The role of ozone in the treatment of the acute phase of ischemic heart disease

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Abstract

The present paper describes the role of ozone in the treatment of the acute phase of ischemic heart disease. Two studies are presented: the first mechanically induced myocardial ischemia in rats; the second involved 42 consecutive patients with an outcome comparable to the UA/non-Q wave NSTEMI AMI group of the ENACT study, divided randomly into 2 groups with homogeneous quantitative levels (NYHA class II-III) and was evaluated at weeks 8 and 12 of treatment with ozone. Both studies confirm that i) the effectiveness of ozone therapy increases in relation to how early and how long treatment is performed; and ii) that ozone reduces the relative risk both of ischemic (infarct extension) and arrhythmia complications.

Introduction

An experimental study of murine samples involved a control group and a second group in which myocardial ischemia was mechanically induced by ligation, maintained for 25 minutes, of the left anterior descending coronary artery of the left ventricle. Subsequently, the process of reperfusion of the area was immediately begun for an additional 120 minutes.[1]

Sixty minutes before ischemia/reperfusion was induced, the rats in the second group were pre-treated with a mixture of oxygen-ozone by intraperitoneal delivery (Figure 1). The mean arterial blood pressure was recorded at the times shown in Figure 1.

The following calculations were made for the animals immediately after sacrifice: the extension of the necrotic tissue in relation to the muscle mass of the area at risk, expressed as a % (ISLV) relative to the total weight of the left ventricle (ISLV/non-ischemic myocardium).

The area at risk was demonstrated using Evans Blue dye and the infarct size was shown with NBT staining. With regard to function, the entities of the following parameters were evaluated: oxidative stress, using nitrotyrosine which is known to be the most specific marker of peroxynitrite; the extent of the inflammation, using the CD68 cluster of differentiation which is specific for macrophage activity; immune status, using CD4 and CD8 which are specific for lymphocyte activity; of the apoptotic cells by assaying the activity of the caspase 3 proteases, which are known to be cell death modulators.

A comparative evaluation of the biological samples of the infarcted myocardial tissue from the control group *vs* the group of rats which was pre-treated with ozone showed that nitrotyrosine precipitated less intensely in the infarcted heart + ozone (Figure 2). Figure 3 also shows that the activity of caspase 3 (an indicator of cell apoptosis) is reduced by pre-treatment with ozone; the same behavior is seen following the comparative assessment of the infarcted myocardial tissue from the two groups in Figure 4 with regards to macrophage (CD 68) and lymphocyte (CD 4; CD8) activity.[2]

The experimental study presented shows that ozone is capable of significantly reducing infarct extension in mice (Figure 5).

A survey of the most recent and accredited literature confirms that the oxygen-ozone mixture has also been used in humans in the treatment of cardiovascular diseases and in particular in cardiac-output,[3] on hematology parameters,[4] on the reperfusion of organs and systems.[5-9]

However, to date, no study has been able to convincingly correlate the therapeutic efficacy of the oxygen-ozone mixture with the prevention, or at least reduction, of the myocardial damage that follows each ischemic event: the reduction of the area at risk in the early phase of acute myocardial infarction (AMI) and on the eradication of arrhythmia, which is the leading cause of death in the first hour following an AMI.

Our experience is prompted by a pan-European survey of acute coronary syndromes,[10-12] which is significantly due to the number of patients: 3092 enrolled cases in 17 European countries. The ENACT study has also revealed that chest pain was found to be caused: by unstable angina (UA) in 45% of cases; by non-Q wave NSTEMI AMI in 15% of cases; by Q-wave transmural AMI in 39% of cases.

During the follow-up performed at 8 months after the termination of the ENACT study, it was observed that 9% of the entirety of the two groups defined in the ENACT study as *unstable angina* (UA) and as non-Q wave NSTEMI AMI and which was 62% of the enrolled popula-

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tion and were stratified as medium-low risk and therefore treated with standard drug therapy, without using an invasive approach (aorto-coronary bypass) or a mini-invasive one (angioplasty) with thrombolysis, evolved towards overt myocardial infarction (QT-AMI).

