MERS, SARS, and emerging Coronaviruses: theoretical considerations and a proposal for critical care parenteral oxygen/ozone therapy

BACK TO HOME

Abstract

SARS (Severe Acute Respiratory Syndrome) is a global disease of significant lethality with a variable incidence and prevalence base. Of massive public health importance because of the unpredictability of its cycles and its lethality, SARS presents supremely challenging problems in light of its pathogenic capacity and mutational potential.

MERS (Middle East Respiratory Syndrome) belongs to the same Family as SARS and is more recent on the world stage. Much uncertainty remains as to its modes of transmission and the nature of its animal reservoirs. Its lethality, however, is well established.

There are neither vaccines nor antiviral agents available for SARS nor MERS, only supportive and often intensive measures such as cardiopulmonary assistance and the maintenance of physiological homeostasis.

Ozone, via a number of possible mechanisms enumerated in this paper, possesses recognized anti-viral properties. The technology of interfacing oxygen/ozone mixtures with biological fluids has long been mastered, as has been the technology of administering this gaseous mixture to the systemic circulation.

Ozone, because of its special biological properties, has theoretical and practical attributes to make it a viable candidate as a MERS and SARS inactivator, through a variety of physicochemical and immunological mechanisms. Bereft of all known therapeutic strategies, a proposal is herewith made for the parenteral administration of calibrated oxygen/ozone gaseous mixtures in the critical care of MERS, SARS, and related Coronavirus infections.

The Family of Coronaviruses

The SARS and MERS viruses belong to the viral family *Coronaviridae*. Which includes two genera, coronavirus and togovirus, each showing similar replication mechanisms and genomic organization but distinct genomic lengths and viral architecture. First identified in the 60's, this family identifies itself by large, enveloped, positive-stranded RNA virions. Their appearance is characteristically distinct, with envelopes endowed with host cell membrane-tropic petal shaped spikes (peplomers). The

large, amply spaced peplomers on the virion surface suggests a coronal (crown-like) appearance.

Prior to SARS and MERS, *Coronaviridae* were responsible for relatively mild cold-like syndromes in humans corresponding to their predilection for the ciliary epithelium of the trachea, nasal mucosa, and alveolar cells of the lungs. At times they were only rarely implicated in serious respiratory illnesses in frail older adults (Falsey 2002). SARS and MERS represent a quantum leap in *Coronaviridae* infectivity by way of their significant lethality. Widely seen in nature, coronaviruses infect a spectrum of animal hosts and are responsible for avian infectious bronchitis, murine hepatitis, and porcine gastroenteritis, among others. Of probable significance to humans is that animal coronaviruses are able to penetrate into the central nervous system.

SARS and MERS: Virion architecture and molecular biology

The SARS virion differs from other members of the *Coronaviridae* family in its genomic composition. The other viral structures, however, are similar, including virion architecture, and the fundamental composition of structural and non-structural proteins.

The software for viral replication is the nucleic acid core, a single strand long chain RNA nucleotide. The core is surrounded by the nucleic acid coat or capsid. The capsid is rigid and determines the shape of the virus; it is made of repeating units called capsomeres. The SARS viral nucleocapsid is tubular with a helical symmetry.

An envelope that forms the outer layer of the virion and maintains intimate contact with host bodily fluids surrounds the nucleocapsid. As such, it is sensitive to the composition and alterations in its milieu, such as temperature, pH, and ionic balance. The viral envelope is formed at the time of budding, an intricate process in which the nucleocapsid exits the host cell. In order to do this, it fuses with the host cell membrane, appropriating its components to form its own envelope. It is known that the lipid composition of viral membranes reflects the lipid composition through which the particles exit. Viral envelopes are composed of lipid bilayers associated with a union of carbohydrates and proteins, glycoproteins, and lipids and phosphates, phospholipids. Up to 60% of the lipid component of the envelope is composed of phospholipid and the remainder is mostly cholesterol. This lipid-carbohydrate envelope is closely articulated with the peplomers, which determine attachment and penetration into host cells.

