INTERNATIONAL JOURNAL OF OZONE THERAPY

formerly RIVISTA ITALIANA DI OSSIGENO-OZONOTERAPIA

The Official Journal of
WFOOT - World Federation of Oxygen-Ozone Therapy,
FIO - Italian Federation of Ozone Therapy,
SEOT - Spanish Association of Ozone Therapy,
Hellenic, Indian, Slovach and Chinese National Societies

VOLUME 11 - No. 2 - OCTOBER 2012

CENTAURO S.r.l., BOLOGNA

ISSN 1972-3539

ASOCIACION MEDICA ARGENTINA DE OXIGENO OZONOTERAPIA

World Federation Oxygen - Ozone Therapy

IV World Congress of Oxygen – Ozone Therapy

October 30th to November 1st 2013 - Buenos Aires, Argentina

Conferences and workshop about new topics in Oxygen-Ozone Therapy:

- Phlebology and ginecology
- Odontology
- Veterinary medicine
- Stem cells
- Musculo-Skeletal Pathology
- Epidural o2 o3 Injection
- Legal

www.ivwcoot.com.ar // info@ivwcoot.com.ar

INTERNATIONAL JOURNAL OF OZONE THERAPY

formerly RIVISTA ITALIANA DI OSSIGENO-OZONOTERAPIA

THE OFFICIAL JOURNAL OF WFOT - WORLD FEDERATION OF OXYGEN-OZONE THERAPY,
FIO - ITALIAN FEDERATION OF OZONE THERAPY, SEOT - SPANISH ASSOCIATION OF OZONE THERAPY,
HELLENIC, INDIAN, SLOVACH AND CHINESE NATIONAL SOCIETIES

Index

Cover: Emil Nolde - *Marsh Landscape with Thunder Clouds* (1930), watercolour on paper, 33 × 45 cm - Album di Cea - http://www.flickr.com

Indexed in: EMBASE, Elsevier (http://www.scopus.com), Google Scholar (http://scholar.google.com)

DIAGNOSI & RIABILITAZIONE FORMATO FAMIGLIA.

Sì, proprio così.

A Brescia è nato un Centro Polifunzionale in grado si soddisfare i bisogni di tutta la famiglia.

Dove il rapporto umano viene prima di tutto. E dove specialisti e fisioterapisti di alto livello si incontrano con metodi, sistemi e tecnologie avanzatissimi.

- Laserterapia
- Correnti Analgesiche
- Massoterapie
- Rieducazione e Riabilitazione
- **Ozonoterapia**
- Visite Specialistiche
- Tac
- Ecografie
- Ecodoppler
- Tecar
- **Spine-Lab**

NUOVO A BRESCIA

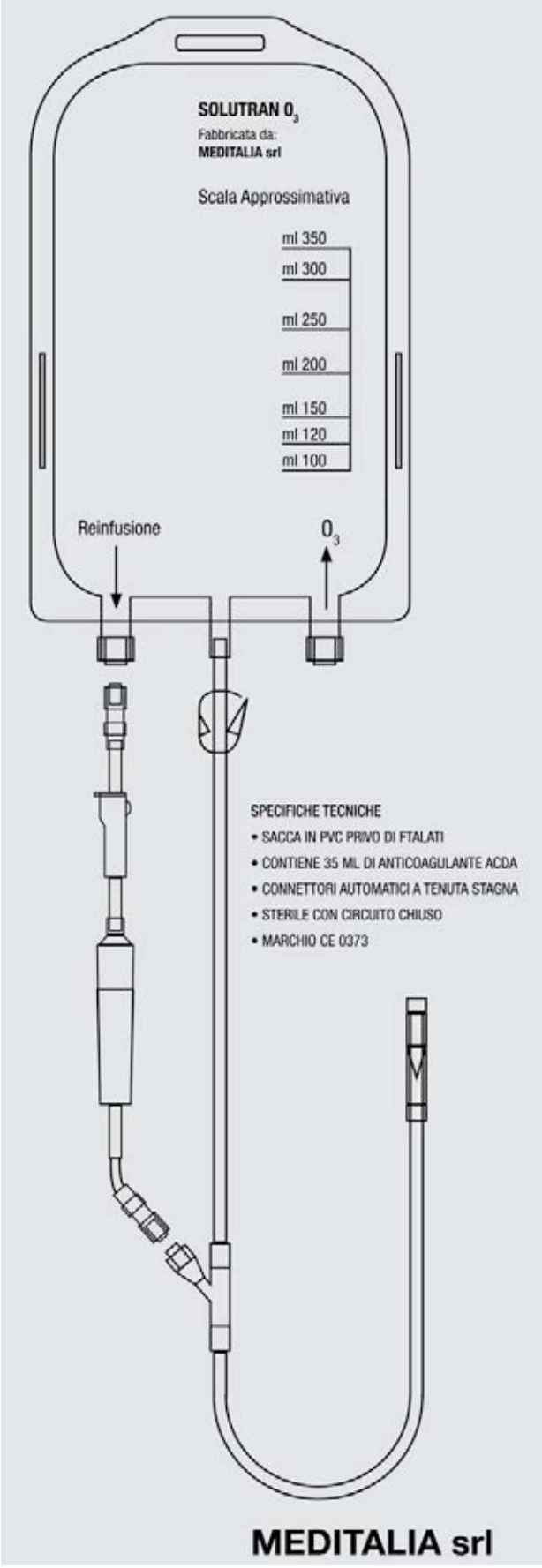
PO Oberdan Poliambulatorio

X Ray service

POLIAMBULATORIO OBERDAN / info@poliambulatorioberdan.it
Via G. Oberdan, 140
25128 BRESCIA

Tel. 030 3701312
Fax 030 3387728


In collaborazione con
Terme di Angolo
www.termediangolo.it
salute · wellness · beauty · fitness
©iStockphoto.com/Paha_L



Kit ozonoterapia Solutran 0_3

COMUNICAZIONE:
La Società Meditalia, produttrice di materiale plastico, ha sentito l'esigenza come altre società, di preparare un kit per Autoemo in Ozonoterapia con materiale privo di ftalati.
È stato quindi superato l'ostacolo della Circolare Ministeriale 1999 che proibiva l'uso di sacche standard nell'Autoemo perché non testate per quanto riguardava il rilascio di ftalati.
Oggi gli Ozonoterapisti devono porre la loro attenzione sul confronto **plastica-vetro**. Per fare questo è necessario partire da una realtà: nella prima parte l'Autoemo è da considerarsi **"prelievo di sangue intero da paziente"** e come tale è preferibile seguire le Disposizioni Ministeriali che regolano la trasfusione.

Perché sacche -si:
1) Registrate specificatamente per Autoemo.
2) Prelievo a caduta fisiologico.
3) Prelievo reimpostato in ml.
4) Prelievo continuamente basculato. Questo implica l'utilizzo di una piccola bilancia automatizzata

Perché flacone-no:
1) Prelievo forzato (vuoto d'aria interno).
2) Possibilità di collabire la vena del paziente.
3) Emolisi significativa dei globuli rossi.
   Il sangue intero infatti entrando velocemente sbatte violentemente contro le pareti rigide del flacone.
4) Procedimento che si allontana dal circuito chiuso.
   Cito due decreti ministeriali, tra i tanti, emanati e pubblicati nella Gazzeta Ufficiale:
   1) 26-01-2001 – prende in considerazione il materiale da usare - sacche con inserito anticoagulante.
   2) 17-05-2011 al punto T4 indica la procedura da seguire per il prelievo.

Per quanto detto e citato il flacone è stato abbandonato già dagli anni '70 nel settore trasfusionale sia a livello nazionale che internazionale.
A voi Gentili Medici Ozonoterapisti riflettere sulla prima parte dell'Autoemo, **il prelievo di sangue intero**.

Fabio Malipiero
Tecnico di pratiche emoinfusionali
Tel. 339 3861725
e-mail: fabio.malipiero@tiscali.it

Ozone Therapy Attenuates Doxorubicin-Induced Hepatotoxicity in Sprague Dawley Rats

L. DELGADO ROCHE[1], Y. HERNÁNDEZ-MATOS[1], M. RAVELO GONZÁLEZ[2], E. ACOSTA MEDINA[3]

[1] *Center of Studies for Research and Biological Evaluations, Pharmacy and Food Sciences College, University of Havana; Havana, Cuba.*
[2] *Pharmaceutical Laboratories LABIOFAM, Havana, Cuba.*
[3] *Center of Advanced Studies; Havana, Cuba*

Key words: ozone, doxorubicin, hepatotoxicity, oxidative stress.

SUMMARY - *Doxorubicin is an effective chemotherapeutic drug; however, its hepatotoxicity compromises its therapeutic index. Doxorubicin-induced hepatotoxicity is thought to be caused by reduction/oxidation cycling of drug to generate oxidative stress and hepatocyte death. Because ozone therapy can activate antioxidant systems, the aim of the present study was to investigate the therapeutic efficacy of ozone-oxidative preconditioning against doxorubicin hepatotoxicity. Male Sprague Dawley rats were utilized in the study. The animals were divided into three groups (n=10); (1): intraperitoneally treated with 2 mg/kg of doxorubicin during 50 days twice a week; (2): treated with 0.3 mg of ozone/oxygen mixture (50 µg/mL of ozone/6 mL) by rectal insufflation once a day during 20 sessions in alternated days, and (3): treated with oxygen and NaCl 0.9% solution. Hepatic oxidative stress biomarkers and serum transaminases levels were spectrophotometrically determined. The damages to livers were examined by light microscopy with eosin/hematoxylin staining. Also, the survival percent and body weight were determined. A preservation of liver morphology in the ozonized animals was accompanied with a reduction of total death and body weight loss. Furthermore, ozone therapy induced a significant ($p<0.05$) reduction of lipid and protein oxidation, meanwhile a significant ($p<0.05$) increment of superoxide dismutase and catalase activities was observed. Our results suggest that ozone attenuates doxorubicin-induced hepatotoxicity through an increase of antioxidant enzymes and a reduction of biomolecules damages. The integrative therapy with ozone could be a future opportunity for correcting the chronic oxidative stress present in oncology patients and reducing the doxorubicin-induced hepatotoxicity.*

Introduction

Doxorubicin (DOX) is an anthracycline antibiotic which is used to treat a wide range of cancers [1]. Like most of the anticancer drugs, DOX causes various toxic effects, the commonest of which is the dose-dependent hepatotoxicity [2]. DOX toxicity induces disruption in basal metabolism in the liver [3].

Cellular damage induced by DOX is mediated by the formation of an iron-anthracycline complex that generates free radicals, which in turn, causes severe damage mediated by oxidative stress (OS) [4]. DOX localizes to the mitochondria and is highly susceptible to enzymatic reduction to generate superoxide radicals ($O_2^{\bullet-}$) and reactive oxygen species (ROS), which alter mitochondrial function [5].

Therapeutic strategies, designed to augment cellular endogenous defense systems have been identified as a promising approach to combat OS-associated disease conditions [4]. Evidence that antioxidant enzymes, nitric oxide pathways and other subcellular activities could be modulated by low doses of ozone is now proven and could support the effects of ozonetherapy in many pathological conditions such as hepatic and renal ischemia-reperfusion [6], diabetes mellitus [7], disc hernia [8], retinitis pigmentosa [9] and coronary artery disease [10,11].

In the light of more recent pharmacological

Abbreviations:
DOX: doxorubicin
OS: oxidative stress
$O_2^{\bullet-}$: superoxide anion radical
Ozone-OP: ozone-oxidative preconditioning
O_3/O_2: ozone/oxygen mixture
AST: aspartate aminotransferase
ALT: alanine aminotransferase
SOD: superoxide dismutase
GSH: glutathione
AOPP: advanced oxidation protein products

knowledge, ozone can be considered a pro-drug which at certain non-toxic doses can induce a rearrangement of the biochemical pathways with the activation of a second messenger in a cascade with a multiple system action [8,12,13].

The present study was designed to evaluate the effects of ozone-oxidative preconditioning (Ozone-OP) on DOX-induced hepatotoxicity in Sprague Dawley rats. Particularly, we examined the status of antioxidant enzymes activity, biomolecules damages, transaminases, and also the histological characteristics of the liver tissue in ozonized animals. Our results allow us to conclude that Ozone-OP prevents DOX-induced hepatotoxicity through an increase of antioxidant enzymes activity, a reduction of biomolecules damages and transaminases levels, with a consequent preservation of liver tissue integrity.

Materials and Methods

Reagents

All reagents were purchased from Sigma-Aldrich (St Louis, MO, USA), except DOX, which was kindly provided by the manufacturer, the Center of Drug Research and Development, Havana, Cuba.

Animals

Adult male Sprague Dawley rats, weighing 250-300 g were obtained from the National Center for Laboratory Animals Production (CENPALAB, Mayabeque, Cuba) and then adapted to laboratory conditions (60% humidity, 25 ± 1 ºC) for at least 1 week before the experiments. The rats were housed in groups of five and exposed to 12 h light/darkness cycle, with free access to food and water. Animal studies were performed with approval of Pharmacy and Food Sciences College Institutional Animal Ethical Committee. All procedures were in accordance with the guidelines stipulated by the Institutional Animal Care Committee and the European Union Guidelines for animal experimentation.

Ozone generation

Ozone was generated by OZOMED equipment manufactured by the Ozone Research Center (Havana, Cuba). Ozone was obtained from medical grade oxygen, and was used immediately upon generation and represented only about 3% of the gas mixture (O_3+O_2). The ozone concentration was measured by using a built-in UV spectrophotometer set at 254 nm.

Experimental design

Three groups of 10 rats were used in the study. The first group was treated with 6 mL of O_3/O_2 containing 50 µg/mL of ozone by rectal insufflation once a day during 20 sessions in alternated days. Prior to ozone insufflation the rectum was stimulated to eliminate the excrements. After 20 sessions of ozone, the rats were intraperitoneally treated with 2 mg/kg of DOX during 50 days twice a week (ozonized group). The second group was similarly treated with DOX (DOX group), while the third group was insufflated with oxygen (under identical conditions to ozone) and then intraperitoneally treated with 2 mL/kg of NaCl solution (control group). At the end of the study, the animals were anesthetized with ketamine hydrochloride (5 mg/kg i.m.), and euthanized with an overdose of sodium pentobarbital (90 mg/kg, i.v.) (Abbott Laboratories, Mexico SA de CV, Mexico). Then, vascular system was perfused with ice cold NaCl 0.9% solution and livers were used for OS biomarkers determination (n=5) and histopathological study (n=5).

Samples collection

Blood samples for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) determination were obtained after 12 h overnight fast, at 24 h after the last ozone administration. These samples were immediately centrifuged at 3000 g, at 4°C for 10 min. Finally, the serum was collected and aliquots were stored at –80 °C until analysis.

Liver homogenate preparation

Livers were macerated and homogenized in ice-cold KCl-histidine buffer pH 7.3, in a tissue homogenizer (Edmund Bühler LBMA, Germany). Homogenized tissue was then centrifuged at 4500 g for 20 min at 4ºC and the supernatants were collected and stored at –80ºC until redox biomarkers determinations.

Histopathology

The livers samples were rinsed in PBS, pH 7.4, and fixed in 10% formaldehyde solution during 24h.

Samples were then embedded in paraffin. Five-micrometer tissue sections were cut, air-dried on glass slides with different grades of alcohol/xylol, deparaffinized and rehydrated. Finally, tissue sections were stained with eosin and hematoxylin (HE) under standard procedures. The sections were analyzed in an optic microscope Olympus BX51.

Marker of liver injury

Plasma ALT and AST levels were measured using a commercial kit obtained from Finlay Laboratories (Havana, Cuba).

Determination of oxidative stress biomarkers

All biochemical parameters were determined by spectrophotometric methods using a Pharmacia 1000 Spectrophotometer (Pharmacia LKB, Uppsala, Sweden) and a microplate reader (SUMA, Center of Immunoassay, Havana, Cuba).

Total proteins concentration

Total proteins levels were determined using the method described by Bradford [14] with bovine serum albumin as standard.

Antioxidant enzymes activity

SOD activity was determined by using RANSOD kit (catalogue no. SD 125, Randox Labs, Crumlin, UK). The method employs xanthine and xanthine oxidase to generate superoxide anion radicals ($O_2^{\bullet -}$), which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. SOD activity was measured by the inhibition degree of this reaction [15].

Catalase activity was determined by following the decomposition of hydrogen peroxide (H_2O_2) at 240 nm at 10 s intervals during 1 min 15.

Non-enzymatic antioxidants

After precipitation of thiol proteins, the reduced glutathione (GSH) levels were measured according to the method of Sedlak and Lindsay with Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid), and the absorbance was measured at 412 nm. Purified GSH was used to generate standard curves [16].

Biomarkers of biomolecules damage

The advanced oxidation protein products (AOPP) were measured as described previously [17]. Briefly, samples in PBS pH 7.40, 10 mM (1 mL) were treated with 50 µL of potassium iodide 1.16 M followed by the addition of 100 µL of acetic acid. The absorbance was immediately read at 340 nm. AOPP concentration was expressed as µM of chloramines-T. Concentration of malondialdehyde (MDA) was determined using the LPO-586 kit obtained from Calbiochem (La Jolla, CA, USA). In the assay, the production of a stable chromophore after 40 min of incubation at 45 ºC was measured at 586 nm. For standards, freshly prepared solutions of malondialdehyde bis (dimethyl acetal) were employed and assayed under identical conditions [18].

Statistical analysis

Statistical analysis was performed using the SPSS program for Windows (version 11.5, SPSS Inc.). Bartlett's Box-test was used to test the homogeneity of variance. Differences between groups were determined by student's t-test (two-tailed). Data were expressed as the mean ± standard deviation (SD). A P-value of <0.05 was considered statistically significant.

Results

Survival and body weight behaviour

The survival behaviour is shown in Figure 1. In the ozone-treated group there was a 90% survival, while in the group only treated with DOX the survival was lower (70%) than in control and ozone groups. Meanwhile, in Figure 2 is shown that ozone was able to reduce the loss of body weight associated to DOX administration. Although, the body weight of ozonized rats was lower ($p<0.05$) than controls, it was significantly ($p<0.05$) higher in comparison with the group only treated with DOX. The body weight did not vary significantly in any experimental group before DOX administration (data not shown). Macroscopic and microscopic examination of organs did not show any relevant disease or abnormalities during Ozone-OP.

Histopathology

Microscopic analysis of liver sections from control rats showed a normal morphology (Figure 3A). Meanwhile, significant tissue injury was seen

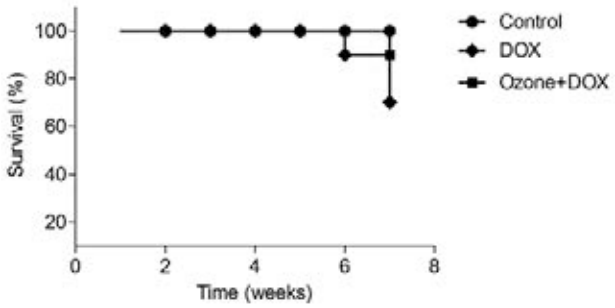


Figure 1 Corresponding survival for each experimental group. The survival percent in DOX group was of 70%, while in ozonized animals was of 90%.

