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Research Article

Thiol Homeostasis in The Crystalline Eye and Influence on It Irradiation by Decimeter Electromagnetic Radiation (Experiments on Rats)

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Abstract:

Electromagnetic radiation (EMR) in the microwave range, even at non-thermal intensity, causes significant biochemical and physiological changes in living organisms, which are supposed to be associated with their possible oxidative effect. This work is devoted to the study of the mechanism of realization of the EMR effect in the eye lens at the level of redox state elements, based on the fact that this organ is the most suitable model: it functions semiautonomously and has a well-organized system of antioxidant protection. The transparency of the lens is maintained by preserving the redox balance, in which the homeostasis of thiol compounds of protein and non-protein nature plays an important role. Our experiments were performed on rats using 460 MHz EMR for exposure at non-thermal intensities (power flux density between 10 and 30 μW/cm2). It has been shown that chronic exposure to EMR for up to two weeks caused changes in the redox state of the lens, which manifested in changes in the level of lipid peroxidation processes and the content of thiols of various natures. The substructures of the lens (cortical and nuclear regions) reacted to EMR exposure in different ways. Depending on the EMR intensity, pro- and antioxidant characters were revealed in their reactions. The dynamics of the oxidative reaction of lens substructures were also different under high- and low-intensity exposure. The character of the kinetics of changes in the products of oxidative reactions (malondialdehyde and lipid hydroperoxides) and reducing agents (non-protein and protein SH groups) in the lens of the irradiated organism suggested the role of the enzymatic thiolation-dethiolation system to preserve the redox balance in the substructures of the lens. In addition, the results on changes (kinetics) in the content of various protein SH-groups, i.e., hidden inside the protein molecule and exposed on its surface, during EMR exposure, as well as the data available in the literature, allow us to put forward suggestions about the supramolecular mechanism of homeostasis regulation, in particular, thiol homeostasis regulation in such high-protein structures as the lens, which can be realized by aggregation-disaggregation of protein molecules (crystallins in the case of the lens). Our results can serve as a basis for developing a new non-invasive approach to cataract prevention using low-intensity microwave radiation.

keywords: electromagnetic radiation; eye lens; thiols; cataract

Introduction

The data available in the literature indicate that the lens can be a good, convenient model for studying the oxidative action of an external factor, in particular, nonionizing EMR [5, 6, 7]. It is already known that ensuring the transparency of the lens is associated with the balance of its redox state. A high level of endogenous thiols, especially glutathione, plays a vital role in maintaining the reduced state of lens proteins [8, 9]. Along with this, to maintain the function of the lens, two systems of internal repair (glutaredoxin and thioredoxin systems) are constantly working, which deiolate mixed disulfides of the protein-non-protein thiol type or protein-protein disulfides formed during oxidative stress [10, 11]. We have previously shown that exposure UHF EMR in rats modifies the activity of lipid peroxidation (LPO) in the lens [12, 13]. Since the LPO level is closely related to antioxidant protection in tissues, including the content of endogenous reduced thiols, the latter are predominantly oxidized by lipid peroxidation products, thereby protecting other functional groups and molecules from oxidation. The redox shift state of the lens (in one direction or another), which can occur under the action of low-energy radiation, can serve as a change in the conditions for the development of free radical pathologies, in particular, a pre-cataract state [14, 15]. Based on these data, we set a goal – to find out how changes in

LPO processes (caused by exposure to non-ionizing electromagnetic radiation) in the lens of the eye are related to changes in the content of thiols.

