NOVATEUR PUBLICATIONS
JournalNX- A Multidisciplinary Peer Reviewed Journal
ISSN No: 2581 - 4230
VOLUME 8, ISSUE 7, July -2022

EFFECT OF FLAVONOIDS SAFORAFLAVONOLOSIDE AND NARCISSIN ON THE FUNCTIONAL STATE OF RAT HEART MITOCHONDRIA IN AN EXPERIMENTAL MODEL OF ISCHEMIA

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ABSTRACT

In this research it has been investigated the effect of sophoraflavonoloside isolated from Crocus sativus L. plant, narcissine flavonoid isolated from Alhagi canescens (Regel) B. Keller & Shap plant on functional disorders of rat cardiac mitochondria under conditions of experimental ischemia. Flavonoids were extracted from plant materials using alcohol-water (70%). They were re-extracted with organic solvents and separated into fractions, and pure glycosides of flavonoids were isolated from the ethyl acetate fraction using column chromatography. Using differential centrifugation, mitochondria were isolated from the hearts of healthy and ischemic rats. Animals with ischemia received orally narcissine 10 mg / kg and saforaflovonoloside (SFL) 10 mg / kg for 7 days. They have

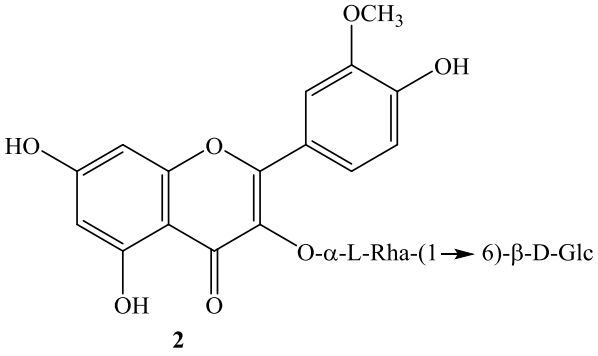
been shown to restore the swelling of the mitochondria of the heart of ischemic rats, lipid peroxidation (LPO), and the formation of malondialdehyde (MDA).

Keywords: ischemia; mitochondria; Ca^{2+} -dependent pores; saforaflavonoloside; narcissine

INTRODUCTION

The outer and inner membranes of mitochondria contain various ion channels and transporters, one of which is Ca2+ dependent pores with high conductivity (mitochondrial permeability transition pore-mRTP), which plays a key role in the activity of human and animal cells, metabolism and the development of various pathological conditions [8,9]. Since this pore is very sensitive to cyclosporine A (CsA), it is also known as the CsA sensitive pore. The CsA-sensitive pore (mRTR) was first studied in isolated mitochondria by R.A.Haworth and D.R. Hunter in the 1970s, and it led to a sharp increase in the permeability of the inner mitochondrial membrane for water-soluble molecules to 1500 Da [6]. This increase in conductivity is caused by Ca2+ ions, which are inductors of this mRTR pore, therefore it is also called a Ca2+-dependent megapore. MPTP is a channel that physiologically regulates the release of Ca2+ ions from the matrix and plays an important role in the homeostasis of Ca2+ ions and Ca2+ signaling between the cytosol and the matrix. This megapora ensures the transition of mitochondrial membranes to a highly permeable state in various pathologies. In pathological conditions, mRTR passes into an open conformational state. This conformational state causes the release of cytochrome c and pro-apoptic protein molecules from the mitochondrial matrix into the cytosol of the cell, which activates the enzyme system caspase, which, in turn, activates the mechanism of apoptosis [11]. In addition, an increase in the concentration of Ca2+ ions in the cytoplasm of the cell stimulates the release of tanotogenic (self-destructive) factors from mitochondria. In turn, the lipolysis process in the organelle membrane is activated and the normal functioning of the respiratory chain is disrupted. The accumulation of excess Ca2+ ions in the mitochondrial matrix can lead to the opening of a nonspecific pore with high permeability.

