Effect of Ozonized Normal Saline on Biochemical Parameters of the Liver in Malignant Growth

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Injection of ozonized normal saline to animals with transplanted sarcoma-45 normalized energy metabolism in the liver, which is important for this organ's function under conditions of malignant growth.

Key Words: *ozone; sarcoma-45; phospholipids; lipid peroxidation; macroergic nucleotides*

Tumor cell metabolites and products of degradation affect oxygen homeostasis by inducing chronic tissue hypoxia. Oxygen deficiency leads to energy deficiency, involving disorders in metabolism, function, and structure of organs. Disorders in liver function can be regarded as a manifestation of tumor effect on the organism. Malignant growth is associated with the loss of antioxidants, specifically, vitamin E, by hepatocytes and accumulation of lipid peroxides in them [7]. Ozone affects the pro- and antioxidant system by modifying the plasma membrane lipid layer and stimulating energy metabolism in tissues [3,6].

We studied the effect of ozonized normal saline on lipid peroxidation (LPO), phospholipid composition of liver tissue, and level of macroergic nucleotides as markers of energy metabolism during development of a malignant tumor in experimental animals.

MATERIALS AND METHODS

Experiments were carried out on 75 outbred female rats weighing 180-200 g. Tumor was induced by transplantation of sarcoma-45 (Tumor Bank of the Oncology Research Center): a subcutaneous injection of 0.5 ml cell suspension in Hanks' solution (1:3) in the right thigh. The animals were divided into 5 groups: 1) intact controls; 2 and 4) untreated animals with tumors developing for 21 and 28 days, respectively; 3 and 5) with tumors at the same terms, injected intraperitoneally with ozonized normal saline (1.5 ml) for 5 days. In group 5 the same amount of saline was injected into the tumor. Ozonized normal saline was prepared by bubbling an ozone-oxygen mixture through 0.9% NaCl at a rate of 1 liter/min at an ozone concentration of 3000 μg/liter gaseous mixture. Ozone was obtained by using a special generator. The ozone concentration was monitored routinely: in the gaseous phase spectrophotometrically at a wavelength of 254 nm and in liquid by iodometric titration. A single ozone dose was 0.6-0.7 μg.

At the end of experiment, lipid peroxides were analyzed in liver tissue homogenates: diene conjugates by ultraviolet spectrum of lipid solution absorption at a wavelength 233 nm in optic density units, triene conjugates at a wavelength 277 nm in optic density units [9], and Schiff bases by fluorescence intensity at excitation wavelength 365 nm and emission wavelength 420 nm in arbitrary units of fluorescence [8]. All results were calculated to correspond to the total lipid level (in mg) analyzed by standard Lachema kits. Phospholipids were identified by chromatography on Siluful plates using a chloroform:methanol:water mixture (65:25:4) [2]. The detected fractions were assessed by the content of inorganic phosphorus [5] and the results expressed in percent. The concentrations of adenyl and guanyl nucleotides were measured by ion-ex-

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TABLE 1. Content of the Main Phospholipid Fractions in Rat Liver Homogenate (*M±m*)

Group	Lisophosphatidylcholine	Sphyngomyelin	Phosphatidylcholine	PEA
1	9.28±0.06	14.02±1.10	41.86±1.69	34.25±1.93
2	6.77±1.22	12.07±1.09	42.53±0.49	38.63±1.42
3	28.39±0.61**	23.46±1.42**	27.42±3.29**	23.98±2.20**
4	7.66±1.07	10.72±1.02	44.85±2.03	36.59±1.81
5	7.58±0.97	16.99±1.61**	46.45±2.69	28.98±1.57**

Note. Here and in Tables 2 and 3: $p<0.05$: *in comparison with intact rats, **in comparison with control.

change chromatography with ecteol cellulose as ion exchanger and expressed in μg/g wet tissue [1].

RESULTS

Thin-layer chromatography detected in liver tissue homogenate the following phospholipid fractions in quantitatively significant amounts: phosphatidylcholine, phosphatidylethanolamine (PEA), lisophosphatidylcholine, and sphyngomyelin. There were no changes in the phospholipid spectrum at both terms of tumor growth in comparison with intact controls (Table 1). No accumulation of lipid peroxides was detected at the early terms (21 days) of tumor development (Table 2). After 28 days, in group 4 rats the levels of primary (diene conjugates) and final (Schiff base) lipid peroxides significantly increased, indicating activation of free-radical reactions in hepatocytes caused by sarcoma-45. The detrimental effect of lipid peroxides in this group of rats was manifested by impaired energy processes involving a decrease in ATP and GTP levels, an increase in AMP+ADP/ATP ratio, and a decrease in the GDP/GTP ratio.

