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EFFECT OF PLANT PHOTOSENSITIZER PSORALEN ON MITOCHONDRIAL STRUCTURES IN INFLAMMATORY PROCESSES

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ABSTRACT

The effects of the plant photosensitizer psoralen in various combinations on experimental chronic inflammatory conditions and its mitochondrial structures in the liver have been demonstrated in this paper. Liver mitochondrial suffocation in chronically inflamed rats was found to be 140.0 ± 6.5% higher than in healthy rats. Under conditions of chronic inflammation, high permeability pores of hepatic mitochondria were found to inhibit increased permeability of mPTP by 35.7 ± 2.5% and psoralen by 22.6 ± 1.5% compared with those of chronically inflamed rats. However, under similar conditions, it can be seen that the complex effect of UV-irradiation and psoralen reliably inhibits hepatic mitochondrial suffocation by 50.0 ± 4.5% and maintains the homeostasis of calcium ions in the matrix. Decreased ATP synthesis under the influence of various disorders in the mitochondria alters the cycle of potassium ions in the membrane. Examination of the activity of K+ ATP-channels of isolated liver mitochondria revealed the following results. Experimental chronic inflammation was found to be 21.1 ± 1.2% more active when the K+ ATP-duct permeability of their liver mitochondria was exposed to UV-irradiation than in rats with chronic inflammation. Specific administration of psoralen has been shown to increase mitoK+ ATP -channel activity by 13.1 ± 0.3% compared with rats with chronic inflammation. The complex effect of psoralen and UV-irradiation led to the activation of mitochondrial K+ ATP-channel activity by 30.2 ± 2.4% compared with those of chronically inflamed rats. From the results obtained, it can be concluded that the complex effect of psoralen and UV-radiation showed the highest effect on the structures in the mitochondrial membrane under experimental chronic inflammatory conditions.

Key words: mitochondria, mitochondrial pore, mitoK+ ATP-channel, psoralen, photodynamic therapy.

INTRODUCTION

The functional activity of the mitochondria determines the vital activity of cells and the whole organism. Experimental studies have shown that mitochondrial dysfunction plays an important role in the development of various pathological conditions. The view of the mitochondria as the main organelles that control energy metabolism is now complemented by the view that they are organelles that contain the factors that determine the fate of its cell.

Mitochondria play an important role in various aspects of cell physiology [2]. One of the main features of mitochondria is the formation of transmembrane potential in its inner membrane and its use in ATP synthesis and cation transport. Changes in the permeability of the mitochondrial membrane lead to a decrease in the membrane potential and a sufficient change in its fluorescence and light transmittance using ion-selective electrodes [16,6].

Animal cell mitochondria are the most suitable model for studying the mechanism of action of biologically active substances. On the other hand, mitochondria and structures localized in them, primarily high-permeability pores of the respiratory chain and mitochondrial membrane (permeability transition pore, PTP, megapora, Ca^{2+} -bound megacanal) are the target for exposure to biologically active substances [9,19]. Currently, the role of mitochondrial PTP in cell vital activity and physiological processes, as well as in the development of various pathologies is being actively discussed. It is known that mitochondrial megaporas - mPTP play a key role in the development of various cell pathologies, as well as cell death - necrosis and apoptosis. The formation of reactive oxygen species in the cell and the over-activation of free radical oxidation processes are considered as a universal mechanism for the development of various pathologies [22]. It is also known that mitochondria are the main source of reactive oxygen species in the respiratory chain [10].

It is known that many physiological processes of mitochondria and cells are controlled at the level of mitochondrial megaporas (mPTP) [3,7]. Impairment of mitochondrial function leads to the development of various pathologies: the formation of reactive oxygen species (ROS), dysfunction of ion channels, lipid peroxid activation, oxidation of membrane thiol groups, etc. [4,18]. In this regard, modern research pays special attention to the effect of mPTP management mechanisms on potential drugs.

Photodynamic therapy (PDT) is a photochemical therapy in which oxygen interacts with a photosensitizer (PS) and light of a certain wavelength to form singlet oxygen, which in turn reacts very rapidly with cellular structures, damaging them and even killing them. leads to Photodynamic therapy has a wide range of effects, both directly, at the cellular level, and indirectly - the effects of

pathological tissue on vascular injury and immunomodulation are known [8]. By studying the mechanisms of PDT, it is possible to increase the effectiveness of its application in clinical practice.

