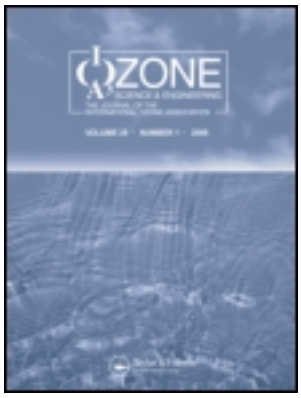

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Ozone in Medicine: The Low-Dose Ozone Concept—Guidelines and Treatment Strategies

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I. INTRODUCTION

Ozone in Medicine: The Low-Dose Ozone Concept—Guidelines and Treatment Strategies

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The low-dose ozone concept with its moderate oxidative stress represents an ideal hormesis strategy. Dose-response and concentration-effect relationships in the context with specific applications allow one to fix concentration ranges with therapeutical benefit. Based on the well-known reaction mechanisms of ozone, its biochemical and pharmacological effects, international guidelines have to be defined concerning physiological and ozone resistant materials, indications, applications and the effective concentration and dosage range in dependence on the specific indications. Following the international regulations of ozone concentrations outdoors and indoors, as working site concentrations by WHO (World Health Organization) and in conformance with the Medical Device Directives (MDD) for quality assurance and control, some European Medical Societies for the Use of Ozone have set up a draft for the essential requirements for the treatment procedures, including: (a) production of Medical Ozone; reactivity of O_3 and ozone-resistant materials; (b) ozone-free surroundings (WHO regulations) by the integration of effective catalytic systems; (c) ozone-resistant and physiologically indifferent materials used in disposables for MAH, intra-articular, intramuscular and other topical injections (for rectal insufflation, topical treatment as transcutanous gas bath and/or disinfection); (d) medical device directives for safety of the patient; (e) ozone measurement as requirement for concentrations and dosages; and, (f) concentration, dosages, and treatment frequency are listed in dependence on the indication and disease as well as on the underlying mechanism of action.

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Keywords Ozone, Ozone Therapy, Guidelines, Hormesis, Treatment Concepts, Concentration, Dose

INTRODUCTION

When used in specific diseases and conditions, medical ozone produces the same or similar therapy results worldwide. Improper application in the form of erratic methods and doses is the most frequent cause of ineffectiveness and adverse effects—and is always the cause of violent controversies.

For this reason, the medical societies for ozone application have set up treatment protocols as basis for standards and guidelines, revised and published as a result of the most recent research and 30 years of experience (Beck et al. 1998; Knoch et al. 2009). They have been used in the standardization of applications, indications, concentrations, doses, and frequency of treatment as based on the mechanism of action and the pharmacology of ozone.

1. In its pharmacological effect, medical ozone follows the principle of hormesis: low concentrations (or doses) show a high efficacy, which decreases with increasing concentration, finally reversing into a questionable and even toxic effect (Figure 1). The effect/concentration relationship for the systemic application of ozone—in the form of a standardized major ozone autohemotherapy and rectal ozone gas insufflation results in the following concentration ranges: concentrations of 10–40 μg ozone/ml ozone/oxygen mixture represent those levels that are physiologically effective and recommended for systemic application. In the high concentration range of 60–100 μg/ml the antibiotic effect of ozone has a wide range of applications in the treatment of infected wounds, diabetic foot, decubitus

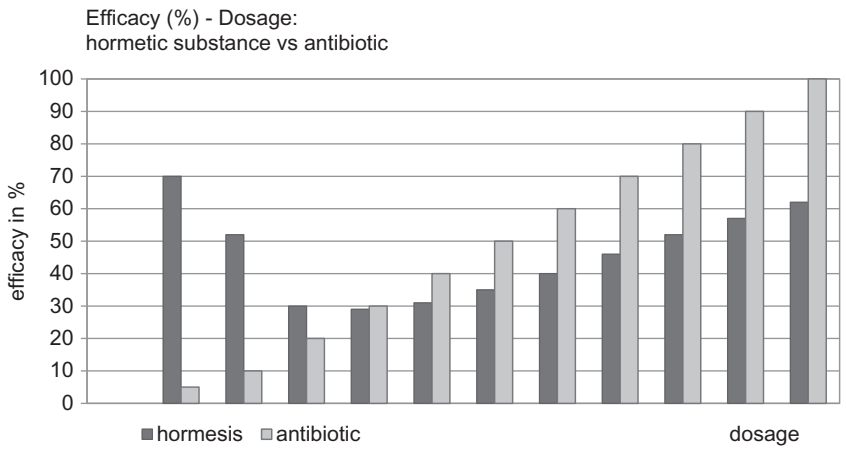

FIGURE 1. Dose-response relationship: hormetic substance vs. antibiotic (schematic): High efficacy at low doses and/or concentrations (e.g., ozone). The higher the dose, the more the efficacy decreases, thus becoming less effective in the middle-dose range and finally causing adverse or even toxic effects in the high-dose range. The efficacy of substances such as antibiotics is low at low doses and higher when the doses are increased.

ulcers and burns, but completely restricted to the topical forms of application.

2. Principally, ozone is applied complementary to a corresponding basic therapy. Diabetes, Type 2 diabetes, chronic inflammatory diseases such as inflammatory vascular disease, in particular diabetic angiopathia, chronic hepatitis forms, and chronic intestinal conditions belong to the classic indications within the low-dose ozone concept.

Chronic oxidative stress (pathologically increased values for malone dialdehyde MDA; hydrogen peroxide, H_2O_2; total hydroperoxide, TH, etc.) and an anti-oxidant deficit (disbalanced superoxide dismutase and subgroups of SOD as well as catalase CAT and others) are phenomena common to all the diseases listed. Here, in particular, low-dose ozone, as a hormetic substance, shows a regulatory effect on pathological processes.

OZONE PEROXIDE, THE PHARMACOLOGICALLY ACTIVE SUBSTANCE, AND ITS UNDERLYING MECHANISMS OF ACTION

Oxidative Stress *and* Ozone?

Does ozone treatment make sense at all if there is already an insufficiency of the cellular antioxidant system? Doesn't this increase H_2O_2, MDA, and TH (total hydroperoxides) as oxidative stressors? Thus reducing and/or destroying the antioxidant system which is already working beyond its capacity in these indications?

In a pharmacological context, we have been able to find clear answers to this and other questions, particularly from research over the last 10 years: in fact, we exert a regulatory influence on a disturbed oxidant/anti-oxidant balance (Figure 5a-b). Oxidative stress decreases in measurable form is shown by reduction of H_2O_2 (Figure 6), and a corresponding regulation of the antioxidants, as shown by SOD, GSH and G-6PDH (Figures 6 and 7). The oxidative stress markers MDA (Figures 8 and 9) and TH (Figures 10 and 11) decrease simultaneously.

"Ozone peroxides" assume the role of physiologically active ozone metabolites: Due to the selective reaction

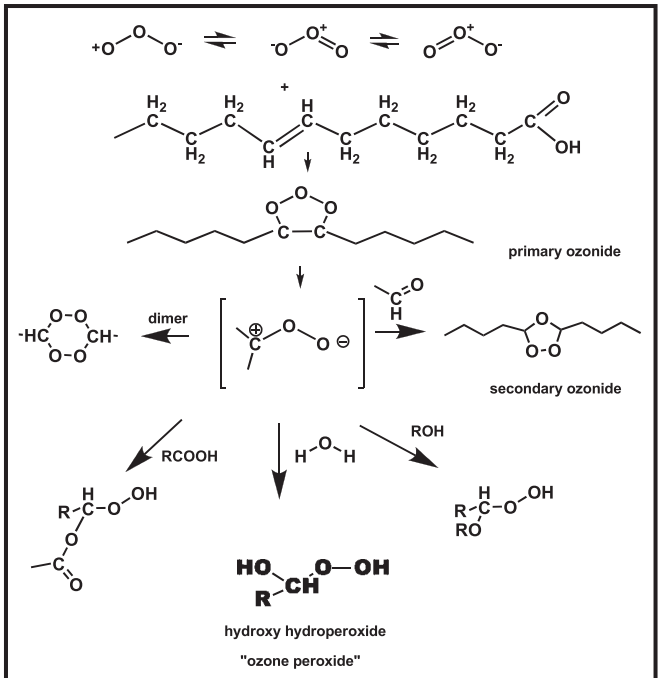

FIGURE 2. Ozone reaction with isolated double bonds, i.e., ozonolysis according to Crigée (1953, 1975). The hydroxyhydroperoxides, here "ozoneperoxides," are understood as the pharmacologically active substances.

