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ABAMEKTIN VA TIAMETOKSAM PESTITSIDLARINING JIGAR MITOXONDRIYASIGA TA’SIRI

Annotatsiya

Hozirgi kunda pestitsidlar butun dunyo bo‘ylab qishloq xo‘jaligida keng qo‘llaniladi. Pestitsidlar zararkunandalar uchun asosiy nishon bo‘lsa-da, ular sut emizuvchilarga, xususan, jigar mitoxondriyalariga salbiy ta’sir ko‘rsatadi. Ushbu maqolada abamektin va tiametoksam pestitsidlarining mPTP (mitochondrial permeability transitional pore) ochilishi va Fe^{2+}/sitrat bilan indusirlangan lipidlarning perekisli okdlanishi (LPO) natijasida kalamush jigar mitoxondriasi bo‘kishiga ta’siri *in vitro* sharoitda o‘rganilgan. Tadqiqot natijalari shuni ko‘rsatadiki, abamektin jigar mitoxondriyasi mPTP o‘tkazuvchanligiga va LPOga tiametoksamga nisbatan faol ta’sir ko‘rsatadi.

Kalit so‘zlar: Jigar, mitoxondriya, abamektin, tiametoksam, mPTP, LPO.

ВЛИЯНИЕ ПЕСТИЦИДОВ АБАМЕКТИНА И ТИАМЕТОКСАМА НА МИТОХОНДРИИ ПЕЧЕНИ КРЫСЫ

Аннотация

В настоящее время пестициды широко используются в сельском хозяйстве по всему миру. Несмотря на то, что пестициды являются основной мишенью для вредителей, они также оказывают негативное воздействие на организм млекопитающих, в частности на митохондрии печени. В данной статье описывается изучение влияния пестицидов: абамектина и тиаметоксама, на процесс набухания митохондрий печени крыс, обусловленный открытием mPTP (mitochondrial permeability transition pore) и перекисным окислением липидов (ПОЛ), индуцированным Fe^{2+}, в условиях *in vitro.* Полученные данные свидетельствуют о том, что повреждающее действие абамектина более активно, чем тиаметоксама, на проницаемость mPTP и ПОЛ.

Ключевые слова: Печень, митохондрии, абамектин, тиаметоксам, мПТП, ПОЛ.

THE EFFECT OF ABAMECTIN AND THIAMETHOXAM PESTICIDES ON ISOLATED RAT LIVER MITOCHONDRIA

Annotation

Nowadays, pesticides are widely used chemicals in agriculture all around the world. Even though, pesticides are the pivotal target for the pest, they also have negative impact on mammalian organism, particularly on liver mitochondria. This research article describes the study of the the effect of pesticides: abamectin and thiamethoxam on the swelling process of rat liver mitochondria by opening the mitochondrial permeability transition pore (mPTP) and Fe^{2+}- induced lipid peroxidation (LPO) under *in vitro* conditions. The obtained data indicates that damaging effect of abamectin is more active than thiamethoxam on the opening mPTP and LPO.

Keywords: Liver, mitochondria, abamectin, thiamethoxam, mPTP, LPO.

Introduction. The liver is the main organ of xenobiotic biotransformation, and its mitochondria play a key role in energy metabolism. Disturbances in the mitochondrial function of hepatocytes induced by pesticides can lead to a decrease in bioenergetic activity and the initiation of cytotoxicity processes. Mitochondria are not only the center of cellular energy production, but also an important element in the regulation of apoptosis, calcium homeostasis and oxidation-reduction balance. Disturbances in their function can lead to the development of oxidative stress, changes in the permeability of mitochondrial membranes and the initiation of mitochondria-dependent cell death pathways. This disturbance may occur as a result of effecting pesticides on organism. At present, large quantities of pesticides are produced globally and are widely applied in agriculture to protect crops from pests, with annual production reaching about 1 million tons. On average, pesticide concentrations in cultivated fields are estimated at 300 g per hectare of farmland, or 30 mg per square meter [1]. Such extensive use has adverse environmental consequences, as pesticides can enter living organisms through different routes and accumulate in tissues as residues. These residual compounds may disrupt the biochemical and physiological functions of liver mitochondria, leading to various alterations. Exposure to pesticides can markedly impair mitochondrial function in rat liver by inducing oxidative stress, disrupting oxidative phosphorylation, and provoking structural alterations. Such mitochondrial disturbances may compromise cellular integrity and contribute to the development of hepatotoxicity [2]. One of commonly used pesticides widely used in modern agriculture are abamectin and thiamethoxam.

