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OZONE ENTERS ITS AGE OF ENLIGHTENMENT: Ozone therapies today, and tomorrow

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Abstract

The science of ozone is poised to achieve major advances in medicine. Ozone, the first sentinel gaseous layer separating earth from outer space, protects life from solar electromagnetic radiation; at the same time, in the very depths of our organisms, ozone works at molecular and cellular levels to inactivate microorganism invaders via one of the most potent weapons known to biology: oxidation.

Ozone, thus, as a prominent member of the oxygen's atom family, is gathering respectability because it is increasingly understood, not as a freak of nature to be shunned, but as a fundamental player in the very architectures of our metabolism and our physiology.

Anti-pathogen actions are ozone's current most validated properties. Properly handled to safeguard healthy tissues, ozone/oxygen mixtures easily inactivate the bacterial, viral and fungal species that invade wounds, providing clinicians with new tools to combat diabetic, pressure and venous stasis ulcers, infected wounds, and burns.

Ozone, interfaced with blood or other fluids and administered systemically, is reported to have metabolic, physiological and immunological effects that, with new research and understanding, may make it a significant treatment modality in the therapy of systemic infections, cardiovascular conditions, and in clinical disorders implicating immunological malfunctions such as certain types of cancer.

Introduction

Today's awareness of medical ozone is decidedly on an upward trajectory. Finally loosening the chains of ozone's rigid association with toxicity, a new openness sees ozone in its greater, and greener perspectives, as one of nature's most fascinating molecules.

Ozone is much more than one of the earth's most powerful oxidants: it is a supremely clean oxidant, belonging to a family whose most prominent member is oxygen, intrinsic to earthly life.

This paper summarizes the scientific underpinnings of ozone-based medical therapies so as to provide a clearer rationale for clinical practice. Only if derived from sound scientific principles can ozone therapies assert themselves in true partnership with other medical sciences.

Based on ozone's established properties, some forward-looking views on ozone's medical future are offered, for the near and longer term.

Ozone exerts its chemical force for biological missions

All energies, in order to be useful, need to be properly harnessed. The same holds true for ozone.

Ozone's core attribute is its potent capacity to attract electrons from molecules. In exchange, it jettisons one of its oxygen atoms and assumes a more relaxed energetic state, the oxygen we all depend upon.

This dance of electrons, as simple as this may appear on its surface, attains great complexity when involving biological systems. Indeed, while ozone dynamics are generally understood for many inorganic transactions, the same cannot be said for ozone interactions with the vastly complex biochemical universe within us.

What is the advantage of confronting an organism with an element that has an avid hunger for the electrons in its system? The question is poignant enough when talking about a situation involving an organism in a state of health. The same question becomes especially interesting when examining an organism afflicted with a pathological condition. Can an oxidizing challenge ever be beneficial, by way of therapeutically coaxing the organism in the direction of physiological balance and better health?

Electron cascades in the harmony of life

Much of life centers on energy transfers. This is not to ignore its spiritual dimensions, but on a metabolic level, organisms depend on the constant withdrawal of energy from molecules. Energy transfers involve intricate cascading electron exchanges. In this electron marketplace, the addition of ozone to an organism's metabolic functions – as for example, the administering of ozone to blood – account for biochemical actions that are far from instantaneous. Complex molecules, whose electrons are siphoned off by ozone, undergo many chemical alterations in the form of transitional compounds (e.g., zwitterions, molozonides, cyclic ozonides, lipid and protein peroxides) that, with lifespans of microseconds to hours, may have unique biological activities of their own.

In addition, ozone, in its interactions with tissue water, is instrumental in generating hydrogen peroxides, hydroxyl and superoxide radicals, and dihydrogen trioxide, among others, all dynamically related as members of the same extended oxygen family (Halliwell 2007). Their effects, although modulated by buffers and antioxidants, are nevertheless cause for attention because of free radicals' capacity for altering cellular organelles and genetic structures (Olinescu 2002). A question is posed at this juncture: can the temporary creation of free radicals in the body – in this case via systemic ozone administration - manifest therapeutic value? Can the induction of activated molecules perform useful functions such as stimulating bodily systems – including immune networks and anti-oxidant defenses – to assist the organism in its quest for repair and health?

Mammalian cells have evolved to use oxygen as their ultimate electron acceptor. An essential feature of humans, which allows for the very use of ozone therapies, is the discrepancy of higher

versus lower life forms for processing oxidative challenges. While viruses have nil, and bacterial organisms have few anti-oxidative defenses, mammalian cells, on the contrary, because of their supreme dependence on oxygen, have evolved complex buffering schemes capable of deflecting the disruptive products of oxygen metabolism. This endows them with a high degree of resistance to the oxidative challenges encountered in ozone therapies. Indeed, without these cellular defenses, most forms of ozone therapeutics could not be performed.