Material and methods

The experiments were carried out on 3- month-old male rats which were irradiated using a "Volna-2" generator (460 MHz). The technique of the experiment was described in more detail in the article by Abbasova and Gadzhiev [16]. Experiments with low-intensity and relatively high-intensity irradiation were carried out at a power flux density of 10 and 30 μW/cm2 . The values of the specific absorption rate (SAR) of electromagnetic energy averaged over the entire animal body were estimated as 5 and 15 mW/kg for two intensity modes, respectively. For each specific exposure, the rats were divided into three groups of six rats each, i.e., one control group (falsely irradiated) and two experimental groups, accordingly, low intensity and relatively high-intensity exposed. Experimental groups were exposed to EMR 20 min daily for 1, 3, 5, 7, 10, and 14 days. After an appropriate radiation load, the lenses of the control and experimental groups were isolated for studies in compliance with the rules of working with experimental animals. To determine the content of thiols in lens homogenates, a modified Sedlak-Lindsay method based on the Ellman reaction was used [17]. Concentrations of readily available (RA) (the sum of low molecular weight thiols and superficially located protein thiols) and hidden (masked in the protein structure) thiols in the cortex and nucleus of the lens, which were then recalculated by 1 mg of protein (nmol/mg protein). Statistical data analysis was performed using the SPSS software package for Windows version 22.0. The differences between the control and experimental measurements were examined using the t-test for paired samples.

Results

We studied the content of readily available (RA) (the sum of low molecular weight thiols and superficially located protein thiol groups, which can also be called cytoplasmic) and hidden (masked in the protein structure) thiol groups in the cortex and nucleus of the lens of rats during chronic irradiation for a period of up to 14 days. Changes in the content of cytoplasmic thiols in the cortex and nucleus of the lens at different exposures to high-intensity irradiation are shown in Figs.1 and 2.

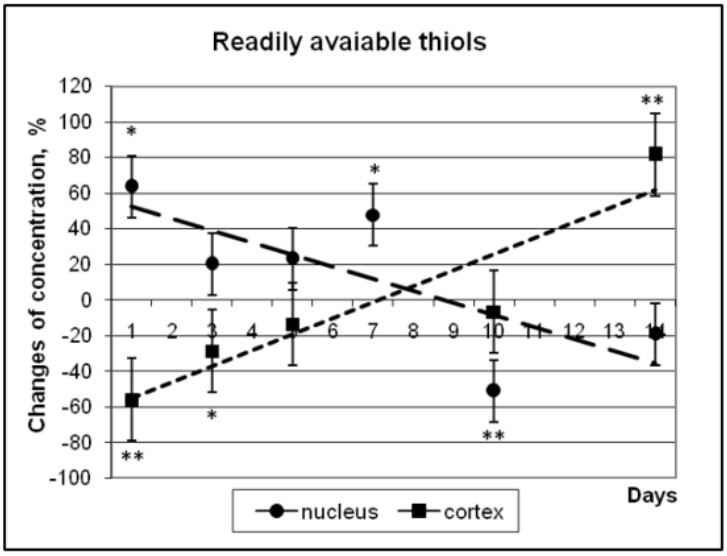

Figure 1: Changes in the content of readily available thiols in the lens substructures of rats exposed to high intensity irradiation with EMR 460 MHz

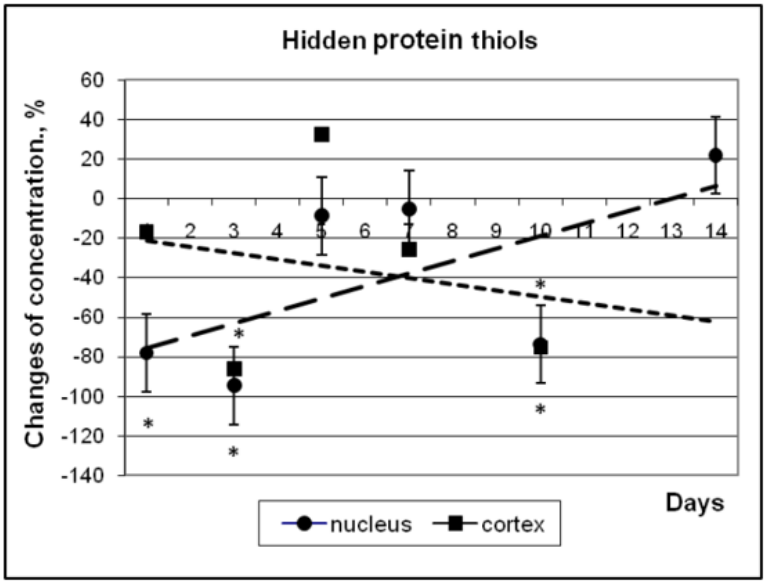

Figure 2: Changes in the content of hidden thiols in the lens substructures of rats exposed to high intensity irradiation with EMR 460 MHz.

