Inflammation, Vol. 30, Nos. 1–2, April 2007 (© 2007)
DOI: 10.1007/s10753-007-9021-7

Ozonotherapy in an Induced Septic Shock. I. Effect of Ozonotherapy on Rat Organs in Evaluation of Free Radical Reactions and Selected Enzymatic Systems

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Abstract—The confirmed advantageous effects of oxygen/ozone therapy in several clinical conditions stimulated experimental studies on effects of the therapy in rats with an induced septic shock. The studies were conducted on adult male rats of Wistar strain. Four groups of the animals, each of 15 rats, included: I—control group, (C); II—animals intraperitoneally administered with O_2/O_3 (CO), III—rats given of *Escherichia coli* endotoxin (lipopolysaccharide—LPS) (CL), IV—rats administered with the lipopolysaccharide plus administered with the oxygen/ozone mixture (OL). Activities of catalase and superoxide dismutase and of free radical reactions were estimated. The exposure to LPS augmented activities of SOD and of catalase in liver, lungs and heart. In all the examined organs LPS induced significant changes in levels of free radicals. Except of the lungs, parallel administration of the rats with LPS and ozone/oxygen revoked development of the alterations. The obtained results point to a strong, stabilizing and regenerative effect of ozonotherapy.

KEY WORDS: septic shock; ozonotherapy; oxidative stress; free radicals; rats.

INTRODUCTION

Endotoxin represents the generally recognized agent which precipitates the chain of pathophysiological events leading to development of septic shock [1]. Septic shock is a sequele of infection with Gram-negative bacteria and frequently results in death of the host. Intravenous infusion of endotoxin provides the most popular experimental model of septic shock [2, 3]. Mediators of the shock development seem to include, i.a., reactive forms of oxygen.

The oxygen/ozone therapy involves administration of O_2/O_3 gas mixture to body cavities, intraarterially, in the form of autohemotransfusion or under foil tents, etc. The intense supply of oxygen promotes supply of the gas to anoxic sites and, in parallel, inhibits development of bacteria, viruses and fungi, improving results of the principal therapy. Therapeutic doses of O_2/O_3 mixture activate enzymes responsible for body protection from processes linked to overproduction of superoxides [4, 5]. Ozone stimulates transmembraneous flow of oxygen, induces enzymes such as superoxide dismutase, catalase or peroxidases and makes oxygen utilization in the mitochondrial respiratory chain more effective.

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Intraperitoneal administration of the ozone/oxygen mixture in conditions of an induced septic shock (both septic shock and oxygen therapy are known to provoke pro-oxidative status and production of high amounts of free radicals) requires that the systems are evaluated which protect the body against free radicals. The most significant line of body protection against free radicals involves enzymatic mechanisms linked to activity of superoxide dismutase, catalase and glutathione peroxidase. Superoxide dismutase (SOD) represents the only enzyme for which free radicals are a direct substrate [6, 7]. In rat liver SOD comprises as much as 0.1–0.3% of total protein [8].

Present study aimed at evaluating whether and, if so, in which way treatment with O_2/O_3 mixture in an induced septic shock affects free radical reactions and the enzymatic systems which protect the host from damage in the septic shock-induced anoxia. Activities of processes determining efficiency of the free radical scavenger system, including superoxide dismutase and catalase, were selected as indices of efficiency of the system, directly preventing the host from the action of free radicals. Free radical reactions were also directly estimated in selected organs and tissues. Rats were chosen as a model animal since the species is most frequently employed in experiments on ozonotherapy and septic shock [9–11].

MATERIALS AND METHODS

Animals

The experiments were performed on adult (4-month-old) male rats of Wistar strain, originating from Experimental Animal Breeding Laboratory of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wroc^{3}aw, Poland. The average body weight of the rats amounted to 172 ± 8.0 g. The animals were kept in plastic cages, one rat per cage, at room temperature, at the 12 h L/12 h D illumination cycle and were allowed free access to the Murigran pelleted chow and tap water.

