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CRYSTALLINE LENS: REDOX CHANGES IN RESPONSE TO THE ACTION OF NON-IONIZING ELECTROMAGNETIC RADIATION

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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 its 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 semi-autonomously 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/cm^2). 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.

Key words: electromagnetic radiation, eye lens, thiols, cataract

INTRODUCTION

Numerous studies on various biological systems show that the non-ionizing electromagnetic radiation (EMR) of the microwave range, even at non-thermal intensity, causes significant physiological changes in living organisms [5, 16]. Interest in these studies is due to the lack of specific biochemical and biophysical mechanisms of EMR action, although a huge amount of experimental data

has been accumulated on biological systems of various levels and complexity.

The results of experiments with animals exposed to microwave radiation obtained in recent years indicate the oxidative nature of the radiation effects on organisms [19, 20]. In particular, the data obtained at our laboratory (Laboratory of Radiation Physiology) concerning the chronic effect of decimeter radiation on the processes of lipid peroxidation (LPO) and the antioxidant system in some structures of the brain and eye make a serious contribution to the experimental substantiation of the free radical mechanism of EMR action [3, 6, 9].

The data available in the literature on the redox properties of the eye lens, the mechanisms of regulation of the processes underlying the preservation of the transparency of the lens, and the changes that occur as a result of aging and diseases (especially cataractogenesis) indicate that the lens can be a good, convenient model for studying the oxidative effect of an external factor, in particular, non-ionizing EMR [2, 3, 12].

We consider the following points to be the main arguments in favor of such a statement: 1) in the body, the lens functions (semi) autonomously, so it's *in vitro* studies can be brought as close as possible to *in vivo* conditions; 2) the biochemistry of the lens is subordinated to the preservation of a simple physiological function – maintaining the transparency of the crystalline substance by regulating the redox balance (protein thiols protection from oxidation and aggregation of protein molecules); 3) the processes of oxidation and reduction (of proteins and lipids) occurring in the lens prevail over other cellular processes (for example, syntheses), i.e. the lens can be considered as a system of redox reactions; 4) the pathogenesis (cataractogenesis) of the lens is well studied and its indicators can be used as parameters of the model that is the basis for studying the influence of the oxidative factor, in our case, EMR of the decimeter range [10].

It is already known that ensuring the transparency of the lens is associated with the balance of its redox state. High levels of endogenous thiols, particularly glutathione, play a vital role in keeping lens proteins in a reduced state [11, 13]. Along with this, two internal repair systems (glutaredoxin and thioredoxin systems) are constantly operating to maintain the function of the lens, which dethiolate mixed disulfides of the protein-non-protein thiol type or protein-protein disulfides formed under oxidative stress [14, 18].

We have previously shown that irradiation of rats with decimeter-range EMR modifies the course of lipid peroxidation (LPO) in the lens [4, 9]. Since the level of LPO is closely related to antioxidant protection in tissues, including the content of endogenous reduced thiols, the latter is primarily oxidized by LPO products, thereby protecting other functional groups and molecules from oxidation. The shift of the redox state (redox balance) of the lens, which can occur under the influence of low-energy radiation in one direction or another, can serve to modify the conditions for the development of pathologies of free radical nature, in particular the pre-cataract state [17, 21].

The purpose of this work was to find out whether changes in the process of LPO in the lens under the action of non-ionizing EMR are interrelated with changes in the thiol content of the lens. The expected results will allow us to start studying the possibility of using radiation with decimeter electromagnetic waves for the prevention of such a socially important disease as cataracts.

MATERIAL AND METHODS

The experiments were carried out on 3-month-old male rats, which were irradiated using a "Volna-2" generator (Russia, 460 MHz). The technique of the experiment was described in more detail in the article by Abbasova and Gadzhiev [6]. Experiments with low-intensity and relatively high-intensity irradiation were carried out at a power flux density of 10 and 30 $\mu W/cm^2$. The values of the specific absorption

rate (SAR) of electromagnetic energy averaged over the entire animal body were estimated as 5 and 15 μW/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, respectively, low-intensity and relatively high-intensity exposed. Experimental groups were exposed to EMR for 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 [15]. 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 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. The changes in the content of cytoplasmic thiols in the cortex and nucleus of the lens at different exposures are demonstrated in the Fig. 1 for relatively high-intensity irradiation.

The level of RA-thiols in the lens cortex, which had fallen after the 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 had increased after the 1st day of irradiation gradually fell to the control level on days 8–9, with a further decrease with respect to the control by ~30% (see the left diagram in Fig.). Such changes in the content of RA-thiols were 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. 1, right diagram). 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 high-intensity irradiation (the data has not been 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, and 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 veins of animals. Experimental retinal dystrophy of a moderate degree 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 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).

