THE EFFECT OF SOPHOROFLAVONOSIDE 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,

Many factors have been identified that increase the permeability of the inner membrane of cardiomyocyte mitochondria in ischemia/reperfusion. It is very important to study the disorders of heart muscle cells in the conditions of ischemia at the level of mitochondria and to study the mechanisms of molecular action of pharmacological drugs on them. Also, the mechanisms of opening the conformation of the high permeability pore of heart mitochondria in ischemia-reperfusion were studied. However, the effect of sophoroflavonoside (SFL) flavonoid on the amount of malondialdehyde (MDA) product of lipid peroxidation (LPO) in cardiac mitochondrial membrane under ischemia conditions and the process induced by Fe^{2+}/citrate has not been studied.

The purpose of the study. The purpose of this study was to study the effects of SFL flavonoid on the amount of LPO product MDA and Fe^{2}+/citrate permeability of the inner and outer membrane of rat heart mitochondria 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). We used 0.1 ml of 0.1% solution of adrenaline to induce ischemia model (IM) in our experiments. In experimental ischemia, rats

were divided into groups to study disruption of mitochondrial ion channels and metabolic processes. Group I - control (n=5); Group II – ischemia (n=5); III group IM+SFL (n=5):

In order to induce experimental ischemia in group II, III laboratory animals, 0.1 ml of 0.1% adrenaline solution at a dose of 100 mg/kg body weight was injected subcutaneously for 3 days. Electrocardiograms were performed to determine the pathophysiological changes in cardiac function in rats induced by the experimental ischemia model. After making sure that the ischemia model was formed in the experimental animals, SFL flavonoid 10 mg/kg was administered orally for 7 days to 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 PLP 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 SFL flavonoid on the formation of LPO product MDA in heart mitochondria of ischemic rats 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 production 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). When group III animals with ischemia were subjected to pharmacotherapy with SFL once a

day for 7 days, the amount of MDA in mitochondria isolated from their hearts was 3.1±0.2 nmol mg/protein, and it was found that it increased by 64.5±4.5% compared to group II. Thus, SFL enhanced the antioxidant system by reducing the intensity of the PLP process in cardiac mitochondria under ischemic conditions.

In order to further elucidate the inhibitory effect of flavonoid on membrane LPO under ischemic conditions, in our next experiment, Fe^{2}+/citrate-induced swelling of rat heart mitochondria was studied. 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. When pharmacotherapy of group III animals with SFL, which was called ischemia model, it was found that their mitochondria inhibition with Fe^{2}+/citrate was 75.0±3.7% compared to group II values.

In conclusion, SFL flavonoid restores cardiac mitochondrial damage under 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.