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CLINICAL & BASIC RESEARCH

Ozone-Oxidative Preconditioning Prevents Doxorubicin-induced Cardiotoxicity in Sprague-Dawley Rats

Livan Delgado-Roche,[1] Yanet Hernández-Matos,[1] Emilio A. Medina,[1] Dalia Á. Morejón, Maité R. González,[2] *Gregorio Martínez-Sánchez[3]

التعرض السابق للأكسدة بالأوزون يحمي ضد التسمم القلبي الذي يحدثه دواء دوكسوروبسين في جرذان سبراق داولي

ليفان. روشي، يانت هيرناندیزماتوس، اميليو . مدينا، داليا . مورجون، ماتي جونزالس، جريجوريوا مارتينيزسانشيز

ABSTRACT: ***Objectives:*** Induced dilated cardiomyopathy is the main limitation of the anti-cancer drug doxorubicin, which causes oxidative stress and cardiomyocyte death. As ozone therapy can activate the antioxidant systems, this study aimed to investigate the therapeutic efficacy of ozone-oxidative preconditioning against doxorubicin-induced cardiotoxicity. ***Methods:*** The study was carried out from September 2013 to January 2014. Sprague-Dawley rats were randomly distributed in the following treatment groups: Group 1 were treated with 2 mg/kg intraperitoneal (i.p.) of doxorubicin twice a week for 50 days; Group 2 were treated with 0.3 mg of ozone/oxygen mixture at 50 µg/mL of ozone per 6 mL of oxygen by rectal insufflation and then treated with doxorubicin; Group 3 were treated as Group 2 but only with the oxygen, and Group 4 were treated with oxygen first, and then with sodium chloride i.p. as the control group. ***Results:*** The results showed that ozone therapy preserved left ventricle morphology which was accompanied by a reduction of serum pro-brain natriuretic peptide levels. The cardioprotective effects of ozone-oxidative preconditioning were associated with a significant increase (P <0.05) of antioxidant enzymes activities and a reduction of lipid and protein oxidation (P <0.05). ***Conclusion:*** Ozone-oxidative preconditioning prevents doxorubicin-induced dilated cardiomyopathy through an increase of antioxidant enzymes and a reduction of oxidised macromolecules. This establishes the background for future studies to determine if ozone therapy can be used as a complementary treatment for attenuating doxorubicin-induced cardiotoxicity in cancer patients.

Keywords: Ozone; Doxorubicin; Dilated Cardiomyopathy; Cardiotoxins; Oxidative Stress.

الملخص: الهدف: يعد اعتلال عضلة القلب التوسعي هو أحد الآثار السمية لدواء السرطان دوكسوروبسين، إذ يسبب هذا الدواء إجهادا تأكسديا وموتا للخلايا القلبية. وبما أن العلاج بالأوزون يمكن أن ينشط أنظمة الجسم المضادة للأكسدة، عملت هذه الدراسة لتقويم الفعالية العلاجية للتعرض السابق للأكسدة بالأوزون ضد التسمم القلبي الذي يحدثه دوكسوروبسين. **الطريقة:** أجريت الدراسة بين سبتمبر 2013 إلى يناير 2014م. وقسمت جرذان سبراق دالي إلى المجموعات التالية: عولجت المجموعة الأولى بحقن دوكسوروبسين في الصفاق بجرعة 2 مجم لكل كجم مرتين في الأسبوع لمدة 50 يوما متواصلة. وأعطيت المجموعة الثانية عن طريق المستقيم خليطا من الأكسجين والاوزون قدره 0.3 مجم (50 ميكروجرام لكل مل من الأوزون لكل 6 مل من الأكسجين) ثم عولجت بالأوزون. وعولجت المجموعة الثالثة بمثل ما عولجت به المجموعة الثانية، ولكنها أعطيت أكسجين فقط. وأعطيت المجموعة الرابعة الأكسجين أولا ثم أعطيت كلوريد الصوديم بالحقن الصفاقي، وعدت كمجموعة ضابطة. **النتائج:** وجد أن العلاج بالأوزون قد حافظ على مورفولجيا البطين الأيسر للقلب، وصاحب ذلك نقص في تركيز البيبتيد المدر للصوديوم. وارتبطت الحماية التي وفرها التعرض المسبق للأوزون للقلب بزيادة معنوية ($P < 0.05$) في نشاط الأنزيمات المضادة، ونقص في أكسدة الدهن والبروتين ($P < 0.05$). **الخلاصة:** يمنع التعرض المسبق للأكسدة بالأوزون اعتلال عضلة القلب التوسعي الذي يسببه دوكسوروبسين، وذلك عن طريق زيادة نشاط الانزيمات المضادة للأكسدة وتقليل الجزئيات الكبيرة المؤكسدة. تقدم هذه الدراسة خلفية لدراسات مستقبلية تقرر عما إذا كان بالإمكان استخدام العلاج بالأوزون كعلاج مكمل يقلل من آثار سمية دواء دوكسوروبسين عند مرضى السرطان.

