BioGecko Vol 12 Issue 03 2023

ISSN NO: 2230-5807

EFFECTS OF ULTRASOUND WAVES ON RAT LIVER MITOCHONDRIA

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Abstract: This article presents information on the increase of lipid peroxide oxidation (LPO), the sharp decrease of cytochrome-c-oxidase and succinate dehydrogenase activities in rat liver mitochondria under the influence of ultrasound waves. Alteration of LPO resulted in impaired functional activity of membrane-bound cytochrome -c -oxidase and succinate dehydrogenase. This, in turn, affects the antioxidant defense system. The extract of horsetail leaf and the oil extract of biosep used in the studies showed antioxidant properties and led to a certain degree of restoration of their activity.
Key words: liver, mitochondria, lipid peroxide oxidation, extract, biosep, ultrasound wave, cytochrome-c-oxidase, succinate dehydrogenase.

Relevance of the topic: Currently, ultrasound waves are widely used in medicine for diagnostic purposes. While the methods of ultrasound waves are effective methods in biological and medical research, according to some literature, ultrasound waves cause various biological effects. These effects can be mechanical, thermal and physico-chemical [1, 2].

Currently, a lot of information on the biological effects of ultrasound waves has been collected, but most of this information is the result of observing the effects of ultrasound waves used for therapeutic purposes.

Taking into account the above, it is of great importance to study the formation of free radicals as a result of the influence of external factors on the body and the related antioxidant system.

It is known that detoxification processes are carried out in all organs, especially in the liver. Liver damage affects metabolism. The emergence and development of a number of pathological conditions in humans and animals occurs as a result of the activation of LPO in cells [3].

In membranes, LPO is under normal conditions controlled by the antioxidant defense system. As a result of metabolism in living organisms, oxidized products - "free radicals" and peroxide compounds of other organic and inorganic substances are formed. Under the influence of adverse factors, this process develops rapidly. Free radicals damage cells and disrupt the function of the immune system. This leads to various infectious diseases and degenerative diseases, including cancer and cardiovascular diseases. The following groups of protein free radicals are known in the body - peroxides, hydroxides, peroxides of various lipids, radicals of hydrochlorides, etc. The reactive oxygen species produced by stress causes increased LPO in the inner and outer membranes of mitochondria, leading to tissue and organ damage [3, 4, 5].

In the body, LPO products affect a number of processes, that is, they inhibit the activity of membrane-dependent enzymes, increase their permeability, and eventually lead to its degradation [6].

Taking into account the above, the aim of the work is to study the effect of ultrasound waves on rat hepatocytes at the molecular level. First of all, it is necessary to study the effect of ultrasound waves on LPO, membrane-bound enzymes, and certain activities of enzymes of the antioxidant system in liver mitochondria.

Cytochrome-c-oxidase (cytochromoxidase) or cytochrome-c-oxidoreductase, also known as cytochrome aa3 and terminal oxidase, which transports electrons in the aerobic respiratory chain complex IV, is responsible for the transfer of electrons from cytochrome-c to oxygen due to the formation of water. catalyze[7]. Cytochrome-c-oxidase is present in the inner mitochondrial membrane of all eukaryotes, commonly known as complex IV, and is also found in the cell membranes of many aerobic bacteria [8]. Cytochrome-c-oxidase enzyme participates in the transport of electrons from complex III to complex IV in the mitochondrial membrane [6, 9]. As a result of the influence of various external factors, there is a change in the activity of cytochrome-c-oxidase enzyme dependent on the mitochondrial membrane, a decrease in membrane potential and ATF synthesis [10].

Succinate dehydrogenase (SDG) is widespread in plant and animal cells, is located in the inner membrane of mitochondria and is one of the main enzymes of energy metabolism [11, 12, 13].

SDG catalyzes the oxidation of succinate to fumarate in the Krebs cycle, and the resulting electrons are transported to complex III of respiration to reduce oxygen and produce water [14].

