the functional activity of leucocytes, particularly neutrophils and lymphocytes. Thus they can increase the physiological protection and the level of resistance of an organism.

The main physiological mechanisms that underline the influence of the millimeter-waves are the changes in activation of sympathetic-adrenaline system, the increase in immunoreactivity and the non-specific resistance and activation of endogenous regulatory immune system [20]. It is of high interest to study the changes in morphological indicators of the blood under hypomotile conditions, because the blood system is connected to the all working systems of the body. Therefore, the aim of the present work is to study the changes of morphological indicators in the blood of animals that have been pretreated by the millimeter-waves electromagnetic radiation under the condition of hypokinesia.

2. Materials and Methods

Experiments were carried out on rabbits of the same weight (2.5-3 kg) and under the same care and nutrition survey conditions. 10 rabbits have been taken and two experimental setups have been used to achieve our goals. In the first setup, the changes in morphological indicators of leukopoiesis were studied under dynamics of hypokinesia. In the second setup, before hypokinesia the animals were treated during 20 days by EMR.

In order to restrict the movements of the animals, they were placed in a special wooden box for 20 days, 22 hours for each day. 2 hours were used to take blood samples and for the feed. For the irradiation treatments, a Russian-made G4-141 generator (Istok, Fryazino, Russia) was used with the frequency of 42.2 GHz (range of frequencies of G4-141 is 38.5÷53.57 GHz). The exposure time was 30 minutes per day during 20 days. A whole-body EMR exposure of rabbits was conducted in the far-field zone of antenna (50 cm). Incident power density (IPD) at the location of the object was less than $10 \mu\text{W}/\text{cm}^2$.

For animals, that have been under normal conditions and the dynamics of hypokinesia, and for those that were pre-irradiated and then kept under hypokinesia, the following parameters have been studied: the total number

of leukocytes, the leukocyte formula, the absolute numbers of different leukocytes, the cellular content of the bone marrow leucoplasts, the maturation index of bone marrow neutrophils. The number of leukocytes was determined in Goryaev's chamber. For the derivation of leukocyte formula, blood smears were prepared, which was fixed by ethylene alcohol for 10 minutes and then was stained by azure eosin (Romanovsky stain method). 200 cells have been counted in the smears under immersion regime.

3. Results and Discussion

The investigations have shown that in the initial period (5-10 days), the hypomotility causes distributional leukocytosis. The number of leukocytes was increased by about 28% ($p < 0,001$) compared to the initial stage. In the leukocytes formula lymphocytosis, neutrophils, basophilic, monocytosis and eosinophilia were observed (Figure 1). These changes are results of the activation of blood distributional simpatic mechanisms.

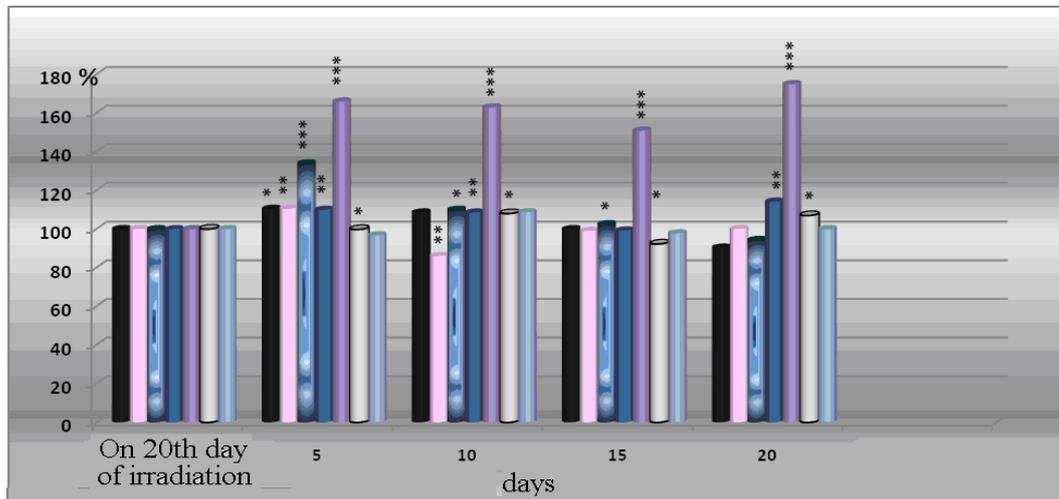


Figure 1. The changes of peripheral white blood indicators under hypomotile dynamics

*- $p < 0.05$, **- $p < 0.01$, $p < 0.02$, ***- $p < 0.001$

■ Numbers of leukocytes in 1mm^3 blood, □ and neutrophil, ■ segmented neutrophils, ■ Eosinophilis, ■ Basophilis, □ Monocytes, ■ Lymphocytes

The increase of the numbers of granulocytes and lymphocyte is apparently due to their quick export to the peripheral blood, by the stressor mobilization of granulocytes of bone marrow reserve and spleen, and of lymphocytes of thymus gland [21]. Under the stress the latter provide the cell composition of the peripheral blood [21].

