

Ozone Administration Reduces Reperfusion Injury in an Isolated Rat Heart Model

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ABSTRACT *Background:* Accumulating clinical experience with ozone administration for conditions associated with ischemia has been encouraging. The aim of our study was to determine the effect of ozone on reperfusion injury in an isolated rat heart model. *Methods:* Isolated rat hearts were perfused with modified Krebs-Henseleit buffer solution via ascending aorta cannulation. After 15 minutes, perfusion was stopped and global ischemia was maintained for 30 minutes, following which perfusion was restarted, and continued for 40 minutes. Baseline hemodynamic measurements (heart rate, left ventricular developed pressure (LVDP), dP/dt , and coronary flow) were taken prior to ischemia, and every 10 minutes after reperfusion was started. Eleven hearts were treated with ozone during reperfusion and eight hearts served as controls. In the treatment group, after 5 minutes of reperfusion, ozone was administered in distilled water via a side arm for 5 minutes. *Results:* Preischemic baseline hemodynamic measurements and coronary flow were similar in the two groups. Hearts treated with ozone during reperfusion exhibited better recovery than did controls. Mean (\pm SE) percent recovery for treatment and control groups, respectively, was: LVDP $69 \pm 2\%$ vs $51 \pm 6\%$ ($p = 0.04$); dP/dt $68.9 \pm 13.3\%$ vs $53.7 \pm 20.4\%$ ($p = 0.05$); and LVDP \times HR $61.4 \pm 3.3\%$ vs $44.4 \pm 3.5\%$ ($p = 0.02$). *Conclusion:* In the isolated rat heart model, treatment with ozone during reperfusion enables better recovery than in controls. Although the mechanism by which ozone exerts its beneficial effect is not identified, it is possibly due to reduction in reperfusion injury. doi: 10.1111/j.1540-8191.2007.00419.x (*J Card Surg* 2007;22:339-342)

Myocardial reperfusion injury is defined as the death of myocytes, alive at the time of reperfusion, as a direct result of one or more events initiated by reperfusion. The principal mediators of this phenomenon are oxygen radicals, neutrophils, and components of the complement system that can overwhelm cellular defenses and induce tissue damage.¹⁻³ In recent years extensive efforts have focused on therapeutic strategies that might modify this phenomenon. Substances that may alter the interaction between neutrophils and endothelium such as perfluorochemical⁴ and adenosine⁵ have been suggested, but without clinical application as yet. Others examined the effects of various free-radical scavenging enzymes such as superoxide

dismutase; however, the efficacy of these agents on enhancing myocardial salvage in experimental models was inconsistent.⁶

Ozone gas was discovered in 1848.⁷ During World War I, ozone was first used on the battlefield to prevent infection during local medical procedures as well as prophylactically to control wound infections.⁷ Ozone is considered one of the most powerful oxidants available; hence, its use as a disinfectant with bactericidal as well as virucidal features.⁸ Clinical experience with ozone to date has been for management of conditions associated with ischemia, inflammation, and infections, as well as pressure sores, peritonitis, peripheral vascular disorders in diabetic and nondiabetic patients, strokes, and others.⁹ Ozone has been administered via several routes, including local irrigation, intravenous, intraarterial, intramuscular, subcutaneous, intraarticular, and as enemas.¹⁰ The aim of this study was to determine the effects of ozone on reperfusion injury in an isolated rat heart model.

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MATERIALS AND METHODS

The study was reviewed and approved by the animal care facility of the Hebrew University and is in compliance with the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals," published by the National Institution of Health.¹¹

Experimental model

Twenty-six Sprague-Dawley rats, weighing 270 to 320 g were heparinized (sodium heparin 500 units intraperitoneal) and anesthetized 30 minutes later with pentobarbital (30 mg intraperitoneal). The hearts were excised and immediately placed in ice-cold heparinized normal saline. The ascending aorta was then cannulated and retrograde perfusion of the nonworking heart (Langendorff method) was initiated with modified Krebs-Henseleit (KH) buffer solution maintained at 37 °C and at a constant pressure of 90 cmH₂O. The perfusate was aerated with a mixture of 95% O₂ and 5% CO₂, equilibrated at a pH of 7.3 to 7.4. A latex balloon-tipped catheter, connected to a pressure transducer and HP Recorder (7758B System), was inserted into the left ventricular cavity (through a small cut in the left atrium) to assess cardiac contractile function. The pulmonary artery was cut open to guarantee unrestricted coronary flow.