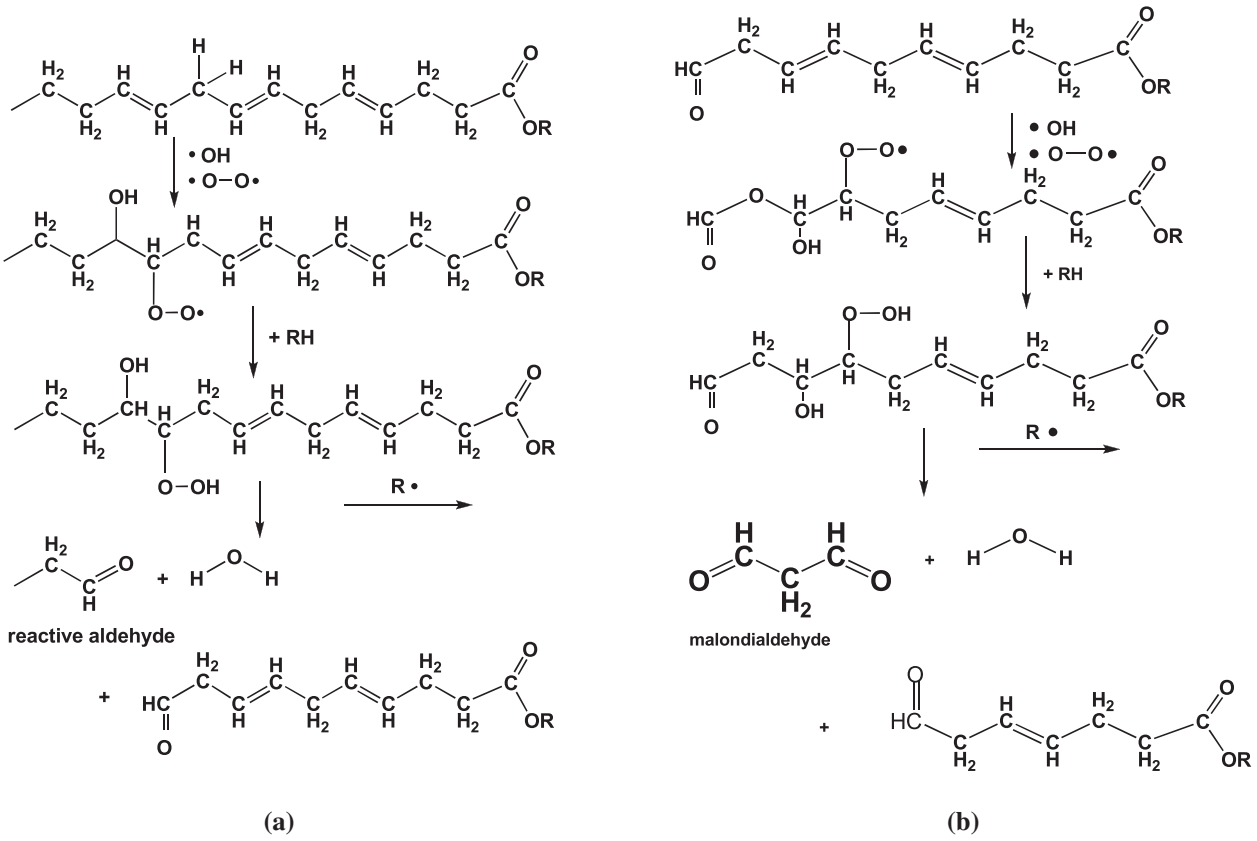

FIGURE 3. (a–b): Malone dialdehyde (MDA) formation by oxygen radicals + polyunsaturated fatty acids (PUFA).

behavior of ozone, the 1,3-dipolar (electrophilic) addition to the isolated C=C double bonds of essential fatty acids, according to classical ozonolysis as described by Criegée (1953, 1975), is the dominant reaction under physiological conditions with pH values $\leq$7,4.

Ozonolysis only takes fractions of a second, preferably forming short-chain hydroxy-hydroperoxides in an aqueous medium (Figure 2) here simply designated as "ozone peroxides," which are obviously responsible for the pharmacological effect in systemic ozone treatment.

Aldehydes, as secondary products, are only demonstrable in slight quantities, which means that malone dialdehyde MDA as measure for oxidative stress during extracorporeal blood treatment with ozone is negligible and stays within a physiological range (here see also: Bocci et al. 2005).

A short-term slight increase is possibly the result of oxygen (ozone/oxygen gas mixture) reacting with polyunsaturated fatty acids (PUFAs) in the form of autoxidation (Figure 3). These kinds of oxidations with free radicals such as OH, superoxide, peroxidic radicals, or oxygen itself, characterize also basic biological reactions for oxidative stress, which can be measured as an increase of the corresponding parameters in the blood (MDA, TH, H_2O_2 . . .). Note that this is not to be confused with ozonolysis: although ozone reacts in a highly selective way with isolated double bonds, this is not, or is far less, the case with polyunsaturated fatty acids, i.e., especially conjugated double bonds.

"Ozone Peroxides" as Second Messenger Molecules

As reactive oxygen compounds, membrane-associated "ozone peroxides," could act as second messengers via cysteine residues and/or reduction through glutathione GSH in a less aggressive way than the superoxide radicals ·O-O$^-$ and H_2O_2, and take over regulation of the anti-oxidants, i.e., without SOD and catalase demand as in the oxidative stress processes of relevant pathological conditions (Figures 4a–c).

Short-chain hydroxy-hydroperoxide with its low tendency to radical reactions could initiate the regulation of anti-oxidant protective mechanisms as redox signal e.g., via the nuclear factors NFkB in stress and inflammation processes via Nrf2 (Gough 2009; León et al. 1998; Viebahn-Hänsler 2006). Figures 5a–b present a schematic survey.

H_2O_2 formed from ozone peroxides, as proposed by other authors (e.g., Bocci et al. 2011), will probably not fulfill this function, as the H_2O_2 content is, as a rule, pathologically increased in the patients concerned; the corresponding

