Effect of Ozone on Lipid Peroxidation in Rat Hearts Isolated against the Background of Clinical Death

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Clinical death of outbred albino rats ensues after rapid blood loss due to a cut in the coronary artery. Five minutes later, the isolated heart is perfused with ozonized Krebs-Henseleit solution. The activity of the antioxidant system in the heart is increased compared with that during routine oxygenation. The intensity of lipid peroxidation assessed by the intensity of chemiluminescence and the amount of lipid peroxidation products is significantly decreased during ozonization.

Key Words: *ozone; isolated heart; clinical death; lipid peroxidation*

Correction of the states caused by oxygen deficiency is a key concern in modern medicine. Among other forms of hypoxia, circulatory-hemic hypoxia caused by rapid blood loss during surgery and trauma is characterized by a high lethality and a high percentage of complications [6]. Therefore, increasing the oxygen supply to tissues suffering from hypoxia or reducing the oxygen demand are crucial strategies in reanimation practice. Ozone therapy has recently been used for the correction of hypoxic disorders. The detoxification properties of ozone and its ability to saturate the body with oxygen and to affect the metabolism by boosting the oxygen-dependent reactions have been studied [7,10].

Lipid peroxidation (LPO) is known to occur in tissues suffering from oxygen deficiency [1,4,5]. It can be caused by incomplete reduction of oxygen during the blockade of the final link of electron transfer, the accumulation of fatty acids in cardiomyocytes, and an increase of the sympathoadrenal influences of catecholamines.

Bearing in mind the prooxidant properties of ozone, we attempted to assess the effect of ozonized perfusion solution on LPO and the antioxidant system during the restoration of cardiac function after severe hypoxia.

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MATERIALS AND METHODS

Experiments on outbred albino rats weighing 180-200 g were performed under Nembutal anesthesia (25 mg/kg). Clinical death was caused by rapid bleeding via a cut in the carotid artery. Langendorff-Falen retrograde perfusion of the isolated heart with Krebs-Henseleit solution was started 5 min after the cessation of heart contractions and respiration. During the first 7 min the temperature of the solution was 32°C and then it was raised to 37°C. The hearts were perfused for 60 min.

Series I included 15 hearts isolated from intact animals (control). Series II (n=15) consisted of hearts isolated in the 5th minute of clinical death. Series III hearts (n=15) were perfused with oxygenated Krebs-Henseleit solution for 60 min. Series IV hearts (n=15) were perfused with ozonized solution. Ozone was added in a gaseous phase concentration of 48 μg/liter during a 5-min period starting from the 5th minute of perfusion. Four ozonization sessions were performed. Each session was followed by a 10-min perfusion with a solution aerated with a 95% O_2/5% CO_2 mixture.

The choice of ozone concentration was based on earlier *in vivo* and *in vitro* experiments [2]. Ozone

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TABLE 1. Lipid Peroxidation in Rat Heart Isolated upon Clinical Death and during Perfusion ($M\pm m$, n=15)

Series	I_{max}, cps/mg TL	Photosum, cps/mg TL	Diene conjugates, optical density/mg TL	MDA, optical density/mg TL	Schiff bases, rel. units/mg TL	Superoxide dismutase, arb. units/mg protein×min
Control	217.5±10.9	1854.2±33.8	22.00±2.14	3.29±0.11	215.9±9.3	3.58±0.34
Clinical death	1567.5±95.6*	10850.1±191.4*	16.53±3.53	12.27±1.79*	307.9±19.6*	2.71±0.21*
Perfusion with oxygenated solution	1261.6±71.5*	5985.7±109.4*,**	19.34±4.56	5.83±1.54**	510.8±22.6*,**	4.81±0.49**
Perfusion with ozonized solution	890.5±40.6*,***,+	4103.3±170.1*,**	2.36±0.71*,***,+	3.89±0.21**	302.7±15.9+	6.18±0.35*,***,+

Note. TL = total lipids. $p<0.05$: *significance of differences compared with the control; **compared with clinical death; +compared with perfusion with oxygenated solution.

was obtained by passing a barrier discharge current through medical grade oxygen. The ozone concentration was controlled spectrophotometrically at wavelength 254 nm.