Some authors agree on the hypothesis that this unfavorable consequence could be influenced by the compressive action of the perifocal edema around the area of necrosis, the extension of which is favored by oxidative stress (ROS) and the synthesis and release of mediators of reactive inflammation (cytokines) into the bloodstream. This mechanism is the cause of the prolongation of the ischemia of the myocardial tissue, which in turn facilitates the recurrence of necrosis in the same tissue and infarct extension.

Myocardial ischemia is the cause of cellular electrical instability, causes QT prolongation and causes severe arrhythmia. Clinical evidence and authoritative literature document the existence of a direct correlation between QT prolongation and arrhythmia that can lead to sudden death.

Taking action as early as possible on the myocardial territory surrounding the area of necrosis and which is characterized by an extensive area of edema, in turn surrounded by a wider zone of ischemia, may be a winning therapeutic strategy in preventing extension of AMI stratified as having a low-risk prognosis *quoad vitam* (61%).

The design of the study conducted at the Second University of Naples Resuscitation Unit was prospective, double-blind with parallel groups of 42 consecutive patients with an outcome comparable to the UA/non-Q wave NSTEMI AMI group of the ENACT study, divided randomly into 2 groups with homogeneous quantitative levels (NYHA class II-III) and was evaluated at weeks 8 and 12 of treatment with ozone (Tables 1-3).

The S Group made use of traditional standard therapy consisting of: analgesics, O_2 therapy by nasal inhalation, heparin/LMWH, antiaggregant drugs, β-blockers, calcium channel blockers, nitrates and statins. In the O_3 group, treatment with a mixture of O_2-O_3 was added to the abovementioned standard therapy.

The primary endpoint was to verify the effectiveness of ozone on recovery in the acute phase of AMI, the hypoperfused area of risk (ischemic penumbra) by myocardial scintigraphy.

The secondary endpoint used QTc analysis to verify the effectiveness of ozone in the control of arrhythmia. The patients were monitored using Holter ECG and the analysis concerned ECG tracings recorded 60 minutes before and after autotransfusion with ozone.

The QT interval was measured every 10 minutes, its correction relative to the heart rate was obtained using Bazett's formula: QTc=QRS/RR. Myocardial scintigraphy before and after ozone treatment: at T_0 and at the 12th week of treatment.

Table 1. Patients' overview.

	S	O_3
Patients (n)	22	20
M:F	16:4	18:4
Age year (mean)	57.7	57.7
Hours from pain onset	6.4	6.4
Previous AMI	3	4
Previous CABG	2	2

M, male; F, female; AMI, acute myocardial infarction; CABG, coronary artery bypass graft.

Table 2. Analysis of efficacy results on short-term complications (8th week).

	S	O_3
CV death	1	0
Q-wave AMI	3	1
Arrhythmia	8	3
Vagal stimulation	0	5
PCI/PTCA	1	1

CV, cardiovascular; AMI, acute myocardial infarction; PCI/PTCA, percutaneous coronary intervention/percutaneous transluminal coronary angioplasty.

Table 3. Analysis of efficacy results on long-term complications (12th week).

	S	O_3
CV death	0	0
Q-wave AMI	4	1
Arrhythmia	4	2
Vagal stimulation	0	8
PCI/PTCA	1	1

CV, cardiovascular; AMI, acute myocardial infarction; PCI/PTCA, percutaneous coronary intervention/percutaneous transluminal coronary angioplasty.