During the mentioned period, in myelogram has been observed a moderate reduction of the number of young neutrophils and the increase in the number of lymphocyte. The maturation index of neutrophils was 0.6.

On the 15th day under hypomotile conditions, the total amount of leucocytes dropped by 15% as compared to the data for 10th day, but it was higher by 123% ($p < 0,001$) from the initial level. The absolute number of lymphocytes was 97%. The high level of matured neutrophils, eosinophilis, basophilis and monocytosis were maintained.

On the 20th day of the investigation, the number of leucocytes returned to the initial level (102%). In the leucocyte formula a reduction of the absolute number of lymphocytes was observed (86% ($p < 0.001$)). The

numbers of eosinophilis and monocytosis dropped compared to the results of previous days and have been maintained on normal level. The number of mature neutrophils and basophilis was maintained at high level, 127% ($p < 0,001$), 204% ($p < 0.001$), respectively.

In the myelogram it was observed a decrease in the numbers of young and mature neutrophils which is a result of the suppression of proliferation processes of white spot. The lymphocyte number was increased up to 18, as compared to the normal level 8 and the maturation index of neutrophils was 0.6 (Table 1).

Along with the increase of the duration of exposure of hypomotile condition, a gradual reduction in the number of leucocytes was observed and during 25-30 days it was on the level lower than the initial one (90%, $p < 0,05$; 88%, $p < 0,01$, respectively). A reduction of the absolute numbers of lymphocytes, eosinophilis, neutrophils, monocytes in the leucocytes formula was observed. Only the number of basophilis was persisted on the high level. In the myelogram, on 30th day, the low level of young and mature neutrophils was retained.

Table 1. The Changes of Peripheral White Blood Indicators under Hypomotile Dynamics

Indicators	Initial data	The days of investigation					
		5	10	15	20	25	30
Numbers of leukocytes in 1mm ³ blood	8400±270	10800±245 p<0.001	11600±381 p<0.001	10400±305 p<0.001	8600±221	7600±199 p<0,05	7425±189 p<0,01
Band neutrophil	42±6	54±1.8 p<0.001	29±2 p<0.001	26±1.9 p<0.001	22±1.6 p<0.001	38±2.1 p<0,01	38±2.1 p<0,01
Segmented neutrophils	2772±121	4698 ±146 p<0.001	4147±123 p<0.001	4550±142 p<0.001	3526±136	2622±129	2450±126 p<0.01
Eosinophilis	168±7	162±10 p<0.001	232±14 p<0,001	208±11 p<0.001	172±10	152±9 p<0.01	148±9 p<0.01
Basophilis	84±5	162±9 p<0.001	203±14 p<0.001	208±13 p<0.001	172±9 p<0.001	152±7 p<0,001	148±7 p<0.001
Monocytes	546±10	756±17 p<0.001	725±16 p<0.001	728±16 p<0.01	580±12	456 ±10 p<0.001	481±11 p<0.001
Lymphocytes	4788±156	4968 ±112	6264±205 p<0.001	4680 ±114	4128±111 p<0.001	4180±113 p<0.001	4158±112 p<0.001

The initial levels of the observed parameters have been recovered after 2 weeks from the end of hypomotile conditions. The high level of basophilis numbers during 30 day under hypomotile dynamic conditions, is probably due to the increased amount of interleukin1, influence of under the which the histamine excretion takes place from basophilis and fertile cells. The latter expands the small arteries, increases the transparency of capillaries, which has adaptive significance for the organs blood supply under the hypomotile conditions.

According to the literature data, the hypomotility, as a stress factor, reduces the organism resistance, the amount of lysozyme, compliments and induces movements in the immune system [1]. It has been shown that in the case of 9 days hypocinesia the functional activity of neutrophils and lymphocytes was notably decreased. At the same time, the activity of hydrolytic enzymes in neutrophils increases which can cause the development of cytolytic processes.

