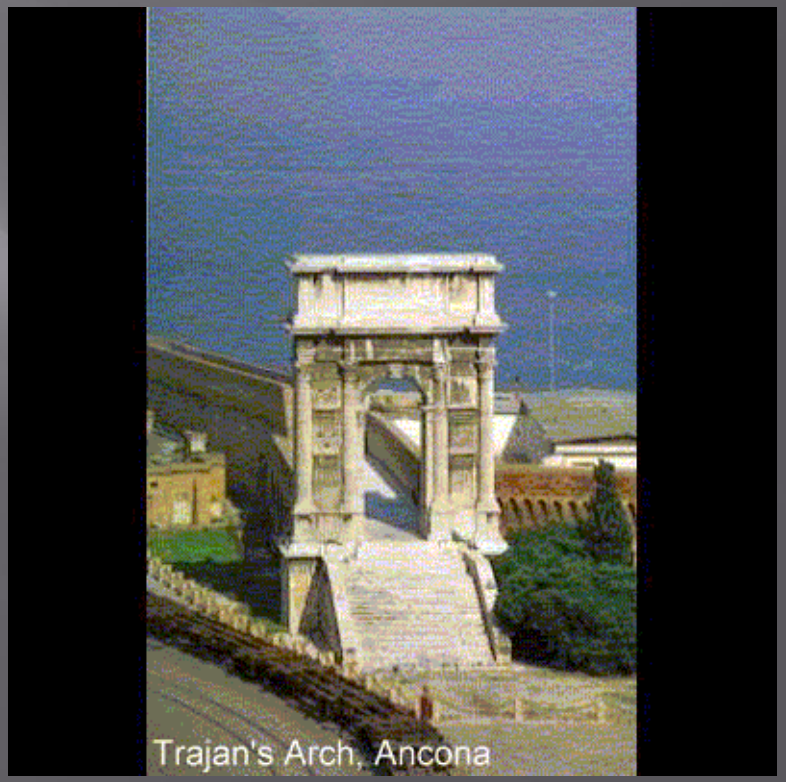
THE DEVELOPMENT OF OZONE THERAPY FROM THE EMPIRICISM TO SCIENCE

LAMBERTO RE

UNIVERSITY OF ANCONA

CLINICAL PHARMACOLOGY & TOXICOLOGY

WWW.LAMBERTORE.COM



INTRODUCTION

Our first interest on ozone therapeutic potential started in 1993 after a discussion on the hypothesis of a positive conditioning induced by low ozone concentrations against the oxidative stress.

Subsequently the same Colleague Leon Fernandez O.S. and Prof. Bocci proposed the basic evidence of some biochemical effects induced by low ozone doses (***Mediators-Inflamm. 7(4): 289-94, 1998***).

The theory was based upon the fact that the exposure to low, non-toxic, ozone concentrations could increase the efficacy of the endogenous antioxidant system by increasing the production or the activity of some enzymatic isoforms.

Similarly to the ischemic preconditioning in which it is scientifically proved that *repetitive brief ischemia* plays an important role in the acquisition of late-phase cardio protection against ischemia/reperfusion injury in rats (***Yamashita et al, Br J Pharmacol, 131(3): 415-422, 2000***), it could be speculate that *repetitive brief oxidative stress* induced with low ozone doses could ameliorate the cell defenses mechanisms against reactive oxygen species (ROS).

The hypothesis was supported by other data reported by Rao and Shaha (***Free Radic Biol Med, Nov 15; 29 (10): 1015-1027, 2000***) demonstrating the formation of multiple isoforms of glutathione S-transferase after the exposure to H_2O_2.

Molecular hypotheses

Due to our experience as pharmacologist and with previous research devoted mainly to the study of the molecular events underlying the pharmacological action of drugs, our group was initially attracted by a pure scientific curiosity.

The main questions addressed to:

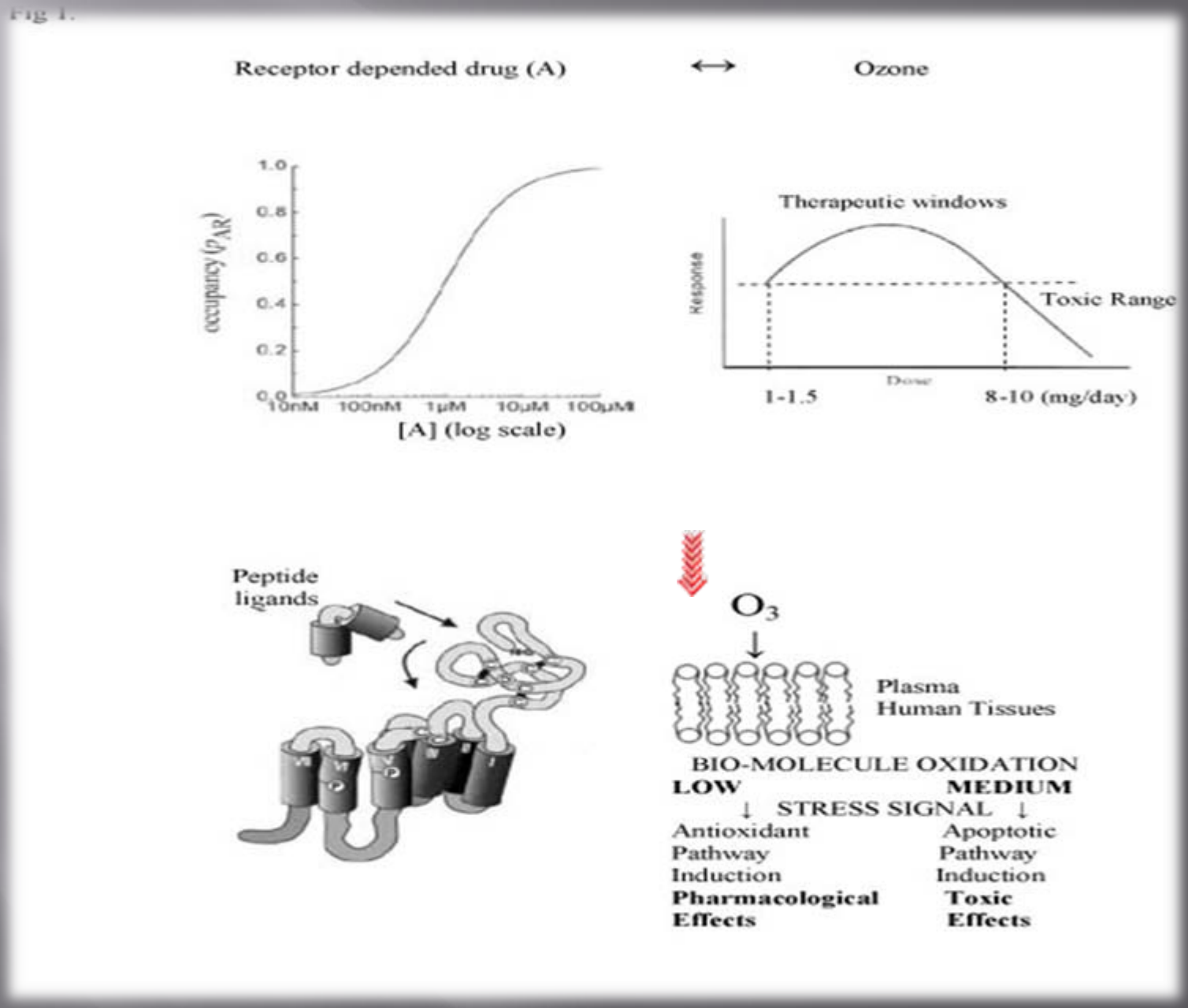
Why many epidemiological data reported the evidence of a benefit of this gas in different, apparently unrelated, pathologies?

Was there a dose-effect relationship?

Was this therapy completely safe?

Why an agent known for its strong oxidative potential could induce a benefit other than its intrinsic bactericidal action?

Fig 1.



L Re, Martínez-Sánchez G. Drug Response (2010) in press

Interaction with Conventional Medicine

In the last years Ozone Therapists have been negatively focused for the lacking of rigorous scientific data concerning the use of Medical Ozone.

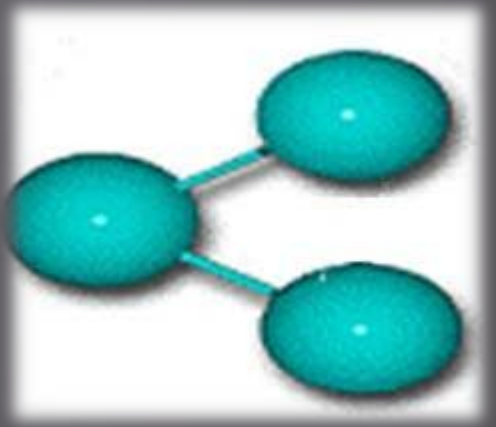
Conversely, looking at the wide epidemiological data mainly obtained following the Evidence Based Medicine (EBM) protocols we think that this therapy could represent a very important and useful complement in many medical fields.

Without any doubt, most of the problems arise from the first empiric use and approach to Ozone by the same Ozone Therapists!

What is Ozone?

Ozone (O_3 - PM 48) is an allotropic form of oxygen.

It represents an extremely unstable molecule characterized by 3 atoms of oxygen.



To temperature environment is a gas, colorless with sharp and prickly odor.

It is an extremely important mixture for the life on the earth and one of the fundamental components of the atmosphere.

As stated above emphasis and attention has been focused on the use of medical ozone.

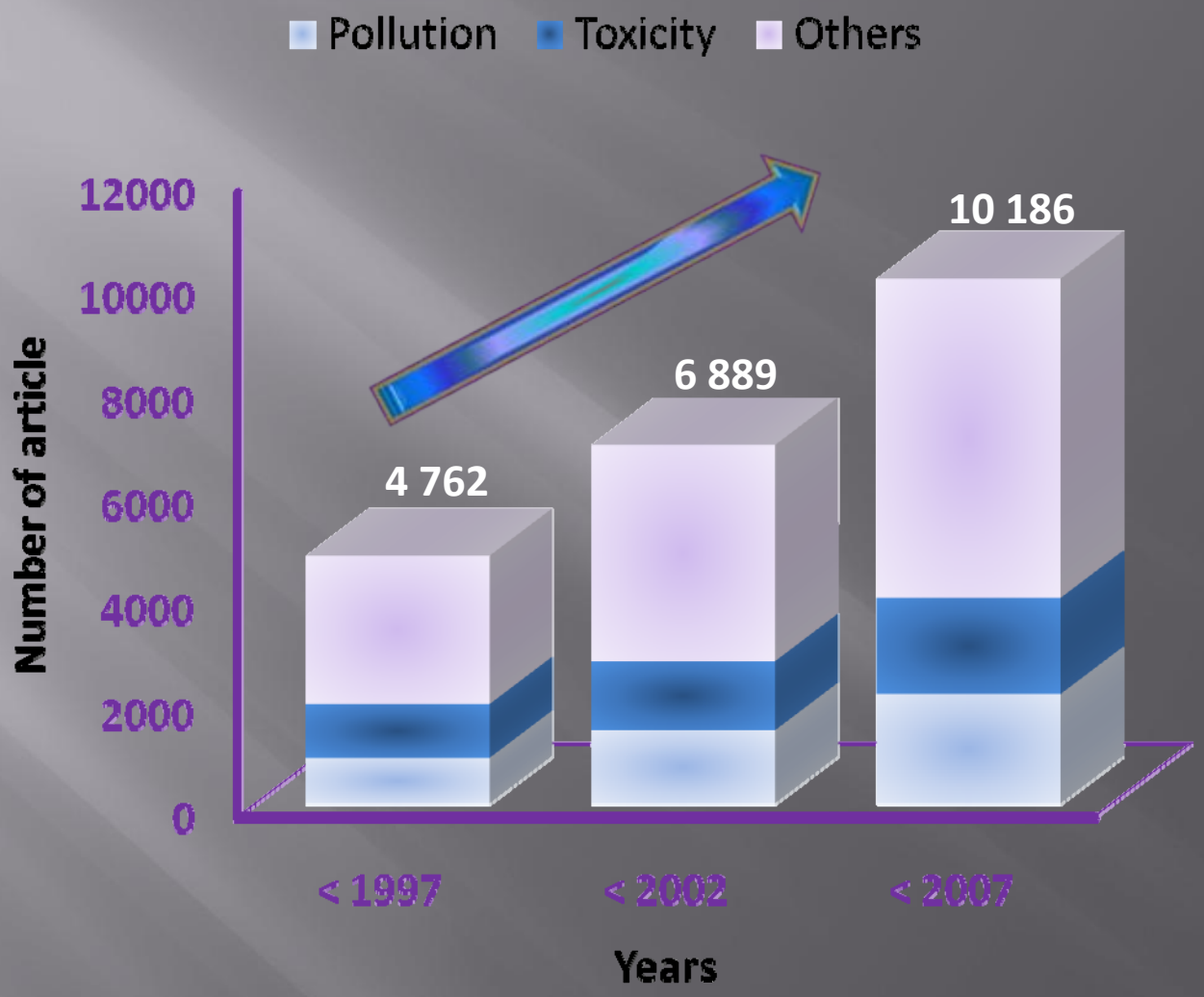
Despite ample clarification confusion still persists concerning its potential toxicity as an oxidant agent versus the reported clinical efficacy.

This confusion is a major factor preventing a more widespread acceptance.

Furthermore the use in specialities so diverse as neurology, orthopaedy, internal medicine, sport medicine, endocrinology and others makes difficult to categorize ozone as a therapeutic agent.

This may cause conflicts between the different fields of application and the various medical areas.

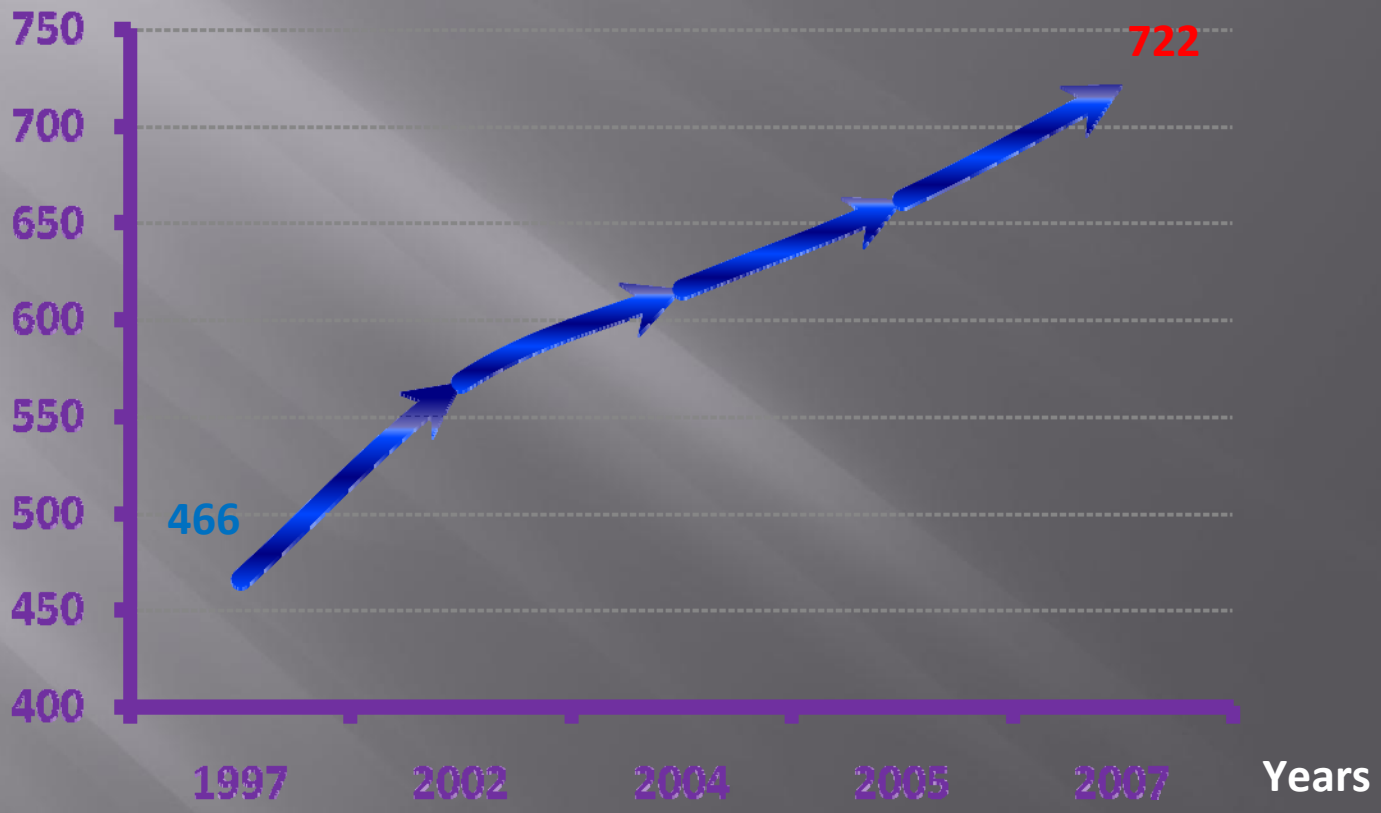
NUMBER OF ARTICLES PER YEAR, RELATIVE TO "OZONE
(CUMULATIVE)



