

THE DYNAMICS OF CHANGES IN THE KINETICS OF LIPID PEROXIDATION IN RAT BRAIN MITOCHONDRIA IN DIFFERENT AGES AND VEGETABLE PREPARATIONS' EFFECT ON THIS PARAMETER

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ABSTRACT

In the process of aging of organisms, lipid peroxidation is activated in cellular mitochondria. Activation of lipid peroxidation is shown in the accumulation of malondialdehyde in the mitochondria. This process has been studied on the mitochondria of the liver and brain cells. In the present time investigated number of natural antioxidants, which reduce the concentration of oxidants. In this paper, the property supramolecular complex of antioxidant PRE-1 studied on MDA in vitro and in vivo conditions. This compound shows antioxidant property in different age categories rat's mitochondria and reduces the concentration of MDA

KEYWORDS: aging, antioxidant, malondialdehyde – MDA, lipid peroxidation, supramolecular complex.

INTRODUCTION

Oxidative damage to macromolecules that are part of various systems of living organisms increased with age, which suggests a connection of this process with aging and age-related diseases (Gladyshev 2015). The observed weakening of antioxidant protection during aging is associated with mitochondrial dysfunction and the development of programmed cell death (Wang 2009). At the end of the twentieth century, the mitochondrial theory of aging (Harman 1995) was formulated and since mitochondria and its individual components have been a promising target for gerontological research and the search for anti-aging drugs.

In this regard, the purpose of our research was to study changes in lipid peroxidation (LOPs) in vitro kinetics experiments in rat brain mitochondria of different age and the influence of this complex process plant, conventionally designated as PRE-I.

MATERIALS AND METHODS

Experiments were performed on 3-, 6-, 9-, 12- and 15-month-old male rats. Isolation of brain homogenate mitochondria and evaluation of free radical oxidation on the accumulation of lipid peroxidation of the final product - the MDA were performed according to the procedures described in the manual (Eshchenko 2006). Brain tissue homogenates were prepared in 50 mM tris HCl buffer (pH 7.4) containing 50 mM NaCl in 1:2 ratio.

The protein of mitochondria was determined by the biuret method (Gornal 1949). Experiments with animals were carried out in compliance with all the necessary rules of humane treatment of animals.

In a preliminary screening concentration of the antioxidant effect of the complex PRE-I was the most effective dose of 10 μM, which was selected as a work.

RESULTS AND DISCUSSION

Table 1 presents data on the changes in the content of MDA in brain mitochondria depending on rats of various ages. As seen from the table, correlation on the increasing age of the experimental animals and increases the amount of MDA in the mitochondria, which may indicate a decrease in activity of the antioxidant system (AOS). Thus, when compared to the initial values of the amount of MDA in 3-month-old rats, then after 3 months basal level of MDA increased by 40%. In more mature rats, this increase is even more significant and 9-month-old rats increased the basal level of MDA in average more than 5 times and at 12- and 15-month – 7 times and 7.5 times respectively as compared with values in a group of 3 month old animals.

Table 1: The accumulation of MDA depending on the age of the rats (n = 8, M ± m; nmol MDA / mg protein).

Age of rats. months	Incubation time. Minutes				
	Start	5 min	10 min	15 min	30 min
3	3.47±0.10	10.16±0.31	13.78±0.11	20.80±0.62	33.90±0.097
6	4.84±0.22	18.09±0.89	18.95±0.79	23.31±1.03	24.59±1.00
9	19.59±0.82	50.48±2.16	79.50±3.09	84.44±2.78	84.28±3.53
12	24.26±0.62	70.19±2.53	100.90±3.37	112.40±3.85	127.48±4.86
15	25.84±1.14	125.84±4.59	139.4±5.47	143.61±5.76	144.30±5.93

