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The Influence of Some Isoquinoline Alkaloids on the Dysfunction of Rat Heart Mitochondria Under Conditions of Oxidative Stress

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In this article, the effects of 1-(4-dimethylaminophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-24) and 1-(4-methoxylphenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (F-4) isoquinoline alkaloids on the swelling process of rat heart mitochnndria under condition of oxidative stress and citrate-Fe^{2+}-dependent lipid peroxidation were studied. The oxidative stress (OS) model in rats was induced by oral administration of $PbCl_2$ salt at a dose of 10 mg/kg once daily. After inducing OS, rats of groups III and IV were administered isoquinoline alkaloids F-24 and F-4, with their addition to animal feed, at a dose of 30 mg/kg once a day for 7 days, respectively. In the OS medel groups, only a small number of rats died (10%). It was found that the inhibitory effect of isoquinoline alkaloid F-4 on the swelling of heart mitochondria under OS conditions is more active than that of isoquinoline alkaloid F-24. Under OS conditions, isoquinoline alkaloids F-24 and F-4 had an inhibitory effect on Fe^{2+}/citrate-induced lipid peroxidation of rat heart mitochondrial membranes. The main reasons for the opnning of mitochondrial permeability transition pore (mPTP) under OS conditions are the development of stress, pro-oxidants, induxtion of lipid peroxidation, and oxidation of thiol groups in the mPTP complex.

Keywords: Heart, Isoquinoline alkaloids, LPO, Mitochondria, mPTP, Oxidative stress.

Oxidative stress is a pathophysiological process associated with production of reactive oxygen species (ROS) in excessive amount in cells[1]. Increased production and accumulation of ROS in cells causes an imbalance between antioxidant defense systems[2]. First, low levels of ROS play a vital physiological role in signaling of intracellular pathways, however if generation of it developed, it causes cell and tissue damage[3]. Second, it appears as a result of harmful in a direct way effects on biological structures such as lipids, proteins, nucleic acids[2,4]. Endogenous ROS are produced as a byproduct of oxygen metabolism, while exogenous oxidative stress can be induced

by environmental stresses such as ionizing of some drugs or X-ray radiation, carcinogens[5]. Chronic inflammatory processes in the body improve oxidative stress and ROS generation[6].

Mitochondria is the the important source of free radicals in the cells. Apart from the main function of mitochondria in the synthesis of ATF, it also participates in the biosynthesis of nucleic purines, lipids, steroidogenesis, amino acids, hemes, acids. In addition, it controls intracellular calcium homeostasis and regulates cell, division thermogenesis, and programmed deathof cell[7]. During strong oxidative metabolism mitochondria generates ROS, roughly 1-2% of the molecular oxygen received by cells is changed into ROS during the physiological respiration. In fact, the main components of free radicals are mainly the superoxide anion, the products of mitochondrial respiration, which are formed during the flow of electrons in complexes I, II and III of the electron transport chain[8]. It has been estimated that the concentration of $O^{2\bullet-}$ in the mitochondrial matrix is 5-10 times higher than in the cytosol or nucleus[9]. Hypoxia, cytokines, LPO (Lipid perioxidation), dysfunction of membrane ion transport systems, or changes in mitochondrial membrane potential are stimuli which are induced by oxidative stress in mitochondria[7,10]. When the antioxidant defense system is impaired in mitochondria, damaging process of biomolecules (DNA, proteins, and lipids) due to ROS can lead to mitochondrial dysfunction and the release of pro-apoptotic proteins from the structure which is known as intermembrane space. As a result, mitochondrial swelling increases, through the mPTP, cytochrome c protein released into the intermembrane space. The activity of antioxidant enzymes decreases and free radicals increase. Mitochondrial dysfunctions associated with oxidative stress can be corrected using biologically active compounds[11]. One of these biologically active substances is isoquinoline alkaloids, which are currently being studied with great interest *in vitr*o and *in viv*o experiments on ion transport systems and mitochondrial membrane permeability of rat smooth muscle cells[12,13]. However, the effects of F-24 and F-4 alkaloids on the dysfunction of rat heart mitochondria under oxidative stress conditions have not been studied.

From this point of view, in this study, we aimed to study the effects of F-24 and F-4 isoquinoline alkaloids on rat heart mitochondrial swelling and LPO process under oxidative stress conditions. Isoquinoline alkaloids F-24 and F-4 which were selected for research were presented by Sh.N.Zhurakulov, a scientist of Academy of Sciences of Uzbekistan of the Institute of the Chemistry Plant of Substances.