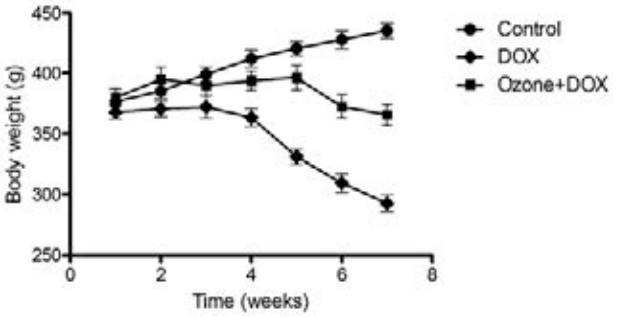


Figure 2 Body mass changes during the experimental protocol and observation period. The reduction of body mass in DOX group was significantly ($p<0.05$) respect to controls and ozonized animals.

in DOX group (Figure 3B), showing dilated and hyperemic sinusoids where necrosis was evident. Conversely, only slight damage was observed in ozone-treated animals with a normal morphology of hepatic lobuli (Figure 3C).

Oxidative stress biomarkers

Table 1 shows the effect of Ozone-OP on biomolecule oxidation in hepatic tissue. MDA and AOPP concentrations were measured as surrogate markers of lipid and protein damage, revealing a significant ($p<0.05$) increase of these variables in the group only treated with DOX. Meanwhile, in ozonized rats there was a significant reduction ($p<0.05$) of biomolecules damages in comparison to non-ozonized animals. Furthermore, ozone was able to preserve the activity of SOD and catalase. The activity of these enzymes in the Ozone-OP group did not differ from control animals, while a significant ($p<0.05$) increase was observed respect to the animals only treated with DOX. On the other hand, the GSH levels in the ozone group were significantly higher ($p<0.05$) than in DOX-treated animals. In these group there was a significant ($p<0.05$) decrease of the non-enzymatic antioxidant GSH.

Hepatic damage markers

As shown in Table 2 the degree of hepatic damage, evaluated by the serum levels of AST and ALT, increased significantly ($p<0.05$) in the group only treated with DOX, while Ozone-OP was able to diminish both transaminases, although statistical differences ($p<0.05$) respect to controls were observed.

Discussion

DOX-induced hepatotoxicity has long been a serious side effect in treating human cancers, which limits the clinical dosage of DOX. Consequently, developing a palliative treatment that can attenuate DOX toxicity 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. Nowadays, scientific evidence connected the modulation of different biomarkers (e.g. antioxidant enzymes, nitric oxide pathways, 2,3-diphosphoglycerate) as a consequence of applying low ozone doses. Those facts support some of the current clinical applications of ozone therapy [8,19]. It is important to highlight that the biological effect of rectal insufflation of Ozone has been demonstrated extensively both experimentally and clinically [20]. Furthermore, preclinical studies demonstrated its low toxicity [21]. For this reason, the application of Ozone by rectal way has been now extended to treat many diseases. Because of the oxidative preconditioning effect of ozone therapy, a cycle of 20 treatments will be enough to sustain the effect for approximately 3 months, depending on the OS status of the patients [11].

The histopathological evaluation of liver sections demonstrated the deleterious effects of DOX on hepatic morphology. On the contrary, Ozone-OP prevented DOX-induced hepatic damage. Accordingly with these findings, the serum AST and ALT levels were significantly reduced in the animals treated with ozone compared with the rats only treated with DOX. Furthermore, a higher survival index (90%) and a lower body weight loss were observed in ozonized rats compared with DOX group. All of this demonstrates

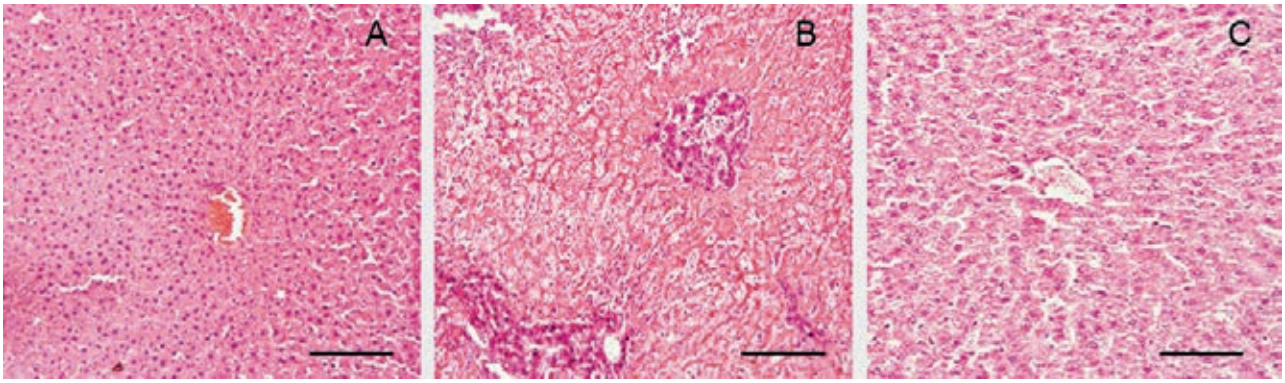


Figure 3 Representative microphotograph of microscopic analysis of liver sections. Control rats showed a normal morphology (Figure 3A). Meanwhile, Figure 3B shows significant tissue injury in DOX group, showing dilated and hyperemic sinusoids where necrosis was evident. Conversely, only slight damage was observed in ozone-treated animals with a normal morphology of hepatic lobuli (Figure 3C).

Table 1 Effects of ozone-oxidative preconditioning on liver redox biomarkers.

Liver redox biomarkers	*Control*	*DOX*	*Ozone + DOX*
MDA, μM/mgPr	5.25 ± 0.62	18.61 ± 2.11*	7.12 ± 0.71
AOPP, μM of chloramines/mgPr	9.54 ± 0.83	21.35 ± 2.97*	13.42 ± 1.12#
CAT, U/L/min/mgPr	1109.46 ± 17.81	785.23 ± 24.72*	1087.50 ± 23.91
SOD, U/mL/min/mgPr	65.12 ± 2.47	30.64 ± 5.88*	61.34 ± 3.31
GSH, mM/mgPr	3.19 ± 0.65	1.08 ± 0.38*	2.97 ± 0.24

Table shows the means ± SD of redox biomarkers. The parameters were expressed per milligrams of protein (mgPr).
Asterisks and number symbol represent statistical differences ($p<0.05$) between the same set.
MDA: malondialdehyde, *AOPP*: advanced oxidation protein products, *CAT*: catalase, *SOD*: superoxide dismutase, *GSH*: glutathione, *DOX*: doxorubicin.

Table 2 Effects of ozone-oxidative preconditioning against liver damage.

Experimental group	*AST (U/L)*	*ALT (U/L)*
Control group	49.15 ± 1,59 a	21.19 ± 3.59 a
DOX group	221.78 ± 9.23 b	97.46 ± 3.44 b
Ozone + DOX group	64.25 ± 3.84 c	41.08 ± 2.67 c

Table shows the means ± SD of transaminases. Different letters represent statistical differences ($p<0.05$) between groups.
DOX: doxorubicin, *Ozone-OP*: ozone-oxidative preconditioning.

the hepatoprotective effect of ozone-OP against DOX-induced toxicity. In order to further examination of mechanisms responsible of cardioprotective action of Ozone-OP we evaluated its effect on OS biomarkers. The induction of lipids and proteins peroxidation by DOX administration has been well documented in the literature [4,22]. In our study we observed a significant increase of MDA and AOPP, surrogate markers of lipid and protein oxidation. Both, MDA and AOPP were downregulated by ozone in comparison to those animals only treated with DOX. Improvement of MDA in the ozonized animals is indicative of the antioxidant effect of ozone therapy, and it is of major importance due to the central role of lipid oxidation in the hepatotoxicity mediated by DOX [2]. Our results are in accordance with other reports in which antioxidants were used to prevent DOX toxicity [2,3,23].

AOPP is a biomarker of OS correlating tightly with the degree of monocyte activation and inflammatory cytokines increment [24]. Moreover, AOPP appeared to act as true inflammatory mediators since they are able to trigger the oxidative burst and the synthesis of cytokines [25]. The effect of ozone regulating AOPP may be considered as an indirect index connecting with the control of the DOX-induced inflammatory process.

DOX increases hepatocyte susceptibility to OS by reducing antioxidants and therefore reducing the ability of cells to inactivate ROS. In the present study, the attenuation of DOX-induced OS and tissue injury might be attributed to the increase of hepatic GSH and also the activity of SOD and catalase. Ozone-OP may promote a moderate OS which, in turn, increases antioxidant endogenous systems protecting against liver damage [26,27].

On the other hand, Ajamieh and coworkers demonstrated that the protective mechanism mediated by Ozone-OP involves protein synthesis [28]. Elevated ROS concentrations induce gene expression in many cells, whose products exhibit antioxidant activity.

A major mechanism of redox homeostasis is based on the ROS-mediated induction of redox-sensitive signal cascades that lead to increased expression of antioxidants [29]. Furthermore, León and coworkers showed that ozone protected Ca^{2+}-ATPase from inactivation by OS, regulated accumulation of adenosine and blocked the xanthine/xanthine oxidase pathway for ROS generation. The above mentioned mechanisms may be involved in the protective effect of ozone against DOX hepatotoxicity.

Conclusions

Data presented in this paper are indicative of potentially hepatoprotective effect induced by the treatment with low doses of ozone against DOX toxicity. Also, the integrative therapy with ozone could be a future opportunity for correcting the chronic OS present in malignant tumors and attenuating the DOX-induced hepatotoxicity. In addition, the present work establishes the antecedent for future studies in order to determine the clinical efficacy of ozone in oncology patients.

Acknowledgements

The authors want to thank the technical assistance of Rafael Pérez and Dalia Rosa Álvarez.

References

1 Blum RH, Carter SK. Adriamycin. A new anticancer drug with significant clinical activity. Ann Intern Medicine. 1974, 80: 249-259.
2 Kalender Y, Yel M, Kalender S. Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats: The effects of vitamin E and catechin. Toxicology. 2005; 209: 39-45.
3 Deepa PR, Varalkshmi P. Protective effect of low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced cardiac and hepatic toxicity. Chem Biol Interac. 2003; 146: 201-210.
4 Murkhejee S, Banerjee SK, Maulik M, et al. Protection against acute adriamycin-induced cardiotoxicity by garlic: Role of endogenous antioxidants and inhibition of TNF-α expression. BMC Pharmacol. 2003; 3:16-21.
5 Kim DS, Woo ER, Chae SW, et al. Plantainoside D protects adriamycin-induced apoptosis in H9c2 cardiac muscle cells via the inhibition of ROS generation and NF-kappaB activation. Life Sci. 2007; 80: 314-323.
6 Ajamieh H, Merino N, Candelario-Jalil E, et al. Similar protective effect of ischaemic and ozone oxidative preconditionings in liver ischaemia/reperfusion injury. Pharmacol Res. 2002; 45: 333-339.
7 Martínez-Sánchez G, Al-Dalain SM, Menéndez S, et al. Therapeutic efficacy of ozone in patients with diabetic foot. Eur J Pharmacol. 2005; 523: 151-161.
8 Re L, Mawsouf MN, Menéndez S, et al. Ozone therapy: clinical and basic evidence of its therapeutic potential. Arch Med Res. 2008; 39: 17-26.
9 Copello M, Menéndez S. Retinitis Pigmentosa patients treated with ozone therapy during 20 years. Cuban experiences. Rev Española Ozonoterapia. 2011; 1: 13-22.
10 Delgado-Roche L, Martínez-Sánchez G, Díaz A, et al. Effects of ozone therapy on oxidative stress biomarkers in coronary artery disease patients. Int J Ozone Ther. 2011; 10: 99-104.
11 Martínez-Sánchez G, Delgado-Roche L, Díaz A, et al. Effects of ozone therapy on hemostatic and oxidative stress index in coronary artery disease. Eur J Pharmacol. 2012; 691: 156-162.
12 Bocci VA. Scientific and medical aspects of ozone therapy. State of the art. Arch Med Res. 2006; 37: 425-435.
13 Bocci VA, Zanardi I, Travagli V. Ozone acting on human blood yields a hormetic dose-response relationship. J Transl Med. 2011; 9: 66.
14 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 1976; 72: 248-254.
15 Boehringer Manheim. Biochemical Information. A revised biochemical reference source. Enzymes for routine. 1st ed, Germany, 1987, p. 15-6.
16 Sedlak J, Lindsay RH. Estimation of total protein bound and non protein sulfhydryl group in tissue with Ellman's reagent. Anal Biochem. 1968; 25: 192-205.
17 Witko-Sarsat V, Friedlander M, Nguyen-Khoa T, et al. Advanced oxidation protein products as novel mediators of inflammation and monocytes activation in chronic renal failure. J Immunol. 1998; 161: 2524-2532.
18 Erdelmeier I, Gerard D, Yadan JC, et al. Reactions of N-methyl-2-phenyl-indole with malondialdehyde and 4-hydroxy-alkenals. Mechanistic aspects of the colorimetric assay of lipid peroxidation. Chem Res Toxicol. 1998; 11: 1184-1194.
19 Steppan J, Meaders T, Muto M, et al. A metaanalysis of the effectiveness and safety of ozone treatments for herniated lumbar discs. J Vasc Interv Radiol. 2012; 21: 534-548.
20 Martínez-Sánchez G, Re L. Rectal administration and its application in ozonetherapy. Int J Ozone Ther. 2012; 11: 41-49.
21 Díaz-Llera S, González-Hernández Y, Mesa JE, et al. Induction of DNA primary damage in peripheral blood leukocytes and exfoliated colorectal epithelial cells in rats treated with O_3/O_2 mix. Int J Ozone Ther. 2009; 8: 217-221.
22 Ibrahim SS, Barakat MA, Helmy HM. Role of selenium in attenuating cardiac and hepatic damages induced by the antitumor agent, doxorubicin. Life Sci J 2010; 7: 162-172.
23 Quiles JL, Huertas JR, Battino M, et al. Antioxidant nutrients and adriamycin toxicity. Toxicology. 2002; 180: 79-95.
24 Witko-Sarsat V, Nguyen-Khoa T, Jungers P, et al. Advanced oxidation protein products as a novel molecular basis of oxidative stress in uraemia. Nephrol Dial Transplant. 1999; 14: 76-78.
25 Martínez-Sánchez G, Giuliani A, Pérez-Davison G, et al. Oxidized proteins and their contribution to redox homeostasis. Redox Rep. 2005; 10: 175-185.
26 León OS, Menéndez S, Merino N, et al. Ozone oxidative preconditioning: a protection against cellular damage by free radicals. Mediat Inflamm. 1998; 7: 289-294.
27 Candelario-Jalil E, Mohammed-Al-Dalain S, León OS, et al. Oxidative preconditioning affords protection against carbon tetrachloride-induced glycogen depletion and oxidative stress in rats. J Appl Toxicol. 2001; 21: 291-301.
28 Ajamieh HH, Berlanga J, Merino N, et al. Role of protein synthesis in the protection conferred by ozone-oxidative-preconditioning in hepatic ischaemia/reperfusion. Transplant Int. 2005; 18: 604-612.
29 Dröge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002; 82: 47-95.

Livan Delgado Roche, MD
222 St. and 27A No. 21425
La Coronela, La Lisa
Havana 13600
Cuba
Tel.: 537-2719538.
E-mail: ldelgado@ifal.uh.cu

FEDERAZIONE ITALIANA DI
OSSIGENO-OZONOTERAPIA

PRIMO ANNUNCIO

VI CONGRESSO NAZIONALE DELLA FEDERAZIONE ITALIANA DI OSSIGENO-OZONO TERAPIA

Firenze 9-11 maggio 2013

Organizzazione Scientifica:
Dr. G. Pellicanò

Segreteria Organizzativa:
Scaramuzziteam
Tel. +39 055 494949
Fax +39 055 476393
www.scaramuzziteam.com

A Technically Feasible Treatment for Peritoneal Carcinomatosis

V. BOCCI[1], I. ZANARDI[2], J.C. PÉREZ OLMEDO[3], V. TRAVAGLI[2]

[1] *Department of Physiology,* [2] *Department of Pharmaceutical Chemistry and Technology, University of Siena; Siena, Italy*
[3] *Unidad de Ozonoterapia Sanatorio Marescot; Pontevedra, España*

Key words: abdominal carcinomatosis, malignant ascites, intraperitoneal administration, chemicals and drugs, ozone, reactive oxygen species, antioxidants

SUMMARY - *Peritoneal and pleural carcinomatosis are late complications of a primary tumour mainly developed in abdominal organs. Peritonectomy followed by hyperthermic intraperitoneal chemotherapy (HIPEC) represents a feasible and effective intervention, which however has considerable risks and cannot guarantee the cure. It appears reasonable to propose and discuss an alternative treatment based on intraperitoneal application of ozone either as a gas or dissolved in physiological solution. Light and flexible silastic catheters have been implanted into the peritoneal cavities. Two distinct protocols have been used. Protocol* a) *has been based on five daily successive sessions. For each session the ozone concentration was of 50 µg/mL with a gas volume of 2000-2500 mL, hence a total ozone dose of 100-125 mg has been used. Protocol* b) *used at the morning the insufflations of gaseous oxygen-ozone mixture at low ozone concentrations and in the afternoon the intraperitoneal infusion of a sterile lipid emulsion in ozonated NaCl 0.9% solution (42 °C). All the four patients presenting peritoneal carcinomatosis, ascite and multiple metastasis from colon, ovary and pancreas tumors, showed a prolonged survival after treatment based on protocol* a). *The single case treated with protocol* b)*, even if it is clinically irrelevant, it has been significant only for improving technical details. Ozone, not only possesses direct cytotoxic activity on peritoneal neoplastic cells, but* via *its messengers stimulates a number of important biological activities among which immune-stimulation as well as both activation and up-regulation of antioxidant enzymes. In comparison to chemotherapeutic drugs, ozone displays only a local oxidant activity without multi-organ toxicity. Moreover, it is an inexpensive drug and it is easy to use in precisely defined dosages. Based on these technical data, it is hoped that this paper may interest a group of oncologists for both optimizing the methodology and exploring the most effective scheme of ozone delivery.*

Introduction

Peritoneal (involving both visceral and parietal membranes) carcinomatosis represents the late stage of neoplasms originally present in the ovary, colon, appendix, stomach, pancreas and liver [1,2]. Very rarely it may originate from ovarian cells disseminated in the peritoneum during embryogenesis. Neoplastic cells, through the lymphatic lacuna present within the diaphragmatic peritoneum [3] can also invade the pleural membranes inducing also an abundant pleural effusion. Consequently, it is a fairly common complication of frequent human tumors. On the other hand, both peritoneal and pleural mesothelioma are rare tumors linked to asbestos exposure. Sugarbaker [4] was the first to propose a surgical intervention for removing neoplastic agglomerates spread within the abdomino-pelvic cavity, while Spratt et al. [5] have previously indicated the feasibility of the intraoperative hyperthermic intraperitoneal chemoperfusion (HIPEC). These approaches, although effective, are understandably complex and aggressive because peritonectomy may take 8-15 hours and it represents an imposing stress which may be overcome by patients not over 60 years old. Moreover, HIPEC aims to destroy the remaining cancer cells by intraperitoneal hyperthermic (42-43 °C) perfusion with a physiological liquid containing cisplatin realized by an extravascular double perfusion system activated by peristaltic pumps. The initial surgical cytoreduction is complemented by chemotherapy which can use drugs at concentrations 3-5 times higher than those used for intravenous infusion. In particular, HIPEC is painful and it cannot insure the total destruction of neoplastic stem cells.

