LIPID PEROXIDATION AND BLOOD GAS COMPOSITION DURING OZONE THERAPY AFTER RESUSCITATION

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Ozone therapy is currently attracting the attention of clinicians and experimental medical scientists. The use of ozone in lung pathology, infectious diseases, surgery (osteomyelitis, burns, ulcers), oncology, physiotherapy, and stomatology has been discussed in the literature [13]. The chemical properties of ozone as a powerful oxidizing agent and its ability to saturate the tissues rapidly with oxygen enable it to be used to correct hypoxic disturbances by parenteral administration [6]. However the introduction of this method into practice must be preceded by a study of therapeutically effective concentrations of ozone and the responses of biological systems to it. In aqueous solutions, including biological fluids, ozone can decompose by a radical mechanism [7], creating the risk of intensified spontaneous lipid peroxidation (LPO). The high selectivity of ozone relative to compounds with double bonds, namely aromatic amino acids, peptides, polyunsaturated fatty acids [12], is noteworthy. In experiments with erythrocytes, a response predominantly of membrane proteins, followed by juxtamembranous lipids and nonprotein-bound lipids to ozone has been discovered [2].

This paper describes a study of the effect of ozone on the state of LPO, on some parameters of carbohydrate metabolism, the acid—base balance (ABB) and blood gases, in the postresuscitation period after hemorrhagic shock.

EXPERIMENTAL METHOD

Experiments were carried out in vitro on whole dog's blood taken 15 min after resuscitation of the animal from hemorrhagic shock by Wiggers' method, Blood (50 ml) was introduced into an oxygenator through which a mixture of ozone and oxygen, with an ozone concentration of 0.048 mg/liter was passed at the rate of 1 liter/min, The ozone was produced in a special electrode ozonizer for use with the Iskra-1 apparatus for local d'arsonvalization, The partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2) was determined on a Radelkis gas microanalyzer, and levels of buffer bases (BB) and base excess (BE) were calculated from Siggaard—Andersen nomograms [8]. Concentrations of bound and dissolved oxygen were determined by calculation. The lactate and pyruvate levels were measured as in [4]. LPO activity was estimated from concentrations of molecular products: diene conjugates (DC) [3], malonic dialdehyde (MDA) [13], and Schiff bases (SB) [9]. Total antioxidative activity (AOA) was measured on a model system as inhibition of induced chemiluminescence [7]. Blood from an intact animal served as the control. The date were subjected to analysis of variance and also to multivariate linear regression analysis, using dialogic regression programs on an SM-4 computer.

EXPERIMENTAL RESULTS

The period of the first 10-20 min after hypoxia is the early stage of recovery of the functions of the body after the beginning of resuscitation [5], when disturbances which took place during hemorrhagic shock are still mainly preserved. This was confirmed by blood analysis in the postresuscitation period [2], when despite recovery of the gas composition, pH and BB remained low, BE was at its highest level, and the lactate level also was high. AOA was significantly depressed, but

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TABLE 1. Parameters of ABB and Blood Gas Composition (M ± m, n = 10)

Blood sample	pO_2, mm Hg	pCO_2, mm Hg	Concentration of bound O_2, %	Concentration of dissolved O_2, %	BB, mM	BE, mM	pH
Initial (control)	97,89±8,72	19,35±0,99	16,76±0,98	0,302±0,028	49,32±1,64	—5,85±1,54	7,31±0,028
Postresuscitation	109,30±8,60	19,54±2,79	17,11±1,37	0,304±0,031	37,70±2,83*	—11,33±0,99*	7,24±0,085
Ozonized	211,90±0,904**	17,14±1,79	20,12±0,918	0,650±0,079**	38,13±3,16	—8,9±1,80	7,43±0,034**

Legend. Here and in Table 2: *p < 0.05 compared with control, **p < 0.05 compared with postresuscitation blood.

TABLE 2. Parameters of LPO and Carbohydrate Metabolism (M ± m, n = 10)

Blood sample	DC, nmoles/ml	MDA, nmoles/ml	SB, relative units/ml	AOA, relative units	Lactate, μmoles NADH/ml	Pyruvate, μmoles NADH/ml
Initial (control)	65,4±11,8	1,98±0,21	41,8±8,57	0,61±0,008	2,41±0,24	0,120±0,011
Postresuscitation	60,9±9,0	2,11±0,21	39,5±7,67	0,41±0,043*	4,03±0,46*	0,154±0,017*
Ozonized	58,8±19,14	2,13±0,19	40,5±4,92	0,47±0,012	3,64±0,50	0,299±0,014**

the LPO level was the same as in intact animals, Meanwhile dependence of changes in individual physicochemical blood parameters on the initial state and on the conditions of the recovery period were established on a regular basis.