However, studies on changes in rat liver mitochondria cytochrome -c -oxidase and SDG enzyme activity under the influence of ultrasound waves have been little studied [15, 16, 17].

The purpose of the study: the effect of ultrasound waves on the activity of LPO, succinate dehydrogenase and cytochrome-c-oxidase of rat liver mitochondria and the corrective effect of horsetail leaf extract and biosep oil extract were studied.

Research methods and materials. The studies were conducted on purebred white female laboratory rats weighing 150-220 g. Mindrey DP-50 Vet ultrasonic waves device designed for animals was used in this work. Rats were exposed to 7.5 mHz for 5 minutes.

For the experiment, the rats were divided into separate model groups for the effect of ultrasound waves and their correction:

Group I healthy (control) (n=5)

Group II was exposed to ultrasound waves for 5 minutes (n=5-6)

Group III ultrasound waves + mulberry extract (n=5-6)

Group IV ultrasound waves + biosep (n=5-6)

In the experiment, after exposure to ultrasound waves for 5 minutes, group III rats were given 1 ml per body weight extract of horsetail leaf once a day for 5 days through a special probe, and group IV rats were given 1 ml of biosep oil extract pre-orally for 5 days.

The activities of LPO, succinate dehydrogenase and cytochrome-c-oxidase in liver mitochondria were studied 1, 3, 5, 10 and 15 days after administration of horsetail leaf extract and biosep oil extracts to ultrasound-exposed rats.

Rat liver mitochondria were isolated using W.C.Schneider's differential centrifugation method. 0.25 M sucrose - TKM buffer solution was used to isolate mitochondria from liver tissue. A 1:10 homogenate of tissues was prepared and centrifuged at 1000 rpm for 10 minutes. The sediment was discarded and the liquid part was centrifuged at 12000 rpm for 10 minutes. Detection of peroxidation of lipids is based on the reaction between malondialdehyde (MDA) and thiobarbituric acids (TBK), which results in the formation of a colored trimethine complex at high temperature and acidic pH [18]. The complex is measured in a UV/VIS spectrophotometer at a wavelength of 532 nm.

A spectrophotometric method was used to determine the activity of the cytochrome-c-oxidase enzyme, in which the rate of oxidation of cytochrome-c regenerated by dithionite was determined [19]. Enzyme activity was determined in spectrophotometer at a wavelength of 550 nm. 2.2 ml of 0.2 M potassium phosphate buffer with pH 7.0 and 0.2 ml of $2*10^{-5}$ molar reduced cytochrome c were added to a 3 ml cuvette. The reaction was initiated by the addition of mitochondria suspended in 0.25 M sucrose in Tris-HCl buffer. Enzyme activity was expressed in μmol/min.mg protein.

SDG activity was determined in a UV/VIS spectrophotometer at a wavelength of 540 nm. Determination of this enzyme activity is based on the reduction of tetrazole salts, where N+ ions pass from the substrate through $FAD^{+}$to tetrazole salts. The peculiarity of tetrazole is that it is easily reduced by forming compounds - formazins, which are brightly colored, soluble in water, but insoluble in acetone. To determine enzyme activity, the following incubation medium is required: 0.2 ml of 0.2 M magnesium chloride solution, 0.2 ml of 33 mM ATPsolution, 0.4 ml of 0.2 M phosphate buffer (pH 8.0). 0.2 ml of mitochondrial suspension was added to 0.8 ml of incubation medium and incubated at 37^0C for 10 minutes. The reaction was continued by adding 0.1 ml of sodium succinate solution. 0.4 ml of 0.1% nitrotetrazol blue is added and the samples are incubated at 37^0C for 30 minutes.

The reaction was stopped by adding 3.5 ml of acetone. The precipitate was removed by centrifugation at 3000 rpmfor 10 min. The optical density of the solution was measured at a wavelength of 540 nm. Enzyme activity is expressed in mg protein in nmol/min[20].

