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Lipid Spectrum of Membranes and Lipid Peroxidation and Proteins in the Liver Echinococcosis in Humans

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Abstract

One of the major issues in Medical Parasitology is Echinococcosis which causes the Hydatid disease of the liver. It causes significant useful changes in liver prompting to nearby entanglements related with liver disappointment and in addition loss of essentials of human body all in all. The weight of these cysts on liver tissue causes ischemia prompting to interruption of metabolic procedures in it. This is the reason why studies on revealing and improving dysfunctions in the mechanisms of oxygen-dependent processes allow to explain and adjust the characteristics of toxic liver damage in general. Study on these issue also allows to assists closely to the fundamental research in general biological problems, such as free radical oxygen and nitrogen species, peroxidase modification of lipids and proteins, function of biological membranes, etc. The consequences of our review demonstrate that LE causes profound practical changes with respect to the advancement of oxidative stress in the body. In perspective of the over, the increase and advancement of lipid peroxidation designs that we have found in LE pathogenesis procure a specific significance. Through our research we understand that hydatid ailment of the liver is because of initiation of free radical responses, which prompts to infringement of the digestion system of proteins and lipids in the structure of cell films, metabolic scatters showing a vital part of oxidative procedures in the pathogenesis of echinococcosis.

Introduction

Echinococcosis is an important problem in Medical Parasitology. Hydatid disease of the liver causes profound functional changes in liver leading to local complications associated with liver failure as well as loss of vitality of human body in general [1].

The pressure of hydatid cysts on liver tissue causes ischemia leading to disruption of metabolic processes in it. For this reason studies on revealing and improving dysfunctions in the mechanisms of oxygen-dependent processes allow to explain and adjust the characteristics of toxic liver damage in general [2]. Addressing these issues is closely related to fundamental research in general biological problems, such as free radical oxygen and nitrogen species, peroxidase modification of lipids and proteins, function of biological membranes, etc. [3,4].

The results of our study show that LE causes deep functional changes associated with the development of oxidative stress in the body. In view of the above, the intensification and development of lipid peroxidation patterns that we have discovered in LE pathogenesis acquire a particular significance.

In this pathogenesis the oxidation source for the wastes of different free radicals may be those free radicals that are produced as a result of compression of liver tissues by hydatid cysts caused by hypoxic exposure in LE. The above mentioned changes also lead to disruptions in the metabolism of proteins. In this respect the changes that we have discovered in protein oxidation processes acquire a particular significance. The most important consequence of oxidative modification of proteins is the blockage of certain enzymes [5]. In these pathogenic functions the disruption of free radical processes primarily prevent the normal functioning of cell membranes. Due to the above mentioned reasons from the groups of membrane lipids we have studied the qualitative and quantitative changes of erythrocyte membrane phospholipids in hydatid cyst of the liver. The results show that echinococcosis is accompanied by a decrease in the amount of phosphatidylcholine (PC), phosphatidylserin (PS), phosphatidyletanolamine (PE), cardiolipine (CL) and an increase of lisophosphatidylcholine (LPC), phospholipid (PL). These changes lead to serious disorders of the functioning of cells [6,7].

Thus our research reveals that hydatid disease of the liver is accompanied by activation of free radical reactions, which leads to violation of the metabolism of proteins and lipids in the structure of cell membranes, metabolic disorders indicating an important role of oxidative processes in the pathogenesis of echinococcosis.

Materials and Methods

The study was conducted in hospitalized patients with liver echinococcosis. In order to confirm the diagnosis, the size, location, and focal lesions all patients were performed CT and abdominal ultrasound during the preoperative examination. Blood samples were taken 1 day prior to surgery and 3, 5 and 10 days after surgery. Donors served as controls.

After fasting whole blood samples were collected from peripheral veins into tubes with EDTA for some studies, with subsequent centrifugation and secretion of plasma.

The method of determining peroxides and hydroperoxides

The basis of the method for determining lipid peroxidation is the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA), which runs at a high temperature and acidic pH and finalizes in the formation of stained trimethyl complex containing one molecule malonic dialdehyde and two-TBA. The intensity of the color is measured spectrophotometrically at a wavelength of 535 nm. The number of hydroperoxides was determined from the intensity of the color reaction

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in the presence of ammonium tiotsinata, spectrophotometry was carried out at 480 nm [8].

The method of determining oxidative modifications of protein (OMP)

For quantitative determination of OMP product we used the method based on reaction of the oxidized protein and amino acid residues of 2,4-dinitrophenylhydrazine (DNPH) to form 2,4-dinitrophenylhydrazone, the number of which was determined spectrophotometrically [9,10].