Note: The average concentration of readily available thiols for the control group was 473±31 nmol/mg protein in the nucleus and 464±39 nmol/mg protein in the cortex. The average value of concentration for hidden intramolecular protein thiols in the control group, calculated from the difference between the total number of thiols and readily available thiols, was 565±126 nmol/mg protein in the nucleus and 296±100 nmol/mg protein in the cortex. Dotted lines indicate the general trend of changes in different substructures and were carried out using linear approximation of experimental points.

The changes in the content of cytoplasmic thiols in the cortex and nucleus of the lens at different exposures are demonstrated in the Fig. for relatively highintensity irradiation. The level of RA-thiols in the lens cortex, which has fallen after 1st day of irradiation, gradually increased, reached the control level on days 7-8, and increased by ~60% over the control by further irradiation; in the nucleus, on the contrary, the level of thiols that has increased after 1st day of irradiation gradually falls to the control level on days 8-9 with a further decrease in with respect to control by ~30% (see in Fig.1). Such a nature of changes in the content of RA-thiols was correlated with changes in the LPO process in the same tissues. The dynamics of changes in the content of hidden thiols in the cortex and nucleus of the lens under relatively high-intensity irradiation were opposite to the changes in RA-thiols (Fig.2). With a linear approximation of the time dependence of the experimental data, it can be seen that the initial decrease in the level of hidden thiols by 80% in the nucleus was replaced by a gradual increase until it was restored to control at the end of irradiation. In the lens cortex, at the beginning of exposure to EMR,

there was also a decrease in the level of hidden thiols (initially by ~20%), which developed further with the continuation of irradiation, and by the end of exposure reached ~60% lower level than the control. An important result was that the assessment of the total amount of thiols, both for the cortex and for the nucleus, showed a stable level during the entire irradiation period, which was about 20% lower than the control level. Exposure to irradiation at low intensity led to a pattern of changes in thiols of various types in the cortex and the nucleus of the lens, in general, opposite to the picture with highintensity irradiation (the data has not given here). The decrease in the number of readily available thiols in the lens cortex was compensated with an increase in hidden protein thiols under low-intensity irradiation. In the nucleus, the nature of changes in easily accessible and hidden thiols was the same as in the cortex, but these changes were more moderate. Experimental studies were carried out in several stages. First of all, the parameters of the amplitude of the evoked potential (EP) of individual components (total, positive, negative) were recorded in all studied structures in intact animals. Then, in accordance with Noel's methodology, an experimental model of retinal dystrophy was created by injecting MIAA into the ear vein of animals. Experimental retinal dystrophy of a moderate degree was formed within 28-30 days. 30 days after injection EP was recorded again and a corresponding decrease in the amplitude parameters of EP in each structure was observed. The decrease was 40-50% in CS and LGB, and 20-25% in the VC compared to the control. Then curcumin was added to animal food for 30 days. After that, the EP parameters were recorded again. From the results obtained, it became known that the amplitude parameters of EP in all structures after taking curcumin partially increased. However, the positive effect of curcumin on the amplitude parameters of EP in CS and LGB structures was much less than in VC (Fig.1 and 2).

