The Mechanism of the Anti-Inflammatory Effect of Ozonated Physiological Saline

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Abstract

The ozonated 0,9% sodium chlorid solution barbotaged in a standard bottle by ozone-oxygen mixture with ozone concentration of 1200 mcg/L for 15-20 minutes at flow rate 1 L/min produces a positive effect on the clinical course of acute PID and on some indices of the homeostasis in the given group of patients when introduced intravenously.

The fungicidal and bactericidal effects of ozonated saline have not been established during the experiments in vitro on pure cultures of Staphilococcus aureus CCM 885 gram-positive bacteria, Escherichia coli M-17 gram-negative bacilli and Candida albicans yeast-like fungi.

The study of the spontaneous (SCL) and induced chemiluminescence (ICL) parameters of neutrophiles in patients with acute PID testifies to the ability of ozonides to stimulate the function of polymorphonuclear leukocytes.

Introduction

Acute uterine appendages inflammation or pelvic inflammatory disease (PID - according to International classification) is the most frequent pathology among the women of reproductive age, it is detected in 60-65% of gynecological patients, and 20-30% of them need hospital treatment.

The complex conservative treatment of acute PID includes therapeutic methods which service not only to eliminate the causative agent of disease, but also to correct the metabolic and immunological disturbances.

Materials and Methods

The aim of the experiment in vitro was to investigate the bactericidal properties of ozonated physiological saline solution produced by barbotage method by introducing an ozone-oxygen mixture with ozone concentration of 1200 mcg/L into the saline. The investigation was conducted on pure cultures of S. aureus, E. coli and C. albicans. 18 hour broth cultures were sowed out by lawn method on growth agar in Petri dishes, after that 20,0 ml of ozonated

saline were added to cultures, the equal volume of not-ozonated saline was used as the control. The results were evaluated after 18 hours. The experiment was conducted three times. As a criterium for evaluation of results it was considered a formation (or no formation) of so called sterile spots.

The aim of clinical experiment series was to study the influence of ozone on the oxygen-dependent metabolism of neutrophiles in patients with PID. We examined 25 women with acute internal genital inflammation which underwent antibacterial therapy in combination with ozonetherapy. The control group included 8 healthy women and 8 patients with the above pathology which received the complex conventional treatment. Blood samples for biochemilumenescence analysis were taken from the elbow vein in standard conditions during the whole 5 day treatment session in order to determine the spontaneous (SCL) and induced chemiluminescence (ICL).

Results and Measurements

No one of experiments in vitro showed a formation of sterile spots, accordingly, the solution to be investigated did not cause death of microorganisms included into the experiment. As the experiment was conducted on gram-positive, gram-negative bacteria and Eucaryotae microbes i.e. microorganisms with different organization of bacterial cell wall and cell as a whole, the received results can be interpreted as valid for a wide spectrum of microorganisms.

In the course of clinical investigations the following data were received (Table I, II).

Table I : The indices of SCL of neutrophiles in patients with PID in case of conventional treatment and on the background of ozonetherapy in comparison with healthy donors

Treatment days	The control group		The test group
Before treatment	2,4 $\pm$ 0,02	$p > 0,05$	2,1 $\pm$ 0,08
1	2,5 $\pm$ 0,05	$p > 0,05$	3,8 $\pm$ 0,07
2	2,1 $\pm$ 0,03	$p < 0,01$	6,06 $\pm$ 0,09
3	1,3 $\pm$ 0,02	$p < 0,01$	4,9 $\pm$ 0,06
4	1,1 $\pm$ 0,02	$p < 0,01$	3,8 $\pm$ 0,05
5	0,9 $\pm$ 0,01	$p < 0,01$	3,7 $\pm$ 0,05
In health	0,5 – 1,5		

Table II : The indices of ICL of neutrophiles in patients with PID in case of conventional treatment and on the background of ozonetherapy in comparison with healthy donors

Treatment days	The control group		The test group
Before treatment	0,7 $\pm$ 0,01	$p > 0,05$	0,78 $\pm$ 0,02
1	0,68 $\pm$ 0,01	$p < 0,01$	2,1 $\pm$ 0,04
2	0,54 $\pm$ 0,01	$p < 0,01$	2,7 $\pm$ 0,03
3	0,43 $\pm$ 0,01	$p < 0,01$	2,3 $\pm$ 0,02
4	0,3 $\pm$ 0,02	$p < 0,01$	2,3 $\pm$ 0,01
5	0,3 $\pm$ 0,02	$p < 0,01$	2,2 $\pm$ 0,01
In health	0,5 – 1,0		

Note: The values of SCL are expressed as impulses per second , ICL as impulses per second.

