OZONE, A PHYSIOLOGICAL GAS, IS CREATED IN VIVO

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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 = 3/2 O2 + 143 KJ/mole). It has a bond angle of 127° ± 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 neutraliza-
tion 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 pro-
duced 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 capac-
ity 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, ulcer-
ative 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 experimenta-
tion 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 peptidoglycan
shell is thick and rigid. By contrast, Gram-negative bacteria possess a thin peptidoglycan 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
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, Paramyxoviridae, 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 perozone, 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, cholesterol, 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.

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