The genome composition and sequence of the SARS virus has been identified (Marra 2003; Rota 2003). Marra et al. described a viral genome configuration of 29,727 nucleotides in length, within which exists a gene order similar to other coronaviruses. However, because the genetic composition of SARS does not closely resemble any of the three known classes of coronaviruses, they propose a new and fourth class of coronaviruses, the SARS-CoV. Postulated, is a hypothesis that an animal virus recently mutated to successfully infect humans, or that the SARS virus mutated from a common human coronavirus.

Rota et al. reported a nucleotide sequence of 29,727 in SARS-CoV, with 11 open reading frames. Phylogenetic analyses and sequence

comparisons showed that the SARS virus is not closely related to any of the previously characterized coronaviruses.

Virion structural proteins are essential elements in determining the morphological and functional dimensions of the SARS virus. Coronavirus structural proteins include the N nucleocapsid phosphoprotein which binds to viral RNA; the membrane glycoprotein M which forms the shell of the internal viral core and is responsible for triggering viral assembly; the protein E associated with the virion envelope; the spike glycoprotein S which binds to specific cellular receptors and elicits cell-mediated immunity; and the hemagglutinin-esterase glycoprotein HE forming small spikes on the coronavirus envelope (Knipe 2001).

SARS: Viral replication

The viral replication cycle follows the pattern seen in mammalian viruses and may be divided into several stages (Cann 1997; Evans 1997; Knipe 2001). The coronavirus attaches to the membrane of the host cells by binding the S and HE proteins of its peplomers to receptor glycoproteins or glycans.

Once cell entry is achieved, the virion sheds its envelope to commence its replication in the host cell cytoplasm. It binds to cellular ribosomes and released viral polymerase begins the RNA replication cycle. Newly formed nucleocapsids continue their assembly with the acquisition of new envelopes by means of budding through membranes of the cell's endoplamic reticulum.

Virions are then released into the general blood and lymphatic circulation, ready to infect new cells, other organ systems, and new hosts.

SARS: Clinical findings

Recently, the clinical manifestations of SARS have been comprehensively described (Peiris 2003). In this study of 50 hospitalized patients, fever, chills, myalgia, and dry cough were the most frequent presenting complaints. Also reported, were rhinorrhea, sore throat, and gastrointestinal symptoms.

Radiological examination showed evidence of pulmonary consolidation approximately 5 days after the onset of symptoms. Laboratory examination showed leucopenia and lymphopenia, despite the presence of fever; also anemia, thrombocytopenia, liver enzyme elevations (alanine aminotransferase), and skeletal and heart muscle enzyme elevation (creatinine phosphokinase). All these features point to severe systemic inflammatory insults.

The incubation of SARS is 2 to 10 days, and in some patients perhaps longer. The respiratory route achieves viral transmission where it may infect the new host through aerosol and droplet contact with mucosal surfaces of the mouth, nose, throat, and probably the conjunctiva. SARS virions have been found in feces and the importance of this route of transmission is being evaluated, as it is known that several animal coronaviruses use this propagation venue. Moreover, since it is appreciated that SARS particles remain viable on fomites for 48 hours or

longer, any eradication effort must address the infectivity of objects in the environment.

The syndrome progresses to severe disease with respiratory distress and oxygen desaturation requiring ventilatory support in over a third of patients, approximately 8 days after symptom onset. Mortality has been noted to vary according to transmission clusters, ranging from 3 to 20%. This suggests that the etiology of SARS depends upon a heterogenous population of viral quasispecies with variable degrees of virulence.

SARS and MERS: Genetic creativity

As is the case in the majority of RNA viruses, coronaviruses mutate at a high rate (Steinhauer 1986). Within any one afflicted individual, coronaviruses particles do not show a homogeneous population. Instead, they function as a pool of genetically variant strains known as quasispecies. This is due to the high error frequency of RNA polymerases, the presence of deletion mutants, the high frequency of RNA recombination and point mutations, and the occurrence of defective-interfering RNA (DI RNA). The net result of these diverse and complex mechanisms is the continuous spawning of novel virions and divergent quasispecies. Some of the genetic creations will find themselves at an advantage in negotiating new host antibody responses and pharmacological antiviral countermeasures; and they will propagate accordingly, thus expanding their ecological terrain. Other genetic creations will be too lethal to their hosts, work against their own survival, and will prove to be non-adaptive. If we can speak of a viral psychology, an efficient survival balance aims somewhere between defeat by host defenses on one hand, and viral suicide through aggressive lethality on the other.

