Effects Of Ultrasound Waves On Rat Hepatocyte Mitochondria Antioxidant Enzymes - Superoxide Dismutase And Catalase And Search For Their Correction Methods

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Abstract

After exposure to ultrasound, the activity of antioxidant enzymes – superoxide dismutase(SOD) and catalase significantly decreased on the 1st and 3rd days, and in the groups corrected with horsetail and biosep extracts, these drugs showed their antioxidant properties. That is, on the 10th and 15th days, it was found that the activity of the enzymes was restored to a certain extent. The results of the conducted research showed that the antioxidant property of the extract of sagot was more effective than that of the extract of biosep.

Keywords: mitochondria, hepatocyte, enzyme, extract, biosep, ultrasound wave, buffer, catalase, superoxide dismutase

Introduction

The formation of free radicals in the body of the rat under the influence of ultrasound leads to a change in the activity of the antioxidant system that protects the body from these free radicals. Antioxidants are any compounds that reduce the formation of free radicals [1].

The body has a system of antioxidant enzymes, which include superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione transferase, and peroxidases. Enzymes of the antioxidant system catalyze biochemical reactions in the body and convert the resulting toxic products - peroxidation of lipids and free radicals into harmless substances.

Superoxide dismutase (KF 1.15.1.1)(SOD) is the main antioxidant enzyme, which ensures reduction of O2•- free radical to H2O2 [2]. SOD catalyzes the reaction in two steps. First, an electron is released from the free radical O2•- and transferred to the metal in the active center of the enzyme. As a result, a free O2 molecule is formed. The extra electron is then transferred to the second molecule of superoxide, which forms H2O2.

The complete reduction of H2O2 to water and molecular oxygen is catalyzed by the enzyme catalase (KF 1.11.1.6) [3].

2H2O2→2H2O+O2

Catalase belongs to the class of oxidoreductases and catalyzes the hydrolytic cleavage of O-O bonds in hydrogen peroxide. It is present in almost all systems where electron transport is carried out with the participation of
cytochromes, that is, in systems where hydrogen peroxide, which is toxic to the cell, is formed. The enzyme is mainly located in the cell's peroxisome and cytoplasm. In the human body, a high amount of catalase enzyme has been detected in erythrocytes, as well as in the liver and kidneys[4].

Also, in the literature, the effect of ultrasound on the enzymes of rat liver mitochondria was studied, and it was determined that the deep inhibition of enzymes was observed on the 1st and 3rd days after the effect of ultrasound [5, 6, 7, 8]. This condition, in turn, depends on the change in peroxygen oxidation of membrane lipids in rat liver mitochondria, which causes its increase [6]. Changes in the peroxygen oxidation of lipids(LPU) lead to disruption of the structure of the mitochondrial membrane and the activity of enzymes. In membranes, LPU is under normal conditions controlled by the antioxidant defense system [9, 10, 11].

Taking into account the above, in our studies, we determined the activity of antioxidant enzymes - SOD and catalase in the dynamics of the mitochondria of the rat liver under the influence of ultrasound.

Research methods and materials. The studies were conducted on purebred white female laboratory rats weighing 150-220 g. Experimental objects were irradiated for 5 minutes in the wave range of 7.5 mHz by Mindrey DP-50 Vet ultrasound apparatus designed for animals.

Rats were divided into separate model groups for UV exposure and their correction:
- Group I healthy (control) (n=5)
- Group II 5 minute exposure to ultrasound (n=5-6)
- Group III ultrasound + shotut extract (n=5-6)
- Group IV ultrasound + biosep (n=5-6)

During the study, after a 5-minute exposure to ultrasound, group III rats were given 1 ml per body weight once a day for 5 days through a special probe, and group IV rats were given 1 ml of Biosep drug preorally.

1, 3, 5, 10, and 15 days after the administration of rattan and biosep extracts to rats exposed to ultrasound, the activities of SOD and catalase enzymes in their liver mitochondria were studied.