After evaluating a number of other approaches reviewed by Barni et al. [6], a valid conclusion is that the combination of cytoreductive surgery

with perioperative HIPEC yields an improved survival as compared with systemic chemotherapy. In selected groups of patients, median survival varies from 25 to 60 months and the 5-year survival ranges from 20 to 45%, while morbidity varies from 20 to 40%. However, it appears reasonable to select patients according to the existing independent prognostic variables [7]. Consequently, in order to also extend the treatment in older or debilitated patients, a new approach based on a strongly oxidant gas which can be locally efficacious in the absence of detrimental effects for the whole organism will be suggested.

Reasons to use ozone

During the last two decades we have evaluated the possibility of using ozone as a cytotoxic agent and a biological response modifier [8]. However, serious evidence that the classical ozonated autohemotherapy is beneficial to cancer patients is lacking because firstly randomized, double-blind clinical trials have not been performed and, secondly because ozone acting on blood *ex vivo* is totally exhausted during the following blood infusion into the donor's patient and it cannot act on either primary or metastatic neoplastic cells present in different organs [9]. On the other hand, since 1980 Sweet et al. have shown that ozone *in vitro* inhibits the growth of a variety of human cancer cells [10]. Such an observation is relevant only for intraperitoneal or/and intrapleural (mesothelioma) neoplastic cells proliferating on these serous membranes, which closely resemble the situation *in vitro* where the stratified cells, overlaid with a thin layer of tissue culture fluid, were exposed for 8 days to the gas mixture composed of ozone (0.8 μg/mL) and aqueous vapour. As it happens on the pulmonary epithelial lining during ozone exposure [11], it must be clarified that this gas does not directly attack the cell membrane because it firstly dissolves in the surfactant film layer and reacts with hydrosoluble and lipophylic antioxidants and unsaturated fatty acids. In spite of this instantaneous reaction, the concentration of the ozone messengers, namely H_2O_2, (among Reactive Oxygen Species, ROS) and toxic 4-hydroxy-2,3-trans-nonenal (4-HNE) as the major lipid oxidation products (LOPs) are locally very high and deleterious for cancer cells [10,12]. On the whole, these cells have scarce antioxidant defences against these compounds able to cause either apoptosis or necrosis. Owing to a brief half-life of ozone messengers, partly quenched by natural antioxidants present in the peritoneal or pleural fluids, it appears necessary to slowly administer the oxygen-ozone gas mixture or ozone dissolved in physiological solution during about 60 min at least twice daily. It needs to be emphasized that H_2O_2 via activation of the NFkB, can also activate resident macrophages, neutrophils and lymphocytes, which can release cytokines such as IFN-γ, IL-1 and TNF-α [13]. Another relevant peculiarity is that both ozone and its messengers do not display multi-organs toxicity because they are rapidly quenched by antioxidants present in plasma and lymph.

The aspects of the paradoxical ozone reactivity and mechanism of action have been extensively reviewed [8] and it can be speculated that ozone used in appropriate dosages may act as a therapeutic agent in peritoneal and pleural carcinomatosis.

Materials and Methods

Ozone administration methods

A few technical aspects need to be examined for the ozone administration in the peritoneal-pleural cavities. After the fundamental patient's agreement, one or even better two light and flexible silastic catheters can be implanted into the cavities under strict aseptic technique by an experienced surgeon. Either a silicone or another ozone-resistant catheter can be used, because rubber or other materials commonly used are not ozone-resistant and can disintegrate. To start with, a typical catheter used for chronic peritoneal dialysis (Braun, Milan) has been used but subsequently a lighter catheter (Vygon, Italy, code 296.10) has appeared more practical. One of us (J.C.P.O.) has used a nasal pharyngeal catheter apparently ozone-resistant.

Ozone administration schedules

One of us (J.C.P.O.) has treated four patients affected by colon, ovary, and pancreas neoplasms presenting ascite with concomitant parenteral chemotherapy and intraperitoneal ozone, with a protocol that is based on five daily successive sessions. The ozone concentration used is of 50 μg/mL with a gas volume of 2000-2500 mL hence a total ozone dose of 100-125 mg. He has informed us that intraperitoneal (IP) administration of 50 μg/mL of gaseous ozone represents a painful technique and therefore it is performed under general anaesthesia in the operating room under continuous control of the anaesthesiologist. In a few patients he has also carried out two successive cycles. Although patients treated with both chemotherapy and ozone showed a prolonged survival, no conclusion can be drawn by a mixed therapy. Moreover, both the ozone concentration and total dose are extreme and may be toxic. For this reason, it was felt that a different preliminary experience ought

to be investigated. Such an approach is based on the experimental groundwork that, in order to induce an adaptation to the stress of ozone therapy, it is better to "start low", slowly increasing the ozone concentration. Thus, the following schedule has been used: a) for the morning (9-10 am), gaseous oxygen-ozone mixture as reported in Table 1; and b) for the afternoon (5-6 pm) with a sterile lipid emulsion in ozonated saline [14-16]. As excessive IP ozone doses elicit undesirable pain, it is preferred not to exceed an ozone concentration of 20 μg/mL and in case of the lipid emulsion in ozonated saline the maximum ozone concentration is of 10 μg/mL.

All the patients gave a full informed consent for the therapy.

Administration modalities of ozone at low concentrations

a) The gas volume (about 98% O_2 and 2% O_3) is delivered as ozone is generated by corona discharge and the concentration is accurately measured in real time by assessing the absorption at 253.7 nm within the Hartley band. Medical oxygen is used and the gas mixture is filtered through a 0.22 μm membrane to prevent infection. Also the volume is precisely measured via a 50 mL syringe operated at an interval of two minutes. The initial volume of 500 mL can be progressively increased up to 2 litres. There is no need to evacuate the exhausted gas because oxygen is slowly absorbed with the advantage that the hyper-oxygenation of the peritoneal microenvironment inhibits the proliferation of neoplastic cells, which thrive only in hypoxia.

The initial concentration is of 5 μg/mL and it is progressively increased of 2.5 μg/mL up to a maximum of 20 μg/mL at the seventh session. A total dose of 40 mg administered in one hour is easily acceptable because ozone instantly reacts with antioxidants and biomolecules present in the peritoneal fluid and therefore its actual concentration remains very low.

b) Ozonation of physiological saline is performed by bubbling O_2-O_3 at the desired O_3 concentration (10 μg/mL) for 20 min, possibly keeping the saline at 42 °C and infusing it at the same temperature together with the extemporaneously mixed lipid emulsion. The mixing allows the formation of H_2O_2 (8-10 μmol), a small amount of dissolved ozone and the end product of the peroxidate lipids that will decompose into hydroperoxide and alkenals toxic for neoplastic cells.

Keeping in mind that the peritoneal surface is about 1.7 m^2, the maximum volume of either gas or ozonated saline can be of 2500 mL, aiming to cover both the pelvic and diaphragmatic surfaces. The infusion of ozonated saline at 42 °C is slowly performed *via* one or two catheters in about 60 min while the patient lies on her/his back. Although ozone is a potent disinfectant, the outmost care must be exercised to prevent bacterial contamination of the catheter. Almost needless to say that the primary tumour or macroscopic neoplastic agglomerates had to be surgically removed for improving the ozone treatment. In order to maintain the potency of the antioxidant system of biological fluids in critical patients, the RDA oral administration of both a multivitamin complex and two doses of N-acetylcysteine (1200 mg daily) is suggested. The slow infusion of either the gas mixture or the ozonated saline does not increase the abdominal pain caused by the carcinomatosis.

Results and Discussion

Five cancer patients have been intraperitoneally treated with ozone. Four patients have been classified at IV-V in a ranking of I-V, and they have been already treated with intensive intravenous chemo-

Table 1 The proposed daily schedule for the intraperitoneal (IP) ozone administration

Day	*O_3 μg/mL*	*O_2-O_3 mixture volume mL*	*O_3 total dose mg*
1	5	2000	10
2	7.5	2000	15
3	10	2000	20
4	12.5	2000	25
5	15	2000	30
6	17.5	2000	35
7	20	2000	40
8-30	20	2000	40

therapy. All patients presented malignant ascite due to colon (n=2), ovarian (n=1) and pancreatic cancer (n=1), classified at IV stage. Moreover, the single patient presented both IP and intrapleural carcinomatosis due to an ovarian tumor. Although the single case is clinically irrelevant, it has been also critical for improving technical details. As a consequence of intraperitoneal ozone application, our patient and the four patients subject of the present preliminary investigation improved, as evidenced by the marked decrease of the tumour markers. Apart from such results, no conclusions can be drawn at the moment. As a matter of fact, while ozone useful actions as blocking some UV rays in the stratosphere or displaying bactericidal activity in contaminated water are well known, its proficiency in vascular diseases and in orthopaedics remains almost unknown in spite of well-documented results [17,18]. On the contrary, the deleterious effects of ozone in the pulmonary systems after chronic inhalation are known by everyone but, as it has been clarified [19], this is due to the monthly cumulative ozone dose active on the alveolar lining volume (only about 30 mL) which contains only a minimal antioxidant capacity. In contrast, the plasma possesses a great antioxidant reservoir. That is the reason explaining why therapeutic ozone concentrations can be safely used in Medicine. While judicious blood ozonation does not yield side effects, it procures [8]:

a) blood circulation and oxygen delivery improvements to ischemic tissue;

b) metabolic enhancements by improving oxygen delivery;

c) cellular antioxidant enzymes upregulation, and HO-1 and HSP-70 induction via the activation of Nrf2 bound to the antioxidant/electrophile response element (ARE/EpRE) in the cell nucleus;

d) a more or less direct toxic effect on cancer cells;

e) mild activation of the immune system and enhanced release of growth factors from platelets;

f) a beneficial improvements of coenesthesis in most of the patients, probably by stimulating the neuro-endocrine system.

As previously mentioned, during the common blood ozonation within the therapeutic range (0.42÷1.68 mM) with the successive infusion into the donor's patient, if in most cases improves the quality of life, it does not block metastatic progression. The same results had been obtained in HIV-AIDS patients [20]. In contrast with other claims, our negative results are now well-understood because ozonation of blood *ex vivo* fully exhaust ozone which cannot become in contact with both tumour cells or even free pathogens in the circulation [21] because protected by the plasma antioxidants. Indeed ozone reacting with blood biomolecules has a lifetime of seconds inducing the release of ROS and LOP messengers, which are the true therapeutic effectors. On the other hand, in the case of a direct ozone administration into the peritoneal or pleural cavities, ozone and mostly its messengers readily can interact with neoplastic cells and resident leukocytes. In such a case it is reasonable to postulate biological effects such as the death of neoplastic cells due to H_2O_2 and aldehydic compounds or *via* the activation of neutrophils and macrophages. Obviously, ozone and its derivatives need to be almost constantly present justifying the daily double administration for at least a month. The evaluation of a number of tumoral markers will permit to decide whether the therapy must be continued or not. Such a long period of hospitalization is comparable with that of peritonectomy and the successive HIPEC. There are a few similarities between the photodynamic therapy reviewed by Barni et al. and the ozone therapy approach but it is felt that the latter can be precisely quantified and may be more effective.

A previous result achieved in normal rabbits implanted with VX2 carcinoma HNSCC tumor cells, treated for 5 consecutive days with IP injection of 160-240 mL ozone (equivalent to 8-12.0 mg) showed that 7 out of 14 were cured [22]. In this study the experimental tumor had been implanted only two weeks before the ozone treatment and therefore the situation does not compare with the slow progression of human tumours. It was expected that ozone injected into the peritoneal cavity may have activated resident macrophages and neutrophils which may have switched on an immune-mediated reaction with prompt tumour rejection. In the control group (medical O_2 only) also 3 rabbits (out of 13) were also cured, suggesting the need to be cautious in interpreting an exceptional results similar to many others obtained in mice with IL-2 or endostatin later not confirmed in humans [23]. In contrast with the experimental tumors in rabbits, human neoplasms, when discovered, have already had time to paralyze the immune system by releasing immune suppressive compounds. On this basis, above all lack of toxicity, it appears useful to evaluate the ozone protocol in IP and intrapleural carcinomatosis, even if today there is a lack of validated clinical efficacy.

In conclusion, while the proposed IP ozone administration appears technically feasible, it is clear that only a controlled randomized clinical trials in human peritoneal carcinomatosis can clarify this matter. It is hoped that this paper may interest a group of oncologists, who can have our enthusiastic help in terms of technical knowledge of ozone concentrations and dosages.

References

1 Sugarbaker PH, Schellinx ME, Chang D, et al. Peritoneal carcinomatosis from adenocarcinoma of the colon. World J Surg. 1996; 20: 585-591.
2 Chua TC, Robertson G, Liauw W, et al. Intraoperative hyperthermic intraperitoneal chemotherapy after cytoreductive surgery in ovarian cancer peritoneal carcinomatosis: systematic review of current results. J Cancer Res Clin Oncol. 2009; 135: 1637-1645.
3 Abu-Hijleh MF, Habbal OA, Moqattash ST. The role of the diaphragm in lymphatic absorption from the peritoneal cavity. J Anat. 1995; 186: 453-467.
4 Sugarbaker PH. Peritonectomy procedures. Ann Surg. 1995; 221: 29-42.
5 Spratt JS, Adcock RA, Muskovin M, et al. Clinical delivery system for intraperitoneal hyperthermic chemotherapy. Cancer Res. 1980; 40: 256-260.
6 Barni S, Cabiddu M, Ghilardi M, et al. A novel perspective for an orphan problem: Old and new drugs for the medical management of malignant ascites. Crit Rev Oncol Hematol. 2011; 79: 144-153.
7 Cavaliere F, De Simone M, Virzì S, et al. Prognostic factors and oncologic outcome in 146 patients with colorectal peritoneal carcinomatosis treated with cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy: Italian multicenter study S.I.T.I.L.O. Eur J Surg Oncol. 2011; 37: 148-154.
8 Bocci V, Borrelli E, Travagli V, et al. The ozone paradox: ozone is a strong oxidant as well as a medical drug. Med Res Rev. 2009; 29: 646-82.
9 Bocci V. Ozone. A new medical drug. Dordrecht, The Netherlands: Springer, 2011.
10 Sweet F, Kao MS, Lee S. Ozone selectively inhibits growth of human cancer cells. Science. 1980; 209: 931-933.
11 Pryor WA. How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? Free Radic Biol Med. 1992; 12: 83-88.
12 Poli G, Schaur RJ, Siems WG, et al. 4-hydroxynonenal: a membrane lipid oxidation product of medicinal interest. Med Res Rev. 2008; 28: 569-631.
13 Bocci V, Luzzi E, Corradeschi F, et al. Studies on the biological effects of ozone: 3. An attempt to define conditions for optimal induction of cytokines. Lymphokine Cytokine Res. 1993; 12: 121-126.
14 Klein MD, Coran AG, Drongowski RA et al. The quantitative transperitoneal absorption of a fat emulsion: implications for intraperitoneal nutrition. J Pediatr Surg 1983; 18: 724-731.
15 Mahedero G, Morán JM, Salas J, et al. Absorption of Intralipid and interferences from nutrients infused into the peritoneal cavity of the rat. Am J Surg. 1992; 164: 45-50.
16 Driscoll DF. Lipid injectable emulsions: Pharmacopeial and safety issues. Pharm Res. 2006; 23: 1959-1969.
17 Di Paolo N, Bocci V, Salvo DP, et al. Extracorporeal blood oxygenation and ozonation (EBOO): a controlled trial in patients with peripheral artery disease. Int J Artif Organs. 2005; 28: 1039-1050.
18 Andreula CF, Simonetti L, De Santis F, et al. Minimally invasive oxygen-ozone therapy for lumbar disk herniation. Am J Neuroradiol. 2003; 24: 996-1000.
19 Bocci V. Is it true that ozone is always toxic? The end of a dogma. Toxicol Appl Pharmacol. 2006; 216: 493-504.
20 Bocci V, Venturi G, Catucci M, et al. Lack of efficacy of ozone therapy in HIV infection. Clin Microbiol Infect. 1998; 4: 667-669.
21 Burgassi S, Zanardi I, Travagli V, et al. How much ozone bactericidal activity is compromised by plasma components? J Appl Microbiol. 2009; 106: 1715-1721.
22 Schulz S, Häussler U, Mandic R, et al. Treatment with ozone/oxygen-pneumoperitoneum results in complete remission of rabbit squamous cell carcinomas. Int J Cancer. 2008; 122: 2360-2367.
23 Bocci V. Does ozone really "cure" cancer? Int J Cancer. 2008; 123: 1222.

Prof. Velio Bocci
Department of Physiology, University of Siena
Via A. Moro 2
53100 Siena, Italy
Tel.: +39 0577 234226
Fax +39 0577 234219
E-mail: bocci@unisi.it

Prof. Valter Travagli
Department of Pharmaceutical Chemistry and Technology
Via A. Moro 2
53100 Siena, Italy
Tel.: +39 0577 234317, Fax +39 0577 234333
E-mail: travagli@unisi.it

JORNADA EUROPEA DE OXIGENO-OZONOTERAPIA

(Taller teórico-práctico.Policlinic San Joan D´Espí.Barcelona)

1 DICIEMBRE 2012

ORGANIZA:

Asociación Científica Española de Aplicación de OXÍGENO-OZONO TERAPIA
ACEOOT

SECRETARÍA TÉCNICA E INFORMACIÓN

Asociación Científica Española de aplicación de Oxígeno-Ozono Terapia (ACEOOT).
Dirección Postal: C/ Pasión, 13-3º. 47001 Valladolid
Teléfono: 685 534 510
Fax: 983 350 254
Email: secretaria.ozono@hotmail.es
Web: http://www.aceoot.org

COLABORA:

SOLICITADOS CRÉDITOS DE FORMACIÓN MEDICA CONTINUADA AL CONSEJO GENERAL DE COLEGIOS OFICIALES DE ESPAÑA

Editorial

Journal of Experimental and Integrative Medicine 2012; 2(3):189-191

Are we ready for a medical ozone challenge?

Bernardino Clavo[1], Norberto Santana-Rodriguez[2]

[1]Radiation Oncology, and [2]Experimental Surgery,
Research Unit of the Dr. Negrin University Hospital, Las Palmas, Spain.