مفتاح الكلمات: الأوزون؛ دوكسوروبسين؛ اعتلال عضلة القلب التوسعي؛ السموم القلبية؛ الإجهاد التأكسدي.

Advances in Knowledge

- *Ozone therapy can activate antioxidant systems. This study was the first to investigate the therapeutic efficacy of ozone-oxidative preconditioning against doxorubicin-induced cardiotoxicity.*

Application to Patient Care

- *The results of this study suggest that ozone therapy could be used to attenuate doxorubicin-induced cardiotoxicity as a complementary treatment for cancer patients. These results require further pharmacological and toxicological investigations.*

[1]Center of Studies for Research & Biological Evaluations, Pharmacy & Food Science College, University of Havana, Cuba; [2]LABIOFAM Pharmaceutical Laboratories, Havana, Cuba; [3]Medical Center Beauty Benefit, Osimo, Ancona, Italy

**Corresponding Author e-mail: gregorcuba@yahoo.it*

Doxorubicin (DOX) is an anthracycline antibiotic which is used to treat a wide range of cancers.[1,2] Like most of the anti-cancer drugs, DOX causes various toxic effects, the commonest of which is dose-dependent cardiotoxicity.[3] Studies have reported DOX-induced cardiac abnormalities in a significant number of patients.[4] In both animal and human models, DOX induces dilated cardiomyopathy (DCM), cardiac muscle wasting and congestive heart failure.[5,6]

Cellular injury induced by DOX is mediated by the intermediary iron-anthracycline complex that generates free radicals, which in turn causes serious damage due to oxidative stress (OS).[2] DOX localises to the mitochondria and is highly susceptible to enzymatic reduction, generating superoxide radicals ($O_2^{\bullet -}$) and reactive oxygen species (ROS) which alter mitochondrial function.[7] DOX increases the susceptibility of the cardiomyocytes to OS by reducing superoxide dismutase (SOD) activity, therefore reducing the ability of the cardiac cells to deactivate the ROS.[8] DOX-induced cardiotoxicity is also mediated by impaired calcium ion (Ca^{2+}) fluxes, the suppression of cardiac-specific genes expression and myofibrillar and mitochondrial degeneration in the cardiomyocytes.[3,9]

In patients with DCM, the left ventricle dysfunction causes an augmentation of the circulating levels of amino-terminal pro-brain natriuretic peptide (pro-BNP). This peptide is a sensitive biomarker of myocarditis and congestive heart failure.[10] In this context, it is possible to evaluate the efficacy of cardioprotective drugs by measuring proBNP levels.

Therapeutic strategies that focus on increasing cellular endogenous defence systems have been identified as a valid approach against OS-associated diseases such as DCM.[2] There is proven evidence that antioxidant enzymes, nitric oxide pathways and other subcellular activities could be modulated by low doses of ozone and could support the effects of ozone therapy in many pathological conditions such as hepatic and renal ischaemia-reperfusion injuries,[11] diabetes mellitus,[12] Parkinson's disease[13] and coronary artery disease.[14,15] In light of more recent pharmacological findings, ozone can be considered a prodrug which, at non-toxic doses, can induce a rearrangement of the biochemical pathways with the activation of second messengers in a cascade with multiple system actions.[13,16,17]

The present study was designed to evaluate the effects of ozone-oxidative preconditioning (ozone-OP) on DOX-induced cardiotoxicity in Sprague-Dawley rats. In particular, the animals were examined for antioxidant enzyme activity, biomolecular damage, systemic levels of pro-BNP and the histopathological characteristics of the cardiac tissue.