Oxidative stress is the imbalance between free radical production and antioxidant defense (Lane 2002). The oldest evolutionary electron donor designed to defend against reactive oxygen species is catalase. Catalase makes possible the conversion of hydrogen peroxide, a byproduct of metabolism, to water and oxygen. Other electron providers crafted to harmonize cellular metabolism include the peroxidases, glutathione, vitamins C and E, and uric acid, among others.

Ozone effects on bacterial organisms.

Werner von Siemens developed the first ozone generator in 1857 (Viebahn 2007). Essentially, this consisted of an oxygen chamber subjected to an intense electrical field. Kleimann, shortly thereafter, carried out the first bacteriological studies on pathogenic bacteria (Rilling 1987).

Pioneering clinical applications first came during the First World War when externally-applied ozone/oxygen mixtures were administered to battlefield wounds. Ozone fought infections, and via its vasoactive properties, encouraged wound repair. Equipment failures, however, due to ozone oxidative action on rubberized treatment envelopes, impeded progress in this area until the development of ozone-resistant plastics many decades later.

Today, there are over 3000 ozone-based municipal water purification systems worldwide, a constantly growing number. This represents a clear testimony to ozone's potent antimicrobial properties.

Exposed to ozone, all bacterial species fare poorly. Bacterial envelopes are composed of invaginating multilayers whose components are ozone-reactive. Surrounding the bacterial cytoplasm is a phospholipid proteinaceous cytoplasmic membrane, itself englobed by a structurally-stabilizing peptidoglycan shell. In acid-fast bacteria (e.g., *mycobacterium tuberculosis*), up to one half of the capsule contains complex lipids.

Ozone acts on bacterial cell membranes via the oxidation of their lipid and lipoprotein components, whose multiple chemical bonds then assume new angular configurations incompatible with viable bacterial architecture. There is evidence for interaction with proteins as well (Mudd 1969). In one study exploring the effect of ozone on *E. coli*, ozone penetrated through cell membranes, reacting with cytoplasmic contents, cleaving the circular plasmid DNA, thus impairing bacterial procreation (Ishizaki 1987). Higher organisms have developed mechanisms for protecting DNA and RNA, and for repairing them when disrupted, which could provide a partial explanation for why, in clinical treatment using ozone at doses prescribed, ozone is toxic to pathogens and not to the patient (Cech 1986).

Given adequate time of exposure and intensity of concentration, any and all bacterial species – except perhaps the super hardy *Deinococcus radiodurans* and similar organisms - invariably succumb to ozone action, a fact that endows ozone therapy with one of its most solid scientific foundations.

Ozone's antiviral actions

Viruses are parasites at the genetic level, separated into families based on their structures, types of nucleic genome, and modes of replication. Recently, there has been ever increasing interest in ozone's potential for viral inactivation *in vivo*. Long established is ozone's *in vitro* neutralization of viruses and it stands to reason that this capacity would be studied in living systems. *In vivo* ozone applications, however, present special challenges.

All viruses are susceptible to ozone; yet differ widely in their susceptibility. In one study, poliovirus resistance was 40 times that of coxsackievirus (Roy 1982). Analysis of viral components showed damage to polypeptide chains and envelope proteins impairing viral attachment capability, and breakage of viral RNA. Other researchers suggested that, in ozonation, it is the viral protein capsid that sustains damage (Riesser 1977). Viruses, unlike mammalian cells, have no enzymatic protection against oxidative confrontation.

Lipid-enveloped viruses are especially sensitive to ozone challenge, implicating that lipid alteration is a salient mechanism for their viral death. Viruses containing lipid envelopes include the *Hepadnaviridae* (Hepatitis B), the *Flaviviridae* (hepatitis C, West Nile virus, yellow fever); the *Herpesviridae*, a large family grouping the Simplex, Varicella-Zoster, Cytomegalovirus, and Epstein-Barr viruses; the *Orthomyxoviridae* (influenza); the *Paramyxoviridae* (mumps, measles); the *Coronaviridae* (SARS); the *Rhabdoviridae* (rabies); the *Togaviridae* (Rubella, encephalitis); the *Bunyaviridae* (Hantavirus); the *Poxviridae* (smallpox); the *Retroviridae* (HIV), and the *Filoviridae* (Ebola, Marburg), among others. Indeed, once the virion's lipid envelope becomes fragmented, its DNA or RNA core cannot progress in its life cycle.