The intensity of lipid peroxidation depending not only on age, but also the kinetics of this process: the incubation mitochondria 9-12 months rats with inductors LPO basic gain MDA observed during the first 10 - 15 minutes and at 15 months rats MDA accumulation rate and higher maximum MDA accumulation is observed in the first 5 minutes of incubation, further increase in the number of MDA becomes less noticeable (plateaus), which can be explained by many factors, including activation of the AOS. An exception is the kinetics of growth of MDA among the youngest age groups - in 3 months rats: in this case, the amount of MDA in the course of the incubation time increases exponentially and compared with the initial values increased nearly 9 times. In other age groups MDA growth upon incubation with inducers of LPO does not exceed the fivefold value (maximum increase was 5.6 times). It should be noted that the kinetics of accumulation of MDA in 6-month old rats has double step – 4.5 min after incubation of MDA concentration reaches a plateau and remains practically constant until the 10th minute of incubation, while further incubation MDA quantity starts to increase again gradually and reaches a plateau by 30th minute, increasing as compared with 10 minutes incubation in 1.4 times. In the case of 12 monthly animals, the accumulation of MDA occurs in the entire time range of incubation, only the rate of accumulation of MDA changes, but these values never reach the plateau. If we compare the rate of accumulation of MDA in the linear section (the first 5-10 min), then with age, the rate of MDA also increases, significantly increasing by 15 months. So, in comparison with 3-month-old animals, in 6-month-old rats in the first 5 minutes the speed

increases almost 2 times and 9-months - more than 4.5 times in the first 5 minutes and a 10 minute increase becomes substantially 6- multiple. In 12-month-old animals LPO rate increases 7-7.5 times as compared to 3 monthly animals and 15 month animals MDA accumulation rate in the first 5 minutes of incubation in 15-times higher than 3 month in the same time period, it should be noted that by 10 minutes the speed is 11 times greater than the same values in 3 month-old rats.

One of the approaches in the fight against age-related changes is to use of effective antioxidants that can regulate the system of free radical production, the accumulation of oxidation products and postpone the start time of age-related changes. In the next series of experiments, we investigated the effect of the plant complex PRE-I, which is a supramolecular complex of glycyrrhizic acid and flavonoid quercetin, on the accumulation of MDA in the mitochondria of rats of different ages. Data are shown in Figures 1 and 2.

Figure 1 shows that in 3 month old animals, the PRE-I complex showed pronounced prooxidant properties - during the first 5 minutes of incubation compared to control, the level of MDA increased almost 2.6 times, further incubation led to an even greater increase in MDA - at 10 minutes, the level of MDA increased by more than 3.5 times compared with the control values, with further incubation, the prooxidant effect several times decreased and compared to the control values at the 15th and 30th minutes of incubation in the presence of complex GUT MDA was increased 2.4 and 1.6 times correspondingly.

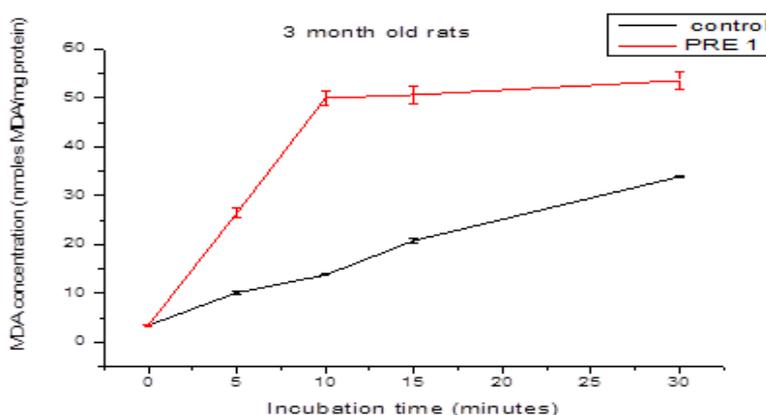


Figure 1: The effect of 10 μ M of the supramolecular complex PRE-1 on the kinetics of lipid peroxidation and the rate of accumulation of MDA in brain mitochondria of 3 month old rats. The baseline value of MDA in the control group (n = 8, M ± m; nmol MDA / mg protein) was taken as 0.