Methods

The study was carried out from September 2013 to January 2014. All reagents were purchased from Sigma-Aldrich Corp. (St. Louis, Missouri, USA), except for the DOX, which was provided by the manufacturer (Center of Drug Research and Development, Havana, Cuba). Adult male Sprague-Dawley rats (n = 40) weighing 250–300 g were obtained from the National Center for Production of Laboratory Animals (CENPALAB, Mayabeque, Cuba) and adapted to laboratory conditions (60% humidity and 25 ± 1 °C) for at least one week before the experiments. The rats were housed in groups of five and exposed to a 12-hour light/darkness cycle with free access to food and water. Ozone was obtained by an OZOMED® device (Ozone Research Center, Havana, Cuba). Ozone was generated from medical-grade oxygen and was used immediately upon generation. The ozone represented only about 3% of the oxygen plus ozone gas mixture. The ozone concentration was measured by using a built-in ultraviolet (UV) spectrophotometre set at 254 nm.[15]

Four groups of 10 rats were assigned to different treatments [Table 1]. Prior to ozone/oxygen insufflation, the rectum was stimulated to eliminate excrement. DOX was administered twice a week for 50 days to both the ozonised and the oxygen group after 20 sessions of ozone or oxygen, respectively. On the final day of DOX administration, the animals were processed as previously described in order to conduct the biochemical and histological analysis.[18] The animals were anaesthetised with 5 mg/kg of ketamine hydrochloride intramuscularly and euthanised with an overdose of 90 mg/kg of sodium pentobarbital

Table 1: Experimental design and effects of ozone therapy on the pro-BNP levels of the experimental groups of Sprague-Dawley rats

Experimental groups	Mean ± SD of pro-BNP levels in pg/mL*
Control[a,b]	2.01 ± 0.23
DOX[c]	48.95 ± 1.78†
Oxygen plus DOX[a,c]	50.01 ± 0.99†
Ozone-OP plus DOX[c,d]	8.62 ± 0.84†‡

pro-BNP = pro-brain natriuretic peptide; SD = standard deviation; DOX = doxorubicin; ozone-OP = ozone-oxidative preconditioning.

*[a]Rectal insufflation of oxygen (6 mL) once on alternating days for 20 sessions; [b]An intraperitoneal injection of 0.9% sodium chloride (2 ml/kg) twice a week for 50 days; [c]An intraperitoneal injection of DOX (2 mg/kg) twice a week for 50 days; [d]Rectal insufflaction of ozone/oxygen mixture (0.3 mg) with 50 µg/mL of ozone per 6 mL once on alternating days for 20 sessions. *Values higher than 5 pg/mL are considered pathological. †Statistical differences (P <0.05) compared to control group. ‡Statistical differences (P <0.05) compared to the DOX and oxygen plus DOX groups.*

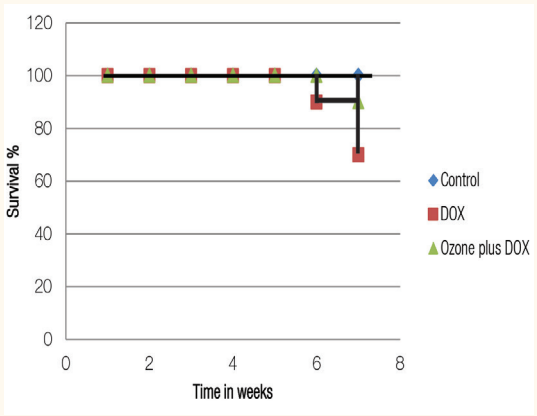

Figure 1: The corresponding survival rates for each experimental group of Sprague-Dawley rats. The survival rate in the DOX group was 60% in comparison to 90% for the ozonised rats.
DOX = doxorubicin.

intravenously (Abbott Laboratories S.A. de C.V., Mexico City, Mexico). The cardiovascular system was subsequently perfused with a solution of ice-cold 0.9% sodium chloride (NaCl). The hearts were harvested and used to determine biomarkers of OS (n = 5) and for histopathological analysis (n = 5).