Abamectin is widely used both in agriculture as an insecticidal agent and in veterinary and human medicine for the treatment of helminthic infections, functioning as an anthelmintic. Its insecticidal composition predominantly includes avermectin B1a (≥80%) and avermectin B1b (≤20%). Avermectins represent a relatively recent group of macrocyclic lactones derived from microbial fermentation. These compounds are naturally synthesized by the soil bacterium *Streptomyces avermitilis* under both natural and industrial conditions. The insecticidal action of abamectin is mediated through its interaction with γ-aminobutyric acid (GABA) receptors, impairing neurotransmission, which induces irreversible paralysis and subsequent death in lepidopteran insect species [3].

The extensive application of pesticides in contemporary agriculture has led to increasing concern about their potential toxic effects on non-target organisms, including humans. Neonicotinoids, in particular, have attracted considerable attention due to their selective affinity for insect nicotinic acetylcholine receptors and their initially presumed lower toxicity to mammals. Thiamethoxam, a second-generation neonicotinoid, is widely employed for controlling pests across various crop systems. Nevertheless, accumulating evidence indicates that Thiamethoxam may elicit toxicological effects beyond its intended insecticidal activity. Despite its design to exhibit reduced toxicity in vertebrates, multiple studies have demonstrated that Thiamethoxam exposure can induce oxidative stress, impair organ function, and negatively affect reproductive health in mammalian models [4].

Based on the aforementioned background, the aim of our study was to investigate the effects of abamectin and thiamethoxam on the opening of the mPTP and lipid peroxidation, induced by Fe^{2+}/citrate under *in vitro* conditions.

Materials and Methods

Materials

All reagents and chemicals used for this study were of the highest grade of purity commercially available and pesticides abamectin and thiamethoxam were obtained from Sigma (Sigma Aldrich, USA).

Animals

All experiments were performed according to the International Helsinki Declaration (CIOMS; the council for international organizations of medical sciences) (1985) and the “Regulations on the procedure for the use of laboratory animals in scientific research” (2019) of the Institute of Biophysics and Biochemistry. 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. Male Wistar rats weighing approximately 200 g were used for this study. All experimental animals housed in plastic cages with well-ventilated tops at room temperature with a 12-hour light/dark cycle, while water and food were provided *ad libitum*.

Isolation of mitochondria from rat liver

Rat liver mitochondria were isolated using the method of differential centrifugation [5]. The experimental rats were sacrificed by decapitation, the liver was removed and placed in a buffer solution with pH 7.4 (250 mM sucrose, 10 mM Tris-HCl, 1 mM EDTA). The liver mass was weighed and passed through a micro press with a hole diameter of 1 mm for grinding. The crushed liver tissue was added to the cooled buffer solution in a ratio of 1:6 and homogenized by using a special homogenizer (HG-15A). The resulting homogenate was centrifuged (temperature 0-2 °C) and the centrifugation process was carried out in 2 stages. At the first stage, centrifugation took 7-8 minutes at a speed of 1500 rpm. At the same time, cellular remains and nuclear fractions precipitated. At the next stage, the supernatant liquid was poured into a centrifuge tube and centrifuged at 6000 rpm for 15 minutes. After this, the supernatant was separated, the mitochondria pellet was washed in the isolation EGTA-free medium and stored in a freezer in an ice container.