The kinetics of accumulation of lipid peroxidation in 3 months rats in the presence of complex kinetics different from the control group and corresponded LPO kinetics in Group 9 and 15 month rats. In the first 5 minutes of incubation in the experimental group MDA accumulation rate was increased, in contrast with control values by 3.4 times in the subsequent 5 minutes there is an even greater acceleration and velocity in the presence of LPO complex increased almost 7 times, which is reflected in a dramatic increase in the number MDA. However, upon further incubation the presence of complex leads to a decrease in the rate of accumulation of MDA and compared to control values of the same period of incubation (10-15 minutes of incubation) the rate in the experimental group is reduced by 14 times compared with the same values in the control group. This inhibition is reflected in the reduction of pro-oxidant effect of the complex. With further incubation of the control indicated a significant increase in the number of MDA than in the experimental group, the presence of complex leads to a decrease in the rate of accumulation in MDA 4.5 times, compared with the control. Thus, in the case of 3 month old animals in the presence of the supramolecular complex from 10 to 15 minutes of incubation in response to an avalanche-like increase in the amount of MDA,

AOC is most likely activated and the peroxidation process is stabilized.

The PRE-I complex had a different effect on the mitochondria of the older age groups (Fig. 2). In all age groups of adult rats (6-15 months) PRE-I significantly reduced the amount of MDA, accumulated as a result of incubation with inductors LPO. At 6 months rats PRE-I significantly affect the kinetics of accumulation MDA: in the presence of the complex, the two-step process is practically not observed, while in the first 5 minutes the number of MDA is reduced by 20% compared to control values in the same age group, MDA main growth is observed only on the 10th minute of incubation, the values in the test group exceeded the control values by 20% - i.e. during this period the complex acts as prooxidant, however with further incubation again manifest its antioxidant properties and the final total amount of MDA after 30 minutes of incubation is reduced by 11% compared with control values for this age group. It is important to note that in the control group at the 10th minute, the formation of the second stage of the increase in the number of MDA occurred and it can be assumed that at this moment the activity of AOS is regulated and at this stage influenced by the complex.

