Effect of Ozone on the Liver in Experimental Chronic Hepatitis

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Short-term administration of ozonized physiological solution to rats with chronic toxic hepatitis reduces the content of total lipids in the liver tissue, activates antioxidant enzymes, normalizes the level of diene conjugates, and reliably increses adenosine nucleotides, the number of normal hepatocytes being increased 2.2-fold. The data suggest that ozone can be used as a stimulator of regeneration of the pathologically altered liver.

Key Words: *experimental chronic hepatitis; ozone; regeneration*

Chronic liver diseases occupy a significant place in the structure of gastroenterologic pathology; therefore the search for new ways of combating these diseases especially those based on the stimulation of regeneration assumes great importance. There are data on the efficiency of ozonetherapy for treatment of hepatitis [13]. The detoxication properties of ozone and its ability to activate metabolic processes in the organism in hypoxia and stimulate compensatory ultrastructural rearrangement of cell organelles are well documented. Optimal ozone concentrations have been established which do not damage membrane structures, but activate pro- and antioxidant systems and modify the lipid bilayer, thereby enhancing the formation of macroergic compounds and improving the functioning of the organ [2,5].

The aim of the present study is to analyze the short-term influence of ozonized physiological solution on the structure and metabolic parameters of rat liver in chronic toxic hepatitis.

MATERIALS AND METHODS

Experiments were carried out on nonpedigree rats weighing 180-200 g. Chronic toxic hepatitis was induced by subcutaneous injections of 0.3 ml 65% tetrachloromethane solution in vegetable oil (4 injections per week, 30 injections). On day 4 after termination of tetrachloromethane treatment, experimental rats (n=15) were injected intraperitoneally with 1 ml ozonized physiological solution for 2 days. Physiological solution was aerated with an ozone-oxygen mixture containing 300 μg/liter ozone at a flow rate of 1 liter/min. The concentration of ozone was determined spectrophotometrically at 254 nm. Fifteen nontreated rats served as controls.

The experimental animals were sacrificed under Nembutal narcosis (25 mg/kg) on day 2 after administration of ozonized physiological saline. Untreated animals were killed on day 6 after termination of tetrachloromethane treatment. The state of lipid peroxidation (LPO) and antioxidant protective system in the liver was analyzed. The analysis included measurement of Fe^{2+}- and hydrogen peroxide-induced chemiluminescence (pulses/sec) [4]. Diene and triene conjugates were measured at 233 and 277 nm, respectively [12], malonic dialdehyde (MDA) in the reaction with 2-thiobarbituric acid [11] (optical density/g total lipids), and Schiff bases were assayed by the intensity of fluorescence at 365 nm excitation and 420 nm emission wavelengths (rel. units/g total lipids) [9]. The superoxide dismutase (SOD) activity was assayed as described previously [10] and expressed in arb.units/g protein/min. Catalase activity was measured as described elsewhere [8] and expressed in μmol H_2O_2/g protein/min). The

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TABLE 1. Effect of Ozone on Lipid Peroxidation and Antioxidant Enzymes in the Liver of Rats with Chronic Hepatitis ($M\pm m$, n=15)

Series	Chemiluminescence	Diene conjugates	Triene conjugates	MDA	Shiff bases	SOD	Catalase	Total lipids
Intact rats	417.7±64.4	0.74±0.03	0.439±0.027	1.55±0.04	122.2±6.3	9.23±0.19	0.45±0.14	0.069±0.005
Control	690.9±78.4*	1.19±0.05*	0.635±0.041*	1.58±0.06	388.1±4.9*	4.05±0.31*	0.31±0.06	0.346±0.019*
Ozonetherapy	489.2±37.2	0.25±0.01*,**	0.691±0.039	1.01±0.05	390.0±3.7	10.12±0.17**	0.98±0.10*,**	0.045±0.002*

Note. Here and in Table 2: $p<0.05$: *in comparison with intact animals, **in comparison with controls.

content of total lipids was measured using Lachema kits and adenosine nucleotides as described previously [1]. For morphological and morphometric evaluation of the number of normal hepatocytes per test-square liver samples were fixed in 10% neutral formalin, embedded in paraffin, and then 5-6-μ-thin sections were stained with hematoxylin and post-stained with eosin. Cells with round or oval-shaped well-structured nucleus, distinct nucleoli, intact membrane and sufficient cytoplasm protein content were assigned to normal hepatocytes. All calculations were carried out with a defined reliability level at $p<0.05$.

RESULTS

Biochemical analysis revealed an elevation of total lipids in the liver of rats with chronic hepatitis (Table 1). In liver homogenates of control rats 2 days after termination of tetrachloromethane treatment we observed a boost of induced chemiluminescence, the magnitude of maximal burst being increased 2-fold (Table 1). This attested to enhanced free radical reactions in liver microsomes in response to the toxic action of tetrachloromethane. The increase in primary (diene conjugates) and final (Schiff bases) LPO products 1.5- and 2-fold, respectively (Table 1) results from the imbalance of pro- and antioxidant systems. The active LPO reactions beyond the adequate control of the antioxidant system give rise to structural and metabolic changes in cell membranes affecting energetic processes. In our experiments, the content of adenosine nucleotides in the liver of control rats was reliably lowered (Table 2), which attested to disturbed energy metabolism.

The number of normal hepatocytes per test-square of the sample in nontreated animals with chronic hepatitis was 7.1±0.65, the normal value being 13.6.

Intraperitoneal administration of ozonized physiological saline as soon as after 48 hours reduced the content of total lipids. These findings were in conformity with previous data on the hypolipidemic effect of ozone and the dynamics of lipid metabolism under condition of stimulated liver regeneration [7] and electron-microscopic observations in rat liver after ozone treatment. The normalized content of total lipids attested to improvement of structural and metabolic parameters of the liver. Our experiments demonstrated for the first time that ozone treatment activates the antioxidant enzymes SOD and catalase in affected liver, which results in the inhibition of free radical reactions. This manifested itself in the inhibition of chemiluminescence and a decrease in the content of primary LPO products (diene conjugates). Interruption of the initiation stage of free radical reactions may therefore be attributed to the ability of ozone to maintain the natural antioxidant system of the organism [6]. Modulation of LPO processes affects metabolic reactions. Normalization of enzymatic reactions promotes the accumulation of adenosine nucleotides, especially ATP. This suggests that short-term ozone treatment optimizes biochemical processes in the pathologically changed liver, which is necessary and very important for liver regeneration.

Two days after ozone treatment, the number of normal hepatocytes increased 2.2-fold in comparison with the control and attained 16.3±1.45 ($p<0.01$).

Our findings indicate that short-term administration of ozonized physiological saline normalizes metabolism and stimulates regeneration of the pathologically changed liver, implying that ozone can be used for these purposes [3].

TABLE 2. Effect of Ozone therapy on the Content of Adenosine Nucleotides in the Liver of Rats with Chronic Hepatitis ($M\pm m$, n=15)

Series	Adenosine nucleotides			
	AMP	ADP	ATP	AMP+ADP+ATP
Intact rats	2.147±0.115	1.533±0.079	1.884±0.156	5.564±0.148
Control	1.943±0.106	1.158±0.098*	1.676±0.121	4.775±0.234*
Ozone therapy	2.410±0.119	1.502±0.089	1.227±0.174**	6.139±0.195**

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