MATERIALS AND METHODS

Experimental studies were conducted on mongrel white male rats weighing 180-200 g. Laboratory animals were fed in standard rational vivarium conditions. Research on experimental animals was performed on the basis of the international Declaration of Helsinki developed by the Council for International Organizations of Medical Sciences (CIOMS; the council for international organizations of medical sciences) (1985) and the "Regulations on the use of laboratory animals in research work" carried out by at the Institute of Biophysics and Biochemistry (2019). This Regulation has been developed on the basis of the recommendations of the Council of Europe Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes: Strasbourg, Council of Europe, 51 pp; 18.03. 1986, ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) NC3Rs (2013) and the University of Arizona Department of Animal Care Manual "Handling, restraint, and techniques of laboratory rodents", May 2001 (http:www .ahsc.arizona.edu/uac).

The studies were carried out under in vivo conditions. A $PbCl_2$ salt was used to induce an oxidative stress model in rats.

Rats allocated for the experiment were divided into several groups: group I - control (n=7), group II - OS induced by $PbCl_2$, (n=7), group III - OS induced by $PbCl_2$ +F-24, (n=7) and group IV - OS induced by $PbCl_2$ +F-4, (n=7). Groups II, III and IV were perorally administered $PbCl_2$ once a day for 7 days at a dose 10 mg/kg. After inducing OS, rats of groups III and IV were administered isoquinoline alkaloids F-24 and F-4, with their addition to animal feed, at a dose of 30 mg/kg once a day for 7 days, respectively. In the OS model groups, only a small number of rats died (10%).

Rat heart mitochondria were isolated by differential centrifugation[14]. Kinetic analysis of mitochondrial swelling (0.3-0.4 mg/ml protein) was determined by spectrophotometer (spectrophotometer V-5000) at 540 nm in an open cell (volume 3 ml) with continuous stirring of the mitochondrial suspension at 26°C [15].

To study the LPO process in the mitochondrial membrane, the Fe^{2+}/citrate complex was used. This complex is based on swelling and changes in the volume of mitochondria as a result of LPO in the membrane. The change in volume was determined photometrically[16].

Statistical processing of the results obtained and drawing of images was carried out using the Origin 8.6 computer program (USA). In the experiments, the kinetic analysis of mitochondrial swelling was calculated as a percentage of the maximum, and the arithmetic mean of 5 different experiments was also calculated.

RESULTS AND DISCUSSION

In order to tackle the heart disease progression, signaling molecules that cause oxidative stress should be used as the main target.

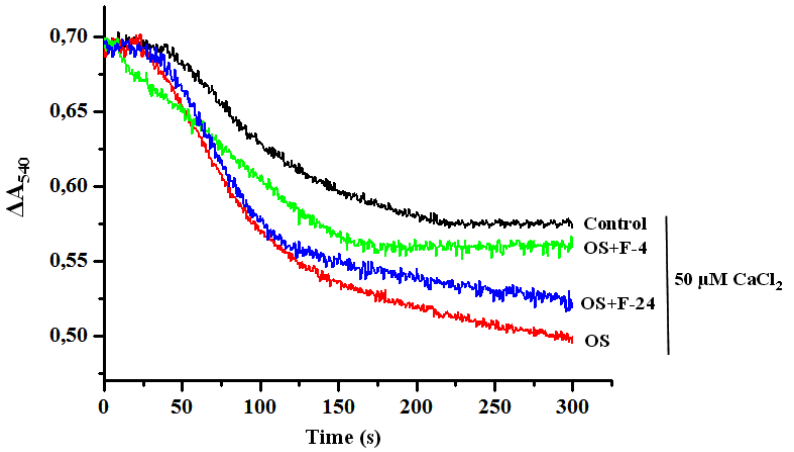

Fig. 1. Influence of isoquinoline alkaloids F-24 and F-4 on the swelling of rat heart mitochondria under $PbCl_2$-induced OS (original post).