Ozone therapy is gaining credence and is being used by an increasing number of clinicians to treat ever more diverse types of patients. Previous reviews have highlighted potential mechanisms-of-action arising out of clinical experience as well as theoretical bases of this therapy in various diseases [1]. However, ozone therapy is still viewed with skepticism in mainstream medicine, especially since there has been scant evidence from stringent clinical trials.

In this issue of the Journal of Experimental and Integrative Medicine, Re *et al* [2] review the basic mechanisms underlying ozone therapy, and the barriers that need to be overcome if this therapy is to establish its place within orthodox medicine. Re *et al* describe why ozone cannot be considered a drug, *i.e.* it doesn't act on specific receptors; it doesn't follow pharmaco-dynamic or pharmaco-kinetic models; and optimum schedules/doses for administration cannot be established based on any calculated "half-life". Additionally, ozone has many non-linear effects, as described by the authors according to the concept of "hormesis" (*i.e.* a cellular adaptive response to specific stimuli). So, it is crucial to achieve an appropriate ozone-concentration sufficient to induce a calculated oxidative stress while avoiding toxic levels (which are different for different tissues)[3]. Ozone "only acts" as a modulator or pro-drug and, by inducing secondary messengers, will enhance the subsequent adaptive responses. These actions are better achieved by the recent theoretical concepts regarding administration "start at low ozone concentration and increase slowly". According to this concept, the best final concentration and dose will depend on different diseases, on the route of administration, and the patient's characteristics (such as basal pro/anti-oxidant status)[1]. The advantage is that ozone can be used in a "personalized medicine" approach. However, this can also be a considerable limitation in the progress of ozone therapy since, with this personalized approach, it is more difficult to establish standard protocols and to evaluate outcomes such as in a clinical trial. The existing clinical experiences with ozone therapy have been obtained using different protocols, different ozone concentrations and gas-volumes, as well as range of administration schedules. All these preclude a systematic evaluation and comparison of studies to help clinical decision making. However, major efforts have been made over recent years to merge ozone therapy procedures. The most relevant are:

(1) The "Madrid Declaration on Ozone therapy" in June 2010 [4]. This represents the first consensus document on ozone therapy. It was developed by the relevant specialists, initially from 11 countries (with subsequent addition of more countries). The consensus document, which will be updated regularly, was translated into 10 different languages and supported by 24 international Ozonetherapy Associations.

(2) The foundation of the International Scientific Committee of Ozonetherapy in October 2010. Presided over by the eminent Prof. Velio Bocci, this is an independent International Scientific Board whose objectives include standardization of clinical practice in the use of ozone, together with harmonization and unification of criteria among different scientific societies [5].

The advent of this preliminary consensus, and the setting-up of the International Scientific Board, will take ozone therapy to the next level in clinical practice, with more homogeneous and rigorous criteria.

The review by Re *et al* [2] also describes a major handicap in conducting clinical trials with ozone therapy, *i.e.* the lack of interest by the pharmaceutical industry. Ozone is inexpensive and, unlike conventional medications, it is not patentable and it cannot be conveniently packaged and marketed. Ozone needs to be generated and administered *in situ* [3]. We need to remind ourselves that, nowadays, most biomedical research depends on funding from the pharmaceutical industry. The administrative procedures to initiate a clinical trial or for obtaining approval from the national regulatory agencies are complex and

expensive. Occasionally, trials supported and conducted by the pharmaceutical industry have a risk of methodological bias. Nevertheless, "rules are rules" and these regulatory procedures were created to protect patients from unethical research practices, whether from industry or from individual researchers. Currently it is increasingly difficult to conduct research without the support of pharmaceutical companies. This places constraints on funding for research units, or even contracting of external specialized companies, who accept the responsibility for the conduct and budgeting for the numerous administrative procedures necessary in setting-up and performing a clinical trial.

Currently, few clinical trials with ozone therapy have been registered. For example, in the database of the U.S. National Institutes of Health in May 2012 they constitute only 6 clinical trials [6]. This situation is difficult to solve, even more so if drug companies see ozone therapy as the "enemy".

However, all is not lost. While awaiting more specific clinical evidence, it would be better to reinforce the concept that ozone could be useful as an adjuvant treatment in several diseases, in close collaboration with "official" medicine. The main role of ozone therapy is not to replace pharmacotherapies but to improve the clinical results that they can offer in several diseases. In the United States, over one hundred thousand people die each year from adverse effects of medications that are unrelated to clinical error [7]. We should highlight the potential role of ozone to prevent, decrease or ameliorate toxicity induced by several drugs such as cisplatin [8], methotrexate [9] or procedures such as surgery or radiation therapy [10, 11]. These collaborative efforts should be of interest to the pharmaceutical industry rather than being seen as a threat, *i.e.* the consequence of these efforts could improve the outcomes of standard drug therapy and even increase indications for several drugs or medical interventions. For example, many articles have described the effect of ozone preconditioning in the protection against damage mediated by free radicals or ischemia-reperfusion; these effects would be very relevant for organ transplantation. A final field to explore could be the use of ozone therapy in the management of symptoms, with a focus on quality-of-life improvement [10, 11].

Needless to say, options for ozone therapy will depend on evidence from well-addressed clinical trials. If ozone is presented as a potential partner (rather than a threat) would ozone therapy receive support from the pharmaceutical industry, at least in setting-up clinical trials teams? Perhaps not; and, as such, other ways to continue ozone-research need to be explored. Following the steps taken in other fields of "official" medicine, perhaps scientific associations that support ozone therapy and/or collaboration among the ozone-device manufacturers (which are usually small start-up companies with very limited budgets) should play more important roles in supporting short, relatively inexpensive, well-designed, clinical trials.

We agree with Re *et al* [3] in that the medical use of ozone is now ready for a rigorous scientific evaluation. But are we, the practitioners, sufficiently geared-up to meet the challenge?

Acknowledgements

National funding via the Instituto de Salud Carlos III was made available to BC (grant #EC 07/90024) and NS (grant #PI 10/01485) to support the therapeutic use of ozone. Editorial assistance was by Dr. Peter R Turner of tscimed.com.

References

1. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. Med Res Rev 2009; 29:646-82.
2. Re L, Malcangi G, Martinez-Sanchez G. Medical ozone is now ready for a scientific challenge: current status and future perspectives. J Exp Integr Med 2012; 2:193-6.
3. Bocci VA. Why orthodox medicine has not yet taken advantage of ozone therapy. Arch Med Res. 2008; 39:259-60.
4. Madrid Declaration on Ozone therapy. Available via http://www.aepromo.org/declaracion_madrid/Madrid_declaration.pdf (Accessed 16 June 2012).
5. International Scientific Committee of Ozonetherapy. Available via http://www.isco3.org (Accessed 16 June 2012).
6. U.S. National Institute of Health. Clinical Trials. Available via http://clinicaltrials.gov/ct2/results?term=ozone+therapy (Accessed 16 June 2012).
7. Starfield B. Is US health really the best in the world? JAMA. 26 2000; 284:483-5.
8. Gonzalez R, Borrego A, Zamora Z, Romay C, Hernandez F, Menendez S, Montero T, Rojas E. Reversion by ozone treatment of acute nephrotoxicity induced by cisplatin in rats. Mediators Inflamm 2004; 13:307-12.

DOI:10.5455/jeim.170612.ed.014

Journal of Experimental and Integrative Medicine 2012; 2(3):189-191

9. Kesik V, Uysal B, Kurt B, Kismet E, Koseoglu V. Ozone ameliorates methotrexate-induced intestinal injury in rats. Cancer Biol Ther. 2009; 8:1623-8.

10. Clavo B, Suarez G, Aguilar Y, Gutierrez D, Ponce P, Cubero A, Robaina F, Carreras JL. Syndrome of brain ischemia and hypometabolism treated by ozone therapy. Forsch Komplementmed. 2011; 18:283-7.

11. Clavo B, Santana-Rodriguez N, Lopez-Silva SM, Dominguez E, Mori M, Gutierrez D, Hernandez MA, Robaina F. Persistent PORT-A-CATH(R)-related fistula and fibrosis in a breast cancer patient successfully treated with local ozone application. J Pain Symptom Manage. 2012; 43:e3-6.

Key words:
Adjuvant treatments; Clinical trials; Medical ozone; Ozone therapy; Symptom management

Correspondence:
B. Clavo
Department of Radiation Oncology,
Chronic Pain Unit and Research Unit
Dr. Negrin University Hospital,
C/ Barranco la Ballena s/n,
35010 Las Palmas (Canary Islands), Spain.
bernardinoclavo@gmail.com, bernardinoclavo@terra.es

Received: May 30, 2012
Accepted: June 11, 2012
Published online: June 17, 2012

J Exp Integr Med 2012; 2:189-191
DOI:10.5455/jeim.170612.ed.014

Invited Review

Journal of Experimental and Integrative Medicine 2012; 2(3):193-196

Medical ozone is now ready for a scientific challenge: current status and future perspectives

Lamberto Re[1,2], Giuseppe Malcangi[1], Gregorio Martinez-Sanchez[1]

[1]Medinat srl Clinic, Camerano; [2]Molecular and Clinical Pharmacology, University of Ancona, Ancona; Italy.

Abstract

The aim of the present review is to clarify some of the basic mechanisms underlying ozone therapy. Indeed, after its empiric use started at the beginning of the last century, science is now ready to give a chance to the more and more medical doctors working in this field. Unfortunately, the lack of a full recognition by the health authorities and some ostracism against it is, up to date, the major obstacle for its full medical acceptance. Anyway, in the last years and thanks to the contributions of several scientists, most of the mechanisms characterizing the bio-humoral activity of ozone have been scientifically outlined. The built up of randomized clinical studies is going on slowly despite the lack of funds and the difficulties bound mainly to the huge variability of the ozone action.

The thousand and thousand medical doctors involved in the use of ozone as emerging therapy, must be fully educated about the properties of this gas in the aim to counteract scientifically the criticisms of colleagues devoted to other field of medicine and not expert of the ozone pharmacological properties. Is for this reason that we encourage all the professionals to deeply increase the knowledge related to the scientific data produced and published on the international literatures in the field of the ozone therapy.

For the future we suggest the use of ozone not in alternative but as a complement of the most appropriate pharmacological treatments also in the aim to reduce some side effects derived from a chronic drug use. The lack of a well-defined binding site for the ozone molecule could suggest the introduction of virtual receptors for the supposed biological activity of ozone acting mostly throughout second messengers pathways.

Key words:
Complementary therapy;
Ozone; Ozone therapy

Correspondence:
Lamberto Re;
Vía Fazioli 22,
60021 Camerano, Italy.
lambertore@univpm.it

Received: April 7, 2012
Accepted: May 1, 2012
Published online: June 7, 2012

J Exp Integr Med 2012; 2:193-196
DOI:10.5455/jeim.070612.ir.012

Introduction

When many decades ago we started our scientific work at the Department of Clinical Pharmacology of the Medical Faculty of the Ancona University, we were fascinated in discovering the most intimate mechanisms of the drug action. During these years our prevalent interest was to investigate how cells communicate and how neuronal mediators are released with a highly recognized technique like Patch Clamp [1, 2], The results were encouraging and some pioneering work [3] anticipated important discoveries regarding some new ion channels whose encoding genes were named Slick and Slack [4].

This preface is essential to understand our aim also in different field of medicine like "**ozone therapy**" (OT) that must be, as has been in the past, joined to science following the most rigorous protocol to validate any new proposed therapeutic potential. This was the reason and the condition that we put over any other things when we decided more than 20 years ago to share our science with the OT. Indeed, the jump to this new field of interest occurred in one period were only a few papers dealing on ozone mechanisms appeared on indexed scientific journals.

The most criticism that we received that time was mainly addressed to the fact that this new field, OT, was apparently out of any reasonable interest either of the pharmaceuticals or of the clinicians. Anyway, we followed because we thought, and we already think, that scientific challenge must be devoted to any field of possible interest, without any exclusion that can be eventually stated only after and not before the demonstration of any scientific data supporting it.

One more reason that stimulates us to follow in the field of OT was the lack of any therapeutic potential against some rare illnesses and the possibility that a deep scientific characterization of the ozone potential could be useful, almost in reducing symptoms, in many conditions so far orphan of adequate pharmacological treatment. More, regarding the autonomic nervous system, looking at the complexity of its organization, with

cholinergic receptors represented both at the preganglionic (nicotinic) and postganglionic (muscarinic or adrenergic) sides, appeared not easy a therapeutic intervention following the conventional schemes. In this context, we can imagine OT not against but as a promising allied of the orthodox medicine treatment.

The fact that ozone interaction with the biological environment is not ruled by the classical *Lineweaver-Burk* plot was not a reason so strong to convince us to leave the challenge, as some colleagues dealing in the pharmacological field suggested to us too many times. Indeed, new concepts or models must be outlined to fit the conditioning mechanisms described for the ozone activity. The pharmacological approach to patients is rationally conducted with drugs and surgery methodologies underwent, before marketing, to the most rigorous scientific and clinic characterization. The fact is one of the most relevant criteria for drug registration and diffusion from the health authorities all around the world. Ozone was introduced to the medical attention more than two decades ago, mainly used as a support therapy in the aesthetic and rare illnesses fields. Unfortunately, beside the wide use in many countries, its characterization is still lacking of a deepening clinical evaluation throughout well-defined randomized and blind studies. The problems behind a more large acceptability of the technique are mainly due to the difficult in classifying its supposed pharmacological effects and, because of its brief lifetime, to evaluate some pharmacokinetic parameters. Furthermore, if we consider that it is also difficult to grant researches that can't warrant an adequate marketing interest, we can have an idea of a very complicated pattern reflecting negatively against ozone. One of the first international reports of ozone activity was done at the beginning of the past century [5] and described its action as antiseptic at high doses. Later on the concepts of low doses started to be the more appropriate for such agent that, as known, is a strong oxidant and considered very toxic for living organisms and very dangerous when breathed [6]. The hypotheses that low ozone doses, non-toxic and well tolerated by biological tissues which possess an own antioxidant defences, seems to be now supported by some experimental data. In this context some works of the toxicologist Edward Calabrese concerning hormesis could be indicative of such action [7]. Although not completely accepted, the theory is well known to most of the pharmacologists working with *in vitro* assays. Indeed, it could be easily proven that while higher doses of a drug could activate a receptor, low doses could inhibit it. Looking to the wide basic and clinical studies reported in the next session, it could be not without sense the theory proposed for the ozone mechanism [8, 9].

Current status

By a pure pharmacological point of view ozone can't be considered a drug. However, looking at the multiple second messenger cascades that it activates could be classified as a physio-pharmacological agent. In other words, some physiological mechanisms are involved as an adaptive response from the biological environment but not as a direct consequence of a binding reaction.

This makes more difficult the efforts of scientists in the evaluation of the molecular events underlying its clinical efficacy. The physiological formation of an ozone-like mediator during inflammation is indicative of the striking potential of ozone as a new bio-molecule. The fact imposes hard efforts to clarify the hypothesized mechanism following new strategies with newly constructed randomized-standardized clinical trials. Moreover, the mechanisms of action of ozone on blood bio-molecules with the generation of several messengers responsible of its biological effects have been well clarified since 2002.

Ozone used in appropriate doses is characterized by the formation of reactive oxygen species and lipid peroxides allowing them to become late and long-lasting messengers. The paradoxical concept that ozone eventually induces an antioxidant response capable of reversing a chronic oxidative stress is common in the animal and vegetal kingdom; it is already supported by findings of an increased level of antioxidant enzymes during OT. Those facts can include ozone as a hormetin.

After ozone administration an acute oxidative stress has occurred that leads to a number of phenomena such as up-regulation of antioxidant enzymes. An example of this dose dependent induction was evident in a study in rabbits, even when was imperceptible for the authors the hormetic behavior. A second look to their results shown a significant increase in level of manganese superoxide dismutase (MnSOD) and myeloperoxi-dase after treatment, only in the central doses (Fig.1)[10].

When ozone treatment was combined with different drugs, used as a model of tissue damage, it was evident that optimal doses of ozone could avoid the drug damage. For example, in a rat model

 DOI:10.5455/jeim.070612.ir.012

Journal of Experimental and Integrative Medicine 2012; 2(3):193-196

of nephrotoxicity induced by cisplatin, central doses of ozone reduce the renal damage (serum creatinine decrease), at the same time ameliorating the SOD activities (Fig.2)[11].

Other animal models using combination of drug and ozone treatment showed similar results. For example, ozone reduces methotrexate-induced intestinal injury in rats. The mechanism of protection involves also an overexpression of SOD and glutathione peroxidase in tissue (intestine)[12]. The same mechanism is involved in the protection observed in nephrotoxicity induced by acetaminophen [13].

The integrative effect of OT is also evident in clinical studies. For example, in a multicentre randomized, double-blind controlled trial in patients with acute low back pain due to lumbar disc herniation was found that paravertebral injections of ozone seems to safely and effectively relieve pain, as well as reduce both disability and the intake of analgesic drugs [14]. In addition, two recent meta-analysis studies showed that ozone treatment of herniated discs is an effective and extremely safe procedure. Pain and function outcomes are similar to the outcomes for lumbar discs treated with surgical discectomy, but the complication rate is much lower (< 0.1%) and the recovery time is significantly shorter [15, 16]. Ozone treatment also reduces the microorganism resistance and consequently makes more efficient the antibiotic therapy [17, 18]. During treatment of diseases that involve a chronic disruption of redox status (oxidative stress), up-regulation of antioxidant enzymes is far more beneficial than oral antioxidant supplementation. However, the key to reach the optimal effect of ozone depend on the doses.

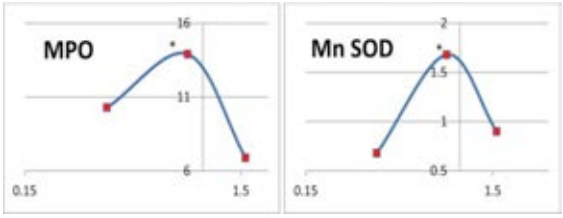


Figure 1. Hormetic shape of antioxidant enzymatic activities after 90 days of ozone treatment in rabbits. Dose log (0.36, 0.85 and 1.57 mg/kg) *vs* enzymatic activities. Animals were treated 3 times a weeks.
MPO; myeloperoxidase was assayed in lung tissues and expressed in U per g of tissues. **MnSOD**; manganese superoxide dismutase, was assayed in liver homogenates and expressed as U per mg of protein. *significant differences ($p < 0.05$) compared to control group (animals treated with O_2) and low and high O_3 doses. Data were represented using values took from Martinez *et al* [10] Doses were calculated from total dose to mg/kg taking 1.65 kg as median body weight of animals.

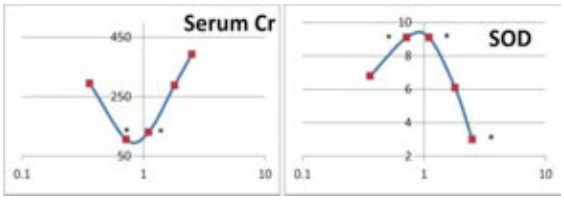


Figure 2. Hormetic shape of serum creatinine (**Cr**) and superoxide dismutase (**SOD**) after 15 days of ozone treatment and cisplatin-induced nephrotoxicity in rats. Ozone was administered by rectal way 15 days after cisplatin-induced nephotoxicity. Dose log (0.36, 0.72, 1.1, 1.8 and 2.5 mg/kg) *vs* Cr or enzymatic activity.
Cr was expressed in μM. SOD was assayed in kidney homogenates and expressed as U per mg of protein. *significant differences ($p < 0.05$) compared to control group (animals treated with O_2). Data were represented using values taken from Borrego *et al* [11].