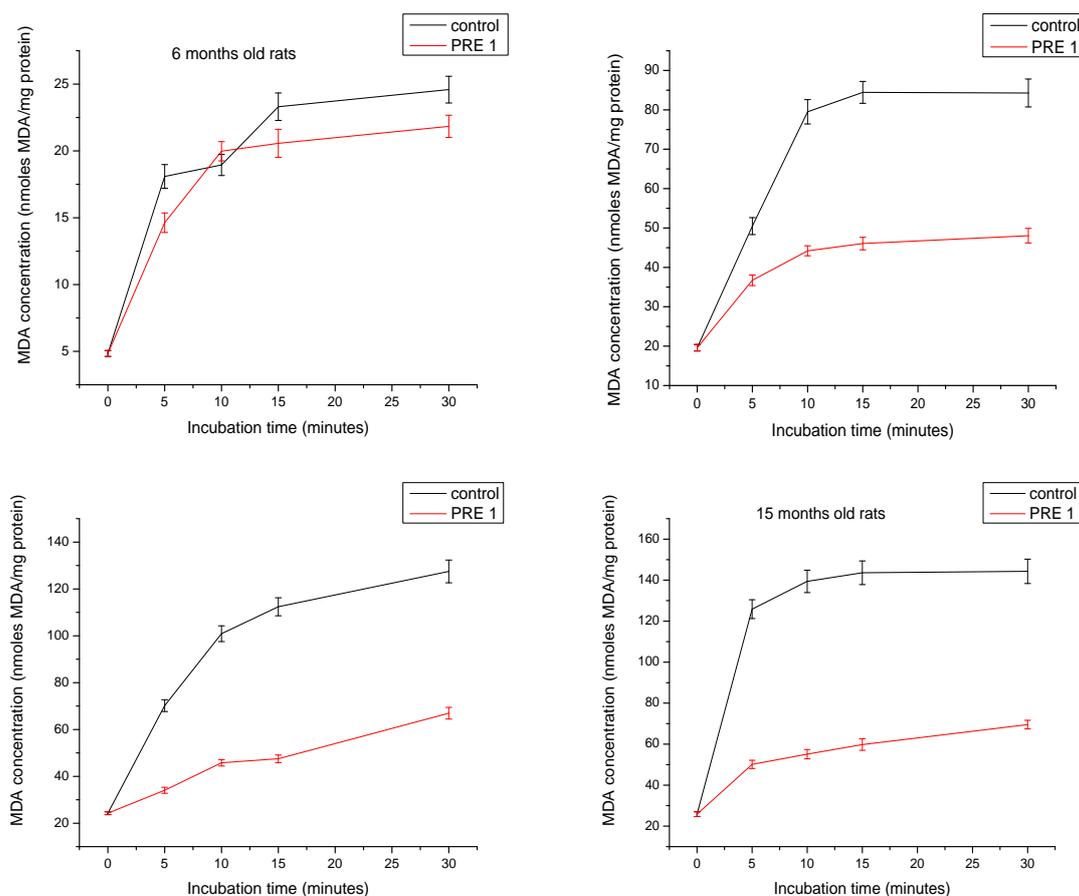


Figure 2. The effect of 10 μ M of the supramolecular complex PRE-1 on the kinetics of lipid peroxidation and the rate of accumulation of MDA in brain mitochondria of rats of different ages. The baseline value of MDA in the control group ($n = 8$, $M \pm m$; nmol MDA / mg protein) was taken as 0.

In the group of 9-month-old rats PRE-I complex showed a pronounced antioxidant effect after 30 minutes of incubation, the total amount of MDA was reduced by 43% in contrast with the control group. In the presence of the complex, observed decrease in MDA accumulation rate in the first 5 minutes 1.8- times in contrast with the control and with time the inhibition became more noticeable and the 10th minute of incubation LPO rate was 2.4 times lower than in the control. At the same time, the overall kinetic picture did not change - both in the control and in the presence of the complex in 9-month-old animals after the first 12-15 minutes of incubation, there was practically no increase in the amount of MDA.

In the group of 12-month-old animals PRE-I antioxidant effect of the drug becomes more pronounced - in comparison with control values after 30 minutes of incubation the amount of MDA was reduced by 47.5%. The overall picture of MDA accumulation in the presence of the complex has not changed - the MDA accumulation occurred during the time of incubation with inductors, only the process speed changed. It should be noted that in this age group, the antioxidant effect of the PRE-I complex depended on the incubation time — in the first 15 minutes of incubation, the antioxidant activity increased and reached its maximum by the 15th minute, inhibiting the accumulation of MDA by 58% compared to control, but with further incubation (apparently due to the activation of peroxidation processes) the effectiveness of the drug was several reduced. It is possible that this is due to excessive activation of prooxidant processes and decreased activity of AOS with age. In the first 5 minutes, the complex significantly reduced the rate of lipoperoxidation - by 5.7 times compared with the control, then the rate of accumulation of MDA increased and in the experimental group by the 10th minute was only 3.6 times slower compared to the control, that is, the complex could not as effectively reduce the speed of LPO, as in the initial stages. Perhaps this is an increase in the speed of LPO can be explained by a decrease of the antioxidant effectiveness of the complex by the end of the incubation period.

In the group of 15-month-old animals, the PRE I complex during the first 10 minutes of incubation of mitochondria with POL inducers, on average, reduced the rate of accumulation of MDA by 4 times compared with control values. In this age group, the antioxidant effect of the complex was the most significant - in the end, after 30 minutes of incubation in the experimental group, the number of MDA was reduced by 52% compared to control. It should be noted that the greatest effect was observed in the first 15 minutes of incubation - that is, in the initial stages of the activation of the lipid peroxidation process: compared with control values, the MDA level was reduced by an average of 60%. However, with time of incubation complex cannot so effectively restrain processes LPO - starting with 10

minutes of incubation (II step process) the rate of MDA accumulation increases and exceeds the speed control at this stage almost 3 times, which leads to a reduction of the antioxidant effect.

CONCLUSIONS

Summarizing obtained in this series of studies results, it can be asserted that the efficiency of the antioxidant complex PRE-I exhibits the most pronounced properties in the group of 15-month old rats at lower age of experimental animals with 15 to 6 months and severity proportionally reduced antioxidant effect.

Thus, with aging, occurs activation of free-radical lipid peroxidation (LPO) of mitochondria, which contributes to the disruption of normal biochemical processes in mitochondria (Mazunin 2010, Sukarnik 2002). Age-related dysfunction of the mitochondria leads to a steady increase in the imbalance between its pro - and antioxidant components. The resulting oxidative stress destabilizes vital macromolecules and processes in membranes, cytoplasm and in the cell nucleus, which may lead to age-related pathologies (Koopman 2012). An application in these cases the antioxidants of plant origin helps to eliminate the imbalance and has a beneficial effect on the mitochondria of old animals.

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