Ozone: Physical and physiological properties

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 ($O3 \rightarrow 3/2\ O2 + 143$ KJ/mole). It has a bond angle of $127 \pm 3°$, which resonates among several forms, is distinctly blue as a gas and dark blue as a solid; (4) O4 is a very unstable, rare, nonmagnetic pale blue gas which readily breaks down into two molecules of oxygen.

Ozone (O3), a naturally occurring configuration of three oxygen atoms, has a half-life of about one hour at room temperature, reverting to oxygen. A powerful oxidant, ozone has unique biological properties. Since medicinal ozone is administered by interfacing it with blood, basic research on ozone's biological dynamics have centered upon its effects on blood cellular elements (erythrocytes, leucocytes, and platelets), and to its serum components (proteins, lipids, lipoproteins, glycolipids, carbohydrates, electrolytes).

The effects of ozonation on whole blood are extraordinarily complex and are far from adequately elucidated. If the biochemical configuration of serum - with its proteins, including enzymes, immunoglobulins, clotting factors; its hormones, vitamins, lipoproteins and cholesterol; its

carbohydrates including glucose, and electrolytes, among others (Dailey 1998) can be compared to an orchestra, ozone administration can be likened to the introduction of a novel and powerful musical instrument, affecting the interactions of all the other instruments.

Even though an in-depth analysis of ozone's multifaceted effects upon the panoply of blood constituents is beyond the intent and scope of this article (The reader is referred to Bocci 2002; Sunnen 1988, 2009), the following points of research interest are significant:

Erythrocytes have been extensively studied in relation to ozone administration. Many studies that have used erythrocyte suspension in physiologic saline (Kourie 1998; Fukunaga 1999) have found hemolysis at relatively low ozone dosages (10 to 30 ug/ml). When ozone is administered in whole blood, however, the dynamics of ozone interaction are such that hemolysis begins to be observed at significantly higher doses, implying a buffering action of blood constituents. Moreover, the functionality of erythrocyte enzymes is maintained, suggesting a protective role of antioxidant systems (Cross 1992). There is some evidence that ozone administration may stimulate erythrocyte formation and release (Hernandez 1999).

Leucocytes, intimately connected to immune function, show good resistance to ozone because they possess enzymes that protect them from oxidative confrontation. These enzymes include superoxide dismutase, glutathione, and catalase. A promising area of research centers on cytokine and interferon stimulation in ozone administration and its implication for enhancing immune function (Paulesu 1991; Bocci 1994; Larini 2001). A classical adage of ozone therapy is that lower ozone dosages are stimulating to immune action while higher dosages become inhibitory (Viebahn 1999). Further research will need to clarify the parameters of this phenomenon, as well as the effects of ozone infusion upon different types of leucocytes in relation to the disease under treatment.

Ozone: Antipathogenic properties

Recently, there has been renewed interest in the potential of ozone for viral inactivation in vivo. It has long been established that ozone neutralizes bacteria, viruses, fungi, and parasites in aqueous media. This has prompted the creation of water purification processing plants in numerous major municipalities worldwide. Ozone's unique physicochemical and biological properties, and environmentally-friendly aspects, have since been applied to a panoply of industrial uses such as the packaging of pharmaceuticals, the fumigation of homes and buildings (sick building syndrome), the treatment of indoor air in operating rooms and nursing homes, and the disinfection of large scale air conditioning systems in hospitals (Rice 2002).

