

Study of thiol components of antioxidant protection in the lens of developing rats exposed to electromagnetic irradiation in the newborn period

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Received: March 12, 2025; Reviewed: May 03, 2025; Accepted: June 20, 2025

Currently, non-ionizing electromagnetic fields are an essential part of the environment, which is becoming increasingly important due to their widespread use in various spheres of human activity. Studying the influence of the electromagnetic factor on living organisms is an urgent task and is interesting both from a medical and social point of view. People are most exposed to the effects of alternating electromagnetic fields in various frequency ranges, since they directly penetrate into people's lives and work activities through household electronic equipment, radio and television systems, mobile communications, medical diagnostic devices, etc. In the Laboratory of Radiation Physiology (Institute of Physiology, Baku, Azerbaijan), it has been studying the mechanisms of the effect of decimeter electromagnetic radiation (EMR) on a living organism for more than ten years, and significant results have been obtained in experimental studies indicating the oxidative nature of the effects of radiation; shifts in oxidant-antioxidant ratios in various organs - brain, blood and lens - have been detected. In particular, experiments with prenatal exposure have shown that changes in the redox state of organs (in other words, the formation of a new status) in early postnatal development depend on the stage of intrauterine development at which radiation was applied. In this paper, we aimed to study how exposure of the newborn to electromagnetic radiation as an oxidative stress factor will affect the antioxidant response of the organism (organ) in subsequent development, as well as to show the possibility of a preadaptation effect to oxidative stress. The thiol parameters in the eye lenses of 30, 45, and 60-day-old white rats exposed to 460 MHz EMR in the newborns were studied. Based on measurements of the content of easily accessible and hidden protein thiols, it is shown that there are differences in the quantitative ratio of the two types of thiols and their response to a single test exposure to EMR between irradiated rats at birth and intact rats of the appropriate age. Thus, in contrast to intact rats, the proportion of hidden thiols in the total thiol content in the lens increases with age in rats exposed at birth. Another important difference is that a single test exposure in all 3 age groups of intact rats leads to a sharp drop in the level of both types of thiols in the lens, whereas in rats exposed at birth, the test exposure does not affect the level of thiols. The main conclusion is that the exposure of the newborn to electromagnetic radiation as an oxidizing factor enhances its antioxidant protection from oxidative effects in further development (at least in the example of the eye lens), in other words, we observe the effect of preliminary adaptation by decimeter electromagnetic radiation.

Keywords: *Electromagnetic radiation, white rats, eye lens, thiols*

INTRODUCTION

As a result of the widespread use of electromagnetic waves in industry, communications (TV, radio,

telephones, etc.), medicine and other branches of technology, EMI has become an integral part of our everyday life over the past hundred years. However, to this day, no scientific organization has been able to unequivocally state that low-intensity EMI does not pose a danger to living organisms in general and to their visual organs in particular (Gadzhiev, 2013). The effect of high-frequency non-ionizing EMI on biological objects has been studied in the laboratory of radiation physiology for over ten years (Abbasova and Gadzhiev, 2022; Gadzhiev and Ibragimova, 2023; Ibragimova et al., 2021). Data have been obtained on various organs and tissues indicating that the induction of oxidative stress by radiation at different stages of the body's development (prenatal period, prepubertal and pubertal periods) and in its different functional states (stress, physical activity, etc.) can lead to physiological and biochemical changes, the harmfulness of which to health is not excluded. In our previous work, we studied rats from neonatal to the period of independence (Gadzhiev and Ibragimova, 2023). Continuing our research in this area of scientific knowledge, our next goal was to study the effect of electromagnetic radiation with a frequency of 460 MHz on the dynamics of the content of two types of thiols (easily accessible - EA-thiols and hard-to-reach thiols hidden in the protein structure - HR-thiols) in the lens of the eyes of rats at an older age before puberty. The results of this study are presented in this article.