The extraction, quantification and fractionation of phospholipids (PL)

PL extraction was carried out according to Folch metod. Fractionation was performed by individual PL [8,9] uplink dimensional thin layer chromatography us ing the solvent system chloroform-methanol-ammonia (65: 35: 5 v/v). PL spots were identified by the appropriate standard of witnesses (Sigma, USA). Lipid phosphorus mineralization was carried out in a medium of sulfuric and nitric acids. Quantitative determination of lipid phosphorus was performed spectrophotometrically at a wavelength of 830 nm.

The erythrocyte membrane separation

Whole blood was centrifuged at 3000 rev/min for 15 minutes. Packed red blood cells suspended in buffer, 1:50 or 1: 100. Then centrifuged at 15,000 rev/min K-24 for 20 min. The precipitate was washed with buffer: 0.8 g $NaHCO_3$, 0.37 g EDTA, 0.18 g NaCl - dovadili to 1 liter with distilled water [11].

Statistical treatment

The mathematical processing of the data was performed with of "SigmaPlot 11.0" software, using special manuals for medical and biological statistics.

Results and Discussion

The study of free radical oxidation of lipids with liver echinococcosis

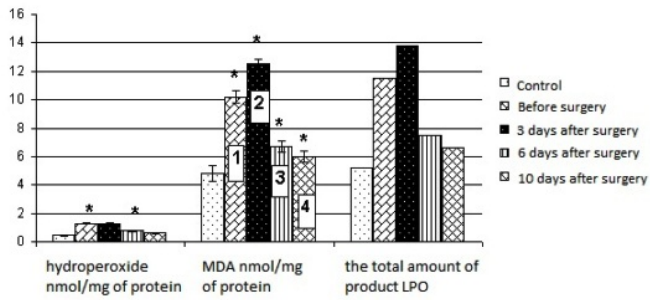
Lipid peroxidation was studied by us in enzyme (NADPH dependent) and non-enzymatic (ascorbate-dependent) oxidation systems. The survey revealed the presence of a certain fixed level of intensity of free radical reactions in the blood of healthy people.

The study of the process of lipid peroxidation at LE has revealed an increase in the content of malondialdehyde and hydroperoxides in the membranes of red blood cells.

The content of MDA before surgery with ascorbate and NADFH-dependent peroxidation was 10.2 ± 0.45 and 7.3 ± 0.2 respectively in the membranes of red blood cells of patients with hepatic echinococcosis. A similar pattern is observed in the hydroperoxide, where hydroperoxides at performance EF increased in the case of ascorbate peroxidation and NADFH-dependent peroxidation. On the first day after surgery there were also increased levels of malondialdehyde and hydroperoxide, which decreased by the 10th day of the operation. (Figures 1 and 2). The increase of these numbers is one of the causes for damage to the hepatocyte cells.

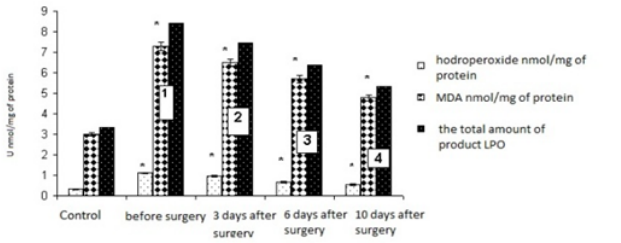
The study of oxidative modification of proteins in the liver echinococcosis

The results of the study (Figure 3), in patients with liver



Note: * P <0.05
1. Significance of differences compared to the control
2. The significance of differences compared to 1
3. The significance of differences compared to 2
4. The significance of differences compared to 3

Figure 1: The content of the products in the membrane lipid peroxidation of red blood cells in patients with hepatic echinococcosis (ascorbate dependent peroxidation).



Note: * P< 0.01
1. Significance of differences compared to the control
2. The significance of differences compared to
3. The significance of differences compared to 2
4. The significance of differences compared to 3

Figure 2: The content of the products of lipid peroxidation in the membranes of red blood cells in patients with hepatic echinococcosis (NADPH-dependent peroxidation) (N=15).

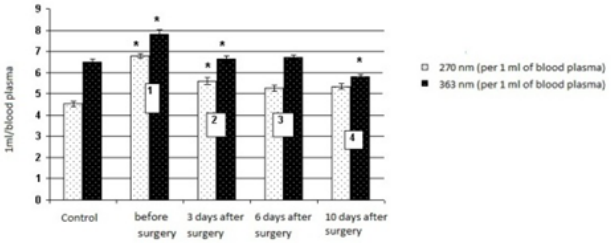
echinococcosis show that there is a tendency to increase the intensity of oxidative modification of proteins at a wavelength of 270 nm and 363 nm, indicating a statistically significant increase in the aliphatic aldehyde and ketone dinitrophenylhydrazone.