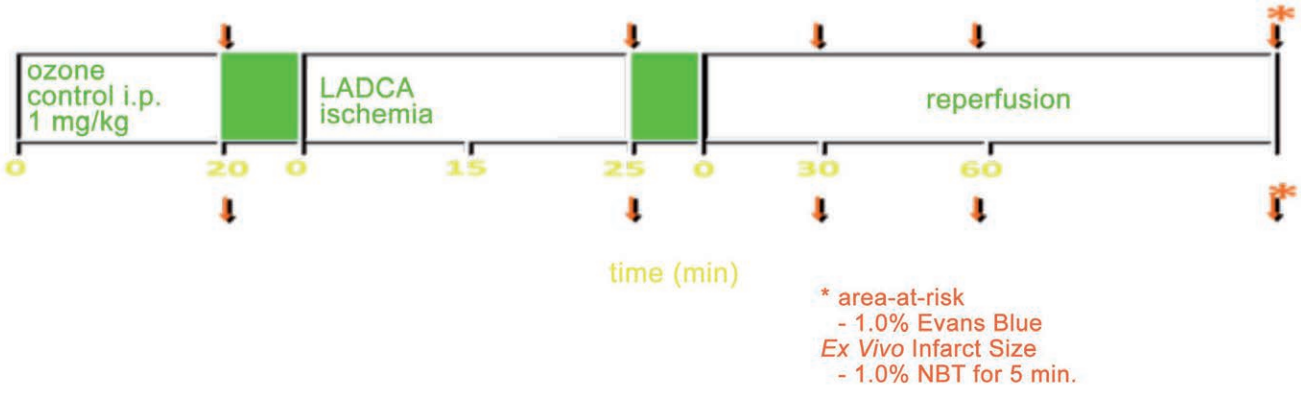

Figure 1. Mean arterial blood pressure recorded at different times.

Materials and Methods

Ozone was generated by the MEDICAL 95 CPS Computerized Photometric System manufactured by Multiossigen.

Recruitment criteria were the following: informed consent, patients who presented for observation in the ICU <6 h from onset of pain, patients with persistent pain >30 minutes who were non-responders to sublingual glyceryl trinitrate.

On the other hand, exclusion criteria were: patients who could benefit from CABG, PTCA or pharmacological thrombolysis, patients with NYHA class IV heart failure, patients who had recently (<3 months) undergone PCI or PTCA.

The O_3 group underwent *ozonation* according to the following regimen:[13,14] sampling of 200 mL of blood over 15 minutes in a sterile vacuum blood collection tube containing citrate (ACD); immediate reinfusion of 20 mL of blood to prevent clotting in the connecting pipe; exposure of 180 mL of blood to a mixture of 180 mL O_2-O_3 (O_3 concentration of 30 µg/mL) for 3 minutes until the blood took on a bright red color; the blood was reinfused following this treatment. After any post-AMI hemodynamic stabilization, treatment was carried out at 6 h, and

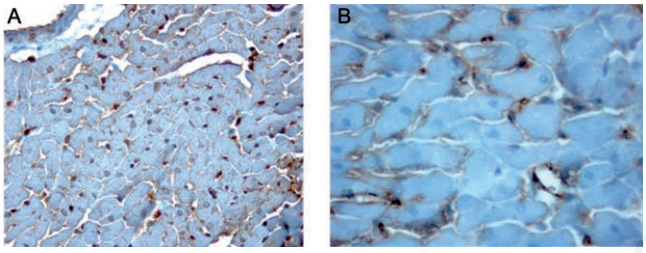

Figure 2. Comparative evaluation of the biological samples of the infarcted myocardial tissue from the control group *vs* the group of rats.

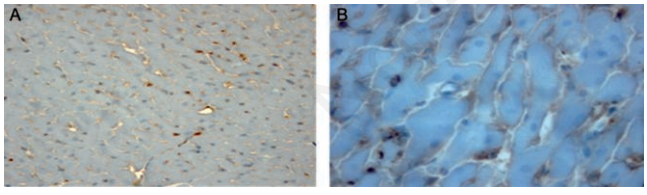

Figure 3. Activity of caspase 3 reduced by pre-treatment with ozone.