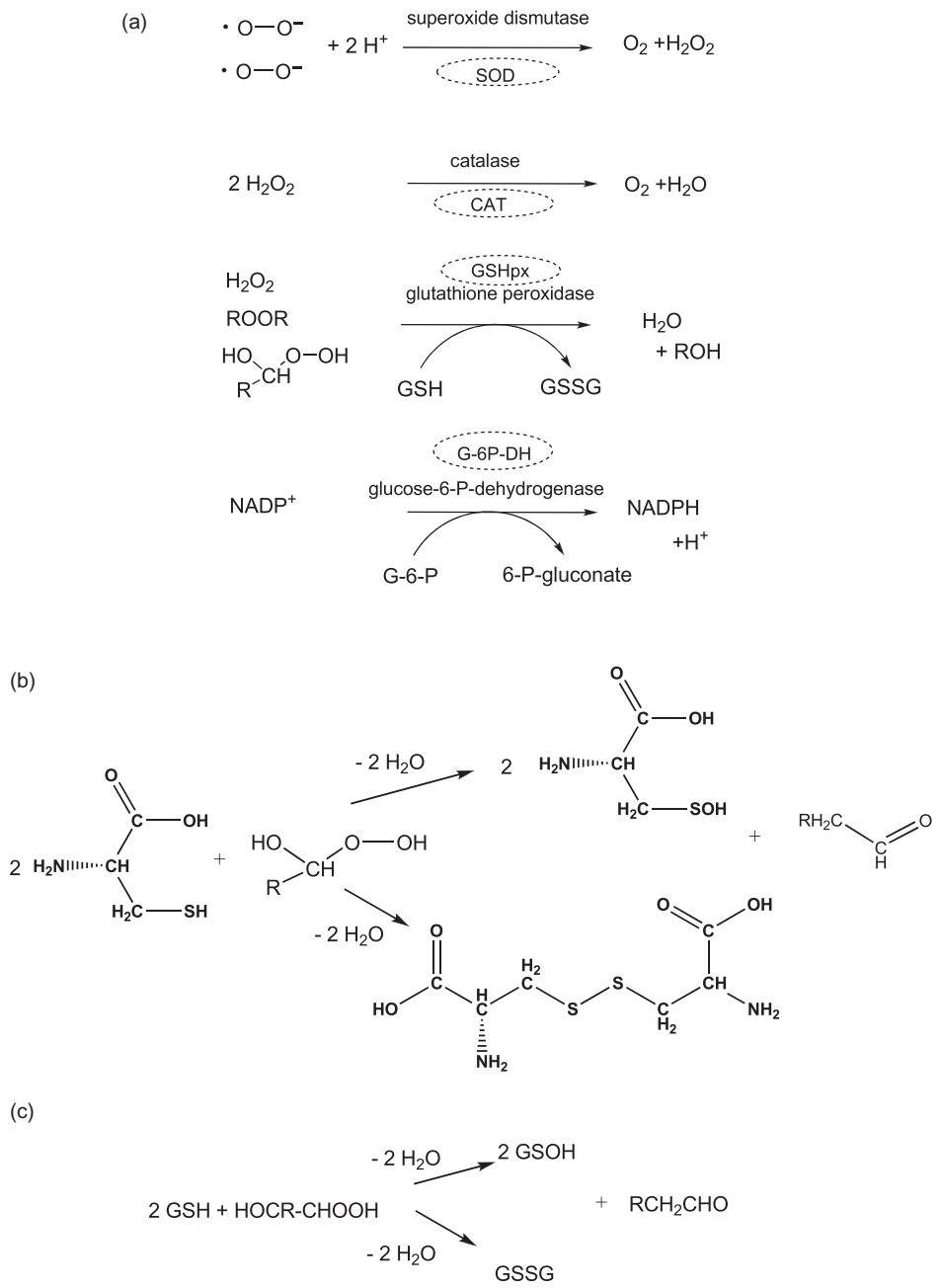

FIGURE 4. (a) The biological anti-oxidant system controlling the reactive oxygen species ROS, i.e., the oxidative stress situation. The "ozone peroxides" are controlled by the glutathione system (not by catalase); (b) Reaction of "ozone peroxides" with cysteine (residues); and, (c) glutathione.

signal transduction is insufficient, and there is a deficit in anti-oxidant enzymes.

THE LOW-DOSE OZONE CONCEPT

The effects of medical ozone as a hormetic substance can be assigned to those defined by hormesis (Rattan 2008). In living organisms, single or repetitive administration of otherwise potentially dangerous or toxic substances in small doses increases their homeodynamics (homeodynamic space), i.e., their self-regulatory capacity. Or, alternatively, moderate oxidative stress stimulates the protective mechanisms of cells and organs and is biologically useful.

Long-term ozone inhalation (e.g., for 8 h and using ozone concentrations up to 1 ppm in animal studies) produces oxidative distress, with a corresponding increase in the formation of reactive oxygen compounds, lipoperoxides,

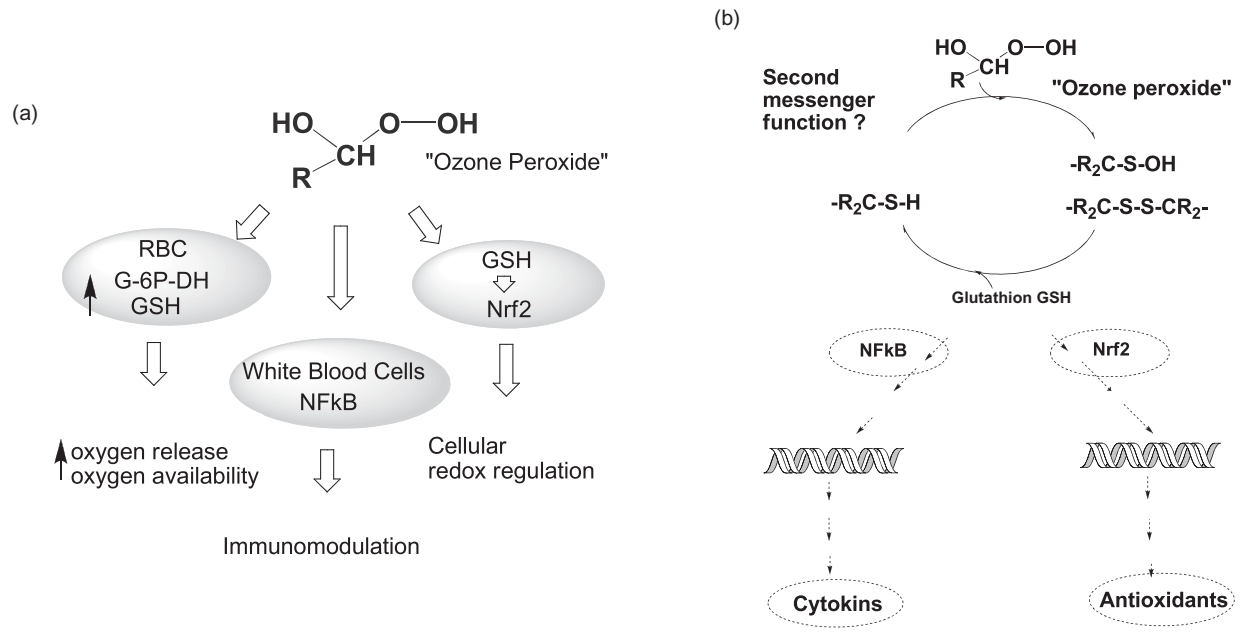

FIGURE 5. (a) Pharmacological effects of medical ozone via ozone-produced peroxides. The three effects: (1) improved oxygen release by the red blood cells (RBCs); (2) Immunomodulation through activation of the white blood cells (WBCs); and, (3) Regulation of cellular anti-oxidants. (b) "Ozone peroxide" and its second messenger function by signal transduction. Cysteine residue and glutathione oxidation processes induce the cytokine- or anti-oxidant regulation via Nrf2 or, NFkB, respectively.

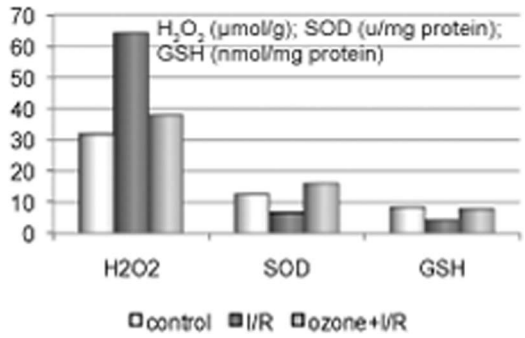

FIGURE 6. Protective effect of ozone on injury associated with hepatic ischemia reperfusion in an animal model. Pro-oxidant H_2O_2 and anti-oxidants SOD and GSH are regulated by pretreatment with ozone in the form of 10 daily rectal insufflations followed by 90 min of ischemia and 90 min of reperfusion (Peralta et al. 1999).

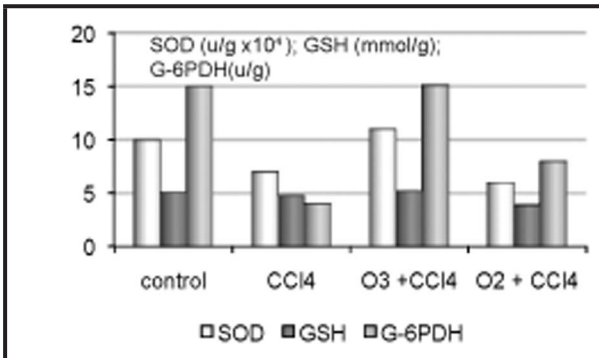

FIGURE 7. Protection against hepatocellular damage by free radicals induced by CCl4. Preventive ozone application (15 daily rectal insufflations; animal model) before administering CCl4. Liver seems to be completely protected in the ozone group: SOD, GSH and G-6PDH show physiological values, whereas the CCl_4- group as suppressed anti-oxidants (as well as the O_2-group; León et al. 1998).

cytokines, increasing infiltration of neutrophils and activated macrophages inducing and maintaining inflammatory processes. Biological response in the form of superoxide and OH radicals finally ends in a chronic inflammatory process with disfunction and downregulation of the cellular anti-oxidants.

