The Experimental Grounds for Safe and Effective Dosage of Medical Ozone used in the Treatment of Abortion

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Abstract

Blood samples of 15 patients with threatened abortion, 15 healthy non-pregnant women and 15 pregnant women without pathology were investigated. The following indices were determined: the antioxidative plasma activity (by method of biochemiluminescence), the level of diene conjugates (by method of spectrophotometry) and the Schiff's bases (after D.I.Flether).

The influence of ozone on the above indices depended on the used concentration of ozone. The ozone concentration of 400 mcg/L induced a significant increase (by 2,6 times) in the antioxidative plasma activity. Meanwhile, the level of lipid peroxidation molecular products had a tendency to decrease. Higher ozone concentrations (800 to 1200 mcg/L) led to reverse processes.

Introduction

For over 100 years ozone has been successfully used in various fields of medicine, but till 1993 in literature there were no references to ozone applications in the treatment of obstetric complications, that was why for safe clinical applications of medical ozone there was a need for experimental grounds of the present method of treatment.

The aim of our investigation was to establish in vitro a range of ozone concentrations being safe for the homeostasis of patients with threatened abortion for the purpose of its further using in clinical conditions.

Materials and Methods

As a criterium in vitro for safety and efficiency of the given ozone concentrations it was considered intensification of the antioxidative defense system (AODS) and a decrease in the molecular products of lipid peroxidation (LP). The choice was based on the objectivity and informativity of the above indices as well as their importance for prognosis of obstetric pathologies including threatened abortion (2,3,5,6).

45 blood samples were investigated, 15 of them were taken from the patients with threatened abortion. As the control we used blood samples of 15 healthy non-pregnant women and 15 pregnant women without pathology.

20 ml of blood were taken from the elbow vein in standard conditions: at eight a.m., on an empty stomach, into the heparinized tube. Each sample was divided into 4 equal parts, the first one was used for the control as intact, as for the others 0,4 ml of ozonated physiological saline solution were added to each. Ozonization was carried out by barbotage method by introducing an ozone-oxygen mixture with ozone concentration of 400, 800 and 1200 mcg/L into 10 ml of sterile 0,9% sodium chlorid solution. Medical oxygen was delivered into ozone generator at flow rate 1 L/min, treatment time was 15 seconds that was enough for saturation of 10 ml physiological saline solution with ozone. The duration of ozone saturation of different saline volumes was determined on the laboratory grounds of Nizhny Novgorod Medical Academy, the method used for that purpose was based on the spectrophotometry detection (λ = 254 nm) of ozone concentration stabilization at an outlet port of the ozone generator and at an outlet port of the bottle with physiological saline. The correlation 5 ml blood – 0,4 ml ozonated 0,9% NaCl solution defined the clinical application of ozone (5 L – an average volume of circulating blood in human body, 0,4 L – a standard volume of bottles for intravenous infusions). As ozone is very unstable in water as well as in solutions, the ozonated saline was added to blood samples immediately after ozonization. The most important indices of LP were determined before and after the ozonated saline was added to blood samples. The total antioxidative activity (AOA) of blood serum was evaluated according to the change in light sum of induced chemiluminescence within 30 seconds by means of the biochemiluminometer BChL-06 equipped with software. The value of AOA was expressed as relative units (rel.U). The level of diene conjugates (DC) was evaluated according to ultraviolet absorption spectrum of methanol-hexane lipid solution at λ = 233 nm by method of F.S. Shenstone and was expressed as nanomoles per mg of total lipids (nmol/mg of total lipids). The level of Schiff's bases was determined according to fluorescent radiation by means of the fluorimeter ACO-1 at exitation wave λ = 365 nm and emission wave λ = 420 nm. The concentration of Schiff's bases (SB) was expressed as relative units of fluorescence per mg of total lipids (rel.U/mg of total lipids). The level of malonic dialdehyde was determined in reaction with 2-thiobarbituric acid according to formation of coloured trimethylenic complex with maximum absorption at λ = 532 nm.

Results and Measurements

3 experiment series were conducted: in the first series blood samples of 15 healthy pregnant women with 8-10 week pregnancy terms were investigated, in the second one – 15 healthy non-pregnant women of fertile age, in the third one – 15 patients with threatened abortion within the gestational period of 8–22 weeks. The analysis of initial LP values pointed to a considerable difference between them inside all 3 groups (Tables I, II, III). So, the patients with threatened abortion (3^{d} experiment series) showed the level of primary LP products - diene conjugates – at 6,72$\pm$1,07 nmol/mg of total lipids that was credibly 2,4 times ($p<0,05$) higher than in healthy pregnant women and 1,8 times ($p<0,05$) higher as compared with non-pregnant women. In the above mentioned series (1^{st} and 2^{nd}) the values of DC were lying within 2,78$\pm$0,74 nmol/mg of total lipids and 3,75$\pm$0,89 nmol/mg of total lipids, respectively (Table I). The level of end LP products – Schiff's bases – in patients with threatened abortion was likewise 2,1 times ($p<0,05$) higher than in pregnant women without pathology and 1,2 times ($p<0,05$) higher than in non-pregnant women and was equal to 497,5$\pm$70,9 rel.U/mg of

total lipids. In the 1st and 2nd series the above index was valued at 241,84±35,5 rel.U/mg of total lipids and 412,0±30,6 rel.U/mg of total lipids, respectively (Table II). The index SB : DC appeared to be the lowest in the 3d experiment series and was by 15% ($p<0,05$) lower than in the 1st series and by 33% ($p<0,05$) lower than in the 2nd one that pointed to the active LP process in patients with threatened abortion (Table III), at the same time, the patients showed a low level of AOA at 0,065±0,01 rel.U (Table IV) that was 2,3 times ($p<0,05$) lower than in healthy pregnant women and 1,8 times ($p<0,05$) lower than in non-pregnant women.