Left ventricular developed pressure (LVDP = difference between LV systolic and diastolic pressures), maximal pressure derivative during contraction (dp/dt), coronary flow, and heart rate were all recorded and analyzed.

Experimental protocol

After 15 minutes of perfusion, baseline hemodynamic parameters were measured. Perfusate flow was then stopped, creating a state of global ischemia, which was maintained for 30 minutes at 37 °C. The hearts were then allowed to reperfuse for 40 minutes during which hemodynamic parameters were collected every 10 minutes.

Experimental groups

Hearts were randomly divided into two groups, 13 in each group. Hearts were excluded if preischemic hemodynamics were marked by: heart rate below 200/min, LVDP < 80 mmHg or > 250 mmHg. Five hearts were excluded in the control group and two in the treatment group because of preischemic hemodynamic values. Thus, there were eight hearts in the control group and 11 in the treatment group.

In the treatment group, after five minutes of reperfusion, ozone was perfused via a side arm for five minutes. Ozone was administered in distilled water at a rate of 0.17 cm³/min for a total of 0.85 cm³ of ozone. Ozone was produced in a Dr. Hansler generator PM84 (Humazona™, Humares, GmbH Karlsruhe, Germany) at a concentration of 30 µg/mL on the morning of the experiment.

Statistical analysis

Statistical analysis was performed using JMP software (SAS Institute Inc., Japan). Heart rate, LVDP, dp/dt, and left ventricle work load (LVDP × HR) were compared between the treatment and the control groups at baseline (preischemic), and at 10, 20, 30, and 40 minutes after reperfusion using Mann-Whitney rank sum test.

RESULTS

Table 1 summarizes the recovery of the various parameters following ischemia and 40 minutes of reperfusion.

Preischemic baseline measurements of coronary flow, LVDP, and heart rate were similar between the two groups. Hearts in the ozone treatment group exhibited a significantly better postischemic recovery of LVDP, dp/dt and the pressure and rate product LVDP × HR, which is considered as the work index in the Langendorff system ($p < 0.05$; Table 1). Figure 1 presents the recovery of the work index during the 40 minutes of reperfusion. No change was detected during the actual administration of ozone. The improvement in the treatment group was gradual, with the significantly improved recovery ($p < 0.05$) appearing at a later phase (30 and 40 minutes of reperfusion, Fig. 1).

COMMENT

Ozone is used for medical management of various clinical conditions, including ischemic and inflammatory disorders. Anecdotal reports of clinicians who employ ozone therapy testify to its apparent efficacy. However, because of lack of well-designed studies, ozone therapy remains in the realm of alternative medicine.

Although inhaled free ozone is deleterious to the respiratory system,¹² when ozone is bubbled into a fluid

Table 1
Recovery After Reperfusion

	Control	Ozone	P
HR			
Preischemia	272 ± 16	286 ± 17	ns
Postischemia	250 ± 22	257 ± 22	ns
% Recovery	91 ± 4	89 ± 5	ns
LVDP			
Preischemia	110 ± 7	120.6 ± 7	ns
Postischemia	56 ± 8	83 ± 5	0.05
% Recovery	51 ± 6	69 ± 2	0.04
dp/dt			
Preischemia	1.19 ± 0.42	1.43 ± 0.41	ns
Postischemia	0.63 ± 0.32	0.98 ± 0.33	0.02
% Recovery	53.7 ± 20.4	68.9 ± 13.3	0.05
LVDP × HR			
Preischemia	29,885 ± 2457	35,238 ± 3471	ns
Postischemia	13,395 ± 1637	22,057 ± 2950	0.01
% Recovery	44.4 ± 3.5	61.4 ± 3.3	0.02

Hemodynamic recovery following 30 minutes of global ischemia (37 °C) and 40 minutes of reperfusion (mean ± SE). HR = heart rate (beat/min); LVDP = left ventricular developed pressure (mmHg).