To determine the pro-BNP levels, ethylene-diamine-tetra-acetic acid (EDTA)-anticoagulated blood samples (1 mL) were obtained by penetrating the retro-orbital plexus with a capillary tube after a 12-hour overnight fast and 24 hours after the final administration of DOX. The samples were immediately centrifuged at 3,000 g at 4 ˚C for 10 min. Finally, the serum was collected and aliquots were stored at -80 ˚C until analysis.

The hearts were treated as previously described.[19] They were placed in 0.1 mol/L of ice-cold tris(hydroxymethyl)aminomethane-hydrochloric acid (tris-HCl) buffer solution (pH 7.6) with 1.0 mmol/L of EDTA and 0.2 mmol/L of butylated hydroxytoluene. They were macerated before homogenisation at 3,000 rpm^{-1} for 10 min in a tissue homogeniser (Edmund Bühler GmbH, Tübingen, Germany). The homogenised tissue was then centrifuged at 4,500 g for 20 min at 4 ˚C and the supernatants were collected and stored at -80 ˚C until the OS biomarker determination.

The hearts were cut transversally in order to enhance observation of the four chambers. The samples were then rinsed in an ice-cold phosphate-buffered saline (PBS) solution (pH 7.4) and fixed in a solution of 10% formaldehyde for 24 hours. Samples were subsequently embedded in paraffin as previously described.[19] Tissue sections of 5 µm were cut, air-dried on glass slides with different grades of alcohol/xylene, deparaffinised and then rehydrated. Finally, the tissue sections were stained with haematoxylin and eosin via the standard procedures.[18] The sections were analysed using a BX51 optic microscope (Olympus® Europa Holding GmbH, Hamburg, Germany).

The serum levels of pro-BNP were quantified using an electrochemoluminescence immunoassay kit (Elecsys® NT-proBNP, Roche DiagnosticsGmbH, Basel, Switzerland). All biochemical variables were analysed using spectrophotometric methods with a 1000 Spectrophotometer (Pharmacia LKB, Uppsala, Sweden) and a microplate reader (SUMA, Center of Immunoassay, Havana, Cuba). Total protein concentrations were assayed with bovineserum albumin (#A7906, Sigma-Aldrich Corp., St. Louis, Missouri, USA) as the standard, using the method described by Bradford.[20] The SOD activity was determined using a RANSOD kit (Randox Laboratories, Crumlin, UK) and measured by the inhibition degree of the reaction.[19] Catalase (CAT) activity was determined spectrophotometrically by following hydrogen peroxide (H_2O_2) decomposition at 240 nm at 10-second intervals for one min.[21]

The advanced oxidation protein products (AOPPs) were measured as described previously.[18,22] Samples in a PBS solution (pH 7.4) at 10 mM were treated with 50 µL of potassium iodide at 1.16 M followed by the addition of 100 µL of acetic acid. The absorbance was immediately read at 340 nm. The concentration of AOPPs was expressed in µM of chloramine-T. The concentration of malondialdehyde (MDA) was determined using the LPO-586 assay kit obtained from Calbiochem (La Jolla, California, USA). The production of a stable chromophore after 40 min of incubation at 45 °C was measured at 586 nm. For standards, freshly prepared solutions of MDA bis (dimethyl acetal) were employed and assayed under identical conditions.[18,23]

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version 11.5 (IBM Corp., Chicago, Illinois, USA). Levene's test was used to analyse the homogeneity of variance. The differences between the four groups were determined by analysis of variance (ANOVA) followed by the Bonferroni *post-hoc* test. Data were expressed as means ± standard deviation (SD). A P value of <0.05 was considered statistically significant. Growth curves were analysed using the comparison of the slope of a regression line fitted to each individual.

This animal study was performed in line with international ethical guidelines and with the approval of the Institutional Animal Ethics Committee of the College of Pharmacy & Food Sciences at Havana University (approval protocol #2012AS671211).

