OZONICS INTERNATIONAL

200 East 33rd Street, # 26J New York, NY 10016-4831 Tel. 212-6790679 Fax 212-6798008

OZONE, A PHYSIOLOGICAL GAS, IS CREATED IN VIVO

Gerard Sunnen, M.D.

Introduction

The idea that a gas could be generated in the body to regulate various physiological functions would have been hard to imagine, even unthinkable, until the discovery of nitric oxide's role in the body's vital functions.

A gas, with a half-life of only a few seconds, generated in vivo, as an essential component of the nervous, the immune, and the cardiovascular systems?

When Solomon Snyder announced that his laboratory discovered nitric oxide's crucial role in neurotransmission, *Science* magazine, in 1992, declared it the "molecule of the year." Since then, nitric oxide (NO) has been recognized to exert multidimensional functions. Indeed, NO has been documented to have a role in vasodilatation, inflammation, neurotransmitter and immune action, angiogenesis (blood vessel growth), smooth muscle relaxation, and apoptosis (programmed cell death) (Wink 1996, Lincoln 1997, Laskin 1999, Ignarro 2000).

Nitroglycerin, a century-old medication for angina pectoris has been found to exert its beneficial vascular action via nitric oxide formation. By way of its creation through the arginine-nitric oxide pathway and its vasorelaxing properties, nitric oxide is implicated in the continuous modulation of blood pressure, and in erectile function.

In the nervous system, nitric oxide acts as a neurotransmitter (Snyder 1996). It regulates circadian rhythms, assists in memory formation, and influences the release of pituitary hormones.

Nitric oxide, at the molecular level, is a bactericide. Cytokine-activated macrophages produce nitric oxide as one component of immune offense against bacteria, viruses, and nascent cancer cells. Nitric oxide exerts its antipathogenic functions by disrupting bacterial enzymes, by interfering with bacterial metabolic pathways such as the Krebs cycle, and by disorganizing bacterial genes and mitochondrial function.

Another gas, carbon monoxide (CO), despite its injurious effects when breathed, and its toxic reputation, also functions as an intercellular messenger, regulating calcium influx into cells (Dawson 1994). CO, generated by heme oxygenase enzymes, is involved, much like NO, in neurotransmission and in blood vessel physiology. A signaling molecule, carbon monoxide influences gene expression, inflammation, and cell growth (Dulak 2003).

Gases, in the nineties, have thus established themselves as a novel category of biological modulators and are in the process of revolutionizing medicine.

Ozone: A universal bactericide and virucide

Another such gaseous agent is ozone.

The oxygen atom exists in nature in several forms: (1) as a free atomic particle (O), it is highly reactive and unstable; (2) Oxygen (O2), its most common and stable form, is colorless as a gas and pale blue as a liquid (3) Ozone (O3), has a molecular weight of 48, a density one and a half times that of oxygen and contains a large excess of energy in its molecule (O3 é 3/2 O2 + 143 KJ/mole). It has a bond angle of 127º ± 3∞, resonates among several hybrid forms, is distinctly blue as a gas and dark blue as a solid. Ozone is the first layer that separates the earth's biosphere from outer space (4) O4 is a very unstable, rare, nonmagnetic pale blue gas that readily breaks down into two molecules of oxygen.

Ozone in the body may have a protective role against pathogenic invaders much as it has in the stratosphere against lethal ultraviolet rays. The fact that reactive oxygen species (ROS) are produced by immune system cells during infectious processes has been appreciated for a long time (Babior 2000; Kourie 1998; Valentine 1995). ROS, including the hyroxyl radical, nitric

oxide, and hydrogen peroxide, were thought to be toxic by-products of metabolic redox reactions requiring rapid neutralization by enzyme systems. Ozone had hitherto been seen as a molecule capable of inducing the formation of ROS but certainly not as a molecule specifically produced by the body to fight infections. The crucial role of ozone in the task of staving off invading microorganisms had not been as fully explained as in the following landmark study.

An under-publicized article with momentous implications (Wentworth 2002, Max 2002) documented that ozone is indeed produced in the body in the context of immune function. Ozone synthesis is triggered by antigen-antibody reactions generated by activated neutrophils. In this model, antibodies, provided with appropriate starting materials, are capable of creating singlet oxygen, a most powerful oxidant. The singlet oxygen combines with oxygen to form ozone, itself an oxidant, whose electron-extracting capacity is only second to fluorine. It can also combine with water to form the hydroxyl radical (OH) and hydrogen peroxide.