After five injections of ozonized saline at earlier stages of tumor growth (group 3), the liver content of lisophosphatidylcholine increased 3-fold and the levels of phosphatidylcholine and PEA decreased 1.5-fold. The content of sphyngomyelin increased 2-fold. This indicated that the solution activated phospholipase A_2, catalyzing oxidation of phosphatidylcholine and PEA with production of lisophosphatidylcholine. The increase in sphyngomyelin fraction may be regarded as a compensatory reaction of hepatocytes to chaotropic effect of lisophosphatidylcholine resultant from activation of a liver enzyme CDP-choline: ceramide-cholinephosphate transferase. A significant decrease in the content of Schiff bases (Table 2) in comparison with intact controls confirms activation of membrane lipid oxidation with participation of phospholipase A_2.

After 5 injections of saline, the absolute content of ATP decreased and the AMP+ADP/ATP ratio increased, while the content of GTP sharply increased in group 3 (Table 3). A decrease in ATP level resulted from impairment of energy processes in hepatocytes. Comparison of ATP levels in groups 2 and 4 showed that without treatment ATP level decreased within a week, while ozonized saline, causing restructuring of hepatocyte membranes, restored the functional activity of the plasma membrane enzymes responsible for production of macroergic compounds. A 2.5-fold increase in the content of GTP even in comparison with intact controls indicated activation of Krebs cycle, in which GTP is formed at the stage of succinyl-CoA in succinate during substrate phosphorylation. GTP can be utilized directly glucogenesis, fatty acid activation, and protein production or liberate one phosphate ADP group with production of ATP as a result of enzymatic reaction catalyzed by nucleoside diphosphate kinase (Table 3).

Injection of ozonized saline according to two protocols did not result in accumulation of phospholipid lysoforms in group 5, in comparison with group 3. The content of PEA decreased only by 20%, and the level of sphyngomyelin increased by 20%. Treatment of these animals resulted in complete normalization of LPO parameters (Table 2).

The levels of ATP and GTP increased in comparison with untreated rats, although did not reach the values in intact animals. This indicates partial recovery of energy metabolism, which can be explained by incomplete repair of membrane structures of hepatocytes at this terms of experiment.

TABLE 2. Content of Lipid Peroxides in Rat Liver Homogenate (*M±m*)

Group	Diene conjugates	Triene conjugates	Schiff's bases
1	0.35±0.06	0.16±0.08	90.75±1.38
2	0.36±0.05	0.17±0.06	91.47±1.87
3	0.38±0.09	0.11±0.01	80.07±0.99**
4	0.53±0.03*	0.17±0.04	113.67±0.50*
5	0.40±0.08**	0.12±0.04	101.91±1.22**

TABLE 3. Content of Macroergic Nucleotides and Their Ratios in Rat Liver Homogenate (*M±m*)

Group	ATP	GTP	AMP+ADP/ATP	GDP/GTP
1	1.63±0.14	0.28±0.07	2.07±0.25	2.28±0.32
2	1.66±0.08	0.28±0.05	2.07±0.15	2.22±0.29
3	1.25±0.10**	0.67±0.11**	3.24±0.36**	1.12±0.12**
4	1.12±0.06*	0.09±0.01*	2.47±0.22	2.00±0.29
5	1.45±0.15**	0.13±0.02	2.48±0.22	2.99±0.35**

Therefore, injection of ozonized normal saline to animals promoted the recovery of the plasma membrane lipid layer and activated energy processes required for the detoxication and synthetic functions of the liver.

REFERENCES

1. G. N. Ivanova and L. N. Rubel', *Zh. Evoluts. Biokhim.*, **5**, No. 3, 279-287 (1969).
2. M. Kates, *Lipidology Technologies* [in Russian], Moscow (1975).
3. K. N. Kontorshchikova, *Lipid Peroxidation in Correction of Hypoxia by Physicochemical Factors* [in Russian], Author's Synopsis of Doct. Biol. Sci. Thesis, St. Petersburg (1992).
4. V. S. Mosienko, L. I. Zagoruiko, and M. S. Povzhitkova, *Eksp. Onkol.*, **4**, No. 4, 60-64 (1982).
5. V. I. Svetashev, *Lipid Analysis by Micromethod* [in Russian]. Author's Synopsis of Doct. Biol. Sci. Thesis, Vladivostok (1973).
6. I. M. Solopaeva, K. N. Kontorschikova, and S. P. Peretyagin, *Byull. Eksp. Biol., Med.*, **122**, No. 8, 673-675 (1996).
7. V. S. Shapot, *Biochemical Aspects of Tumor Growth* [in Russian], Moscow (1975).
8. D. L. Fletcher, C. J. Dillared, and A. Y. Tappel, *Anal. Biochem.*, **52**, 497-499 (1973).
9. F. S. Shenstone, in: *Ultraviolet and Visible Spectroscopy of Lipids*, New York (1971), pp. 77-79.