Viruses that do not have an envelope are called "naked viruses." Made of a DNA or RNA nucleic acid cores, and a nucleic acid protein coat, or capsid, they are generally more resistant to ozone challenge than lipid-coated virions. Some naked viruses include: *Adenoviridae* (respiratory infections), *Picornaviridae* (poliovirus, coxsackie, echovirus, rhinovirus, hepatitis A), *Caliciviridae* (hepatitis E, Norwalk gastroenteritis), and *Papillomaviridae* (Molluscum contagiosum). Ozone interacts with the viral proteins of naked viruses, forming protein hydroxides and peroxides, leading to viral demise.

Possible mechanisms for ozone's antiviral action in bodily fluids:

A major avenue for ozone administration is the "internal route," namely a direct interfacing of ozone/oxygen mixtures with bodily fluids that, in contemporary practice, mainly involves blood. Ozonating blood is used for a number of pathological conditions, mostly chronic viral infections (e.g., hepatitis B and C, herpes), but also for a number of non-infectious clinical situations. In view of ozone's demonstrated antiviral actions *in vitro*, what are its possible modes of antiviral action *in vivo*,

given the fact that *in vivo* ozone concentrations require downward adjustments to safeguard the integrity of blood's constituents?

1. The denaturation of virions through direct ozone contact. Ozone, via this mechanism, disrupts viral envelope lipids and lipoproteins. Lipid bonds are reconfigured, fragmenting the viral envelope. At doses administered in hematogenous ozone therapy, research will need to gauge the relative contribution of this direct mechanism.
2. Ozone may directly alter structures jutting from the viral envelope that enable attachment to host cells. Peplomers, the viral glycoproteins protuberances that bind to host cell receptors are likely sites of ozone action. Peplomer alteration impairs docking to host cell membranes, foiling viral penetration.
3. Introduction of ozone in blood induces the formation of circulating serum lipid and protein peroxides. While these peroxides are not demonstrably toxic to the host in quantities generated by ozone therapy, they nevertheless possess oxidizing properties of their own that persist in the bloodstream from seconds to several hours. Peroxides created by ozone administration may serve to further reduce viral load via the above mechanisms, and via the engagement of immune factors.
4. Immunological effects of ozone have been reported. Cellular and humoral systems have been studied. Cytokines (e.g., interferons, interleukins, colony stimulating factors, tumor necrosis factors) are intercellular signaling molecules manufactured by several types of cells that regulate the functions of other cells. Mostly released by leucocytes, they are important in mobilizing immune response. Ozone, via unknown mechanisms, has been found to induce the release of cytokines (Bocci 2005). Cellular (e.g., natural (NK) killer cells) activation has been reported as well (Larini 2001). Ozone is reported to be an immuno-stimulant in low doses and immuno-inhibitory at higher levels (Werkmeister 1985, Varro 1974, Zabel 1960, Bocci 2000).
5. Ozone's modification of virion architecture may leave some virions structurally intact yet sufficiently dysfunctional so as to be nonpathogenic. This attenuation of viral functionality, through alterations of the viral envelope and possibly the viral genome itself, may soften or nullify virulence. The creation of dysfunctional circulating viruses by ozone could offer unique therapeutic possibilities. In view of the fact that so many mutational viral variants exist in any one afflicted individual (e.g., hepatitis C, HIV), the creation of an antigenic spectrum of crippled or fragmented virions could function as a host-specific autovaccine.
6. An exciting research direction suggests that the virucidal – and bactericidal - properties of antibodies are predicated upon their ability to catalyse highly active forms of oxygen, including ozone (Marx 2002; Wentworth 2002). In this model, activated neutrophils are capable of generating singlet oxygen, a most potent oxidant. Singlet oxygen paired with oxygen forms ozone, and with water yields the hydroxyl radical (OH), and hydrogen peroxide. Ozone and hydrogen peroxide combine to form peroxone, another highly active pathogen destroyer. Endogenously created oxygen reactive species, including ozone, thus become fundamental immunological agents for pathogen inactivation. Could exogenously administered ozone augment the antimicrobial functions of leucocytes?
7. Periodic oxidative challenges such as practiced in ozone hemotherapy may jolt the network of immune systems, and anti-oxidant systems, to upregulate their general reactivity. For any immune

system riposte to happen, however, there must be the presence of a modicum of immune functionality. In end stage HIV and hepatitis C infections, for example, where the immune system is all but moribund, immune reactivity may have become exhausted. In these situations, any attempt at immune stimulation, including ozone administration, may be all but futile, a fact which makes the use of early ozone interventions clinically prescient.

