Determination of the optimal concentration of ozone–containing mixture for safe use in clinical conditions

M. V. Kostylev1, G. V. Terekhov1, I. M. Savytska1, E. V. Simulyk1, O. Yu. Furmanov1, N. Yu. Grigorieva2

1Shalimov National Scientific Centre of Surgery and Transplantation, Kyiv,
2Scientific and Methodological Centre “Medical Innovative Technologies”, Kyiv

Abstract

Objective. To study the effect of ozone during hyperbaric oxygenation with an ozone–containing mixture on the respiratory system and stress response organs of experimental animals, to select the optimal parameters of ozone generation and supply by the POS–1 apparatus to perform this procedure in a confined space without harming the health of the patient and medical staff.

Materials and methods. Experimental studies were performed on 20 white laboratory rats divided into two groups. In group 1, 10 animals breathed the ozone–containing mixture for 7 days, each session lasted 15 minutes. In the 2nd group, 10 animals breathed the ozone–containing vapour–water mixture for 7 days, each session also lasted 15 minutes. All animals were kept in a semi–hermetic box with a volume of 0.042 m3 with an ozone supply of 500 mg/h, which exceeds the maximum permissible concentration (0.16 mg/m3) by a factor of 2. The pressure of the gas mixture in the box with the animals was equal to atmospheric pressure. The animals were withdrawn from the experiment on the 3rd, 7th and 14th day by injecting an excessive dose of 5.0% sodium thiopental solution. The trachea, lungs, liver, kidneys, spleen, heart, stomach, pancreas, and adrenal glands were autopsied and taken for histological examination. The ozone concentration in the room during the experiment was measured using a SAMI 100S ozone concentration meter.

Results. Exposure to the ozone–containing mixture did not cause pathological changes in the organs and systems of the experimental animals, as well as a significant stress response, which proves the safety of the technique in confined spaces. The method of treatment of infectious and purulent complications proposed in previous studies does not harm the health of the patient and medical staff and can be recommended for use in clinical practice without additional measures for the utilisation of ozone produced by the POS–1 apparatus.

Conclusions. The optimal parameters of ozone production and supply in the ozone–containing mixture for hyperbaric oxygenation in a confined space without harming the health of the patient and medical staff have been proposed. The ozone–containing steam–water mixture can be recommended for use in clinical practice in conditions of massive tissue infection as a factor of external physical influence with pronounced bactericidal and bacteriostatic properties. The concentration of residual ozone in the room after the therapeutic procedure does not exceed the permissible level and does not harm the health of the patient and medical staff.

Key words: hyperbaric oxygenation; ozone; ozone–containing mixture; steam–water ozone–containing mixture; swimming test.

EXPERIMENTAL INVESTIGATIONS

Ozone is a modified form of oxygen, its molecule consists of three atoms. Under normal conditions, this gas is blue in colour and has a pungent odour that can be detected even at a dilution of 1:100,000. The solubility of ozone in water is 10 times higher than that of oxygen and strongly depends on the purity of the water, as impurities catalyse the decomposition of ozone [1]. The ozone molecule is unstable and at sufficient concentrations in the air under normal conditions spontaneously turns into oxygen in a few tens of minutes with the release of heat. Contact of ozone with even small amounts of organic matter, some metals or their oxides dramatically accelerates its transformation [2, 3].

Ozone has a much higher oxidising capacity than dinuclear oxygen, which in many chemical reactions leads to the formation of oxygen free radicals that determine its toxic properties [4].

According to the national hygiene standards, the orders of the Ministry of Health of Ukraine of 2020, the maximum one–time maximum permissible concentration (MPC) of ozone is 0.16 mg/m3, the MPC of ozone in the air of the working area is 0.1 mg/m3. At the same time, the threshold of human olfaction is approximately 0.01 mg/m3, i.e. a person can smell ozone when its concentration in the air is 10 times lower than the MPC [4–7].

The use of ozone in medicine is due not only to its bactericidal, fungicidal and virucidal properties, but also to the fact that it is a strong oxidising agent [3, 8].

According to therapists’ observations, the duration of many diseases is significantly reduced if dosed ozone therapy
is added to standard treatments, either externally or orally, intravenously or extracorporeally [9–11].

When used externally (on the skin and wound surface), enterally and parenterally in the therapeutic concentration range, ozone has no toxic effect on the human body [8].