Such different recoveries (reconstructions) in neutrophils are considered as a suppression of body's natural defense forces [22,23]. Under the influence of limited motile activity, the energy systems in leucocytes, decreased.

Thus, analyzing the literature data and the data obtained in our work it is supposed that under the initial stage of the hypomotility influence the body mobilizes its recovery

and defense mechanisms, which provide the vital activities at the expense of the us of the functional reserves, but the long-term impact induces the strain in regulatory mechanisms and the reduction of reserve capacities of the organism.

Therefore, in the next series of experiments, for the rectification of the negative effects of hypokinesia the animals have been pretreated by the millimeter waves during 20 days.

According to the literature data, the millimeter waves increase non-specific resistance of the body; they are able to alter the function of the immune system, which is among the main mechanisms of the organism's initial stage recovery [24].

From the analysis of the obtained results it follows that after the 20 days irradiation the standards of the morphological indicators of leukopoiesis have been increased and, on that background, the significant changes in the indicators of leukopoiesis have not been observed.

On the 5th day of hypomotility a moderated increase in number of leukocytes (110.5%, p <0,001) has been observed as a result of the increased number of granulocytes, which apparently is a result of the stressor mobilization of the bone marrow's reserve of the granulocytes. The numbers of lymphocyte and monocytes were on the initial level (Figure 2).

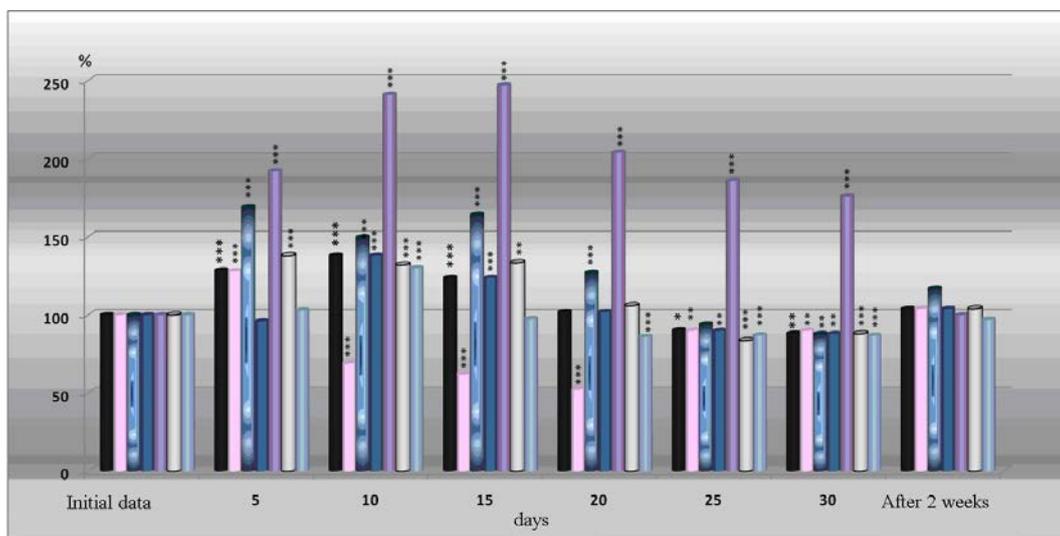


Figure 2. The changes of peripheral white blood indicators in irradiated animals under hypomotile dynamics

*- p<0.05, **- p<0.01, p<0.02, ***-p<0.001

■ Numbers of leukocytes in 1mm³ blood, □ Band neutrophil, ▤ Segmented neutrophils, ▥ Eosinophilis, ▦ Basophilis, ▧ Monocytes, ▨ Lymphocytes

On the 10th day of the investigation the number of leucocytes was not changed significantly (108.6%). In the leukocytes formula, a moderated increase of the numbers of basophils, monocytes, neutrophils and lymphocytes was observed. At the same time, in the myelogram the numbers of young and matured neutrophils were not changed and the increase in the number of lymphocytes has been observed.

On the 15th day of the investigation the white blood indicators in peripheral blood were determined at basal level, and only at high level of basophil numbers were

maintained (150% ($p < 0,001$)), which has an important adaptation denotation.