Mitochondria of rat liver were isolated using the method of differential centrifugation by W.C.Schneider[12] and the modified method of Kuzmina et al.[13]. 0.25 M sucrose - TKM buffer solution was used to isolate mitochondria from liver tissue. First, a 1:10 homogenate of tissues was prepared and centrifuged at 1000 rpm for 10 minutes. The sediment was discarded and the supernatant was centrifuged at 12000 rpm for 10 minutes. The resulting precipitate was washed twice with 0.25 M sucrose TKM buffer and used to determine the activity of SOD and catalase enzymes.

Activity of SOD (KF 1.15.1.1) enzyme Misra and J. Fridovich (1972). λ=540 nm in a spectrophotometer according to the method. determined in wavelength [14]. The essence of the method is based on the reduction of superoxide anions, nitrotetrozole blue, which are formed as a result of aerobic action and reduce the amount of NADN, phenosine metasulfate (FMS). Enzyme activity is expressed in U/ml of protein.

Catalase activity was determined by the spectrophotometric method at a wavelength of λ =410 nm. The essence of the method is based on the formation of a stable color complex of H2O2 with ammonium molybdate reagent. Color intensity was measured in a spectrophotometer against a sample containing 2 ml of H2O instead of H2O2. Catalase activity in liver mitochondria is expressed in µKat/mg protein [15].

The amount of protein in mitochondria was determined according to the Lowry method[16]. The difference between the results obtained from the control, experiment and experiment+shotut, experiment+biosep groups was calculated by t-test, where the value of p<0.05, p<0.01 represents statistical reliability.

Results obtained and their analysis. The results of the study show that after exposure to the liver of rats at 7.5 mHz wave range for 5 minutes through the Mindrey DP-50 Vet ultrasound device, the activity of SOD enzyme in the hepatocyte mitochondria in rats of this group on days 1, 3, 5, 10 and 15 was correspondingly higher than that of the control decreased by 47.4±1.04%, 42.4±0.6%, 34.7±0.4%, 31.4±0.5%, 29.8±0.3% (Picture.1).
1. Effects of shotut i and biosep extracts on mitochondrial SOD enzyme activity of ultrasound rat hepatocytes (depending on the dynamics of 1, 3, 5, 10 and 15 days) (U/mg protein) (*p<0.05, **p <0.01 n=5-6)

This, in turn, indicates that the activity of the antioxidant enzyme SOD in mitochondria is impaired under the influence of ultrasound (Table 1). A sharp decrease in the activity of SOD enzyme in the liver mitochondria of rats of this group was observed on the 1st and 3rd days after exposure to ultrasound, and it was found that it decreased by 47.4±0.3% and 42.4±0.4%, respectively.

Table 1 Effects of shotut i and biosep extracts on mitochondrial SOD enzyme activity ultrasound rat hepatocytes (depending on the dynamics of 1, 3, 5, 10 and 15 days) (U/mg protein) (*p<0.05, **p<0.01 n=5-6)

<table>
<thead>
<tr>
<th>№</th>
<th>Experience groups</th>
<th>n</th>
<th>day 3-</th>
<th>5-day</th>
<th>10-day</th>
<th>15-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5</td>
<td>1.33±0.01</td>
<td>1.32±0.01</td>
<td>1.29±0.02</td>
<td>1.34±0.01</td>
</tr>
<tr>
<td>II</td>
<td>ULTRASOUND</td>
<td>6</td>
<td>0.70±0.01</td>
<td>0.76±0.02</td>
<td>0.83±0.02</td>
<td>0.92±0.02</td>
</tr>
<tr>
<td>III</td>
<td>ULTRASOUND+shotut</td>
<td>5</td>
<td>0.84±0.02*</td>
<td>0.90±0.02</td>
<td>0.95±0.02</td>
<td>1.04±0.03**</td>
</tr>
<tr>
<td>IV</td>
<td>Ultrasound + biosep</td>
<td>5</td>
<td>0.79±0.02</td>
<td>0.86±0.02</td>
<td>0.91±0.01</td>
<td>0.99±0.02</td>
</tr>
</tbody>
</table>

Note: (*p<0.05, **p<0.01 n=5-6)

A significant effect of the extract on the activity of liver mitochondrial SOD enzyme was found in group III rats corrected with barley extract (Picture 1). On days 1, 3, 5, 10, 15, its activity was 10.2±0.1%, 10.6±1.1%, 9.3±1.0%, 9±7.6%, respectively, compared to the II group and it was found to be higher by 8.7±0.6%. It was observed that the enzyme activity in the mitochondria of the hepatocytes of this group was significantly restored by the 15th day (Picture 1).

