THE EFFECT OF NARCISSIN FLAVONOID ON THE PEROXIDATION PROCESS OF HEART MITOCHONDRIA MEMBRANE LIPIDS IN EXPERIMENTAL ISCHEMIA

Mirzaolimov E. I.
Namangan State University, 316 Uychi Street, Namangan 160119,

Pozilov M. K.
M. Ulugbek National University of Uzbekistan,
4 University Street, Tashkent 100174.

Nishanbaev S. Z.
Acad. Institute of Plant Substances Chemistry named after S.Yu.Yunusov,
77 Mirzo Ulugbek Avenue, Tashkent 100170.

Abdullaev G. R.
Namangan State University, 316 Uychi Street, Namangan 160119,

Various experimental models of myocardial injury have shown the relationship between the accumulation of free fatty acids, the activity of intracellular phospholipases and the preservation of myocardial energy metabolism at the level of mitochondria. In conditions of ischemia, the functional activity of myocardial cells is disturbed by changes in the mitochondrial membrane. Mitochondrial membrane ion transport system disorders, including mPTP permeability increase, lead to disruption of regulation of Ca^{2+} ions between cytosol and matrix. As a result, the release of cytochrome c from the matrix leads to the development of apoptosis processes. In addition, as a result of the violation of myocardial contraction processes, heart contraction rhythms can be lost.

The purpose of the study. The purpose of this study was to investigate the effect of narcissin flavanoid on the amount of MDA, the LPO product of rat heart mitochondria, and on Fe^{2+} /citrate permeability of the inner and outer membrane under conditions of experimental ischemia induced by adrenaline.

Methods and techniques. Male white rats weighing 200-250 g were used for the experiment. Scientific research on experimental animals was carried out based on the rules of the Institute of Biophysics and Biochemistry "Regulation of bioethics on the procedure for using laboratory animals in scientific research" (February 22, 2019).

Currently, there are many methods of inducing experimental myocarditis ischemia models, and the most widely used models of adrenaline are used in experiments. We used 0.1 ml of 0.1% solution of adrenaline to induce ischemia model (IM) in our experiments. Rats were

divided into groups to study the disruption of mitochondrial ion channels and metabolic processes in experimental ischemia. Group I - control (n=5); Group II – ischemia (n=5); Group III - IM+narcissin, (n=5). In order to cause experimental ischemia in laboratory animals of group II, III, 0.1 ml of 0.1% solution of adrenaline 100 mg/kg body weight of animals was injected subcutaneously for 3 days. Rats subjected to experimental ischemia model were subjected to electrocardiogram to determine pathophysiological changes in cardiac function. After making sure that the ischemia model was formed in the experimental animals, narcissin flavonoid 10 mg/kg was injected orally for 7 days in group III. After that, the experimental animals were electrocardiogrammed again. Mitochondria were isolated from the rat heart tissue by differential centrifugation after it was determined that the recovery process was observed in their cardiogram.

Isolation of LPO products was carried out in the presence of thiobarbituric acid (TBA). The reaction was stopped by adding 0.220 mL of 70% trichloroacetic acid IM. After this step, the mitochondrial suspension was centrifuged at 4000 rpm for 15 min. Then 2 ml of supernatant was taken and 1 ml of 75% TBA was added. 2 ml of H^2O and 1 ml of TBA were added to the control tube. The mixture was incubated in a water bath for 30 min. After cooling, the change in optical density at a wavelength of 540 nm was determined.

In determining the amount of MDA, the molar extinction coefficient ($\varepsilon=1,56^{\times}10^5$ M^{-1} $см^{-1}$)) in the formula was used: nmol MDA/mg protein=D/1.56x30.

Also, the Fe2+/citrate system was used to study the process of LPO in the mitochondrial membrane. Under the influence of this system, the mitochondrial membrane lost its barrier function, as a result, the size of the organelle increased and the mitochondria collapsed.

The amount of protein in mitochondria was determined by the Lowry method. In the experiments, the kinetics of mitochondrial decay was calculated as a percentage of the maximum, as the arithmetic mean value of 4-5 different experiments was calculated. The difference between the values obtained from control, experiment and experiment+study material was calculated by t-test. In this case, $P<0.05$ and $P<0.01$ values represent statistical reliability.

Results: In the conditions of experimental ischemia, the increase of cardiac mitochondrial stress can in turn hydrolyze the lipids located in the inner and outer membrane. In order to determine this, in our next experiment, the effect of narcissin flavonoid on the formation of MDA, a product of LPO, in the heart mitochondria of rats with ischemia was studied. According to the obtained results, the amount of LPO product MDA in the heart mitochondria of the control group was 2.3±0.2 nmol mg/protein and was taken as 100%. MDA formation in mitochondria isolated from heart tissue of rats subjected to experimental ischemia (group II) was found to be 4.58±0.2 nmol mg/protein, which increased by 99.1±3.3% compared to control (group I).

It was found that the amount of MDA in the mitochondria isolated from their hearts was 3.7±0.3 nmol mg/protein and increased by 38.5±2.8% compared to the values of group II. Thus, narcissin enhanced the antioxidant system by reducing the intensity of the LPO process in cardiac mitochondria under ischemic conditions. In order to further elucidate the inhibitory effect of narcissin flavonoid on membrane LPO under ischemic conditions, in our next experiment, Fe^{2}+/citrate-induced swelling of rat heart mitochondria was studied. In this case, the inducer Fe^{2}+/citrate accelerates mitochondrial membrane LPO and disrupts its barrier function, as a result of which organelle size increases and mitochondria collapse. In experimental ischemia, it was found that heart mitochondria with Fe^{2}+/citrate increased by 110.7±6.8% in pathological group II compared to control. Ischemia can be associated with an increase in LPO of the mitochondrial membrane of the heart of rats, its ion transport systems are disturbed. When narcissine pharmacotherapy of group III animals called ischemia model, it was found that their mitochondrial damage with Fe^{2}+/citrate was inhibited by 57.1±3.5% compared to group II.

In conclusion, it can be said that narcissin flavonoid restores cardiac mitochondrial damage in ischemic conditions. It acted as an inhibitor of the increase in cardiac permeability transition pore (mPTP) under ischemic conditions, and the LPO product showed antioxidant activity by reducing the amount of MDA.