Up to now, ozone doses are empirically established based on the clinical experience of the different school of OT. In general doses are ranged in 3 intervals: low, medium and high and it is recommended to begin from low doses to high doses depending by the clinical evolution of the patients. In the future, this therapy will be close connected with a diagnostic of the redox status of the patients. In addition, the mathematical tool based on the hormetic response of ozone to calculate the doses should be developed in order to optimize the therapeutic response. As stated above, we must always take into account that the pharmacological models used to define the mechanism of action of drug are usually based upon the *Michaelis–Menten* kinetics. We know that either the metabotropic or the voltage operated receptors are characterized by an extracellular binding site for the drug interaction and an intracellular transduction mechanism [19].

Usually, *in vitro* assays are very useful to determine with the above model, or with the linearization of the *Lineweaver-Burk* plot, the pharmacodinamic behaviour of drugs and make easier the evaluation of the affinity constant for each ligand-receptor interaction. In the case of ozone or other substances orphan of a stereo chemical ligand site we can postulate the presence of a "**virtual receptor**" characterized by some cellular components like lipids or other membrane substrates. Many scientific data are consistent with a first interaction between ozone molecule and double-binds of membrane fatty acids and the subsequent formations of second messengers like hydroperoxides or lipoperoxides [20]. These products could in turn activate a cascade of molecular events responsible of the modulating

effects induced by the ozone molecule at the intracellular environment and mainly in the mitochondria. By a certain point of view we can state that ozone do not fit any pharmaco-dynamics or pharmaco-kinetics model but simply acts as a pharmaco-modulator due to an adaptive response following its oxidative reactions. These effects must be clearly stated also in the case of the positive effects of ozone in the disk herniation avoiding referring to terms like lysis or disk disruption that, even occurring, do not represent the main mechanism responsible of the pain relief and could induce some confusion to the patients as well.

Conclusion

In conclusion, the hormesis model could fit well the wide reports describing some biochemical activities induced by ozone. Ozone could not be used as a regular drug, of which appear to be very far, but as a conditioning agent that could be helpful in modulating some key functions and finally ameliorate the physical status of the patients. In this context, the term ***ozohormesis*** *or* ***ozormesis*** could better explain some positive action of the gas simply as complement or as integrative support to the inalienable orthodox treatment.

Competing interest statement

Authors declare that no support from any organisation for the submitted work was received. Authors have no financial relationships with any organisations that might have an interest in the submitted work in the previous three years and no other relationships or activities that could appear to have influenced the submitted work.

References

1. Penner R, Neher E. The patch-clamp technique in the study of secretion. Trends Neurosci 1989; 12:159-63.
2. Re L, Cola V, Fulgenzi G, Marinelli F, Concettoni C, Rossini L. Postsynaptic effects of methoctramine at the mouse neuromuscular junction. Neuroscience 1993; 57:451-7.
3. Re L, Moretti V, Rossini L, Giusti P. Sodium-activated potassium current in mouse diaphragm. FEBS Lett 1990; 270:195-7.
4. Bhattacharjee A, Kaczmarek LK. For K^+ channels, Na^+ is the new Ca^{2+}. Trends Neurosci 2005; 28:422-8.
5. Bocci V. Ozone A New Medical Drug, Springer, Dordrecht, The Nederlands, p 295, 2005.
6. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. Med Res Rev 2009; 29:646-82.
7. Calabrese EJ. Hormesis and medicine. Br J Clin Pharmacol 2008; 66:594-617.
8. Martinez-Sanchez G, Perez-Davison G, Re L, Giuliani A. Ozone as U-shaped dose responses molecules (hormetins). Dose Response 2011; 9:32-49.
9. Bocci VA, Zanardi I, Travagli V. Ozone acting on human blood yields a hormetic dose-response relationship. J Transl Med 2011; 9:66.
10. Martinez Y, Guanche D, Zamora Z, Hernandez F, Alonso Y, Gonzalez Torres Y. Efecto de la mezcla ozono/oxígeno en el balance redox tisular en un estudio de toxicidad subcrónica en conejo. Retel 2009; 20:15-22.
11. Borrego A, Zamora ZB, Gonzalez R, Romay C, Menendez S, Hernandez F, Montero T, Rojas E. Protection by ozone preconditioning is mediated by the antioxidant system in cisplatin-induced nephrotoxicity in rats. Mediators Inflamm 2004; 13:13-9.
12. Kesik V, Uysal B, Kurt B, Kismet E, Koseoglu V. Ozone ameliorates methotrexate-induced intestinal injury in rats. Cancer Biol Ther 2009; 8:1623-8.
13. Demirbag S, Uysal B, Guven A, Cayci T, Ozler M, Ozcan A, Kaldirim U, Surer I, Korkmaz A. Effects of medical ozone therapy on acetaminophen-induced nephrotoxicity in rats. Ren Fail 2010; 32:493-7.
14. Paoloni M, Di Sante L, Cacchio A, Apuzzo D, Marotta S, Razzano M, Franzini M, Santilli V. Intramuscular oxygen-ozone therapy in the treatment of acute back pain with lumbar disc herniation: a multicenter, randomized, double-blind, clinical trial of active and simulated lumbar paravertebral injection. Spine (Phila Pa 1976) 2009; 34:1337-44.
15. Steppan J, Meaders T, Muto M, Murphy KJ. A metaanalysis of the effectiveness and safety of ozone treatments for herniated lumbar discs. J Vasc Interv Radiol 2010; 21:534-48.
16. Magalhaes FN, Dotta L, Sasse A, Teixera MJ, Fonoff ET. Ozone therapy as a treatment for low back pain secondary to herniated disc: a systematic review and meta-analysis of randomized controlled trials. Pain Physician,2012; 15:E115-29.
17. Belianin II. Decreased resistance of multiresistant mycobacteria to isoniazid during the treatment of experimental tuberculosis with ozone and isoniazid. Zh Mikrobiol Epidemiol Immunobiol 2004; 3:95-8.
18. Belianin II, Martynova LP, Shmelev EI. Dissolved ozone treatment-induced change in the resistance of multi-resistant mycobacterial strain to isoniazid and rifampicin. Probl Tuberk 2002; 1:46-9.
19. McFadzean I, Gibson A. The developing relationship between receptor-operated and store-operated calcium channels in smooth muscle. Br J Pharmacol 2002; 135:1-13.
20. Bocci V, Valacchi G, Rossi R, Giustarini D, Paccagnini E, Pucci AM, Di Simplicio P. Studies on the biological effects of ozone: 9. Effects of ozone on human platelets. Platelets 1999; 10:110-6.

DOI:10.5455/jeim.070612.ir.012

Effectiveness of Hyaluronic Acid and O_2-O_3 Injections in Lateral Ankle Sprains without Tear of Anterior Talofibular Ligament

M. MORETTI

Poliambulatorio Oberdan; Brescia, Italy

Key words: hyaluronic acid, oxigen-ozone, ankle sprains, tear, talofibular ligament

SUMMARY - *The Ankle Strain (AS) is one of the most common injuries in sport competitions. Hyaluronic Acid (HY) is a well know remedy for AS and also the O_2-O_3 injections (O_2-O_3 I) can be a valid aid in our experience. We selected 20 athletes who have had an episode of AS without tear of anterior talofibular ligament to determinate the effectiveness of the HY and O_2-O_3 I injections to treat this common injury.*

The lateral Ankle Sprain (AS) is a common injury that occurs in sports like soccer, basketball, tennis and volleyball. The recovery time to return to the competitions is often more than one month and this is a problem for the athletes that need to return to compete as soon as possible [1]. The AS are usually treated with not always effective remedies like rest, ice, N. S. A. I. D. and physical therapy [2, 3-4]. The problem derived from AS is often the reactive peroneal tendons tenosynovitis (PT) as show in Figure 1. AS often cause re-sprain during the first three months because the recovery is often incomplete [4]. In high grade AS the pain is given by the edema of the soft tissues and often by the edema of the bone spongy and in these cases the time recovery is long. But we must consider that the professional athletes need to quickly return to the competitions and so the sport physicians are often forced to send the players on the court too early. In our experience and in scientific literature Hyaluronic Acid (HY) is a safe and effective remedy for AS [5,6,7]. We haven't found any article about the use of Oxygen and Ozone injections (O_2O_3I) in AS but in our experience has resulted a good, safe and quick remedy.

Aim of the study

Our aim is to evaluate if O_2O_3I and HA can be a good remedy for AS without tear of anterior talofibular ligament (LT) but with PT in only 14 days and if a so quick return to the competion can be followed by a re-sprain.

Materials and Methods

20 athletes were selected to evaluate if HA and O_2O_3I can be a safe, quick and effective method to treat the AS. All the athletes were underwent to ultrasound exam to evaluate the grade of AS. Only athletes without tear of LT were selected for our study. All the ecographic exams had to show the presence of PT as shown in the Figure 1. The athletes selected were all males. The AS was treated within three days after the injury. All the athletes declared the intensity of their pain with VAS scale. The treatment consisted in three injection in 14 days with HA with low molecular weight and O_2O_3I (5 ml with 16 mg/ml). Every injection was practiced at a distance of 3-4 days apart. The pain

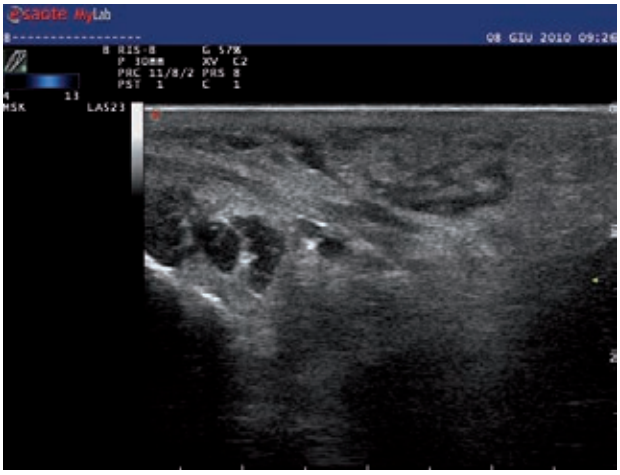
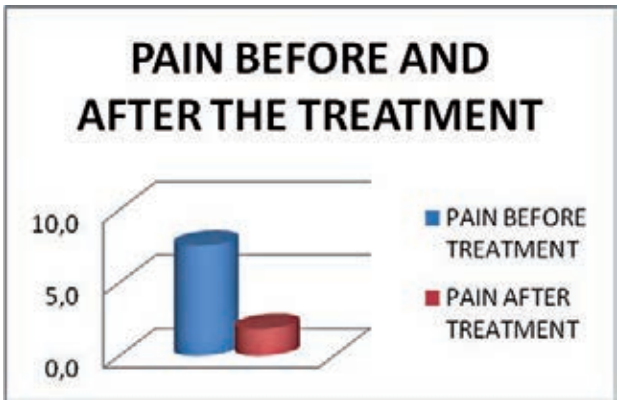
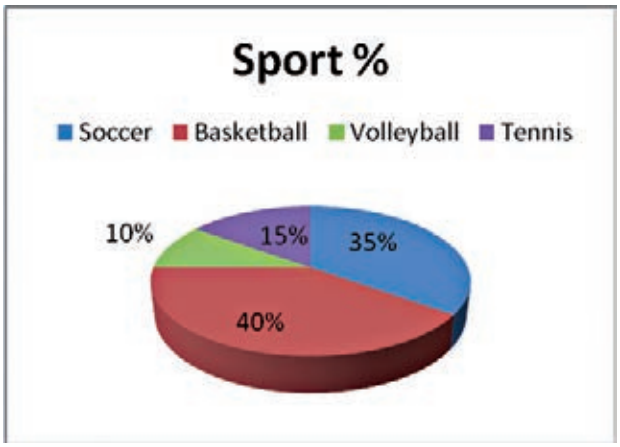


Figure 1 Ecographic image of tenosynivitis of peroneal tendons with edema of soft tissues.



Graph 1 Pain before and after the treatment.



Graph 2 Percentage of sports played by the sample.

Table 1 Result of the study. Here, the values of the pain before the treatment are shown.

Athlete	*Age*	*Pain Before Treatment*	*Pain After Treatment*
1	19	8	2
2	21	8	1
3	20	8	2
4	22	7	3
5	24	9	2
6	27	7	1
7	26	8	3
8	24	8	2
9	20	6	1
10	32	7	4
11	29	8	2
12	31	6	1
13	30	8	1
14	34	7	2
15	27	7	2
16	21	7	2
17	23	8	0
18	22	9	2
19	31	7	1
20	39	8	3
Mean	26,1	7,6	1,9
SD	5,5	0,8	0,9

was evaluated two days after the third treatment. it was examined whether there have been re-sprain in the athletes at a distance of three months.

Results

The mean age of the sample selected was 26,1±5,5. The mean value of the pain before the treatment was 7,6±0,8. The mean pain after the treatment was 1,9±0,9. The results are shown in Table 1 and in Graph 1. The percentage of sports played by the sample examined are shown in Graph 2. Basketball and Soccer were the more played sports.

Only three of the athletes of the sample had a case of re-sprain, that is 15%.

Conclusions

This quick protocol with HY and O_2O_3I injections can be a safe and effective remedy for AS without tear of LT with low percentage of resprain. An experiment on a larger sample is however necessary and it will be interesting in future also evaluate the effectiveness in AS with injury to the LT not only about the recovery time but also in the detection of resprain of the ankle.

References

1 Petrella MJ, Cogliano A, Petrella RJ. Original research: long-term efficacy and safety of periarticular hyaluronic acid in acute ankle sprain. Phys Sportsmed. 2009; 37 (1): 64-70.
2 Swain M, Henschke N. Therapeutic ultrasound is not clinically beneficial for acute ankle sprains. Br J Sports Med. 2012; 46 (4): 241-2. Epub 2012 Jan 12.
3 Dhillon S. Tramadol/paracetamol fixed-dose combination: a review of its use in the management of moderate to severe pain. Clin Drug Investig. 2010; 30 (10): 711-38.
4 van Middelkoop M, van Rijn RM, Verhaar JA, et al. Resprains during the first 3 months after initial ankle sprain are related to incomplete recovery: an observational study. J Physiother. 2012; 58 (3): 181-8.
5 Tiemstra JD. Update on acute ankle sprains. Am Fam Physician. 2012 Jun 15; 85 (12): 1170-6.
6 Petrella MJ, Cogliano A, Petrella RJ. Original research: long-term efficacy and safety of periarticular hyaluronic acid in acute anklesprain. Phys Sportsmed. 2009; 37 (1): 64-70.
7 Petrella RJ, Petrella MJ, Cogliano A. Periarticular hyaluronic acid in acute ankle sprain. Clin J Sport Med. 2007; 17 (4): 251-7.
8 Kemler E, van de Port I, Backx F, et al. A systematic review on the treatment of acute ankle sprain: brace versus other functional treatment types. Sports Med. 2011; 41 (3): 185-97. Review.

M. Moretti, MD
Poliambulatorio Oberdan
Via Oberdan, 140
25128 Brescia, Italy
Tel.: +39 030 9701312
E-mail: dr.marcomoretti@libero.it

Oxygen-Ozone Therapy in Cervical Disc Herniation
A Case Report

M. BONETTI

Servizio di Neuroradiologia, Istituto Clinico Città di Brescia; Brescia, Italy

Key words: intraforaminal infiltration, oxygen-ozone, medical ozone, herniated disc, cervical pain

SUMMARY - *This paper assesses the therapeutic efficacy of CT-guided intraforaminal injection of an oxygen-ozone mixture in patients with cervical disc herniation C6-C7*[1-21].

Introduction

The cervical disc herniation is less common than lumbar disc herniation at a ratio of 1 to 5 and occurs predominantly in relation to the mechanical and biochemical changes that accompany aging, but ultimately this disease is becoming increasingly common among young people especially related to trauma in flexion-extension (whiplash injury).

Discs that are usually to be affected are C5-C6 and C6-C7.

In this paper we report the case of a patient with great herniated cervical disc C6-C7 completely resolved after oxygen-ozone intraforaminal TC guided. Because clinical examination RM comforted control to a month after treatment.

Materials and Methods

MM, male, 25-years-old bearer of great paramedian preforaminal right cervical hernia C6-C7 (Figure 1ABC) is treated with oxygen-ozone performed under CT guidance, at the Specialist Clinic Oberdan using a Computed Tomography (CT) Esaote (Soon Hitachi - 4 slice).

For the production of mixture O_2-O_3 we used an apparatus Alnitec Futura 2 with photometric detector of the concentration of ozone.

The infiltration was performed with 22G needles Terumo cord from (color code black) of a length of 9 cm.

We have infiltrated the patient using a mixture O_2-O_3 at a concentration of 20 ug/ml, injecting at level intraforaminal-periganglionic 2 cc. of gas mixture.

The treatment was conducted by carrying out an initial assessment of the TC district cervical confirming what has been shown on MRI, namely: great preforaminal-paramedian right disc herniation C6-C7, was carried out by measuring TC to set the entry point on the skin with lateral approach, posterior to the neurovascular bundle nerve in the neck, and then we measured the distance from the point of entry to the skin foramen right.

After skin disinfection and local anesthesia with ethyl chloride spray we positioned the spinal needle. The descent of the needle towards the foramen was monitored with CT scans in a thin layer. Once you have verified the correct needle placement was done by injecting 2 cc of gas mixture to 20 g/ml. At this point has been removed and the spinal needle through a further CT scan has occurred the correct distribution of the gaseous mixture at the level of the foramen of the root ganglion and C7 right.

The patient was held at our center for about two hours after treatment, at the end of the observation period the patient was asked to reassess the pain and paresthetica and motor deficit. Similar clinical monitoring was performed at a distance of 10 additional days during which the patient we reported complete resolution of the pain symptoms.

After one month of treatment we performed an MRI control (Figure 2) where it shows complete disappearance of disk herniation. Clinically, the patient does not complain of any discomfort.

