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Hepatitis C and Ozone Therapy

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Abstract

Hepatitis C (HCV) is a global disease with a worldwide expanding incidence and prevalence base. Of massive public health importance, hepatitis C presents supremely challenging problems in view of its adaptability and its pathogenic capacity. The unique strategies that HCV utilizes to parasitize its hosts make it a formidable enemy and therapeutic interventions need considerable sophistication to counter its progress. Ozone, because of its special biological properties, has theoretical and practical attributes to make it a potent hepatitis C inactivator.

History of the virus. A form of hepatitis became recognized in the 1970's that resembled serum hepatitis B, and to a lesser extent infectious hepatitis A. It had, however, novel features, among them a distinctive serological profile. In 1989, the genome of hepatitis C was deciphered.

It is possible, by means of extrapolation from the genetic evolution of a virus to approximate its age. Sequence genetic analysis points to the diversification of different HCV genotypes 200 to 400 years ago. Ancestors to these genotypes probably date back 100,000 or so years when viruses co-evolved with modern humans. Further analysis of genetic viral trees and Old and New World primates take the primordial forms of these viruses to primate speciation periods some 35 million years ago.

Today, in the context of human population growth, migration, and global travel, the hepatitis C virus has widened its territories, geographically and demographically. There is every indication that the evolution of this virus is currently showing an accelerated phase.

Virion architecture and molecular biology. The HCV particle is composed of a nucleocapsid containing its genome, an RNA single strand composed of approximately 9600 nucleotides, and its protein coating. An envelope that allows attachment and penetration into host cells surrounds the nucleocapsid. The genome encodes structural proteins, designated as core (C), envelope 1 (E1), envelope 2 (E2), and P7 (uncertain function), providing for virion architecture, and nonstructural proteins, mainly enzymes essential to the virion's life cycle, designated as NS2, NS3, NS4A, NS4B, NS5A, and NS5B. Proteases generate structural and nonstructural proteins. Helicases unwind viral nucleic acid. Polymerases replicate RNA. Within this genome is located a hypervariable region implying an area of intensive genetic fluidity and mutational potential. HCV displays great genotypic flexibility that makes for sophisticated evasiveness to host defenses.

An envelope, a lipid bilayer associated with a glycoprotein union of carbohydrates and proteins surrounds the nucleocapsid. Up to 60% of the lipid component of the envelope is phospholipid and the remainder is mostly cholesterol. It possesses projections called peplomers that facilitate attachment to host cells. One protein on peplomers of the HCV particle thought to be instrumental in the attachment process is designated CD-81.

The sequence of nucleotides within the HCV genome shows significant variations. Strains obtained from different parts of the world, for example, may differ substantially in their structural and nonstructural protein compositions. This has spawned a system of classification of the HCV family into 6 genotypes and approximately a hundred subtypes (designated a, b, c, etc.). Genotypes vary from each other by a factor of 30% and subtypes by about 20%. Genotypes 1 to 3 have global distribution. Genotype 4 and 5 are found mainly in Africa, and genotype 6 is distributed in Asia. Importantly, genotype and subtype differences have shown varying susceptibility to antiviral



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therapies.

Within any one afflicted individual, HCV 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 replication error inherent in the function of the polymerase enzymes. Herein lies one of HCV's important armaments. Continuously generated genetic diversity provides it with a great advantage in negotiating host immune defense and therapeutic drug strategies.

The Hepatitis C viral life cycle

Circulating virions enter host cells by binding to cell surface receptors. In the case of HCV the host cell is a hepatocyte. However, bone marrow, kidney cells, macrophages, lymphocytes, and granulocytes may also be trespassed.

Once cell entry is achieved, the virion sheds its envelope. Binding to cellular ribosomes, released viral polymerase begins the RNA replication cycle. Newly formed nucleocapsids continue their assembly by acquiring envelopes whose ingredients are appropriated from budding through cellular endoplasmic reticulum membranes. Newly formed virions may number in the range of 10 billion daily. The average life span of virions is in the order of a few hours.

Virions are then released into the general blood and lymphatic circulation, ready to infect new cells and eventually new hosts, mainly through bodily fluid transmission. Hepatitis C RNA as measured by polymerase chain reaction (PCR) may show 10 million or more virions per milliliter. As little as 0.0001 ml of blood may be sufficient to impart infection. The evolution of hepatitis C is characterized by phases of accentuated viremia punctuated by periods of relative quiescence. The timely detection of these viremic waves may point to novel therapeutic strategies.