Table I : The influence of different ozone concentrations used in physiological saline on the level of diene conjugates (DC) in vitro

Series	Intact blood	Ozone concentrations		
		400 mcg/L	800 mcg/L	1200 mcg/L
1. DC in healthy pregnant women, nmol/mg of total lipids	2,78±0,74	2,23±0,72	2,22±0,65	3,05±0,86
2. DC in healthy non-pregnant women, nmol/mg of total lipids	3,75±0,89	4,94±0,96	4,64±0,87	4,06±0,89
3. DC in patients with threatened abortion, nmol/mg of total lipids	6,72±1,07	5,51±0,58	9,81±0,22	11,22±1,73

P1-3<0,05
P2-3<0,05

Table II : The influence of different ozone concentrations used in physiological saline on the level of Schiff's bases (SB) in vitro

Series	Intact blood	Ozone concentrations		
		400 mcg/L	800 mcg/L	1200 mcg/L
1. SB in healthy pregnant women, rel.U/mg of total lipids	241,8 ± 35,5	242,7 ± 68,8	247,1 ± 55,4	350,2 ± 89,4
2. SB in healthy non-pregnant women, rel.U/mg of total lipids	412,0 ± 30,6	836,5 ± 100,2	1001,7±140,2	1412±107,9
3. SB in patients with threatened abortion, rel.U/mg of total lipids	497,5±70,9	438,9±109,3	768,8±112,5	856,6±121,3

P1-3<0,05
P2-3<0,05

Having investigated in vitro the influence of ozone on the levels of primary and end LP products and AOA we established that the dynamics of the present indices depended on the ozone concentration, it was particularly obvious in the experiments on blood samples of the patients with threatened abortion. So, the blood samples treated with ozonated physiological saline at ozone saturation concentration of 400 mcg/L showed a tendency towards a decrease in DC and SB and a significant increase in AOA by 2,6 times ($p<0,01$) (Tables I, II, IV). It was also pointed out that higher ozone saturation concentrations (800 mcg/L and 1200 mcg/L)

without producing any effect on AOA caused a credible combinative increase in the primary and end LP products in patients with threatened abortion. So, at ozone saturation concentration of 800 mcg/L the level of DC reached 9,814±0,22 nmol/mg of total lipids, and the level of SB – 766,8±112,5 rel.U/mg of total lipids; at ozone concentration of 1200 mcg/L the above indices were 11,22±1,73 nmol/mg of total lipids and 856,6±121,3 rel.U/mg of total lipids, respectively (Tables I, II). So, in the course of experiments it was established that in patients with threatened abortion the LP processes increased and the AOA decreased. The LP processes in healthy women occured on the minimal level. According to the above indices the healthy non-pregnant women took an intermediate position. The blood samples of healthy pregnant women were most resistant to ozone in vitro, the blood samples of non-pregnant women were a little more sensitive, and the action of ozone on the blood samples of patients with threatened abortion was at its maximum. The manner of action of ozone on the level of LP and AOA in patients with threatened abortion depended on the dose of ozone: ozone concentration of 400 mcg/L induced a considerable increase in AOA and a decrease in the molecular products of LP. Higher ozone concentrations (800 mcg/L and 1200 mcg/L) caused reverse processes. On the basis of the received results ozone saturation concentration of 400 mcg/L was accepted as the optimal one for clinical applications owing to its positive influence on LP and AOA processes of blood plasma.

Table III : The influence of different ozone concentrations used in physiological saline on the index Schiff's bases : diene conjugates (SB/DC) in vitro

Series	Intact blood	Ozone concentrations		
		400 mcg/L	800 mcg/L	1200 mcg/L
1. SB/DC in healthy pregnant women	86,6±5,7	108,8±6,3	110,8±5,1	114,8±10,1
2. SB/DC in healthy non-pregnant women	109,9±9,8	169,3±11,2	215,7±15,3	347,7±10,2
3. SB/DC in patients with threatened abortion	73,9±2,1	80,0±3,3	78,2±4,4	77,8±10,1

P1-3<0,05
P2-3<0,05

Table IV : The influence of different ozone concentrations used in physiological saline on the total antioxidative activity (AOA) of blood serum in vitro

Series	Intact blood	Ozone concentrations		
		400 mcg/L	800 mcg/L	1200 mcg/L
1. AOA in healthy pregnant women, rel.U	0,15±0,07	0,164±0,06	0,16±0,03	0,154±0,04
2. AOA in healthy non-pregnant women, rel.U	0,12±0,05	0,174±0,03	0,18±0,04	0,12±0,04
3. AOA in patients with threatened abortion, rel.U	0,065±0,01	0,174±0,02	0,06±0,01	0,07±0,04

P1-3<0,05
P2-3<0,05

Conclusion

Thus, before clinical applications of ozone it was experimentally established and pathogenetically verified that in case of gestational complications ozonetherapy should be used within a narrow range of ozone concentrations (400-800 mcg/L of an ozone-oxygen mixture) that is a part of therapeutical range accepted by the Russian school of ozonetherapy. Further clinical investigations have absolutely proved the applicability of ozonetherapy in the treatment of such gestational complications as threatened abortion, gestosis, pregnancy anemia (4,5,6) as well as for prophylaxis of intrauterine fetus infection and fatness-associated pregnancy complications.

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