EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 6
05-08
Year: 2017

AGE-RELATED ALTERATIONS OF MITOCHONDRIAL FUNCTIONAL ACTIVITY OF RAT LIVER AND THE INFLUENCE OF HERBAL PREPARATIONS ON THEM

S.N. Dolimova, Sh.N. Kuziev*, G.M. Mukhamedzhanova and G.B. Umarova

National University of Uzbekistan named after M.Ulugbek, Biology Faculty, Tashkent, Uzbekistan.

***Corresponding Author: Sh.N. Kuziev**
National University of Uzbekistan named after M.Ulugbek, Biology Faculty, Tashkent, Uzbekistan.

Article Received on 21/03/2017 **Article Revised on 11/04/2017** **Article Accepted on 01/05/2017**

ABSTRACT
It has been studied influence of herbal preparations "Megaferon" and MASGK on respiration processes and oxidative phosphorylation of liver mitochondria in rats of different ages and on the model of "accelerated aging". It has been established that with the age of the animal it is observed uncoupling of the processes of respiration and phosphorylation of liver mitochondria, as well as inhibition of electron transfer in the respiratory chain. Similar data were obtained on the model of "accelerated aging" caused by irradiation of rats. Preparations created on the basis of natural compounds have a beneficial effect on the studied parameters and helped to restore the conjugation of mitochondria of "old" and irradiated rats.

KEYWORDS: liver mitochondria, ROS, quercetin, rutin, energetic metabolism, oxidative phosphorylation, mtDNA damage.

1. INTRODUCTION

Metabolic direction has been intensively developing in medicine during the last twenty years, aiming theoretical and applied analysis of metabolic processes at various levels as the basis or background for many diseases. Particularly, representations about the role of violations of cellular energy metabolism are actively forming during the most diverse pathological processes. Energy metabolism, both at the level of the entire organism and at the level of an individual cell is a complex of processes that are organized in the most complicated manner in space and time and provide virtually all aspects of vital activity of living matter.

The key parts of this complex are mitochondria - structures that are present in the cytoplasm of all cells, and which carry out the vital functions for each cell. With considering of the foregoing, it is clear that violations of cellular energetic metabolism, which are primarily based on mitochondrial insufficiency, lead to a wide range of clinical symptoms.[1,2]

Based on data of the roles of genetic, structural and functional disorders of mitochondria in the development of diseases, by the end of the XXth century, the concept of a new class of so-called "mitochondrial" diseases was formulated. Originally, this class of diseases included disorders of the nervous system, sensory organs, skeletal muscles and cardiovascular system. However, in the subsequent information on the role of mitochondrial dysfunction in the development of gastroenterological disorders, these diseases were also attributed to the class of "mitochondrial".[3]

According to the mitochondrial hypothesis of aging, mitochondrial efficiency decreases with age, which leads to an increase in the concentration of reactive oxygen species – *ROS*. It is established that mitochondrial dysfunction is one of the earliest signs of many neurodegenerative diseases. Thereby, mitochondria are a promising target for the search for various geroprotective agents.

Mitochondrial hypothesis of aging is a special case of a free radical theory. Mitochondria have their own instrument for repairing of DNA damages by exogenous and endogenous agents, in which free radicals, toxins, and drugs are most often used. Great importance in the mtDNA damage is the proximity to the electron transport chain and the lack of histones that protect DNA.[4] Oxidative damage of DNA causes a change in the bases, the appearance of AP-sites and other types of damages.

In this connection, a constant search is being conducted for compounds that have not only antioxidant activity, but also the ability to stimulate the functional state of mitochondria.

The goal of this research was to study the effect of flavonoid quercetin (Q) and its supramolecular complex with glycyrrhizin acid (GA + Q) on oxidative phosphorylation and respiration of liver mitochondria of

intact rats of various ages and rats on the model of "accelerated aging".

2. MATERIAL AND METHODS

White outbred rats of age 3, 6, 9, 12, 15 months were used in the research. Such strong antioxidants as quercetin (Q) and rutin (R) were used in experiments.[5] All animals were divided into 13 groups. The first group is "control" (3 months old), the second group is "adults", corresponding to the age of 6 months, the third group is "adult" rats receiving Q, the fourth is "adult" rats receiving R, the fifth group is "elderly", corresponding to the age of 12 months, the sixth and seventh groups – "elderly" rats, who received Q and R, respectively, the eighth – "old", corresponding to the age of 15 months, the ninth and tenth group -"old" rats, receiving respectively Q and R.