The combination of ozone and hydrogen peroxide is called peroxone. Peroxone is more lethal to microorganisms than either agent alone. In the above experiment, it was shown that ozone, in tandem and in combination with hydrogen peroxide, could account for the inactivation of 95% of Escherichia coli bacteria under study.

At the molecular level, ozone thus becomes a pivotal factor for fighting microorganisms. Additionally, ozone functions as a signaling agent by stimulating production of nuclear factor kappa B, interleukin 6, and tumor necrosis factor a. Ozone's capacity for cytokine induction has been amply documented (Bocci 2005).

While the body's production of ozone, the hydroxyl radical, and peroxone may be of paramount importance in eliminating bacteria, viruses, and cancer cells, this phenomenon could, however, in autoimmune diseases (e.g., rheumatoid arthritis, ulcerative colitis, multiple sclerosis, systemic lupus erythematosus), work against the host. In these conditions, in a normal process gone astray, these oxidants may have some responsibility in tissue destruction.

Ozone: Mechanisms of bactericidal and virucidal action

The mechanisms by which nitric oxide exerts antipathogenic action have benefited from more recent intensive experimentation than those implicating ozone. Nonetheless, the action of ozone against pathogens has been known for well over a century and considerable scientific data about this unique property has cumulated accordingly.

Bacteria. Although the inhibitory and lethal effects of ozone on noxious organisms have been observed since its discovery by Schonbein in 1840, the mechanisms for these actions still needs deeper clarification. Ozone is a strong bactericide needing only a few micrograms per milliliter for measurable action. At a concentration of 1 mg/liter H2O at 1?C, ozone rapidly inactivates coliform bacteria, Staphylococcus aureus, and Aeromonas hydrophilia (Lohr 1984).

A partial list of organisms susceptible to ozone inactivation includes both aerobic and anaerobic bacteria: *Bacteroides, Campylobacter, Clostridium, Corynebacteria, Escherichia, Klebsiella, Legionella, Mycobacteria, Propriobacteria, Pseudomonas, Salmonella, Shigella, Staphylococcus, Streptococcus, and Yersinia.* Indeed, all bacteria, including Mycobacteria known for their robust cell walls succumb to ozone's killing action.

The cell envelopes of bacteria are composed of intricate multilayers. Covering the bacterial cytoplasm to form the innermost layer of the envelope is the cytoplasmic membrane, made of phospholipids and proteins. Next, a polymeric layer built with giant peptidoglycan molecules provides bacteria with a stable architecture. In Gram-positive organisms, the pepticoglycan shell is thick and rigid. By contrast, Gram-negative bacteria possess a thin pepticoglycan lamella on which is superimposed an outer membrane made of lipoproteins and lipopolysaccharides. In acid-fast bacteria such as *Mycobacterium*, up to one half of the cell envelope is formed of complex lipids and glycolipids. The high lipid content of these ubiquitous bacteria may explain their sensitivity, and eventual demise, subsequent to ozone exposure

The outermost bacterial layer is the polysaccharide capsule. In many bacterial species, the capsule, by way of its stickiness, enables adherence to host tissues. The capacity of Streptococcus mutans to accrete to tooth enamel, for example, is due to its capsular properties.

The most cited explanation for ozone's bactericidal effects centers on disruption of cell membrane integrity through oxidation of its phospholipids and lipoproteins. There is evidence for interaction with proteins as well (Mudd 1969). In one study exploring the effect of ozone on E. coli, evidence was also found for ozone's penetration through the cell membrane, reacting with

OZONICS INTERNATIONAL
200 East 33rd Street, # 26J New York, NY 10016-4831 Tel. 212-6790679 Fax 212-6798008

cytoplasmic contents, and converting the closed circular plasmid DNA to open circular DNA, which would presumably diminish the efficiency of bacterial procreation (Ishizaki 1987). Capsular polysaccharides may be possible sites for ozone action.