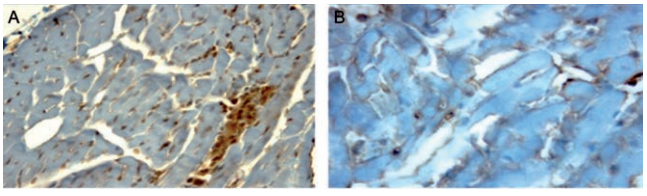

Figure 4. Comparative assessment of the infarcted myocardial tissue from the two groups with regards to macrophage (CD 68) and lymphocyte (CD 4; CD8) activity.

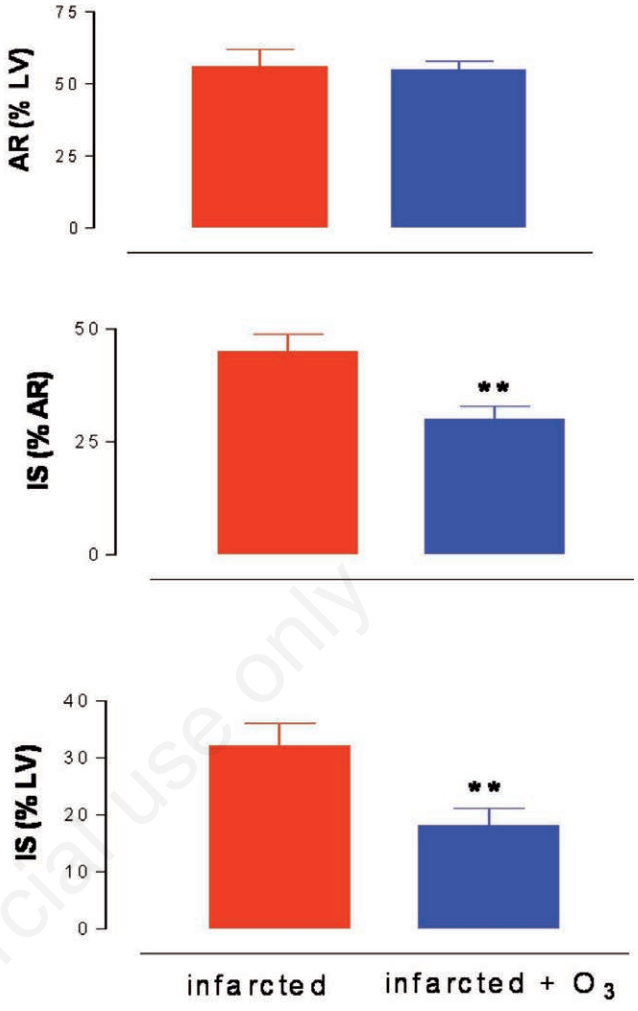

Figure 5. Ozone is capable of significantly reducing infarct extension in mice.

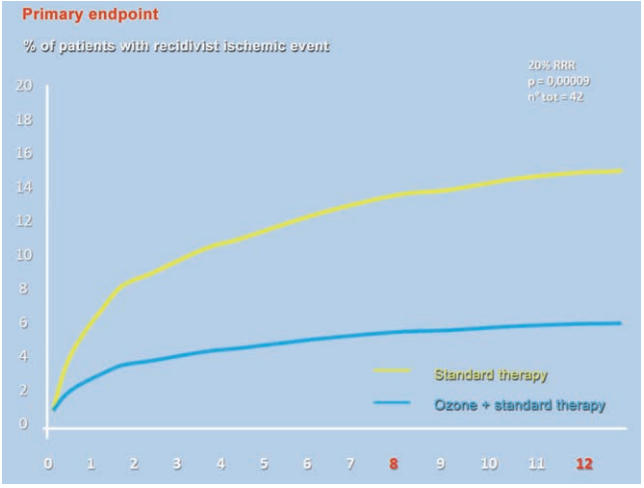

Figure 6. Difference in prognosis tends to increase with the passage of time: at week 12 the gap is significantly enlarged.