Among the reasons for the bactericidal effect of ozone, the most commonly mentioned is the violation of the integrity of bacterial cell membranes caused by the oxidation of phospholipids and lipoproteins. There are also data on the interaction of ozone with proteins. It was found that ozone penetrates into the microbial cell, reacts with cytoplasmic organelles and converts a closed DNA plasmid into an open DNA form, which reduces bacterial proliferation [3, 12–14]. The bactericidal properties of distilled ozonated water with an ozone concentration of 4 mg/l were tested. Under in vitro conditions, the growth of colonies of Staphylococcus aureus, Escherichia coli, Proteus, Klebsiella was completely inhibited at the levels of 102 – 104 colony forming units (CFU)/ml. If the number of microorganisms approached 105 – 107 CFU/ml, their incomplete inactivation occurred [2, 15].

It has been found that ozone can inactivate the virus both extracorporeally and inside cells. Many antibiotic–resistant infections are inactivated by ozone in concentrations that are non–toxic to human cells [16].

Therapeutic doses of ozone reduce the intensity of lipid peroxidation (LPO). At the same time, the antioxidant defence system is rapidly activated, which ozone stimulates indirectly [17]. The importance of these results lies in the evidence of the safety of the ozone concentrations used. Regulation of the processes of lipid peroxidation and restoration of the balance between it and the antioxidant defence system is probably one of the most important mechanisms of effective ozone therapy [18].

Ozone does not destroy tissues and cells; it restores or increases normal cellular oxidation that has been reduced by disease. Blood in the presence of ozone can absorb 2–10 times more oxygen than under normal conditions, as oxygen dissolves in plasma. The tropism of ozone and its fixation by tissues have been proven. During ozone therapy, blood serum and red blood cells are saturated with oxygen [19].

Given the above effects, ozone therapy is increasingly being used in medical practice. Ozone affects several links in the pathogenesis of many diseases simultaneously, primarily by regulating the dynamic balance of lipid peroxidation and antioxidant activity. The use of ozone therapy improves the oxygen supply to tissues, activates oxygen–dependent processes in them, reduces vascular tone and inhibits the atherosclerotic process [20].

The authors have carried out experimental studies of the possibility of humidifying an ozone–containing gas mixture so that during the procedure of hyperbaric oxygenation with such a gas mixture there is no threat to the health of the patient and medical personnel.

The aim of the study is to investigate the effect of ozone during the procedure of hyperbaric oxygenation with an ozone–containing mixture on the respiratory system and stress response organs of experimental animals, to select the optimal parameters of ozone generation and supply by the POS–1 apparatus for performing this procedure in a confined space without harming the health of the patient and medical staff.

**Materials and methods**

The basic model of the POS–1 apparatus, which produces an ozone–containing mixture, and its modified model for producing a steam–water ozone–containing mixture, created by the staff of the Scientific and Methodological Centre "Medical Innovative Technologies" (Kyiv, Ukraine), were used for the study. The possibility of using these models is confirmed by the "Conclusions of the State Sanitary and Epidemiological Expertise" No. 12.2–18–2/10782 dated 15.05.2020 and No. 12.2–18–1/11376 dated 22.05.2020. The parameters of the created devices provide the possibility of preparing an ozone–containing mixture with a capacity of (7 ± 0.4) 1/min at an ozone concentration of (0.16 ± 0.03) mg/m³.

The modified model of the POS–1 apparatus consists of an ozone generator and a Bobrov flask for humidifying the gas mixture. It has already been tested experimentally and clinically to prove its bactericidal properties for the treatment of massively infected and purulent wounds using the method of hyperbaric oxygenation with an ozone–containing mixture.

The research programme was developed by the team of authors and approved at a joint meeting.

To investigate the effect of ozone–containing and steam–water ozone–containing mixtures, experiments were conducted on 20 white laboratory rats divided into two groups. In group 1, 10 animals breathed the ozone–containing mixture without humidification for 7 days, each session lasted 15 minutes. Animals in group 2 (n=10) breathed a steam–water ozone–containing mixture for 7 days, each session also lasted 15 minutes. All animals were kept in a semi–hermetic box with a volume of 0.042 m³ with an ozone supply of 500 mg/h, which exceeds the MPC (0.16 mg/m³) by 2 times. The pressure of the gas mixture in the box with animals was equal to atmospheric pressure.

The ozone concentration in the room during the experiment was measured using a SAMI 100S ozone concentration meter. The volume of the room is 34.5 m³.

After each procedure, animals of both groups underwent a forced swimming test with a load at the same time of day (from 10:00 to 11:00) immediately after the breathing procedure. After completion of the swimming test, the behavioural response of animals to stress (motor activity, spatial orientation, hunger and reaction to external stimuli) was subjectively assessed during 10 min of observation.