Ozone effects on wound pathogens

At dosage concentrations used in topical therapy, ozone essentially inactivates all bacterial species. This holds true for oxygen-dependent aerobic organisms, for oxygen-independent anaerobic bacteria well-known for causing gangrene, and for facultative species capable of thriving in either medium. Ozone's universal antibacterial action makes it an agent of choice in the management of wound infections colonized by bacterial species belonging to diverse groups.

A partial list of bacterial families found in infected wounds and susceptible to ozone inactivation includes the *Enterobacteriaceae*, a large group of Gram-negative microorganism families whose natural habitat is the intestinal tract of humans (*Escherichia coli*, *Salmonella*, *Enterobacter*, *Shigella*, *Klebsiella*, *Serratia*, and *Proteus*). Other ozone-sensitive bacterial species include *Streptococci*, *Staphylococci*, *Legionella*, *Pseudomonas*, *Yersinia*, *Campylobacteri*, and *Mycobacteria*.

Fungi are frequent inhabitants of infected wounds. One study of fungal infections of burn wounds (Moussa 1999) found colonization mainly by *Candida* and *Aspergillus*. Fungal organisms neutralized by exposure to ozone also include *Histoplasma*, *Actinomycoses*, and *Cryptococcus*. The multilayered cell walls of fungi are composed of approximately 80% carbohydrates, and 20% proteins and glycoproteins. Ozone may target fungi wall's numerous disulfide bonds, making this a privileged site for ozone inactivation. Ozone also has the capacity to diffuse through fungal membranes into the organismic cytoplasm, thus disrupting vital cellular functions.

Protozoan organisms are occasionally found in infected wounds. Protozoan species disrupted by ozone include *Giardia*, *Cryptosporidium*, and free-living amoebas, namely *Acanthamoeba*, *Hartmonella*, and *Negleria*. Spores of *Bacillus cereus* and *Bacillus megaterium* were susceptible to ozone exposure at 5 minutes (Broadwater 1973). Several authors have demonstrated ozone's capacity to penetrate through the walls of *Giardia* cysts causing structural damage (Widmer 2002, Wickramanayake 1984, Finch 1993).

Medical conditions responsive to externally applied ozone/oxygen mixtures.

In view of ozone's above-mentioned antimicrobial properties, the following are conditions beneficially influenced by this unique drug therapy, utilized as a sole agent, or as an adjunct to other treatment modalities:

Poorly-healing wounds are frustratingly difficult to master. Some of these wounds are apt to regress if treatment continuity is interrupted, even for a short time. Poorly-healing wounds, aside from their chronicity, imply infestations with multiplicities of different microorganisms.

Human skin is home to myriad pathogenic microorganisms, all normally kept at bay. In tissue injury, however, these same pathogens can become invasive. In poorly healing wounds, oxygen-avoidant

bacteria (e.g., *Bacteroides*, *Clostridium*) may fester at deeper levels of the dermis, insulated from the antibacterial action of surface oxygen. Aerobic bacteria such as *Staphylococcus epidermis*, *Corynebacteria*, and *Propionobacteria*, normally free-living on epidermal layers, are also capable of remarkably aggressive colonization once skin integrity is breached.

Diabetic and decubitus skin ulcers. Approximately 15 to 20% of the estimated 25 million Americans afflicted with diabetes mellitus will require hospitalization during the course of their illness for associated leg ulcerations, infections or gangrene. About 15% of these patients, often after prolonged intensive management, will suffer lower leg amputations.

Diabetic ulceration is promoted by poor circulation and neuropathy. One study (Anandi 2004) reported bacterial culture results for 107 patients with diabetic leg lesions. Bacterial families cultures included: *E. coli*, *Klebsiella*, *Pseudomonas*, *Proteus*, *Enterobacter*, *Clostridium perfringens*, *Bacteroides*, *Prevotella*, and *Peptostreptococcus*.

Decubitus ulcers arise when patients maintain a bodily position for prolonged periods of time. The pressure on skin contact points compresses dermal arterioles, impairing tissue perfusion. Ulceration develops, a fertile ground for pathogens. At times, the denudation of dermal tissues reaches the bone, leading to osteomyelitis. Treating diabetic and decubitus ulcers requires multidisciplinary approaches, including surgical, topical and systemic interventions. Topical antibiotics often fail to penetrate far enough into the wound and not infrequently cause secondary dermatitis. Furthermore, topical as well as systemic antibiotics can only address a portion of the spectrum of infectious microorganisms cultured from such wounds, and are often found to have been surpassed by bacterial resistance (e.g., β -lactam antibiotic resistance, as in methicillin-resistant staphylococcus aureus - MRSA).