In the lens cortex, at the beginning of exposure to EMR, there was also a decrease in the level of hidden thiols (initially by ~20%), which developed further with the continuation of irradiation, and by the end of exposure reached ~60% lower level than the control. An important result was that the assessment of the total amount of thiols, both for the cortex and for the nucleus, showed a stable level during the entire irradiation period, which was about 20% lower than the control level. Exposure to irradiation at low intensity led to a pattern of changes in thiols of various types in the cortex and the nucleus of the lens, in general, opposite to the picture with highintensity irradiation (the data has not given here). The decrease in the number of readily available thiols in the lens cortex was compensated with an increase in hidden protein thiols under low-intensity irradiation. In the nucleus, the nature of changes in easily accessible and hidden thiols was the same as in the cortex, but these changes were more moderate.

Discussion

The results of the total exposure of organisms to relatively high- and low-intensity EMR revealed shifts in the redox state in the lens, respectively, in the direction of oxidation and in the direction of reduction. And, apparently, one of the ways to realize the shift of the redox balance is the transition between different types of thiols. The level of LPO can be considered an indicator of the redox state of the tissue [6]. Because the rate of accumulation of its products depends on the balance between the speed of this process and the antioxidant ability of the medium to destroy its products. The increase in LPO indicates a shift in the redox state towards greater oxidation of the cellular environment; this occurred under high-intensity irradiation. A decrease in the rate of LPO, as it occurs with low-intensity irradiation in the lens, indicates a shift towards lower oxidation, i.e. greater reduction. Just as with high oxidation of the tissue environment, when we talk about oxidative stress, with high recovery of the environment, for some time they began to talk about reductive (restorative) stress. References to reports on the phenomenon of reductive stress in relation to other tissues (liver, muscles) can be found in the article by Clanton et al. [18]. Apparently, there are systems of protection against reductive stress in the cells, which are able to mask the excess of reductive agents, and various thiols. With low-intensity irradiation in the lens, we are faced with just such a situation [12]. With a reduced level of LPO, open protein thiols pass into a disguised (hidden) state when they are unable to restore oxidized LPO products. Thus, there is a transition from one type of thiol to another under the influence of highintensity irradiation. Such transformation of protein thiols in tissues, in particular, in the lens under the action of oxidative factors is discussed in the literature and the regulation of these processes by thiolation and dethiolation reactions using certain enzymes is an important subject in the study of the lens [19]. Based on our results and literature data, we can discuss the development of a new noninvasive non-drug method of cataract prevention by exposure to low-intensity decimeter EMR to change two factors, namely redox shift and protein aggregation leading to loss of lens transparency [20, 21]. The transition of protein thiols from one state to another under the influence of a physical factor allows us to put forward speculation suggestion about a supramolecular mechanism for regulating homeostasis (in particular, thiol homeostasis) in such high-protein structure as the lens, which can be realized by aggregationdisaggregation of protein molecules - crystallins. There is a certain threshold size of protein aggregates (molecular weight - about 107Da), above which such aggregates, with sufficient concentration, cause significant scattering of light falling on the lens, which manifests itself in the loss of transparency of the latter. It can be assumed that the proteins of the lens at the physiological norm are represented by their small aggregates within those limits that do not affect transparency. At the same time, these aggregated molecules hide their SH groups. Under the action of oxidative-damaging factors, with the development of oxidative stress, perhaps at some certain stage of this development, the path of antioxidant protection implemented by disaggregation of the supramolecular protein structure, comes into effect, as a result of which previously hidden SH-groups can act as additional reducing agents. When the threat of oxidative damage to cellular structures or lens enzymes disappears, protein molecules can form high-molecular aggregates again without compromising the transparency of this visual structure.

Conclusions

It has been established that shifts in the redox state are detected in the substructures of the lens (in its nuclear and cortical parts) as a result of irradiation of the body with non-ionizing EMR of a certain intensity. The data obtained indicate that one of the ways to realize the shift of the redox balance in the lens is most likely the transition between different forms of protein SH groups. A suggestion is put forward about the supramolecular mechanism of regulation of thiol homeostasis in the eye lens, which allows via aggregation-disaggregation of SH containing protein molecules of crystallins to protect themselves from oxidative-damaging factors.

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