MATERIALS AND METHODS

The objects of the study were 192 Wistar rats (Wistar line) aged from birth to 60 days (male pups were studied). These animals were equally divided into 3 age groups: 30-day, 45-day and 60-day rats. According to the conditions of the planned studies, the animals were irradiated with high-intensity EMR in a metal chamber. The physiotherapeutic apparatus "Volna-2" (the apparatus meets the technical requirements for use in medical purposes) was used as a source of radiation. The rats were irradiated under the following conditions: the output power of the apparatus was 60 W, the energy flux density was 30 mW/cm², the frequency was 460 MHz, the duration of irradiation was 20 minutes. Based on the objectives of our experiment, each age group we identified was divided in turn into 4 subgroups of 16 individuals each. The first subgroup in these groups consisted of animals of the control (intact) group, which were not irradiated but were subjected to the same "irradiation" procedures as the experimental rats, but only with the apparatus turned off (i.e., sham irradiation). The second subgroup included animals that were irradiated only on the day of euthanasia (conditionally "irradiated control group") of the corresponding age group. The third subgroups included rat pups that were irradiated only at birth (age up to 48 hours). Finally, the fourth subgroups were made up of animals that were irradiated at birth and on the day of euthanasia. After the expiration of the designated period, all animals of one of the groups were euthanized (first 30-day-old, then 45-day-old, and finally 60-day-old). After the decapitation of the animals, a pair of lenses were removed from their eyes, then the tissue to be examined was dissected in the cold and a homogenate was prepared in the appropriate concentration of physiological solution (for further analytical procedures). The irradiation procedures and the killing of rats were carried out in the first half of the day under natural light. The handling of animals and the process of their sacrifice complied with the requirements of the international convention on the protection of animals. The modified Sedlak-Lindsay method based on the Ellman reaction was used to determine the thiol content in the prepared lens homogenate (Sedlak and Lindsay, 1968). The concentration of easily accessible (the sum of low-molecular thiols and superficially located protein thiols) and hidden (masked in the protein structure) thiols in the cortex and nucleus of the lens was determined using a Spekol-221 spectrophotometer (manufacturer "Carl Zeiss Industrielle Messtechnik GmbH"). These results were then recalculated to determine the thiol content per 1 mg protein (nmol/mg protein). Statistical analysis of the data was performed using the MATLAB software package (version 8.6) in the Windows operating environment. Analysis of variance and comparison of indicators in the studied age groups, as well as between subgroups within these groups, were performed using a two-sample t-test (Glanz, 1998).

RESULTS AND DISCUSSION

As we can see, the content of both easily accessible and hidden thiols in the lens of animals subjected to experiments for 30 days decreases (Table). Judging by the average values, the amount of easily accessible thiols decreased by 34.4% in rat pups irradiated only on the day of euthanasia compared to the intact group. At the same time, the level of EA-thiols decreased by 62.8% in rat pups irradiated at birth, and by 63.7% in rat pups irradiated twice (on the day of birth and on the day of euthanasia) (the differences are reliable at a significance level of $P<0.05$). If we compare the experimental animals with the rat pups of the "irradiated control group", we will see a more moderate decrease in EA-thiols (by 43.3% in rat pups irradiated only on the day of birth, and by 44.6% in rat pups irradiated twice). There were almost no differences between the experimental groups (a decrease in EA-thiols by 2.4% in rat pups irradiated twice). Latent thiols changed according to a very similar scenario. The amount of HR-thiols in the lens of the eye in rat pups irradiated only on the day of euthanasia compared to the intact control group decreased by 51.6%. In rat pups irradiated once at birth, the level of HR-thiols decreased compared to the control by 75.6% and was 71.1% lower in rat pups irradiated twice (the differences are reliable at the significance level of $P<0.01$). In addition, compared to the second subgroup, both in rat pups irradiated only on the day of birth and in rat pups irradiated twice, a decrease in HR-thiols by half was observed (by 49.5% and 50.7%, respectively). Finally, compared with rats irradiated only on the day of birth, the amount of latent thiols in the lens of the eyes of rats irradiated both at birth and on the day of euthanasia decreased by 2.4% (a decrease similar to the values of EA-thiols).