Analyzing of Opening of Mitochondrial Permeability Transition Pore

One of the main factors that lead to mitochondrial swelling is an opening of mPTP. The kinetics of mitochondria swelling (protein 0.3-0.4 mg/ml) was determined on a spectrophotometer (spectrophotometer V-5000) by changing the optical density at 540 nm in an open cell (volume 3 ml) of the mitochondria suspension at 26°C with constant stirring. To determine the permeability of mitochondrial PTP, the following incubation medium was used: 200 mM sucrose, 20 μM EGTA, 5 mM succinate, 2 μM rotenone, 1 μg/ml oligomycin, 20 mM Tris, 20 mM HEPES and 1 mM KH_2PO_4, pH 7.4 [6]. Mitochondrial suspension was preincubated for 2 minutes after which the solution of $CaCl_2$ was added and the change was monitored for another 4 minutes at 0.5 minutes interval.

Measuring of Lipid Peroxidation

The Fe^{2+}/citrate system was used to study the process of lipid peroxidation in the mitochondrial membrane. Under the influence of this system, the mitochondrial membrane lost its functional state, as a result of the membrane's destruction, the size of the organelle increased and the mitochondria swelled. This change in volume was determined photometrically at 25°C by continuous mixing with the following composition of the incubation medium. Incubation medium (μM): sucrose - 125, KCl -65, HEPES -10, pH 7.2; The number of mitochondria - 0.5 mg / ml; Mitochondria were incubated for 2 min in a medium containing 2 mM citrate, after which 50 μM Fe^{2+} were added [7].

Statistical evaluation

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.

Results and discussions

The effect of Abamectin and Thiamethoxam on mPTP

The effects of studied pesticides are shown as a summary in figure1. First of all, it is demonstrated that in the absence of Ca^{2+} ions in the incubation medium, the mitochondrial swelling is not observed and marked as an intact. To conduct the experiment the $CaCl_2$ at the dose of 30 μM was used as an inductor to induce mitochondrial swelling which was shown as a control. Figure 1 demonstrates the influence of abamectin on the opening of mPTP in three different concentrations. In the presence of abamectin at the concentration of 100μM in the incubation medium, the mitochondrial swelling was increased by 23.6% compared to control. It was demonstrated that abamectin at the concentrations of 150 and 200 μM grew the parameters to 28 and 40.8%, respectively.

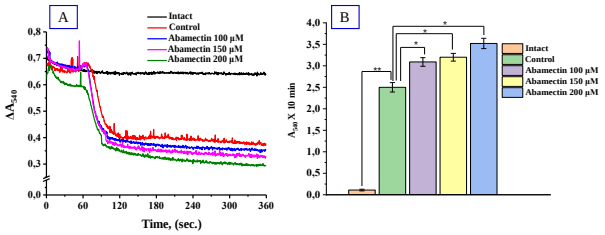

Figure 1. The effect of thiamethoxam on the swelling of rat liver mitochondria A) Original post B) *P<0.05; **P<0.01

The obtained data on figure 1B demonstrates the effect of thiamethoxam in three different concentrations. This pesticide did not cause the opening of mPTP at the concentration of 10 µM. However, thiamethoxam at the concentration of 50 and 100 µM, at the presence of Ca^{2+} ions, the results showed growths of swelling, which are 19% and 21.78% higher compared to control, respectively.