Animals were withdrawn from the experiment on days 3, 7 and 14 by injection of an excessive dose of 5.0% sodium thiopental solution. The trachea, lungs, liver, kidneys, spleen, heart, stomach, pancreas, and adrenal glands were autopsied and taken for histological examination. The detected changes were evaluated by comparing them with the changes detected in the autopsy of healthy white rats kept in a vivarium under general conditions.
The selected tissues were fixed in a 10% solution of neutral formalin, dehydrated in ethyl alcohol solutions of increasing concentrations, embedded in paraffin according to the conventional method, and histological sections (5 μm thick) were made and stained with hematoxylin and eosin and picofuchsins using the Van Gieson method.

Histological studies were performed using a Leica DM 500 microscope with a computer console and the possibility of morphometric studies. Morphometric data processing was carried out using a video analyser and the computer program "Paradise". The preparations were photographed using a Leica ICC50 HD camera.

The digital material was processed using the methods of variation statistics. For statistical evaluation of the significance of the difference in the obtained digital indicators, the Student's T–test and Microsoft Excel and Microcal Origin software were used. Differences with p values <0.05 were considered statistically significant.

Results

The results of swimming tests and behavioural reactions of white rats of both groups did not differ from each other.

Measurements of the ozone concentration in a 34.5 m³ room during the experiment showed that it was at the "moderate" level at the end of each study and ranged from 0.3 to 1.0 ppm. After opening the semi–hermetic box, the smell of ozone was felt in the room for several minutes at a concentration of 0.05 mg/m³, which did not exceed the MPC, so there was no need for additional ozone disposal measures.

Morphological studies performed on day 3 showed that inhalation of a non–humidified ozone–containing mixture did not cause any significant changes in the liver, kidneys, spleen, adrenal gland, and pancreas. However, epithelial desquamation from the tracheal mucosa and lymphocytic infiltration of the mucosa and submucosa increased (Fig. 1).

In the lungs, some alveolar sacs were stretched, some were closed and compressed. In the heart, there was an uneven blood supply to the myocardium. Increased desquamation of the surface epithelium was observed in the stomach.

After 3 days, no pathological changes in the studied organs were observed in animals breathing the steam--water ozone mixture, only in one rat, increased desquamation of the bronchial epithelium was noted in the lungs. The trachea was unremarkable (Fig. 2).

After 7 days, in animals breathing a non–humidified ozone–containing mixture, hepatocytes showed a decrease in glycogen levels, kidneys had no pronounced features, and the area of white pulp in the spleen increased relative to normal. In the trachea, moderate dystrophic changes in the epithelial cells of the mucous membrane and damage to the ciliary layer were noted (Fig. 3). Bronchial epithelial desquamation was increased in the lungs. Lymphocytic infiltrates were found in the tracheal submucosa and parenchyma. Moderate cardiomyocyte edema was noted in the myocardium. The adrenal gland had no pronounced features. In the stomach, increased desquamation of the epithelium from the surface of the mucous membrane was observed. The pancreas had no pronounced features.

After 7 days, in animals breathing a steam–water ozone–containing mixture, the liver, kidneys, spleen, heart, adrenal gland, stomach, and pancreas had no pronounced features. In the trachea, the mucous membrane was preserved, the epithelial plate underwent dystrophic changes, and increased desquamation of its cells was noted in some areas (Fig. 4). In the lungs, the bronchial epithelium was preserved, the ciliated layer was less dense than normal. In some areas, there were signs of moderate stromal edema.

After 14 days in animals breathing a non–humidified ozone–containing mixture, moderate manifestations of granular dystrophy were observed in the liver, pronounced

![Fig. 1. Trachea of a white rat 3 days after inhalation of of a non-humidified ozone-containing mixture. Hematoxylin and eosin staining. × 100.](image1)

![Fig. 2. Trachea of a white rat 3 days after inhalation of of a steam and water ozone mixture. Hematoxylin and eosin staining. × 100.](image2)
haemorrhage in the cortical and cerebral substances in the kidneys, subcapsular edema in the spleen, and an increase in the area of white matter. In the trachea, the epithelium was mostly preserved, the ciliated layer was partially damaged, the mucosa’s own plate and submucosa were abundantly infiltrated with lymphocytes. In the heart, there were marked diffuse atrophic changes in cardiomyocytes. In the adrenal gland, there was a pronounced cerebral haemorrhage, the cortical substance was unremarkable. The pancreas was unchanged. Increased desquamation of the surface epithelium was observed in the stomach.