A similar picture is observed in animals subjected to experiments for 45 days – the content of both easily accessible and hidden thiols in the lens decreases. Only here we observe an insignificant decrease in EA-thiols (compared to the intact control group). At the same time, the content of hidden thiols decreases almost to the same extent as in animals observed for 30 days (the difference in the percentage of the decrease is 15-20%). In addition, differences are revealed between the third and fourth subgroups of animals. In animals irradiated only at birth, the decrease in the content of both easily accessible and hidden thiols becomes more pronounced compared to the twice irradiated subgroup (by 25.3% and 21.4%, respectively). Thus, it can be assumed that 45-day-old rat pups, when re-irradiated, show signs of adaptation to radiation. The content of EA-thiols and HR-thiols in the intact control group was lower than in 30-day-old animals (the differences were reliable at a significance level of $P<0.05$). At the same time, no reliable differences were found between the indices of the second subgroups (irradiated control subgroup) when comparing 30-day and 45-day animals. The most interesting was the dynamics of the thiol content in the group of animals subjected to experiments for 60 days. Here, in both experimental subgroups, a decrease in the content of EA-thiols and HR-thiols was also observed compared to both control groups. In rats irradiated once at birth, the average value of the EA-thiol index decreased compared to the control by 40.0% and by 41.2%; this indicator was lower in rats irradiated at birth and on the day of euthanasia. Similar percentage differences were also revealed for HR-thiols (by 42.7% and 41.5%, respectively). However, the experimental subgroups hardly differ from each other in this indicator (the differences are insignificant at the significance level of $P=0.05$). In absolute values, the indicators of both EA-thiols and HR-thiols are close to the indicators that were determined in 30-day-old animals. Compared with the animals irradiated for 45 days, the content of easily accessible thiols in the control group hardly changed (the differences are insignificant). However, the indicators of HR-thiols differ significantly (the differences are significant at $P=0.05$) both from the values for the first subgroup of 30-day-old animals and from the values for the first subgroup of 45-day-old animals. For other subgroups, the differences between the compared indicators are also insignificant. Visualization of the actual data we obtained using a diagram most realistically demonstrates the observed trends (diagram).

Table. Average values of concentration of easily accessible (EA-thiols) and hidden (HR-thiols) thiols (nmol/mg protein) in the lens of the eyes of white rats (aged 30, 45 and 60 days) under different variants of their irradiation with EMR of 460 MHz frequency

Subgroups	30-day-old animals		45-day-old animals		60-day-old animals	
	EA-thiols	HR-thiols	EA-thiols	HR-thiols	EA-thiols	HR-thiols
Control intact	1021±131	1718±195	690±83	1211±139	650±78	890±103
Control irradiated	670±75	832±96	560±77	749±92*	600±69	820±91
Experimental irradiated 1 time	371±41*	410±37*	320±39	420±43*	390±37*	510±66*
Experimental irradiated 2 times	380±43**	420±49**	401±41	510±64*	382±45*	521±61*

*, ** – confidence level for differences between control and irradiated samples, respectively $p < 0.05$ and $p < 0.01$