Data from MedLine PubMed, October 2007

AVERAGE IN NUMBER OF ARTICLES PER YEAR, RELATIVE TO "OZONE IN MEDICINE"

(LAST TEN YEARS. ARTICLES NOT RELATED TO OZONE POLLUTION OR TOXICITY)

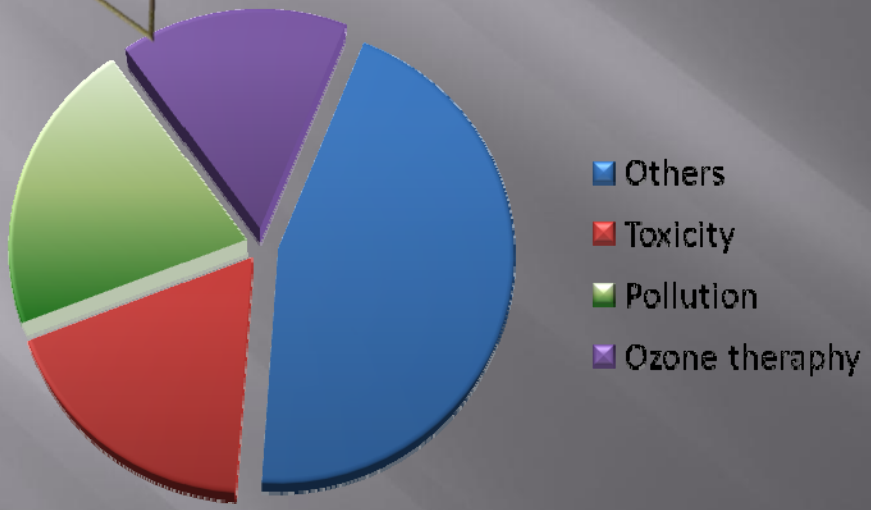


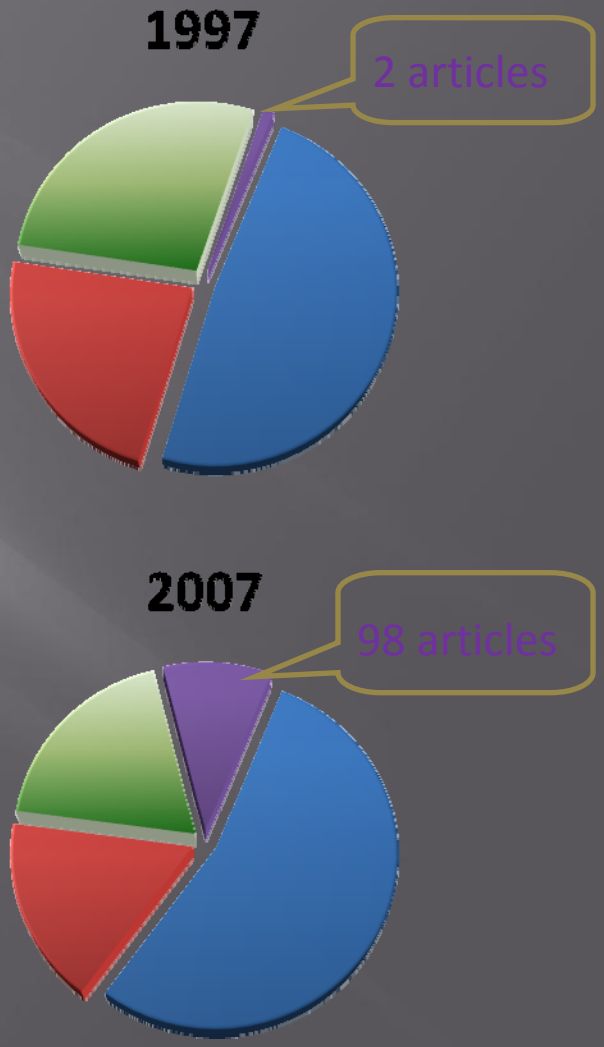
Data from MedLine PubMed, October 2007

EVOLUTION IN NUMBER OF ARTICLES RELATIVE TO "OZONE / CLINICAL TRIALS"

(ALL RECORDS AND COMPARATIVE EVOLUTION BETWEEN 1997 AND 2007

Includes **243** papers on Clinical Trials



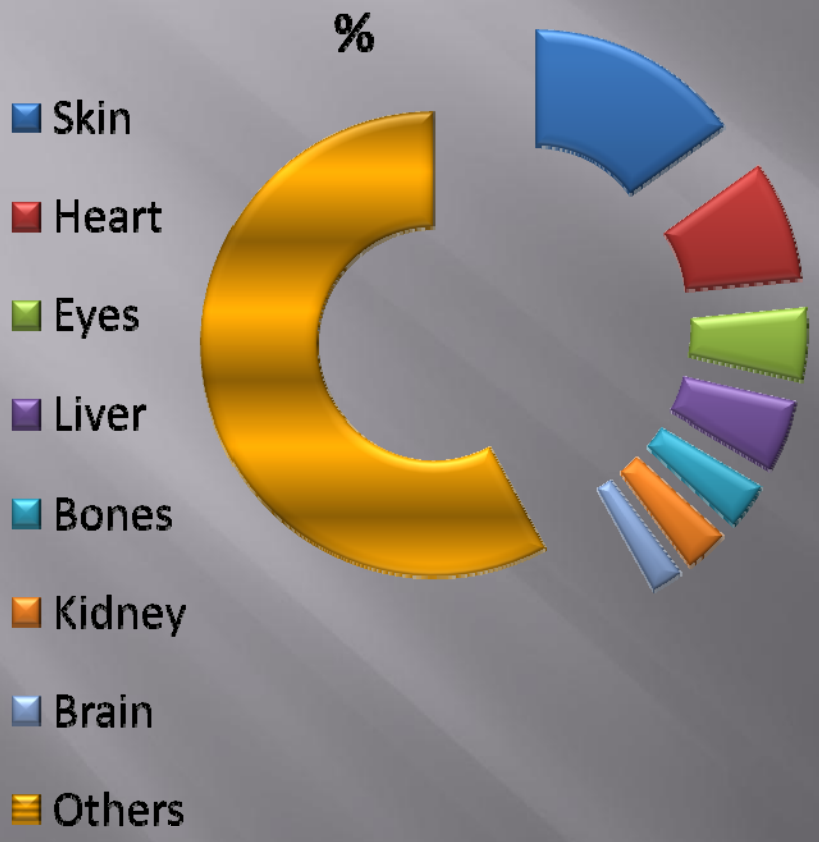


Data from MedLine PubMed, October 2007

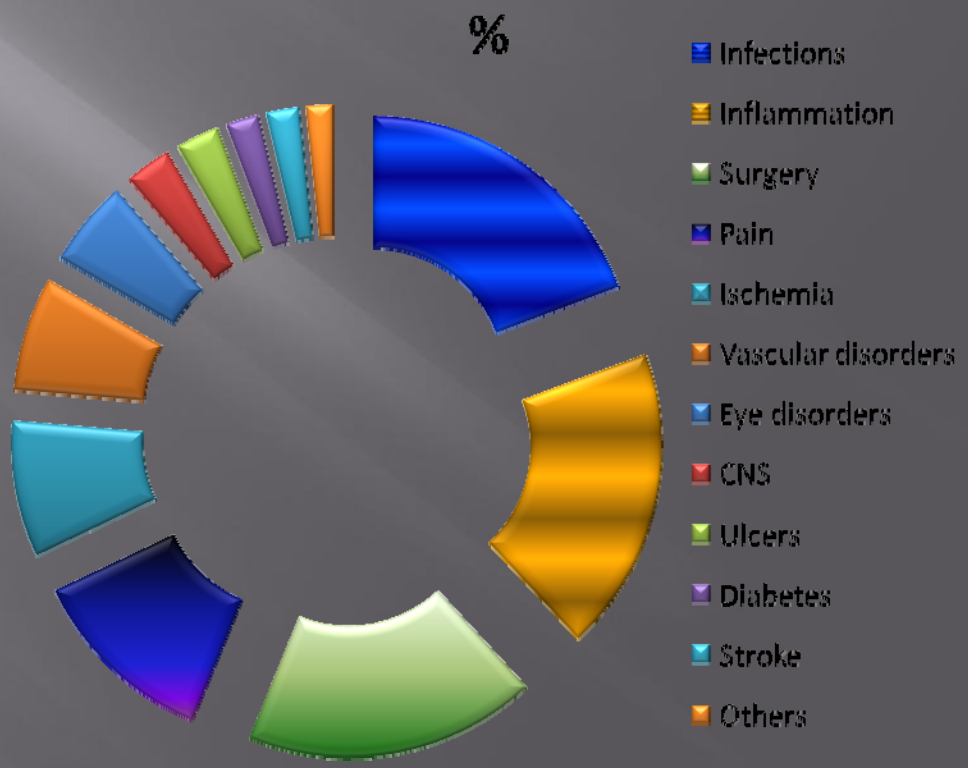
MAIN TARGET DISEASES / ORGANS OF "OZONE THERAPY

(CUMULATIVE NUMBER OR ARTICLES UP TO OCTOBER 2007)

Main Target Organs



Main Target Diseases



Data from MedLine PubMed, October 2007

Ozone is commonly associated with intervertebral disc herniation (***Andreula et al., Am J Neuroradiol. 2003 May;24(5):996-1000***) and only recently was introduced in the field of stomatology.

Regarding disc herniation and the so called disc conflict, most of the ozone actions are bound to its peculiar effects on the bio-humoral environment.

The mistaken view to consider ozone a simple mechanistic agent causing the lyses and the reduction of the herniated disc must be, to our opinion, argued and discussed.

The application of ozone in neuro-radiology is considered scientific and successful based on the possibility to estimate statistically the reduction or the disappearance of the anatomical protrusion.

We think that this is not proof enough.

Indeed, recent works indicate that the symptoms are not strictly bind to the volume of the herniated disc.

Moreover, the anatomical presence of an herniated disc or its removal with surgery, not always is correlated with pain.

The positive effects induced by ozone therapy, usually long lasting and not simply bind to the period of the treatment, indicate a different action exerted by this gas.

A follow up considering the status of the patient following the treatment could better indicate the efficacy or not of the more invasive ozone treatments or surgery compared to the minimal invasive techniques based on the neuro-humoral action of ozone such as para-vertebral or percutaneous injections.

At the light of the more recent pharmacological knowledge we can consider ozone as a pro-drug which, at certain non-toxic doses, can induce a rearrangement of the biochemical pathways with the activation of second messengers in a cascade with a multiple system action.

Ischemic Preconditioning (IPC) represents the best similarity in this context.

Evidence that antioxidant enzymes, nitric oxide pathways and other sub-cellular activities could be modulated by low ozone doses is now well assessed and could support the surprising effects of ozone in many pathological conditions.

Furthermore the data reported by Wentworth *et al*. (***Science 2002; 298: 2195-2199***) are scientifically indicative of some pharmacological actions.

Indeed, the authors demonstrate the physiological presence of an ozone-similar mediator during inflammation, indicating ozone as a new bio-molecule with striking effects which must be considered and studied following new strategies with newly constructed randomized-standardized clinical studies.

Other evidences related to the protective action induced by low Ozone concentrations demonstrated the modulation of the intracellular calcium.

The cytosolic calcium could be considered as the common final pathway of the cellular activation and an impairment of its intracellular levels could promote damage.

A low calcium level represents a further element in supporting the idea of a protection against the oxidative cell damage either in the chronic (physiological) or in the acute (pathological) ageing.

Similarly to the IPC the acronyms OzoneOP was introduced to define the effects induced by the Ozone Oxidative Preconditioning.

Ozone and Diabetes

Basic Studies

Diabetes produces a large number of changes in vessels that affect the reactivity of smooth muscle and endothelium. Vascular endothelium appears to be a vulnerable target for hyperglycemia-induced metabolic changes. Activation of the polyol pathway, non-enzymatic glycosilation of proteins and the increase of reactive oxygen species (ROS) play an important role in diabetes complications.

Ozone has been used as a therapeutic agent and beneficial effects have been observed. However, so far only a few biochemical and pharmacodynamic mechanisms have been elucidated.

Some studies (*Mediators of Inflammation 1998; 7:289-294; Free Radical Research 1999;31:191-196*) confirmed that controlled ozone administration may promote an oxidative preconditioning or adaptation to oxidative stress, preventing the damage induced by ROS.

Given that diabetes is a disorder associated with oxidative stress, it was postulated that ozone treatment might protect antioxidant systems and maintain at a physiological level other markers of endothelial cell damage associated with diabetic complications. A study using streptozotocin (STZ) as a diabetes inducer was designed to test the protective effect promoted by ozone.

Ozone treatment improved glycaemia control, the increase of aldose reductase, the fructolysine content, the advanced oxidation protein products, the pancreas integrity and prevented the oxidative damage. Furthermore, increased nitrite and nitrate levels with respect to STZ group occurred, but without changes when compared to non-diabetic controls. The results of this study show that repeated administration of ozone in non-toxic doses might play a role in the control of diabetes and its complications (*Pharmacological Research 2001;44: 391-396*).

In addition, ozone antioxidant properties preserved β-cells functions and reduced hyperglycaemia. Taken together, these results suggest that this approach may represent a potential complement in the treatment of diabetes and its complications.

Clinical Studies

Because ozone therapy can activate the antioxidant system, influencing the level of glycaemia and some markers of endothelial cell damage at a pre-clinical level, a study to investigate the therapeutic efficacy of ozone treatment in patients with type 2 diabetes and diabetic foot was done in the aim to compare the ozone effects with respect to the antibiotic therapy.

A randomized controlled clinical trial was performed with 101 patients divided into two groups: one (n=52) treated with ozone (local and rectal insufflation of the gas) and the other (n=49) treated with topical and systemic antibiotics.

The efficacy of the treatments was evaluated by comparing the glycaemia index, the area and perimeter of the lesions, the biochemical markers of oxidative stress and the endothelial damage in both groups after 20 days of treatment.

Ozone improved glycaemia control, prevented oxidative stress, normalized levels of organic peroxides and activated superoxide dismutase.

The effect of ozone in the treatment of patients with neuroinfectious diabetic foot can be ascribed to its action as a superoxide scavenger. Superoxide is considered a link between the four metabolic routes associated with diabetes pathology and its complications.

In the study the healing of the lesions improved resulting in fewer amputations than in the control group (40%).

There were no side effects. These results reinforce the opinion that medical ozone treatment could be a complementary therapy in the treatment of diabetes and its complications (***European Journal of Pharmacology 2005; 523:151-161***).

Ozone and SOD

Many studies indicate that, after reoxygenation of the liver, oxygen free-radical formation may initiate the cascade of hepatocellular injury, necrosis/apoptosis, and subsequent infiltration of inflammatory cells. Although ROS can arise from a number of sources, xanthine oxidase (XO) is frequently implicated as a significant source of these toxic oxygen species.

The IPC is an inducible and potent endogenous mechanism by which repeated episodes of brief ischemia/re-perfusion (I/R) confer a state of protection against subsequent sustained I/R.

On the other hand, it has been demonstrated that ozone at low doses is able to promote an OzoneOP through the increase and preservation of antioxidant endogenous systems.

Superoxide is one of the most relevant radicals in biological regulation. Many regulatory effects are mediated by hydrogen peroxide and other ROS that are chemically derived from superoxide (*Physiol Rev 2002; 82: 47-95*).

Although SOD could protect against liver I/R injury, the administration of SOD does not protect the liver against I/R damage (*J Surg Res 1997;73:160-165*).

The protein SOD degrades rapidly when administered parenterally. Gene delivery has been used to increase protein expression in the cell (*Pathology 1998; 30:335-347*).

OzoneOP is able to promote a moderate oxidative stress which, in turn, increases antioxidant endogenous systems protecting against liver damage (*J Appl Toxicol 2001;21: 291-301*). The protective mechanism mediated by OzoneOP may involve protein synthesis. Elevated ROS concentrations induce in many cells the expression of genes whose products exhibit antioxidative 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.

To investigate the influence of the inhibition of protein synthesis on the protective actions conferred by OzoneOP in hepatic I/R, rats were treated with cicloheximide in order to inhibit protein synthesis before OzoneOP treatment. Plasma transaminases, malondialdehyde + 4-hydroxyalkenals (MDA + 4-HDA) and morphological characteristics were measured as an index of hepatocellular damage; Cu/Zn-SOD, Mn-SOD, catalase (CAT), total hydroperoxides (TH) and reduced glutathione (GSH) levels as markers of endogenous antioxidant system were evaluated.