There is hypertrophy of the myocardial structure in pathological conditions and a decrease in the strength of myocardial contraction in the death zones of individual cardiomyocytes under the influence of fibrosis. Then there is a gradual increase in heart failure, and this process, in turn, is accompanied by such changes as a violation of energy metabolism in cardiomyocytes, a violation of the transport of Ca2+ ions, increased production of reactive oxygen species, a transition to the open conformation of mRTP [5, 10, 12]. However, the question of the mRTR function remains one of the issues that has not yet been fully clarified, i.e. mechanisms aimed at preserving the vital activity of damaged cells or programmed cell death, where the role of mitochondria is not fully understood. Currently, an increase in the intensity of the mitochondrial membrane of the heart, a decrease in ATP synthesis, a change in the activity of antioxidant enzymes and an increase in the permeability of mRTR in ischemic conditions are being studied [10,13]. Studies on the pharmacological correction of disorders of the mitochondrial membranes of the heart with plant compounds in ischemia are continuing. However, the effect of sophoraflavonoside (kaempferol-3-O-(-D-sophoroside) (1) [3,4] isolated from the plant Crocus sativus L. of the family Iridaceae and the flavonoid narcissin, (isoramnetin-3-O-(-D-rutinoside) (2) isolated from the plant Alhagi canescens (Regel) B. Keller & Shap on functional disorders of rat cardiac mitochondria in experimental ischemia has not yet been studied.



2

Fig. 1. The structural formula of the flavonoids saphoraflavonoside (SFL - 1) and narcissin (2).

Materials and methods

The following reagents and pharmacological preparations were used in the experiments: Epinephrine (Ellara Russia), EGTA, EDTA, KH2PO4, K2HPO4, Hepes, KCl, MgSO4, succinate, (Sigma-Aldrich, USA); oligomycin, rotenone, sucrose, tris-HCl, CaCl2 (Serva, Germany). All reagents used have the qualification of chemical purity for the experiment.

Isolation and study of the structure of flavonoids.

Flavonoids were extracted from vegetable raw materials using water-alcohol (70%). The resulting water-alcohol extract was condensed on a rotary evaporator and diluted with distilled water (in a ratio of 1:1), re-extracted with organic solvents (gasoline, chloroform, ethyl acetate and n-butanol) in order of increasing polarity. Using column chromatography (adsorbent silica gel 150/200 mesh, KSK company "Tianjin Sinomed Pharmaceutical" (China)) and gel chromatography (Sephadex LH-20 sorbent from GE Healthcare Bio-Sciences AB (Sweden)) individual flavonoid glycosides were isolated from the ethyl acetate fraction. The chemical structure of individually isolated flavonoid glycosides was determined using IR, 1H and 13C NMR spectroscopy.

The experiments were carried out in vivo on white mongrel male rats weighing 180-200 g. The animals of the experimental group were divided into 4 groups: control group I (healthy), experimental group II (ischemia model), experimental group III (ischemia + narcissin), experimental group IV (ischemia + SFL). To create a model of ischemia in rats in experimental groups II, III and IV, they were injected subcutaneously for 3 days in 0.1 ml of 0.1% solution of 100 mg/kg of epinephrine hydrochloride per animal body weight. An electrocardiogram was performed to detect pathophysiological changes in cardiac function in rats susceptible to ischemia. After confirming that the experimental animals had formed a model of ischemia, they were administered orally the flavonoid narcissin in group III 10 mg/kg and the flavonoid SFL in group IV 10 mg/kg for 7 days. After that, the experimental animals were given an ECG again. After it was determined that a recovery process was observed on their cardiogram, the mitochondria of rat heart tissue were obtained by differential centrifugation.

Isolation of mitochondria from the heart.

Mitochondria were isolated from the rat heart by differential centrifugation [1]. The composition of the isolation medium is as follows: sucrose 300 mM, tris-HCl - 10 mM, EDTA - 2 mM, albumin 0.2% pH 7.4.

After the cooled heart was cleaned of fat and other blood vessels, it was dried using filter paper and its mass was determined. The heart tissue was homogenized using a Teflon pestle in an isolation medium in a ratio of 10:1 [1]. The homogenate was poured into a centrifuge solution and placed on the rotor.

At the first stage, the deposition of heavy aggregates in a centrifuge lasted 7 minutes at a rotation speed of 1500 rpm. At the second stage, centrifugation was carried out at 6000 rpm for 20 min. Before the experiment, mitochondria were washed in a medium free of EDTA and albumin. Mitochondria were pipetted in a special container and stored in a container with ice in the freezer (0 ± 2 °C).