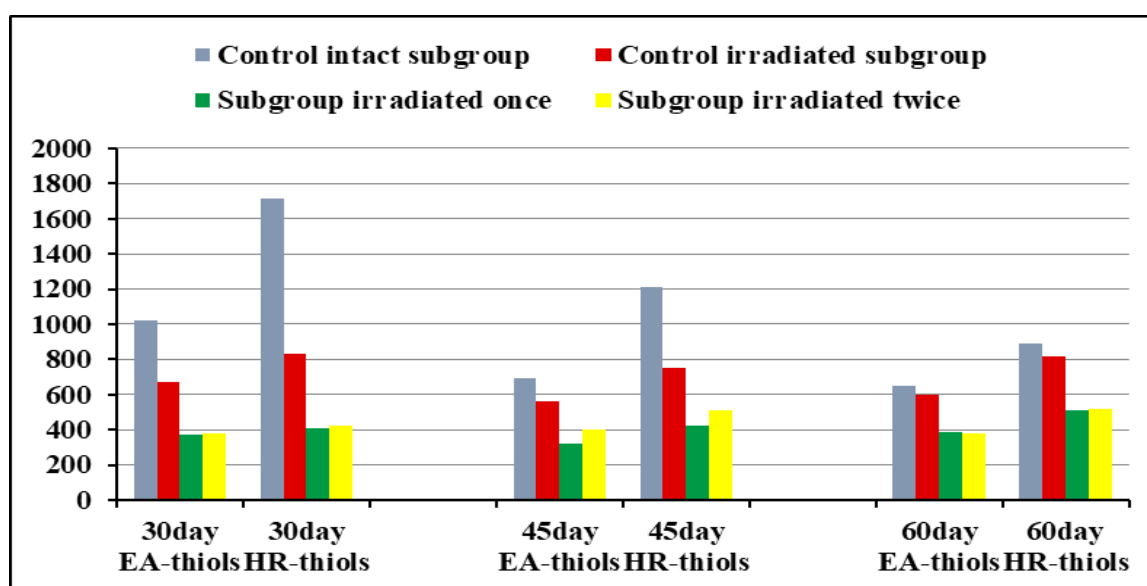


Diagram. Indicators of readily accessible and hidden thiols in the lens of the eyes of white rats of different ages after their irradiation with decimeter-range EMR. Note: the numbers on the vertical axis are the concentration of thiols (nmol/mg protein).

Thus, the analysis of the obtained data allows us to draw some conclusions regarding the behavior of thiols in the lens of the eye of young rats when their organism is exposed to decimeter-range EMR. In the animals that were subjected to irradiation in our experiment, the content of both easily accessible and hidden thiols in the lens of the eye decreased after birth, but this decrease was somewhat stabilized by the end of the 2nd month of their life. The short-term effect of EMR exposure of the animals on the day of euthanasia significantly affects the concentration of both types of thiols in the lens, but this effect is almost not observed in animals at the end of 2 months of age. At the same time, observation of the remote

effects of EMR after one, one and a half, and two months from the moment of irradiation shows a strong drop in the content of both types of thiols. This indirectly confirms the protective role of thiols against oxidative factors. At the same time, experiments show that in 45-day-old animals the corresponding adaptation mechanism is not yet fully formed, which affects the abrupt growth of thiol content during repeated irradiation on the day of euthanasia. Two-month-old animals react moderately to such an oxidative stress factor, which allows us to speak about more formed adaptation mechanisms. In addition, within the framework of these mechanisms, hard-to-reach thiols are better restored to their close-to-normal

content in the lens, and this is observed in all age groups. As we have already noted above, there is a lot of scientific data indicating various types of effects of EMR on living organisms (Grigoriev, 2000; Yakymenko et al., 2016; Yurekli et al., 2006; Shahbazi et al., 2018). At the same time, there are many works concerning the specifically harmful effects of EMR and the oxidative stress it causes on nerve tissue and tissues of the visual analyzer, but there are still more (Hossmann and Hermann, 2003; Panakhova, 2019; Zhang, 2017). In our previous works, we noted that changes in thiol homeostasis in the lens of the eye during EMR irradiation may indicate the participation of the latter in compensatory reactions for restoring the redox balance (Gadzhiev, 2013). In addition, a comprehensive analysis of changes in thiols and LPO reactions after irradiation with decimeter-range EMR allowed us to assume that changes in thiol homeostasis in this context may reflect the antioxidant properties of thiols in LPO reactions (Ibragimova et al., 2023). Our results obtained during the last experiment confirm the conclusions that changes in the oxidation-reduction balance in the lens of the eye largely depend on thiol homeostasis, and thiols play an important role in the mechanism of antioxidant protection in case of redox balance disturbance. However, to more accurately elucidate the mechanism of this protection, it is necessary to study the behavior of thiols in the lens of the eye in adulthood.

CONCLUSIONS

Based on the data from the study of the contents of easily accessible and hidden protein thiols, it was shown that there are differences in the quantitative ratio of the two types of thiols and their response to a single test exposure to EMR between rats irradiated at birth and intact rats of the corresponding age. Unlike intact rats, the proportion of hidden thiols in the total thiol content in the lens increases with age in rats irradiated at birth. Another important difference is that in all three age groups of intact rats, a single irradiation leads to a sharp drop in the level of thiols of both types in the lens, whereas in rats irradiated at birth, test irradiation does not affect

the level of thiols. The main conclusion is that the effect of electromagnetic radiation on a newborn organism as an oxidizing factor strengthens its antioxidant protection from oxidative effects in further development (at least in the example of the lens of the eye); in other words, we observe the effect of preliminary adaptation to electromagnetic radiation in the decimeter range. The presented results are still preliminary and for more specific conclusions we plan to conduct additional studies in a similar format (meaning longer experiments, as well as studies of female individuals). Accordingly, we plan to use more powerful statistical methods of data analysis.

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