The amount of protein in mitochondria was determined by Lowry's method. The difference between the results obtained from control, experimental and experimental+mulberry, experimental+biosep groups was calculated by t-test.

The obtained results and their analysis.

According to the results of the study, when ultrasound waves in the range of 7.5 MHz were exposed to the liver of rats for 5 minutes through the Mindrey DP-50 Vet ultrasound machine, the amount of LPO product MDA in the liver mitochondria membrane of this group of rats on 1, 3, 5, 10 and 15 days was lower than that of the control (group I) 72.73±0.7%, 47.02±0.4%, 21.39±0.2%, 19.76±0.2%, 7.91±0.1% respectively was found to have increased to.

This indicator indicates the acceleration of the LPO process in mitochondrial membranes on days 1, 3, 5, 10 after exposure to ultrasound waves (table-1). This, in turn, leads to disruption of membrane structures and changes in LPO in rat liver mitochondria. It was observed in the experiments that the LPO in the liver mitochondria of the groups of rats corrected with the extract of the birch leaf and the extract of the biosep oil was restored to a certain extent.

Table -1

Effects of mulberry leaves and biosep extracts on MDA content of ultrasound-treated rat liver mitochondria (1, 3, 5, 10, and 15 days dependent dynamics) (nmol/min mg protein)

	eriencegroups		**y**	**y**	**y**	**lay**	**lay**
	rol		±0.033	±0.035*	±0.033	±0.040	±0.032
	sound		±0.017*	±0.032*	0.017*	0.023*	±0.011*
	sound+mulberry		±0.011*	±0.020*	±0.017*	±0.011*	±0.024*
	sound+biosep		±0.027*	±0.024*	0.027*	±0.026*	±0.014*

Explanation: (*p<0,05, n=5-6)

The amount of MDA in liver mitochondria in rats of group III, corrected with mulberry leaf extract, compared with the values of group II, was 44.85 ± 0.9% on the 1st day and 27.38 ± 0.3% on the 3rd, 5th day, respectively. f, 10th and 15th days y It was established that it decreased by 25±0.2%, 10.18±0.2% and 9.61±0.9%.

In group IV rats, which were corrected with Biosep oil extract, the MDA content on days 1, 3, 5, 10 and 15 after exposure to ultrasound waves was 26.06±0.3%, 13.69±0.1%, 5, 8±0.1%, 4.79±0.05% and 5.09±0.3% compared with group II, which shows its significant decrease.

In groups (groups III and IV) corrected with herbal antioxidant preparations, i.e. mulberry extract and biosep fatty extracts, lipid peroxidation in rat liver mitochondria compared with group IV, lipid peroxidation in liver mitochondria of rats of group III was 18.79% on day 1, 13.7% on day 3, 3.5% on day 5 day and had a high correlation effect of 5.4% on day 10 and 4.5% on day 15.

The activity of enzyme cytochrome-s-oxidase, dependent on the mitochondrial membrane of hepatocytes, compared to the group of crys etoy 44.5±0.3%, 40.7±0.2%, 35±0.2% , 31±1.8%, 29.1±1.9%, corresponding to 1, 3, 5, 10 and 15 days.This indicates the inactivation of cytochrome-c-oxidase enzyme activity in mitochondria under the influence of ultrasound waves. In this group of rats, a sharp decrease in the activity of the cytochrome -c -oxidase enzyme in the liver mitochondria compared to the control was detected on the 1st and 3rd days after exposure to ultrasound waves, i.e., it was found to be decreased by 44.5±0.3% and 40.7±0.2%, respectively.