External ozone therapy in ulcers provides dual functions of broad-spectrum microorganism destruction and circulatory stimulation. Serial applications with extended times of ozone exposure allows for deeper penetration of tissue layers where anaerobic bacteria are more apt to reside.

Gas gangrene is also known as necrotizing fasciitis, myositis, and myonecrosis. Feared because of its galloping evolution and the irreversible destruction of affected tissues, gas gangrene may be a rapidly fatal complication of traumatic injuries such as automobile accidents, war traumas, surgical wounds, burns, and diabetic and decubitus ulcers.

Several bacterial species are implicated in this condition, the most common being *Clostridium* and toxin-producing Group A Streptococcus families. Other bacterial species cultured in gas gangrene include *Enterobacteria*, *E. coli*, *Proteus*, *Staphylococcus*, *Vibrio*, *Bacteriodes*, and *Fusiforims*.

Feeding on glycogen and sugars, these anaerobic and facultative bacteria produce lactic acid and gases such as methane, carbon dioxide and hydrogen. Their life-threatening toxins cause severe local tissue breakdown, hemolysis, renal failure and shock.

Emergency external ozone application to necrotizing lesions is an important adjunct to the multidisciplinary intensive interventions these impressively destructive wounds demand. The effectiveness of ozone is tempered, however, by the extent of tissue injury at depths that may be beyond its reach. Faced with these situations, several authors recommend the addition of hematogenous ozone administration for its contribution to enhancing blood oxygenation and

improving the rheological profile of blood.

Lymphedema. The lymphatic system is essential for proper fluid equilibration within the body, and most importantly for adequate defense against infections. Lymphedema implies a blockage to lymphatic drainage, often secondary to trauma, surgical procedures, and infections.

Increasingly common is lymphedema resulting from surgical removal of lymph nodes following surgery for breast cancer. The affected arm in these patients is likely to be chronically swollen and exercises are often prescribed to develop collateral circulation. Most important, however, in the absence of lymphatic drainage, is the proneness to infection following even minor injuries to the affected hand and arm.

Superficial injuries in limbs with lymphatic compromise are commonly addressed with intensive topical care. Systemic antibiotics are added when local measures fail. Timely topical ozone/oxygen treatment, on the other hand, instituted as soon as an injury is noted, may abort infectious developments and ultimately avert the repeated use of topical and systemic antibiotics.

Fungal skin infections. Fungal families frequently implicated in skin lesions include *Candida*, *Aspergillus*, *Histoplasma*, *Actinomycoses* and *Cryptococcus*, all susceptible to growth inhibition and by ozone.

Obstinate nail afflictions caused by *Candida albicans*, *Trichophyton* and *Epidermophyton floccosum* are therapeutically assisted by topical ozone treatment. Ozone, with extended exposure, penetrates the affected areas, including the nails proper. With repeated administration, ozone is capable of inactivating all species of fungi mentioned above.

Burns. Colonizing organisms in infected burns may show a broad spectrum of infectious families, and thus may be ideally suited for ozone therapy.

Viral cutaneous conditions. Herpes viruses are widespread in the human population. In herpetic lesions, fluid accumulates between the dermis and epidermis, producing vesicles that rupture, releasing virions. Broken vesicles are then open to bacterial suprainfections.

Herpes lesions have been extensively studied with reference to topical ozone administration (Mattassi 1985, Olwin 1997). Ozone, in these cases, neutralizes herpes virions by direct action, thus inhibiting bactericidal suprainfections, and stimulating the healing of tissues through circulatory prompting. It is postulated that ozone, conjointly administered topically and systemically, may have beneficial effects on the peripheral neurons harboring these viruses via activating immunological pathways.

Radiodermatitis. Exposure to ionizing radiation may occur during nuclear accidents and as side effects of radiation therapy. Clinical findings are commensurate with the type, amount, and length of radiation exposure. Radiation erythema, and acute and chronic radiodermatitis may follow.

Tissues damaged by radiation are vulnerable to infection. Preventive external ozone therapy in radiodermal injuries decreases the probability of developing bacterial complications.

Frostbite. Prolonged exposure to cold leads to the formation of ice crystals within tissues. Loss of

sensation occurs and tissues become hard to the touch. Extended exposure leading to irreversible tissue damage, and improper therapeutic tissue rewarming contribute to the development of dry gangrene which may evolve to wet gangrene.