The studies were performed on adult animals since such animals are more sensitive to ozone than the young ones, as demonstrated by Canada et al. [12]. The dose and procedure of ozone administration were consistent with the concentration and timing of oxygen/ozone administration in the clinical ozonotherapy [13].

Treatment

The applied in our study intraperitoneal route of administration of the oxygen/ozone mixture warranted high surface area of absorption and prevented direct contact between ozone and morphotic elements of blood. Considering the ozone half-life of approximately 30 min in the in vitro conditions, at 403 K [13], the route seemed appropriate for testing effects of the oxygen/ozone mixture on function of individual organs since it restricted the potential for a direct induction of alterations in blood by the O_2/O_3 mixture [3, 14, 15]. On the other hand, the applied dose of O_2/O_3 mixture has been sufficiently high to permit detection of any therapeutic effects on selected organs and enzymatic systems [16–18].

The applied dose of endotoxin was so selected in preliminary experiments that the LPS-exposed animals were capable to survive at least one day.

The animals formed the following four groups, each of 15 rats:

I control animals (C), given a single intraperitoneal dose of 1 cm^3 saline;
II experimental group (CO), in which the rats were administered only with O_2/O_3 mixture (around 95% oxygen and around 5% ozone) for ten consecutive days; 1 cm^3 of the mixture, at the concentration of 54 mg/dm^3 (around 150 μg/kg body weight), was given in a single dose between hours 9^{00} and 10^{00} A.M.;
III experimental group (CL), in which the rats were given a single 1 cm^3 dose of *Escherichia coli* endotoxin (20 mg/kg body weight), i.e. LPS (serotype O 127:B8; Sigma Chemical) injected to the tail vein;
IV experimental group (OL), in which the rats were given intravenously LPS (as in group III) and, intraperitoneally, the oxygen/ozone mixture (as in group II).

Animals of CO and OL groups received oxygen/ozone mixture for ten consecutive days and, moreover, in the tenth day rats of the control group were given physiological saline while rats of CL and OL were treated with endotoxin. The animals were sacrificed 24 h after treatment with selected xenobiotic Following autopsy, liver, kidneys, lungs and walls of cardiac ventricles were taken for biochemical studies.

The autopsies were performed always in the morning to avoid problems reflecting circadian variation of enzymatic activities [19, 20].

The oxygen/ozone mixture was produced in the AK1 apparatus (Kriometrum, Poland) from a pure medical oxygen in a silent discharge chamber.

The studies were performed after obtaining consent of the Local Ethical Committee.

Methods of Enzymatic Studies

Activity of superoxide dismutase was estimated in homogenates of liver, kidneys, lungs and heart using the adrenalin technique of Misra and Fridlovich [21] while activity of catalase was estimated according to Aebi et al. [22].

Chemiluminescent studies aimed at detection free radical (FR) reactions were conducted recording the scanty photon luminescence in 20% aqueous extracts of liver, kidneys, lungs and heart, as described by Madej et al. [23]. Duration of the record in a sample warmed up to 323 K was 30 min. Impulses originating from the weak photon luminescence were scored using the electronic type PEL-5 recorder with the P-12 FQ 51 photomultiplier.

Statistical Analysis

The results were subjected to statistical analysis employing the Statistica 5.1 software (StatSoft), to calculate means, standard deviation and significance of differences between the means on the basis of the lowest significant difference (LSD) in the ANOVA unifactorial analysis of variance.

RESULTS

Signs of endotoxic shock developed in all animals of CL and OL groups immediately or few minutes after administering *Escherichia coli* endotoxin. They manifested in the form of circulatory failure (cyanosis of oral mucosa and of conjunctivae, accelerated pulse and respiration). The observed signs even if progressing in a similar way in CL and OL groups were evidently less pronounced in the group administered with LPS plus O_2/O_3 mixture.