Detection of swelling of cardiac mitochondria.

The kinetics of mitochondrial swelling (0.3–0.4 mg/ml) was determined by changing the optical density of a suspension of mitochondria in an open cell at 540 nm (volume 3 ml) with constant stirring at 26 °C. The following incubation medium was used to determine the permeability of mitochondrial PTP: 200 mm sucrose, 20 mm EGTA, 5 mM succinate, 2 mm rotenone, 1 mg/ml oligomycin, 20 mM Tris, 20 mM HEPES, 1 mM KH_2PO_4, pH 7.4 .

Determination of lipid peroxidation products in mitochondria.

The separation of POL products was carried out in the presence of thiobarbituric acid (TBC). The reaction was stopped by adding 0.220 ml of 70% trichloroacetic acid to the incubation medium. After that, the suspension of mitochondria was centrifuged at 4000 rpm for 15 minutes. Then we received 2 ml of the infusion fluid and poured 1 ml of 75% TBC. 2 ml of H_2O and 1 ml of TBC were added to the control solution. The mixture was incubated for 30 minutes in a water bath.

After cooling, a change in the optical density at a wavelength of 540 nm was detected. When determining the amount of MDA, the molar extinction coefficient was used in the formula (ε=1.56×105 M-1 cm-1): nmol MDA / mg of protein = D / 1,56x30.

The Fe^{2+}/citrate system was also used to study the process of POL in the mitochondrial membrane. Under the influence of this system, the mitochondrial membrane lost its barrier function, which led to an increase in the size of organelles and swelling of mitochondria.

This volume change was detected photometrically. Incubation medium: Sucrose - 125 mM, KSI - 65 mM, HEPES - 10mM, pH 7.4; Concentrations: $FeSO_4$ - 50 microns, citrate – 2 kM; mitochondrial content 0.5 mg/ml.

STATISTICAL ANALYSIS

Statistical processing of the obtained results and drawing of images were carried out using the OriginPro 7.5 computer program (Microsoft, USA). In the experiments, the kinetics of mitochondrial swelling was determined as a percentage of the maximum by calculating the arithmetic mean of 6 experiments. The difference between the values obtained in the control, experiment and experiment + study substance was calculated by the t-test. In this case, the values $P < 0.05$ and $P < 0.01$ represent statistical reliability.

RESULTS AND DISCUSSION

A very important morphological sign of mitochondrial damage is their swelling. Mitochondrial swelling is observed, for example, in myocardial cells with heart failure, as well as in many infectious, hypoxic, toxic and other pathological processes. In particular, the following experiment was conducted to determine the corrective effect of flavonoid compounds on mitochondrial swelling in experimental ischemia. A concentration of 20 microns of Ca^{2+} ions was used as an induction of swelling of cardiac mitochondria. Swelling of the mitochondria of the heart using 20 microns of $CaCl_2$ in healthy rats (group I) was taken as a control (100%). According to the results, mitochondrial swelling in animals of group II, called the ischemic model, increased by 94.1± 5.5% compared to the control (group I) (Fig. 2). Experimental ischemia leads to an increase in the load of Ca^{2+} in cardiac

mitochondria and loss of membrane stability, which ensures a state of high permeability of mPTP. It was found that in experimental group III animals with induced ischemia, who received the flavonoid narcissin (10 mg/kg) and group IV animals who received the flavonoid SFL (10.0 mg/kg) once a day for 7 days, the swelling of the mitochondria of the heart significantly decreased. At the same time, the swelling of the mitochondria of the heart under the influence of narcissin and SFL was inhibited by 42.5 ± 1.8% and 63.4± 2.5%, respectively, compared with the ischemic model (group II) (Fig. 2).