Topical ozone therapy can be effective in halting the evolution of frostbite via the intensive oxygenation of tissues, the encouragement of blood flow, and the prevention of opportunistic infections.

Advantages of topical ozone therapy. Ozone exerts its anti-pan-pathogenic actions through entirely different mechanisms than antibiotic agents. The latter must be constantly upgraded to surmount pathogen resistance and mutational strategies. Ozone, on the other hand, offers oxidative challenges that pathogens are incapable of circumventing. In addition, there is evidence that ozone directly inactivates bacterial toxins, while antibiotics do not. Indeed, toxins are major contributors to bacterial tissue destruction.

MRSA infections constitute emergency situations. Immediate external ozone applications in high concentrations, in tandem with systemic interventions, may decidedly favor clinical success.

A caveat, however, is in order: ozone has limited penetration into wounds, a few millimeters at most. Admittedly, ozone wound penetration can be increased with longer exposures, higher ozone concentrations, and adequate humidification of the gaseous mixture. Yet, the clinician must carefully gauge the extent of tissue involvement, especially the depths of its reach, so as to recruit adjunctive wound therapies in a timely fashion.

Ozone therapies in systemic conditions.

Ozone, in addition to its clearly documented antimicrobial properties, possesses other attributes that are being applied to a variety of medical disorders. These reported and yet understudied properties, observed in the context of the systemic administration of ozone - most commonly via blood - encompass biochemical, vascular, and immunological dimensions.

Biochemical properties associated with ozone center on its reported stimulation of glycolysis via the activation of the citric acid cycle and the mitochondrial respiratory chain (Viebahn 2007; Bocci 2005).

Cardiovascular effects of ozone privilege its vasodilatory effects, due to ozone-mediated nitric oxide release. Erythrocytes exposed to ozone reportedly become more pliable, thus favoring their rheological comportment thus enhancing circulatory fluidity. In addition, the increased red blood cell glycolysis rate shifts oxyhemoglobin curves to favor the release of oxygen to tissues (Viebahn 2007).

Immunological effects of systemically administered ozone are perhaps the most complex to fathom. Reported effects include the stimulation of lymphocytes (Bocci 2005), the production of interferons and other cytokines (Larini 2001), and the stimulation of (NK) killer cells.

The role of reactive oxygen family members, including ozone, has been strongly upgraded with findings relative to their intimate – and crucial – role in the body’s defenses against microbial challengers. Exogenously administered ozone is also said to provide oxidative challenges to the organism that upregulate its production of antioxidant reserves. Whether exogenous ozone has the capacity to implicate itself in these dynamics is yet to be determined.

Medical ozone in the future

1. Ozone in infection control

Hospital and clinic environments are fertile grounds for microorganism proliferation, often breeding bacterial species highly resistant to mainstream antibiotics. Nosocomial infections contribute to unacceptable morbidity and mortality in health care settings. Ozone disinfection will increasingly be applied to the decontamination of health care facilities. Some commercial enterprises, for example, capitalizing on ozone’s gaseous nature – and therefore high penetrability into hard-to-reach spaces - are currently developing technologies for the rapid disinfection of operating room theaters.

2. Ozone in wound care

Ozone will become a standard therapeutic option in integrated wound care. Clinicians will routinely recruit externally-applied ozone technologies when devising treatment protocols for all manner of skin ulcers, traumatic wounds, surgical lesions, burns, gangrene and MRSA.

Oxygen, externally-applied, has now been approved for chronic wound therapy – diabetic, pressure, and venous insufficiency skin ulcers - by the Food and Drug Administration (FDA), in recognition of the fact that oxygen accelerates wound healing. It is foreseen that the approval for the addition of ozone to oxygen will follow in due time.

3. Hyperbaric oxygen combined with ozone

The beneficial effects of oxygenation in selected clinical situations have long been established and forms the basis for the use of hyperbaric oxygen treatment for conditions such as carbon monoxide poisoning, decompression sickness, crush injuries, thermal burns, necrotizing fasciitis, gas gangrene, blood sepsis, diabetic leg ulcers, osteomyelitis, and radiation dermatitis, among others. Basically, pressurized oxygen therapy is beneficial in most conditions where the oxygen supply to tissues is deficient and where bacterial infections fester. It is in this last scenario that ozone, as an added ingredient to hyperbaric oxygen, could greatly enhance its antibacterial power.

Envisaged, is the use of hyperbaric oxygen/ozone in the treatment of infections of the extremities. Patients, in these situations, would not *in toto* be enclosed in a chamber in the presence of ozone, thus avoiding ozone exposure to respiratory tissues; only their arms, legs or torsos would be exposed to this potent anti-infectious mixture in specially configured enclosures.

