The role of mitochondrial bioenergy and system glutathione in deficiency of Coenzyme Q and Complex I

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Abstract. Cytokine Coenzyme Q10 (CoQ10) is an insoluble coenzyme that is a component of the electron transport chain and is involved in oxidative phosphorylation in mitochondria. By a decrease in CoQ_{10}, the energy requirements of the cell are not fully covered: cell division slows down, and the processes of tissue renewal and regeneration are disrupted. In our work, we used a model of mice CoQ_9 Knockin (R239X). In homozygous mutant mice, a strong decrease in CoQ_7 protein and the accumulation of dimethoxyubiquinone are observed. An impairment of hydrogen sulfide metabolism was previously identified as a significant pathomechanism of primary Coenzyme Q deficiency. The experimental results showed that the disruption of sulfide and glutathione metabolism in Coq9R239X mice is directly dependent on CoQ levels. Therefore, changes in dietary sulfur-containing amino acids do not alter sulfide metabolism and the glutathione system in $Coq9^{R239X}$ mice. As our experiments have shown, an increase in total glutathione is observed only in symptomatic brain tissue. It can be assumed that this is due to oxidative stress in the symptomatic brain tissue, which is often found with mitochondrial dysfunction.

1 Introduction

The biosynthesis of coenzyme Q (CoQ) is a complex process that occurs in the inner membrane of mitochondria and is present in all mammalian tissues [1]. Coenzyme Q is a redox lipid that has a major role in electron transport and oxidative phosphorylation [2, 3]. Coenzyme Q deficiency causes mitochondrial disease with heterogeneous clinical manifestations [4, 5]. It was found that a violation of sulfide metabolism can be identified as a significant pathomechanism of primary Coenzyme Q deficiency [6]. CoQ deficiency leads to a decrease in enzyme levels of the sulfidequinone oxidoreductase (SQOR), which causes damage in the mitochondria of sulfide oxidation [7]. Sulfidequinone oxidoreductase oxidizes H2S and forms persulfide, which is bound to protein. As a result, electrons are transferred through flavin adenine dinucleotide to CoQ, then to the electron transport chain (ETC) [8, 9].

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Since mitochondria are both the main producers and targets of reactive oxygen species (ROS), it is believed that among the many antioxidants that protect mitochondria from ROS is glutathione (GSH) [10]. Glutathione protects mitochondria from free radicals and regulates oxidative processes inside the cell [11]. It has been shown that GSH deficiency occurs before neuropathological disorders, and GSH depletion can lead to impaired mitochondrial function [10,12]. It is known that dietary restriction of sulfur-containing amino acids (SAAR) or the addition of N-acetyl-L-cysteine (NAC) can affect sulfide metabolism, both strategies have shown therapeutic benefits in various conditions [12].

Preclinical studies have shown that diets limited to sulfur-containing amino acids have many beneficial effects, including increasing life expectancy and preventing the development of various diseases. The restriction of sulfur-containing amino acids, methionine, and cysteine is a common dietary restriction for numerous regimes. Therefore, modulation of the presence of sulfur-containing amino acids in the diet can change the course of the disease. In this regard, we conducted real studies of the effect of sulfide metabolism and the glutathione system with CoQ deficiency and mitochondrial complex I deficiency in *Coq9*^{R239X} mice models.

2 Materials and Methods

Two types of homozygous mice *Coq9*^{R239X} and *Ndufs4*$^{-/-}$ were used. In collaboration with the Ingenious Targeting Laboratory (USA), the Coq9R239X mouse model was previously created by combining the genetic background of C57BL/6N and C57BL/6J mice and was identified at the University of Granada, Laboratory of Molecular Biology. *Ndufs4*$^{-/-}$ mice were previously obtained by removing the exon of the 2nd gene of *Ndufs4* mice. Mice were studied for 1 month. All experiments were performed according to a protocol approved by the Ethical Committee for the Care and Use of Animals of the University of Granada.

CoQ_9R239X/+ mice (n =3-4for each group) were crossed to obtain CoQ_9+/+, CoQ_9R239X/+, CoQ_9R239X/R239X (CoQ_9R239X). CoQ_9+/+ mice were used as a control group. The first experimental group - homozygous mice *Coq9*^{R239X}. In the second experimental group (*Coq9*^{R239X}+SAAR), the restriction of sulfur-containing amino acids at the rate of 1-3 g/kg of body weight per day was used in the diet of *Coq9*^{R239X} mice. In the third test group *(Coq9*^{R239X}+NAC), N-acetyl-L-cysteine (NAC) was added to water 2 g/200 ml in the diet of *Coq9*^{R239X} mice. To study the deficit of mitochondrial complex I, we used a month-old, *Ndufs4*$^{+/+}$ as a control group of the mice, and *Ndufs4*$^{-/-}$ as an experimental group (n =3-4 for each group). Experiments on mice were carried out at the age of 1 to 2 months. The study was performed on kidney and muscle homogenates. The work was carried out using the Western blotting method, and the fluorometric method for determining glutathione. All *statistical analyzes* were performed using scientific software Prism 6. One-way ANOVA with Tukey's post hoc test was used to compare differences between the three experimental groups.