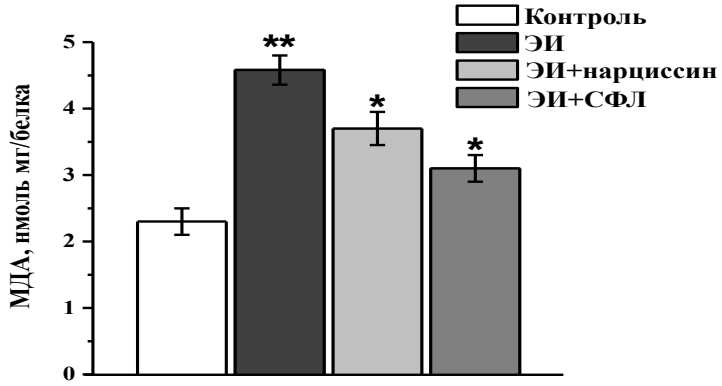


Fig. 2. The effect of narcissin and SPL on mitochondrial swelling of the rat heart in experimental ischemia (EI) (*P<0.05; **P<0.01; n=6).

Consequently, the flavonoids narcissin and SFL in ischemic conditions significantly suppressed the swelling of the mitochondria of the heart with the help of Ca2+ ions. This indicates that the high permeability of cardiac mPTP has been modified to improve their functional state.

При in experimental ischemia, increased swelling of the mitochondria of the heart can lead to hydrolysis of lipids located on the inner and outer membranes, respectively. To determine this, in our next experiment, the effect of the flavonoids narcissin and SFL on the formation of MDA, the product of the SEX of the mitochondria of the heart of ischemic rats was studied (Fig. 3). According to the results obtained, the content of MDA in the product of SEX in the mitochondria of rats of the control group was 2.3 ± 0.2 nmol mg/protein, which was 100%. In rats with experimental ischemia (group II), the formation of MDA in mitochondria isolated from cardiac tissue was 4.58± 0.2 nmol mg/protein, which is 99.1± 3.3% more than in the control (group I) (Fig. 3).

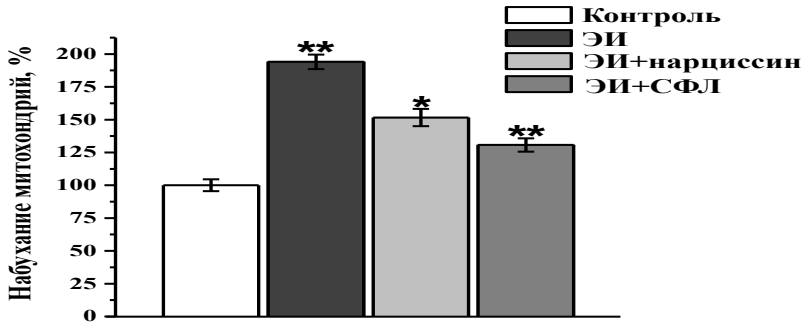


Fig. 3. The effect of narcissin and SFL on the amount of MDA, product GENDER
митохондрий сердца крысы при экспериментальной ишемии (*P<0.05; **P<0.01; n=6).

During pharmacotherapy of animals of groups III and IV with narcissin and SPL ischemia once a day for 7 days, the content of MDA in their mitochondria was 3.7 ± 0.3 and 3.1±0.2 nmol mg/protein and

increased by 38.5±2.8% and 64.5± 4.5%, respectively, from the indicators of group II (Fig. 3). Thus, narcissin and SPL enhance the antioxidant system by reducing the level of POL in the mitochondria of the heart in the conditions of ish ai. Moreover, the antioxidant activity of the flavonoid SFL in ischemic conditions was significantly high compared to narcissin.

In order to further demonstrate the inhibitory effect of the flavonoids narcissin and SPL on the membrane floor under ischemic conditions, in our next experiment, induced swelling of rat heart mitochondria using Fe2+/citrate was studied (Fig. 4). In this case, the Fe2+/citrate inducer accelerates the increase of the mitochondrial membrane FLOOR and disrupts its barrier function, which leads to an increase in the size of organelles and swelling of mitochondria. In experimental ischemia, the swelling of the mitochondria of the heart using Fe2+/citrate was 110.7±6.8% higher in the II pathological group than in the control. The increased sex of the mitochondrial membrane of the rat heart caused by ischemia may be associated with a violation of its ion transport systems [6]. With narcissin pharmacotherapy of group III animals with an ischemia model, it was found that their mitochondrial swelling using Fe2+/citrate was suppressed by 57.1±3.5% compared to group II. It was found that the swelling of the mitochondria of the heart in group IV rats treated with SFL was suppressed and restored by 75.0 ± 3.7% compared to group II (Fig. 4).