Viruses. All viruses are susceptible to ozone's neutralizing action. Viruses differ in their susceptibility to destruction by ozone. In one study, poliovirus resistance was 40 times that of coxsackie virus. Relative ozone susceptibility in ascending order was found to be: poliovirus type 2, echovirus type 1, poliovirus type 1, coxsackie virus type B5, echovirus type 5, and coxsackie virus type A9. In pure water, at maximal solubility of ozone and room temperature, echovirus type 29 is inactivated in one minute, poliovirus type 1 in two, type 3 in three, and type 2 in seven minutes (Roy 1982).

Susceptible viruses include all major and minor viral families: *Flaviviridae, Filoviridae, Hepnaviridae, Herpesviridae, Orthomyxoviridae, Retroviridae*. *Coronaviridae, Togaviridae, Rhabdoviridae, Bunyaviridae, Pramyxoviridae*, and *Poxviridae*. Non-enveloped susceptible viral families include: *Adenoviridae, Picornaviridae, Papillomaviridae, Caliciviridae, Astroviridae, and Reoviridae.*

Lipid-containing viruses are sensitive to treatment with ether, assorted organic solvents, and ozone, indicating that disruption or loss of lipids results in impaired or destroyed infectivity. Some viruses containing lipid envelopes include the *Flaviviridae* (Hepatitis C), the *Orthomyxoviridae* (Avian influenza), the *Retroviridae* (HIV), and the *Herpesviridae*, a large family grouping the Simplex, Varicella-Zoster, Cytomegalovirus, and Epstein-Barr viruses.

Non-enveloped naked viruses have a protein outer layer made of protein surrounding their nucleic acid core, the capsid. Ozone has well-documented action on proteins. The formation of protein peroxides disrupts capsid integrity. Without a capsid, the virion cannot survive (Riesser 1977).

Ozone: A conceptual shift

Ozone, for over a century, has shown a potential for universally inactivating all families of bacterial as well as viral pathogens. Numerous researchers who subjected every known microorganism to varying ozone concentrations and time exposures to determine the parameters of their susceptibilities have documented this.

While exogenously applied ozone has received total investigative focus, little or no attention has been paid to endogenously generated ozone, until now. This is understandable if conceptual blockage is invoked: How can a gas reflexively seen as toxic for decades ever find a role in the body's metabolism, and even more astonishingly, such a crucial role?

The fact that the body has adopted ozone as a molecule central to its immune defense makes perfect sense. It can be generated by redox reactions at the molecular level and because of its universal potency against all pathogens it essentially becomes the sword that fells the body's microorganism trespassers.

Conclusion

Gases have recently opened new dimensions of understanding human physiology. Nitric oxide, the first gaseous molecule to be extensively studied is remarkable for its panoptic range of capacities: Immune system modulation; vasodilatation and cardiovascular regulation; and neurotransmitter function. Of primordial importance is nitric oxide's crucial role in the inactivation of microorganisms and the destruction of nascent cancer cells.

Another gas, carbon monoxide (CO), functions as an intercellular messenger. It regulates calcium influx into cells. CO is involved, much like NO, in neurotransmission and in vascular response. It is a signaling molecule with actions upon gene expression, inflammation, and cell growth.

Ozone, on the other hand, despite its long historical life, has received less attention. The reasons for this are unclear. Perhaps ozone continues to reside in the shadows of a century-old image of toxicity. Indeed, nitric oxide, carbon monoxide, and ozone, all have toxic potential, most directly when breathed. Yet, at the molecular level, they all exert vital functions without which life, as we know it, would not be possible.

Ozone had hitherto been seen as a molecule capable of inducing the formation of reactive oxygen species but not as a molecule specifically produced by the body to fight infection. The crucial role of ozone in the task of staving off invading microor-

ganisms has recently been highlighted.

Singlet oxygen is produced by activated neutrophils and other cellular elements of the immune system. In its bonding with tissue oxygen, ozone is formed. In joining with water, singlet oxygen yields the hydroxyl radical and hydrogen peroxide. Ozone and hydrogen peroxide combine into peroxone, a particularly potent bacterial and viral inactivator.

Ozone neutralizes microorganisms via a spectrum of mechanisms. Most studied is ozone oxidation of bacterial lipids and proteins found in bacterial cell membranes, and viral envelope lipids, phospholipids, cholesterols, and glycoproteins.

To maintain ongoing viability and health, the organism needs to generate a constant sustained defense against microbial invaders. It now appears that ozone, produced at the cellular level, has the capacity to perform this fundamental function.