Therapeutic usefulness is obtained via specific and well experienced forms of application, whereby airways are a taboo in ozone therapy. Single doses at low concentrations produce positive oxidative stress, i.e., equivalent to a single dose of "ozone peroxides," which result in a short-term effect on red and white blood cells involving induction of cell metabolism and bioregulation of enzymatic anti-oxidants. Table 1 shows the hormetic principle of ozone: toxicity versus therapeutical benefit in a shortened version.

Over the last 20 years, the low-dose ozone concept has developed into an established treatment method with a sound scientific basis. Its biochemical action and pharmacological

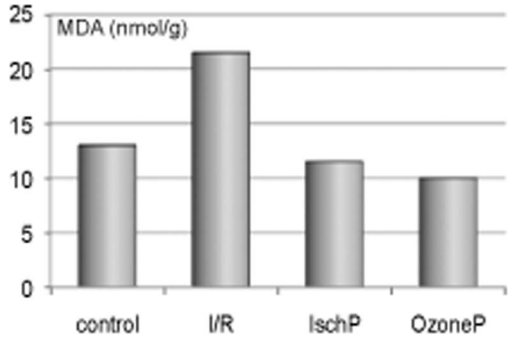

FIGURE 8. Protective effect of ozone in an ischemia/reperfusion animal model by pretreatment with ozone in the form of 15 daily rectal insufflations. Malondialdehyde MDA (as measure for oxidative stress) levels show a similar protection as ischemia preconditioning (Ajamieh et al. 2003).

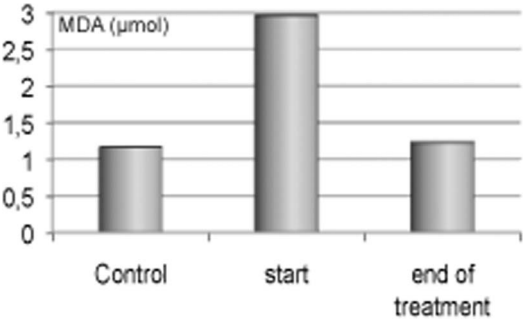

FIGURE 9. Malondialdehyde as plasma marker for oxidative stress in patients with disc hernia (acute) and after 20 days of paravertebral ozone injections (daily). Ozone reduces the oxidative stress, statistically high significance (León Fernández et al. 2012).

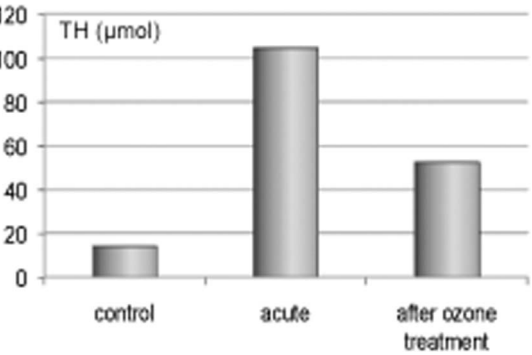

FIGURE 10. Total hydroperoxides TH as plasma marker for oxidative stress in patients with disc hernia (acute) and after 20 days of paravertebral ozone injections (daily). Ozone reduces the oxidative stress, statistically high significant (León Fernández et al. 2012).

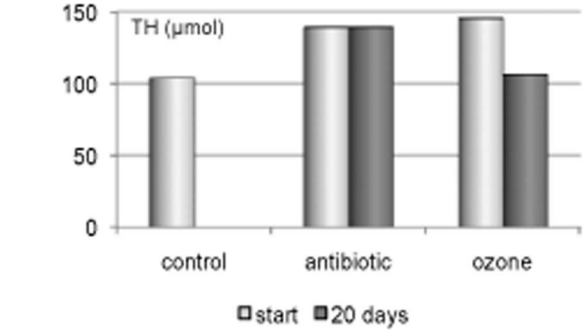

FIGURE 11. Total hydroperoxides as measure for oxidative stress in a diabetic study with 160 patients (60 healthy as control, 49 in the antibiotic group and 51 in the ozone group) decreasing significantly in the ozone group (Martinez et al. 2005).

properties have been well investigated, documented, and published in internationally recognized journals in the context of its classical indications. In the meantime, applied on a worldwide basis, ozone therapy continues to provide good and reproducible results, particularly in diabetic angiopathia, diabetic foot, chronic hepatitis, and chronic intestinal disorders.

However, application techniques and doses still vary to a certain extent; these now require recognized international standardization to establish the optimum forms of ozone application and the most effective concentration range in the relevant indications. This has been one of the major subjects dealt with by the "Medical Society for the Use of Ozone in Prevention and Therapy," in Germany, as one of 9 members (2011) of the European Cooperation of Medical Ozone Societies, over the last decade. These are based on scientific results and more than 30 years of experience (Beck et al. 1998; Knoch et al. 2009, Viebahn-Hänsler 2009).

Definition, Concentration Range, and Preparation

Medical ozone, an ozone/oxygen mixture consisting of purest O_2 and purest O_3, is produced from medical oxygen (in accordance with pharmaceutical legislation) using a medical ozone generator (Medical Devices Directives: 93/42EC or equivalent directives in non-European countries) in a concentration range between 1 and 100 µg/ml (mg/l). Therapeutically, and specific to each application, the concentration and dose ranges are listed in Table 2.

Data on the toxicity of ozone (mitotic index, cell aberrations, chromatic breaks) determined in (intraperitoneal) animal studies using mice show cytotoxicity at 2300 µg/kg, and a slight clastogenic activity at 1400 µg/kg mouse at 70 µg/ml. When administered intraperitoneally on 15 subsequent days, low concentrations of 4, 11, 20, and 35 µg O_3/ml, showed no toxicity, even at volumes of 80 ml/mouse (approx. 30 g body weight) (Fernández et al. 1989). This means that whether ozone is therapeutically useful or toxic is determined by both concentration and dose.

TABLE 1. Ozone and Hormesis: Toxicity Versus Therapeutical Benefit

Toxicity of Ozone in the Respiratory System via Inhalation	Therapeutic Effect in the Specific Forms of Medical Ozone Application
Long-Term Exposure (8 h, etc.) with ozone concentrations up to 1 ppm* (in animals)	Single Dose Low concentration
→ Oxidative DYS-Stress	Low dose → Positive stress
→ ROS (reactive oxygen species), LOP (lipid oxidation products) cytokines	→ Oxidative EU-Stress
→ Increasing infiltration of neutrophils, activation of macrophages	
→ Chronic inflammation	→ Induction of cell metabolism
→→→ Superoxide and OH-radicals.	
→ Disfunction of Anti-oxidants	
→ Downregulation of anti-oxidants	→ Regulation of anti-oxidants

* 1 ppm ≈ 2 mg/m^3

TABLE 2. Application-Relevant Concentration and Dosage Ranges in Ozone Therapy

Application	Ozone Concentration Range	Ozone Volume	Dosage/Ozone Amount Per Treatment
Systemic Treatment			
Major autohemotherapy (MAH)	10–30 μg/ml (max. 40 μg/ml)	50 ml	500–1,500 μg (max. 2000)
Rectal insufflation	10–25 μg/ml	max. 300 ml	3,000–7,500 μg
Minor autohemotherapy	10–20 μg/ml	10 ml	100–200 μg
Topical Treatment			
Wound cleansing	80–100 μg/ml		
Wound healing	10–25 μg/ml		
Injections in pain Syndrome	1–10 μg/ml	1 ml–20 ml	1–200 μg
In combination with local anesthetic	10–20 μg/ml	1 ml–20 ml	10–400 μg

Compared with this, in humans, the doses of 21.4 μg ozone/kg body weight applied (70 kg) in major autohemotherapy and 107 μg ozone/kg body weight in rectal insufflation are low, and can be administered over an extended period of time without any adverse effects. To make full use of the bioregulative function of ozone, low concentrations and small doses are required in the standardized applications (Figure 12). Figure 13 gives a concentration/effect relationship for systemic ozone applications.