A model of "accelerated aging", 6-month-old rats were subjected to a single X-ray irradiation at a dose of 2 Gray on the RUM-17 unit (dose rate 0.87 Gray/min, at 15 mA, 180 kV). The eleventh group consisted of irradiated rats, and the twelfth and thirteenth groups included irradiated animals that received respectively Q and R. Q and R preparations were administered to experimental rats intraperitoneally for 7 days at doses of 30 and 0.35 mg/1000 g of the body weight of the animal, respectively.

Mitochondria were isolated by the generally accepted method of differential centrifugation.[6] The rate of respiration and parameters of oxidative phosphorylation were determined by polarographic method with the help of a platinum rotating electrode under standard conditions in a 1 ml cell at 25°C.[7] As substrates of respiration 5mM malate and glutamate were used. The functional state of strongly coupled mitochondria was determined by Chance.

3. RESULTS AND DISCUSSION

Results of research of respiration and parameters of oxidative phosphorylation of rat liver mitochondria of various ages are presented in Table 1. It was detected that in rats with increasing of age serious disturbances were observed in the process of mitochondrial synthesis of ATP correlating with the degree of "aging" of the animal. Thus, in animals of "adult rats" group, oxygen consumption rate in the third (synthesizing) metabolic state was reduced to 15% compared to the control, in the "elderly" group this value was already reduced to 30%, and in the "old" group this value was only 50% that is, it was reduced 2 times. Values of V4 metabolic state were increased with respect to control to 25 and 14% in the "adult" and "elderly" groups, and in "old" group this value was more than 3 times bigger control value. As a result of these alterations, there was a significant decrease of respiratory control rate (RC, demonstrating intact mitochondria), which argues the uncoupling of oxidative phosphorylation. In the group of "old" rats, the RC value could not be calculated due to the lack of response to the addition of ADP, indicating complete uncoupling of the respiratory chain. This is confirmed by a decrease in the rate of oxygen consumption by a mitochondrial suspension in the presence of a 2,4-dinitrophenol uncoupler, DNP (V_{DNP}) to 30 and 50% compared to the control in the "elderly" and "old" rats, which supposes the electron transfer rate along respiratory chain of mitochondria. In the "adults" group, V_{DNP} was closer to the control one (average 86%), which may indicate a lesser degree of uncoupling. Coefficients of ADP/O and ADP/Δt, reflecting the efficiency and phosphorylation rate (ATP synthesis), were also significantly reduced compared to the control values. Effectiveness of phosphorylation was more reduced in the "adults" group (on 20-23% compared to control) and speed was the lowest in the "elderly" group - 30% less than the control values. In the group of "old" rats these indicators could not be calculated for the same reason as the RC. Another indicator characterizing the state of the mitochondrial respiratory chain is the ratio of V_{DNP}/V4, giving anotation of the degree of energy of these organelles. The most energized were mitochondria of "adult" group, where V_{DNP}/V4 parameter was 70% of the control values, in the "elderly" group this figure was less than the control one by 40%. It should be noted that, in spite of considerable dissociation of oxidative phosphorylation in the "old" group, it was not 100%, as there was an insignificant stimulation of respiration in the presence of a DNF uncoupler, which is confirmed by the value of V_{DNP}/V4 ratio, which is 20% of the control.

Thus, in all investigated age-specific groups, it was observed uncoupling the processes of respiration and phosphorylation of liver mitochondria, as well as inhibition of electron transport. This can be explained by the uncoupling of the respiratory chain by products of lipid peroxidation, the accumulation of which was established by us in previous experiments.[8] It can be suggested that the more dramatic manifestations of oxidation products accumulation in the "old" rat model are the result of both direct oxidation of membrane lipids and the violation of the functioning of respiratory chain complexes (I and III), which in the pathological states themselves become sources of free radicals, thereby further aggravating the accumulation of peroxidation products.

In the next series of investigations, we studied respiration parameters and mitochondrial oxidative phosphorylation of model rats with "accelerated aging".

It is known that the X-ray irradiation of animals in doses of 2-2.5 Gray causes stable alterations in chromatin composition and protein synthesis in liver cells, in directional form, recalling of changes occurring during accelerated aging of animals.[9] Our data presented in table 2 indicate that in irradiated 6-month-old rats, in liver mitochondria, violations of oxidative phosphorylation processes are similar to those occurring in 15-month "old" rats.