Ozone's remarkable capacities for pan-antipathogenic action have been applied to the treatment of poorly healing wounds and burns (Sunnen 1999). A partial list of organisms susceptible to ozone inactivation in these clinical situations includes aerobic and anaerobic bacteria, Bacteroids, Campylobacter, Clostridium, Corynebacteria, Escherichia, Klebsiella, Legionella, Mycobacteria, Propriobacteria, Pseudomonas, Salmonella, Shigella, Staphylococcus, Streptococcus, and Yersinia.

Susceptible viruses include Adenoviridae, Filiviridae, Hepnaviridae, *Herpesviridae*, Orthomyxoviridae, Picornaviridae, Reoviridae, and Retroviridae. Ozone-sensitive fungi include Actinomycoses, Aspergillus, Candida, Cryptococcus, Epidermophyton, Histoplasma, Microsporum, and Trichophyton.

Some viruses are more susceptible to ozone's action than others. It has been found that lipid-enveloped viruses are the most sensitive. This makes intuitive sense, since enveloped viruses are designed to blend into the dynamically constant milieu of their mammalian hosts. This group includes, hepatitis B and C, herpes 1 and 2, Cytomegalus (Epstein-Barr), HIV 1 and 2, Influenza A and B, West Nile virus, *Togaviridae*, Eastern and Western equine encephalitis, rabies, and *Filiviridae* (Ebola, Marburg), among others.

The envelopes of viruses provide for intricate cell attachment, penetration, and cell exit strategies. Peplomers, finely tuned to adjust to changing receptors on a variety of host cells, constantly elaborate slightly new glycoprotein configuration under the direction of portions of the viral genome, thus adapting to host cell defenses. Envelopes are fragile. Ozone and its by-products can thus disrupt them.

Lipid enveloped viruses in aqueous media are readily inactivated by ozone via the oxidation of their envelope lipoproteins and glycoproteins (Akey 1985; Shinriki 1988; Vaughn 1990; Wells 1991; Carpendale 1991). In whole blood, however, ozone’s virucidal actions are buffered by the spectrum of its components and ozone becomes less effective. This situation is further complicated in the case of retroviruses which ensconce themselves within host DNA (Chun 1999), and in Herpesviridae, where virions have the capacity to persist indefinitely in their host through the formation of an episome in the nuclei of the cells that harbor them (White 1994).

Several studies have reported the safety and the benefits of ozone administration in vivo. Wells et al. (1991) showed that ozone-treated HIV-spiked Factor VIII maintained its biological capacity; and that, concomitantly, there was an 11-log reduction in detectable virions. The improvement of liver enzymes in hepatitis C patients after several months of ozone therapy was described (Viebahn 1999; Amato 2000). An 80% hepatitis C viral load reduction in 82 patients using AHT was reported (Luongo et al., 2000).

It is remarkable, however, that to date, no adequate double blinded study has addressed ozone therapy in viral conditions such as hepatitis B and C, HIV, or herpes.

Ozone: Clinical methodology

Ozone may be utilized for the therapy of a spectrum of clinical conditions (Viebahn 1999, Bocci 2002). Routes of administration are varied and include external, and internal (blood interfacing) methods. In the technique of ozone major autohemotherapy (AHT), an aliquot of blood (50 to 300 ml) is withdrawn from a virally afflicted patient, anticoagulated, interfaced with an ozone/oxygen mixture, then re-infused. This process is repeated serially, in a manner consonant with the

treatment protocol until viral load reduction and symptom abatement are observed.

Recently there has been interest in new methods of interfacing oxygen-ozone mixtures with whole blood, serum, and serum components (Bocci 2002, Sunnen and Robinson, 2001).

Another, more experimental and more intensive technique of ozone administration, is called the Extracorporeal Blood Circulation Versus O2-O3 (EBOO), which treats the entire blood volume using a hollow-fiber oxygenator-ozonizer (Di Paolo 2000).

Ozone: Possible mechanisms of anti-viral action

The average adult has 4 to 6 liters of blood, accounting for about 7% of body weight. How can any viral load reduction reported via AHT ozone therapy be explained in the face of a technique that treats relatively small percentages of blood volume, albeit serially?