Preparation and Measurement of Medical Ozone

Contrary to technical and smog ozone, the O_3 used in medicine is produced from pure medical oxygen via silent electrical discharge; it is not acceptable to use oxygen concentrators or oxygen/air mixtures due to their nitrogen component and the consequent possibility of nitrogen oxides being formed in the discharge tube.

As with other pharmaceuticals, medical ozone is a clearly defined molecule with a clearly defined range of action. With a half-life of 55 min in a 50 ml disposable injection syringe (completely siliconized and ozone resistant), medical ozone must be prepared on-site and made especially available for the type of application required.

As the concentration and decomposition rate of ozone is extremely dependent on different parameters such as temperature, pressure, volume flow rate, etc., medical ozone generators have to be equipped with a measurement device to ensure continuous concentration control.

Ozone produced in excess, either as part of the generator gas or after local application, must always be completely reduced back to oxygen to avoid odor and inconvenience to the respiratory tract; correspondingly, the system must be equipped with high-power catalysts (active carbon must not be used due to temperature and combustion hazard). The maximum workplace concentration is 120 μg/m^3 (or 180–240,

FIGURE 12. Major autohemotherapy. Standardized system acc. to the "Medical Device Directives" 93/42 EC. (1) Ozone syringe (Polypropylene); (2) Bacterial filter (0.2 μm); (3) Vacuum bottle (glass); (4) Transfusion set (polyethylene) (color figure available online).

respectively) (European Communities 2008); the maximum immission concentration is: 100 $\mu g/m^3$ (WHO 2006).

Measuring Ozone

Due to a powerful absorption band in the ultraviolet range (Hartley Band) with a maximum absorption at 253.7 nm, a photometric procedure is a method of choice at this wavelength for continuous ozone concentration measurement, and has become an international standard on which other measuring methods base their values and correspondingly apply for calibrating, as summarized by the International Ozone Association (Masschelein et al. 1998).

Quality Assurance

From a quality assurance and quality control point of view, the high reactivity of ozone with organic substances requires a careful selection of materials needed for the different types of medical equipment:

- Only special materials can be used in ozone generators, such as Teflon (PTFE), specially anodized aluminum, V_4A quality stainless steel (in long-term use, V_2A quality is subject to surface changes), glass, and ceramics.
- For application systems only "ozone-resistant" materials such as glass, polyethylene (PE), polypropylene (PP), and PTFE may be considered.
- Other plastics, especially for syringe pistons, must be silicone-coated.
- Medical plasma *flasks* as used for reinfusion should be made of *glass only*. Plasma bags or blood bags made of non-ozone-resistant, soft polyvinylchloride (PVC) must not be used. Reactions between ozone and these materials can occur producing xenobiotic and/or toxic substances, especially during O_3 blood treatment requiring up to 5 min to obtain the proper effect. The substances arising from a decomposition of the softening agents in the plastic, such as hydrogen peroxide or phthalic acid esters are not only able to distort the desired effects of ozone, but also damage the patient's health.
- For preparing and storing ozone-treated water, containers made exclusively of glass are to be used. These must have as small a volume (e.g., 250 ml) as possible; they should be completely filled and well sealed with O_3-resistant material.
- Use sterile, siliconized, 50-ml disposable syringes (measured ozone half-life: 55 min) to transport ozone/oxygen mixtures for use during home visits.

Ozone generators as well as all the disposables used for treatment have to fulfill the MDD (Medical Device Directives) in Europe (93/42 EC) and are labeled with a "CE" sign, including the number of the supervising authorities. Manufacturers must possess detailed and valid certificates.

THERAPEUTIC APPLICATIONS AND INDICATIONS

Major Autohemotherapy (MAH) with Ozone as Systemic Application

Writing guidelines for quality assurance and MAH procedures is only useful if, right from the beginning, incorrect applications are recognized as quality detraction factors that must be avoided at all cost. These are:

1. Application of intravenous injections and transfusions under pressure - (hazard of embolism!)
2. Application of O_3 gas with inadequate or unsuitable materials; e.g., use of non-ozone-resistant blood bags – this may result in the formation of xenobiotic substances
3. Withdrawal of an O_3/O_2 gas mixture via a direct and solid tube connection between the outlet valve of the ozone generator and the flask – (retrograde contamination with blood!), or
4. Repeated reuse of syringes without disinfecting, cleansing and sterilizing them as required – (infection hazard!).

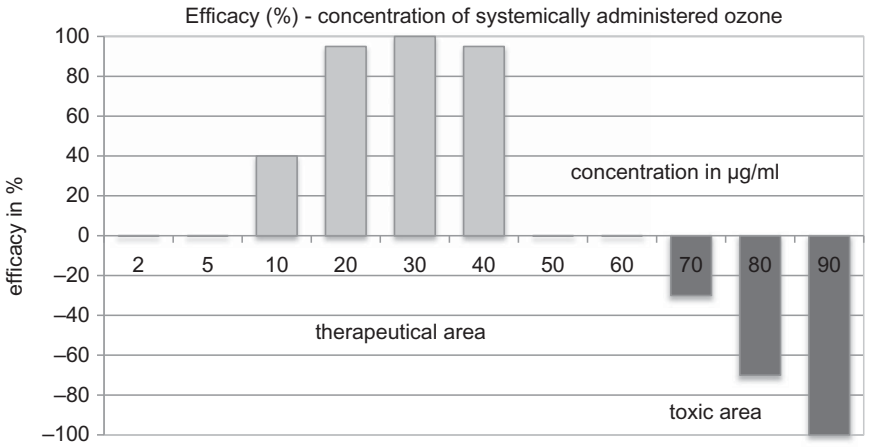

FIGURE 13. Efficacy concentration relationship of systemically administered medical ozone.

MAH Indications and Application Methods

MAH is reserved as a complementary medical concept for special indications. These are:

Arterial circulatory disturbances

- Peripheral arterial circulatory disturbance
- Cerebral circulatory disturbance (poststroke)
- Ocular circulatory disturbances (retinopathies)
- Inner ear circulatory disturbances (acute hearing loss (AHL), tinnitus)

Angiopathia

- Diabetic angiopathia in particular

Virus-caused diseases

- Hepatitis B and C
- Herpes simplex, herpes zoster

General immune deficiency

- As complementary therapy in general asthenia, geriatric and environmental medicine

Complementary concept in oncology

Chronic inflammatory processes in orthopedics and rheumatology

Contraindications

- Glucose-6-phosphate dehydrogenase deficiency (favism, acute hemolytic anemia)
- Hyperthyroidism if not controlled
- The first 3 months of pregnancy
- MAH is *not* indicated in leukemia

Procedure

In MAH, under strict aseptic conditions, 50–100 ml venous blood are withdrawn from the patient into a vacuum flask with sodium citrate as anticoagulant, where the medical ozone/oxygen gas mixture is added to it—extracorporeally in a closed, sterile system—before being re-infused via drip infusion (pressure-free).

The ozone/oxygen mixture must pass through the patient's blood evenly, preferably using what is called the microbubble system to produce a reaction surface as large as possible in the short reaction time (less than 1 s). Thus contact with ozone can take place with nearly all RBCs and nearly all WBCs making the ozone reaction as effective as possible, while the oxygen bubbles through, forming a layer of O_2 gas above the liquid level in the flask, see Figure 12 and Tables 3a–b.

Following contact with the blood outside the body, not one single ozone molecule nor one single oxygen molecule, enters the patient's vascular system. Only the products of a reaction between the ozone and the cellular components of the blood, i.e., activated RBCs and activated WBCs, are infused. In the presence of organic substances such as membrane lipids, the life of a highly reactive ozone molecule is extremely short (<1 sec), i.e., it is reduced prior to re-infusion.