Results

The survival rates of the animals are shown in Figure

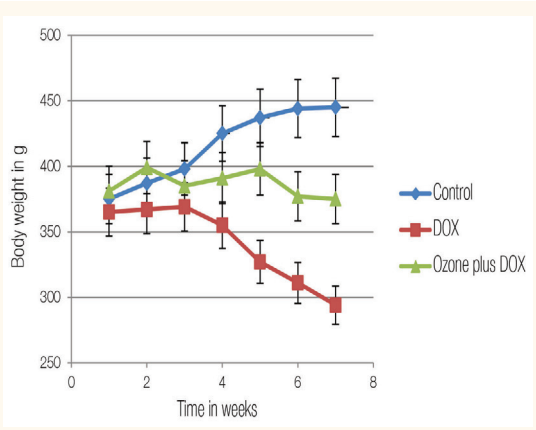

Figure 2: Changes in body weight among the experimental groups of Sprague-Dawley rats. The reduction of body mass in the DOX and oxygen plus DOX groups was significantly higher in comparison to the controls and ozonised rats (P <0.05).
DOX = doxorubicin.

1. The ozone-treated group displayed a 90% survival rate while survival was lower for the groups who were only treated with DOX or with oxygen plus DOX (70% and 60%, respectively). In addition, ozone treatment was able to reduce the loss of body weight which is normally associated with the administration of DOX [Figure 2]. Although the body weight of the ozonised rats was lower than that of the controls (P <0.05), it was significantly higher in comparison to those only treated with DOX or those treated with oxygen plus DOX (P <0.05). Body weight did not vary significantly in any of the experimental groups before the administration of DOX. Macroscopic and microscopic examinations of the organs did not show any relevant disease or abnormalities during ozone-OP.

The effect of ozone-OP on left ventricle dysfunction was determined by evaluating pro-BNP levels. Significantly high levels of serum pro-BNP were observed in the rats who were only treated with DOX and those treated with oxygen and DOX when compared to the control and ozonised rats (P <0.05). It is important to highlight that the rats undergoing ozone treatment demonstrated a significant reduction in pro-BNP levels in comparison to the animals treated with DOX and oxygen pus DOX (P <0.05) [Table 1].

Table 2 shows the effect of ozone-OP on the oxidised macromolecules and antioxidant enzymes in the cardiac tissue homogenates. MDA and AOPPs concentrations were measured as surrogate markers of lipid and protein damage, revealing a significant increase of these variables in the group treated with DOX only and the one treated with oxygen plus DOX (P <0.05). However, in ozonised rats there was a significant reduction of biomolecule damage in comparison to non-ozonised animals (P <0.05). Furthermore, ozone treatment was shown to preserve the activity of SOD and CAT, regulating the CAT/SOD ratio. The activity of these enzymes in the ozone-OP group did not differ from control animals; moreover, a significant increase was observed in respect to the animals only treated with DOX or the ones treated with oxygen plus DOX (P <0.05).

A microscopic analysis of the left ventricle sections from the control rats showed a normal morphology [Figure 3A]. Significant tissue injuries with the subendocardial loss of longitudinal muscular fibres, mild oedema and necrosis were seen in the DOX group [Figure 3B]. Conversely, only minor damage was observed in the ozone-treated animals with a preservation of the morphology of the cardiac muscular fibres [Figure 3C].

Discussion

The results of this study showed that ozone-OP improved DOX-induced DCM in rats. Ozone therapy preserved left ventricle morphology, which

Table 2: Effect of ozone therapy on oxidative stress biomarkers among the experimental groups of Sprague-Dawley rats*

Heart redox biomarkers	Experimental groups			
	Control	DOX	Oxygen plus DOX	Ozone-OP plus DOX
MDA in µM/mg Pr	6.49 ± 0.81	17.34 ± 1.95†	18.21 ± 0.99†	7.02 ± 0.68
AOPP in µM of chloramines/mg Pr	10.32 ± 0.99	22.70 ± 3.21†	20.69 ± 2.11†	14.52 ± 1.26†‡
CAT in U/L/min/mg Pr	1,025.87 ± 19.67	790.76 ± 54.56†	779.45 ± 34.92†	1,049.80 ± 27.43
SOD in U/mL/min/mg Pr	54.36 ± 4.99	32.21 ± 6.12†	34.54 ± 4.21†	60.21 ± 5.33
CAT/SOD in U/mL/min/mg Pr	0.018 ± 0.003	0.024 ± 0.001	0.022 ± 0.008	0.017 ± 0.005

DOX = doxorubicin; Ozone-OP = ozone-oxidative preconditioning; MDA = malondialdehyde; mg Pr = protein concentration; AOPP = advanced oxidation protein products; CAT = catalase; SOD = superoxide dismutase.