Research conducted by the Institute of Plant Chemistry of the Academy of Sciences of Uzbekistan has shown that a number of plants of the local flora are a source of natural compounds with photodynamic properties. One of the plants with similar photosensitizing properties is fig. Scientists have found that fig leaves contain a certain amount of the two most active furanocoumarins - psoralen and bergapten [23]. Under the influence of light of a certain wavelength, psoralen can modify biological molecules in two ways: as a result of oxygen-independent photobiotic reactions and as a result of oxidative photoreactions [11].

Furanocoumarins have an effective effect on the membrane processes of mitochondria. Furanocoumarins have a protective effect on mitochondrial membranes by stimulating membrane potential [5]. The protective and restorative properties of furanocoumarins have been observed in tumors of the breast, lungs, kidneys, liver, colon, cervix, ovaries and prostate gland [1]. Apoptosis, autophagy, participation in the antioxidant cell cycle, effective in activating V-cells have been reported in the literature [1]. In this study, we conducted experiments showing the role of furanocoumarins in the treatment of chronic inflammation. The effects of radiation and psoralen on rats in chronic inflammatory conditions have been studied at the level of hepatic mitochondria.

The purpose of the study

To study the effect of the use of psoralen in different combinations (with UV-irradiation and separately) on the structures of the liver mitochondria in experimental chronic inflammatory conditions.

Materials and Methods

The experiments were initially performed on mature white male rats weighing 180–220 g, quarantined under standard vivarium conditions for 14 days. The “cotton plate” method was used to study anti-inflammatory activity [15]. Clinically healthy rats with clean skin were divided into 5 groups for the study.

The initial radiation dose was determined by determining the minimum erythemal dose (MED). This was found to be 2 minutes. We administered psoralen at a dose of 10 mg / kg. One day after the last administration of the drug (on the eighth day), the animals were removed from the experiment by decapitation under light ether anesthesia. The cotton balls were separated along with the granulation tissue formed around them, weighed on an electronic scale (SINKO, Japan) and dried at 60° C for 3 days until they reached a constant weight. The degree of proliferative stage was assessed by the difference between the mass of the dried

granule and the initial mass of the balloon. The exudative reaction was evaluated by the difference between the masses of the wet and dried bubbles [21].

Table 1

Scheme of experiments in rats using the method of "cotton plate"

Animal group	The scheme used	UV radiation transmission time and exposure distance			Psoralen insertion path and transmission frequency
		First day, the day of surgery	4th day	7th day	
1-group	healthy	-	-	-	-
2-group	untreated	UV-irradiated			Psoralen was not sent
3-group	UV radiation	2 minutes at a distance of 50 cm	2 minutes 30 seconds 50 cm away	3 minutes at a distance of 50 cm	Psoralen was not sent
4-group	Psoralen	UV-irradiated			The drug was administered to the stomach once every 3 days for 7 days
5-group	Psoralen + UV radiation	2 minutes at a distance of 50 cm	2 minutes 30 seconds 50 cm away	3 minutes at a distance of 50 cm	The drug was administered to the stomach once every 3 days for 7 days

The experiments were carried out in accordance with the “Rules for the Use of Experimental Animals” as well as the provisions of the European Convention

for the Protection of Animals Used for Experimental Research or Other Scientific Purposes (ETs № 123, Strasbourg, 03/18/1986).

Mitochondria were isolated from rat liver using the W.C.Schneider [17] method of differential centrifugation. Separation medium composition: 250 mM sucrose, 10 mM tris-chloride, 1 mM EDTA, pH 7.4.

Determination of mitochondrial PTP permeability. Mitochondrial swelling (swelling) kinetics (0.3–0.4 mg / ml) was determined by varying the optical density of the mitochondrial suspension at 26 ° C in an open cell (volume 3 ml) at 540 nm. The following incubation medium (IM) was used to determine the permeability of PTP in mitochondria: 200 mM sucrose, 20 μM EDTA, 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 [7].

Determination of mitochondrial ATP-dependent potassium channel activity. MitoK^+_{ATP} -channel conductivity (0.3–0.4 mg / ml) was determined by varying the optical density at a wavelength of 540 nm in 3 ml cells. IM were as follows: 125 mM KCl, 10 mM Hepes, 5 mM succinate, 1 mM $MgCl_2$, 2.5 mM K_2HPO_4, 2.5 mM KH_2PO_4, 0.005 mM rotenone, and 0.001 mM oligomycin, pH 7.4 [20,12].