The viral culling effects of ozone in infected blood may recruit a variety of mechanisms. Research is needed to ascribe relative importance to these, and possibly other mechanisms of ozone's anti-viral action:

1. The denaturation of virions through direct contact with ozone. Ozone, via this mechanism, disrupts viral proteins, lipoproteins, lipids, glycolipids, or glycoproteins. The presence of numerous double bonds in these molecules makes them vulnerable to the oxidizing effects of ozone that readily donates its oxygen atom and accepts electrons in redox reactions. Unsaturated bonds are thus reconfigured, molecular architecture is disrupted, and breakage of the envelope ensues. Deprived of an envelope, virions cannot sustain nor replicate themselves.
2. Ozone proper, and the peroxide compounds it creates, may alter structures on the viral envelope that are necessary for attachment to host cells. Peplomers, the viral glycoproteins protuberances that connect to host cell receptors are likely sites of ozone action. Even minimal alteration in peplomer integrity through glycoprotein peroxidation could impair attachment to host cellular membranes foiling viral attachment and penetration.
3. Introduction of ozone into the serum portion of whole blood induces the formation of lipid and protein peroxides. While these peroxides are not toxic to the host in quantities produced by ozone therapy, they nevertheless possess oxidizing properties of their own which persist in the bloodstream for several hours. Peroxides created by ozone administration show long-term antiviral effects that may serve to further reduce viral load.
4. The immunological effects of ozone have been documented (Bocci 1992; Paulesu 1991). Cytokines, proteins manufactured by several different types of cells, regulate the functions of other cells. Mostly released by leucocytes, they are important in mobilizing immune reactivity. Ozone-induced release of cytokines may constitute an avenue for the reduction of circulating virions.
5. Ozone's actions on viral particles in infected blood yield several possible outcomes. One outcome is the modification of virions so that they remain structurally grossly intact yet sufficiently dysfunctional as to be nonpathogenic. This attenuation of viral

particle functionality through slight modifications of the viral envelope, and possibly the viral genome itself, not only modifies pathogenicity, but also allows the host to diversify its immune response. The creation of dysfunctional viruses by ozone offers unique therapeutic possibilities. In view of the fact that so many mutational variants exist in any one afflicted individual, the creation of an antigenic spectrum of crippled virions could provide for a unique host-specific stimulation of the immune system, thus designing what may be called a host-specific autovaccine.

6. A very exiting avenue of research suggests that the virucidal properties of antibodies are predicated upon their ability to catalyse highly active forms of oxygen including ozone (Marx 2002; Wentworth 2002). A key element in the microbe-inactivating capacity of antibodies may thus reside in the formation of ozone and other oxygen reactive species integral to antigen-antibody (successful) reactions. Exogenously administered ozone may, in this model, amplify the efficacy of antigen-antibody dynamics.

MERS, SARS, and Ozone: Special considerations and a proposal

MERS and SARS are disease manifestations produced by novel Coronaviruses that have succeeded in finding breaches in the immunological defenses in our contemporary human populations. They appear to have developed an aggressive balance between viral propagation, and lethality.

A universal strategy in mastering infections, whether bacterial or viral, is the culling of pathogenic organisms to the point where they no longer represent an invasive and replicative threat. This may include the elaboration of systems of immune defense capable of neutralizing subsequent viral attacks. Such strategy may be achieved via massive pathogen inactivation on one hand, and via the stimulation of host immune competence on the other.

MERS and SARS are acute, rapidly progressing, pan-inflammatory infections that, predicated upon the quasispecies involved, may present distressful mortality outcomes. A salient clinical configuration in these infections stems from their acute involvement of the respiratory system, in their rapid disruption of blood gas balance. When pO2 and pCO2 are sufficiently compromised, chemoreceptors in the medulla fail.

Antiviral agents and inhibitors to inflammation (steroids) have, thus far, not been effective in significantly softening the virulence of SARS nor MERS.