Free Radicals/Free Radical Reactions

Following infusion of the oxygen-ozone mixture levels of free radicals in studied organs remained at the level of control groups. Administration of LPS augmented this level to 145 and 200% control values in liver and heart, respectively, (Fig. 1) but in kidneys and lungs exposure to the xenobiotic agent significantly decreased free radical pool. Protective effect of the ozone-oxygen mixture in the OL group could be demonstrated in liver, kidneys and heart, in which free radical content returned to control levels. In lungs, the lowered level of free radicals persisted, as compared to the control groups.

Analysis of free radical reactions demonstrated significant differences in free radical levels in all organs between ozone treated animals (CO group) and LPS treated animals while in all the organs except lungs between animals treated with LPS (group CL) and those treated with LPS and ozone (group OL). Nevertheless, free radical content of lungs differed significantly between CO and OL groups.

Superoxide Dismutase

Administration of the oxygen/ozone mixture exerted no influence on activity of superoxide dismutase in any of the examined organs (Fig. 2). Administration of LPS (CL group) augmented the enzyme activity to 135% control values in liver, to 250% in lungs and to 215% in heart while no changes in activity of the enzyme took place in kidneys.

Introduction of ozone therapy in the OL group ablated the increase in activity of superoxide dismutase in liver and heart. In kidneys the activity remained at the control level. In lungs of experimental animals the activity of superoxide dismutase remained high (195% of the control level) but it was lower than that in the CL group.

In cases of superoxide dismutase activities significant differences were confirmed between CO and CL groups in liver, lung and heart, between CO and OL groups in lungs and between CL and OL groups in liver and heart. No significant inter-group differences in activities of the enzyme were disclosed in kidneys.

Catalase

Similarly to the superoxide dismutase activities, also in this case the ozone-treated CO group manifested

no changes in catalase activities in the examined organs (Fig. 3). Exposure to LPS resulted in elevated catalase activities in all organs, i.e. to 185% control values in liver, to 135% in kidneys, to 145% in lungs and to 195% in heart.

Parallel exposure to both the xenobiotic agents resulted in all the organs in such a significant decrease in catalase activities that the latter reached practically the control group level.

Catalase activity differed significantly between CO and CL groups as well as between CL and OL groups in liver, lungs and heart. In kidneys no significant inter-group differences in catalase activity were disclosed.

DISCUSSION

Factors which disturb homeostasis of the body induce a specific response, i.e., a response, which corresponds to the type of stimulus and to stress or the general, non-specific reaction. The action of stress-inducing factor is counteracted by the host response in the form of a release of various agents aiming at elimination or at least restricting the potential for development of injury to the host. Therefore, evaluation of selected systems which participate in neutralizing the stress inducers should resolve whether and to which extent supporting the septic shock-affected host by administration of the oxygen/ozone mixture helps the host in overcoming the induced shock. Observations conducted within this study may support the hypothesis suggesting that appropriate induction of dismutase activity is required for improvement of patients, in whom excess of oxygen free radicals and lipid super-oxides accumulated in the course of active stages of a disease [24, 25].

In a septic shock, administration of oxygen represents one of the most significant elements of the therapy, permitting its augmented utilization by the shock-affected body. Nevertheless, the intense oxygen therapy may also damage the treated body. However, no such negative effects of the therapy can be noted in a septic shock in which an apparently paradoxical effect of bacterial endotoxins is observed, which augment host's resistance to the high, therapeutic partial pressures of oxygen. In this case the protective mechanism