Statistical processing of the obtained results was carried out using computer software OriginPro 7.0 (Microsoft, USA). In the experiments, the kinetics of hepatic mitochondrial suffocation were performed as a percentage of the maximum, by calculating the arithmetic mean of 4 different experiments. The difference between the values obtained from the control and the experiment was calculated on the t-test. In this case, the values of $P<0.05$ and $P<0.01$ represent statistical reliability.

Results and Discussion

In evaluating the anti-inflammatory activity, it can be seen that all the treatment combinations performed stopped the development of the granule. When psoralen was used without UV irradiation, the mass of dry granulation-fibrous tissue was 48.66 ± 5.57 mg, and group II-chronic inflammation was 44.2% lower than in the untreated group. Similarly, the exudate mass was 288.8 ± 24 mg, which was 17.6% lower than that of group II animals (Table 2). When used in combination with psoralen + UV-irradiation, the mass of dry granulation-fibrous tissue was 33.4 ± 3.1 mg, and the mass of exudate was 178.4 ± 14.71 mg, which was 61.5% and 49.2% lower than in the control group, respectively. This means that psoralen was more effective when used with UV radiation than psoralen itself. When exposed to UV radiation itself, the mass of dry granulation-fibrous tissue

was 48.33 ± 3.8 mg, and the mass of exudate was 269 ± 22.37 mg, which is 44.5% and 23.3% higher than in group II, respectively.(Table 2).

Table 2

Effect of psoralen, UV and psoralen + UV treatment regimens on inflammatory stages in rats, n = 6 (M ± m)

Observation groups	Dry granulation-fibrous tissue mass, gr	Exudate mass, gr
Control	87,58±24,1	351,8±36,43
UV radiation	48,33±3,8	269±22,37
Psoralen	48,66±5,57	288,8±24
Psoralen + UV radiation	33,4±3,1	178,4±14,71

P <0.05 relative to the control.

Initially, experiments studied the effects of UV radiation and psoralen on rat liver mitochondrial megaporas (mPTP) in chronic inflammatory conditions. Pathophysiological changes at the level of these mitochondrial membranes can be regulated or corrected using pharmacological agents and plant active substances. The psoralen compound can also pharmacologically modulate molecular changes that occur in liver structures during chronic inflammation.

In experiments, a 20 μM concentration of $CaCl_2$ was used as an inducer to activate high-conductivity pores in the mitochondrial membrane of the liver. According to the results, in the presence of a concentration of 20 μM of $CaCl_2$ in the incubation medium, the rate of suffocation in the presence of mitochondrial Ca^{2+} ions isolated from the liver of healthy group I rats was 0.35 DE540 / 10 min (Fig. 1).

Hepatic mitochondrial obstruction of chronically inflamed group II rats was found to be 0.84 DE540 / 10 min, an increase of 140.0 ± 6.5% compared to the healthy group. This suggests that stress developed in group II rats and that it affected permeability at the mitochondrial membrane level. It is known from the literature that Ca^{2+} ions increase the conductivity of mPTP and transfer it to the open state, matrix swelling is observed [13,14,7].

In experimental group III rats, when exposed to UV radiation for chronic inflammation, their mPTP permeability in the liver mitochondria was found to be 0.54 DE540 / 10 min, inhibiting 35.7 ± 2.5% compared to group II rats (Fig. 1). UV radiation has been shown to partially inhibit the function of the highly

permeable pores of the liver mitochondria resulting from chronic inflammation in rats.

In experimental group IV rats, chronic inflammation was induced and they were injected orally with a 10 mg / kg dose of psoralen on days 1, 4, and 7, respectively. Mitochondria were then isolated from the rats' livers. Their suffocation under the influence of Ca^{2+} ions was recorded as 0.65 DE540 / 10 min, inhibiting 22.6 ± 1.5% compared to group II. From this result, it was found that hepatic mitochondrial mPTP permeability was inhibited by psoralen and manifested by partial recovery in chronic inflammation.

Continuing the experiments, group V rats with chronic inflammation were exposed to UV radiation and on days 1, 4, and 7, 10 mg / kg of psoralen was administered orally through a probe. After that, mitochondria were isolated from the liver of group B rats. The results showed that rats exposed to chronic inflammation were exposed to UV-radiation in a complex way and that the delivery of psoralen reliably inhibited the suffocation of their hepatic mitochondria in the presence of Ca^{2+} ions. The liver mitochondrial permeability of this group of rats was 0.42 DE540 / 10 min and inhibited by 50.0 ± 4.5% compared to group II.