3 Results and Discussion

It is known that sulfide metabolism contributes to oxidative stress during CoQ_{10} deficiency through a change in the glutathione system. The main non-protein thiol in cells is GSH, which is synthesized in the cytosol and imported into mitochondria and other organelles, where it plays an important role in antioxidant protection against reactive oxygen species [13].

Figure 1 (A-D) shows the data for determining the level of glutathione and its enzymes in the brain tissue. As can be seen from Figure 1 A, the GSH indices in the brain tissues of the experimental groups of mice *Coq9*^{R239X}, *Coq9*^{R239X}+SAAR, *Coq9*^{R239X}+NAC are higher

compared to the control group *Coq9*$^{+/+}$. It should be noted that in the *Coq9*^{R239X}+NAC experimental group, the GSH indices significantly exceed the GSH indices of other experimental groups.

When comparing GSSG indices in the brain tissues of experimental groups of mice *Coq9*^{R239X}, *Coq9*^{R239X}+SAAR, and *Coq9*^{R239X}+NAC with the control group *Coq9*$^{+/+}$, only a slight decrease trend was shown, there was no significant change (Fig. 1 B). The ratio of GSSG and GSH indices of the *Coq9*^{R239X}, *Coq9*^{R239X}+SAAR, and *Coq9*^{R239X}+NAC experimental groups is lower compared to the *Coq9*$^{+/+}$ control group (Fig. 1C).

As can be seen from Figure 1 D, the total level of GSH in brain tissues in all experimental groups is significantly higher compared to the control group.

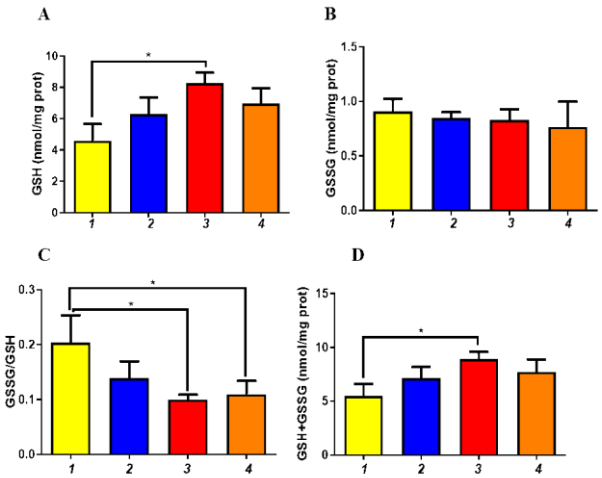

Fig. 1. Measurement of total GSH levels in brain tissue of mice *Coq9*$^{+/+}$ (1-control) and *Coq9*^{R239X} (2-experimental), *Coq9*^{R239X}+NAC (3-experimental mice + addition of N-acetyl-L-cysteine) and *Coq9*^{R239X}+SAAR (4-experimental mice + amino acid content in the diet with sulfur). The GSH indices (A), the GSSG indices (B), the GSSG/GSH ratio (C), Total glutathione (D), *P<0.05; (ANOVA), n = 5-6 for each group.

Figure 2 presents the results of experiments to determine the content of proteins GPx4 and GRd in kidney tissues. The GPx4 and GRd values in the tissues of the experimental groups *Coq9*^{R239X}, *Coq9*^{R239X} + SAAR, *Coq9*^{R239X} + NAC are lower compared to the control group.

The next mouse models obtained during the study were complex I-deficient Ndufs4-/- experimental group and the Ndufs4+/+ control group. In the experiment, the mean percentage of survival of these mouse models, glutathione system, GPx4 and GRd protein activity were studied in brain, kidney, and muscle tissues.

The protein level of GPx4 in the brain tissues of the Ndufs4+/+ control group was reduced by 5% compared to the Ndufs4-/- experimental group. GRd protein activity was increased by 10% in the Ndufs4-/- experimental group compared to the Ndufs4+/+ control group. The protein level of GPx4 in the kidney tissues obtained during the experiment was increased by 12% in the Ndufs4+/+ control group compared to the experimental group. And GRd protein activity increased by 11% in Ndufs4-/- experimental group compared to the Ndufs4+/+ control group.

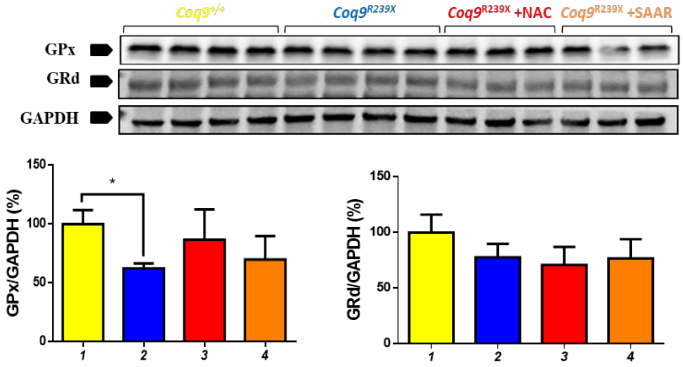

Fig. 2. Western blot analysis of GPx and GRd proteins in the kidney tissues of mice Coq9+/+ (1-control) and Coq9R239X (2-experimental), Coq9R239X+NAC (3-experimental mice + addition of N-acetyl-L-cysteine) and Coq9R239X+SAAR (4-experimental mice + amino acid content in the diet with sulfur). GAPDH was used as a load control. The specific lane for each target protein is indicated by an arrow. *P<0.05; (ANOVA), n =3-4 for each group.