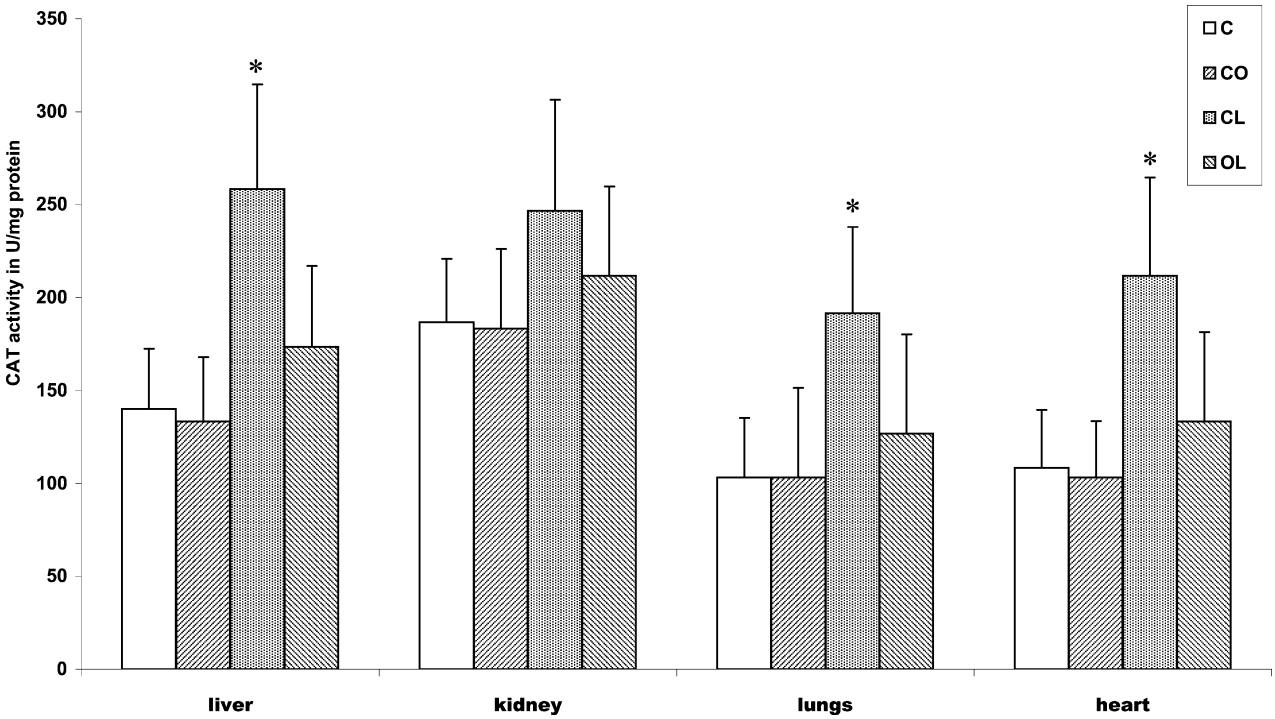

Fig. 1. Free radical level in selected organs of rats exposed to ozone and/or LPS. Results are expressed as the arithmetic mean ± SD. *—Significantly different form control at $P = 0.05$.