OzoneOP increased Mn-SOD isoform and ameliorated mitochondrial damage. Conversely, cicloheximide abrogated the protection conferred by OzoneOP and decreased Mn-SOD activity. Cellular redox balance disappeared when cicloheximide was introduced. Thus, protein synthesis is involved in the protective mechanisms mediated by OzoneOP and ozone treatment preserved mitochondrial functions and cellular redox balance (***Transplant International 2005;18:604–612***).

SOD Linked Pathologies

SOD helps and protects cells from DNA damage, lipid peroxidation, ionizing radiation damage, protein denaturation, and many other forms of progressive cell degradation.

Mutations in the first SOD enzyme (SOD1) have been linked to familial amyotrophic lateral sclerosis (ALS, a form of motor neuron disease).

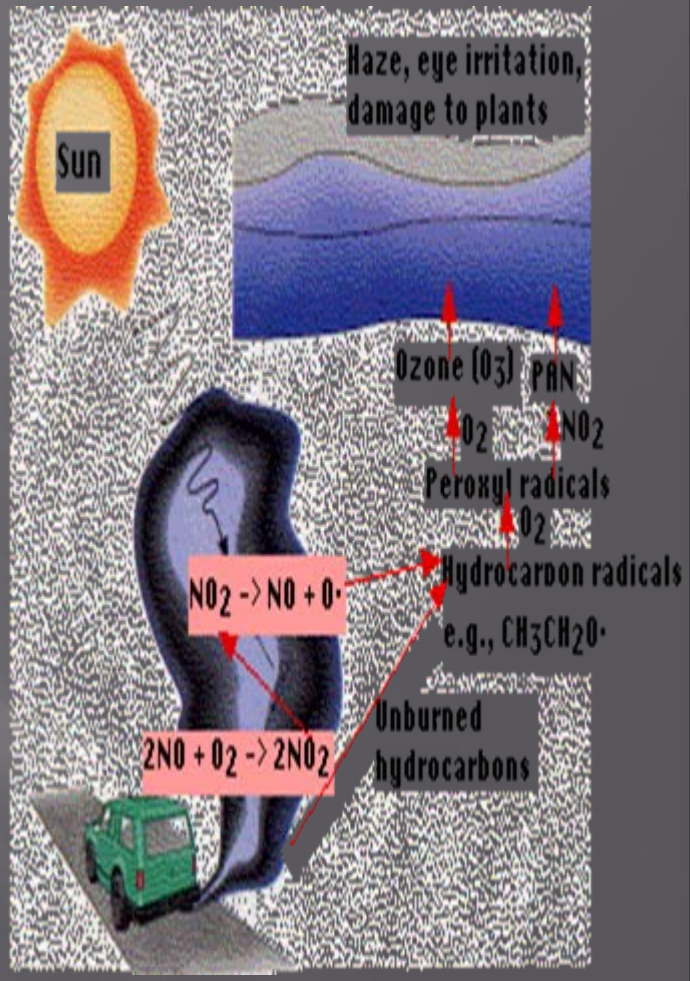
The other two types have not been linked to any human diseases, however, in mice inactivation of SOD2 causes perinatal lethality and inactivation of SOD1 causes hepatocellular carcinoma.

Once more we think that enough evidences are now outlined demonstrating the positive effects induced by Ozone. Taken together, they could represent the key to understand the surprising effects induced by low Ozone doses.

Ozone and Nitric Oxide (NO)

Nitric oxide is a gas. It is highly reactive; that is, it participates in many chemical reactions.

(It is one of the nitrogen oxides ("NOx") in automobile exhaust and plays a major role in the formation of **photochemical smog**.)



But NO also has many physiological functions.
They share these features:
NO is synthesized within cells by an enzyme **NO synthase** (**NOS**). The human (and mouse) genome contains 3 different genes encoding NO synthases.
nNOS (or NOS-1): found in neurons.
iNOS (or NOS-2): found in macrophages, "i" standing for "inducible".
eNOS (or NOS-3): found in the endothelial cells that line the lumen of blood vessels.

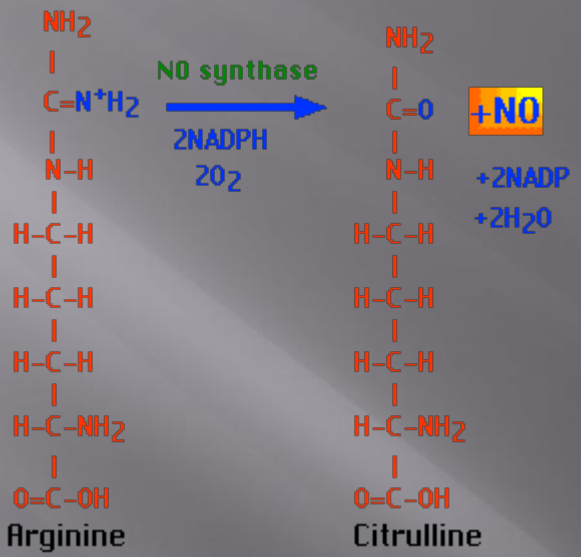
Whereas the levels of nNOS and eNOS are relatively steady, expression of iNOS genes awaits an appropriate stimulus (e.g., ingestion of a parasite).

Liver transplantation is now accepted as the best treatment for end-stage liver disease. Nevertheless, hepatic I/R injury associated with liver transplantation and hepatic resections are unresolved problems in clinical practice.

Many studies indicate that oxygen free-radical formation after reoxygenation of liver may initiate the cascade of hepatocellular injury.

The effects of OzoneOP on NO generation and the cellular redox balance have been studied using the inhibitor of the NO synthesis N^{ω}-nitro-L-arginine methyl ester (L-NAME) (*Liver International 2004;24:55-62*).

All types of NOS produce NO from arginine with the aid of molecular oxygen and NADPH.



NO diffuses freely across cell membranes.

There are so many other molecules with which it can interact, that it is quickly consumed close to where it is synthesized.

The product N^{ω}-nitro-L-arginine methyl ester (L-NAME) is an inhibitor of the NO synthesis and it has been used in the experimental study.

The following parameters were measured:

- plasma transaminases (aspartate aminotransferase, alanine aminotransferase) as an index of hepatocellular injury;
- nitrate/nitrite levels and inducible Nitric Oxide Sintase (iNOS) by immuno-histochemistry as an index of ·NO production;
- SOD, CAT and GSH levels as markers of the endogenous antioxidant system, and finally MDA + 4-HDA, TH and Tumor Necrosis Factor (TNF-α) as indicators of oxidative stress.

A correspondence between liver damage and the increase of ·NO, CAT, TH, GSH and MDA + 4HDA concentrations were observed along with a decrease of SOD activity.

OzoneOP prevented and attenuated hepatic damage in OzoneOP +I/R and OzoneOP+L-NAME+I/R, respectively, in close relation with the above-mentioned parameters.

Immunohistochemistry of iNOS showed that OzoneOP regulated enzymatic activity while TNF-α levels were attenuated in the OzoneOP + I/R group.

These results show that OzoneOP protected against liver I/R injury through mechanisms that promote a regulation of endogenous ·NO concentrations and the maintenance of cellular redox balance. Ozone treatment may have important clinical implications, particularly in view of the increasing hepatic transplantation programs.

There are different experimental results and opinions surrounding NO generation and its function in liver I/R injury as well as its protective effects. Nevertheless, the role of NO as a regulator of important processes in liver I/R is unquestionable.

OzoneOP regulated NO formation in the OzoneOP + I/R group and decreased the liver damage (increases in AST was prevented and those in ALT were attenuated). L-NAME was able to reduce NO generation in sham operated + L-NAME and NO levels were not detectable in L-NAME + I/R group.

Nevertheless, OzoneOP promoted NO formation in OzoneOP + L-NAME + I/R in spite of L-NAME´s presence, but lesser than OzoneOP + I/R.

There was a concomitant increase in transaminase activities in this group (OzoneOP + L-NAME + I/R).

These results suggest that the protection conferred by OzoneOP against the damage in liver I/R is mediated, at least in part, by NO generation.

The contribution of OzoneOP to NO generation may be a consequence of its actions on gene expression.

Punjabi *et at* (***Am J Respir Cell Mol Biol 1994;11:165-172***) and Pendino *et al* (***Am J Respir Cell Mol Biol 1996;14:516-525***) have shown that exposure to ozone causes NO production in macrophages and type II cells of rats, whereas Haddad et al. (***Eur J Pharmacol 1995;293:287-290***) demonstrated iNOS induction in rats.

More recently it has been found that ozone-induced lung hyperpermeability is associated to iNOS and that iNOS mRNA levels are mediated through Tlr-4 which has been identified as the gene that determines susceptibility to endotoxins.

There was a correlative patterns of gene expression in two strains (ozone-susceptible and ozone-resistant, respectively) which support a role of Tlr4 in the regulation of iNOS during ozone exposure in the mouse (***Am J Physiol Lung Cell Mol Physiol 2001;280: L326-L333***).

NO effects

The following reported effects of NO are surprising similar to those induced by the treatments with Ozone at low concentrations.

Throughout our clinical experience over more than 15 years we wondered again when our scientific data well complement the clinical effects observed during patients treatment.

NO on Blood Flow

NO relaxes the smooth muscle in the walls of the arterioles. This diffuses into the underlying smooth muscle cells causing them to relax and thus permit the surge of blood to pass through easily.

Mice whose genes for the NO synthase found in endothelial cells (eNOS) has been "knocked out" suffer from hypertension.

Nitroglycerine, which is often prescribed to reduce the pain of angina, does so by generating nitric oxide, which relaxes the walls of the coronary arteries and arterioles.

Three of the pioneers in working out the biological roles of NO shared a Nobel Prize in 1998 for their discoveries. The award to one of them, Ferid Murad, honoured his discovery that nitroglycerine works by releasing NO. This seems particularly appropriate because Alfred Nobel's fortune came from his invention of making dynamite from nitroglycerine! NO also inhibits the aggregation of platelets and thus keeps inappropriate clotting from interfering with blood flow.

NO on Inflammation

The NO produced by NOS-3 inhibits inflammation in blood vessels. It does this by blocking the exocytosis of mediators of inflammation from the endothelial cells.

NO may also block exocytosis in other types of cells such as macrophages and cytotoxic T lymphocytes (CTL).

NO Killing Pathogens

NO aids in the killing of engulfed pathogens (e.g., bacteria) within the lysosomes of macrophages.

Mice whose genes for the NO synthase found in macrophages (iNOS) have been knocked out are more susceptible to infections by intracellular bacteria like Listeria monocytogenes.

Th1 cells, the ones responsible for an inflammatory response against invaders, secrete NO.

Harmless bacteria, living as commensals at the rear of our throat, convert nitrates in our food into nitrites. When these reach the stomach, the acidic gastric juice (pH ~1.4) generates NO from them. This NO kills almost all the bacteria that have been swallowed in our food.

(Since the dawn of recorded human history, nitrites have been used to preserve meat from bacterial spoilage.)

NO and Longevity

- Mice whose genes for eNos have been knocked out;
- show signs of premature ageing;
- have a shortened life span;
- fail to benefit from the life-extending effect of a calorie-restricted (CR) diet.

Ozone and Purinergic Receptors

The liver is damaged by sustained ischemia in liver transplantation, and the reperfusion after ischemia results in further functional impairment as widely showed before.

In view that OzoneOP protects the liver against ischemia/reperfusion (I/R) injury, a recent study was conducted to investigate the role of A1 adenosine receptor on the protective actions conferred by OzoneOP in hepatic I/R (***Leon-Fernandez et al, in press, Transplant Int, 2007***).

By using a specific agonist and antagonist of the A1 subtype receptor (2-chloro N6 cyclopentyladenosine, CCPA and 8-cyclopentyl-1,3-dipropylxanthine, DPCPX respectively), the authors studied the role of A1 receptor in the protective effects of OzoneOP on the liver damage, NO generation, adenosine deaminase activity and preservation of the cellular redox balance.

Immunohistochemical analysis of Nuclear Factor-kappa B (NF-κB), Tumor Necrosis Factor alpha (TNF-α) and Heat Shock Protein 70 (HSP-70) was performed.

OzoneOP significantly prevented and/or ameliorated ischemic damage.

CCPA showed a similar effect to OzoneOP + I/R group.

A_1AR antagonist DPCPX blocked the protective effect of OzoneOP.

OzoneOP largely reduced the intensity of the NF-κB (p65 subunit), diminished TNF-α production, and promoted a reduction in HSP– 70 immunoreactivity.

The work demonstrated that OzoneOP exerted protective effects against liver I/R injury also promoting an activation of the A1 adenosine receptors (A_1AR).

Adenosine and NO produced by OzoneOP may play a role in the pathways of cellular signaling which promote preservation of the cellular redox balance, mitochondrial function, glutathione pools as well as the regulation of NF-κB and HSP-70.

The effects of OzoneOP on adenosine and NO have a particular importance in cellular signaling processes. Carini et al (*Gastroenterology 2003; 125:* 1480) have proposed that both biomolecules are involved in the mechanisms leading to the development of hepatocyte resistance to I/R injury following early and late hepatic preconditioning.

These protective mechanisms include: preservation of energy sources, mitochondrial functions, pH, ion homeostasis as well as to reduce oxidative injury and caspase activation.

OzoneOP actions preserved mitochondrial integrity (*Transplant International 2005; 18: 604*) and reduced generations of protons and lactate concentrations by anaerobic glycolysis (*J Appl Toxicol, 2001; 21: 297*).

In summary, OzoneOP exerts protective effects against liver I/R injury through activation of A_1AR. In analogy to IPC, adenosine and NO produced by OzoneOP may play a role in the cell signalling pathways which promote preservation of cellular redox balance, mitochondrial function, glutathione pools, regulation of NF-κB and HSP-70, among other effects.

OzoneOP may be considered as a pharmacologic liver preconditioning which might be particularly relevant for improving liver transplantation.

The finding that the effects of OzoneOP are mediated by A_1AR allows considering other potential medical applications for the ozonetherapy mainly in cardiovascular and central nervous systems.

Ozone and Parkinson`s Disease

A recent work evaluated the effects of OzoneOP on an *in vivo* model of rotenone-induced neuro-degeneration in rats.

Oxidative stress has been implicated in numerous pathophysiological situations (***J Neurosci 2004;24:7779-7788***) being considered a unifying factor in the current theories of Parkinson's disease (PD) pathogenesis.

This is because of the links between genetic and potential environmental factors in the onset and progression of the disease. Those environmental toxins that have the strongest association with PD phenotypes either cause high amounts of oxidative stress, such as rotenone, or directly increase the rate of alpha-synuclein aggregation, as with copper and other heavy metals (***J Biol Chem 2001;276:44284-96***).

Furthermore, the aggregation of alpha-synuclein itself can cause oxidative stress (*Free Radic Biol Med 2001;30:1163-1170*) and oxidative stress can in turn cause conformational changes in alpha-synuclein (*Ann N Y Acad Sci 2003;991:93-100*).

Even if the factors initiating the pathogenesis of PD and related neurodegenerative synucleinopathies are still largely unclear, many studies indicate a multiple brain mitochondria dysfunction after systemic treatment with pesticides or rotenone (*Am J Pathol 2007;170(2):658-666; J Biol Chem 2005;280(51):42026-42035; Nat Neurosci 2000;3(12):1301-1306*).

Rotenone is a classical, high affinity inhibitor of complex I, which has been widely used to understand the specific activity of the complex. Rotenone being extremely lipophilic, freely crosses the blood brain barrier and biological membranes, thus rapidly reaching the brain.

Repeated systemic exposure to rotenone has been reported to cause nigrostriatal dopaminergic degeneration in rats, producing an *in-vivo* experimental model of PD (***Antioxid Redox Signal 2005;7:1110-1116***).

The reductions in the activity of complex I of the mitochondrial electron transfer chain (ETC) may play an important role in rotenone-induced dopaminergic neurodegeneration in PD (***J Biol Chem 2004;279:51783-92***).

To evaluate the effect of OzoneOP on PD a study was conducted during a period of four weeks to evaluate the neurochemical effects of repeated exposure to rotenone in rats.

The aim was to test the probable preventive effect of OzoneOP (as an indirect antioxidant) against rotenone-induced neurodegeneration in rats.

It included sixty rats, Sprague-Daley strain, with an average weight of 175 g, divided into four groups:

Group I: The control group.

Group II: The ozone control group. The rats were given 5 ml of 25 μg/ml ozone in oxygen rectal insufflations (0.7 mg/kg). They received 20 sessions: 5 sessions per week for 4 weeks.

Group III: Rotenone was injected subcutaneous in a dose of 2 mg/kg/day every other day for a total of six injections over 11 days.