The values presented in the above Tables show that the initial indices of SCL and ICL in patients of both groups were practically equal ($p > 0,05$). The index of SCL showed a tendency to increase already after first injection of ozone, but the difference from the control was not credible ($p > 0,05$). The credible (as compared with the control) intensification of SCL occured after two injections of ozone. The follow-up infusions did not lead to an activation of the oxygen-dependent cell metabolism, on the contrary, it was established an insignificant decrease in SCL, but its value was still higher than the control. The similar results regarding the effect as well as investigation time were received for ICL of neutrophiles in whole blood: one injection of ozone induced an increase in the index, its maximal increase with follow-up stabilization was registered after two injections of ozone. The abilities of neutrophiles to realize their biocidal potential were demonstrated by functional reserve of phagocytes to be calculated according to formula ICL/SCL, its dynamics is presented in Table III.

Table III : The dynamics of functional reserve of neutrophiles in patients with PID on the background of conventional treatment and ozonetherapy

Treatment days	The control group		The test group
Before treatment	$2,9 \pm 0,05$	$p < 0,1$	$3,7 \pm 0,06$
1	$2,7 \pm 0,02$	$p < 0,01$	$5,5 \pm 0,03$
2	$2,5 \pm 0,03$	$p < 0,01$	$4,4 \pm 0,02$
3	$3,3 \pm 0,02$	$p < 0,01$	$4,6 \pm 0,03$
4	$2,7 \pm 0,01$	$p < 0,01$	$6,0 \pm 0,04$
5	$3,3 \pm 0,04$	$p < 0,01$	$5,9 \pm 0,02$
In health	$8,3 \pm 0,02$		

The values presented in the above Table point to a considerable increase in the functional reserve of neutrophiles on the background of ozonetherapy: after 5 day treatment session the above index established in the test group is credibly higher than in the control although it does not reach the level of healthy donors. The functional reorganization of neutrophile granulocytes is also demonstrated by the kinetics of stimulation response of ICL: appearance time of ICL peak decreases from $15,4 \pm 0,6$ minutes at the beginning of disease to $9,5 \pm 0,5$ minutes by the end of ozonetherapy session ($p < 0,01$). In order to clarify the mechanisms of neutrophile processes some experiment series were conducted. The aim of the first one was to investigate the dynamics of CL indices immediately after administration of ozonated physiological saline. The investigation of blood samples taken immediately after ozone treatment showed that the changes in SCL and ICL were chaotic that made it impossible to draw any conclusions about a tendency of their dynamics. However, already after 24 hours it was established an increase in SCL indices, and after repeated administration of ozone it was received a credible difference. Based on the above results, it was supposed that the stimulating effect on the oxygen-dependent metabolism of neutrophiles depended on blood accumulation of some substance. In further experiments in vitro it was made a plasma elimination model – blood dilution 1:100, the stimulating effect of ozone on SCL and ICL was there not established.

In order to prove the above supposition we conducted isolated experiments on pure neutrophile culture to investigate the influence of plasma of patients treated with ozone on the neutrophile granulocytes of healthy donors. As the control we used plasma of patients with PID treated with conventional treatment and plasma of healthy donors. In the course of an experiment it was established that plasma of patients treated with ozone in all stages of

treatment produced an expressed neutrophile stimulating effect which was considerably higher than in both the controls.

Discussion

The experimental part of our investigation has demonstrated that the ozonated physiological saline produced by barbotage method by introducing an ozone-oxygen mixture with ozone concentration 1200 mcg/L into the saline does not cause in vitro direct destruction of microorganisms and therefore does not produce a bacterio-fungicidal effect. That is why the ozonated physiological saline with the above ozone concentration should be used for treatment of an acute inflammation only as a part of the complex therapy in combination with antimicrobial preparations. The investigation into the indirect effect of ozone has been made in clinical conditions. It is established that ozone is able to increase the respiratory activity of neutrophile granulocytes according to ICL indices. As there is no evidence of stimulating effect after plasma elimination from ozonated blood, we have drawn a preliminary conclusion that the neutrophile stimulating substance is a part of plasma.

The mechanisms of neutrophile function stimulation can be explained as follows: firstly, the use of ozone can increase the opsonic capacity of blood serum, secondly, the contact between ozone molecule and blood serum components can lead to accumulation of neutrophile activating ozonides which do not belong to opsonins according to the traditional interpretation of this concept. As the not-adjustable intensification of the oxygen-dependent metabolsm of neutrophiles can cause intravascular cell activation that is in turn considered a precondition for some pathological situations, the evaluation of CL indices is necessary to select the therapeutical doses of ozone.

Conclusion

The ozone concentration of 1200 mcg/L without producing a bactericidal effect is able to induce an increase in the phagocytic neutrophile activity. This effect is of great importance in the treatment of PID, when the local use of ozone is impossible.

The received results convincingly testify to the real pathophysiological verification of ozone applications for correction of metabolic and immunological disturbances and the applicability of ozonetherapy in the complex treatment of PID.