Ozone, as a molecule with high excess energy, is a universal bactericide and virucide. Small wonder then, that this very molecule would be utilized by the body's immune system as a fundamental weapon against pathogenic attackers.

Research is compellingly needed to understand the deeper mechanisms of ozone and nitric oxide formation in the immune system so that novel antimicrobial therapies may be recruited to respond to the world's increasingly urgent public health needs.

References

Ackey D, Walton TE. Liquid-phase study of ozone inactivation of Venezuelan Equine Encephalomyelitis virus. Appl Environ Microbiol 1985; 50:882-886
Armstrong. Infectious Diseases, First Ed. Mosby, Philadelphia, 2000
Babior BM. Phagocytes and oxidative stress. Am J Med 2000; 109:33-44
Bocci V. Ozone: A New Medical Drug. Springer, 2005
Bocci V. Oxygen-Ozone Therapy: A Critical Evaluation. Kluwer Academic Publishers, Dordrecht, 2002
Bolton DC, Zee YC, Osebold JW. The biological effects of ozone on representative members of five groups of animal viruses. Environmental Research 1982; 27:476-48
Cann AJ. Principles of Molecular Virology, Second Edition. Academic Press, New York, 1997
Cardile V, et al. Effects of ozone on some biological activities of cells in vitro. Cell Biology and Toxicology 1995 Feb; 11(1): 11-21
Carpendale MT, Freeberg JK. Ozone inactivates HIV at noncytotoxic concentrations. Antiviral Research 1991; 16:281-292
Cech T. RNA as an enzyme. Scientific American 1986 Nov; 255(5): 64-76
Clamann H. Physical and medical aspects of ozone. In: Physics and Medicine of the Atmosphere and Space. John Wiley and Sons, New York, 1960, p 151
Dailey JF. Blood. Medical Consulting Group, Arlington MA, 1998
Dawson TM, et al. Gases as biological messengers: Nitric oxide and carbon monoxide in the brain. J Neuroscience 1994; 14:5147-5159
Delves PJ, Roitt IM. Encyclopedia of Immunology. Academic Press: San Diego, 1998
Di Paolo N. Extracorporeal blood oxygenation and ozonation (EBOO) in man. Preliminary report. Int J Artif Organs 01 Feb 2000; 23(2): 131-141
Dulak J, Jozkowicz A. Carbon monoxide: A "new" gaseous modulator of gene expression. Acta Biochimica Polonica 2003; 50(1): 31-47
Dyas A, Boughton B, Das B. Ozone killing action against bacterial and fungal species: Microbiological testing of a domestic ozone generator. J Clin Pathol (Lond) 1983; 36(10): 1102-1104
Evans AS, Kaslow RA (Eds). Viral Infections in Humans: Epidemiology and Control, Fourth Edition, Plenum, New York, 1997
Gumulka J, Smith L. Ozonation of cholesterol. J Am Chem Soc 1983; 105(7): 1972-1979
Hurst CJ. Viral Ecology. Academic Press, New York, 2000
Ignarro LJ (Ed). Nitric Oxide: Biology and Pathobiology. Academic Press, 2000
Ishizaki K, Sawadaishi D, Miura K, Shinriki N. Effect of ozone on plasmid DNA of Escherichia coli in situ. Water Res 1987; 21(7): 823-828
Ivanova O, Bogdanov M, Kazantseva V, et al. Ozone inactivation of enteroviruses in sewage. Vopr Virusol 1983; 0(6): 693-698
Knipe DM, Howley PM. Fundamental Virology, Fourth Edition. Lippincott Williams & Wilkins, Philadelphia, 2001
Laskin JD, Laskin DL. Cellular and Molecular Biology of Nitric Oxide. Marcel Dekker, 1999
Lincoln J, Hoyle CH, Burnstock G. Nitric Oxide in Heath and Disease. Cambridge University Press, 1997
Lohr A, Gratzek J. Bactericidal and paraciticidal effects of an activated air oxidant in a closed aquatic system. J Aquaric Aquat

OZONICS INTERNATIONAL
200 East 33rd Street, # 26J New York, NY 10016-4831 Tel. 212-6790679 Fax 212-6798008