Clinical and laboratory manifestations. Hepatitis C distinguishes itself from other viral hepatropic infections by the low incidence of an acute phase and by the high incidence of progression to chronicity. Hepatitis C often indolently evolves from exposure, to incubation, to pre-icteric, icteric, and convalescent phases. After an incubation period of about 6 weeks, the first and sometimes only symptoms include weakness, fatigue, headache, nausea and vague abdominal pain. The pre-icteric period extends from the onset of symptoms to the appearance of jaundice, ranging usually from 2 to 12 days. The icteric phase corresponds to the declaration of jaundice and darkened urine. The convalescent phase is marked by a gradual disappearance of symptoms.

Chronic hepatitis C is characterized by the presence of HCV RNA and an elevation of liver enzymes for six months or longer. Patients may be asymptomatic for long periods of time. Others experience acute exacerbations with the return of symptoms. Approximately 75% of acutely ill patients continue into a chronic phase accompanied by laboratory evidence of viral presence.

Hepatitis C is distinguished from other viral hepatic conditions by serological and virological determinations. Liver enzymes characteristically affected by HCV infection include serum alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGTP), and alkaline phosphatase. In addition, there may be abnormalities in bilirubin, serum albumin, prothrombin time and platelet density.

Cirrhosis is a diffuse fibrotic disruption of liver tissue architecture is an important sequel to hepatitis C. Within 20 years post HCV infection 20 to 25% of patients will develop cirrhosis. Hepatic decompensation may ensue with ascites as the salient marker.

Hepatocellular carcinoma, another long-term outcome of HCV infection evolves in approximately 5% of patients. Although the mechanisms by which cirrhosis ushers carcinomas are unknown, it is likely that chronic inflamma-



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tion and the sustained pressure of cellular regeneration play important roles.

In up to 10% of patients HCV antibodies are undetectable, as is HCV RNA. Liver enzymes are normalized but liver biopsy may show lingering areas of stagnant inflammation and spotty necrosis. These patients appear to have fully conquered the disease. It is thus possible for host immunocompetence to vanquish HCV infection.

Immunological responses to HCV particles are detected early in the infection, usually within two weeks following exposure. Antibodies to HCV core, nonstructural, and envelope elements appear about within six weeks. A range of cytokines are mobilized. Cellular immunity is activated with broad recruitment of neutrophils, natural killer (NK), macrophages, and CD4 and CD8 T helper cells.

Current and experimental treatment strategies. As of this date the main treatment strategies for hepatitis C include interferons and ribavirin. Interferons are natural cellular products that activate macrophages, neutrophils and natural killer cells. There is controversy as to interferon's biological effects, be they mostly immunoregulatory or directly antiviral. Ribavirin is a guanosine analog that represses messenger RNA formation thus inhibiting viral replication. Interferons and Ribavirin have significant medical and psychiatric side effects.

Treatment response is defined as undetectable viral load six months following therapy. Contemporary detection methods of quantitative HCV RNA determinations are capable of detecting approximately 1000 viral copies per ml. of serum

Resistance to current HCV antiviral therapies is a particularly vexing problem. Experimental antiviral compounds include inhibitors of protease, polymerase and helicase.

Vaccine development needs to take into account HCV's antigenic rainbow and its high mutability. High mutation rates imply a dauntingly diverse and variable array of viral antigenic components. It is estimated, for example, that HCV mutates in its host approximately a thousand times a year. This implies that within any one afflicted individual there exists an awesomely large array of viral quasispecies that in turn create commensurate difficulties relative to the creation of effective vaccines.

Ozone: Physical and physiological properties. Ozone (O₃) is a naturally occurring configuration of three oxygen atoms. With a molecular weight of 48, the ozone molecule contains a large excess of energy. It has a bond angle of 127° and resonates among several hybrid forms. At room temperature, ozone has a half-life of about one hour, reverting to oxygen. A powerful oxidant, ozone has unique biological properties that are being investigated for applications in various medical fields.