After only 5 min, ozonization of the blood mainly corrected the postischemic disturbances present (Table 1) and abolished the acidosis and reduced pCO_2. A tendency was observed for the lactate level to fall and the pyruvate level rose significantly (Table 2). As a result the lactate/pyruvate ratio was reduced by half, indicating intensification of aerobic processes in the blood. pO_2 was doubled, and this was accompanied by similar changes in the concentrations of bound O_2 by 17% and dissolved O_2 by 113% (Table 1). The significant increase in the oxygen concentration, however, was not accompanied by a significant increase in molecular LPO products. This can be explained, first, by the tendency for total AOA to increase and, second, the difference in the responses of the treated blood samples to ozone, cancelling out the mean values. Regression analysis, meanwhile, showed that the changes in DC during ozonization ($\Delta DC\ O_3$) correlated positively with the reaction of SB to postresuscitation conditions (ΔSBP):

$$\Delta DC\ O_3 = -4.981 - 1.314\Delta SBP\ (\rho = 0.762,\ p < 0.001). \quad (1)$$

Whereas the SB concentration in the sample of postresuscitation blood was higher than in the control, after exposure to ozone its concentration fell, and vice versa. As the end product of lipid peroxidation reactions, SB determined the quantity of substrate — unsaturated fatty acids. An increase in the SB concentration therefore causes a decrease in the substrate concentration, and, consequently, a decrease in the rate of formation of new DC molecules as the existing ones break down. Standardization of the blood samples with respect to their SB concentration showed that the changes in DC were dependent on variation of AOA ($\Delta AOA\ O_3$):

$$\Delta DC\ O_3 = 8.75 - 1.134\Delta SBP - 222.08\Delta AOA\ O_3$$

$$(\rho = 0.879,\ p < 0.001), \quad (2)$$

It follows from equation (2) that under the influence of ozone AOA and DC changed in opposite directions, possibly due both to inhibition of LPO when AOA was intensified, and to direct ozonolysis of spontaneous DC. The MDA concentration changed during ozonization ($\Delta MDA\ O_3$) depending on the free O_2 concentration in the treated blood (ΔcO_2bP):

$$\Delta MDA\ O_3 = -0.181 + 0.158\Delta cO_2bP$$

$$(\rho = 0.652,\ p < 0.001), \quad (3)$$

If in the postresuscitation period the bound O_2 concentration was restored or exceeded the control level, MDA formation was enhanced by the action of ozone, but if this parameter remained low, the MDA level, conversely, fell. This means that with complete saturation of the hemoglobin, the oxygen not bound with it participates in oxidative processes. Lowering of the existing MDA level in vitro was due to its transition into SB, With fixed values of the bound O_2 concentration in the blood samples, changes in the MDA concentration correlated positively with pH changes during ozonization (ΔpH O_3):

$$\Delta\text{MDA } O_3 = -0.941 + 0.203\Delta cO_2b + 3.539\Delta\text{pH } O_3$$

$$(\rho = 0.867,\ p < 0.001), \tag{4}$$

Equation (4) confirms data of other workers relating to dependence of LPO on pH of the medium and, in particular, on the fact the MDA formation promotes a physiological pH [14]. The absence of direct correlation between the MDA level and pH during ozonization was due to differences in the bound O_2 concentrations in the samples.

Regression analysis showed that variations in concentration of SB (ΔSB O_3) correlate positively with changes in the lactate concentration in the postresuscitation period (LacP) and the pyruvate concentration during ozonization (Pyr O_3):

$$\Delta\text{SB } O_3 = 14.96 - 11.21\Delta\text{LacP}$$

$$(\rho = 0.862,\ p < 0.001). \tag{5}$$

$$\Delta\text{SB } O_3 = 12.91 - 10.86\Delta\text{LacP} + 19.74\ \text{Pyr } O_3$$

$$(\rho = 0.923,\ p < 0.001) \tag{6}$$

The higher the lactate level in the sample [Eq. (5)] of postischemic blood than in the control, the more ozone is used up on oxidation of lactate and the correspondingly less participates in free-radical reactions. The rise of pH in this case causes degradation of SB molecules [11]. The fact that changes in pyruvate and SB levels are in the same direction [Eq. (6)] is determined by their dependence on oxygen concentration.

The results thus showed that extracorporeal treatment of blood with ozone in a concentration of 0.048 mg/liter corrected postischemic disturbances, produced oxygen saturation, but did not activate LPO When the reactions of blood samples to ozone are evaluated, the initial levels of the parameters must be taken into account.

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