Sci 1984; 4(41/2): 1-8
Matus V, Nikava A, Prakopava Z, Konyew S. Effect of ozone on survivability of Candida utilis cells. Vyestsi AkadNavuk Bssr Syer Biyal Navuk 1981; 0(3): 49-52
Matus V, Lyskova T, Sergienko I, Kustova A, Grigortsevich T, Konev V. Fungi; growth and sporulation after a single treatment of spores with ozone
Max J. Antibodies kill by producing ozone. Science 15 Nov 2002; 298: 1319
Mudd JB, Leavitt R, Ongun A, McManus T. Reaction of ozone with amino acids and proteins. Atmos Environ 1969; 3:669-682
Menzel D. Ozone: An overview of its toxicity in man and animals. Toxicol and Environ Health 1984; 13:183-204
Olinescu R, Smith TL. Free Radicals in Medicine. Nova Science Publishers, Inc. Huntington, New York, 2002
Paulesu L, Luzzi L, Bocci V. Studies on the biological effects of ozone: Induction of tumor necrosis factor (TNF-alpha) on human leucocytes. Lymphokine Cytokine Research 1991; 5:409-412
Razumovskii SD, Zaikov GE. Ozone and Its Reactions With Organic Compounds. Elsevier, New York, 1984
Rilling S, Veibahn R. The Use of Ozone in Medicine. Haug, New York, 1987
Rilling S. The basic clinical applications of ozone therapy. Ozonachrichten 1985; 4:7-17
Smith LL. Cholesterol autoxidation of lipids. Chemistry and Physics of Lipids. 1987; 44:87-125
Snyder S. Drugs and the Brain. Scientific American Library Series, 1996
Rice RG. Century 21 – Pregnant with ozone. Ozone Science and Engineering 2002; 24: 1-15
Riesser V, Perrich J, Silver B, McCammon J. Possible mechanism of poliovirus inactivation by ozone. In: Forum on Ozone Disinfection. Proceedings of the International Ozone Institute. Syracuse, NY, 1977:186-192
Roy D, Wong PK, Engelbrecht RS, Chian ES. Mechanism of enteroviral inactivation by ozone. Appl Envir Microbiol 1981; 41:718-723
Roy D, Engelbrecht RS, Chian ES: Comparative inactivation of six enteroviruses by ozone. Am Water Works Assoc J 1982; 74(12): 660-664
Sunnen G. Ozone in Medicine. Journal of Advancement in Medicine. 1988 Fall; 1(3): 159-174
Sunnen G. Possible mechanisms of viral inactivation by ozone. Townsend Letter for Doctors. Ap 1994: 336
Sweet J, Kao MS, Lee D, Hagar W. Ozone selectively inhibits growth of human cancer cells. Science 1980; 209:931-933
Thanomsub B. Effects of ozone treatment on cell growth and ultrastructural changes in bacteria. J Gen Appl Microbiol 01 Aug 2002; 48(4): 193-199
Valentine GS, Foote CS, Greenberg A, Liebman JF (Eds). Active Oxygen in Biochemistry. Blackie Academic and Professional, London, 1995
Vaughn JM, Chen Y, Linburg K, Morales D. Inactivation of human and simian rotaviruses by ozone. Applied Environmental Microbiology 1987; 48:2218-2221
Viebahn R. The Use of Ozone in Medicine. Odrei Publishers, Iffezheim, 1999
Wells KH, Latino J, Gavalchin J, Poiesz BJ. Inactivation of human immunodeficiency virus Type 1 by ozone in vitro. Blood 1991 Oct; 78(7): 1882-1890
Wentworth P, McDunn JE, Wentworth AD, et al., Evidence for antibody-catalysed ozone formation in bacterial killing and inflammation. Science 13 Dec 2002; 298: 2195-2199
Wink DA, Grisham MB, Mitchell JB, Ford PC. Direct and indirect effects of nitric oxide in chemical reactions relevant to biology. Methods Enzymol 1996; 268:12-31
Wolcott J, Zee YC, Osebold J. Exposure to ozone reduces influenza disease severity and alters distribution of influenza viral antigens in murine lungs. Appl Environ Microbiol 1982; 443:723-731
Yu BP. Cellular defenses against damage from reactive oxygen species. Physiological Reviews 1994 Jan; 74(1): 139-162