The data show an increase in the intensity of the process of oxidative degradation of proteins under the influence of liver echinococcosis. We detected increased intensity of oxidative modification of proteins in blood plasma of patients, which actually reflects the general direction of free-radical processes and in particular the oxidation of proteins throughout the body, including the liver tissue.

The study of the lipid membranes of erythrocytes with liver echinococcosis

Any pathological process is accompanied by morphological and functional changes in the structure of cell membranes. The content and composition of membrane lipids in normal tissues is maintained within relatively constant values and significantly disturbed by various external influences. In this respect, the study of the phospholipid composition of erythrocyte membranes in LE is important.

The results showed a decrease in the content of phosphatidylcholine (PC), with a parallel increase in the number of lysophosphatidylcholine



Note: * P0.01
1. Significance of differences compared to the control:
2. The significance of differences compared to 1
3. The significance of differences compared to 2
4. The significance of differences compared to 3
270 nm-aliphatic aldehyde dinitrophenylhydrazone; 363 nm-aliphatic ketone-dinitrophenylhydrazone

Figure 3: Oxidative modifications of proteins in blood plasma of patients with hepatic echinococcosis (N=15).

(LPC) prior to surgery. The investigation of phosphatidylserine (PS) and phosphatidyletanolamine (PE) also found a decrease in their content in the erythrocyte membrane. It is worth mentioning that the levels of the phospholipid (PL) increase, which are precursors of second messengers, such as inositol triphosphate 1, 4, 5 and diglycerides in signal transduction processes. Cardiolipine (CL) has a major role in the induction of apoptosis in the mitochondria, which significantly decreases the content in erythrocyte membranes with echinococcosis, the latter being associated with oxidative modification of lipids. Sphingomyelin (SPM) study has revealed an increase in their content. The results of the study revealed that changes observed in the content of PL patients with echinococcosis occur prior to surgery and 3-5 days after surgery.

By the 10th day a tendency of normalization of the PL content to donor performance was observed, but it did not reach the levels received from the donors (Table 1).

Conclusion

As our survey shows, hydatid disease of the liver causes profound functional changes associated with the development of oxidative stress. Given above, the intensification of lipid peroxidation with echinococcosis discovered by us is of special significance.

The triggers of activation of free radical oxidation in the liver echinococcosis are free radicals which are formed by compression due to echinococcosis hydatid cysts of the liver tissue caused by hypoxia. The reduction of the activity of antioxidant system affects the normal protein metabolism. The changes in protein oxidation discovered by use plays an important role in the above mentioned plan. The most important consequence of oxidative modification of proteins is the deactivation of enzymes.

From the point of view described above, the results of our studies to determine the qualitative and quantitative content of erythrocyte membrane phospholipids with liver echinococcosis are extremely convincing. According to data obtained, hydatid disease of the liver is

Patients with echinococcosis					
Fraction of phospholipids	**Control (donors)**	**Before the operation 1**	**3rd day after surgery 2**	**6th day after surgery 3**	**10th day after surgery 4**
LPC	10.0 ± 0.5	16.7 ± 0.8*	15.3 ± 1.0**	13.3 ± 1.1**	12.2 ± 0.4
SPM	18.8 ± 1.5	24.1 ± 1.8**	24.1 ± 1.7***	24.3 ± 1.1***	22.5 ± 2.1
PI	8.6 ± 1.2	16.6 ± 1.5*	15.8 ± 1.8**	14.7 ± 1.3**	10.8 ± 0.7
PC	28.3 ± 1.8	18.8 ± 1.6***	20.1 ± 1.7***	20.3 ± 1.8***	22.7 ± 2.0
PS	14.3 ± 1.4	9.6 ± 1.1**	10.8 ± 1.2	12.2 ± 1.1	13.5 ± 1.3
PE	8.5 ± 0.7	6.0 ± 0.8**	7.7 ± 0.6	7.8 ± 0.8	8.4 ± 0.7
CL	11.5 ± 0.5	7.1 ± 0.6*	7.3 ± 0.7*	7.4 ± 0.8*	9.3 ± 0.8

Note: *p<0.001; **-P<0.01; ***-P<0.05.
1. The significance of differences compared to the control
2. The significance of differences compared to the 1
3. The significance of differences compared to the 2
4. The significance of differences compared to the 3

Table 1: The content of phospholipids (PL) (% of phospholipids) in membranes of erythrocytes of patients with hepatic echinococcosis (n=15).

accompanied by a decrease in the content of PC, PS, PE, CL and increase in LPC. The observed changes in the qualitative and quantitative composition of individual phospholipids can lead to serious violations in the functional activity of the cells.

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