Research on ozone's biological dynamics have centered upon its effects on blood cellular elements (e.g., erythrocytes, leucocytes, and platelets), and to its serum components (e.g., proteins, lipoproteins, lipids, carbohydrates, electrolytes). Administering ozone to whole blood shows that beyond a certain threshold there is a rise in the rate of hemolysis. This threshold, depending upon various parameters, begins to be reached at 40 to 60 micrograms of ozone per milliliter and becomes significant when higher levels are attained. Precise ozone dosing capacity is therefore essential in clinical practice and research.

Leucocytes show good resistance to ozone because they have enzymes that protect them from oxidative stress. These enzymes include superoxide dismutase, glutathione, and catalase. Platelets also maintain their integrity after ozone administration. In ozone therapy, the doses applied to blood are gauged to avoid disruption of its cellular elements. Serum components remain viable during ozone therapy. Lipid and protein peroxides, produced in small amounts by ozonation, have demonstrable antiviral properties. Interestingly, ozone tends to stimulate leucocyte function and cytokine production. Ozone increases the oxygen saturation in erythrocytes and enhances their pliability so that capillary circulation is facilitated.



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Ozone: Antiviral properties. Recently, there has surged renewed interest in the potential of ozone for viral inactivation. It has long been established that ozone neutralizes bacteria, viruses, and fungi in aqueous media. This has prompted the creation of water purification processing plants in many major municipalities worldwide.

Ozone's antiviral properties may also be applied to the treatment of biological fluids, albeit in technologically and physiologically appropriate ways. Indeed, it is noted that ozone, administered in dosages designed to respect the integrity of blood's cellular and constituent elements, is capable of inactivating a spectrum of viral families.

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 new glycoproteins under the direction of E1 and E2 portions of the HCV genome. Envelopes are fragile. Ozone and its by-products can disrupt them.

In HCV, viral load appears to be a major factor in the invasiveness and virulence of the disease process. Research is needed to demonstrate conclusively that reduction of viral load in hepatitis C by means of ozone therapy can improve measures of global health (Yamamoto 2000). Normalization of liver enzymes in 14 hepatitis C patients using ozone hemotherapy was reported (Amato et al 2000). Ozone hemotherapy in 82 patients treated for 3 to 6 months showed an 80% reduction in HCV viral load (Luongo et al 2000).

Ozone: Clinical methodology

Ozone may be utilized for the therapy of a spectrum of clinical conditions. Routes of administration are varied and include external and internal (blood interfacing) methods. In the technique of ozone autohemotherapy for hepatitis C, an aliquot of blood is withdrawn from a virally-afflicted patient, anticoagulated, interfaced with an ozone/oxygen mixture, and then re-infused. This process is repeated serially until viral load reduction, laboratory parameters, and clinical improvement are documented.

The average adult has 5 to 6 liters of blood, accounting for about 7% of body weight. How can the viral load reduction observed via ozone therapy be explained in the face of a technique that treats relatively small amount of blood, albeit serially?

Importantly, another, more experimental and more intensive 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). This approach is promising because all blood and lymphatic fluids are interfaced with oxygen/ozone mixtures thus providing integral anti-viral therapy. Research is needed to determine appropriate dosage and treatment duration protocols to determine the therapeutic window parameters of this methodology.

Ozone: Possible mechanisms of anti-viral action

Recently, there has been renewed interest in the potential of ozone for viral inactivation in vivo. It has long been established that ozone neutralizes viruses in aqueous media and it stands to reason that it would be studied for similar applications in living systems. In vivo ozone applications, however, present far greater challenges. Indeed, the technology of medical ozone administration aims to respect the delicate balance of patient safety on one hand and antimicrobial efficacy on the other.

All viruses are susceptible to ozone's neutralizing action. Viruses, however, differ in their relative susceptibility to destruction by ozone. In one study, poliovirus resistance was 40 times that of coxsackievirus. Relative susceptibility in ascending order was found to be: poliovirus type 2, echovirus type 1, poliovirus type 1, coxsackievirus type B5, echovirus type 5, and coxsackievirus 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



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minutes (Roy 1982). Analysis of viral components showed damage to polypeptide chains and envelope proteins, which could result in attachment capability compromise, and breakage of the single-stranded RNA producing replicating dysfunction. Other researchers, in similar experiments, concluded that in ozonation, it is the viral capsid that sustains damage (Riesser 1977). Viruses, unlike mammalian cells, have no enzymatic protection against oxidative stress.