Ozone Concentrations and Dosage in MAH

Based on the results of fundamental research over the last 18 years, the ozone concentrations and required total amounts determined in practice can be given in concrete terms. The concentrations used below are cited in the standard measuring unit of µg/ml (1 µg/ml = 1 mg/l = 1 g/m^3) under ambient conditions on-site /around the patient. Here, care must be taken whether we are discussing:

- µg ozone per ml ozone/oxygen mixture which is delivered by the ozone generator,
- µg ozone per ml blood, or
- the total quantity of ozone in µg per total quantity of blood, or the total quantity of ozone in µg per treatment.

TABLE 3a. Preparation and Performance of Major Autohemotherapy (MAH) Disposables

- Hand disinfectants on an alcohol basis
- Skin disinfectants on an alcohol basis or sterile alcohol
- swabs vacuum-packed in plastic foil
- Sterile cotton wool or gauze swabs
- Hypoallergic injection plaster
- Sterilized covering cloth
- 250 ml sterile vacuum gas flask with microbubble system, with sterile, pyrogen-free sodium citrate, without preserving agents
- Sterile, pyrogen-free transfusion unit with gravity drip chamber and tube clamp
- Sterile, pyrogen-free butterfly (cannula) set
- Sterile, pyrogen-free transfusion set with bacterial filter and tube clamp, for O3 administration in the 250 ml sterile vacuum gas flask
- Sterile, silicone-coated 50 ml disposable syringe, with preconnected bacterial filter
- Mobile (! Independent of patient) ozone supply unit (generator) equipped with a photometer for concentration measurement.

TABLE 3b. Performing MAH, Including Aseptic Procedures

- Disinfect both hands properly using 3–5 ml of a special hand disinfectant, observing the prescribed time to take effect of at least 30 s; in cases of possible contamination with stable viruses (HBV, HCV, HIV), this period should be 5 min. It is the aim of these elaborate precautions to encourage the wearing of protective (surgical) gloves, a preferable measure in all cases.
- Remove the protective cap of the 250-ml vacuum flask; the preferable method is to use both thumbs, pushing up and away from below. Disinfect the stopper with a skin disinfectant by spraying on, allow to dry (requires >1 min to take effect).
- Close clamps and introduce the cannula of the "germ stop" system through the cross marked on the stopper (microbubble system).
- Close clamp of the transfusion set; introduce it through the large circle marked on the stopper by piercing through.
- From the Teflon adapter on the generator, withdraw the O3/O2 mixture with a sterile, silicone-coated 50 ml disposable syringe with a preconnected bacterial filter (after previous loosening the piston of the syringe to overcome possible adhesion). The syringe is filled by the inherent pressure in the unit. Flush out the syringe as required with the gas once more. The remaining ozone is converted back to pure oxygen by the catalyst. In this way, neither the generator nor the syringe comes directly into contact with the patient. Always remember that **dry** ozone is **not** able to act as a microbiocide, inactivate viruses, or disinfect; so bacterial filter and syringe can only be used once.
- Connect the syringe filled with 50 ml gas mixture to the cone of the bacterial filter of the "germ stop" system
- Disinfect the patient's skin properly in the area around the infusion site (arm vein) via spraying on a skin disinfectant and distributing it with a sterilized cotton swab or gauze (in vacuum pack); allow to take effect for at least 1 min. From the butterfly cannula, withdraw approx. 50 ml patient's blood via the infusion system into the vacuum flask and fix butterfly with strip of plaster.
- Withdraw the O3/O2 mixture under vacuum from the disposable syringe via the bacterial filter of the "germ stop" system to ensure a smooth passage through the blood in the form of minute bubbles producing the desired immediate reaction between the ozone and blood cells. After passing through the blood, the remaining oxygen accumulates in the flask above the surface of the liquid.
- Carefully turn over the vacuum flask, remove the gas syringe for de-aeration and pressure-free re-transfusion of the ozone-treated blood, open and regulate the clamp in the transfusion set (60–90 drips/min).
- Remove the intravenous butterfly cannula, dab over the infusion point using a sterile cotton or gauze swab before covering it with a pressure dressing (hypoallergic injection plaster).

Ozone dosage covers a range between 500 μg and maximum 4000 μg ozone per treatment, using a quantity of blood between 50 and 100 ml. The sometimes recommended blood quantity of 200–300 ml is to be *rejected*, as this can present a risk from a hemodynamic viewpoint, especially in elderly or decompensated patients.

For blood treatment, concentrations of 80 μg ozone per ml whole blood and above are also to be *rejected*, on account of the increasing risk of hemolysis (up to 10% at 100 μg ozone per ml whole blood), a decrease in 2,3-diphosphoglycerate (2,3-DPG) and in anti-oxidants. Empirically, in major autohemotherapy (MAH), concentrations between 10 and

TABLE 4a. Major Autohemotherapy—Standard Procedure

Standard Procedure: 50ml of Blood + 50 ml Ozone Oxygene Mixture (or 100 ml of Ozone per 100 ml of Blood)			
Ozone concentration per ml of gas	10–20 μg/ml gas	30 μg/ml gas	Maximum 40 μg/ml gas
per ml blood = biologically relevant concentration	10–20 μg/ml blood	30 μg/ml blood	40 μg/ml blood
Total ozone amount per 50 (100) ml blood	500 –1,000 μg per treatment	1,500 μg per treatment	2,000 μg per treatment

40 μg ozone per ml blood have demonstrably shown themselves to activate cellular metabolism and have immunomodulatory effects as well as a regulatory effect on the intracellular anti-oxidants. The standard procedure for MAH is shown in Table 4a and the treatment protocols for the different indications in Table 4b.

Rectal Ozone/Oxygen Insufflation

This is one of the earliest forms of application in ozone therapy (Aubourg 1936). Based on animal investigations and a comprehensive proctologic study (Knoch et al. 1987), rectal insufflation with an O_3/O_2 gas mixture is increasingly being used as a systemic therapeutic form, and is already being viewed as an alternative to MAH; it is the method of choice in pediatrics.

Indications

Local

- Ulcerous colitis
- Proctitis, stages I and II
- Anal fistulae and fissures

Systemic

- Indications cited for MAH
- Hepatitis B and C
- For immunomodulation (complementary method in oncology)

Method

A rectal insufflation set consists of: An ozone supply container with lock valve, dosing bag with nonreturn valves, connecting tube with luer/luer lock or 50 ml silicone-coated disposable syringe, and rectal catheter.

Dosage

- Systemic: 10–25 μg ozone/ml oxygen gas mixture, volume 150–300 ml; for children: 10–20 μg/ml, volume 10–30 ml
- Local: in ulcerous colitis, high O_3/O_2 concentrations (70–80–100 μg/ml) and small volumes (50 ml) are applied; on cessation of hemorrhage, this is reduced to 30–20 μg/ml, followed by systemic efficacy: 10–20 μg/ml, 150–300 ml volume.

Rectal ozone application is simple, low-cost and practically free of adverse reactions when dosages are adhered to exactly.

As an adjuvant therapy in proctitis and proctocolitis, rectal insufflation is scientifically founded and to be recommended. Rectal O_3 insufflation is being increasingly used in pediatrics, sports medicine, geriatrics, and as a complementary method in oncology (Table 5).

Minor Autohemotherapy with Ozone

As a non-specific, immune stimulant therapy, comparable "autovaccination," not only ozone-specific.

Indications

- Acne vulgaris
- Allergies
- As an adjuvant in cancer therapy
- Immunoactivation

In minor autohemotherapy (MinAH), under aseptic conditions, 2–5 ml blood is removed intravenously and drawn into a sterile, pyrogen-free 30 ml disposable syringe (already containing the ozone-oxygen mixture), where it is mixed with 10 ml of an O_3/O_2 gas mixture, intensively shaken and slowly reinjected intramuscularly in the ventrogluteal region. Ozone concentration: 10–20 μg/ml, Table 6. (Caution: Never fill a syringe containing blood at the ozone generator!)

Topical Ozone Applications

In the local application of an O_3/O_2 gas mixture externally to skin or wounds—already practiced during the First World War—it was the disinfectant and deodorizing effect of ozone that stood in the foreground. It is now known that, with the topical application of O_3/O_2 gas mixtures, from ozone-treated water or ozone cream (ozonides) and beyond, a wound-healing effect is produced, which is being used to an increasingly successful extent.