A significant effect on the activity of the cytochrome c oxidase enzyme was found in the liver mitochondria of group III rats, corrected with mulberry leaf extract (Fig. 1). On days 1, 3, 5, 10, 15, its activity was 5.1±0.6%, 5.4±0.9%, 5.7±3.9%, 8.8±0.6%, 13.4±1.1%, respectively, compared with group II. In this group, a significant recovery of enzyme activity in the mitochondria of rat hepatocytes was observed by the 15th day(Table 2).

Table -2

Effects of mulberry leavesand biosep extracts on cytochrome-c-oxidase enzyme activity of rat liver mitochondria exposed to ultrasound waves (1, 3, 5, 10 and 15-day dynamics dependent) (µmol/min.mg protein)

	eriencegroups		**y**	**y**	**y**	**lay**	**lay**
	rol		9±0.77	2±0.90	2±0.76	4±0.77	3±0.65

	sound		3±0.31*	5±0.42	2±0.43	9±0.50*	3±0.56*
	sound+mulberry		4±0.30	4±0.69*	3±0.99*	5±0.56	9±0.68
	sound+biosep		7±1.03*	3±0.39	9±0.49	5±0.53*	5±0.83*

Explanation: (*p<0,05, n=5-6)

The activity of the cytochrome c-oxidase enzyme in the mitochondria of hepatocytes of group IV rats, corrected with the Biosep fatty extract, showed an increase in the enzyme and amounted to 3.9±1.8%, 4±1.2%, 4.2±3.8%, 4 .2±3.8%, 4.5±0.4%, 14.1±0.6% compared with group II.

According to the results of studies, the enzymatic activity of SDH in rat liver mitochondria under the influence of ultrasonic waves decreased by 37.8±1.5%, 34.57±2.3%, 25.5±3.2%, 16.7±1.6% , 8.6±1.1%(Table 1).

Table -1

Effects of mulberry leaves and biosep extracts on succinate dehydrogenase enzyme activity of rat liver mitochondria exposed to ultrasound waves (1, 3, 5, 10 and 15 day dynamics depend) (nmol/min 1 mg belka) (*R<0.05, n=5- 6)

	eriencegroups		**y**	**y**	**y**	**lay**	**lay**
	rol		±0.055	4±0.026	±0.019	0.011	3±0.011
	sound		±0.014*	±0.023*	±0.015*	±0.022*	±0.016*
	sound+mulberry		±0.024*	±0.016*	±0.016*	±0.020*	±0.047*
	sound+biosep		±0.032*	±0.01*3	±0.028*	±0.041*	±0.026*

Explanation: (*p<0,05, n=5-6)

The results showed that SDH activity decreased in rat liver mitochondria under the action of ultrasonic waves, and it was found that the activity of the enzyme in this group decreased by 37.8±1.5% and 34.57±2.3% in the 1st and 3rd day, respectively, compared with the control group.

A significant effect of mulberry leaf extract on SDH activity in liver mitochondria of group III rats was found (Table 1). On days 1, 3, 5, 10, 15, its activity was 10±0.8%, 10.9±0.9%, 8.9±0.9%, 4.8±0.4% and 4, 7±1.1%. The activity of enzymes in mitochondria of hepatocytes of rats of this group was significantly restored by the 10th and 15th days.

The activity of SDH in mitochondria of hepatocytes of group IV rats, corrected with Biosep oil extract, was 6.2±0.4%, 7.7±0.5%, 7.9±0.6%, 2.4±0.3% , 2.9±0.2% compared with group II.

Based on the results obtained, it was found that the corrective effect of the mulberry extract is more effective than biosept.

Thus, under the action of ultrasonic waves in rat liver mitochondria, an increase in lipid peroxidation, a sharp decrease in the activity of cytochrome s-oxidase and succinate dehydrogenase were observed. Changes in lipid peroxidation led to disruption of the functional activity of membrane-bound cytochrome c-oxidase and succinate dehydrogenase. This, in turn, affects the antioxidant defense system. The oil extracts of mulberry leaves and biosep used in the studies showed antioxidant properties and led to some restoration of their activity.

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