After 14 days, in animals breathing a steam–water ozone–containing mixture, the liver and kidneys were without pronounced features, and a mild subcapsular edema was noted in the spleen. In the trachea, the epithelium was preserved, there was no lymphocytic infiltration. The bronchial epithelium underwent increased desquamation, but was mostly preserved. No pathological changes were noted in the heart. In the stomach, increased desquamation of the surface cells of the mucous membrane was observed. The pancreas and adrenal glands had no pronounced features.

Thus, in animals breathing a non–humidified ozone–containing mixture, in addition to changes in the respiratory system, there were moderate changes in the myocardium, adrenal gland and stomach. No systemic effects were observed in animals breathing a steam–water ozone–containing mixture, and the changes detected were compensatory in nature.

Discussion

After analysing the literature, it was found that no such comparative studies had been conducted. The development of the apparatus for hyperbaric oxygenation with ozone–containing mixture POS–1 and its modification with a humidifier by the team of the Scientific and Methodological Centre "Medical Innovative Technologies" is protected by copyright.

The obtained results of the study of the use of the POS–1 apparatus for the procedure of hyperbaric oxygenation with an ozone–containing mixture proved the absence of pathological changes in the organs and systems of a living organism, as well as the development of stress reactions, which is proof of the safety of the technique in confined spaces. The method of treatment of infectious and purulent complications proposed in previous studies does not harm the health of the patient and medical staff and can be recommended for use in clinical practice without additional measures for the utilisation of ozone produced by the POS–1 apparatus.

The obtained results of the study on the use of the POS–1 apparatus for the procedure of hyperbaric oxygenation with an ozone–containing mixture and a steam–water ozone–containing mixture showed insignificant differences between these two methods, but the following recommendations can be made. The POS–1 device without a humidifier should be used in case of a critical degree of aggressiveness of pathogenic microorganisms (clostridial, anaerobic infection) and the duration of treatment of an infected wound is up to an hour, the POS–1 device equipped with a humidifier – with less virulent microflora and the duration of treatment is from 1 to 1.5 hours during each procedure.

Conclusions

1. Preliminary bacteriological studies on multidrug–resistant clinical bacterial cultures in infected and purulent wounds confirmed the pronounced bactericidal properties of ozone produced by the POS–1 apparatus in the basic configuration and modified version.

2. The optimal parameters of ozone production and supply as part of an ozone–containing mixture for hyperbaric oxygenation in a confined space have been proposed, which allows us to recommend its use in wards, manipulation rooms.
and operating rooms. The residual ozone in the room during a therapeutic procedure does not exceed the MPC and is not harmful to the health of the patient and medical staff.

3. A steam–water mixture containing ozone can be recommended for use in clinical practice in conditions of massive tissue infection as a factor of external physical influence with pronounced bactericidal and bacteriostatic properties.

Funding. The study is a fragment of a research work. Funding is partly from the state budget and partly from the authors.

Authors’ contribution. Kostylev MV: study design, analysis of data, revision of the article; Terekhov GV: study planning, conduct of oxygenation sessions; Savitskaya IM: processing of materials, analysis of data, writing the text; Simulik EV: study design, and operating rooms. The residual ozone in the room during a therapeutic procedure does not exceed the MPC and is not harmful to the health of the patient and medical staff.

3. A steam–water mixture containing ozone can be recommended for use in clinical practice in conditions of massive tissue infection as a factor of external physical influence with pronounced bactericidal and bacteriostatic properties.

Funding. The study is a fragment of a research work. Funding is partly from the state budget and partly from the authors.

Authors’ contribution. Kostylev MV: study design, analysis of data, revision of the article; Terekhov GV: study planning, conduct of oxygenation sessions; Savitskaya IM: processing of materials, analysis of data, writing the text; Simulik EV: study design, and operating rooms. The residual ozone in the room during a therapeutic procedure does not exceed the MPC and is not harmful to the health of the patient and medical staff.

3. A steam–water mixture containing ozone can be recommended for use in clinical practice in conditions of massive tissue infection as a factor of external physical influence with pronounced bactericidal and bacteriostatic properties.

Funding. The study is a fragment of a research work. Funding is partly from the state budget and partly from the authors.

Authors’ contribution. Kostylev MV: study design, analysis of data, revision of the article; Terekhov GV: study planning, conduct of oxygenation sessions; Savitskaya IM: processing of materials, analysis of data, writing the text; Simulik EV: study design, and operating rooms. The residual ozone in the room during a therapeutic procedure does not exceed the MPC and is not harmful to the health of the patient and medical staff.

3. A steam–water mixture containing ozone can be recommended for use in clinical practice in conditions of massive tissue infection as a factor of external physical influence with pronounced bactericidal and bacteriostatic properties.