ABSTRACT

of the dissertation for the doctor of philosophy degree (PhD) specialty «6D060700-Biology»

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"Investigation of the role of oxidative stress in the mitochondrial metabolism changing"

General Characteristics of the Thesis. This work is devoted to the study of the role of oxidative stress in metabolic changes in mitochondria. Along with sulfide metabolism pathways and alteration of CoQ biosynthesis.

The Relevance of the Topic. All aerobic organisms need oxygen to function. Approximately 5% of the oxygen used by the tissues is converted into free radicals. The cell constantly produces active oxygen forms as free radicals, but due to the fact, that their level is low, the cell restrains them with the help of the antioxidant system. However, an increase in the intensity of oxidation of free radicals leads to the pathogenic influence of various factors. Subsequently, it leads to a biochemical disorder in the form of oxidative stress. Active oxygen forms can affect organs and tissues differently. Also, show different stability during the process of oxidative stress.

The increase in free radicals leads to a decrease in ATP levels and a lack of cellular energy. As a result, it causes several disturbances in the process of biosynthesis of the mitochondrial respiratory system. Along with this, mitochondrial dysfunction and the accumulation of mitochondrial mutations in tissues lead to the aging process, as well as to the pathogenesis of several diseases characterized by neurodegeneration. One of these processes is the hydrogen sulfide metabolic pathway and CoQ biosynthesis. Coenzyme Q10 is one of the elements of CoQ biosynthesis, which is part of the mitochondrial respiratory chain. Disruption of Q10 biosynthesis can be result in several mitochondrial diseases. Mitochondrial diseases are a complex of heterogeneous group of pathological situations and hereditary diseases that appeared after disturbances in the activity and structure of respiration of mitochondria and tissue. The well-known disease of Leigh's syndrome can also be noted. During periods of coenzyme Q10 deficiency, hydrogen sulfide metabolism plays a key role. Sulfide exchange between mammalian cells includes transsulfuration (biosynthetic) and pathways (catabolic) of hydrogen sulfide (H_2S) oxidation. Disruption of H_2S oxidation leads to a lack of coenzyme Q and therefore oxidative stress or, in the case of a lack of coenzyme Q, in particular pathogenesis, may play a synergistic role with oxidative stress.

Since oxidative stress is the primary cause or one of the main links in the pathogenesis of most diseases, a special place in the antioxidant system of the organism belongs to glutathione. Glutathione is a component of the defense system that neutralizes the negative changes that appear during the activity of cellular oxygen. Glutathione plays an important role in cell life. By modulating the level of

mitochondrial active oxygen, glutathione can influence the process of cell death. A decrease in the level of glutathione in mitochondria or its disappearance leads to an increase in the level of active oxygen and nitrogen forms, as well as to dysfunction of this organelle, which consequently causes death of the cell and a change in the process of apoptosis into necrosis.

The influence of such processes on mitochondrial metabolism result in a number of diseases and disadvantages. Currently, for the purpose of prevention, many preclinical therapeutic pathways are used. One of them can affect positively on a certain organ, while negatively on the other. The dysfunction of mitochondrial metabolism that appears after oxidative stress will entail the emergence of various mitochondrial diseases and the lack of a cure for this disease is alarming the whole world.

Research Purpose and Tasks. The purpose of this research was to set dietary restrictions on the level of nitrogenous amino acids in the ration of mice groups with a lack of CoQ. To determine the change in the sulfide mechanism pathway after manipulation with N-acetyl-L-cysteine, as well as assessment of glutathione system increase in groups of mice with a Complex I deficiency.

The following **tasks** were set to reach the purpose:

1. Determination of the percentage of vital signs after setting dietary restrictions on the level of nitrogenous amino acids in the ration of mice groups lacking CoQ or after treatment with N-acetyl-L-cysteine.

2. Study of changes in the initial enzyme of the metabolic pathway of hydrogen sulfide - sulfide quinone oxidoreductase (SQOR) and the level of cystathionine γ-lyase (CSE) and cystathionine-β-synthase (CBS) in mitochondrial fractions of the brain, kidneys and muscle tissues after setting dietary restrictions on the level of nitrogenous amino acids in the ration of groups of mice lacking CoQ or after treatment with N-acetyl-L-cysteine.

3. Determination of changes in total glutathione, including the level of glutathione dependent enzymes (GPx and GRd) in the mitochondrial fractions of the brain, kidneys and muscle tissues after setting dietary restrictions on the level of nitrogenous amino acids in the ration of mice groups lacking CoQ or after treatment with N-acetyl-L -cysteine.

4. Comparison of differences in the levels of CoQ9, CoQ10, DMQ9 and in the ratio of DMQ9 / CoQ9 after treatment of brain, kidney and muscle tissues with SAAR and NAC.

5. Determination of the general glutathione system characteristics, including the level of glutathione dependent enzymes (GPx and GRd) and assessment of the percentage of vital activity in groups of mice with a lack of Complex I.

Research Object. Mouse models with CoQ deficiency and complex I were used as research material.