**Values are expressed as mean ± standard deviation with respect to the protein concentration (mgPr). †Statistical differences (P <0.05) compared to control group. ‡Statistical differences (P <0.05) compared to the DOX and oxygen plus DOX groups.*

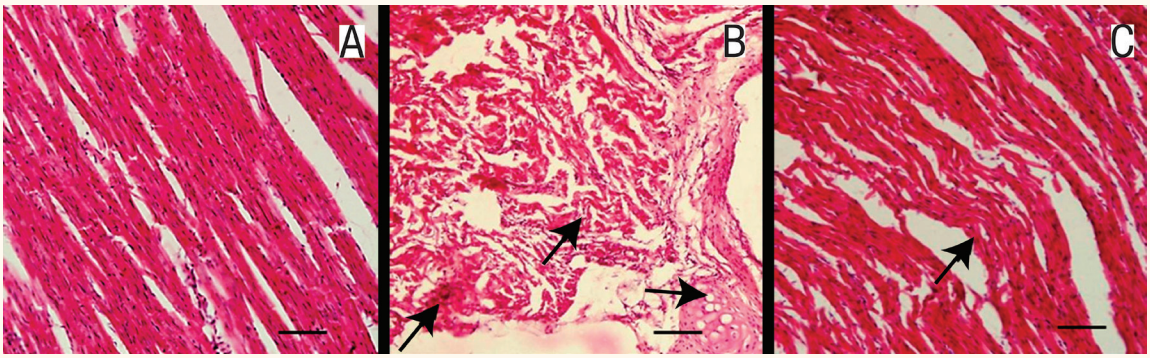

Figure 3 A–C: Effects of ozone therapy on doxorubicin (DOX)-induced cardiotoxicity at x20 magnification and a scale bar of 50 µm. Control rats showed a normal morphology (**A**), whereas the DOX group showed significant tissue injuries (**B**), with evidence of damaged muscular fibres where the necrosis was present (arrows). Conversely, only slight damage was observed in ozone-treated animals with a normal morphology of cardiac fibres (**C**).

was accompanied by a reduction of the serum pro-BNP levels. The mechanism was associated with a significant increase of antioxidant enzyme activities and a reduction of lipid and protein oxidation (P <0.05).

The histopathological evaluation of the heart sections revealed the deleterious effects of DOX on myocardial morphology. Conversely, ozone-OP prevented the loss of muscular fibres, with a preservation of their longitudinal disposition. Also, ozone treatment prevented the development of DOX-induced oedema and necrosis. In accordance with these findings, the serum pro-BNP levels were significantly reduced in the rats treated with ozone compared to those only treated with DOX or with oxygen plus DOX. Furthermore, a higher survival rate (90%) and lower body weight loss was observed in the ozonised group compared to the DOX group. As a whole, these results demonstrate the protective effect of ozone-OP against DOX-induced cardiotoxicity.

In order to examine further the mechanisms responsible for the cardioprotective action of ozone-OP, the effects of ozone-OP on heart OS biomarkers were evaluated. DOX-induced lipid and protein peroxidation has been well documented in the literature.[2,24] In the current study, significant increases of MDA and AOPPs (surrogate markers of lipid and protein oxidation) were observed (P <0.05). Both MDA and AOPPs were decreased by ozone insufflation in comparison to those animals only treated with DOX or with oxygen plus DOX. The reduction of MDA levels in the ozonised animals is indicative of the antioxidant effect of ozone therapy; this is of major importance due to the central role of lipid oxidation in DOX-induced DCM.[24]

AOPPs are closely connected with the degree of monocyte activation and the increase of inflammatory cytokines.[25] The generation of AOPPs is based on the chlorinated oxidation of proteins. In addition, there is evidence that AOPPs act as inflammatory mediators since they are able to trigger an oxidative burst and the synthesis of cytokines.[26] The action of ozone-modulating AOPPs may explain, at least in part, the control of the DOX-induced inflammatory process.