As can be seen from Figure 3, the performance of the GPx4 and GRd proteins in the skeletal muscle of mice from the experimental Ndufs4-/- group is higher compared to the Ndufs4+/+ control group.

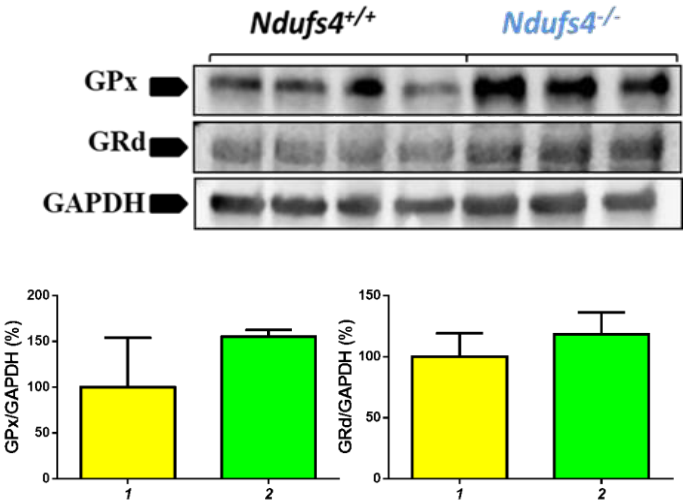

Fig. 3. Western blot analysis of GPx4 and GRd protein in the skeletal muscle of *Ndufs4*$^{+/+}$ and *Ndufs4*$^{-/-}$ mice. GAPDH was used as a load control. The specific lane for each target protein is indicated by an arrow. Data are expressed as mean ± standard deviation. *Ndufs4*$^{-/-}$ mice (2-experimental) versus *Ndufs4*$^{+/+}$ mice (1-control) (t-test; n =3-4 for each group).

The protein level of GPx4 in muscle tissues decreased by 51% in the control group compared to the experimental group. GRd protein activity was increased by 18% in the Ndufs4-/- experimental group compared to Ndufs4+/+ control group. During the activity of antioxidant enzymes glutathione peroxidase and glutathione reductase, a number of changes in brain, kidney, and muscle tissues were revealed. In the brain tissues of Ndufs4-/- experimental mice groups, compared to Ndufs4+/+ control mice groups, increased SQOR level, decreased cystathionine-β-synthase level, increased total glutathione level and GSSG/GSH ratio were found, and no significant difference was observed in the level of

glutathione enzymes. On a global scale, these data confirm that sulfide metabolism affects glutathione levels independently of sulfur amino acid availability. As our experiments have shown, an increase in total glutathione is observed only in symptomatic brain tissue. The main share of this increase was in the oxidized form of glutathione (GSSG), therefore, it can be assumed that this is due to oxidative stress in the symptomatic brain tissue, which is often found with mitochondrial dysfunction. This may explain the increase in life expectancy in preclinical models of Complex I deficiency treated with NAC and vitamin E [13].

4 Conclusion

Tasks were fully fulfilled according to the goals set in the work. Mitochondrial dysfunctions resulting from increased or decreased oxidative stress during mitochondrial metabolism were tested in vivo in CoQ-deficient and Complex I-deficient mouse groups. In the course of work, we found out that a positive effect was observed with the use of treatments used for therapeutic purposes: food containing limited sulfur amino acids and N-acetyl-L-cysteine. Another mitochondrial disorder other than CoQ deficiency is complex I. In mouse models of Lee's syndrome, due to deficiency of complex I (Ndufs4-/-mouse line), an increase in CoQ level and, as a result, an increase in SQOR level were found. It can be assumed that an increase in SQOR will lead to a change in the glutathione system.

The experimental results showed that the disruption of sulfide and glutathione metabolism in Coq9R239X mice is directly dependent on CoQ levels. Therefore, changes in the content of sulfur-containing amino acids in the diet do not lead to changes in the sulfide metabolism and glutathione system in Coq9R239X mice. Dietary restriction of sulfur amino acids in diets of groups of CoQ-deficient mice and treatment with N-acetyl-L-cysteine had a positive effect and increased survival percentages.

As a result of increased oxidative stress in mitochondrial metabolism, various mitochondrial dysfunctions, including CoQ and complex I deficiency, have been found to cause a several of diseases. Thus, it can be concluded that sulfide metabolism is involved in the pathomechanisms of mitochondrial diseases, but its specific modulation may be different depending on the molecular defect.

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