Research Methods. The methods included experimental sampling of animals, Western blot analysis, high performance liquid chromatography with reversed phase, native electrophoresis method, fluorescence spectroscopy (*Bio-Tek Instruments Inc., Winooski, VT, USA*), spectrophotometry (*NanoDrop*), and statistical analysis methods (*GraphPad Prism*).

Scientific Novelty of the Research. For the first time, an in vivo research work was carried out with the aim of preclinical therapy in a mouse model with a CoQ deficiency by sequentially setting dietary restrictions on the level of nitrogenous amino acids in the ration of mice groups with the lack of CoQ or after manipulation with N-acetyl-L-cysteine. It turns out that the lack of CoQ in some cases can affect differently on the mouse tissue. Studies were conducted with therapeutic purposes based on the data taken after research. Their advantageous volume and time of use were determined.

In vivo, an increase in the initial enzyme as sulfide quinone oxidoreductase (SQOR) of the metabolic pathway of hydrogen sulfide was observed in muscle tissues after setting dietary restrictions on the level of nitrogenous amino acids in the ration or after treatment with N-acetyl-L-cysteine, and in kidney tissues increased only after treatment N-acetyl-L-cysteine.

For the first time, an increase in SQOR and total glutathione levels, as well as an increase in the GSSG/GSH ratio, were detected in mouse models with a lack of Complex I in brain tissues compared to experimental $Ndufs4^{+/+}$ groups of mice.

Theoretical Significance of the Research. The results of the dissertation work, particularly the primary determination of the effect of dietary restrictions on the level of nitrogenous amino acids (SAAR) in the diet of groups of mice with the lack of CoQ and dietary restrictions by N-acetyl-L-cysteine (NAC) on the tissues of the brain, kidneys and muscles were made. While experiments carried out in groups of mice with a lack of complex I showed an increase in the glutathione system. The research data taken provide an independent opportunity to conduct an examination of the manipulations used and complement the basis of the theoretical search in this problem.

Practical Relevance of the Research. Based on the results, pathways were developed to treat CoQ-deficient mice and to prevent complex I deficiencies in groups of mice. The made dietary restrictions on the level of nitrogenous amino acids (SAAR) in the diet and manipulation with N-acetyl-L-cysteine in the study, make it possible to justify a new therapy with an additional and independent form of treatment for groups of mice with $Coq9^{R239X}$.

Main statements to be defended:

1. The percentages of vital activity of the CoQ-deficient groups of mice were determined after treatment with dietary restriction.

2. Increases have been shown in the level of SQOR in the brain tissues of experimental $Coq9^{R239X}$ mice after dietary treatments in the level of sulfuric amino acids, as well as in renal tissues after treatment with N-acetyl-L-cysteine and in muscle tissues after treatment with SAAR and NAC.

3. Various effects of levels of cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS) on brain, kidney and muscle tissue were indicated in experimental groups of mice treated with SAAR and NAC.

4. Changes in total glutathione were determined, including the level of glutathione dependent enzymes (GPx and GRd) in the mitochondrial fractions of the brain, kidneys and muscle tissues after setting dietary restrictions on the level of

nitrogenous amino acids in the ration of groups of mice or after manipulation by N-acetyl-L-cysteine.

5. Various effects were indicated after SAAR and NAC treatment of brain, kidney and muscle tissues at the levels of CoQ9, CoQ10, DMQ9, as well as the difference in the ratio of DMQ9 / CoQ9 and mitochondrial respiratory complex I, II, III, IV.

6. The features of the general glutathione system, including the level of enzymes, were determined, and the percentage indicators of vital activity have been assessed in groups of mice with a lack of Complex I.

The author's personal contribution to summarizing the results of the research recommended for defence. The review of the literature sources of the research work, the definition of goals and objectives, as well as statistical analysis and processing of the results of practical work and data collection were carried out with the personal participation of the author.

Connection to the Research Program. This work has the aim at studying changes in oxidative stress in mitochondrial metabolism. The work was carried out at the laboratory project of molecular biology in the central laboratory of Biomedicine of the University of Granada (the head of the scientific project is Professor of the University of Granada L.K. Lopez). Part of the work was carried out in the laboratory of the Department of Biophysics, Biomedicine and Neuroscience at the Al-Farabi Kazakh National University.

Approbation of the Research.

The main principles of the dissertation and the results of the research were presented and discussed at the following international and national scientific conferences:

- International Conference of Students and Young Scientists International Farabi Readings "Farabi Alemi" (2019, Almaty);
- International Scientific Conference "I CONGRESS OF RESEARCHERS FROM THE PTS" (2019, Granada, Spain);
- International Conference of students and young scientists International Farabi readings "Farabi Alemi" (2020, Almaty).
- International Conference of students and young scientists International Farabi readings "Farabi Alemi" (2022, Almaty).

Publications. The main results of the dissertation work have been published in 9 scientific publications, including 1 article in journals based on Web of Science and Scopus, 3 articles in scientific publications recommended by the Committee for Control in the Field of Education and Science of the Republic of Kazakhstan, 5 theses in the materials of international and republican conferences.

Thesis Structure. The dissertation consists of 92 pages and normative references, designations and abbreviations, an introduction, a literary review and materials and methods, research results and conclusions, 211 sources used and 38 figures.