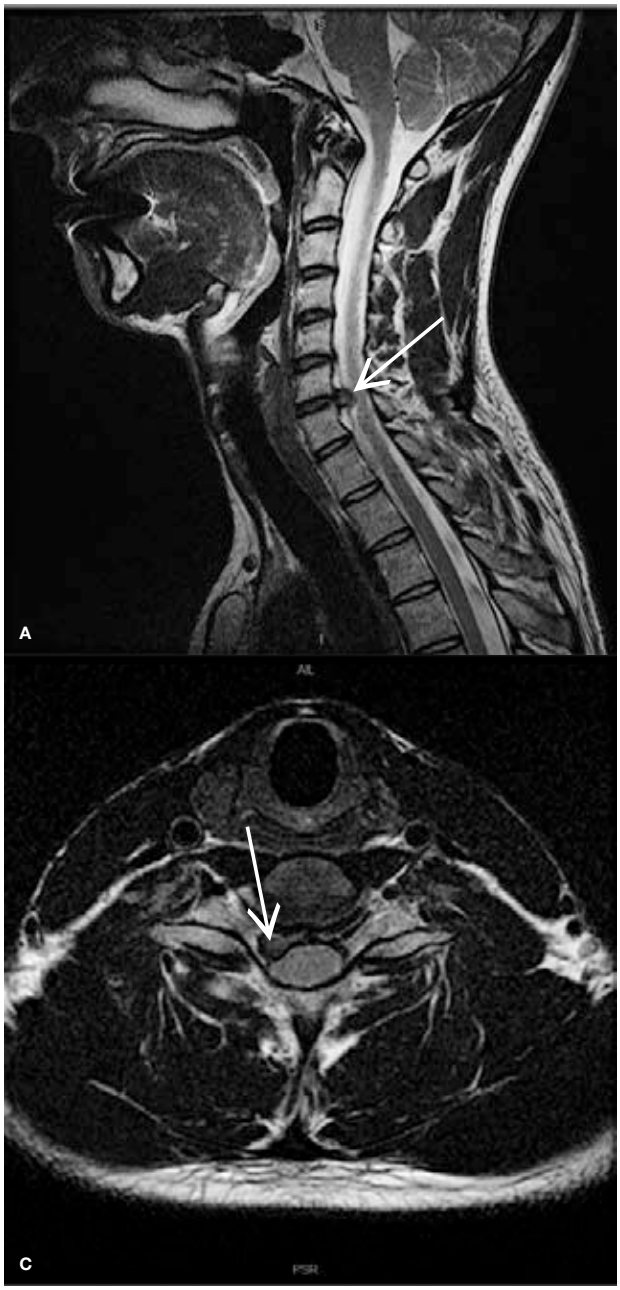


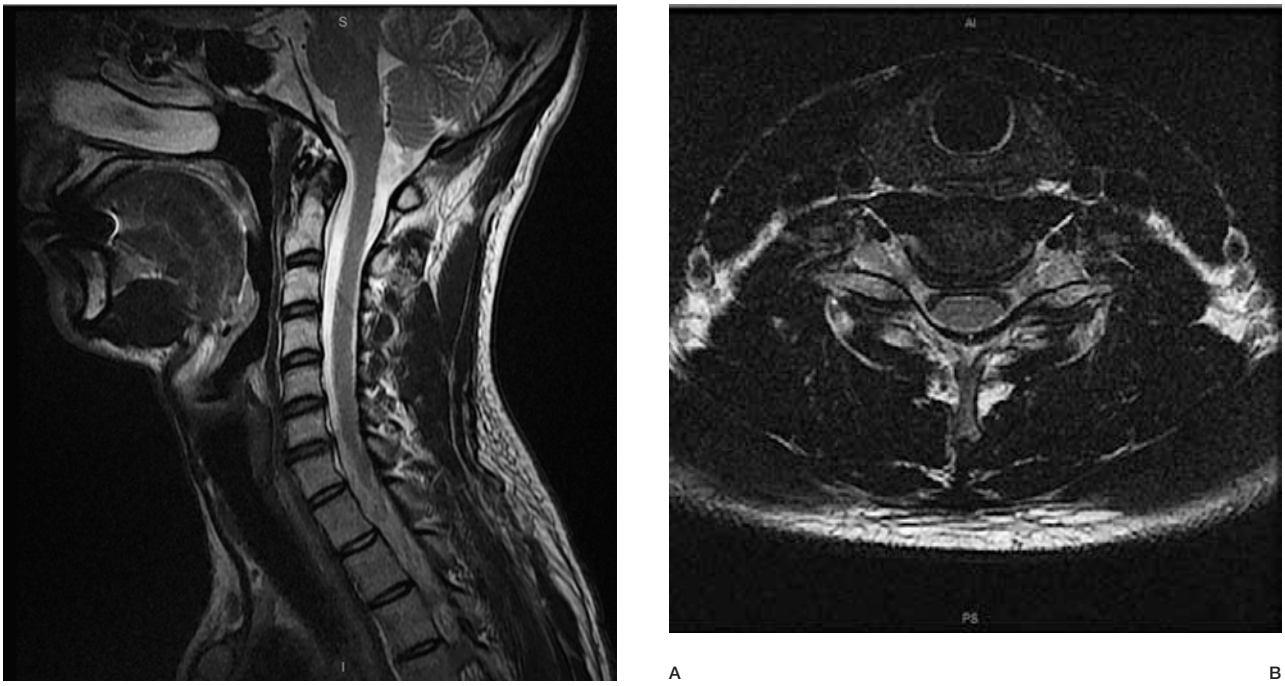
Figure 1 A-C) Right C6-C7 preforminal disc herniation (arrows).

Discussion and Conclusions

The clinical benefit of surgical decompression for cervical disc herniation is often transient even in carefully selected patients. Current surgical techniques vary in relation to extrusion, size and number of herniations and are all highly operator-dependent. Given the very high success rate of oxygen-ozone therapy confirmed by follow-up CT scan, injection of the gas mixture can be deemed an effective non-surgical therapy and the first choice of treatment in patients with cervical disc herniation.

References

1 Andreula C. Ernie discali lombosacrali e patologia degenerativa correlata. Trattamento interventistico spinale con chemiodiscolisi con nucleoptesi con O_3 e infiltrazione periradicolare e periganglionare. Rivista di Neuroradiologia. 2001; 14 (Suppl 1): 81-88.
2 Bonetti M, Cotticelli B, Raimondi D, et al. Ossigeno-ozono



A B

Figure 2 A-B) Complete disappearance of the hernia after CT-guided intraforaminal infiltration of O_2-O_3.

terapia vs infiltrazioni epidurali cortisoniche. Rivista di Neuroradiologia. 2000; 13: 203-206.
3 Bonetti M, Cotticelli B, Valdenassi L, et al. Analisi dei risultati dopo trattamento con O_2-O_3 nelle ernie intra ed extraforaminali lombari. Rivista di Neuroradiologia. 2001; 14 (Suppl 1): 89-92.
4 Bonetti M, Cotticelli B, Valdenassi L, et al. La risonanza magnetica con gadolinio nella valutazione dell'efficacia terapeutica del trattamento con O_2-O_3 nelle ernie discali lombari. Rivista di Neuroradiologia. 2001; 14 (Suppl 1): 109-111.
5 Bonetti M, Fontana A, et al. Intraforaminal O_2-O_3 versus periradicular steroidal infiltrations in lower back pain: randomized controlled study. Am J Neuroradiol. 2005; 26: 996-1000.
6 Bonetti M. Tecnica intraforaminale TC guidata. Rivista Italiana di Ossigeno-Ozonoterapia. 2002; 1: 69-71.
7 De Oliveira Magalhaes FN, Dotta L, et al. Ozone Therapy as a Treatment for Low Back Pain Secondary to Herniated Disc: A Systematic Review and Meta-analysis of Randomized Controlled Trials. Pain Physician. 2012; 15: 115-129.
8 D'Erme M, Scarchilli A, Artale A.M., et al. Ozone therapy in lumbar sciatica. Radiolog Med. 1999; 95 (1-2): 21-4.
9 Fabris G, Tomassini G, Petralia B, et al. L'ossigeno terapia intraforaminale. Rivista di Neuroradiologia. 2001; 14 (Suppl 1): 25-30.
10 Gallucci M, Limbucci N, Zugaro L, et al. Sciatica: treatment with intradiscal and intraforaminal injections of steroid and oxygen-ozone versus steroid only. Radiology. 2007; 242 (3): 907-913.
11 Gautam S, Rastogi V, Jain A, et al. Comparative Evaluation of Oxygen-Ozone Therapy and Combined Use of Oxygen-Ozone Therapy with Percutaneous Intradiscal Radiofrequency Thermocoagulation for the Treatment of Lumbar Disc Herniation. Pain Pract. Epub 2010 Jul 19.
12 Gualandi G, Bonetti M, Pittiani F. Ossigeno-ozonoterapia nel trattamento della patologia dolorosa del rachide lombare: esperienza preliminare. Acta Toxicologica Therapeutica. 1996; 17 (2-3): 261-264.
13 Iliakis E. Ozone treatment in low back pain. Orthopaedics. 1995; 1: 29-33.
14 Iliakis E, Valadakis V, Vynios DH, et al. Ratiolazionation of the activity of medical ozone on intervertebral disc and histological and biochemical study. Rivista di Neuroradiologia. 2001; 14 (Suppl 1): 25-30.
15 Leonardi M, Barbara C, Agati R, et al. Trattamento percutaneo dell'ernia discale lombare con iniezione intradiscale di miscela di ozono. Rivista di Neuroradiologia. 2001; 14 (Suppl 1): 51-53.
16 Muto M, Ambrosanio G, Guarnieri G, et al. Low back pain and sciatica: treatment with intradiscal-intraforaminal O_2-O_3 injection. Our experience. Radiol Med. 2008 Aug; 113 (5): 695-706.
17 Scarchilli A. Tre anni di follow-up nel trattamento delle lombalgie e lombosciatalgie con ozono intradiscale. Rivista di Neuroradiologia. 2001; 14 (Suppl 1): 39-41.
18 Simonetti l, Raffi l, Cenni P, et al. Pharmacological mechanism underlyng Oxygen-Ozone therapy for herniated disc. Rivista Italiana di Ossigeno-Ozonoterapia. 2003; 2: 7-11.
19 Steppan J, Meaders T, Muto M, et al. A metaanalysis of the effectiveness and safety of ozone treatments for herniated lumbar discs. J Vasc Interv Radiol. 2010; 21 (4): 534-48. Epub 2010 Feb 25.
20 Xu L, Li ZL, He XF, et al. Evaluation of the Clinical Curative Effect of an O_2-O_3 mixture to Treat Lumbar Disc Herniation with Different Treatment Sessions. Interv Neuroradiol. 2009; 15 (2): 159-63.

Dr. Matteo Bonetti
Servizio di Neuroradiologia
Istituto Clinico Città di Brescia
Via Gualla 15
I – 25123 Brescia - Italy
Tel.: + 39 030.3701312
Fax: +39 030.3387728
Home: via Verdi 1-25060 Collebeato (BS)
E-mail: matbon@numerica.it

Non-invasive Approaches to Back Pain in Patients with Somatization, 2nd Review

A. BARISELLI, M. BONETTI

Poliambulatorio Oberdan; Brescia, Italy

Key words: somatizazion, low back pain, psychotherapy, ISTDP

SUMMARY - *Somatization implies a tendency to experience and communicate psychological distress in the form of somatic symptoms and to seek medical help for them. So defined, it is neither a disorder nor a diagnostic category but a generic term for a set of experimental, cognitive, and behavioral characteristics of patients who complain of physical symptoms in the absence of relevant medical findings. In our work at the Oberdan Surgery we have noted that patients treated for back disorders, namely low back pain, presents diseases that often cannot be fully explained by a general medical condition or diagnostic tests even though they have painful symptoms resistant to treatment. It is assumed that a certain percentage of these patients may present strong somatoform disorders associated with anxiety. The hypotesis is linked to the fact that chronic disorders are often correlated to somatization and hypochondria. This study set out to demonstrate that the use of non-invasive techniques like intensive short-term dynamic psychotherapy (ISTDP) can yield satisfactory outcomes in terms of quality of life linked to the underlying disorder and general state of health.*

Materials and Methods

We studied a cohort of 98 patients, mostly women (64 women and 34 men) who presented at the Oberdan Surgery in Brescia for low back pain. After history-taking and neurological examination by Dr Matteo Bonetti, patients were offered a psychological consultation combined with administration of psychological questionnaire.

We used *The Symptom Checklist-90-Revised* a questionnaire created by Leonard R. Derogatis is a method to evaluate psychological problems and identify symptoms.

This instrument is also used by psychologists, psychiatrists, mental health, medical, and educational professionals for monitoring the patient's progress or treatment outcome.

Participants are required to respond to 90 minutes using a 5-point rating scale.

Approximately 12-15 minutes is necessary for completion. Testing can be done with a computer, audiocassette, or paper-and-pencil.

Individual of 13 years or older are recommended for accurate test results. Another name for the SCL-90 is the Global Severity Index.

The SCL-R is an established instrument and has over 1,000 independent studies supporting is reliability and validity.

The internal consistency coefficient rating ranged from 0.90 for Depression and 0.77 for Psychoticism. Test-retest reliability has been reported at 0.80 to 0.90 with a time interval of one week.

All nine primary subscales are well correlated with the Minnesota Multiphasic Personality Inventory.

The SCL-R was also correlated with the IIP, 0.73, and the SAS, 0.69 (Pearson).

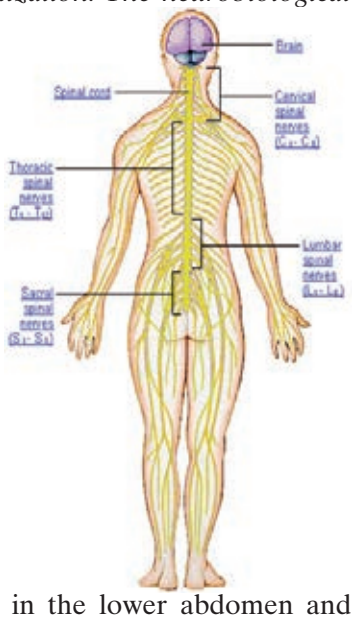
The work was carried out in test mode re-test, with an interval of 3 months after the first administration. Within this period, patients performed 5 sessions of Brief Psychotherapy scondo the model ISTDP (Short Term Dynamic Psychotherapy Intesive), which is a method strives to develop an effective therapeutic relationship within a short period of time.

In contrast to classical psychoanalysis, I.S.T.D.P sets itself the task of solving particular problems in a limited number of sessions.

STDP is clinically effective for patients with somatization

Short-term dynamic psychotherapy (STDP) formats specifically help a patient to examine trauma and oss-related emotions that result in somatization, depression, anxiety, and self-defeating behaviors. Case-series videotaped research.

An example of somatization: The neurobiological path of anger



The inhibitory forces goes down

The path of the somatic rage goes up. Same system

– Somatically begins in the lower abdomen and then gets up with a feeling of warmth, or as a "volcano".
– Get to the chest and then neck.
– It goes to his arms with an impulse to seize and exercise some violence.
– Crosses c8-c7-c6-c5 from the low to the high hand.
– When this occurs, move any unconscious anxiety and somatization the feelings that you can prove they are not converted or somarized but when this feeling is inhibited or suppressed,we can talk about the process of somatization.

STDP is efficacious in controlled trials and meta-analyses

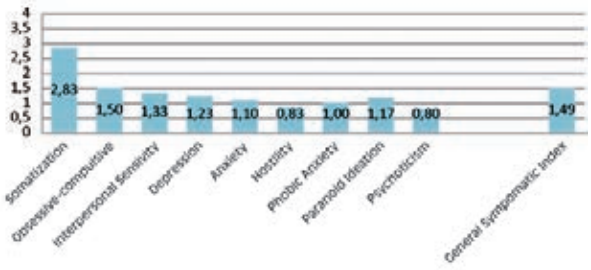
In 1995, Anderson and Lambert, conducted a meta-analysis of 26 controlled studies and found STDP to be superior to minimal treatment controls and wait lists including in samples with somatization.

It was found to be as effective in removing anxiety and depressive symptoms as cognitive behavioral therapy. A recent meta-analysis, using more strict inclusion criteria, yielded the same findings. In a recent randomized controlled trial of symptomatic patients with personality disorders, STDP brought significant symptom reduction while cognitive therapy did not, suggesting that STDP may have added benefits in more resistant and complex symptomatic patients.

Our review has likewise found STDP to be superior to minimal treatment or waitlist controls and that the gains are maintained in follow-up averaging over 1 year.

Results

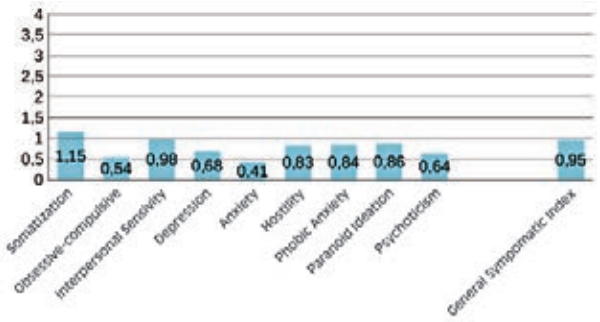
Here are the results in the first session:



Other scales:

Neuroticism	1,11
Sleep disorders	3,33
Discomfort	1,41
Cognitive difficulty performance	1,38

During the second session:



Other scales:

Neuroticism	0,82
Sleep disorders	1,23
Discomfort	0,81
Cognitive difficulty performance	0,64

Discussion

An immediate outcome was a marked reduction in axiety (from 1,97 points to 0,85) and somatization (from 2,43 to 1,56), together with a considerable decrease. In most of the remaining indices.

It is also noteworthy that the "sleep disorders" scale showed a marked improvement: most patients

reported that their quality of sleep improved notably following the psychology sessions. Thanks to the identification of psychological components making up the unresolved neurotic core giving rise to the conflict.

This underlying unresolved situations is readily discharged through. The expression of physical distress sometimes becoming chronic.

We find these components also with reference to previous work of *Fishbain DA, Lewis JE, Gao J, Cole B, Steele Rosomoff R. "Is chronic pain associated with somatization/hypochondriasis? An evidence-based structured review" e di Bacon NM, Bacon SF, Atkinson JH, Slater MA, Patterson TL, Grant I, Garfin SR. "Somatization symptoms in chronic low back pain patients"*. It is reiterated as confirmed by the precedent of how to find work in the form of pain chronic cycles of the psychological dynamics underlying notable that may affect compliance (see results within the scale "Hostility") and the success of therapies.

Patients viewed by us are strong somatic components added to a framework anxious / depressive consistent, nonetheless anyone of those assisted were administered a drug therapy targeted to the component of psychological distress, and instead leverage only on the psychological work.

In the study group, as a result, 32 of the 94 patients (34.37%) have decided to continue the work started with a course of psychotherapy such as "short", which is still active.

Not occur for hours on the physical suffering such that they can get them to request additional clinical diagnostic tests.

It is assumed for so that the symptomatology detected both in reality purely psychological, but that is present for the majority of these cases in the form of a request for medical care, as also shown in the work of Lipowski ZJ. Somatization: "the experience and communication of psychological distress as somatic symptoms".

STDP is cost-effective and reduces health care utilization

STDP has been shown to reduce healthcare utilization and to be cost-effective in treating patients with dyspepsia, irritable bowel syndrome, depression, and self-harm and treatment resistant conditions. Of specific cost figures cited in reviewed papers, 27 out of 34 showed cost savings with STDP including reduction in total costs, medication costs, disability, hospital, and physician use.