DOX increases cardiomyocyte susceptibility to OS by reducing antioxidants and, therefore, reducing the ability of cardiac cells to inactivate ROS.[27] In this respect, SOD and CAT are two enzymes which act to detoxify ROS and ameliorate DOX toxicity.[28] An increase in SOD enzymatic activity is usually interpreted as beneficial in the context of the antioxidant system. Notwithstanding, it has been documented that a rise in SOD activity, without a concomitant rise in the activity of CAT and/or glutathione peroxidase (GPx), might be detrimental.[29] This is because of the production of H_2O_2 by SOD, which is cytotoxic and has to be scavenged by GPx or CAT. Hence, a contemporary increment in GPx and/or CAT enzymatic activity is crucial for the globally beneficial effect of increased SOD activity. In the present study, the attenuation of DOX-induced OS and tissue injuries might be attributed to the increase in both myocardial SOD and CAT activities, as shown by the rate of CAT/SOD.

DOX-induced cardiomyopathy has long been a serious side-effect in the treatment of human cancers using this drug as it limits the clinical dosage.[30] OS is generally held to be the mediating mechanism in the multiple biological processes leading to DOX cardiotoxicity.[31] Consequently, developing a palliative treatment that can attenuate DOX cardiotoxicity is of major importance. In this context, ozone therapy, administered prior to DOX, may represent a promising approach for correcting OS levels and diminishing the toxic side-effects of this anthracycline antibiotic.

Therapeutic strategies using antioxidants and

iron-chelators to protect the heart against DOX damage have been used. Traditional antioxidants, like N-acetylcysteine or tocopherols, have not been very successful in the prevention of DOX-induced cardiotoxicity.[32,33] Dexrazoxane, an iron chelator that possesses potent antioxidant properties, is currently approved by the Food and Drug Administration (FDA) for the prevention of DOX cardiotoxicity; however, due to the high incidence of side-effects such as myelosuppression, its use has also been limited to the advanced stages of some malignant disorders.[2,34]

Currently, a body of evidence links the modulation of different biomarkers (e.g. antioxidant enzymes, nitric oxide pathways or 2,3-diphosphoglycerate) when low ozone doses are applied. These facts support many of the current clinical applications of ozone therapy.[15,35,36] The biological effect of rectal insufflation of ozone has been demonstrated extensively both experimentally and clinically in many diseases.[15] Pre-clinical studies have demonstrated its low toxicity.[37] Due to the oxidative preconditioning effect of ozone therapy, a cycle of 20 treatments will be enough to sustain the effect for approximately three months, depending on the OS status of the patients.[15]

Once the rectal *mucosa* comes into contact with ozone, there are several reactive intermediaries that act as signaling molecules, including H_2O_2, aldehydes and other inorganic and organic peroxides.[38] These reactive intermediaries have different diffusion rates according to their liposolubility and molecular dimensions. H_2O_2 is the ROS that most easily crosses the cell membranes. This ability makes H_2O_2 the most probable candidate of the observed effect of ozone treatment. Another group of ozone intermediaries that could be related to this are the long-chain aldehydes, such as hexanal, heptanal and nonenal aldehydes.[38] The subsequent pathway involving the ozone preconditioning may be likened to the nuclear factor erythroid-2 (Nrf2)/electrophile-responsive element (EpRE) activation pathway.[39]

Nrf2 is a redox-sensitive transcription factor regulating the expression of a number of cytoprotective genes.[40] The effect of ozonised serum in an *ex vivo* experiment demonstrated a dose effect activation of Nrf2 and the subsequent induction of haeme oxygenase 1 and nicotinamide adenine dinucleotide phosphate quinone oxidoreductase in endothelial cell cultures.[39] Nrf2 is a key protein located within the cells and is activated by an Nrf2 activator. Once released, it migrates into the cell nucleus and binds to the deoxyribonucleic acid at the location of the EpRE, which is the master regulator of the entire antioxidant system located in all human cells. However, Nrf2 has emerged as an important contributor to chemoresistance in cancer therapy.[41] Future studies should examine the interaction between the pharmacological effects of DOX (the inhibition of tumour growth, reduction of cell proliferation and inductor of apoptosis) and its toxicological effects (cardiotoxicity) in the presence of ozone.

Conclusion

In summary, the results of this study suggest that ozone-OP prevents DOX-induced DCM through an increase of antioxidant enzymes and a reduction of oxidised macromolecules, avoiding the DOX-induced pro-BNP increase. The results of this study require further pharmacological and toxicological investigations. The present work establishes the background for future studies in order to determine the pre-clinical/clinical efficacy or interaction of ozone in oncological interventions using DOX among humans.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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