Group IV: The study group (OzoneOP). The rats were given 5 ml of 25 μg/ml ozone in oxygen rectal insufflations (0.7 mg/kg). They received 20 sessions 5 sessions per week for 4 weeks. After 10 sessions of ozone administration (two weeks) rotenone was injected s.c. at a dose of 2 mg/kg/day every other day for a total of six injections over 11 days.

The rats were observed daily for the development of any signs of toxicity throughout the treatment period. Repeated rotenone treatment caused a marked decrease in both the food intake and the locomotor activity and induced muscle relaxation of both fore and hind limbs accompanied with high mortality rate in comparison to the other treatments.

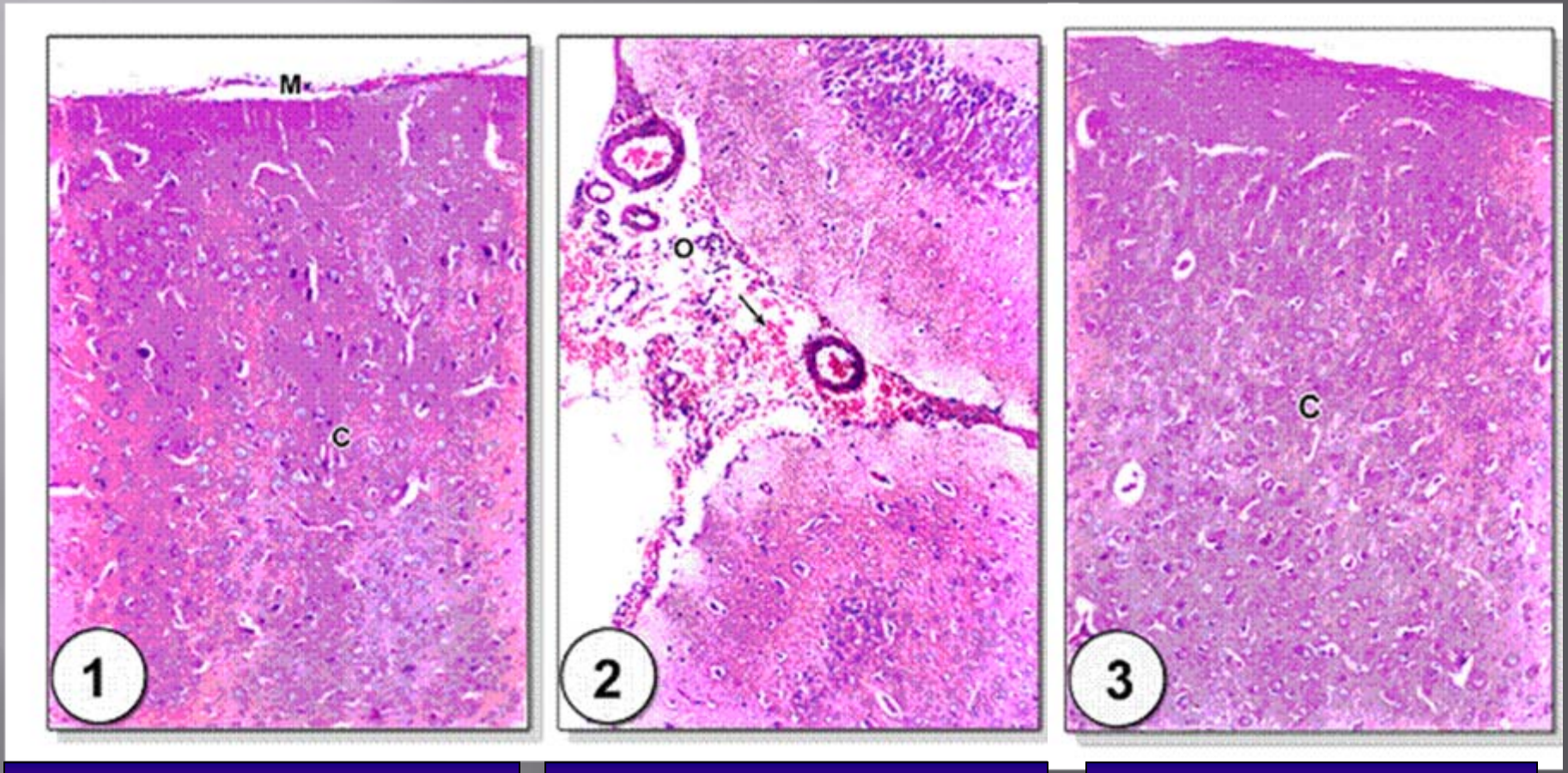
Ozone therapy remarkably increased food intake and rate of weight gain compared to the control group and prevented the mortality of the animals. Repeated treatment with rotenone significantly ($p<0,05$) decreased the levels of dopamine and norepinephrine in both the cortex and striatum. OzoneOP significantly ($p<0,05$) minimized the declining effect of rotenone on the levels of the two transmitters in the cortical and the striatal regions.

In addition, rotenone treatment increased the level of NO, MDA, oxidized glutathione and protein carbonyls of brain cortex and striatum. Rotenone-treated animals exhibited a significant ($p<0,05$) decrease in the level of GSH, ATP and depressed enzymatic activity of SOD.

OzoneOP significantly ($p<0,05$) antagonized the disturbing effect of rotenone on the tested parameters in the cortical and the striatal regions.

Repeated rotenone treatment induced remarkable histopathological abnormalities in brain cortex which are manifested as inflammatory, hemorrhagic and neurodegenerative effects (Fig. 2) in comparison to normal control (Fig. 1).

OzoneOP remarkably attenuated the undesirable histopathological damage induced by rotenone (Fig. 3).



A transverse section of cerebral cortex of control rat showing the normal structure of the meninges (M) and cerebral cortex (C). H& E, x : 40

A transverse section of cerebral cortex of rat injected with rotenone showing sever hemorrhages (arrow), edema (o) and hyperemic blood vessels in the meninges covering cerebral cortex. H& E, x: 40

A transverse section of the cerebral cortex of rat injected with rotenone and ozone showing normal histological structure in the cerebral cortex and surrounding meninges. H& E, x: 40

The study (*Mawsouf, N. et al, Arch Med Res, 2007*) suggests that oxidative stress plays an essential role in rotenone toxicity and that OzoneOP may offer a remarkable protective effect against rotenone induced brain toxicity.

The data presented in this paper are indicative of potentially positive effects induced by treatment with low doses of Ozone. Particularly, an OzoneOP approach could be considered as a positive complement to the actual pharmacological therapies addressed to some pathologies such as diabetes and neurodegenerative disorders promoting the regulation of endogenous NO concentrations and the maintenance of an adequate cellular redox balance.

European Journal of Pharmacology 603 (2009) 42–49

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and Analgesia

A single subcutaneous injection of ozone prevents allodynia and decreases the over-expression of pro-inflammatory caspases in the orbito-frontal cortex of neuropathic mice

Carlo Fuccio [a], Carlo Luongo [b], Paola Capodanno [b], Catia Giordano [a], Maria Antonietta Scafuro [a,b], Dario Siniscalco [a], Biagio Lettieri [b], Francesco Rossi [a], Sabatino Maione [a,*], Liberato Berrino [a]

[a] Department of Experimental Medicine, Section of Pharmacology "L. Donatelli", Faculty of Medicine and Surgery, Second University of Naples, Via Costantinopoli, 16 80138 Naples, Italy
[b] Department of Anaesthesia and Intensive Care, Second University of Naples, Via De Crecchio, 80138 Naples, Italy

ARTICLE INFO

Article history:
Received 20 October 2008
Accepted 26 November 2008
Available online 6 December 2008

Keywords:
Caspases
Orbito-frontal cortex
Allodynia
Ozone therapy
(Mouse)

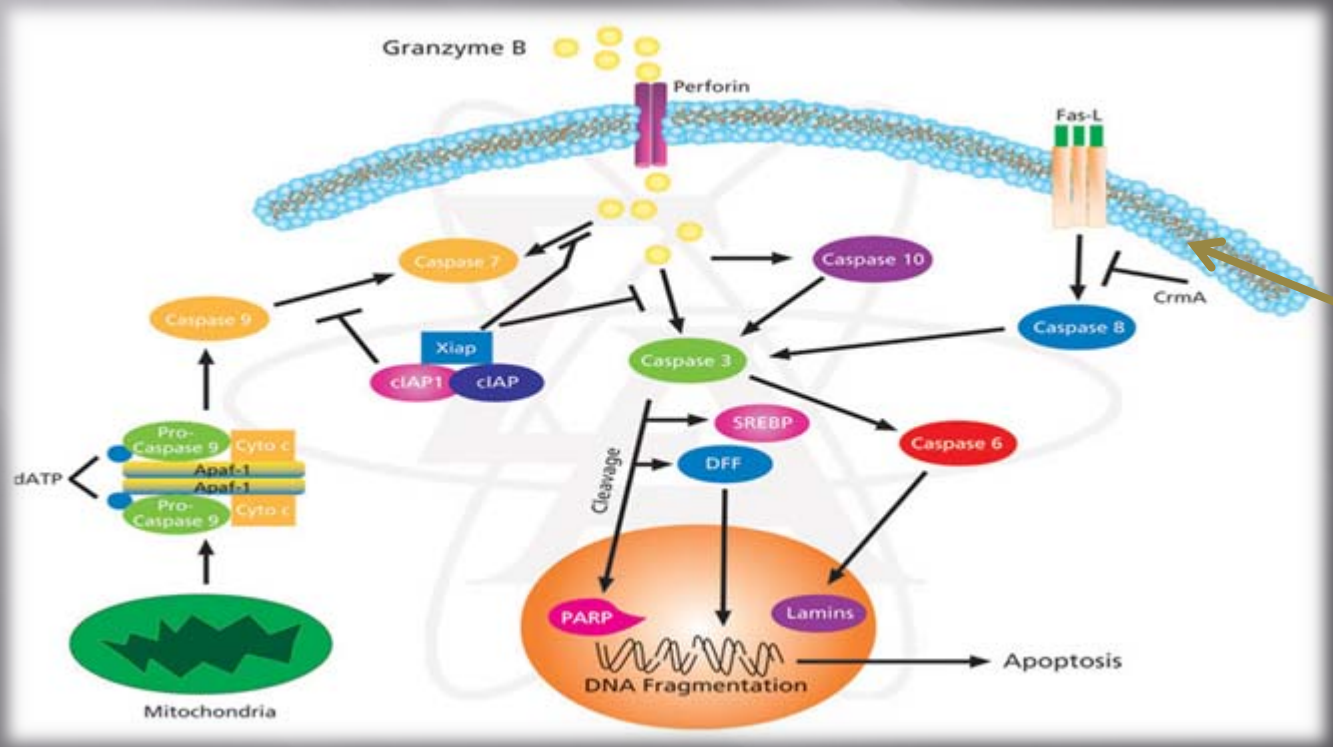
ABSTRACT

The neuropathic pain model consisting of the spared nerve injury of the sciatic nerve was used in the mouse to examine whether peripheral neuropathy is capable of generating over-expression of pro-inflammatory and pro-apoptotic genes in the orbito-frontal cortex, together with allodynia and hyperalgesia. RT-PCR analysis showed increased expression of *caspase-1*, *caspase-12* and *caspase-8* genes in the orbito-frontal cortex 14 days after spared nerve injury of the sciatic nerve. Conversely, the expression of *caspase-3* was decreased by spared nerve injury of the sciatic nerve in the same brain area. A single subcutaneous injection of ozone performed 12 h after the surgical procedure decreased mechanical allodynia and normalized the mRNA *caspase-1*, *caspase-12* and *caspase-8* gene levels, but did not the decrease *caspase-3* level, 14 days post-spared nerve injury. Ozone also reduced IL-1β staining in the orbito-frontal cortex in neuropathic mice. This study provides evidence that a single subcutaneous administration of ozone decreased neuropathic pain type behaviour, normalized the expression of pro-inflammatory caspases and reduced IL-1β staining in the orbito-frontal cortex astrocytes in SNI mice. These preliminary data show that peripheral neuropathy induced over-expression of pro-inflammatory/pro-apoptotic caspases in the orbito-frontal cortex and that ozone, by mechanisms that are as yet unknown, can regulate the expression of the genes that play a pivotal role in the onset and maintenance of allodynia.

We show here that ozone, injected subcutaneously (Muto et al., 2004; Re et al., 2008), prevented the increased mRNA levels of *caspase-1*, *caspase-8* and *caspase-12* in the orbito-frontal cortex of SNI mice and prevented the development of allodynia in the same mice.

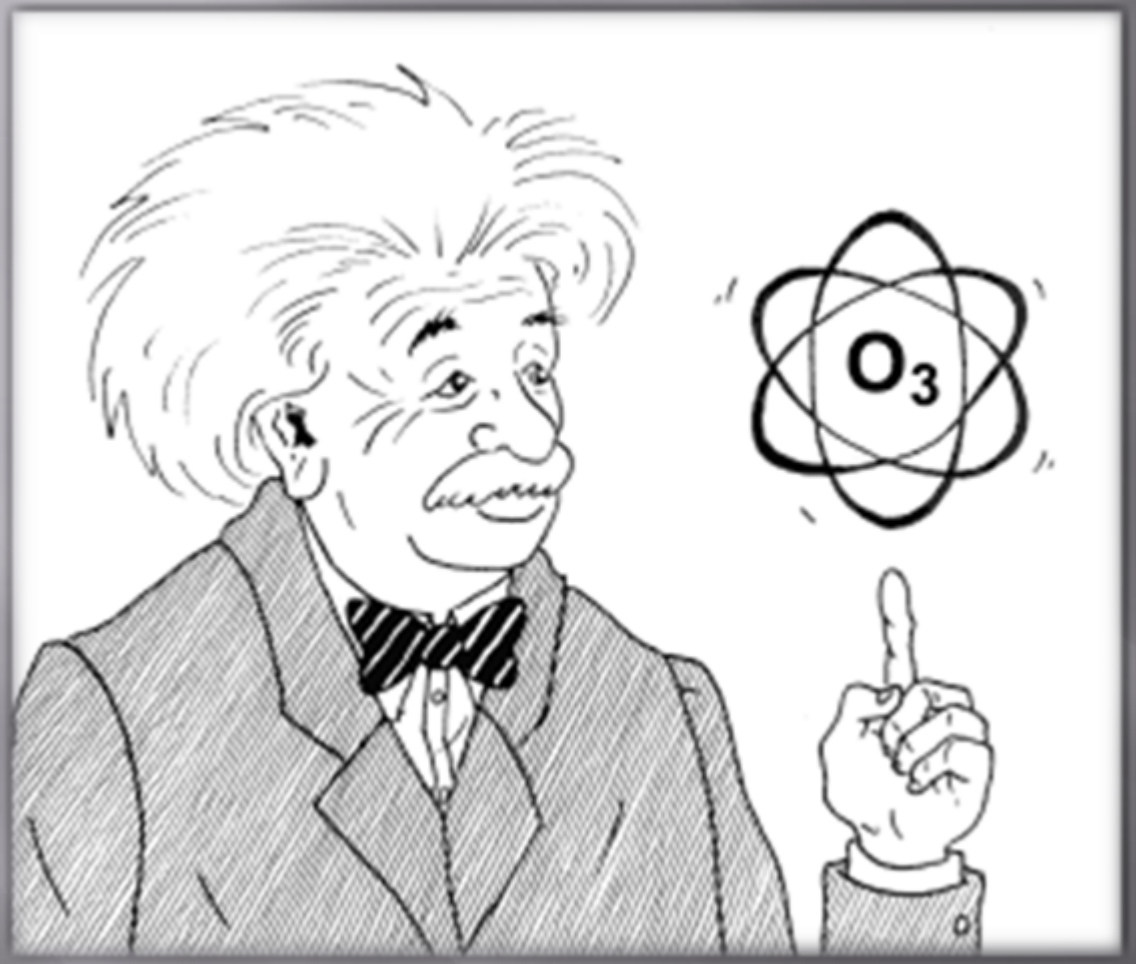
Collectively, these preliminary data indicate that ozone can be an effective practice for preventing the development of neuropathic pain through complex as yet unexplored mechanisms and, among these, through the modulation of specific pro-inflammatory or pro-apoptotic caspases in the brain.

Caspase Cascade



Ozone block the synthesis of caspases 1, 8, 12

Eur J Pharmacol 2009, 603:42-49



Somebody still ask ..

It will be or not?

Some Clinical Data

In the second part of this presentation I would like to introduce my personal experience in the field of ozone as therapeutic tool in the so called Evidence Based Medicine.

It is now time that Science must recognize the big potential of ozone evaluating what ozone is at molecular level and how ozone works in terms of biological effects.

We are familiar with the drug construction, i.e. with all those procedures requested to market a drug.

Chemical identification, Toxicology, Pharmaco-dynamics, Pharmacokinetics, Clinical studies (Phase I, II and III) and finally Pharmaco-epidemiology post marketing (Phase IV).