From the data given in table 2 it follows that at raying of the rats at a dose of 2 Gray, the changes in the functional state of mitochondria characteristic for spontaneous aging are observed. Under the influence of ionizing radiation at model rats with "accelerated aging", it occurs the uncoupling of the processes of respiration and phosphorylation in the liver mitochondria, reduction of the rate of electron transport through the respiratory chain, which leads to a violation of the energy-transforming function of mitochondria. In turn, inadequate production of ATP initiates disorganization of all energy-consuming biochemical processes in liver cells and metabolic violations in this important organ of detoxification and in the body as a whole as a consequence.[10]

Q and P were used as corrective agents and it was found that both compounds render a positive effect on the state of oxidative phosphorylation of rat liver mitochondria. Effectiveness of the drug depended on the rats' age. Thus, in the "adults" rats, R was the most effective - its use led to the normalization of the functioning of the respiratory chain components and the entire oxidative phosphorylation process (Table 1).

Table 1: Influence of supramolecular complexes on respiration and oxidative phosphorylation state of rats' liver mitochondria of different ages (n=7; M±m)

Experiment conditions	Oxygen consumption rate, ngO_2/min·mg protein			RC	ADP/O	ADP/Δt	V_{DNP}/V_4
	V_3	V_4	V_{DNP}				
Young,3 m old	98,95±2,6	15,97±1,8	99,14±5,4	6,20±0,54	2,74±0,07	133,33±1,5	6,14±1,24
Adults,6 m old	85,38±1,7	20,28±1,6	87,20±4,6	4,21±0,45	2,13±0,02	111,80±2,0	4,30±±0,61
Adults+Q	95,27±2,3	19,31±0,7	96,00±6,2	4,93±0,31	2,34±0,04	117,65±1,7	4,97±0,52
Adults+R	97,42±3,4	19,00±1,2	97,84±5,3	5,13±0,53	2,40±0,08	125,00±1,5	5,15±0,64
elderly, 12 m	71,64±2,8	18,28±1,1	69,02±2,7	3,92±0,41	2,33±0,03	93,02±1,1	3,78±0,39
elderly+Q	89,40±3,1	17,60±0,7	91,00±1,5	5,08±0,39	2,61±0,02	111,80±1,2	5,17±0,30
elderly+R	78,80±3,2	17,40±1,3	80,80±3,1	4,53±0,56	2,47±0,04	105,26±2,3	4,64±0,57
elderly, 15 months	51,45±2,9	51,45±2,9	52,00±1,2	-	-	-	1,01±0,09
elderly+Q	72,17±1,7	22,73±2,1	76,16±2,4	3,17±0,4	1,87±0,02	90,90±1,4	3,35±0,46
elderly+R	60,70±2,3	31,15±1,5	62,80±3,3	1,95±0,17	1,50±0,02	87,72±1,3	2,02±0,21

Table 2: Influence of supramolecular complexes on respiration and oxidative phosphorylation of rat liver mitochondria with the model of "accelerated" aging(in% to control, n=7; M±m)

Experimental conditions	Oxygen consumption rate			RC	ADP/O	ADP/Δt	V_{DNP}/V_4
	V_3	V_4	V_{DNP}				
Control, 6 months	100	100	100	100	100	100	100
Radiation 6 months	60,1	322.17	52.45	-	-	-	16,45
Radiated 6 m+Q	72.94	142.33	76.82	50.64	68.25	68.18	54.56
Radiated 6 m+R	61.34	195.05	63.34	31.45	54.74	65.80	32.90

Oxygen consumption rates in 3 (V3) and uncoupled (V_{DNP}) did not virtually differ from the corresponding values in the control group (on the average 98.5% of the control). RC, reflecting the mitochondrial integrity was 17% less than the control, but 15% more than in the untreated group. Effectiveness and rate of phosphorylation was also closer to the control values and were 88% and 94%, respectively, from the control level.

Influence of Q in this group was less effective, V3 and V_{DNP} were 96-97% from control, V4 exceeded control value to 20%, which affected in decrease of RC coefficient (on average 80% of control values), efficiency and rate of phosphorylation, and as well as the degree of energy was reduced to 10-15% in regard to control.

However, in the other two age-specific groups, Q was more effective. It is possible that at an older age, not only the lipid but also the protein part of the membrane structures are exposed to damaging effects, while Q interacts favorably with enzymes and also acts as a powerful interferon inducer prevents the action of pathogenic factors.