TABLE 4b. Major Autohemotherapy MAH—Treatment Concept

Indication	Ozone Concentration	Ozone Volume	Ozone Amount	Treatment Frequency	Number of Treatments
Arterial Circulatory Disorders					
Cerebral and peripheral stage II	15–20 μg/ml	50 ml	750–1,000 μg	2x per week	Series of 10 treatments, 2–3× per year
Stages III and IV	20–30 μg/ml	50 ml 100 ml	1,000–1,500 μg 2,000–3,000 μg per 100 ml of blood	Daily at first, later 2× per week	
Immune Regulation, Oxidative Stress Regulation					
Revitalization, general immune deficiency, infection prevention	20–25 μg/ml	50 ml	1,000–1,250 μg	2x per week	Series of 10 treatments, 2–3× per year
Geriatrics AMD	15–20 μg/ml	50 ml	750–1,000 μg	2x per week	Series of 10 treatments, 2× per year
Virus-Caused Diseases					
Hepatitis acute stage	30 (max. 40) μg/ml	50 ml 100 ml	1,500 (2.000) μg 3.000 (4,000) μg	Daily in the beginning, then 1× per week, 2× per month	As per control
Chronic form (B/C)	10–20 μg/ml	50 ml 100 ml	500–1,000 μg 1,000–2.000 μg	2× per week, then 1×per week, then 2× per month daily	6–12 months
Herpes zoster Acute	40 μg/ml	50 ml	2,000 μg		As per control
Postacute	20–30 μg/ml	50 ml	1,000–1,500 μg	2× per week	As per control
Complementary Oncology					
Prevention of side effects of chemotherapy and/or radiation	10–15 μg/ml	50 ml	500–750 μg	2–3× per week	If possible 10 treatments before starting chemo- or radiation therapy
During chemotherapy	10–15 μg/ml	50 ml			
Or as complement to a biological concept	10–15 μg/ml	50 m	500–750 μg	2× per week	As per control and in compliance with the patient
Inflammatory Processes					
Rheumatoid arthritis acute stage	30–35 μg/ml	50 ml (100 ml)	1.500–1.750 μg per 50 ml blood (3.000–3.500 μg per 100 ml of blood)	Daily	As per control
non acute stage	20–25 μg/ml	50 ml	1.000–1.250 μg	1x per week, then every 2nd week	In compliance with the patient
Angiopathia, diabetic angiopathia	20–25 μg/ml	50 ml	1.000–1.250 μg	2x per week, later 2x per month	In compliance with the patient

Indications

- External ulcers (ulcus cruris, decubitus ulcers)
- Burns, superinfected
- Skin lesions (wounds)
- Local infections (smear infections, herpes simplex, herpes zoster, mycosis)
- Eye injuries and infections.

Application forms

- Ozonized water (acute treatment: e.g., injuries, burns, ulcers, as intraoperative rinsing)
- Pressure-free application in ozone-resistant plastic bags, in the form of transcutaneous O_3 gas bath (e.g., ulcus cruris, immune vasculitis)

TABLE 5. Rectal Ozone Application—Treatment Concept

Indication	Ozone Concentration	Ozone Volume	Ozone Amount	Treatment Frequency	No. of Treatments
Local Effect					
Ulcerate colitis	70–100 μg/ml	50 ml	3,500–5,000 μg	daily at first, later 1–2× per week	3–5, then like proctitis
Proctitis, particularly stage I	10–25 μg/ml	300 ml (150 ml)	3.000–7.500 μg (1.500–3.750 μg)	2–3× per week	4 weeks as percontrol
Anal fistulae	10–40 μg/ml	10–50 ml	Insufflation into the fistular passage	Daily at first, later 2×per week	As per control
Systemic Effect (Rectal Insufflation as Alternative to MAH)					
General immune activation, infection prevention, elderly	15–20 μg/ml	300 ml	4,500–6,000 μg	2× per week	Series of 10 treatments, 2–3× per year
Arterial circulatory disorders (stage II)	20 μg/ml	300 ml	6,000 μg	2× per week	Series of 10 treatments, 2× per year
Diabetic angiopathia	20–25 μg/ml	300 ml	6,000–7,500 μg	2× per week	In compliance with the patient
Complementary oncology					
Before chemo-/ radiation therapy	15 μg/ml	300 ml (150 ml)	4,500 μg 2,250 μg	daily	6–10 treatments
During and after chemotherapy or radiation	15 μg/ml	300 ml (150 ml)	4,500 μg 2,250 μg	2× per week	In compliance with the patient
Without chemotherapy or radiation	15 μg/ml	300 ml	4,500 μg	2× per week	In compliance with the patient
Virus-Caused Diseases and Inflammations					
Hepatitis, esp. chronic form (B/C)	25 μg/ml	300 ml	7,500 μg	Daily in the beginning, then 2× per week, 2× per month	As per control but mostly 6–12 months
Herpes zoster	25 μg/ml	300 ml	7,500 μg	2× per week	As per control
Rheumatoid arthritis	20–25 μg/ml	300 ml	6,000–7,500 μg	2× per week	In compliance with the patient

TABLE 6. Minor Autohemotherapy

Indication	Ozone Concentration	Ozone Volume	Ozone Amount	Treatment Frequency
Acne, furunculosis	10–20 μg/ml	10 ml	100–200 μg	1× per week (max 2× per week
Allergies	20 μg/ml	10 ml	200 μg	1× per week
Additional Cancer therapy	10–20 μg/ml	10 ml	100–200 μg	1× per week

- Subatmospheric ozone gas application under an ozone-resistant suction cup according to Werkmeister (1995) (e.g., decubitus)
- O_3 gas application in the low-pressure plastic boot ("Rokitansky boot") (e.g., diabetic gangrene)
- Ozone cream (ozonides) for long-term treatment: e.g., lesions, burns

Ozonized Water

In topical applications, the use of ozonized water is now gaining in importance. Ozone is present in water in molecular form, i.e., as O_3, presenting a physical solution. When using bidistilled water (aqua bidestillata) and a high-quality ozone generator, a maximum saturation of approx. 20 μg ozone per ml of water at room temperature can be obtained. It reacts immediately on skin contact, contrary to ozonides (such as ozone cream), which have a long-term effect.

Indications

- Local infections
- Ulcus cruris
- Decubitus ulcers
- Mycosis, mycotic infections
- Herpes simplex and herpes zoster (also including subcutaneous ozone injections where required)
- Burns, also superinfected burns
- Intraoperative rinsing
- Eye injuries and infections
- Surgical scars (healing: primary or secondary)
- Edemas of traumatic or bacterial origin

Methods and dosage

For 5–15 min, allow an ozone/oxygen gas mixture at an O_3 concentration > 100 (10 min) or 60–80 μg/ml (15 min) to pass in the form of small bubbles through 1-l aqua bidestillata with a water column of approx. 40 cm. In bidistilled water, the half-life of ozone is approximately 10 h at room temperature, the concentration remaining approximately 18–24 μg/ml at 20 °C (68 °F). In the refrigerator, ozonized bidistilled water can be kept for several days.

Over dosage is not possible, as the dose is limited by the solubility of ozone in water, approximately 24 μg/ml for aqua bidestillata. Ozonized water is basically applied on account of its pain-relieving, disinfectant and anti-inflammatory effects, as well as its tissue-activating properties in acute and chronic injuries with and without infection. In these indications, it is being applied with increasing success. Here, especially, the elimination of perifocal edema is in the foreground. Ozonized water is also being used intraoperatively for rinsing (disinfection), as in hand surgery, in dental medicine and particularly in oral surgery. The healing time for primary scars is shortened and irritation-free. In a number of cases, long-term treatment can be continued using peroxidic oils (Table 7).

Ozone Cream (Ozonides and Peroxides)

As reaction products of O_3 and unsaturated fatty acids, ozone peroxides and ozonides also stimulate wound healing. This could be clearly demonstrated for burns and mechanical injuries in an animal study. Peroxidic oils are used in the long-term treatment of injuries, burns and local infections such as skin and nail mycosis, as well as in the follow-up treatment of ulcus cruris and decubitus ulcers.