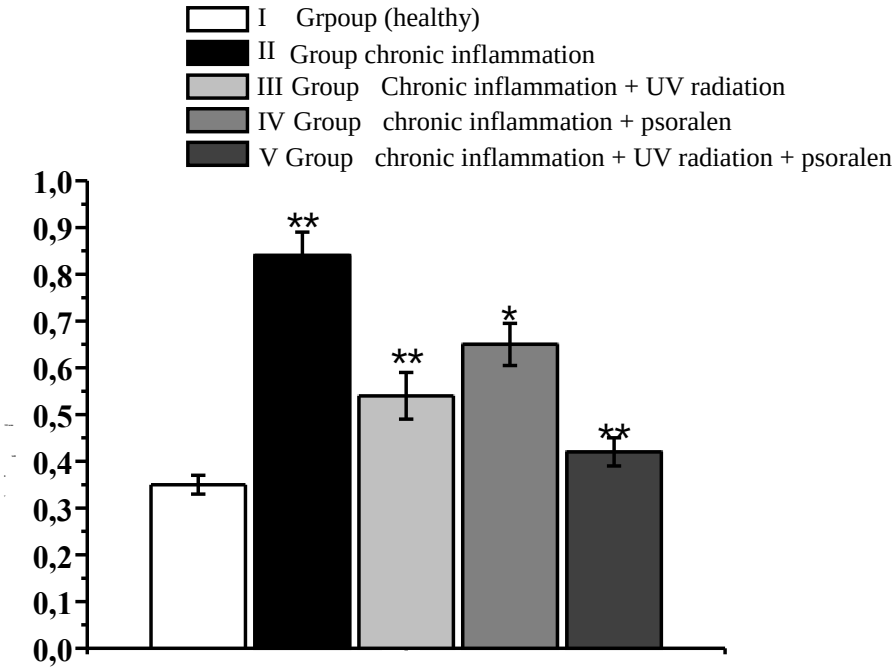

Figure 1. psoralen, UV-irradiation and their complex effects on the highly conductive pores of rat liver mitochondria under conditions of chronic inflammation (* P <0.05; ** P: <0.01; n = 6).

Such changes in the permeability of the mitochondrial membrane that occur in chronic inflammatory processes may also affect other ion-transport systems located in them. Another such ion-transport system located in the mitochondrial membrane is ATP-dependent potassium channels, which can exhibit their functional activity under conditions of physiological concentration of ATP. Decreased ATP synthesis as a result of disruption of electron transport in the respiratory chain and separation of oxidative phosphorylation processes caused by various disorders in the mitochondria alters the cycle of potassium ions in the membrane.

At present, there are no data on changes in the functional activity of K^+_{ATP} - channels of rat liver mitochondria in chronic inflammatory conditions and in cases of UV radiation and psoralen delivery. For this purpose, in our next experiment, rats with chronic inflammation were exposed to UV radiation and psoralen, and their liver mitochondria were isolated. Examination of the activity of K^+_{ATP} - channels of isolated liver mitochondria revealed the following results (Fig. 2). We know that a concentration of 200 μM of ATP in isolated mitochondria leads to a partial inhibition of its functional activity.

The results showed that the activity of K^+_{ATP} -channel of liver mitochondria of group II rats with chronic inflammation caused by inhibition of ATP at a concentration of 200 μM in the incubation medium was inhibited by 45.7 ± 3.4% compared to control (Fig. 2). Hence, the mitochondrial mitochondrial mitoK^+_{ATP} - channel activity of rats may reduce the cycle of potassium ions under the influence of inflammation.

In experimental group III rats, when exposed to chronic inflammation under the influence of UV radiation, it was found that their liver mitoK^+_{ATP} -channel permeability is 21.1 ± 1.2% more active than in group II (Fig. 2). UV radiation has been shown to partially increase liver mitoK^+_{ATP} -channel activity, which results from chronic inflammation in rats.

In experimental group IV rats, chronic inflammation was induced and they were injected orally with a 10 mg / kg dose of psoralen on days 1, 4, and 7, respectively. After that, mitochondria were isolated from the liver of rats and their mitoK^+_{ATP}-channel activity was found to be 13.1 ± 0.3% more active than in group II (Fig. 2).