In all these phases a crucial role is played by the so called randomized standardized clinical studies in blind and double blind fashion.

We know that, also considering very useful all the above studies in the aim to built up a most safe drug in term of human health, the same procedures sometime fail to give us a drug absolutely free from side effects.

Indeed, following the marketing, serious ADR's could be observed in some cases (see Statins, COX 2 inhibitors, etc).

In the last years Ozone Therapists have been negatively focused for the lacking of rigorous scientific data concerning the use of Medical Ozone.

In this context, taking into account the considerations discussed above, we only partially agree with some of the points argued by the critics.

Without any doubt, most of the problems arise from the first empiric use and approach to Ozone by the same Ozone Therapists!

Usually the criticism indicate the Ozone Therapist as a *poisoner* considering ozone like a venom.

In this context however, we can't forget that every drug (from ancient Greek pharmakon) must be considered a venom!

But we can consider aspirin, antibiotic or the same water as venoms?

Furthermore, we can't forget that for every drug the pharmacologists evaluate a parameter to establish the threshold of toxicity and therapeutic (The Therapeutic Index)!

So every substance, when improperly used, could promote damage and create toxicology instead of benefit.

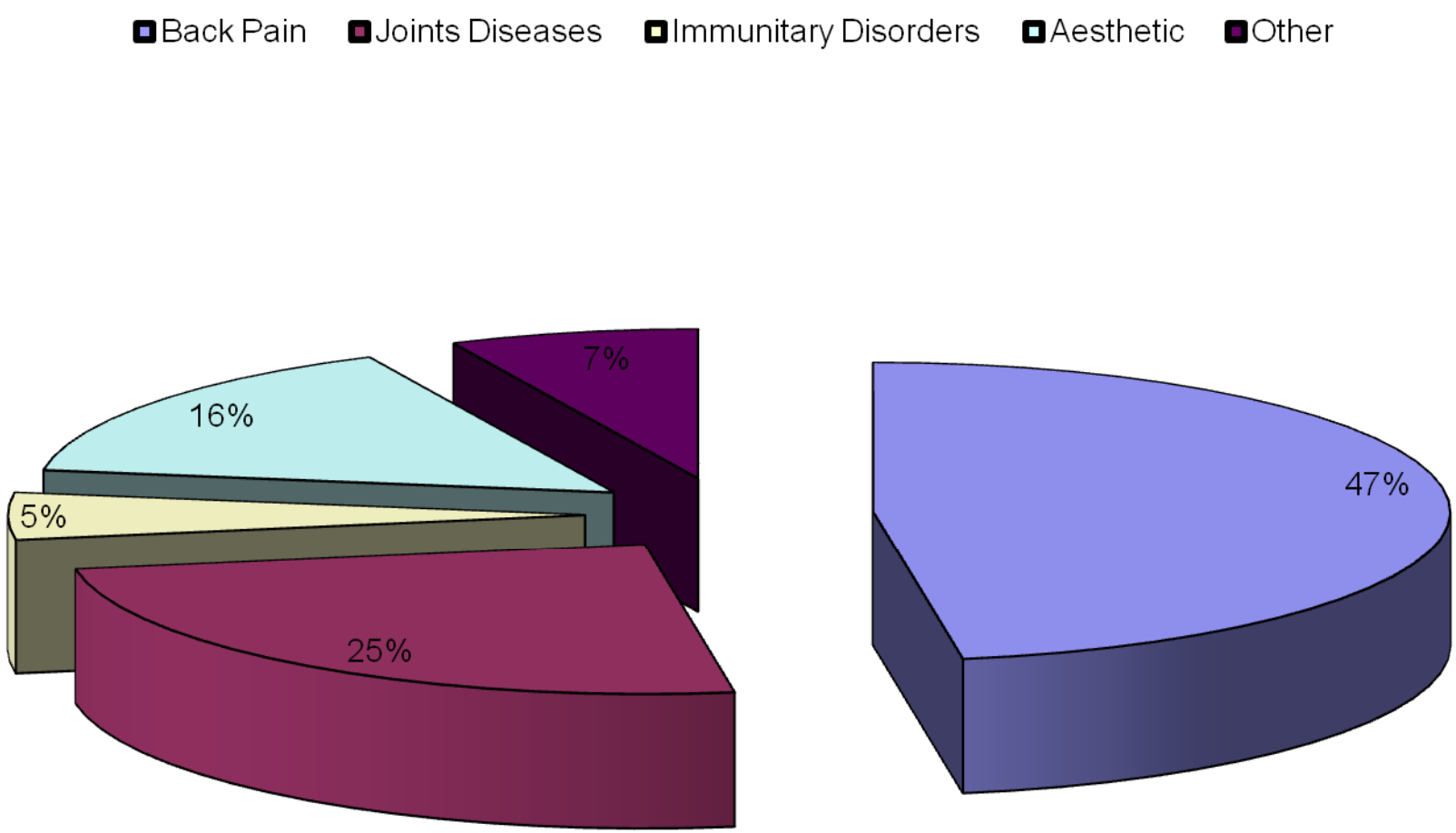
At the light of our clinical experience and at the very important literatures published during the last decade, we are now ready to claim a consideration and a respect which failed since too much time.

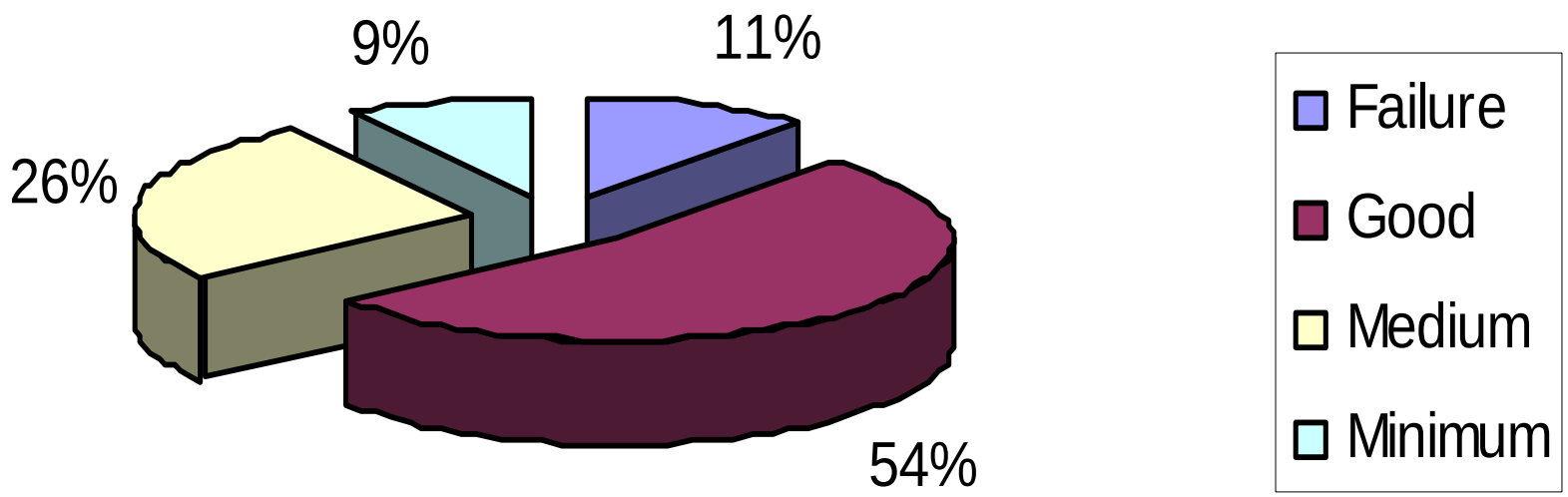
In the years 2005/2006 a total of 947 patients were treated with ozone therapy in our clinic.

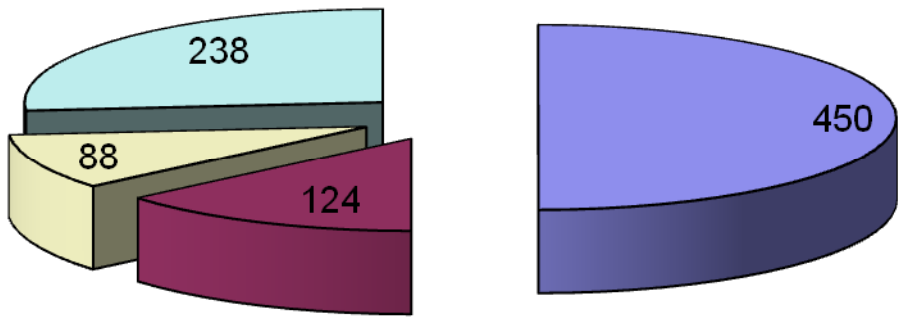
8460 treatments were performed in the same period (mean 9 sessions per patient).

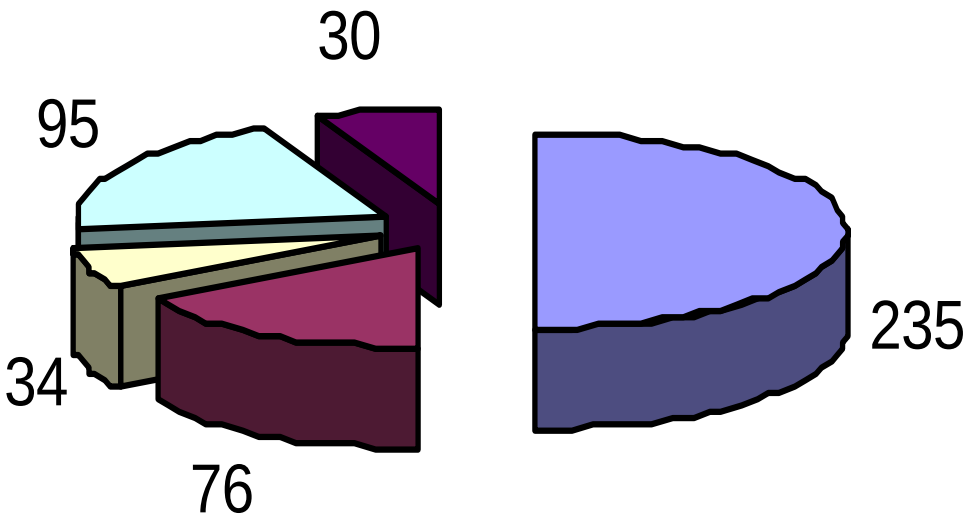
The main pathologies were:

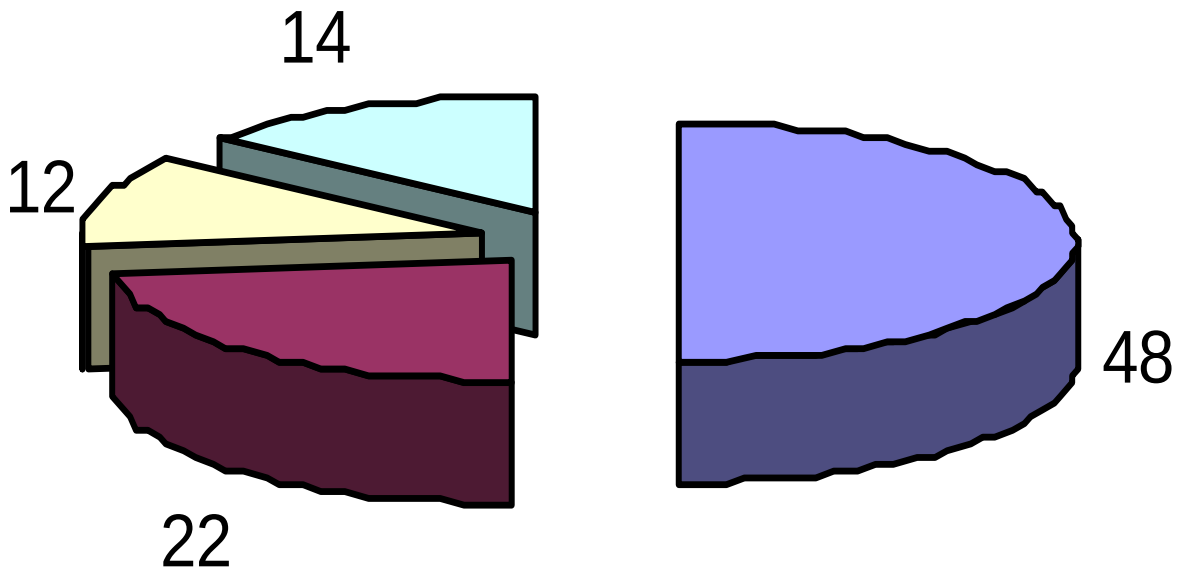
Back pain	450
Joints diseases	235
Immunitary disorders	48
Aesthetic	148
Others	66