Topical Treatment as Transcutanous Gas Immersion or "Bath" (Bagging)

Transcutaneous "ozone gas immersion" is a method of choice in extensive, deep topical infections. Here, after moistening the extremity to be treated or the area over the organ concerned, direct O_3 gas immersion is applied inside a sealed,

TABLE 7. Topical Applications

Indication	Ozone Concentration	Form Of Application	Treatment Time	Treatment Frequency
Decubitus ulcers	80–100 μg/ml in the beginning	Low pressure suction cup	2–10 min	Daily first, then 1–2× per week
After wound cleansing	20–30 μg/ml			
Diabetic gangrene	80–100 μg/ml in the beginning	Low pressure boot (or plastic bag)	10–20 min	Daily first, then 1–2× per week
After wound cleansing	20–30 μg/ml			
Ulcus cruris	80–100 μg/ml in the beginning	Plastic bag (not low pressure!)	10–20 min	Daily first, then 1–2× per week
Wound cleansing				
Wound healing	20–30 μg/ml	compresses + rinsing with ozone water	1–5 min	several times daily
Burns stage 1 and 2	20–30 μg/ml	Plastic bag, compresses + rinsing with ozone water	10–20 min 1–5 min	First 1–2× per day, several times per day

ozone-resistant plastic bag, or in a slight vacuum using a special, low-pressure "boot" or under a low-pressure cup.

Indications

- Skin lesions, burns, superinfected wounds (surgical scars), diabetic foot, phlegmons (erysipela)
- Large-surface, open and deep chronic ulcers, possibly infected, and decubitus ulcers.

In transcutaneous ozone gas immersions or low-pressure applications used in the treatment of infected ulcers, the O_3/O_2 mixture is initially applied at higher concentrations (70–100 μg/ml) after moistening of the area to be treated. Its microbicidal and virostatic effect already takes place at lower concentrations (< 40 μg/ml). Once wound healing has actually started, the concentration can again be reduced (< 20 μg/ml), thus making full use of the metabolically stimulant and immunomodulatory effect of ozone as the healing process continues.

Low-pressure ozone treatment is not a monotherapy, as the other forms of wound treatment must be continued at the same time, according to the condition of the wound and in conformity with current rules. The considerable local hyperemic effect due to the mildly subatmospheric conditions plus the properties of ozone contributes to the healing process (Table 7).

Intra-articular ozone injections

Intra-articular ozone injections in acute and chronic, painful joint conditions represents a complementary treatment method providing rapid pain relief, decongestion, a reduction in inflammation, and an improvement in motility. It involves mainly knee and shoulder joints presenting chronic pathological symptoms.

Indications

- Symptoms of the rheumatic and degenerative type, diseases and injuries of the joints (arthrosis, arthropathias)
- Active gonarthrosis, acute diseases of the shoulder joints involving partially restricted functional movement (shoulder stiffness)
- Chronic shoulder joint conditions with calcification and painfully restricted movements in the final stage.

Intra-articular ozone injection is being applied with increasing success, particularly in orthopedic clinics, where inflammatory and degenerative diseases of the bones and joints as well as posttraumatic conditions (i.e., following sport injuries) and surgery of the large joints are involved, cases in which additional MAH has a supportive and stabilizing function.

Performance

Prior to ozone application, the standard injection points can be infiltrated with a slow-acting local anesthetic. For intra-articular ozone injections, a volume of approx. 20 ml for shoulder and knee is used, with ozone concentrations of 10–20 μg/ml and 2–11 μg/ml for peri-articular and subcutaneous infiltrations (Table 8).

Intra-articular injection of an ozone/oxygen gas mixture must be considered as being an intervention in a sterile system (joint), to be carried out under especially strict aseptic precautionary measures (Table 9). In addition, the ozone unit must be cleaned every day after use as hygiene requires (see manufacturer's instructions), and additionally disinfected by wet wiping with corresponding agents (surface disinfectants) when contamination with blood has occurred.

Regular maintenance of the unit must also be counted as hygienic safety. The aseptic steps recommended agree, for the most part, with those issued by the "German Orthopedics and Traumatology Association," the Orthopedic Physicians' Professional Association, and the "Guidelines on Intra-articular Injection Procedures" published by the Hospital Hygiene Work Group. These guidelines continue to be the officially recognized standard for medical experts and law courts, and should carefully be adhered to.

Subcutaneous and Intracutaneous Application

Indications

- Pain management
- Trigger points, acupuncture points
- Herpes zoster

TABLE 8. Intra-articular Injections—Treatment Concept

Indication	Form Of Application	Ozone Concentration	Ozone Volume	Ozone Amount	Treatment Frequency
Arthrosis	Intra-articular	7–20 μg/ml	1–20 ml	7–400 μg	1–2× per week
	Peri-articular	2–11 μg/ml	2–5 ml	4–55 μg	
Knee joints	Intra-articular	10–20 μg/ml	5–20 ml	50–400 μg	1–2× per week
Shoulder joints	Intra-articular	10–20 μg/ml	5–20 ml	50–400 μg	1–2× per week
Finger joints	Intra-articular	10–20 μg/ml	1–2 ml	10–40 μg	1–2× per week

TABLE 9. Aseptic Procedure for Intra-articular Ozone Injections (Beck et al. 1998)

For intra-articular injections, special aseptic precautionary measures are necessary to prevent infection! [see e.g., "German Orthopaedics and Traumatology Association" (Deutsche Gesellschaft für Orthopädie und Traumatologie) Guidelines] Caution: To avoid general or local infections and skin lesions at injection site and surrounding area:

- Hygienic hand disinfection using an RKI/DGHM listed (or equivalent) alcohol-based detergent. Always allow > 30 s for it to take effect. In cases of suspected HBV or HCV, etc., virus carriers, allow for 5 min before treating or, even better: use (sterile) disposable surgical gloves!
- Alternatively: after surgical hand disinfection, put on sterile surgical gloves, sterile protective clothing, and use sterile cloth covers around the injection site (e.g., when in contact with the patient's skin over the area under treatment).
- Disinfection of skin at treatment site using an RKI/DGHM listed (or equivalent) alcohol-based disinfectant: spray liberally over site and gently rub in with a sterile gauze swab. Allow > 1 min to take effect.
- From the Teflon valve of the supply unit, remove the prescribed quantity of medical ozone/oxygen gas using a sterile, silicone-coated 50 ml disposable syringe with a preconnected bacterial filter.
- Using a long, thin, sterile disposable cannula, e.g., 0.8 × 40 mm (size 2 metric) or 0.6 × 60 mm, inject the ozone/oxygen gas mixture.
- Immediately cover the injection site with a (sterile) quick-action wound dressing.

- Trigger points in combination with local anesthetics (neural therapy)
- Tonanalgetic systems

Ozone concentrations: 2–5 without local anesthetic, 10–15 μg/ml with local anesthesia.

Intradiscal injections

Intradiscal injections in herniated discs may only be performed when monitored by imaging systems as found in hospital departments where these are available (Alexandre et al. 2012, this issue).

FINAL COMMENTS

These guidelines for the use of medical ozone have been set up and evaluated on the basis of nearly 40 years experience and on relevant scientific literature as published in national and international journals and handbooks.

- The German "Medical Society for the Use of Ozone in Prevention and Therapy" was founded in 1971 as a nonprofit organization.
- Together with the sister societies in Switzerland, Austria and Italy the "European Cooperation of Medical Ozone Societies" EUROCOOP was founded in 2002.
- The 9 medical societies in 2011 consisted of the following countries: Switzerland, Austria, Germany, Spain, Romania, Egypt, Turkey, Japan, and Indonesia. Its aim has been to standardize ozone therapy to the greatest possible extent. In the same way, the member societies will aim at providing mutually valid training and development guidelines, making provisions to the fullest possible extent for all indications and application methods of ozone therapy. In the long run, a European Standard Qualification could become a uniform objective.
- Every 2nd year an International Ozone Congress is held, organized by one of the member societies.

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