Because of their galloping symptomatology, MERS and SARS require proactive emergency viral culling. With an estimated 10 billion viral particles generated daily – a reproductive magnitude commonly observed in viremic episodes in enveloped viruses such as HIV – it is suggested that systemic ozone administration be used intensively. This treatment intensity is in contrast to the ozone treatment of chronic viral conditions, such as hepatitis B and C. Whereas the latter conditions have been addressed with parenteral treatments ranging from once daily to once weekly, MERS and SARS may require more accelerated attention, either with autohemotherapy, or with EBOO. Suggested are treatments administered twice daily till symptoms begin to abate.

MERS and SARS: disinfection/sterilization of the environment

The recent findings that the SARS virus has the capacity to remain infectious on fomites for up to several days indicates that it is a hardier organism than most of its other lipid enveloped colleagues.

Predictably, disinfectants such as bleach, phenol, and formaldehyde have been found to be effective in deactivating the SARS virus; detergents, however, were less capable. Caustic liquid agents have the disadvantage of faring poorly in decontaminating complex medical equipment and the hospital room milieu of Coronavirus patients.

Ozone, in light of its pan-virucidal profile, offers the advantage of existing as a gas, with its attendant ability to disinfect poorly accessible spaces. Moreover, ozone has the distinct benefit of reverting to oxygen, while liquid–based disinfectants are likely to injure the surfaces to which they are applied, and to leave toxic residues. Ozone-mediated environmental decontamination, however, needs to respect stringent protocols to insure that the ambient ozone in the process of sterilizing the target environment has time to revert to its stable parent, oxygen, without inflicting toxicity to the personnel.

Summary and conclusions

SARS and MERS are acute pan-inflammatory multi-system syndromes caused by hitherto unknown coronavirus species. These virions incorporate novel RNA genomes and lipid bilayered glycoprotein envelopes. The SARS and MERS viruses, based upon what is known about *Coronaviridae*, possess high rates of mutation allowing any one infected individual to harbor numerous quasispecies.

Ozone is a naturally occurring energy-rich molecule embodying unique physico-chemical and biological properties suggesting a possible role in the therapy of MERS and SARS, either as a monotherapy or, more realistically, as an adjunct to standard treatment regimens.

This paper outlines six possible mechanisms by which ozone may exert its antiviral actions. Due to the excess energy inherent in the ozone molecule, it is theoretically plausible that ozone, unlike microbial-specific options, will show effectiveness across the entire Coronavirus spectrum. The acute infection phase of MERS and SARS is marked by massive viral replication, with viral flooding of the lymph and blood compartments. This stage presents the most clinical challenge. This paper proposes a method of viral culling via systemically administered oxygen/ozone gas to blood interfacing.

Ozone has unique disinfectant properties. As a gas, it has a penetration capacity that liquids do not possess. In view of the fact that MERS and SARS persist on fomites for up to several days, it is suggested that ozone technology be applied to the decontamination of medical environments.

In conclusion, a proposal is made that oxygen/ozone systemic therapy - demonstrated to be innocuous to humans and animals - be granted research consideration for MERS and SARS. Such therapeutic approach may then be found useful not only in these specific conditions, but also in future Coronavirus epidemics that are certain to emerge.