Conclusions

This study is a review and integration of work started earlier, which is delivering more and more comforting. In these diseases, anxiety, distress or traumatic experiences are a discharge path immediately in the soma (noise). In light of this, the treatment of choice for patients with this type of problem (or conversion) is certainly of mold integrated, as evidenced by the work of Kriegler JS, Ashenberg ZS. "Management of chronic low back pain: a comprehensive approach," where it is clear that combining psychological work to a suitable non-invasive medical intervention. Our patients presented strong somatic components associated with major anxiety/depression. Despite this none of the patients were administrated drugs to manage the psychological impairment, and the treatment focused on psychological and relaxation sessions. After treatment, 32 of the 98 patients enrolled in the study (34,37%) decided to continue the treatment whith short-term dynamic psychotherapy, currently ongoing. Persistent somatization poses a serious clinical, social, and economic problem and hence early identification of potential chronic somatizers should be attempted to avoid its development. Etiology of somatization is multifactorial and so should be its management.

References

1 Ford CV. A clinical approach to the somatizing patient. J Fam Pract. 1985; 21 (4): 294-301.
2 Ford CV. Dimensions of somatization and hypochondriasis. Neurol Clin. 1995; 13 (2): 241-253.
3 Fishbain DA, Lewis JE, Gao J, et al. Is chronic pain associated with somatization/hypochondriasis? An evidence-based structured review. Pain Pract. 2009; 9 (6): 449-67. Epub 2009 Sept 3.
4 Kriegler JS, Ashenberg ZS. Management of chronic low back pain: a comprehensive approach. Semin Neurol. 1987; 7 (4): 303-12.
5 Rickels K, Rynn M. Overview and clinical presentation of generalized anxiety disorder. Psychiatr Clin North Am. 2001; 24 (1): 1-17.
6 Atkinson JH, Slater MA, Patterson TL, et al. Prevalence, onset, and risk of psychiatric disorders in men with chronic low back pain: a controlled study. Pain. 1991 May; 45 (2): 111-121.
7 Schmidt AJ, Arntz A. Psychological research and chronic low back pain: a stand-still or breakthrough? Soc Sci Med. 1987; 25 (10): 1095-1104.
8 Bacon NM, Bacon SF, Atkinson JH, et al. Somatization symptoms in chronic low back pain patients. Psychosom Med. 1994; 56 (2): 118-27.
9 Lipowski ZJ. Somatization: the experience and communication of psychological distress as somatic symptoms. Psychother Psychosom. 1987; 47 (3-4): 160-7.
10 Fink P, Sorensen L, Engberg M, et al. Somatization in primary care. Prevalence, health care utilization, and general practitioner recognition. Psychosomatics 1999; 40: 330-338.
11 Ratner L. Human immunodeficiency virus-associated autoimmune thrombocytopenic purpura: a review. Am J Med. 1989; 86: 262-266.
12 Holloway KL, Zerbe KJ. Simplified approach to somatization disorder. When less may prove to be more. Postgrad Med. 2000; 108: 89-92, 95.
13 Servan-Schreiber D, Tabas G, Kolb NR. Somatizing patients: part II. Practical management. Am Fam Physician 2000; 61: 1423-1428, 1431-1432.
14 Servan-Schreiber D, Kolb R, Tabas G. The somatizing patient. Prim Care 1999; 26: 225-242.
15 Noyes R Jr, Holt CS, Kathol RG. Somatization. Diagnosis and management. Arch Fam Med. 1995; 4: 790-795.
16 Leichsenring F, Rabung S, Leibing E. The efficacy of short-term psychodynamic psychotherapy in specific psychiatric disorders: a meta-analysis. Arch Gen Psychiatry. 2004; 61: 1208-1216.
17 Servan-Schreiber D, Tabas G. Somatizing patients part I: practical diagnosis. Am Fam Physician. 2000; 61: 1073-1078.
18 Abbass AA, Hancock JT, Henderson J, et al. Short term psychodynamic psychotherapies for common mental disorders (Protocol for a Cochrane Review). 2004. In: The Cochrane Library. Chichester, UK: John Wiley and Sons, Ltd.
19 Abbass A. The cost-effectiveness of short-term dynamic psychotherapy. Journal of Pharmacoeconomics and Outcomes. Research. 2003; 3: 535-539.
20 Davanloo H. Intensive short-term dynamic psychotherapy: Spectrum of psychoneurotic disorders. In Davanloo H. Intensive Short-Term Dynamic Psychotherapy. Chichester, England: John Wiley and Sons; 2001.

A. Bariselli, MD
Poliambulatorio Oberdan
Via G. Oberdan, 140
25128 Brescia, Italy
Tel.: +39 030 3701312
Fax: +39 030 3387728
E-mail: bariselli.andrea@gmail.com

Letters to the Editor

Dr Matteo Bonetti
Scientific Director
International Journal of Ozone Therapy

Caro Matteo, Marco e Maurizio,

Vi invio per conoscenza un dialogo recente tra me e Bernardino Clavo conseguente alla pubblicazione di due nostri articoli (*allegati*).

Credo che si debba riflettere tutti insieme su quanto contenuto soprattutto per il bene futuro dell'ozono terapia. Ad oggi le iniziative legislative sembrano affossate e il proliferare di nuovi adepti nel campo dell'ozono, tra i quali alcune figure che fino a qualche anno fa si opponevano strenuamente ad essa, se da un lato ci può far piacere dall'altro ci deve preoccupare per la mancanza di controlli in fase autorizzativa che può permettere di avvicinarsi ed eseguire la terapia anche senza aver acquisito la necessaria preparazione.

Credo inoltre che finalmente dovremmo anche eliminare e contrastare alcuni elementi che ci hanno a volte visti contrapposti rispettando e considerando le varianti tecniche della terapia con ozono tenendo conto delle rispettive specializzazioni mediche. Alcune polemiche passate, che mi hanno a volte visto partecipe ahimè, tendono purtroppo a dividere mentre è fondamentale l'unità tra le varie scuole di pensiero purché improntate alla massima collaborazione e all'aggiornamento costante sui meccanismi dell'ozono di tutte le figure professionali attive nel campo.

Un caro saluto a tutti e Buone Vacanze

Lamberto Re
www.lambertore.com

-------- Messaggio originale --------
Oggetto: Re: Compliments
Data: Mon, 6 Aug 2012
Mittente: Bernardino Clavo
A: Lamberto Re

Hello Lamberto,

I completely agree with you once more.

We have problems ?in front of? and ?inside? the Ozone therapy field.

We have to fight against both problems.

I enjoyed reading your article. Congratulations!.

I hope in the next future we can collaborate closer.

Best regards

Bernardino

-------- Messaggio originale --------
Oggetto: Compliments
Data: Mon, 6 Aug 2012
Mittente: Lamberto Re
A: Bernardino Clavo

Hi Bernardino,

I reply to your comment appeared in JEIM in English because my written Spanish is not so good as the spoken!

Starting from the end of your beautiful Editorial, my personal response is that most of the practitioners are *not ready* for such challenge.

This was also clear from my review addressed to the thousand and thousand MD which usually start to approach superficially to Ozone

Therapy and that we can meet only rarely in our or other congresses.

The problem is that being out of the control from the Sanitary

Authority, any MD can start to practice OT also with minimum, or sometimes without, experience derived by serious and approved Courses.

Indeed, at the moment no rules have been proposed or are requested by the Authority. The erroneous statement that Ozone techniques are very simple to execute magnifies the problem and reduce the credibility toward other Colleagues or Scientists.

In the next future we must address all our efforts to try to reduce the spread of this phenomenon.

A big embrace, Lamberto

Lamberto Re, MD
Via Fazioli, 22
60021 Camerano (AN-IT)
Mob. +39 339 5372593
Fax. +39 071 731347
E-mail lambertore@lambertore.com
WEB www.lambertore.com

Francesco Riccardo MONTI

II prize

The **Francesco Riccardo Monti** prize for life time achievements is a recognition for scientific work done to spread Oxygen-Ozone Therapy practice in Italy and around the world.

FEDERAZIONE ITALIANA DI
OSSIGENO-OZONOTERAPIA

VI Congresso Nazionale
9-11 maggio

2013

FIRENZE

Presidente **Gianantonio Pellicanò** Firenze (Italy)

NEWS

Dr Matteo Bonetti
Scientific Director
International Journal of Ozone Therapy

I send to your attention the publication on ClinicalTrial.gov of the clinical trial proposed from our group many years ago to the Health Service of Marche Region who sponsored the study.

It will be executed at INRCA Institution under the supervision of the Head of the Rehabilitation Unit, Dr Oriano Mercante. Code NCT01709058

http://clinicaltrials.gov/

The operative staff will be composed by:
Lamberto Re, MD
Giuseppe Malcangi, MD
Gregorio Martinez Sanchez, PhD
Marica Bordicchia, PhD

We hope to give our contribute to the best collocation of Ozone Therapy within Science.

Lamberto Re, MD

FEDERAZIONE ITALIANA DI
OSSIGENO-OZONOTERAPIA

Corso teorico pratico in O2-O3
sabato 15 dicembre 2012

Il corso è riservato a **medici ortopedici**, **traumatologi**, **neuroradiologi** e t**erapisti del dolore** che si occupano di ossigeno ozono terapia. Obiettivo del corso è quello di divulgare la pratica dell'ossigeno ozono terapia **secondo le linee guida della Federazione Italiana**, mediante apprendimento teorico e pratico con docenti che si occupano di tali pratiche da oltre 20 anni.

Al termine del corso verrà inoltre fornita documentazione cartacea inerente le modalità di trattamento e come **organizzare** ed **ottimizzare** una **struttura dedicata all'ossigneo-ozono terapia**.

Sede: Poliambulatorio Oberdan . Via Oberdan 140, Brescia

Costo: Euro 250 + IVA (Soci FIO)

Euro 300 + IVA (Non soci FIO)

Partecipanti: Max 20. L'iscrizione si intende perfezionata solo a regolarizzazione del saldo del corso e la compilazione del modulo di iscrizione.

ECM: Evento in fase di accreditamento presso il Ministero della Salute, nel programma di educazione continua in medicina (ECM)

Per info e iscrizioni: www.webfio.it

Segreteria organizzativa e provider ECM:

Programma della giornata

Ore 9,00: Registrazione dei partecipanti

Ore 9,15: M. Bonetti Ozonoterapia: "*Stato dell'arte*", "*Meccanismi d'azione*", "*Il trattamento del mal di schiena con O2-O3: Come, quando e perché?*"

Ore 10,15: M. Moretti "*L'ozonoterapia nel trattamento di ginocchio, spalla, caviglia, tendine d'Achille*"

Ore 11,15: Coffee Break

Ore 11,30: A. Zambello "*La grande autoemo trasfusione: Il razionale e le indicazioni*",

"*Il piede diabetico*"

"*Ozonoterapia in medicina estetica*",

Ore 12,30: Prove pratiche

Ore 13,30: Lunch

Ore 14,30 M. Bonetti: "*Come organizzare un ambulatorio per Ossigeno-ozono terapia*",

Ore 15,00: P. Terlizzi: "*La normativa vigente ASL, cosa fare?*"*,

Ore 15,30: Test di valutazione ECM

Ore 17,30: Chiusura dei lavori

* *certificazione per un ambulatorio di ozono terapia*

World Federation Oxygen - Ozone Therapy

World Federation of Oxygen - Ozone Therapy

PRESIDENT
Dr. Vijam Sheel Kumar †
Kumar Pain Management
& Speciality Centre
D.1/28 Vasant Vihar
110057 New Delhi
Tel.: 91 11 26142282 - 26154106
mail: spineconf@kpmsc.com

VICE-PRESIDENT
Dr. Cosma Andreula
Anthea Hospital
Via C. Rosalba, 35-37
70124 Bari (BA)
Tel.: 080/550411
Fax: 080/5042487
mail: cosmaandreula@tin.it

SEGRETARY GENERAL
Dr. Matteo Bonetti
Istituto Clinico Città di Brescia
Via Gualla, 15
25123 Brescia (BS)
Tel.: 030/3710311
Fax: 030/3710370
mail: matbon@numerica.it

TREAUSER
Dr. Alessandro Fontana,
Istituto Clinico Città di Brescia
Via Gualla, 15
25123 Brescia (BS)
Tel.: 030/3710311
Fax: 030/3710370
mail: saratas@tin.it

CENTRAL OFFICE
X Ray Service s.r.l.
Via Oberdan, 140
25128 Brescia (BS)
Tel.: 030/3701312
Fax: 030/3387728
mail: info@xrayservice.it

Ad Interim President until IVWorld Congress WFOOT - Buenos Aires 2013

Membership fees may be paid as follows:

by bank transfer free of charge credited to the F.I.O. - W.F.O. O. T. account
BANCA CARIGE - Agenzia 2 - BRESCIA - ITALIA - € 125,00

IBAN: IT 35 K061 7511 2020 0000 0624 780 - BIC: CRGEITGG542

Please do not forget to indicate your name on the bank transfer.

Sincerely yours,
Dr. A. Fontana
W.F.O.O.T. (World Federation of Oxygen - Ozone Therapy)

World Federation Oxygen - Ozone Therapy

World Federation of Oxygen - Ozone Therapy

First name . . . . . Family name . . . . . .

Institution . . . . . .

Department . . . . . .

Street and number . . . . . .

Post-code and town . . . . . .

Phone number/ Fax number . . . . . .

Address . . . . . .

City - State - Post-code . . . . . .

Tel. . . . . Fax . . . . . .

e-mail . . . . . .

Date . . . . . .

Signature . . . . . .

Membership fees for 2012: € 125,00 for Full Members
Membership fees may be paid as follows:
by bank transfer free of charge credited to the F.I.O. W.F.O.O.T., account
BANCA CARIGE - Agenzia 2 - BRESCIA - ITALIA
IBAN IT35 K061 7511 2020 0000 0624 780 - BIC CRGEITGG542
Please do not forget to indicate your name on the bank transfer.

Dr. Matteo Bonetti
W.F.O.O.T. (World Federation of Oxygen - Ozone Therapy)

Please type or write in capital letters.
Please return this form to:

CENTRAL OFFICE
X Ray Service s.r.l.
Via Oberdan, 140
25128 Brescia (BS)
Tel.: 030/3701312
Fax: 030/3387728
mail: info@xrayservice.it

Subscription order form

INTERNATIONAL JOURNAL OF OZONE THERAPY *Digital*

I would like to subscribe to *International Journal of Ozone Therapy* for the year 2012 (2 issues, only on line version):

euro 50.-

www.centauro.it

Name (write in block capital)

Address

City / State / Post code

Phone Fax

E-mail

☐ I enclose a cheque / money order payable to Centauro srl

☐ Swift transfer to Centauro srl - Unicredit Banca - Swift code: UNCRITM1BA2

IBAN code: IT 74 D 02008 02435 000002933096

☐ Please charge my

☐ Visa ☐ Diners ☐ Master Card ☐ Carta Si ☐ Amex

Credit Card number

Expiry date

Please compile for your digital subscription activation

User Password

Date Signature

Centauro s.r.l. Via del Pratello, 8 I-40122 Bologna - Italy - Fax +39.051.220099

serena.preti@centauro.it - www.centauro.it

Students: Please enclose a copy of your student registration form

Federazione Italiana di Ossigeno-Ozonoterapia

www.webfio.it

Al **Presidente della FIO**

Il sottoscritto/a . . . . . .

Codice Fiscale . . . . . .

Residente in via . . . . . .

CAP . . . . . Città . . . . . .

e-mail . . . . . Telefono . . . . . Fax . . . . . .

Chiede di essere iscritto alla FIO - Federazione Italiana di Ossigeno-Ozonoterapia.
Allega un breve curriculum vitae (una pagina)

Data . . . . . Firma . . . . . .

Mi impegno al versamento della quota sociale annua di 125,00 €.
Di cui 85,00 € come iscrizione alla FIO e 40,00 € come abbonamento alla Rivista "International Journal of Ozone Therapy", organo ufficiale della FIO, Banca Carige agenzia 2 di Brescia,
IBAN: IT 35K0617511202000000624780, oppure inviare con bollettino postale
C/C nr. 43650316, intestato a F.I.O. (Federazione Italiana di Ossigeno-Ozonoterapia).

Dr Matteo Bonetti
Segretario FIO

Presso: X-Ray Service Srl
Sede amministrativa: Via Leonardo da Vinci, 20 - 25122 Brescia

Sede operativa: Via Oberdan, 140 - 25128 Brescia

Tel.: 030.3701312/030.3387729 - Fax: 030.3387728
e-mail: segreteriafio@webfio.it

FEDERAZIONE ITALIANA DI OSSIGENO-OZONOTERAPIA

Date…….

Re: association membership fee

Dear Colleague,
This is a reminder that the Association *membership* fee for *2012 is € 125,00,*
inclusive of a subscription to the International Journal of Ozone Therapy,
payment by bank draft to Banca Carige - agenzia 2 - Brescia, Italia
IBAN: IT 35 K 06175 11202 000000624780 SWIFT Code: CRGEITGG542, or by credit card

Thank you in advance for your payment.

Yours sincerely,

Dr Matteo Bonetti
FIO Secretary

Objeto: cuota de asociación

Estimado Colega,
quería recordarte que *la cuota* de asociación por *el año 2012 es de € 125,00*, la que incluye la subscripción a International Journal of Ozone Therapy,
con un pago en la Banca Carige - agencia 2 - Brescia, Italia
IBAN: IT 35 K 06175 11202 000000624780 Codigo SWIFT: CRGEITGG542, o con la tarjeta de crédito.

Te agradezco desde ahora por el pago de la cuota.

Cordialmente

Dr Matteo Bonetti
Segreteria FIO

Oggetto: quota associativa

Caro Collega,
desidero ricordarti che *la quota sociale* della FIO è per il 2012 di € *125,00*,
comprensiva dell'abbonamento alla rivista International Journal of Ozone Therapy, *con un bonifico alla Banca Carige - agenzia 2 - Brescia, Italia*
IBAN: IT 35 K 06175 11202 000000624780 o con carta di credito.
oppure inviare con bollettino postale: c/c nr. 43650316, intestato a F.I.O.
(Federazione Italiana di Ossigeno-Ozonoterapia)
Ti ringrazio fin da ora per il pagamento.