On the 15th and 20th days, in blood smears large number of reticular and plasma cells were found, which were not observed in normal circumstances (Table 2). In the equilibrium of hemopoiesis the reticular cells do not participate in homeopoetic processes and they are considered as a reserve. Their mobilization plays an important role, especially when the hemopoiesis is in an intense stage. It is assumed that, under this condition, the blood cells can be intensively generated and differentiated from the reticular cells of bone marrow [25].

Table 2. The Changes of Peripheral White Blood Indicators in Irradiated Animals under Hypomotile Dynamics

Indicators	Initial data	On 20 th day of Irradiation	The days of investigation			
			5	10	15	20
Numbers of leukocytes in 1mm ³ blood	7800±210	9200±222	10200±262 p<0.05	10000±338	9160±236	9200±235
Band neutrophil	78±3	115±6	127±6 p<0.02	100±4	114±4	115±4
Segmented neutrophils	2574±150	3036±148	4080±165 p<0.001	3300 ±139 p<0.05	3114±141	2898±125
Eosinophilis	156±10	161±6	178±7 p<0.02	175±7 p<0.02	160±6	184±8 p<0.02
Basophilis	78±6	92±4	152±5 p<0.001	150±5 p<0.001	138±6 p<0.001	161±8 p<0.001
Monocytes	546±9	644±13	663±15	700±16 p<0.001	595±16 p<0.05	690±17 p<0.05
Lymphocytes	4368±180	5152±260	4998±221	5600±224	5038±220 p<0.01	5152±222

It should be mentioned that the maturation index of neutrophils was not changed during all days of the investigation (0.7), which shows that white spot cells were proliferated and matured without any abnormal processes.

According to the obtained data, the EMR pre-treatments of animals elevate the potential of regulatory systems and prevent the negative effect of hypomotility on blood system, resulting in the disappearance of the changes in indicators of leukopoiesis.

Thus, under stress condition the non-ionizing millimeter waves are able to alter the function of immune system, which is one of the main mechanisms for the correction of the state of the organism. Under various stress factors, the functional changes of the organism are based on the activation of stress realizing systems, including the sympathetic-adrenaline system as well [26]. As a result the production of catecholamines is increased [27]. The histological studies have shown that in the case of limited movement the mass of adrenal glands is increased which is a result of the intensive synthesis of catecholamines.

It has been found that the catecholamines are captured and accumulated in various cells and tissues (in atrium (heart), brain hard membrane, and erythrocytes). Accordingly, under the hypokinesia the activity of the sympathetic-adrenaline system is elevated (strengthened).

The elevated effect of adrenergic on the organs and tissues induces a spasm of peripheral vessels and the development of hypoxia. For pre-irradiated animals under hypomotile condition the activity of the sympathetic-adrenaline system is normalized. The evidence of this is the decrease of the weight of adrenal glands, as well as the decrease of the storage of catecholamines in erythrocytes and brain hard membrane [28]. It was shown that under hypomotile condition the functional activity of the neutrophils and lymphocyte is decreased, and on the

contrary, in neutrophils the activity of hydrophilic enzymes is elevated, which can promote the development of the processes of cytolysis, and, consequently, the tissue damages [27]. Such different by directed recoveries suppress the natural defense forces in body's cells [23]. According to the literature data the millimeter range electromagnetic waves reduce the protease activity in the neutrophils, which is one of the mechanisms of the anti-stress effect [23].

4. Conclusion

Thus, under stress condition the low-power millimeter electromagnetic radiation is able to alter the function of immune system, which is one of the main mechanisms for the correction of the state of the organism. Under various stress factors, the functional changes of the organism are based on the activation of stress realizing systems, including the sympathetic-adrenaline system as well.

Under the influence of non-ionizing millimeter waves in the neutrophils and lymphocytes the activity of succinate dehydrogenase is increased, which is an evidence of the energetic level of these cells [29].

It is known that the fertile cells participate to the regulation of the adaptive processes at cellular level and have a significant influence on the properties of the neutrophils. Under hypomotile condition, the degranulation level of the fertile cells is increased. Under the combined effect of the millimeter waves and hypokinesia, the degranulation level of the fertile cells is decreased, that is why they are considered as regulators [30].

Thus, on the base of obtained results and the literature data it is concluded that the non-ionizing millimeter waves elevate the functional state of the blood cells, the functional potential of the leukocytes, preparing the cells to resist against other agents.

Author Contributions

V.K. designed the research. Ts.A. analyzed data and wrote the paper. E.G. wrote the paper. N.A. performed research. T.A. contributed animals.

Conflict of Interest

No Conflict of Interest.

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