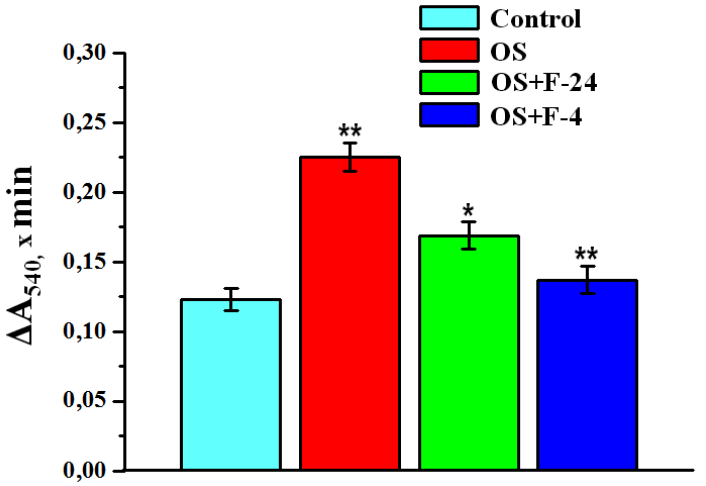

Fig. 2. Influence of isoquinoline alkaloids F-24 and F-4 on the swelling of rat heart mitochondria under OS conditions induced by $PbCl_2$ (*P<0.05; **P<0.01; n=5)

Given the important roles of ROS signaling in both cardiac physiology and disease, ROS signaling is tightly regulated, and intracellular redox homeostasis must be maintained to ensure that physiological ROS signaling can happen while pathological ROS signaling ways are not activated. Intracellular ROS levels are held in check by a complecated array of antioxidant defense systems. Apart from that under the conditions of oxidative stress, because of LPO of the mitochondrial membrane mitochondrial permeability may also change. In this case, the process of mitochondrial swelling is observed, which causes a sharp increase in the permeability of the megachannel (mitochondrial permeability transition pore-mPTP). During OS, mitochondrial swelling also occurs, which was confirmed during experiments. Mitochondrial swelling associated with OS can be inhibited by various biologically active substances. There is evidence of plant substances that inhibit mitochondrial swelling in various pathological conditions. Due to the lack of data on the effect

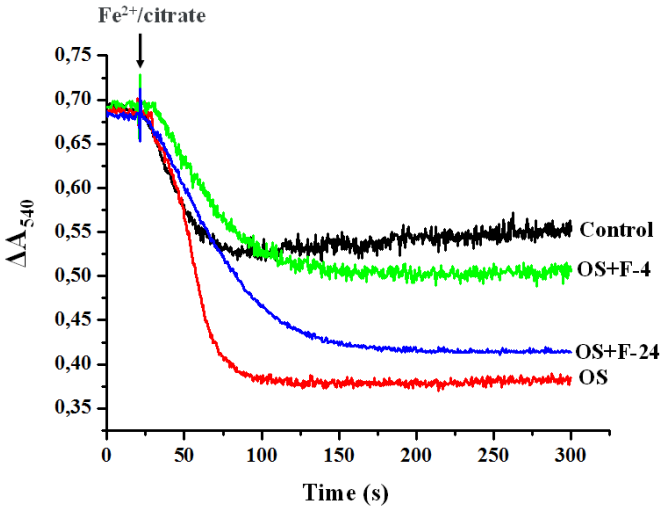

Fig. 3. Effect of isoquinoline alkaloids F-24 and F-4 on the process of lipid peroxidation induced by Fe^{2+}/citrate in rat heart mitochondria under OS conditions (original post)

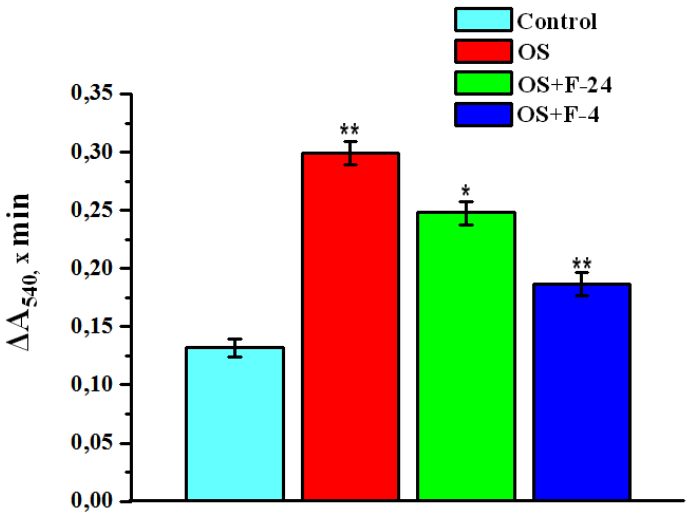

Fig. 4. Influence of isoquinoline alkaloids F-24 and F-4 on the LPO of rat heart mitochondria induced by Fe^{2+}/citrate under OS conditions (*P<0.05; **P<0.01; n=5).