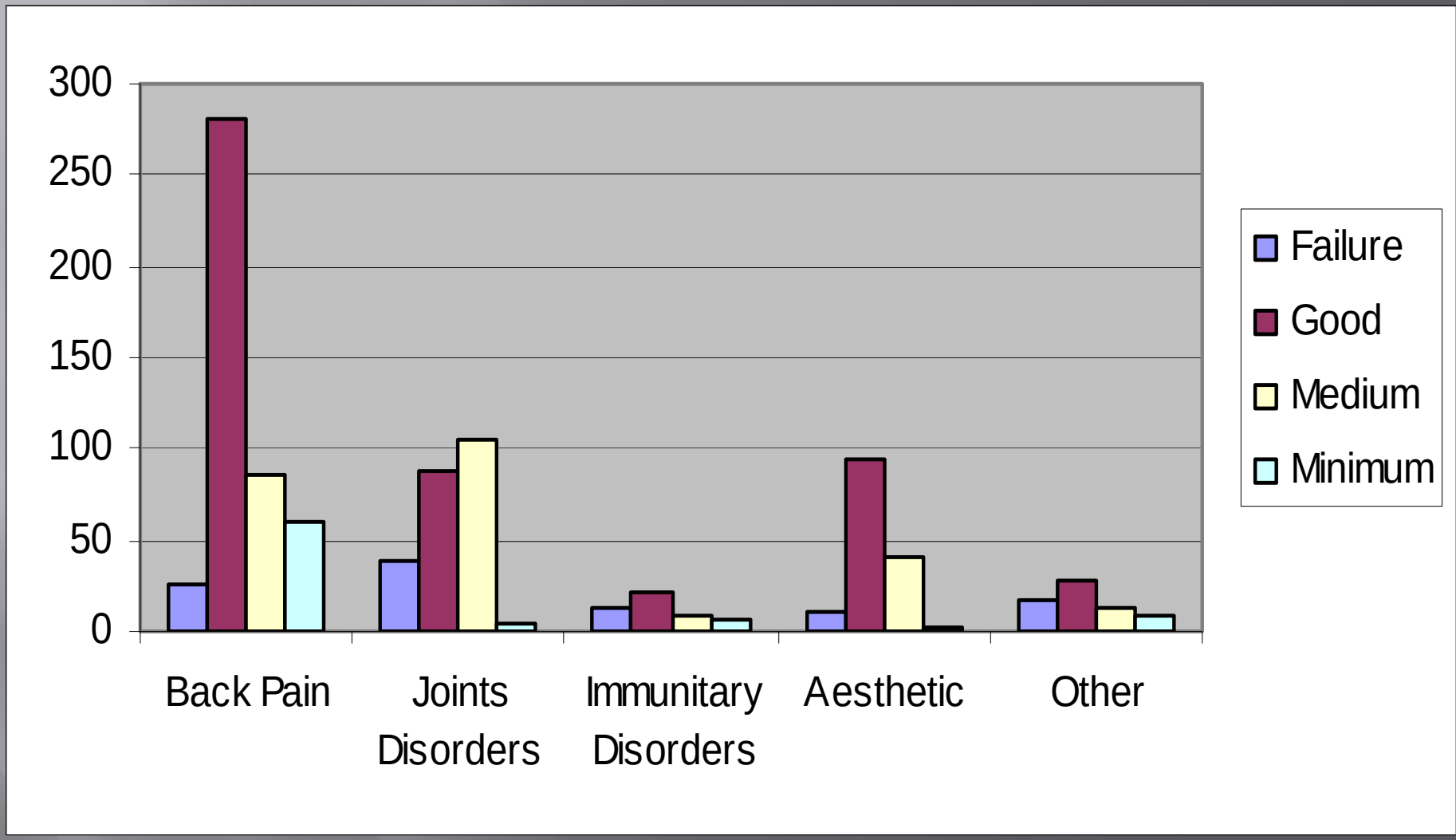

Back Pain
Joints Diseases
Immunitary Disorders
Aesthetic
Other
7%
16%
5%
25%
47%


9%
11%
26%
54%
Failure
Good
Medium
Minimum

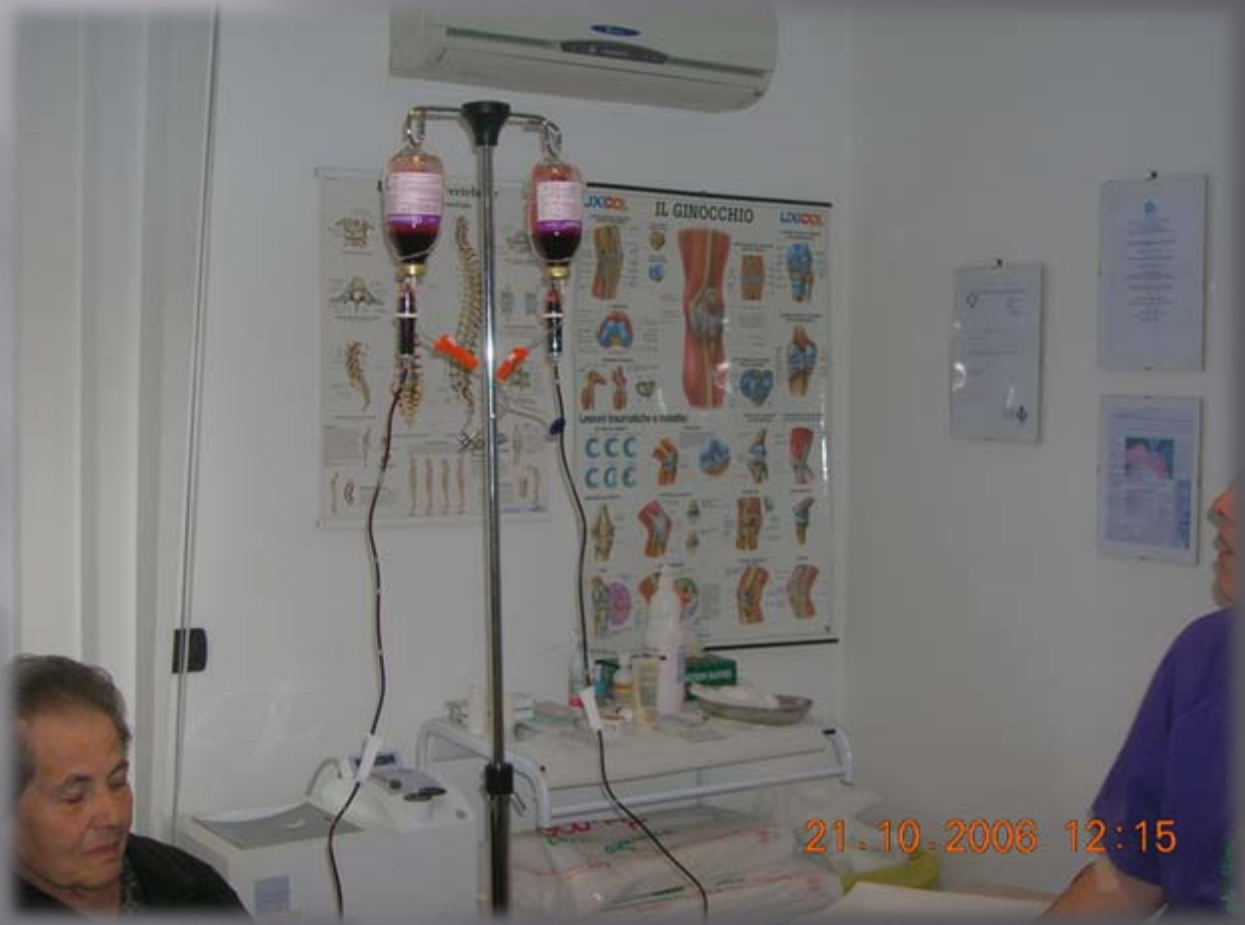

238
88
124
450
Back Pain
Cervical
Dorsal
Lumbar


30
95
34
76
235
Joints Diseases
Shoulder
Hip
Knee
Ankle


14
12
22
48
Immunitary Disorders
Hepatitis
Arthritis
Others

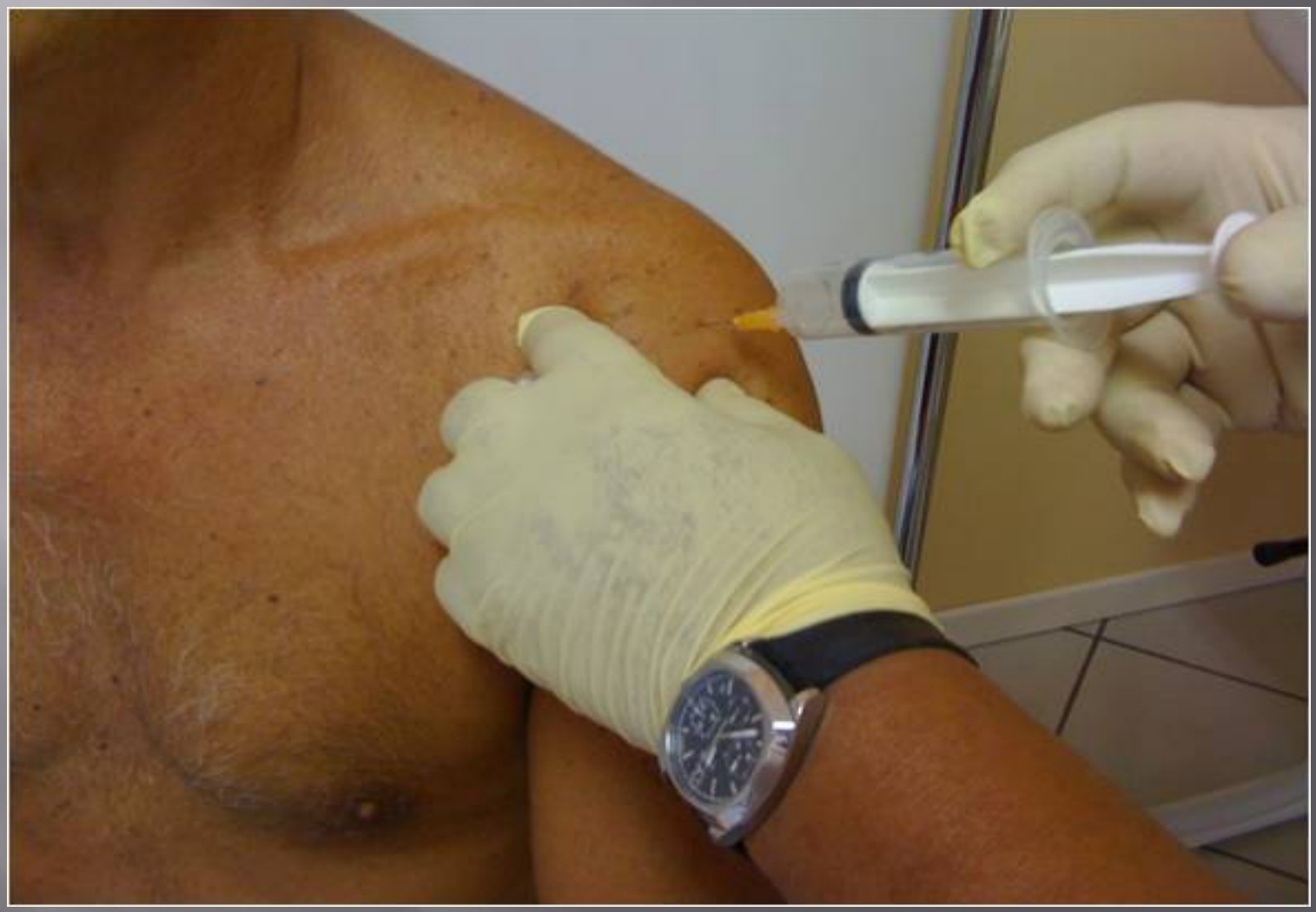

300
250
200
150
100
50
0
Back Pain
Joints Disorders
Immunitary Disorders
Aesthetic
Other
Failure
Good
Medium
Minimum

In the same period a total 828 Major Autohaemo Therapies were done for the various pathologies without any negative or collateral effect …



