RESEARCH OF ANTIOXIDANT AND PROOXIDANT PROPERTIES OF GLYCYRRHETIC ACID DERIVATIVES

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Abstract. *It was studied the effect of new synthetic derivatives of glycyrrhetic acid (GRA) on the functional mitochondrial parameters, as well as the parameters of the CsA-sensitive pore of mitochondrial membranes in the article. For the first time it was shown that GRA derivatives 2-(N-cytisine)-ethyl-3-0-acetyl-18βH-glycyrrhetate (cytisine-ethyl-HC), 2-(N-cytisine)-isopropyl-3-0-acetyl-18βH-glycyrrhetate (cytisine-isopropyl-HC) and N-(2-pyridyl)-3-0-acetyl-11-ketoolean-12-en-30-amide (2-pyridyl-HC-amide) inhibited the activity of the CsA-sensitive pore and have a protective effect on mitochondrial membranes. At the same time, the damaging effect of Ca^{2+} ions and the LPO process decreases. Other GRA derivatives are N-(4-pyridyl)-3-0-acetyl-11-ketoolean-12-en-30-amide (4-pyridyl-HC-amide) and 2-(N-morpholine)-ethyl-3-0-acetyl-18βH-glycyrrhetate (morpholine-HC) uncouples OXPHOS, enhances the damaging effect of inducers of the CsA-sensitive pore on membranes, increasing their permeability to cations*

Keywords: *mitochondria, membrane permeability, CsA-sensitive pore, lipid peroxidation, oxidative phosphorylation (OXPHOS), antioxidants, prooxidants, free radicals, apoptosis, necrosis, glycyrrhetinic acid derivatives.*

Introduction

Modern scientific data suggest that mitochondria and the CsA-sensitive pore are targets for the action of various biologically active substances, pathogens, and pharmaceuticals (Kamburova, 2001; Akinshina, 2001; Dehpour et al., 1999; Чулиев, И.Н и.др, 2005). In this regard, the mechanisms of regulation of the functional state of the Ca^{2+}-dependent CsA-sensitive mitochondrial pore and other Ca^{2+}-dependent intracellular processes by biologically active compounds have recently been actively studied in many laboratories around the world (Zeuzem, 1998). To regulate the functional parameters and state of the CsA-sensitive pore, herbal preparations are often used, the biological and pharmacological activity of which is due to their membrane-active properties. Chemical modification of natural compounds can change their biological and pharmacological properties (Baltina et al., 1992; Tolstikov et al., 1997; Beskina et al., 2000; Beskina, 2002; Kamburova, 2001).

The study of the role of lipid peroxidation (LPO) in the regulation of the most important functions of the cell is of interest for a number of reasons (Vladimirov, 2000). LPO induction in mitochondria leads to a change in membrane permeability, a decrease in membrane potential, uncoupling of OXPHOS and ATP hydrolysis. The effect of LPO on mitochondrial functions is realized both at the level of the direct effect of LPO products on the lipid matrix of membranes, and various indirect effects.

One of the most important mechanisms through which LPO reactions can indirectly regulate mitochondrial functions is the CsA-sensitive pore, the transition of which to the open state is considered as an essential stage of mitochondrial damage during oxidative stress (OS) and associated necrosis or apoptosis. Free radicals are highly reactive compounds that can damage the structure and function of animal and plant cells (Sattorova, I. Y., & Cho'liyev, I. N., 2020). Organisms are constantly exposed to them. First, they are constantly formed as a result of natural metabolic processes occurring in the cell. Secondly, free radicals are formed under the influence of external factors, both natural and anthropogenic or man-made (under the influence of polluted environment, smoking, radiation, household chemicals).

It is known that plant compounds are the main source of biological material in the production of drugs with antioxidant properties (Potapovich., 2003; Tolstikov et al., 1997).

It is known that free radicals are the main cause of many human and animal diseases (Vladimirov, 1998). The body also has an antioxidant system that protects the body from free radicals. Antioxidants are able to neutralize the activity of free radicals, protect cell membrane phospholipids from oxidation (Vladimirov, 1998).

A natural question arises as to whether the glycyrrhetinic acid derivatives that transform the CsA-sensitive pore into a closed configuration have antioxidant properties. In this regard, we studied the effect of glycyrrhetinic acid and its derivatives on the LPO process of mitochondrial membranes. Cumene hydroperoxide (CHP) was used as an LPO inducer.

Results and discussion

As a result of the studies, it was found that glycyrrhetinic acid increases the accumulation of malondialdehyde (MDA) in mitochondrial membranes by 40% (Fig. 1). Similar data were also obtained by other authors. The addition of other derivatives of glycyrrhetinic acid - 2-pyridyl-HC-amide, cytisine-isopropyl-HC and cytisine-ethyl-HC at a concentration of 50 μM prevented the effect of CHP on the level of MDA in isolated liver mitochondria. At the same time, the decrease in the accumulation of MDA was 20%, 40% and 45%, respectively, relative to the control (Fig. 1).