4. Blood Purification

Several authors, some time back, have investigated ozone for sterilizing blood supplies (Wolff

1979, Wehrli 1957). Recent attempts, however, have not been as fruitful. Virally infected whole blood, exposed to ozone for sterilization purposes requires concentration and time exposures incompatible with the acceptable integrity of its cellular elements (e.g., hemolysis) and the functionality of its biological molecules (e.g., protein denaturation). Whether ozone treatment of whole blood could assist in its purification therefore remains controversial. While possibly efficacious in neutralizing selected viral particles suspended in plasma, it is to be determined whether retroviruses such as HIV, for example, once ensconced in the genetic material of blood cells, can ever be cleared by this method (Chun 1999).

Blood derivatives, on the other hand, show promise relative to ozone purification. A landmark study (Wells 1991), demonstrated ozone's capacity to inactivate HIV in Factor VIII derivative, without impairing its functional competence.

5. Ozone-based therapies for cancer

Historically, the logic sustaining the use of oxygen-ozone application to the treatment of carcinomas capitalized on the disturbed metabolism of cancer cells.

Warburg, in 1925, proposed that tumors had higher rates of glycolysis under aerobic conditions than normal cells. Although his theory has subsequently been amended considerably, there is an evolving body of research centering on the biochemical differences between normal and malignant cells (De Vita 1985, Bocci 2002).

Some authors report a peroxide intolerance in tumor cells. Possessing insufficient catalase and peroxidase enzymes, some cancer cells have difficulty processing peroxides. Exposed to ozone, these cells are said to show a significant decrease in lactate content, indicating that ozone may induce metabolic inhibition in some carcinomas (Rilling 1987, Varro 1974).

In one landmark study, cultured cells of different carcinoma types were compared with non-cancerous human lung fibroblasts on exposure to ozonated air. Alveolar adenocarcinoma, breast adenocarcinoma, uterine carcinosarcoma and endometrial carcinoma showed a 40% cell growth inhibition at 0.3 ppm of ozone and 60% at 0.5 ppm. The non-cancerous lung cells were unaffected at these levels. At 0.8 ppm ozone exposure, cancer cell growth inhibition was 90%. Interestingly, it was at this level that the control cell group started to manifest anabolic slowdown. The authors postulate that cancer cells are less able to compensate for the oxidative challenge of ozone than normal cells, possibly by way of reduced functionality of the glutathione system (Sweet 1980).

There are many anecdotal reports but a paucity of controlled data of ozone hemotherapy applied to various cancers (Wolff 1977, Zabel 1960, Wenzel 1983). Several researchers have focused their efforts on using ozone as an adjunct to radiation and chemotherapy (Tietz 1983).

A recent landmark animal study showed the efficacy of ozone peritoneal insufflations in the resolution of squamous cell carcinomas in rabbits (Schulz 2008).

Contemporary approaches to ozone therapeutics in cancer are moving away from the notion of metabolic intolerance of tumors. Rather, they privilege the possibilities for hematogenous ozone to invigorate immune functions.

6. Extracorporeal Ozone Therapy.

An experimental technique of ozone administration makes use of the extracorporeal treatment of the entire blood volume using a hollow-fibre oxygenator-ozonizer (Di Paolo 2000; Bocci 2002). The totality of blood and lymphatic fluids are thus interfaced with ozone/oxygen mixtures in this promising approach.

Extracorporeal ozone therapy is ideally suited for the administration of low and very low ozone dosages over prolonged periods of time. This methodology is reportedly useful in severe peripheral artery disease, and coronary disease (Di Paolo 2005). Possible mechanisms of ozone action may include neoangiogenesis, mediated via nitric oxide pathways (Freedman 2002, and via the oxidation of plaque cholesterols, resulting in their regression.

Theoretically, extracorporeal ozone therapy could also be suitable for the management of acute viral infections when explosive viremia threatens life, (e.g., influenza, Ebola), and for the therapy of chronic viral afflictions (e.g., hepatitis C, HIV) during intensive periodic spikes of viral life cycle recrudescence. Research is needed to determine proper indications and treatment protocols for this innovative ozone methodology that has the potential to have a stellar future in the field of ozone-based therapies.

7. Creative applications for medical ozone

A large recent study demonstrated the benefits of intradiscal ozone injections for back pain (Steppan 2009). Ozone oxidation of disc collagen and proteoglycans shrinks disc volume, thus relieving mechanical pressure on nerve roots.