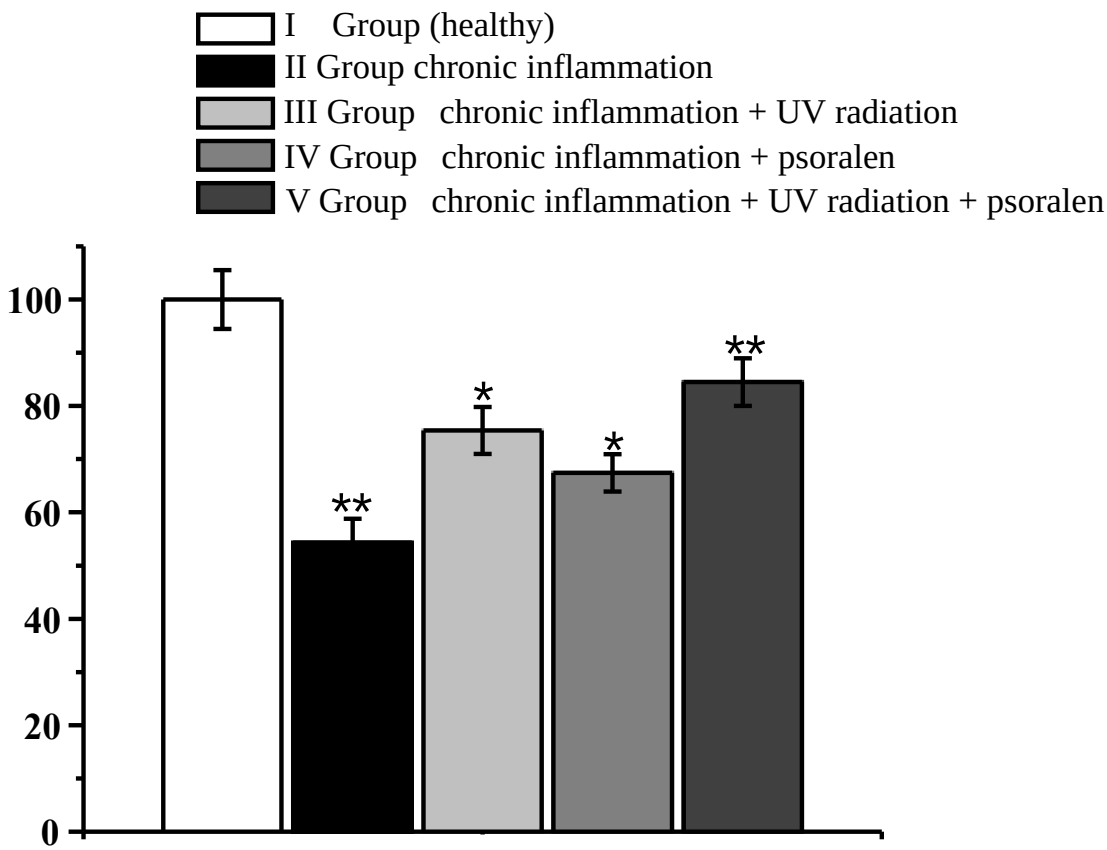

Figure 2. The effect of psoralen, UV-irradiation and their complex on the K^+_{ATP}-channel activity of rat liver mitochondria in chronic inflammatory conditions (* P <0.05; ** P <0.01; n = 6).

Continuing the experiments, group V rats with chronic inflammation were exposed to UV radiation, and on days 1, 4, and 7, 10 mg / kg of psoralen was administered orally through a probe and mitochondria were isolated from the liver. Rats exposed to chronic inflammation were exposed to UV radiation, and the administration of psoralen resulted in their activation of liver mitoK^+_{ATP}-channel activity by 30.2 ± 2.4% compared to group II indicators.

CONCLUSION

1. Mitochondria are the first and most responsive structure within the cell structures in inflammatory processes.

2. In chronic inflammatory conditions, when UV-radiation and psoralen are used separately, the permeability of the liver mitochondrial megaporas - mPTP is partially reduced. However, under the same conditions, the complex effect of UV-radiation and psoralen reliably inhibits this conductivity, allowing water and ions to enter the matrix and reduce its swelling. As a result, damage to the outer membrane of the mitochondria and the release of cytochrome C and proapoptosis

proteins from the matrix to the cytosol and the occurrence of cell apoptosis are prevented.

3. The complex effect of UV-irradiation and psoralen in the conditions of our experimental chronic inflammation activated the permeability of the mitoK^{+} $_{ATP}$-channel in the membrane of the hepatic mitochondria. This is the initial stage of the adaptation process under conditions of cellular hypoxia.

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