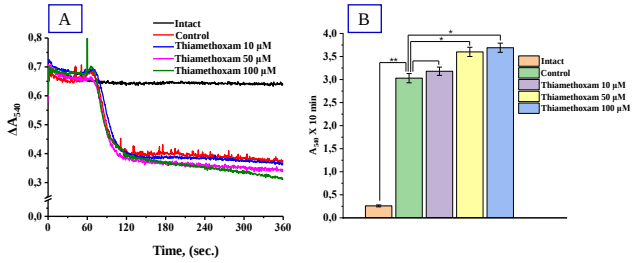

Figure 2. The effect of thiamethoxam on the swelling of rat liver mitochondria A) Original post B) *P<0.05; **P<0.01

LPO related parameters

The Fe^{2+}/citrate system was used to study the LPO process in the mitochondrial membrane. This system is based on the swelling and change in the volume of mitochondria as a result of LPO in the membrane. In our experiments, the swelling of rat liver mitochondria induced by Fe^{2+}/citrate was studied and described on figure 3. In the absence of Fe^{2+}/citrate in the incubation medium, the intact line indicates the absence of the LPO process. However, Fe^{2+}/citrate, being an inducer of LPO, accelerates peroxidation in the mitochondrial membrane and disrupts its barrier function, as a result of which the organ objects increase and swelling of the mitochondria occurs and it was shown as a control. According to our study, the biopesticide abamectin at the concentration of 100 µM increased the LPO for 23.9% compared to control. The following observations related to this study demonstrated that: abamectin at the concentration of 150µM and 200 µM increased the LPO procced to the 28.29% and 39.44%, respectively compared to the control (figure3).

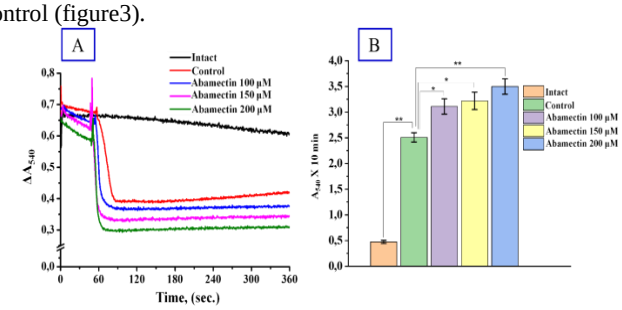

Figure 3. The effect of abamectin on the swelling of rat liver mitochondria caused by LPO induced by Fe^{2+} A) Original post B) *P<0.05; **P<0.01

Continuing the experiment, the next data was obtained, which is described on the figure 4. At the presence of 10µM Thiamethoxam in incubation medium there was an increase to 39.04% compared to control. Following the experiment, there was a growth to a 40.1 and 42.2% while the thiamethoxam was added to the incubation medium at the concentration of 50 and 100 µM.

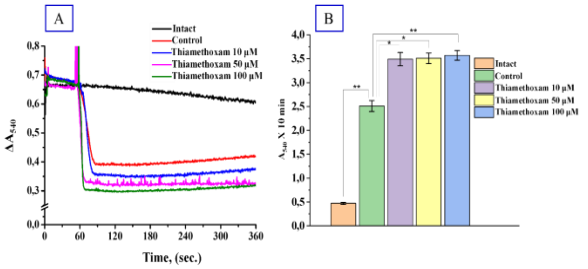

Figure 4. The effect of abamectin on the swelling of rat liver mitochondria caused by LPO induced by Fe^{2+} A) Original post B) *P<0.05; **P<0.01

The obtained results indicate that the effect of the pesticide abamectin in dose-dependent concentrations on mitochondrial damage in rat liver is more pronounced compared to thiamethoxam.

Conclusion. Concentrations of the pesticide abamectin of 100, 150, and 200 µM enhanced Ca2+-induced swelling of rat liver mitochondria and Fe^{2+}/citrate-induced lipid peroxidation in rat liver mitochondria. The pesticide thiamethaxam at a concentration of 10 µM did not affect mitochondrial swelling, but concentrations of 50 and 100 µM partially opened mPTP.

Thiamethaxam enhanced Fe^{2+}/citrate-induced lipid peroxidation in liver mitochondrial membranes, acting as a prooxidant. The pesticides abamectin and thiomethaxam can cause instability of liver mitochondrial membranes *in vitro* conditions.

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