A randomized, double-blinded, placebo-controlled study of ozone therapy for the treatment of sudden sensorineural hearing loss yielded positive results (Ragab 2009). The authors postulated success to be related to ozone's capacity to increase blood flow and oxygenation to the inner ear.

Ozone is finding increasing usefulness in dentistry for the treatment of caries and the management of periodontal disease (Azapazhooh 2008, Lynch 2004).

These are some examples of the way ozone's potential for varied medical applications. The future is likely to see a plethora of creative uses for ozone, not only in internal medicine but also in the greater spectrum of medical subspecialties.

7. Research

Research, vital to ozone's continuing integration into modern medical practice, needs dedicated impetus on several fronts. Knowledge about ozone's inorganic reactions, despite more than a century of experimentation, is still in its infancy. Ozone's impact upon organic molecules (e.g., lipids, proteins, carbohydrates), and especially those that are bioactive (e.g., hormones, immune factors), is open territory to exciting research.

In vitro studies of ozonation on microorganisms in biological fluids are still remarkably sparse. In view of highly variable susceptibilities of different bacterial and viral families to ozone challenge, research is needed delineate relative susceptibilities of bacterial, viral and fungal families to ozone action, and to further characterize the precise mechanisms of its antimicrobial action.

Clinical investigation needs to focus on clear protocols for topical oxygen/ozone mixtures in different pathologies, such as diabetic ulcers and burns. In MRSA and gangrene, can combined topical and systemic ozone administration do better than topical alone? Are certain types of cancers susceptible to ozone action, and can antineoplastic therapies beneficially integrate ozone in their protocols?

The spectrum of reactions systemically administered ozone produces is mind-boggling. Effects on the universe of immune functions, on cardiovascular dynamics, and on metabolism have only now begun to be perceptibly understood.

Current issues related to hematogenous ozone therapies

While investigators have documented that ozone is generated by immune components to combat microbes, these same researchers postulate a negative role for ozone in the formation of atheromatous plaque (Wentworth 2007). Their analysis of plaque constituents show cholesterol derivatives – which they call atheronals – that are associated with a component bearing the signature of ozone. It is thought that the immune system could perceive plaque as a foreign invader, triggering a molecular ozone response by white cells, thus favoring the formation of more plaque. The same is postulated for bodily reactions relative to autoimmune disorders.

Other investigators, on the other hand, report that the hematogenous administration of ozone – as in extracorporeal ozone therapy – results in regression of plaque, presumably via cholesterol oxidation (Di Paolo 2000, 2005).

Conclusions

Today, the most scientifically validated use of medical ozone centers on its capacity for inactivating bacteria, viruses and fungi. Most importantly, ozone, in concentrations used in clinical practice, is able to do so without harming normal cells. The majority of *in vitro* studies till now have centered on addressing microorganism susceptibilities to ozone for purposes of water purification. Fortunately, water disinfection and sterilization research has fairly high extrapolation validity for medical applications.

Topical ozone/oxygen therapy has shown effectiveness and safety in an impressive array of conditions. In this article, the following clinical problems are cited: poorly healing infected wounds including surgical wounds, diabetic, pressure, and venous insufficiency skin ulcers, lymphedema, fungal skin infections, burns, gangrene, complex war wounds, and MRSA. Research continues to be needed to determine optimal treatment protocols for these pathologies.

Research is invited to clarify the actions of systemically-administered ozone on blood constituents, and on the vascular and immune systems. The rewards of this research could well demonstrate ozone's effectiveness in certain viral afflictions, both acute (e.g., influenza) and chronic (e.g.,

hepatitis B and C, HIV); in conditions of tissue oxygen deprivation (e.g., arteriosclerosis; blood sepsis); and in certain forms of cancer. Research into immune system prompting, via judicious ozone use, could explore the course of autoimmune disorders though to have connections to viral activity (e.g., autoimmune diabetes, multiple sclerosis, rheumatoid arthritis, lupus). Anecdotal reports demonstrate an emerging clinical interest for ozone intervention in conditions with unknown etiologies (e.g., chronic fatigue syndrome, fibromyalgia).

A promising modality, extracorporeal ozone therapy, allows for the low and very low-level ozonation of total blood volume. This therapy, currently aimed at peripheral artery disease and coronary disease, could possibly also find clinical applications in infection control and in cancer therapy.

Medical ozone is entering its age of enlightenment. The future of ozone-based medical therapies, in the context of new and enlightened perspectives, is poised to see major inroads into the understanding of its basic science foundation, and to witness clinical contributions in an expanding spectrum of conditions, spanning infectious diseases, cardiovascular conditions, and pathologies implicating the intricate functions of the immune system.

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