BIBLIOGRAPHY

- Ackey D, Walton TE. Liquid-phase study of ozone inactivation of Venezuelan Equine Encephalomyelitis virus. Appl Environ Microbiol 1985; 50: 882-886
- Amato G, Sacchetta A, Borrelli E, et al. Ruolo dell'ozonoterapia mediante grande autoemotrasfusione nel trattamiento delle epatiti croniche post-epatiti virale (II parte), In: Proceedings: I Congresso IMOS, Italia, Siena, 2-4 Nov 2000, p 11
- Bartlett JG. An Epidemic of Epidemics. Medscape Infectious Diseases Feb 28, 2014
- Bocci V. Oxygen-Ozone Therapy: A Critical Evaluation. Kluwer Academic Publishers, Dordrecht, 2002
- Bocci V. Biological and clinical effects of ozone. Br J Biomed Sci 01 Jan 1999; 56(4): 270-279
- Bocci V, Luzzi E, Corradeschi F, Paulesu, et al. Studies on the biological effects of ozone: 5. Evaluation of immunological parameters and tolerability in normal volunteers receiving ambulatory autohaemotherapy. Biotherapy 1994; 7:83-90
- Bocci V. Ozonation of blood for the therapy of viral diseases and immunodeficiencies. A hypothesis. Medical Hypotheses 1992 Sept; 39(1):30-34
- Bocci V. Autohemotherapy after treatment of blood with ozone: A reappraisal. The Journal of International Medical Research 1994; 22: 131-144
- 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
- Buckley RD, Hackney JD, Clarck K, Posin C. Ozone and human blood. Archives of Environmental Health 1975; 30:40-43
- 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
- Clarke LM, Bromberg K. Human Respiratory Viruses. In Armstrong. Infectious Diseases, First Ed. Mosby, Philadelphia, 2000
- Carpendale MT, Freeberg JK. Ozone inactivates HIV at noncytotoxic concentrations. Antiviral Research 1991; 16:281-292
- Cavanaugh D, Brown TD. Coronaviruses and their Diseases (Advances in Experimental Medicine and Biology), Springer 2013
- Chun TW, Fauci AS. Latent reservoirs of HIV; obstacles to the eradication of virus. Proc Natl Acad Sci U.S.A. 1999; 96:10958-10961
- Cross CE, Reznick AZ, Packer L, et al. Oxidative damage to human plasma proteins by ozone. Free Rad Res Commun 1992; 15:347-352
- Dailey JF. Blood. Medical Consulting Group, Arlington MA, 1998
- de Haan CA. Assembly of the coronavirus envelope: homotypic interactions between the M proteins. J Virol 01 Jun 2000; 74(11): 4967-78
- Diadori A, Nuti A, Ferrari G et al. Ozone therapy: a new perspective in ophthalmology. Vision Res 1996; 36 (suppl.):418
- Di Paolo N. Extracorporeal blood oxygenation and ozonation (EBOO) in man. Preliminary report. Int J Artif Organs 01 Feb 2000; 23(2): 131-141

- Evans AS, Kaslow RA (Eds). Viral Infections in Humans: Epidemiology and Control, Fourth Edition, Plenum, New York, 1997
- Falsey AR. Rhinovirus and coronavirus infection-associated hospitalizations among older adults. J Infec Dis 1 May 2002; 185(9): 1338-41
- Fraire AE, Woda BA, Welsh RM, Kradin RL. Viruses and the Lung: Infections and Non-Infectious Viral Linked Disorders, Springer 2014
- Fukagawa NK. Aging: is oxidative stress a marker or is it causal? Proc Soc Exp Biol Med 1999; 222:293-298
- Gastañaduy PA. Update: Severe respiratory illness associated with Middle East Respiratory Syndrome (MERS-CoV). Morbidity and Mortality Weekly Report 2013; 62(23): 480-483
- Goheen SC, O'Rourke L, Larkin EC. Ozone and the peroxidation of polyunsaturated fatty acids in vivo. Environ Res 1986: 40: 47-57
- Gumulka J, Smith L. Ozonation of cholesterol. J Am Chem Soc 1983; 105(7): 1972-1979
- Hernandez F, Menendez S, Wong R. Decrease of blood cholesterol and stimulation of antioxidative response in cardiopathy patients treated with endovenous ozone therapy. Free Rad Biol Med 1995; 19:115-119
- Hurst CJ. Viral Ecology. Academic Press, New York, 2000
- Knipe DM, Howley PM. Fundamental Virology, Fourth Edition. Lippincott Williams & Wilkins, Philadelphia, 2001
- Konrad H. Ozone therapy for viral diseases. In: Proceedings 10th Ozone World Congress 19-21 Mar 1991, Monaco. Zurich: International Ozone Association 1991:75-83
- Ksiasek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003; 348:1947-1958
- Kourie JI. Interaction of reactive oxygen species with ion transport mechanisms. Am J Physiol 1998; 275:1-24
- Lai MM, Holmes KV. Coronaviridae: The Viruses and Their Replication. In: Knipe DM, Howley PM. Fundamental Virology. Lippincott Williams&Wilkins, Philadelphia, 2001
- Lal SK. Molecular Biology of the SARS Coronavirus, Springer, 2010
- Larini A, Aldinucci C, Bocci V. Ozone as a modulator of the immune system, In : Proceedings of the 15th Ozone World Congress, London, UK, 11th-15th September 2001 (International Ozone Association 2001, Speedprint Macmedia Ltd, Ealing, London, UK
- Luongo C, Sammartino A, Lauritano et al. Trattamento e monitoraggio dei potenziali redox nelle membrane cellulari nello studio delle infezioni da HCV. In: Proceedings: I Congresso IMOS, Italia, Siena, 2-4 Nov 2000, p 42
- Maeda J. Membrane topology of coronavirus E protein. Virology 15 Mar 2001; 281(2): 163-169
- Marra MA, Jones SJ, Astell CR. The genome sequence of the SARS-Associated coronavirus. Science. Sciencexpress. 1 May 2003 www.sciencexpress.org
- Max J. Antibodies kill by producing ozone. Science 15 Nov 2002; 298: 1319
- Monto AS. Coronaviruses. In Evans AS, Kaslow RA. Viral Infections in Humans, Fourth edition, Plenum, New York, 1997
- Olinescu R, Smith TL. Free Radicals in Medicine. Nova Science Publishers, Inc. Huntington, New York, 2002