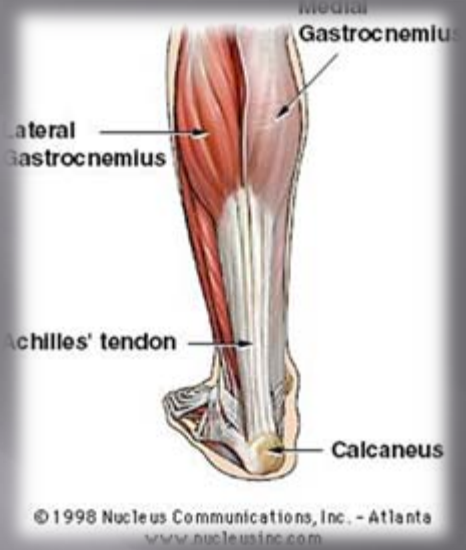
Shoulder Infiltration with ozone

30G 13mm – 10 μg/ml – 20 cc



Achille’s Tendon

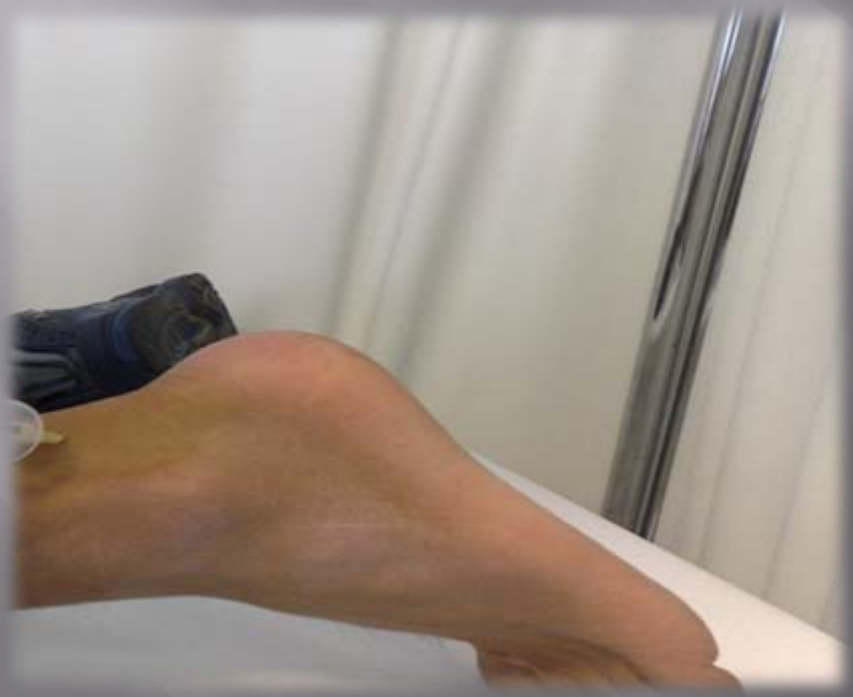
Injuries

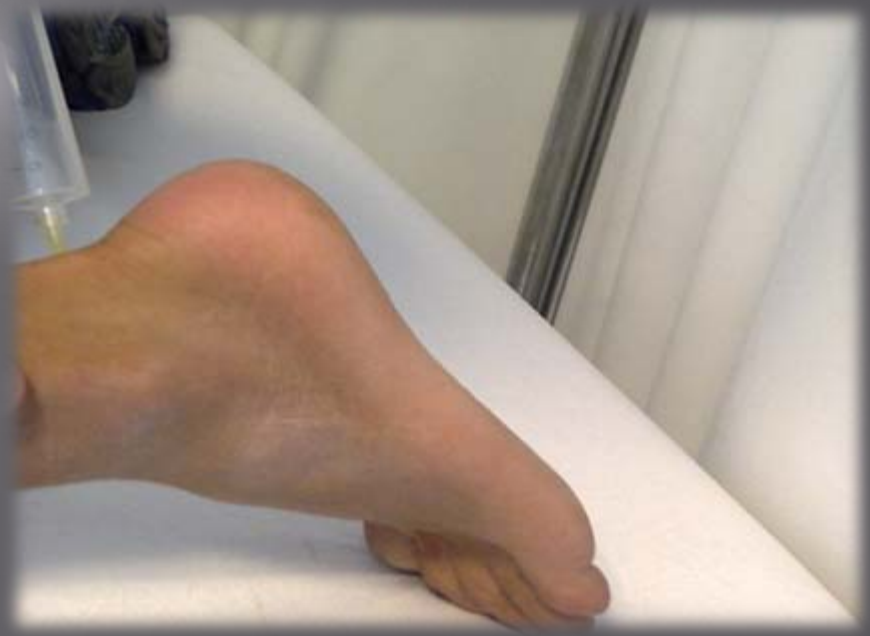


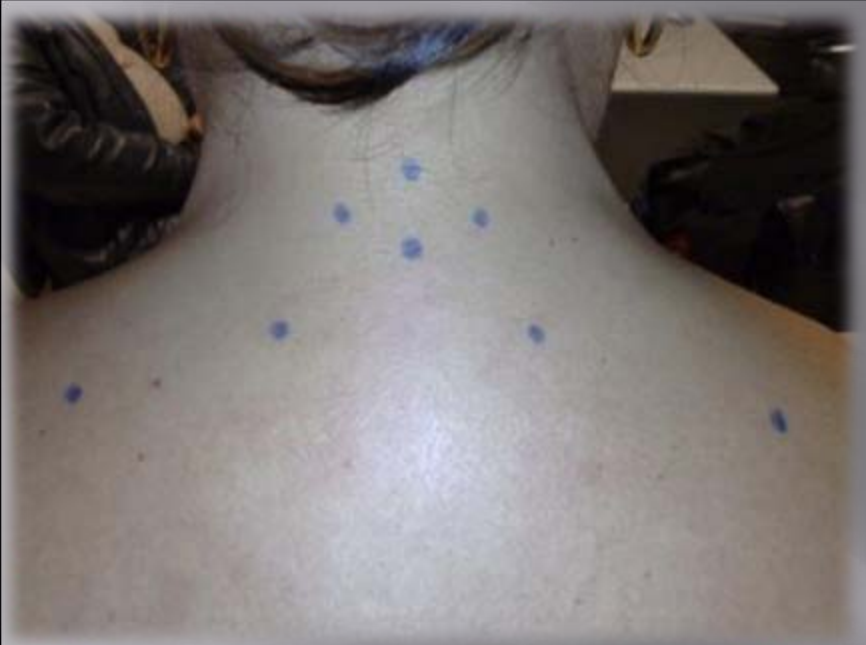
30G 13mm – 27G 6mm

10 μg/ml

20 cc





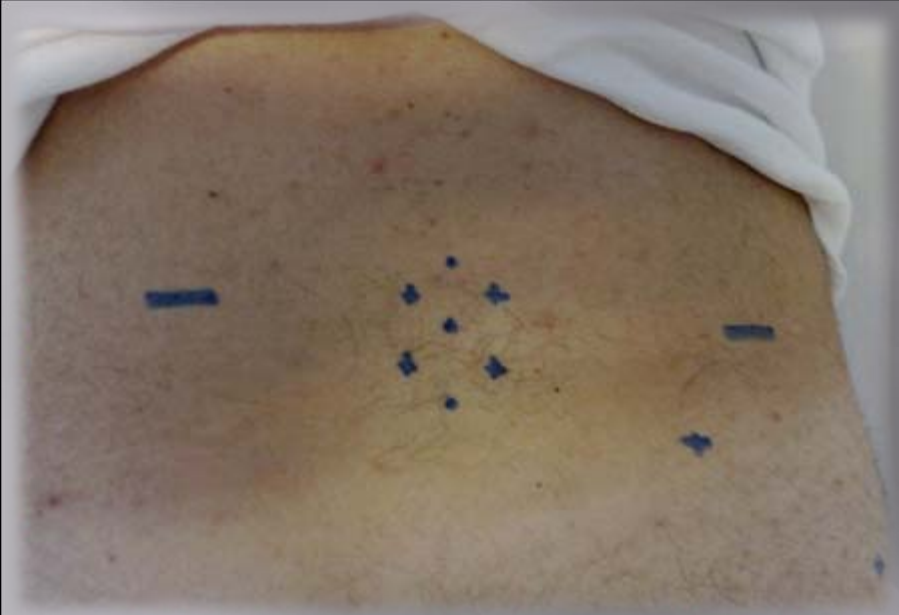


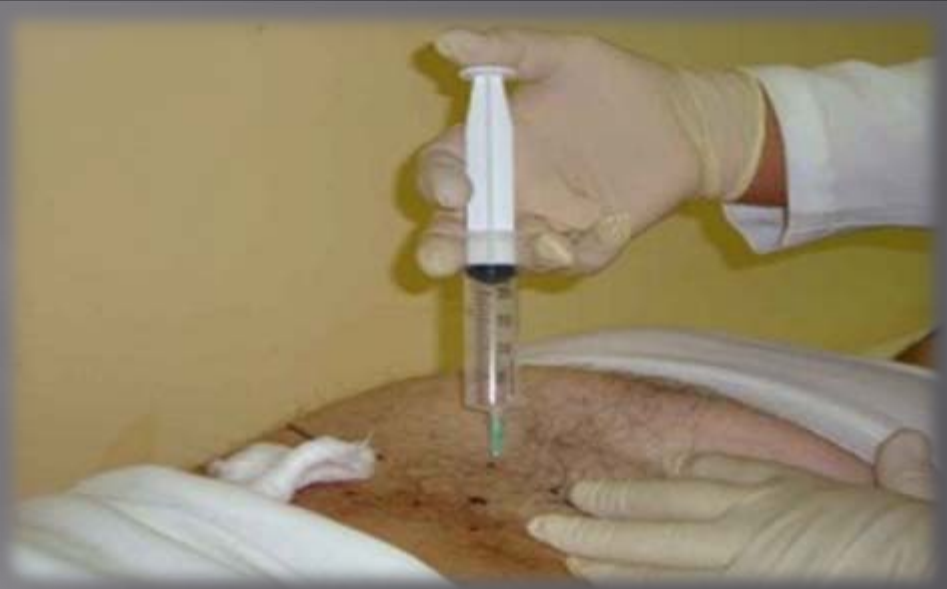
Cervical Discopathy

C5-C6

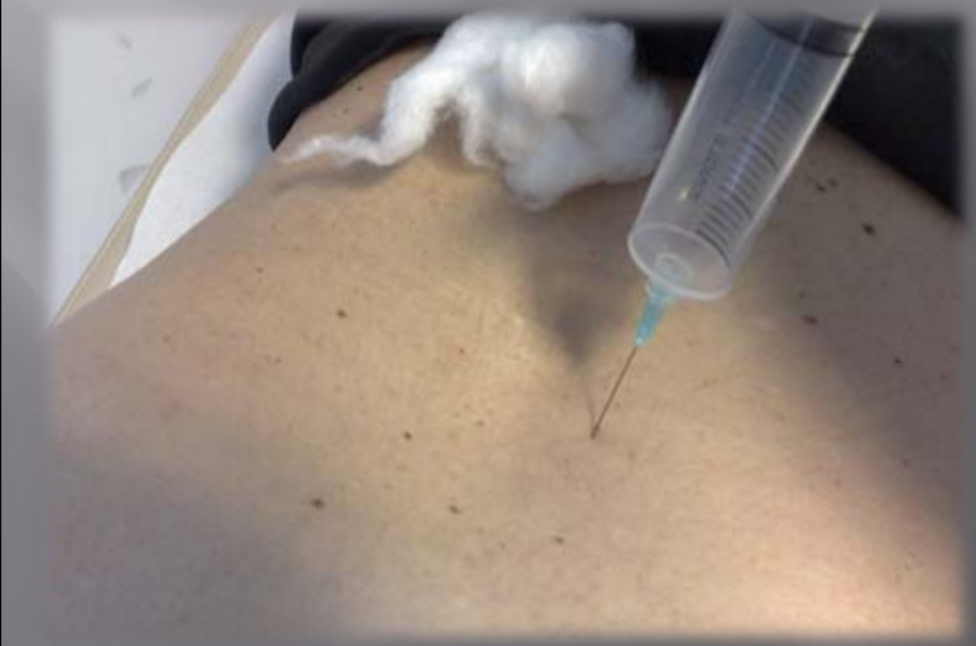
Needle: 30G – 0.3 x 13 mm

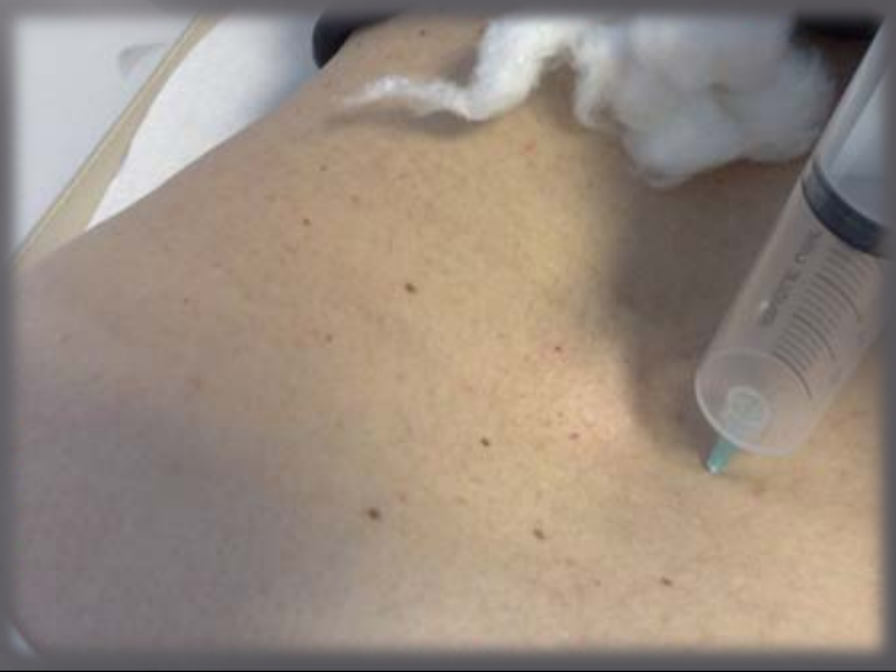
Oxygen-Ozone: 8 µg/ml – 5 ml x 6





Lumbar Discopathy L4-L5 Needle: 23G 0.6x30 Oxygen-Ozone: 12 µg/ml – 5 ml x 6





Plastic Bag

Oxygen-Ozone

70 µg/ml

Skin Ulcer

Ankle Distortion

General Remark

Ozone could be considered as a pro-drug due to the fact that the biological effect induced must be ascribed mainly to the modification of the micro cellular environment and to some secondary agents derived from its biochemical transformations (NO, H_2O_2, etc).

For this reason, the protocols proposed by the different Ozone Associations must be intended as an indicative way of administration taking into account the peculiar response of each patient.

Usually, the result shows a wide variability mainly for the following reasons:

- Temporal occurrence of the traumatic or degenerative disorders: as soon as the treatment is started after the onset of the pathology, as soon and stable will be the therapeutic effect;

- Concomitant intake of drugs: neurological, psychiatric or antidepressant drugs reduce the efficacy of the ozone treatment;

- Age, sex and general status of the patient: usually young patient and female are good responder;

- Nutritional factors: the reduction of some trace elements due to diet abnormality could delay the onset of the benefit from ozone treatment. Particularly, any reduction of mineral (Cu, Zn, Mn, Se mainly) and vitamins (C, E, B1, B6 mainly) must be corrected in the best appropriate way;

- Encourage the patients to modify any deviation from a good life style: suggest weak daily walking, weight reduction when needed, weak sport activity mainly addressed to the best postural equilibrium, reduce alcohol and smoke, balanced diet, drug intake reduced to the absolute request of concomitant pathologies (diabetes, hypertension, etc.);

- Deep and detailed anamnesis and evaluation of the patient status. Familiarity, allergy and clinical history of any past and present trauma;

Moreover, the following points must be taken into account:

• Also if present a rapid onset of wellbeing after the first ozone treatments, inform the patients that this apparent wellbeing could vary during the session and usually during the treatments mostly at the end of the cycle (normally 12-15 sessions) a recrudescence of the pain or symptoms could be usual also if the duration of the crisis is shorter in time if compared with the previous state;

• In the case of apparent no effect of ozone treatment after the first sessions, encourage the patient and inform him in detail about the ozone mechanism: indeed ozone is a conditioning agent and the response will follow the reaching of the anatomical, functional, biochemical equilibrium referred to the peculiar pathology. Ozone is not exclusively a symptomatic agent and mainly its action is etiologic;

• Stimulate the patients to verify any collateral sign not referred to the main signs indicated: e.g. the quality of sleep, the presence or the absence of tiredness, the quality of life by a general point of view. Usually the patients report a wellbeing often not correlated to the main referred problem;

• Ask to the patient or to the familiars mainly in touch with him/her any impression positive or negative compared to the previous state;

• Evaluate the status of the skin and of the cutaneous aspects: hair, nail, general aesthetics;

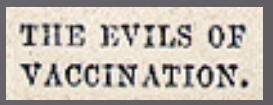
The Vaccination Theory a Surprising Similarity

Edward Jenner and the Discovery of Vaccination

The year 1996 marked the two hundredth anniversary of Edward Jenner's first experimental vaccination--that is, inoculation with the related cow-pox virus to build immunity against the deadly scourge of smallpox.

Edward Jenner (1749-1823), after training in London and a period as an army surgeon, spent his whole career as a country doctor in his native county of Gloucestershire in the West of England. His research was based on careful case-studies and clinical observation more than a hundred years before scientists could explain the viruses themselves. So successful did his innovation prove that by 1840 the British government had banned alternative preventive treatments against smallpox. "Vaccination," the word Jenner invented for his treatment (from the Latin *vacca*, a cow), was adopted by Pasteur for immunization against any disease.

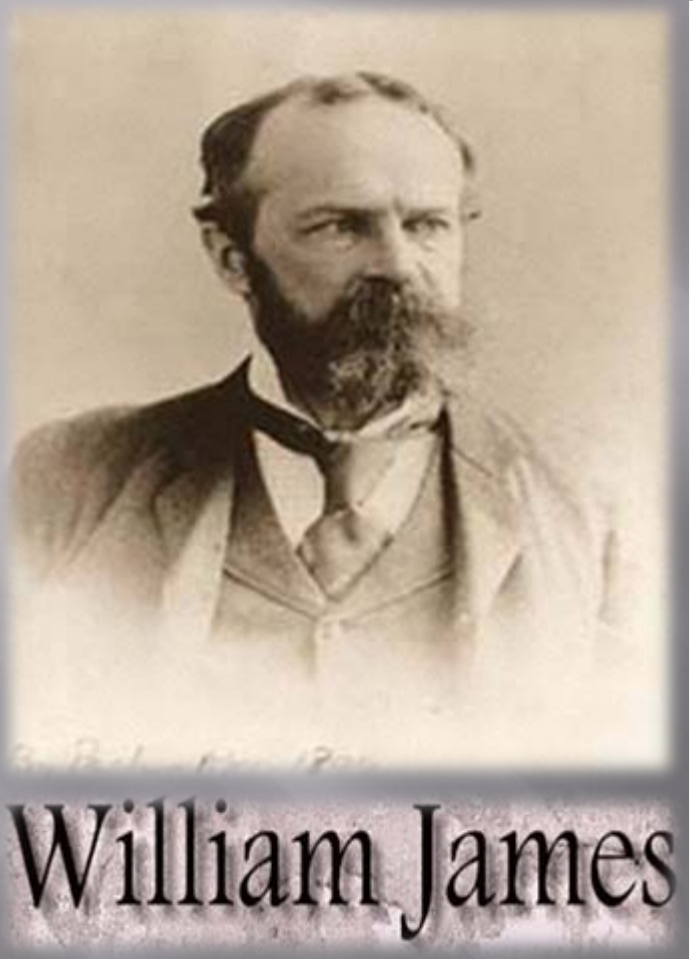
Christian Charles Schieferdecker, M.D.
Dr. C. G. G. Nittinger's Evils of Vaccination.
Philadelphia: the editor, 1856.



Because of the lack of clear scientific explanation of its effects, the frequent side-effects, and contaminated vaccines, vaccination itself remained controversial throughout the nineteenth century. It certainly carried risks for the infants being vaccinated, and this volume, playing on parental fears, argued, inter alia, that vaccination was *nonsensical, unscientific, criminal, and even sinful.*

John Baron, M.D., 1786-1822
The Life of Edward Jenner, M.D., LL.D., F.R.S.
London: Henry Colburn, 1838. 2 volumes.

While still an apothecary's apprentice in the late 1760s, Jenner had been intrigued by possible relationships between smallpox, cowpox, and swinepox. At the time, he was *ridiculed*. By 1780, however, he returned to the idea, as evidenced in the conversation recorded here, and in 1789 he experimented by inoculating his own son, then aged one-and-a-half, with the swine pox, followed by conventional smallpox inoculation.



Every new theory passes through three phases:

1. It is attacked and declared absurd

2. Then it is admitted that it is true and obvious, but insignificant

3. To the end it is recognized the real importance and its detractors demand the honor to have discovered it

Conclusion Remarks

It is time that the Scientific Community start to evaluate Ozone Therapy as a useful complement to the orthodox medical approach, particularly in the case of rare and drug-orphan illnesses.

The incidence of side effects, represented by a number after the comma preceded from at least 5 zeros considering the population treated in the last 40 years, point out the absolute safety of this approach when compared to the side effects of orthodox drug treatment.

It has been estimated that adverse drug reactions (ADRs) are the 4th to 6th largest cause for mortality in the USA (Lazarou J. et al., 1998. Incidence of ADR in hospitalized patients: a meta-analysis of prospective studies. JAMA, 1998, 279 (15) 1000-5.).

They result in the death of several thousands of patients each year, and many more suffer from ADRs.

One of the goals of Ozone Therapy could be that to reduce the appearance of pathologies like Alzheimer, Parkinson, Dementia, etc.

Indeed, the potential amount of population that is going to elderly and to potential expression of oxidative damage (calculation says it will reach 11-16 million Americans in 2050) is enormously more prominent when compared with the population actually suffering of such oxidative impairment (Alzheimer affects today about 4.5 million Americans while Parkinson approximately afflicts one million person in the United States today).

Who claims the absence of standardized, randomized, double bind studies must consider the difficulties to build up serious work without adequate budged. One of the most prominent problems of ozone is its brief life time. Its rapid decomposition make impossible to sell it in the Pharmacies!

No profit no interest

To our opinion, for the respect of the millions people treated all over the world and for all whom interested but still waiting the formal authorization of the Government Health Authorities, a redistribution of the official budget devoted to research must be urgently considered to validate or reject the ozone therapeutic potential.

Back Pain and Disk Herniation

The Question?

Treatment of herniated lumbar disc by intradiscal and intraforaminal oxygen-ozone (O2-O3) injection.

Muto M, Andreula C, Leonardi M.

Neuroradiology OU, AORN Cardarelli, Naples, Italy.

MATERIAL: We report our experience between May 1996 and May 2003 with 2200 patients affected by low back pain or sciatica due to herniated disk treated by intradiscal and intraforaminal oxygen-ozone injection. The patients received medical and physical therapy before treatment for at least 2 months; the patients with conus-cauda syndrome and hyperalgesic sciatica were excluded. We never performed discography before the treatment that was performed under CT guidance or fluoroscopy. CT provided monitoring of gas distribution in the disk and epidural space. RESULTS: No side effects were recorded at short and long-term follow-up. Clinical results were evaluated with the modified McNab method showing an 80% success rate and 20% failure rate in 1750 patients followed up to 6 months while the success rate dropped down at 75% and failure increased at 25% in 1400 followed up to 18 months. CT showed reduction in the size of the herniated disk in only 63% of the followed patients (420 patients). The failure has been mostly related to: calcified herniated disk; spinal canal stenosis; recurrent herniated disk with epidural fibrosis; small descending herniated disk at the level of the lateral recess. Copyright 2004 Masson, Paris

PMID: 15356443 [PubMed - indexed for MEDLINE]

Intradiscal and intraforaminal Injection ..

or

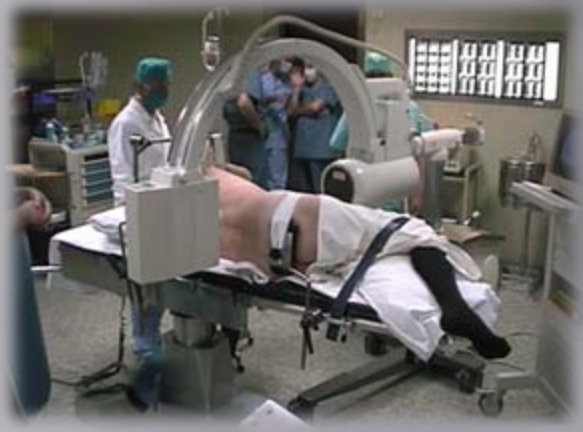
Intramuscular and Paravertebral Injection ?