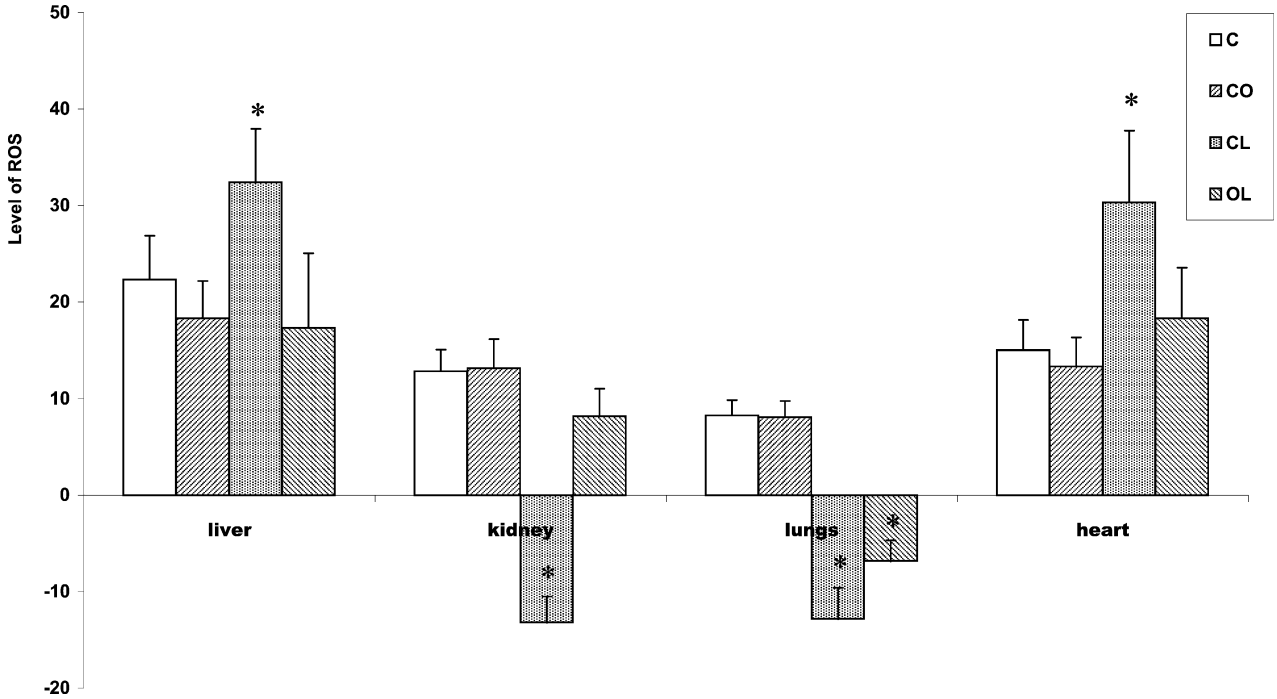

Fig. 2. Superoxide dismutase activity in selected organs of rats exposed to ozone and/or LPS. Results are expressed as the arithmetic mean ± SD. *—Significantly different form control at $P = 0.05$.