Cordialmente

Dr Matteo Bonetti
Segreteria FIO

INTERNATIONAL JOURNAL OF OZONE THERAPY

The Official Journal of the WFOOT - World Federation of Oxygen-Ozone Therapy, FIO - Italian Federation of Ozone Therapy, SEOT - Spanish Association of Ozone Therapy, Hellenic, Indian, Slovach and Chinese National Societies

SCIENTIFIC EDITOR
Matteo Bonetti
Servizio di Neuroradiologia - Istituto Clinico Città di Brescia
via Gualla, 15 - 25100 Brescia
Tel. ..39/030/3710311 - Fax ..39/030/3710370
e-mail: matbon@numerica.it

PUBLISHING STAFF

PUBLISHER
NICOLA LEONARDI
Direttore responsabile
Centauro srl
Via del Pratello, 8
I-40139 Bologna, Italy
Tel: ..39/051/227634
Fax: ..39/051/220099

LANGUAGE EDITOR
Anne Collins
e-mail: collins@iol.it

MANAGING EDITOR
Elisabetta Madrigali
e-mail: elisabetta@centauro.it

ADVERTISING
Serena Preti
e-mail: serena.preti@centauro.it

INSTRUCTIONS FOR AUTHORS

First formulated in 2002 - Revised in Febraury, 2011

The International Journal of Ozone Therapy **(IJOT)** is a clinical practice journal documenting the current state of ozone therapy practice worldwide. **IJOT** publishes original clinical observations, descriptions of new techniques or procedures, case reports, and articles on the ethical and social aspects of related health care. Submissions suitable for the Journal include observational studies, clinical trials, epidemiological work, reports on health services and outcomes, and advances in applied (translational) and/or basic research. The instructions for submission of articles to **IJOT** follow the *Uniform Requirements for Manuscripts Submitted to Biomedical Journals of the International Committee of Medical Journal* editors (ICMJE, http://www.icmje.org), if not otherwise indicated below.

Research and Publication Ethics

For the policies on research and publication ethics not stated in these instructions, the Guidelines on *Good Publication Practice* issued by the Committee on Publication Ethics should be applied.

1. *Author responsibility for originality*: Each author acknowledges responsibility for the integrity of the manuscript, assures the originality of the paper, and guarantees that submitted manuscripts do not contain previously published material and are not under consideration for publication elsewhere. If the submitted manuscript builds on or includes parts of previously published articles, authors are encouraged to enclose copies of the articles with the new submission. The Editor reserves the right to request the original data obtained in the investigation.

2. *Registration of clinical trial research*: Any research that includes clinical trials should be registered with the primary national clinical trial registration authority accredited by WHO (http://www.who.int/ictrp/network/primary/en/index.html) or ICMJE.

3. *Disclosure statement*: This is not intended to prevent authors with potential conflicts of interest from contributing to **IJOT**. Rather, the Journal will place on record any relationship that may exist with disclosed, or competing, products or firms. Disclosed information will be held in confidence during the review process and the Editors will examine any printed disclosure accompanying a published article. Authors are responsible for notifying the Journal of financial arrangements including, but not limited to, agreements for research support including provision of equipment or materials, membership of speaker bureaus, consulting fees, or ownership interests. It is important that disclosure statements be updated promptly to reflect any new relationships that arise after initial submission of the manuscript. If the study is supported by a commercial sponsor, the authors must document the input of the sponsoring agency in study design, data collection, analysis of results, interpretation of data, and report writing. It is important to specify whether the sponsors could have suppressed or influenced publication if the results were negative or detrimental to the product they produce. Authors should also state if the company was involved in the original study design, the collection and monitoring of data, analysis and/or interpretation, and/or the writing and approval of the report.

4. *Statement of human and animal rights*: Manuscripts involving examination of volunteers and patients must include a statement that the study protocol has been approved by an institutional review board (IRB) and that the subjects gave informed consent, thus meeting the standards of the Declaration of Helsinki (revised version) developed by the World Medical Association as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data (http://www.wma.net). With animal subjects, research should be performed based on a National or Institutional Guide for the Care and Use of Laboratory Animals, and ethical treat-

For information:
Centauro s.r.l.
Via del Pratello, 8 - I-40122 Bologna
Tel: ..39/051/227634 - Fax: ..39/051/220099
e-mail: serena.preti@centauro.it
www.centauro.it
SINGLE ISSUE (only digital version): Euro 30,00
Reg. Trib. di Bologna n. 7159 del 20-09-2001

ment of all experimental animals is expected throughout all experiments.
5. *Duplicate/Redundant publication*: **IJOT** only accepts manuscripts describing original research. The editorial office of the **IJOT** does not accept duplicate submission or redundant publication. Redundant (or duplicate) publication is publication of a paper that overlaps substantially with a paper already published in print or electronic media as defined by the updated ICMJE guidelines that cover allegations of scientific misconduct. When submitting a paper, authors should make a full statement to the Editor on all submissions and previous reports that might be regarded as redundant publication of the same or similar work. If authors believe that their manuscript may be considered redundant, they should address this issue in a letter to the Editor accompanying the submission. The authors should also explain in the letter how their report overlaps already published material, or how it differs. Copies of such published material should be included with the submitted paper to help the Editor examine the possibility of redundant publication. If redundant publication is attempted without such notification, authors should expect editorial action to be taken. At the very least, rejection of the manuscript may be expected.
6. *Authorship*: The Editor considers authorship to belong to those persons who accept intellectual and public responsibility for the statements made and results reported. By submitting a manuscript for publication, each author acknowledges having made a substantial contribution to the concept and design of the study, the analysis and interpretation of the results, and the writing of the paper, in addition to having approved the final submitted version. Authorship should not be attributed to Departmental Chairs not directly involved in the study, to physicians or technicians who provided routine services, or to technical advisors. A group study should carry the group name and reference contributing authors in the Acknowledgments.

Peer Review Process

Papers are accepted on the understanding that they are subject to peer review, editorial revision, and, in some cases, comment by the Editor. Manuscripts are examined by independent peer reviewers. Articles and other material published in the Journal represent the opinions of the authors and should not be construed to reflect the opinions of the publisher.

Language

The official language of **IJOT** is English. Most papers in **IJOT** have been written by non-native English speakers, and they will also be read by many non-native speakers. For the purposes of clarity, it is strongly recommended that authors not fluent in English have their manuscript professionally edited for English usage prior to submission. A professional editor will improve the English to ensure that author meaning is clear and to identify problems that require author review. Authors are invited to contact the **IJOT** language editor Anne Collins (*collins@iol.it*) for referral to professional English language editing services. Authors should make contact with and arrange payment to their chosen editing service directly. Please note that the use of such a service is at the author's own expense and risk and does not guarantee that the article will be accepted for publication. Centauro Publishers does not receive any commission or other benefit from editing services. Accordingly, Centauro Publishers neither endorses nor accepts any responsibility or liability for such editing services.

Copyright and Purchase of Offprints

 IJOT offers authors a complimentary hard copy and .pdf file of the issue in which their article is published, for personal use. To purchase offprints of articles published by **IJOT** please contact Centauro Srl for a quotation at *serena.preti@centauro.it*.

Publication Types

Original Research Articles are full-length research papers, which are favored by **IJOT**. Articles cover topics relevant to clinical studies, and may include both basic and experimental work.
Review Articles are comprehensive, state-of-the-art papers focusing on important clinical problems and should address a specific topic in a scholarly manner. Such articles may be invited by the Editor or may be unsolicited reviews.
Case Reports/Technical Notes should be unusually educational and medically important. Although **IJOT** preferentially encourages submission of full-length Original Research Articles, the Journal will consider publication of a limited number of concise Case Reports and Technical Notes.
Editorials are usually invited by the Editor.
Editorial Comments are specific comments on the articles published in **IJOT** and are usually invited by the Editor.
Letters to the Editor contain constructive comments or criticism of a specific paper published by **IJOT**. Letters dealing with subjects of general interest within the field of **IJOT**, or personal opinions on a specific subject within the ambit of **IJOT**, may also be accepted.
All papers should be sent to the Scientific Editor Dr. Matteo Bonetti: *matbon@numerica.it*

Manuscript Preparation

Introductions that Satisfy Reader Expectations

– *Overview:* Once you have a revised draft, you need to ensure that your Introduction frames it, so that your readers will understand where you are taking them. The Introduction should orient readers and motivate them to read the rest of the paper. The Introduction must also make a contract with the reader that a question will be answered. – *Functions:* – To awaken the reader's interest. – To be informative enough to prepare readers to understand your paper. – *Content and Organization:* 1. Common ground ➜ Context ➜ Relevant background ➜ Orients the reader. 2. Disruption ➜ Problem ➜ Gap in Knowledge, Question ➜ Motivates the reader. 3. Resolution ➜ Response ➜ Promise of an answer ➜ Makes a contract with the reader. 1. *Common Ground*: States the consensus, shared understanding, common ground in the field, what's know and not known. Gives the reader context and provides relevant background information, not a literature review. 2. *Disruption:* States the problem/the question that the paper addresses. Conveys the significance, i.e. the cost of leaving problem unsolved, or the benefit of solving it. 3. *Resolution:* Implicitly promises that you will present your answer in the Results and Discussion. Try not to state the answer; that has the effect of closing off the paper rather than leading into it. *When drafting*: Although the Introduction unfolds in the above order, 1-2-3, when you're drafting, write it in a 3-2-1 order. By writing the Introduction in this order, you are sure to work through and clearly set up your main point, the question that it answers, and to include only relevant background information. *When revising*: "The key is to think like a reader -- readers expect and need a sense of structure. Since readers read each sentence in light of how they see it contributing to the whole, when you revise it makes sense to diagnose first the largest elements of the paper, then focus on the coherence of your paragraphs, the clarity of your sentences, and only last on matters of spelling and punctuation. Of course, in reality, no one revises so neatly; all of us revise as we go. But, it is useful to keep in mind that when you revise from the top down, from global structure to sections to paragraphs to sentences to words, you are more likely to discover useful revisions than if you start at the bottom with words and sentences and work up". Booth WC, Colomb GG, Williams JM, et al. The Craft of Research. Chicago: University of Chicago Press; 2008.

Original research should be organized in the customary format, as described below. The text of the manuscript should be submitted as a single document with the following sections (in order):
1. *Author Information Page* (First page) - Full title of the article, authors' names, highest academic degree earned by each author, authors' affiliations, name and complete address for correspondence, address for reprints if different from address for correspondence, fax number, telephone number, and e-mail address.
2. *Acknowledgments and Funding Page* (Second page) - The Acknowledgments section lists all funding sources for the research of the study, and details substantive contributions of individuals. The authors must reveal all possible Conflicts of Interest/Disclosures.
3. *Title Page* (Third page) - The full title, itemized list of the number of tables, the number and types (color or black-and-white) of figures, and three-to-five key words for use as indexing terms should be included. Appropriate key words should be selected from the Medical Subject Heading. The word count of the text should be specified.
4. *Summary* - A summary of up to 250 words should summarize the problems presented and describe the studies undertaken, results and conclusions. Since the abstract must be explicative, the abbreviations must be reduced to a minimum and explained. References should not be cited in the abstract.
5. *Text* - Typical main headings include Introduction, Materials and Methods, Results, Discussion, and Conclusions. Abbreviations must be defined at first mention in the text, tables and figures. The complete names and short addresses of manufacturers of any equipment used in Materials and Methods must be supplied. If animals are used in experiments, state the species, the strain, the number of animals used, and any other pertinent descriptive characteristics. If human subjects or patients are employed, provide a table with relevant characteristics. When describing surgical or neurointerventional procedures on animals, identify the pre-anesthetic and anesthetic agents used, and state the amount or concentration and the route and frequency of administration of each agent. Generic names of drugs must be given. Manuscripts that describe studies on humans must indicate that the study was approved by an Institutional Review Committee and that all subjects gave informed consent. Reports of studies on both animals and humans must indicate that all procedures followed were in accordance with institutional guidelines.
6. *References* - Citations should be listed in order of appearance in the text, and between square brackets [ ]. References must be listed at the end of the text in the order of citation. Journal titles should be abbreviated according to Index Medicus. For citation rules not specified here, authors should refer to the *NLM Style Guide for Authors, Editors, and Publishers* (*http://www.nlm.nih.gov/citingmedicine*). All references must be checked by the author(s).
Journal article: Laredo JD, Bard M. Thoracic spine: percutaneous trephine biopsy. Radiology. 1986; 160: 485-489.
Journal article if the number of authors is more than three: list the first three authors followed by et al.
Geibprasert S, Krings T, Pereira V, et al. Clinical characteristics of dural arteriovenous shunts in 446 patients of three different ethnicities. Intervent Neuroradiol. 2009; 15: 395-400.
Entire book: Valavanis A. Medical radiology: interventional neuroradiology. Heidelberg: Springer Verlag; 1993.
Part of book if number of authors is more than two:
Bonneville JF, Clarisse J, et al. Radiologie interventionnelle. In: Manelfe C ed. Imagerie du rachis et de la moelle. Paris: Vigot Editeur; 1989. p. 761-776.
7. *Tables* - Each table must be typed on a separate sheet and double-spaced, if possible. Tables should be numbered using the Arabic system, followed by a brief informative title. Use type of the same font and size as employed in the text. Include footnotes at the bottom of each table. Tables must be numbered in the order cited in the text. Tables should not duplicate data given in the text or figures.

8. *Figure Legends* - Provide figure legends on a separate sheet. Legends must be double-spaced, and figures must be numbered in the order cited in the text.
9. *Figures* - Figures should preferably be submitted online **in .tiff format** to: *matbon@numerica.it*. The time required to send files will vary depending on the number of figures, but image resolution **must not be reduced** to decrease transmission time. When labeling the figures, please ensure that the label corresponds to the figure number.
Digital images (originals or images acquired by a scanner) must meet the following criteria:
Black-and-white figures: Images must be acquired using the grey scale with a minimum resolution of 300 pixels per inch or 150 pixels per cm. Images must have a base of at least 8.1 cm for one item or a minimum base of 16.9 cm for several items.
Color figures: Images must be acquired using the full color CMYK method with a minimum resolution of 300 pixels per inch or 150 pixels per cm. Images must have a base of at least 8.1 cm for one item or a minimum base of 16.9 cm for several items. The RGB method is recommended for video reproductions only, as the quality of printed figures is poor.
Images must be saved in **.tiff format**. Images in .jpg format are not acceptable as details tend to be lost upon scanning, even at high resolution. Image definition also depends on the enlargement factor. Thus, a large low-resolution image can be proportionally reduced (by 24%) for publication, thereby permitting optimal presentation in print. However, enlargement of a small high-resolution image will highlight all flaws, yielding a pixelated effect.
Do not submit figures already paged in Word, PowerPoint, or other documents, or images inserted in web pages. Such images are of low resolution and are unsuitable for printing.
Figures in .dcm (Dicom) format may be submitted as .dcm or .tiff files. The editorial office will process such images for printing.
Illustrations may be compressed using the StuffIt, Aladdin, or Zip programs.
Do not label an image with arrows, numbers, or letters, but rather indicate on a duplicate copy or a sketch where such indications are desired.
Authors are advised to refer to the Journal guidelines when formatting their work. Otherwise the editorial office may return a submission to the authors for improvement before any Editor is assigned.
If it is not possible to send figures via the Internet, images may be sent by express courier in one of the following formats:
Original x-ray films, slides, or glossy or opaque prints. Clearly indicate on the back of the image the first author's name and the number corresponding to the figure caption and citation in the text.
Figures may be submitted on a CD-ROM or DVD, copied in ISO9006 format legible on both PC and MAC. Floppy disks are not recommended. Clearly indicate on one side of the figure both the top of the figure and the figure number. Do not label the actual image with arrows, numbers, or letters, but rather indicate the top and figure number on a duplicate copy or on a sketch. Do not cut or attach figures with adhesive tape or use paper clips.
If images are sent offline, thumbnails of the images should be uploaded as a submission item online, together with the manuscript, to allow the publishers to reserve space for the original high-resolution images. All material should be sent to the editorial office:

The International Journal of Ozone Therapy
Centauro S.R.L.
Via del Pratello, 8
I-40122 Bologna – Italy
Tel: +39.051.227634
Fax: +39.051.220099
E-mail: marco.leonardi@centauro.it

INTERNATIONAL JOURNAL OF OZONE THERAPY

Conflicts of Interest/Disclosures, Copyright Transfer Agreement, Financial Support, Exclusive Publication Statement, and Author Contribution Form

Manuscript No.: _____ Date: _____

With respect to the article entitled: _____

The authors certify that:

(*Conflicts of Interest/Disclosures*) The corresponding author must disclose all relevant financial, personal, and/or professional relationships with other people or organizations related to the subject of this article. Is there any actual or potential conflict of interest in the subject of this article?

No (___). If yes, please give details in the space below.

_____

(*Copyright Transfer Agreement*) All rights, title, and interest in the manuscript, including copyright ownership, are transferred to Centauro Srl Publisher, Bologna. Authors reserve all proprietary rights (such as patent rights) other than copyright and the right to use parts of this article in their own subsequent work as soon as the article is accepted for publication. The article may not be published elsewhere without permission from the publisher.

(*Financial Support*) If the authors or their institution(s) is/are affiliated with any organization having a financial interest (including the provision of financial support) in the subject of this article, the authors should indicate (a) the name of the industry/institution providing support for the study, (b) the type of affiliation of the organization (stockholder, consultant, donor of honorarium, granting body, source of other financial or material support, provider of equipment, or other support category), and (c) that the authors had complete control of the data and information submitted for publication. Any other non-financial conflict of interest should also be disclosed to the Editor, with the understanding that the information may be published if deemed appropriate by the Editor.

No, I (We) do not have any conflict of interest related to this article (___).

If yes, (a) (_____) (b) (_____) (c) (_____).

(*Exclusive Publication Statement*) None of the material in this manuscript has been published previously or submitted for publication elsewhere prior to appearance in NRJ.

(*Author Contribution Form*) The corresponding author must certify that all authors participated in and contributed sufficiently to the conception and design of the work, and analysis of the data, as well as the writing of the manuscript, and assume public responsibility for the content of the paper. The corresponding author also certifies that all authors have reviewed the final version of the manuscript and approved it for publication.

Name (print) Signature Date

This signed statement must be received before the manuscript can be processed for publication.

Rivista di proprietà EDIZIONI DEL CENTAURO S.r.l. - Edita da CENTAURO S.r.l. - Sede legale: Via del Pratello, 8 – I-40122 Bologna
C.F. e P. IVA 01896531207 – Cap. soc. Euro 15.600.00 i.v. – Reg. Impr. BO – R.E.A. BO n. 397358

Now, webfio.it is ... online ... renewed.

ALNITEC

OZONE IN THE WORLD

EXCELLENT FEATURES: **120 µg/ml**, **9,5 Kg** (Portable Module), extractable from the trolley. No knobs. Totally automatic.

ACTIVE PHOTOMETER: Reads the ozone concentration and keeps it constant always. Checks to work inside strict tolerances with total safety.

10 MEMORIZABLE CONCENTRATIONS: To select 10 selections inside 120 concentrations.

10 SEQUENCES: In a Sequence it is possible to jump from a memorized concentration to another with a touch, to avoid dead times.

BUBBLER H_2O+O_3: With Five Programs to ozonize the water, with a timer. It calculates the concentration of ozone in the water after the bubbling.

BUBBLER OIL+O_3: With a Software Program to ozonize the oil, with a timer.

VACUUM PUMP: Portable. For topical applications. **7 Kg**.

OZONE-PROOF MATERIALS: To avoid contamination of the output gas.

CERTIFICATIONS: MEDICAL DEVICES 93/42/EEC Directive.
EC Quality Assurance System Certificate, according to Annex II of 93/42 EEC Directive.
CE 0476

ALNITEC S.rl. - Via delle Industrie, 14 - 26010 Cremosano (CR) - Italy
Tel. +39 0373 274366 - Fax +39 0373 473581 - www.alnitec.it - e-mail: info@alnitec.it