SPINE Volume 34, Number 13, pp 1337–1344
©2009, Lippincott Williams & Wilkins

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

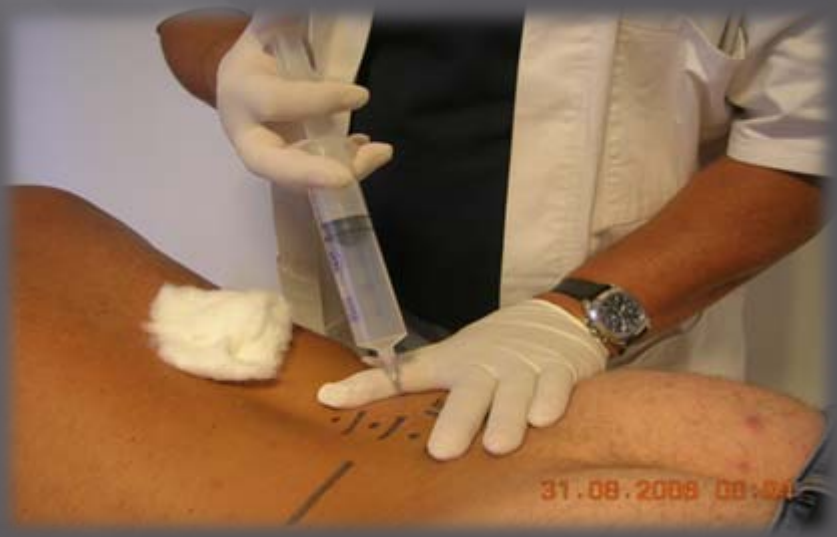
Marco Paoloni, MD,*† Luca Di Sante, MD,*† Angelo Cacchio, MD,† Dario Apuzzo, MD,† Salvatore Marotta, MD,‡ Michele Razzano, MD,‡ Marianno Franzini, MD,§ and Valter Santilli, MD*†

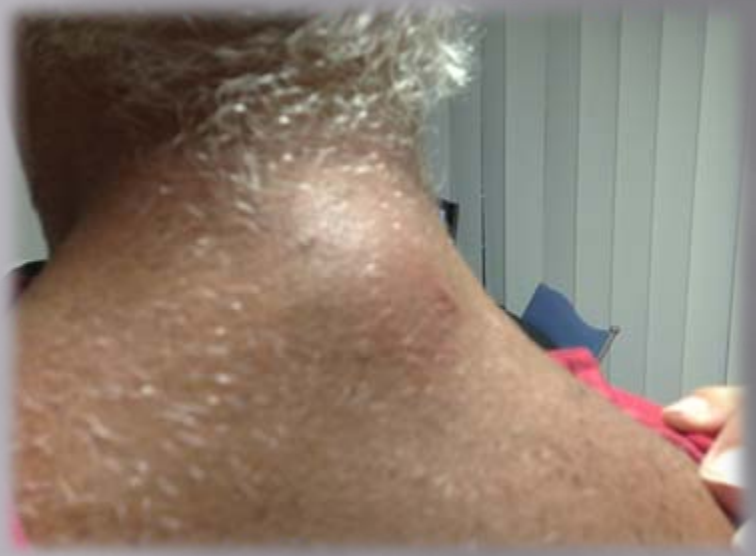


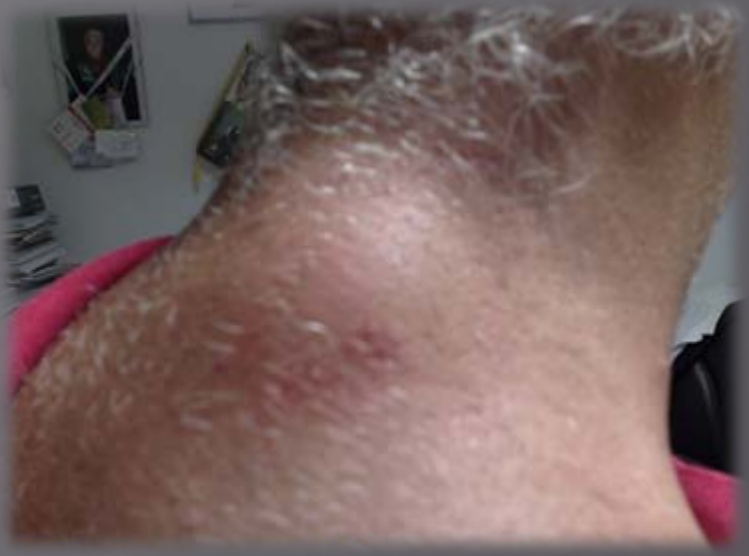
The Response is still controversial !

Invasive
Painfull
Higher Costs
Clinical efficacy 70,3 %
Short Term Failure
Possible Side Effects

Conservative
Minimum Pain
Low Costs
Clinical efficacy 61 %
IM
Clinical efficacy 80 %
PV
Long Term Efficacy

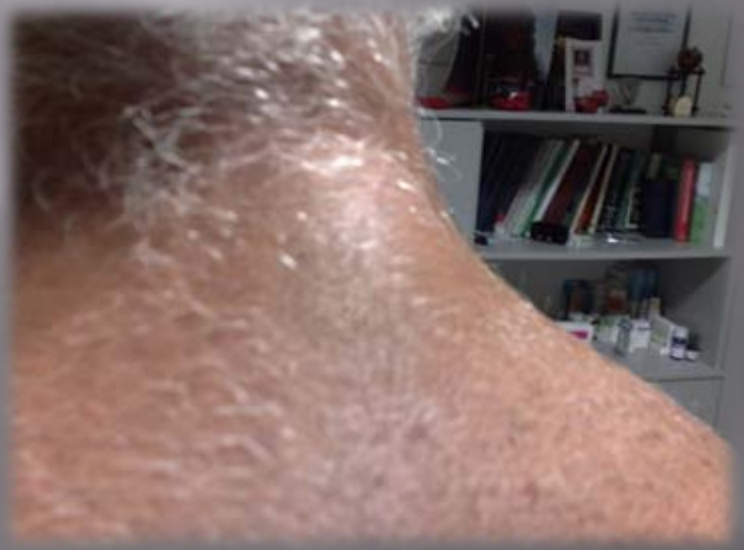


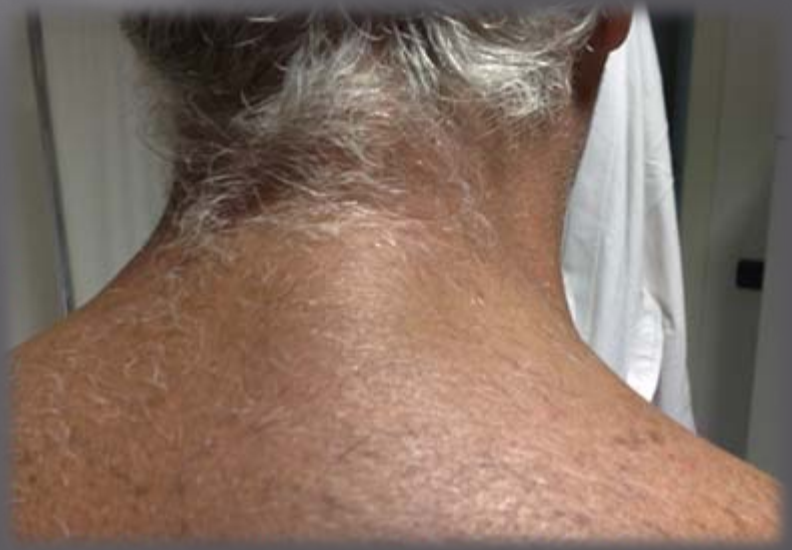




Lipoma 28 June 2007

Lipoma 20 Sept 2007





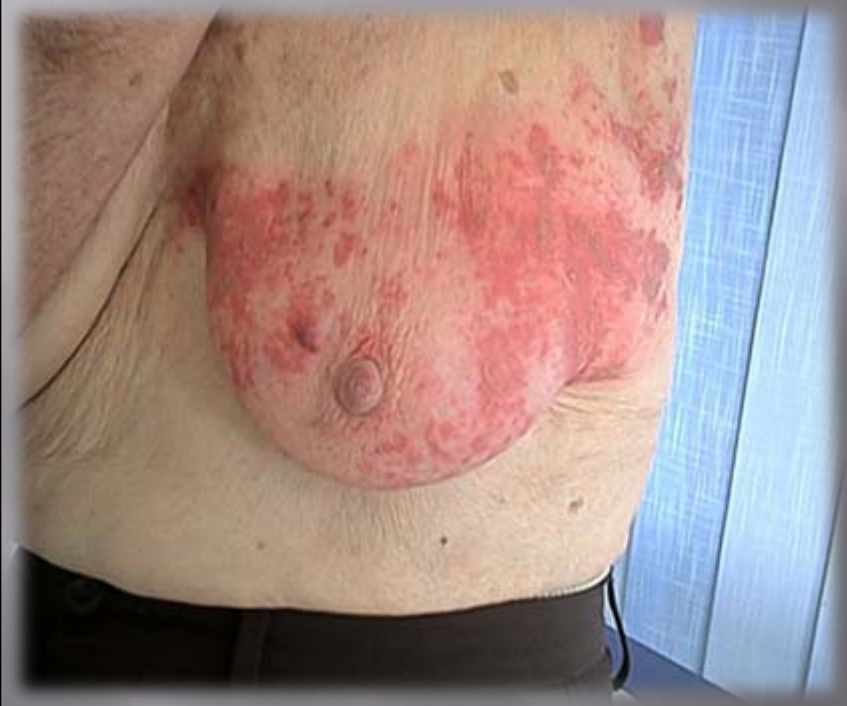
Eye Inflammation – Conjunctivitis

Herpes Zooster

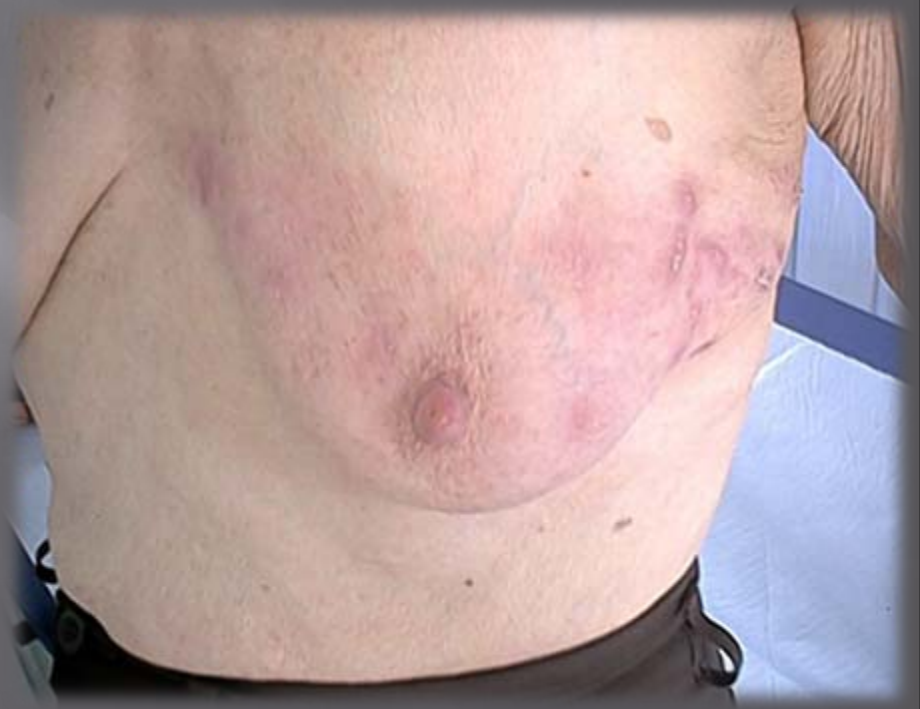
Local Application

Ozonated Oil twice daily

Ozomineral (Trace Elements)



22-09-2005

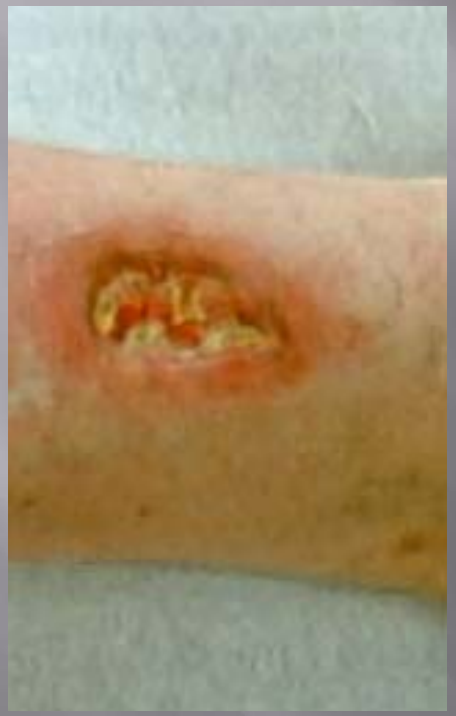


03-10-2005

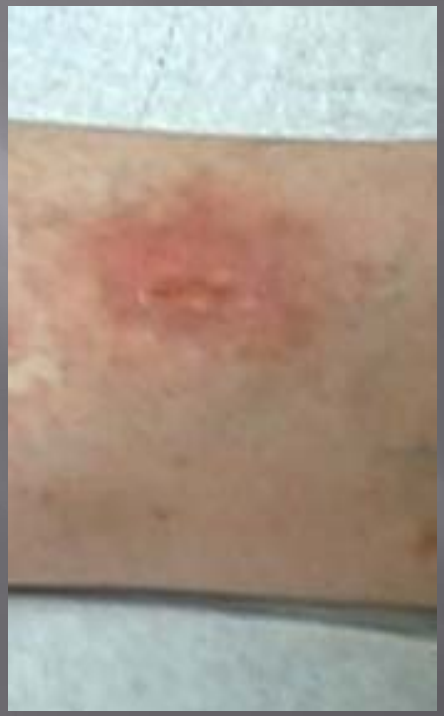
CASE N 1

Leg Ulcer, Female, Age 28

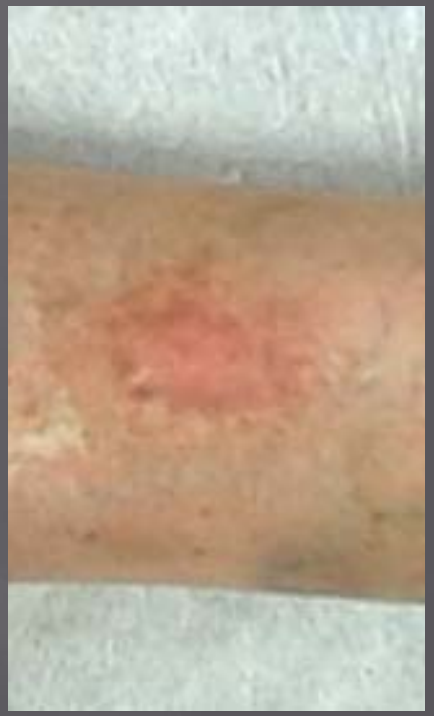
Ozone Bag (40 ug/ml) + Minor Autohaemo + Ozonized Cream



14/4/2001

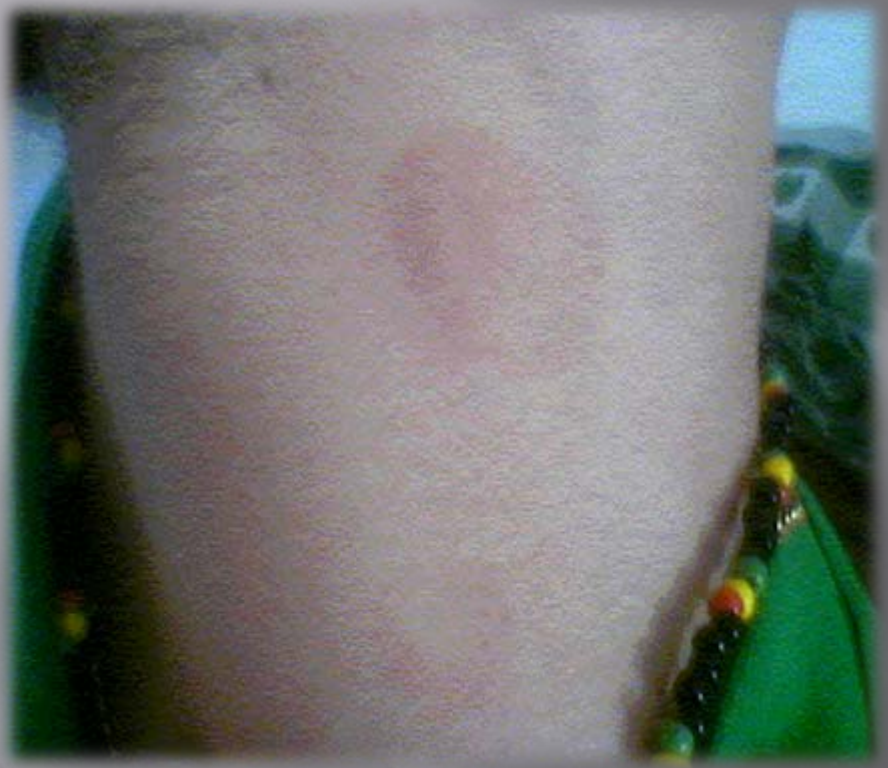


30/6/2001



27/7/2001

Pitiriasis Versicolor




Thank you