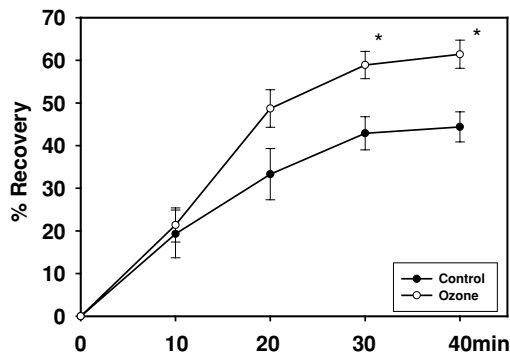


Figure 1. Recovery of the work index (LVDP \times HR) during the 40 minutes of the postischemic reperfusion (mean \pm SE). Closed circle – control, open circle – ozone; * $p < 0.05$.

such as water or blood, it may be used therapeutically.¹³ The modern use of ozone water as a treatment modality was further developed in the 1980s when Dr. Gerhard Wasser developed a means to bubble ozone into distilled water, resulting in an admixture of ozone and oxygen.⁷ Ozone is unstable in air, but at 4 °C and in glass bottles protected from ultraviolet rays, is stable for 5 days without appreciable loss of concentration.

Reports from Germany and Eastern Europe are encouraging. One group from Poland reported intraarterial treatment with ozone to 100 patients with peripheral vascular disease. They showed significant improvement manifested by an increase in ankle/arm index and improvement in intermittent claudication.¹⁴ Groups from Spain and Cuba reported a protective effect of ozone on injury associated with hepatic ischemia reperfusion.^{15,16} These and other reports led us to try and determine the efficacy of ozone in reducing reperfusion injury in ischemic hearts. To our knowledge there are no previous studies dealing with this issue.

We chose the Langendorff isolated rat heart model. This is a well-established model permitting studies of various agents on reperfusion injury.^{17,18} In our study we could demonstrate that hearts treated with ozone for five minutes during reperfusion had a significantly better recovery than had controls. One may argue that the improvement may be due to improved oxygenation in hearts perfused with ozone. Hearts treated with ozone were given $< 1 \text{ cm}^3$ of ozone over five minutes, while the total flow during this time was 50 to 80 cm^3 , with a measured pO_2 of about 500 mmHg. Therefore, the additional amount of oxygen is negligible, although the bioavailability may have been enhanced.

What is the mechanism by which ozone protects from reperfusion injury? One possible mechanism is by enhancement of release of nitric oxide. Treatment with ozone promotes vasodilatation (including facial flushing) and improvement of limb ischemia.¹⁹ Infusion of ozonated blood, by enhancing release of nitric oxide, may induce vasodilatation in ischemic tissues and reduce hypoxia.²⁰ Nitric oxide derived from endothelial cells may be decreased during postischemic reperfusion²¹ and, therefore, enhancement of nitric oxide release by ozone may be a means by which ozone attenuates the extent of injury. Since the effects of reper-

fusion injury are evident in other organs, it is illustrative that injury associated with hepatic ischemia reperfusion has been treated successfully by ozone therapy.²² Ozone therapy in this model was noted to promote regulation of endogenous nitric oxide concentration and maintain cellular redox balance.²³ The interaction of ozone with water produces oxygen radicals.²⁴ Oxygen radicals (especially hydroxyl radical) are involved in reperfusion injury.²⁵ However, reactive oxygen species play a crucial role in the signal transduction of ischemic preconditioning.²⁶ Thus, ozone possibly produces positive and negative effects, and the beneficial hemodynamic effects presented in this study is the net effect of the interplay of these vectors.

Since this is a seminal study, choice of treatment variables such as dosage, timing, and duration of ozone administration were all chosen empirically. Under the conditions described, a beneficial effect of ozone was demonstrated. It remains to be determined whether ozone will have any clinical effect, and what the optimal therapeutic dosage would be. Additionally, the precise mechanism by which ozone exerts its effect has yet to be determined. Potential clinical applications of ozone treatment are in the setting of thrombolytic reperfusion, primary coronary intervention following myocardial infarction, and postcardioplegic arrest in cardiac surgery procedures. Since one cannot extrapolate the isolated heart model to human clinical situations, whole animal experiments are warranted.

Ozone can be generated easily, is safe, and is inexpensive. Preliminary studies of the effect of ozone on red blood cell enzymes²⁷ showed no damaging effect. Subsequently, ozone autohemotherapy was administered to nine patients with Gaucher disease suffering intractable bone pain. All were treated without any serious side effects, and all achieved significant improvement.²⁸ Thus, these preliminary studies, the numerous anecdotal reports of individuals treated reports and the many patients already treated for various conditions, support the use of ozone in clinical practice. Although there is a wealth of clinical experience with ozone treatment, the fact that there are few controlled trials has limited the use of ozone to the field of alternative medicine. The results of this study underscore the importance of further investigation of the potential for ozone in preventing reperfusion injury in an animal model or man.

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