- Olwin JH, Ratajczak HV, House RV. Successful treatment of herpetic infections by autohemotherapy. J Altern Complement Med 1997; 3: 155-158
- O'Neil CA, van der Vliet A, Hu ML, et al. Oxidation of biologic molecules by ozone: the effect of Ph. J Lab Clin Med 1993; 122: 495-505
- 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
- Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet. Ap 19, 2003; 361: 1319-1325
- Razumovskii SD, Zaikov GE. Ozone and its reaction with organic compounds. Elsevier, New York, 1984
- Rice RG. Century 21 – Pregnant with ozone. Ozone Science and Engineering 2002; 24: 1-15
- Romero A, Menendez C, Gomez M, et al. Ozone therapy in the advanced stages of arteriosclerosis obliterans. Angiologia 1993; 45: 146-148
- Roy D, Wong PK, Engelbrecht RS, Chian ES. Mechanism of enteroviral inactivation by ozone. Applied Environmental Microbiology 1981; 41: 728-733
- Shinriki N, Suzuki T, Takama K, et al. Susceptibilities of plasma antioxidants and erythrocyte constituents to low levels of ozone. Haematologia 1998; 29; 229-239
- Siddell SG. The Coronaviruses. Plenum Press, New York, 1995
- Singh SK, Viral Infections and Global Change, Wiley-Blackwell, 2013
- Steiberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997; 272: 20963-66
- Steinhauer DA, Holland GG. Direct method for quantation of extreme error frequencies at selected single base sites in viral RNA. J Virol 1986; 57:219-228
- Sunnen GV. 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
- Sunnen G. Apparatus for the application of ozone/oxygen for the treatment of external pathogenic conditions. Patent pend. PCTUS99-17286, Jul 1999
- Sunnen G, Robinson J. Method and apparatus for ozone decontamination of biological fluids. Patent pend. 10-002943, Jul 2001
- Sunnen G. www.triroc.com/sunnen
- 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
- Vaughn JM, Chen YS, Novotny JF. Effects of ozone treatment on the infectivity of hepatitis A virus. Can J Microbiol 1990; 36: 557-560

- 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
- White DO, Fenner FJ. Medical Virology, Fourth edition. Academic Press, New York, 1994
- Yamamoto M, et al. The effect of ozone on treatment of 4 patients suffering from hepatitis C. Bulletin of Japan Research Association for the Medical Use of Ozone. 1996; 3: 1-2
- Yu BP. Cellular defenses against damage from reactive oxygen species. Physiological Reviews 1994 Jan; 74(1): 139-162

Gérard V. Sunnen M.D.
200 East 33rd St.
New York, NY 10016
Tel. 212-6790679
FAX 212-6798008
gsunnen@aol.com
Triroc.com/sunnen
gsunnen.com