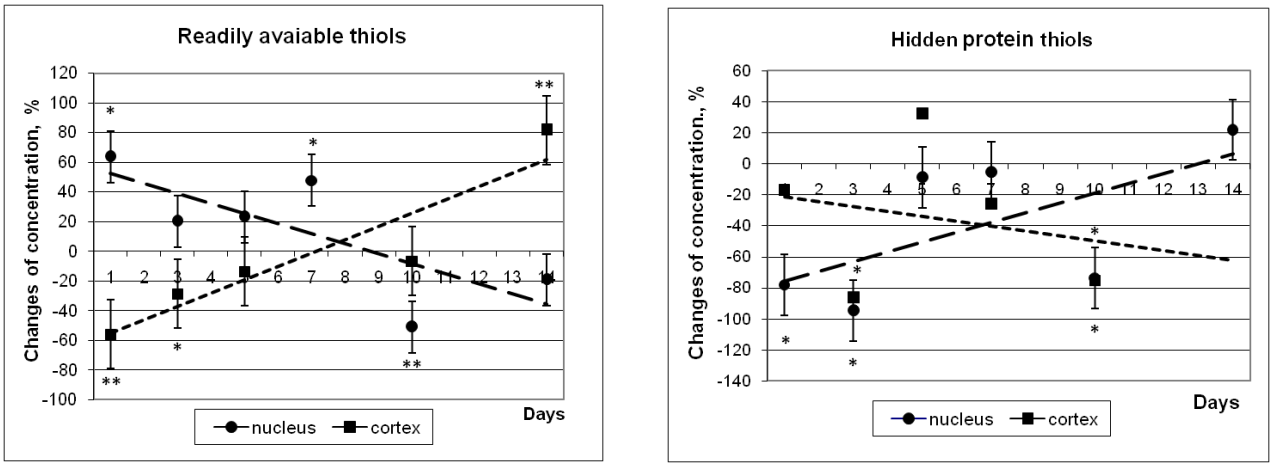

Figure 1. Changes in the content of readily available (left) and hidden (right) thiols in the lens substructures of rats exposed to high intensity irradiation with EMR 460 MHz for various periods of irradiation ("Volna-2" generator, the output power of 60 watts; daily exposure time: 20 min).

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.

Note: Dotted lines indicate the general trend of changes in different substructures and were carried out using a linear approximation of experimental points.

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 high-intensity irradiation (the data has not been 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 in the redox balance is through the transition between different types of thiols.

The level of LPO can be considered an indicator of the redox state of the tissue [3], 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. [8]. Apparently, there are systems of protection against reductive stress in the cells that 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 [4]. 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 high-intensity 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 [7].

Based on our results and literature data, we can discuss the development of a new non-invasive, 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 [1, 2].

The transition of protein thiols from one state to another under the influence of a physical factor allows us to put forward a suggestion about a supramolecular mechanism for regulating homeostasis (in particular, thiol homeostasis) in such a high-protein structure as the lens, which can be realized by the aggregation-disaggregation of protein molecules called crystallins. There is a certain threshold size of protein aggregates (molecular weight: about 107 Da), 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-weight 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 the 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 in 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

crystallins to protect themselves from oxidative-damaging factors via aggregation-disaggregation of SH-containing protein molecules.

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ХРУСТАЛИК ГЛАЗА: РЕДОКС ИЗМЕНЕНИЯ В ОТВЕТ НА ДЕЙСТВИЕ НЕИОНИЗИРУЮЩЕГО ЭЛЕКТРОМАГНИТНОГО ИЗЛУЧЕНИЯ

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Электромагнитное излучение (ЭМИ) микроволнового диапазона, даже нетепловой интенсивности, вызывает значительные биохимические и физиологические изменения в живых организмах, которые, как предполагается, связаны с их возможным окислительным действием. Данная работа посвящена изучению механизма реализации эффекта ЭМИ в хрусталике глаза на уровне элементов окислительно-восстановительного (редокс) состояния, исходя из того факта, что этот орган является наиболее подходящей моделью: он функционирует полуавтономно и обладает хорошо организованной системой антиоксидантной защиты. Прозрачность хрусталика поддерживается за счет сохранения редокс-баланса, в котором важную роль играет гомеостаз тиоловых соединений белковой и небелковой природы. Наши эксперименты были проведены на крысах с использованием ЭМИ частотой 460 МГц для воздействия при нетепловой интенсивности (плотность потока мощности 10 и 30 мкВт/см^2). Было показано, что хроническое воздействие ЭМИ в течение двух недель вызывает изменения в редокс состоянии хрусталика, которые проявляются в изменении уровня процессов перекисного окисления липидов и содержания тиолов различной природы. Субструктуры хрусталика (кортикальная и ядерная области) реагируют на воздействие ЭМИ по-разному. В зависимости от интенсивности ЭМИ в их реакциях проявляется про- и антиоксидантный характер. Динамика окислительной реакции субструктур хрусталика также различается при высокоинтенсивном и низкоинтенсивном воздействии. Характер кинетики изменений продуктов окислительных реакций (малонового диальдегида и гидроперекисей липидов) и восстановителей (небелковых и белковых SH-групп) в хрусталике облученного организма позволяет предположить роль ферментативной системы тиолирования-детиолирования в сохранении редокс-баланса в субструктурах хрусталика. Кроме того, полученные нами результаты по изменению (кинетике) содержания различных SH-групп белка, т.е. скрытых внутри белковой молекулы и находящихся на ее поверхности во время воздействия ЭМИ, а также данные, имеющиеся в литературе, позволяют нам выдвинуть гипотезу о супрамолекулярном механизме регуляции гомеостаза, в частности, регуляции