SOD enzyme activity in the mitochondria of group IV rat hepatocytes corrected with the fatty extract of Biosep was 6.8±0.9%, 7.6±0.8%, 6.2±0.8%, 5.3±0.6%, it was 12.9±0.8% (Picture 1).

As a result of the 5-minute exposure of ultrasound to the liver of rats at a wavelength of 7.5 mHz, the activity of catalase enzyme in hepatocyte mitochondria was 32.2 ± 0.3%, 30.4 ± 30.4%, respectively, in rats of this group on days 1, 3, 5, 10, and 15. decreased by 0.2%, 28.4±2.9%, 24.3±1.7%, 28.9±2.1% (Picture 2).
This, in turn, indicates that the activity of catalase, an antioxidant enzyme of mitochondria, is impaired under the influence of ultrasound (Table 2). A sharp decrease in the catalase enzyme activity in the liver mitochondria of rats of this group was observed on the 1st and 3rd days after ultrasound exposure, and it was found that it decreased by 32.2±2.8% and 30.4±2.3%, respectively.

Table 2 Effects of shotut i and biosep extracts on mitochondrial catalase enzyme activity of ultrasound rat hepatocytes (depending on the dynamics of 1, 3, 5, 10 and 15 days) (µKat/mg protein) (*R˂0.05, **R˂0.01 n=5-6)

<table>
<thead>
<tr>
<th>№</th>
<th>Experience groups</th>
<th>n</th>
<th>1-day</th>
<th>3-day</th>
<th>5-day</th>
<th>10-day</th>
<th>15-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5</td>
<td>32.25±0.59</td>
<td>35.69±0.75</td>
<td>34.66±0.54</td>
<td>37.88±0.93</td>
<td>35.45±0.93</td>
</tr>
<tr>
<td>II</td>
<td>Ultrasound</td>
<td>6</td>
<td>21.83±0.64*</td>
<td>24.83±0.79</td>
<td>24.82±0.57</td>
<td>28.67±0.80</td>
<td>25.22±0.60*</td>
</tr>
<tr>
<td>III</td>
<td>Ultrasound+shotut</td>
<td>5</td>
<td>25.31±0.70*</td>
<td>29.44±0.67*</td>
<td>29.46±0.79</td>
<td>33.33±0.93</td>
<td>31.15±0.75</td>
</tr>
<tr>
<td>IV</td>
<td>Ultrasound + biosep</td>
<td>5</td>
<td>23.15±0.61</td>
<td>26.22±0.55</td>
<td>26.54±0.80*</td>
<td>30.64±0.85</td>
<td>30.64±0.70**</td>
</tr>
</tbody>
</table>

Note: (*R˂0.05, **R˂0.01 n=5-6)

A significant effect of the extract on catalase enzyme activity of liver mitochondria of group III rats corrected with barley extract was found (Table 2). On days 1, 3, 5, 10, 15, its activity was 10.6±0.8%, 12.9±1.0%, 13.3±1.1%, 12.3±0. It was found that it increased by 9% and 16.7±1.4%. It was observed that the enzyme activity in the mitochondria of hepatocytes of this group of rats was significantly restored by the 15th day (Picture 2).

The activity of catalase enzyme in the mitochondria of rat hepatocytes of group IV corrected with the fatty extract of Biosep was 4±0.9%, 4.8±3.4%, 4.9±0.4%, 5.2±0, respectively, compared to the indicators of group II. .4%, it was found to be higher by 15.3±1.3% (Picture 2).

Conclusion: The results of the research showed that after exposure to ultrasound, the activity of antioxidant enzymes - SOD and catalase significantly decreased on the 1st and 3rd days, and in the groups corrected with extracts of sorghum and biosep, these drugs showed their antioxidant properties. That is, on the 10th and 15th days, it was found that the activity of the enzymes was restored to a certain extent. The results of the conducted research showed that the antioxidant property of the extract of sagot was more effective than that of the extract of biosep.
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