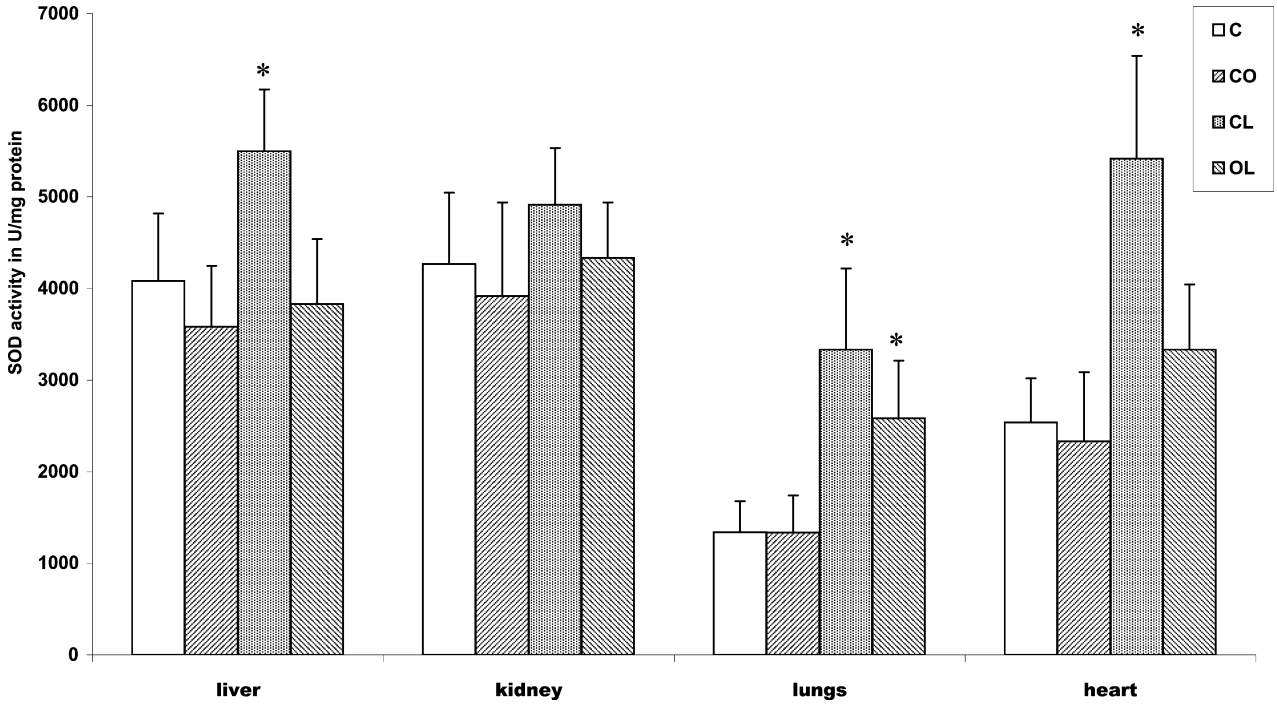

Fig. 3. Catalase activity in selected organs of rats exposed to ozone and/or LPS. Results are expressed as the arithmetic mean ± SD. *—Significantly different form control at $P = 0.05$.

involves induction of synthesis of SOD, glutathione peroxidase and of enzymes which regenerate the reduce glutathione [26, 27].

The mechanism is of a high clinical significance since under shock-accompanying hypoxia implementation of intense oxygen therapy may be harmful, particularly for the pulmonary tissue. The host-protective phenomenon of enzymatic induction has been observed in present study, and has been confirmed by ultrastructural analysis (presented in the subsequent paper in print).

It should also be stressed that the selected by us extra-pulmonary way of administering the oxygen/ozone mixture should be considered in clinical practice as the potentially optimum pathway of intense body oxygenation in a septic shock.

Free radicals were detected in practically all tissues and organs of animals. They participate in several physiological processes in the cell, including respiration, senescence, physical effort associated with normal activity of the body. Nevertheless, in specific, not always fully defined conditions free radicals may damage the cell. This probably happens when peroxidation/anti-oxidation balance becomes disturbed and the disproportional amounts/activities of the so-called free radical scavengers result in pathology at the molecular, cellular, organ and, sometimes, body level.

The most important role in the anti-oxidative system is played by enzymes, the so-called free radical scavengers, which prevent against formation of the reactive hydroxy radical or its precursor, H_2O_2. The radical result in destruction of all cellular components, including DNA [28, 29], lipids and protein [30, 31]. Evaluation of the enzyme activities points to a very complex course of reactions to a trigger so strong as administration of *Escherichia coli* LPS. In CL group, the evident increase of SOD and catalase levels, the natural scavengers of free radicals most probably has been linked to intensified oxygenation of NADH (and NADPH?) in the respiratory chain since high supply of reduced nucleotides was accompanied by, i.a., involvement of active oxygen forms at the levels of cytochromes b and c [32]. This may activate SOD and product of the enzyme, H_2O_2 stimulates, in turn, catalase. Infusion of the oxygen/ozone mixture has lowered free radical levels but this advantageous for the host alteration has never decreased the levels to the control values.

It should be stressed that administration of the oxygen/ozone mixture stabilized the free radical reactions both in the serum and in parenchymatic organs of rats, which may be of a high clinical importance.

CONCLUSIONS

1. Intravenous administration of *Escherichia coli* lipopolysaccharide stimulates in rats free radical reactions and a pronounced increase in SOD and catalase activities.
2. Intraperitoneal infusion of oxygen/ozone mixture reverses free radical reactions and enzymatic reactions to control levels which may be of a clinical significance.

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