тиолового гомеостаза в таких высокобелковых структурах, как хрусталик, который может быть реализован путем агрегации-деагрегации белковых молекул (кристаллинов в случае хрусталика). Наши результаты могут служить основой для разработки нового неинвазивного подхода к профилактике катаракты, использующего низкоинтенсивное микроволновое излучение.

Ключевые слова: электромагнитное излучение, хрусталик глаза, тиолы, катаракта

GÖZ BÜLLURU: QEYRİ-İONLAŞDIRICI ELEKTROMAQNİT ŞÜALANMASININ TƏSİRİNƏ QARŞI REDOKS DƏYİŞİKLİKLƏR

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Mikrodalğa diapazonlu elektromaqnit şüalanması (EMŞ) hətta qeyri-istilik intensivliyində canlı orqanizmlərdə mümkün oksidləşdirici təsiri ilə bağlı olduğu ehtimal olunan əhəmiyyətli biokimyəvi və fizioloji dəyişikliklərə səbəb olur. Bu tədqiqat işi ən müvafiq model kimi göz büllurunda redoks vəziyyətinin elementləri səviyyəsində EMŞ effektinin reallaşması mexanizminin öyrənilməsinə həsr edilmişdir. Göz büllurunun adekvat model olması onun (yarım) avtonom şəraitdə fəaliyyət göstərməsi və çox yüksək səviyyədə təşkil olunmuş antioksidant müdafiə sisteminə malik olması ilə təsdiq olunur. Büllurun şəffaflığı zülal və qeyri-zülal təbiətli tiol birləşmələrinin homeostazının mühüm rol oynadığı redoks balansın saxlanması ilə təmin olunur. Təcrübələrimiz siçovullar üzərində qeyri-istilik intensivlik diapazonunda 460 MHz EMŞ-dan istifadə edərək aparılmışdır (güc axını sıxlığı - 10 və 30 mkVt/sm^2). İki həftə ərzində EMŞ-nın xroniki təsirinə məruz qalan siçovulların göz büllurunda redoks vəziyyətinin dəyişikliklərinə səbəb olduğu göstərilmişdir ki, bu da lipid peroksidləşməsi proseslərinin səviyyəsində və müxtəlif təbiətli tiolların miqdarında dəyişikliklərdə özünü göstərir. Büllurun substrukturlarında (kortikal və nüvə hissələri) EMŞ-nın təsirinə fərqli reaksiya özünü göstərir. EMŞ-nın intensivliyindən asılı olaraq, onların reaksiyalarında pro-və antioksidant xarakter üzə çıxır. Büllurun substrukturlarının oksidləşdirici reaksiyalarının dinamikası da yüksək intensivlikdə və aşağı intensivlikdə fərqlənir. Şüalanan orqanizmin büllurunda oksidləşdirici reaksiyaların məhullarının (malon dialdehidi və lipid hidroperoksidləri) və reduksiyaedici agentlərin (qeyri-zülal və zülal SH qrupları) dəyişikliklərin kinetikasının xarakteri büllurun substrukturlarının redoks-tarazlığın qorunmasında tiollaşdırıcı və detiollaşdırıcı enzimatik sisteminin rolunun olmasına işarə edir. Bundan əlavə, EMŞ-na məruz qalma zamanı müxtəlif zülal SH-qruplarının, yəni zülal molekulunun içərisində gizlənmiş və molekulun səthində yerləşən SH-qruplarının miqdarında dəyişikliklər (kinetikası) üzrə əldə etdiyimiz nəticələr, eləcə də ədəbiyyatda mövcud olan məlumatlar bizə homeostazın, o cümlədən tiol homeostazının supramolekulyar mexanizmi ilə tənzimlənməsi haqqında hipotez irəli sürməyə imkan verir. Bu hipotezə görə supramolekulyar tənzimlənmə büllur kimi yüksək zülal tərkibinə malik olan strukturlarda zülal molekullarının (büllurda kristallin zülallarının) aqreqasiya-deagregasiya yolu ilə həyata keçirilə bilər. Nəticələrimiz kataraktın profilaktikasına aşağı intensivlikli mikrodalğalı şüalanmadan istifadə edən yeni qeyri-invaziv yanaşmanın inkişafı üçün əsas ola bilər.

Açar sözlər: elektromaqnit şüalanması, göz bülluru, tiollar, katarakt

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