Thus, the main reasons for the discovery of mPTP in ischemia are the development of oxidative stress, pro-oxidants, induction of POL, oxidation of thiol groups in the mPTP complex. Due to the strong antiradical properties of flavonoid compounds, they can reduce the amount of free radicals in mitochondria and control the inhibitory properties of CsA by binding to the CyP-D matrix domain. When pathological conditions are activated, mitochondria cannot retain Ca2+ ions [2].

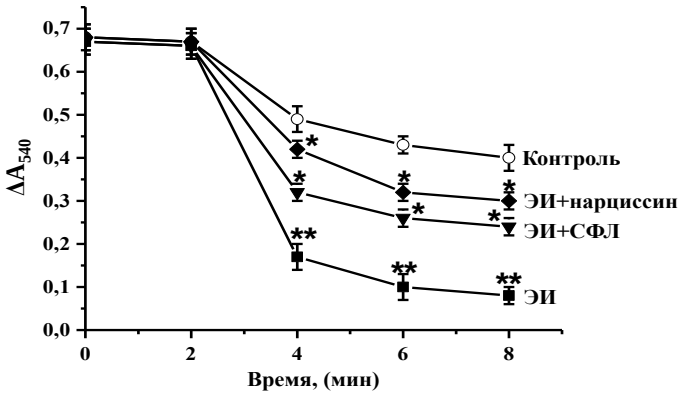


Fig. 4. Effect of narcissin and SPL on swelling of rat heart mitochondria with Fe2+/citrate in experimental ischemia (*P<0.05; **P<0.01; n=6).

There are different ways to release Ca2+ ions from mitochondria. The maximum release of Ca2+ from the organelle matrix is observed when a peculiar permeability of the inner membrane is induced in accordance with the concentration gradient for ions and solutes [14]. At the same time, a sharp increase in the permeability of mPTP can lead to disruption of the energy functions of mitochondria, primarily to a decrease in ATP synthesis, as well as to swelling of the matrix, rupture of the outer membrane, release of cytochrome c and apoptogenic proteins between the membranes [14]. In this regard, the induction of increased permeability of the inner membrane of mitochondria is one of the factors of cell death in various pathological conditions. In the ischemic model, there may be an increase in the permeability of cardiac mitochondria, an increase in the amount of MDA, a POL

product, a violation of membrane stability, a decrease in membrane potential, a decrease in ATP synthesis and a sharp increase in the number of free radicals.

It has been shown that the selected flavonoids narcissin and SFL exhibit cardioprotective properties, correcting cardiac mitochondrial dysfunction in ischemic conditions. Further studies are needed to study the effect of these flavonoids on the ATP synthesis of cardiac mitochondria and the formation of free radicals in pathological conditions.

CONCLUSION

Thus, the flavonoids narcissin and SFL restore cardiac mitochondrial damage in ischemic conditions. In ischemic conditions of the heart, they act as a blocker, suppressing an increase in the permeability of mPTP, by reducing the amount of MDA, the POL product, they exhibit antioxidant activity. In the ischemia model, it was shown that the flavonoids narcissin and SFL have an effective corrective effect on cardiac mitochondrial dysfunction. These results make it possible in the future to use cardiac mitochondria as a mitoprotective agent that protects against the harmful effects of ischemia/reperfusion.

In this study, the effect of sophoraflavonoside isolated from the plant Crocus sativus L., the flavonoid narcissin isolated from the plant Alhagi canescens (Regel) B. Keller was studied & Shap on functional disorders of rat heart mitochondria in experimental ischemia. Flavonoids were extracted from vegetable raw materials using alcohol-water (70%). They were repeatedly extracted with organic solvents and separated into fractions, and pure flavonoid glycosides were isolated from the ethyl acetate fraction using column chromatography. Using differential centrifugation, mitochondria were isolated from the hearts of healthy rats and rats with ischemia. Animals with ischemia orally received narcissin 10 mg/kg and saphoraflovonoside (SFL) 10 mg/kg for 7 days. They have been shown to restore the swelling of the mitochondria of the heart of rats with ischemia, lipid peroxidation (POL) and the formation of malondialdehyde (MDA).

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