Subsequently, we studied the effect of GrK derivatives on the LPO system induced by Fe^{2+}-ascorbate (Fig. 2). Under these conditions, the derivatives of glycyrrhetinic acid - 2-pyridyl-HC-amide, cytisine-isopropyl-HC and cytisine-ethyl-HC at a concentration of 50 μM prevented the effect of Fe^{2+}-ascorbate on the level of MDA in isolated mitochondria by 15%, 30.6% and 50 %, respectively.

Thus, we found that glycyrrhetinic acid derivatives: 2-pyridyl-HC-amide, cytisine-isopropyl-HC and cytisine-ethyl-HC have antioxidant properties and have a protective effect on mitochondria, reducing the damaging effect of CHP and the LPO process.

One of the mechanisms through which LPO reactions can indirectly regulate mitochondrial functions is the CsA-sensitive pore. Possibly, this mechanism is influenced by biologically active compounds: 2-pyridyl-HC-amide, cytisine-isopropyl-HC and cytisine-ethyl-HC on the functions of mitochondria.

We also studied the effect of other derivatives of glycyrrhetinic acid on the LPO process of mitochondrial membranes, using LPO inductors - CHP and the Fe^{2+}-ascorbate system. When studying the effect of morpholine-HC, 4-pyridyl-HC-amide on the lipid peroxidation of mitochondrial membranes, it was shown that these derivatives acted on the state of the mitochondrial pore, increasing the passive permeability of the membranes and reducing the Ca^{2+}-

capacity of mitochondria. Previous experiments with glycyrrhetinic acid showed that it increases the accumulation of MDA in mitochondrial membranes. However, the effects of the compounds of morpholine-HC and 4-pyridyl-HC-amide at a concentration of 50 μM are weaker than the classical LPO inducers - CHP and the Fe^{2+}-ascorbate system (Fig. 1., Fig. 2 and Fig. 3). An increase in the concentration of drugs in the incubation medium led to a further increase in the accumulation of MDA in mitochondrial membranes.

As experiments have shown, in the presence of CHP, an increase in the accumulation of MDA is observed. Against this background, preparations of morpholine-HC and 4-pyridyl-HC-amide (50 μM) led to a further increase in the accumulation of MDA in mitochondrial membranes. Higher concentrations of morpholine-HC and 4-pyridyl-HC-amide in SI led to a further increase in MDA accumulation in mitochondrial membranes by 26% and 30%, respectively. The results obtained confirm our assumption that the morpholine-HC and 4-pyridyl-HC-amide compounds have prooxidant properties (Fig. 1.).

Similar results were also obtained during induction by the Fe^{2+}–ascorbate system (Fig. 2). Subsequently, we studied the effect of morpholine-HC and 4-pyridyl-HC-amide on the LPO system induced by Fe^{2+}-ascorbate (Fig. 2 and Fig. 3). Under the same conditions, the preparations tested by us at a concentration of 50 μM contributed to a further increase in the accumulation of MDA in mitochondrial membranes by 30% and 37%, respectively.

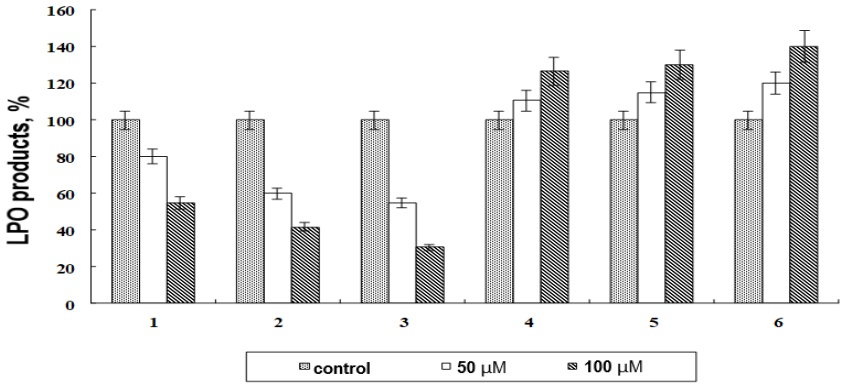

Fig. 1. Effect of glycyrrhetinic acid derivatives on GPA-dependent LPO

1. - 2-pyridyl-GC-amide; 2. - cytisine-isopropyl-HC; 3. - cytisine-ethyl-HC; 4. - 4-pyridyl- GC - amide; 5. - morpholine- GC; 6. - glycyrrhetinic acid. (n=6, P<0.05).