QTc preO3	QTC post O3	QTc preO3	QTc post O3	QTc preO3	QTc post O3	SDNN 1PO3	SDNN1psO3	SDNN2	SDNN2		SDNN 3	SDNN 3
437	437	480	436	420	430	127	58	36,8	35		50	46
428	424	459	424	450	450	100	50	40,6	19,7		26	61
440	384	459	464	450	430	76	41	44	29		19,5	39
444	424	490	485	410	450	72	42	38	25		32	59
470	437	470	471	450	400	64	27	32	30		40	45,7
410	450	458	436	470	450	64	29	29	43		31	49
450	424	480	444	450	430	56	79	30,5	30		24	31
430	426	470	450	480	440	66	39,7	31	23		15	45
460	436	470,75	450	447,5	420	81	45,7125	43	13		23	24
460	436	11,84121	451,1111	23,1455	450	83	16,81423	21	19		37	38
430	400		19,21877		450	75		50	23		78	33
441,7273	425,2727				430	78,54545		47	50		34,13636	36
17,42464	18,61231				450	19,96178		55	31		17,62398	42,225
					436,9231			46	28,51538			10,97138
					15,48366			47	10,02678			
DQTc	DQTc							39,39333				
60	66	2	61	70	50			9,297962				

Figure 7. Verification of the secondary endpoint: the QT interval reduces after treatment with ozone.

repeated at 24, 48, and 96 h from T_0 (onset of pain), then the treatments were repeated twice a week for 12 weeks, with clinical and diagnostic tests at 8 and 12 weeks.

Results and Discussion

It can be seen that in relation to the total number of patients considered (42) that the O_3 group, who used ozone therapy in addition to standard therapy, have an RRR of 20% for recurrent ischemia P=0.00009 *vs* the S group who received traditional therapy alone. The diagram also shows that this difference in prognosis tends to increase with the passage of time from week 8 and at week 12 the gap is significantly enlarged (Figure 6). The corresponding myocardial scintigraphy findings with the data recorded at the 12th week show evident recovery of reperfusion in the myocardial tissue. As regards the verification of the secondary endpoint, the diagram shows that the QT interval reduces after treatment with ozone (Figure 7).

This data prompts the hypothesis that ozone may have a stabilizing effect on the QT interval during the left ventricular recovery phase; this facilitates the eradication of ventricular arrhythmia. The trend is in line with data obtained from induction in rats with a more evident reduction in the RRR of arrhythmia in 32% of patients; indeed, the graph shows greater widening of the gap with the passage of time (from the 8th to the 12th week).

The limited number of patients investigated does not allow us to pass definitive judgment on the effectiveness of ozone therapy in AMI.

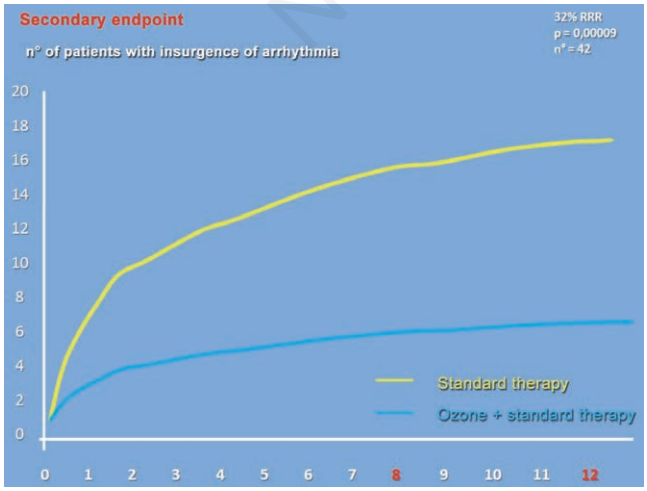

Figure 8. Ozone reduces the relative risk both of ischemic (infarct extension) and arrhythmia complications.

Conclusions

Ultimately, it is observed that: i) the effectiveness of ozone therapy increases in relation to how early treatment is performed (<6 hours from the onset of chest pain) and with its prolongation (the 12th week achieves more effective responses); ii) ozone reduces the relative risk both of ischemic (infarct extension) and arrhythmia complications (Figure 8).[15]

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