At the end of the experiment the intensity of chemiluminescence (CL) induced by Fe^{2+} and H_2O_2 was measured in myocardium homogenate [3]. The results were expressed as the maximum CL (I_{max}) per mg total lipids (cps/mg total lipids) and the CL photosum for 60 sec (cps/mg total lipids). I_{max} reflects the potential capacity of a biological object for LPO, and the photosum reflects the potency of the antioxidant system.

The diene conjugate content was determined from the ultraviolet (233 nm) absorbance spectrum of lipids dissolved in methanol-hexane [11], the malonic dialdehyde (MDA) content from the reaction with 2-thiobarbituric acid [12], and the content of Schiff bases from the intensity of fluorescence at an excitation wavelength of 365 nm and an emission wavelength of 420 nm [8].

The activity of superoxide dismutase, an antioxidant enzyme, was assayed as described elsewhere [9]. The total lipid concentration was measured using Lachema kits.

RESULTS

The rats died when circulatory-hemic hypoxia set in against the background of hemorrhagic shock and hypovolemia 20 min after carotid artery incision (15 min of bleeding, 5 min cessation of heart beats and respiration). In the myocardium, the maximum CL increased 7.7-fold and the CL photosum increased 6-fold during a 60-sec period (Table 1). This indicated an increase in the intensity of free radical reactions and a decline in the total antioxidant defense. The activity of superoxide dismutase decreased 2-fold during this period. The content of the primary molecular product (diene conjugates) did not change compared with the control, while the concentration of the secondary product (MDA) increased almost 4-fold and that of the final product (Schiff bases) increased 1.4-fold. A 2-fold drop of the diene conjugate/Schiff bases index (from 0.101 to 0.053) compared with the control indicated a shift of LPO processes towards the formation of secondary and final products. Evaluation of the ratio of molecular products yielded a temporal characterization of the LPO process.

Analysis of LPO in the myocardium after 60 min of perfusion with oxygenated solution (series III) revealed a significant increase in the activity of superoxide dismutase compared with that during the clinical death period (Table 1). A 2-fold decrease in the CL photosum in the myocardium homogenate indicated a rise of the total antioxidant activity. The maximum intensity of CL and the concentration of diene conjugates remained practically unchanged.

The quantitative ratios of secondary and final molecular products displayed temporal relationships between them, namely, the 2-fold decrease in the MDA content over 60 min coincided with a 2-fold increase in the Schiff base content. It is interesting to note that we did not observe the reperfusion syndrome of intensified initial stages of free radical oxidation in experiments with the use of an oxygenated solution during recovery after circulatory-hemic hypoxia.

Alternate perfusion of the isolated heart with oxygenated and ozonized solutions (series IV) resulted in a more pronounced increase in the activity of the antioxidant system. This manifested itself in a significant decrease of the CL photosum and in an augmentation of the superoxide dismutase activity (Table 1). The result was a decrease in the maximum CL: 2- and 1.5-fold compared with that during clinical death and perfusion with oxygenated solution, respectively. During a 60-min perfusion of hearts obtained from rats during clinical death, the diene conjugate content decreased almost 8-fold and the MDA content decreased 3-fold without there being any increase in the Schiff base content. The diene conjugate/Schiff base index dropped 4-fold compared with

oxygenation (0.032 and 0.008, respectively), indicating a pronounced decline of LPO intensity.

These results point to significant differences in the effects of oxygenated and ozonized perfusion solutions on myocardial metabolism. The involvement of ozone in metabolic reactions promoted an earlier restoration of the antioxidant system of the heart and a significant decrease in the intensity of the initial stages of free radical oxidation and in lipid peroxidation as a whole.

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