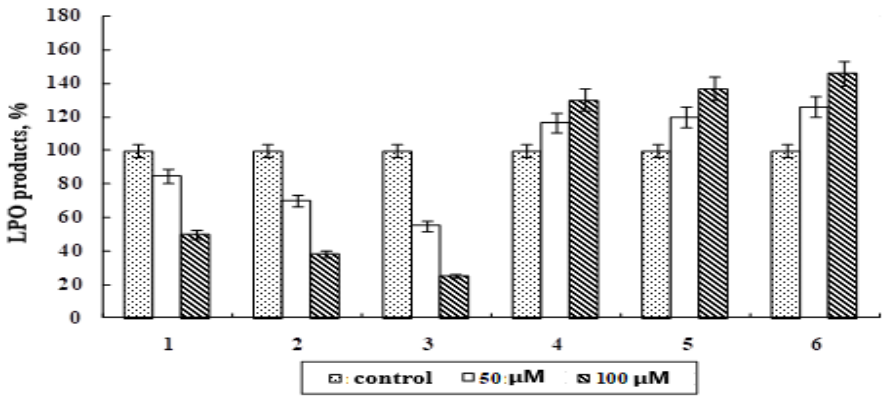

Fig. 2. Effect of glycyrrhetic acid derivatives on Fe-ascorbate dependent LPO

1. - 2-pyridyl-HC-amide; 2. - cytisine-isopropyl-HC; 3. - cytisine-ethyl-HC; 4. - 4-pyridyl-HC-amide; 5. - morpholine-HC; 6. - GrK. (n=6, P<0.05).

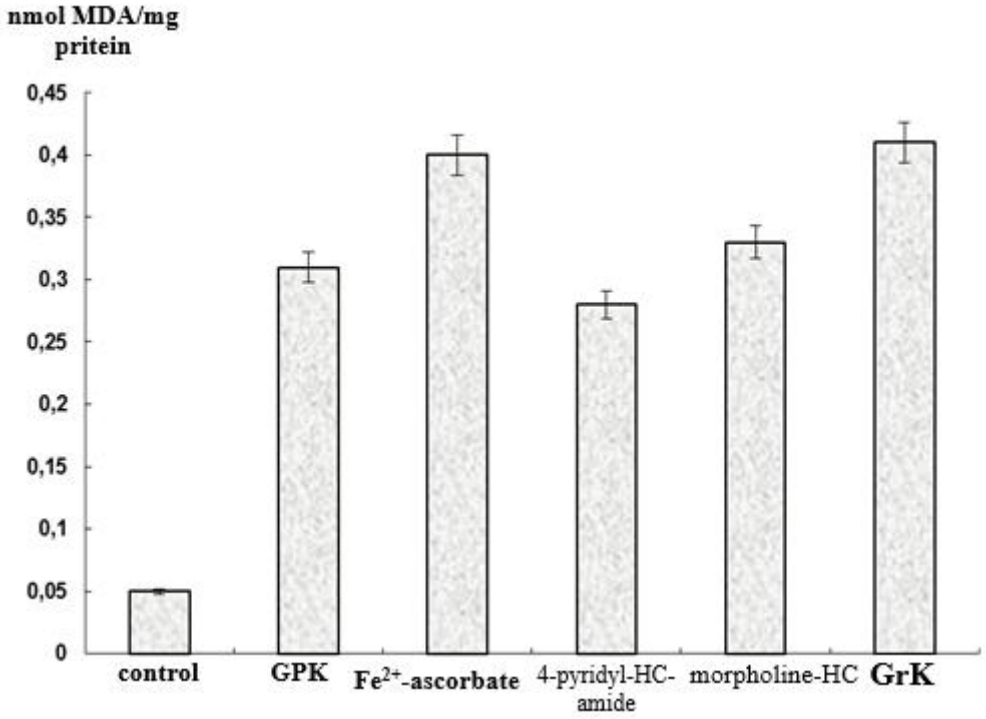

Fig. 3. Influence of GrK derivatives - GrK morpholine-HC and 4-pyridyl-HC-amide on the LPO system*

Notes. The concentration of GrA and its derivatives is 50 μM. (n =6, P<0,05).

It is known that one of the mechanisms of violation of the CsA-sensitive pore is the intensification of lipid peroxidation against the background of a decrease in the activity of antioxidant defense enzymes - catalase and superoxide dismutase. As a result, membrane desensitization and an increase in their permeability to various ions and substances are observed. Some of the compounds we studied reduced the level of MDA in membranes that indicated their antioxidant properties.

Thus, 2-pyridyl-HC-amide, cytisine-isopropyl-HC and cytisine-ethyl-HC have antioxidant properties and have a protective effect on mitochondria, reducing the damaging effect of CHP and the Fe^{2+}–ascorbate system, and other glycyrrhetic acid derivatives: morpholine-HC and 4-pyridyl-HC-amide have pro-oxidant properties, enhancing the damaging effect of CHP and the Fe^{2+}–ascorbate system.

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