of isoquinoline alkaloids on the swelling of heart mitochondria, the following experiments were carried out. First, in our experiment, the effect of F-24 and F-4 isoquinoline alkaloids on rat heart mitochondria contraction under OS conditions induced by $PbCl_2$ was studied. In the experiment, a concentration of 50 μM of $CaCl_2$ was used as an inducer to induce mitochondrial inhibition. In the absence of Ca^{2+} ions in the incubation medium, mitochondrial swelling is not observed. However, when a concentration of 50 μM of $CaCl_2$ was added to the incubation medium, the number of cardiac mitochondria of group I (healthy) rats was 0.123 ΔA_{540}x5 min. (Figures 1 and 2). The swelling of liver mitochondria with the help of Ca^{2+} ions in rats of group II in the state of OS induced with $PbCl_2$ was 0,225 ΔA_{540}x5 min. This indicates an increase in indicators by 82.9% compared to group I.

Thus, administration of $PbCl_2$ to rats at a dose of 10 mg/kg for 7 days led to swelling of heart mitochondria. An extension in the intensity of the process of mitochondrial swelling under the impact of OS with the help of Ca^{2+} ions caused a high value of mPTP permeability. Continuing the experiment, it was revealed that in the third group of rats with OS after administration of the isoquinoline alkaloid F-24 at 30 mg/kg for 7 days, the swelling of rat heart mitochondria was 0,17 ΔA_{540}x5 min., which led to inhibition by 24.4% compared II group (Fig. 2). After pharmacotherapy of group IV rats with OS using the isoquinoline alkaloid F-4, the swelling of their heart mitochondria was 0,14 ΔA_{540}x5 min. This, in turn, led to an inhibition of 39.1% in relation to the indicators of group II (Fig. 2).

Consequently, isoquinoline alkaloids F-24 and F-4 had an inhibitory effect on the swelling of heart mitochondria during $PbCl_2$-induced OS. It was found that the inhibitory effect of isoquinoline alkaloid F-4 on the swelling of heart mitochondria under OS conditions is more active than that of isoquinoline alkaloid F-24.

The opening of the mPTP conformation of the heart under OS conditions may be related to the process of peroxidation of membrane lipids. In order to confirm this hypothesis, in our next experiment, the influence of isoquinoline alkaloids on the Fe^{2+}/citrate-induced LPO process of rat heart mitochondria under OS conditions was investigated. Lipid peroxidation and formation of malondialdehyde (MDA) are stimulated by iron and iron complexes [17].

Under OS conditions, swelling of cardiac mitochondria can, in turn, hydrolyze lipids located in the inner and outer membrane. In our experiment, Fe^{2+}/citrate complex, which is considered to be an inducer of LPO, was used to carry out the process of lipoperoxidation in the mitochondrial membrane.

The optical density of cardiac mitochondria in group I rats with LPO induced by Fe^{2+}/citrate was 0.13 ΔA540x10 min. The optical density of liver mitochondria in group II rats with OS induced by $PbCl_2$ in the presence of Fe^{2+}/citrate was 0.30 ΔA540x10 min, which turned out to be 126.5% higher than the control (Fig. 3).

An increase in the lipid peroxidation process in the mitochondrial membrane of the heart of rats under OS conditions may be associated with, a disruption of ion transport systems[18].

When pharmacotherapy with the isoquinoline alkaloid F-24 of animals of group III with OS induced by $PbCl_2$, it was found that the swelling of mitochondria with Fe^{2+}/citrate was inhibited by 17.05% in contrast to group II. It was studied that in rats of group IV, which received the isoquinoline alkaloid F-4, inhibition of heart mitochondrial swelling was observed by 40.4% compared to group II (Fig. 4).

Consequently, under OS conditions, isoquinoline alkaloids F-24 and F-4 had an inhibitory effect on Fe2+/citrate-induced lipid peroxidation of rat heart mitochondrial membranes. The main reasons for the opening of mPTP under OS conditions are the development of stress, pro-oxidants, induction of lipid peroxidation, and oxidation of thiol groups in the mPTP complex. By inhibiting lipid peroxidation processes, isoquinoline alkaloids can reduce the amount of free radicals in mitochondria and, by binding to the CyP-D matrix domain, control the inhibitory properties of CsA.

CONCLUSION

In conclusion, the isoquinoline alkaloids F-24 and F-4 repair damage to cardiac mitochondria under oxidative stress conditions. In OS, these substances acted as a blocker by inhibiting the

opening of mPTP, and it was also found that they had an inhibitory effect on the lipid peroxidation process caused by Fe^{2+}/citrate.

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Conflict of Interest

All authors declare that they have no conflicts of interest.

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