So within the "elderly" group of rats Q with great efficiency repaired the functions of oxidative phosphorylation, bringing nearer almost all the indicators to the values received on young rats. Oxygen consumption rate in the third and separated metabolic states was less than 10% different from the values of 3-month-old animals; in the untreated group, these values were less than in the young ones on average by 30%, and application of R insignificantly increased the rate of oxygen consumption in this condition. Activation of respiration in metabolic state 4 as before leads to a descent in the intactness of mitochondria-RC coefficient. However, in the case of Q this index was less than in "young" by 20%, and in animals that received rutin-30% (in the group of untreated animals this figure was less than in the "young" by 40%). Effectiveness and rate of oxidation in animals that received Q were also closer to

those of "young" rats than in the case of R. It should be noted that in animals received rutin, a degree of energized mitochondria (V_{DNP} / V4) was less than in untreated animals.

In the groupof "old" animalsboth compounds had a salutary influence on the oxidative phosphorylation state, recovering its conjugation, but in this case the most effective of the two drugs was Q. In the group of untreated animals mitochondria were almost completely separated and did not react to the addition of ADP or 2,4-DNP. In animals received Q, the degree of intactness and energy of mitochondria was 50% of the control values, and in the group received R these values did not exceed 30%. Effectiveness and rate of phosphorylation in the case of Q was averaged 70% of the control values, and in the case of R 55% and 65%, respectively.

An analogous situation was observed in the model of "accelerated aging" (Table 2). Both investigated drugs were effective and contributed to a significant restoration of disturbed processes in mitochondria.

Oxidative stress, violations of ion homeostasis and energetic metabolism play an important role in ontogenesis of aging process and pathogenesis of "elderly diseases". All these processes are closely related to mitochondria, which on the one hand are the main source of ROS pro-oxidants in the cell, and on the other, are most sensitive to free radical oxidation due to a lack of protective histones and DNA-repairing systems in the mitochondrial genome.[11]

4. CONCLUSION

Summarizing the abovementioned facts, it can be said that the derivatives of gossypol and glycyrrhizic acid have a protective effect on mitochondria, preventing the detrimental effect of one of the aging factors-peroxidation products.

REFERENCES

1. Harman D. Free-radical theory of aging.Increasing the functional life span // Ann. N. Y. Acad. Sci., 1994; 717: 1-15.
2. Trifunovic A, Larsson NG. Mitochondrial dysfunction as a cause of ageing. // J Intern Med., 2008; 263: 2.- P. 167-178.
3. Raju R, Jian B, Hubbard W, Chaudry I. The Mitoscriptome in Aging and Disease // Aging Dis., 2011; 2(2): 174-180.
4. Polyakov V.YU., Sukhomlinova M.YU., Fays D. Kak slivayutsya, fragmentiruyutsya i delyatsya mitokhondrii//Biokhimiya. 2003; T. 68. Vyp. 8. S. 1026-1039.
5. Cusimano EM, Knight AR, Slusser JG, Clancy RL, Pierce JD. Mitochondria: the hemi of the cell. // Adv Emerg Nurs J.-2009; 31(1): 54-62.
6. Schneider W. Isolation of mitochondrial from rat liver//J. Biol. Chem., 1948; 176(1): 250-254.
7. Praktikum po biokhimii/pod red. Severina S.Ye. i Solov'yevoy G.A.- M.: Izd-vo MGU, 1989; 403-487.
8. Dolimova S.N., Almetova F.E., Umarova G.B., Turayeva D.T.- Antioksidantnyyesvoystvanekotorykhproizvodnykhg ossipola//Vestnik NUUz.-2011; №2.-S.18-21.
9. Mozzhukhina T.G., Litoshenko A.YA. Sposob modelirovaniya priskorennogo stareniya (№9703849). Resheniye pro vidachu patent vyd. 10.09.98.
10. Krestinina O.V., Kruglov A.G., Grachev D.E., Baburina Y.L., Evtodienko Y.V., Moshkov D.A., Santalova I.M., Azarashvili T.S. Age-dependent changes of mitochondrial functions in Ca2+-induced opening of permeability transitionpore//Biochemistry (Moscow) Supplement. Series A: Membrane and Cell Biology.-2010.
11. Reddy PH, Reddy TP. Mitochondria as a Therapeutic Target for Aging and Neurodegenerative Diseases. // Alzheimer Res.-2011; 6: 231-236.