Lipid-enveloped viruses are sensitive to treatment with ether, organic solvents, and ozone, indicating that disruption or loss of lipids results in impaired or destroyed infectivity. Viruses containing lipid envelopes include the Flaviviridae (hepatitis C, West Nile virus, yellow fever); the Hepadnaviridae (hepatitis B); the *Herpesviridae*, a large family grouping the Simplex, Varicella-Zoster, Cytomegalovirus, and Epstein-Barr viruses; the *Orthomyxoviridae* (avian influenza); the *Paramyxoviridae* (mumps, measles); the *Coronaviridae* (SARS); the *Rhabdoviridae* (rabies); the *Togaviridae* (Rubella, encephalitis); the *Bunyaviridae* (Hantavirus); the *Poxviridae* (smallpox); and the *Retroviridae* (HIV), among others. Indeed, once the virion's lipid envelope becomes fragmented, its DNA or RNA core cannot survive.

The enveloped viruses (e.g., hepatitis C), adapted to the delicate homeostatic milieu of their hosts are usually more sensitive to all physico-chemical challenges than are naked virions. This has been shown for ozone (Bolton 1982). Although ozone's effects upon unsaturated lipids are one of its best documented biochemical actions, ozone is known to interact with other viral constituents. This becomes relevant when ozone inactivation of non-enveloped virions is considered.

Viruses that do not have an envelope are called "naked viruses." They are constituted of a nucleic acid core made of DNA or RNA, and a nucleic acid coat, or capsid, made of protein. Some non-enveloped viruses include: *Adenoviridae* (respiratory infections), *Picornaviridae* (poliovirus, coxsackievirus, echovirus, rhinovirus, hepatitis A), *Caliciviridae* (hepatitis E, Norwalk gastroenteritis), and *Papillomaviridae* (Molluscum contagiosum). Ozone can interact with viral proteins, their constituent amino acids and lipopolysaccharides. Indeed, when ozone comes in contact with viral capsid proteins, protein hydroxides and protein hydroperoxides are formed and viral demise ensues.

In summary, ozone's anti-hepatitis C action in blood may recruit the following mechanisms:

1. The denaturation of virions through direct contact with ozone. Ozone, via this mechanism, disrupts viral envelope lipids, phospholipids and lipoproteins. The presence of numerous chemical double bonds in these unsaturated molecules makes them vulnerable to the oxidizing effects of ozone, which readily donates its oxygen atom and accepts electrons in redox reactions. Broken bonds are thus reconfigured, molecular architecture becomes disrupted, and breakage of the viral envelope ensues. Deprived of an envelope, virions cannot sustain nor replicate themselves.
2. Ozone proper may directly 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. Alteration in peplomer integrity impairs 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 may serve to further reduce viral load.
4. Immunological effects of ozone have been documented. Cytokines are proteins manufactured by several different types of cells that regulate the functions of other cells. Mostly released by leucocytes, they are important in



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mobilizing immune response. Ozone induces the release of cytokines that in turn activate a spectrum of immune cells. Ozone is reported to be an immuno-stimulant in low doses and immuno-inhibitory at higher levels (Werkmeister 1985, Varro 1974, Zabel 1960). Additionally, ozone functions as a signaling agent by stimulating production of nuclear factor kappa B, interleukin 6, and tumor necrosis factor α . Ozone's capacity for cytokine activation has been amply documented (Bocci 2005).

5. Ozone actions on viral particles in infected blood yield several possible outcomes. One outcome is the modification of virions so that they remain structurally 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, modifies pathogenicity and allows the host to increase the sophistication of 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. An exciting 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). In this model, activated neutrophils provided with appropriate starting materials are capable of generating 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 combine with water to form the hydroxyl radical (OH) and hydrogen peroxide. Endogenously created ozone thus becomes a fundamental immunological agent for viral inactivation.

Exogenously administered ozone may, based on this model, amplify the efficacy of antigen-antibody dynamics and assist in the clearing of hepatitis C virions in blood.

Summary

Viruses are far from being static entities. As quintessential intracellular parasites they have developed, through millions of years of cohabitation with their hosts, astoundingly sophisticated structures and propagation mechanisms. They have modified their biological strategies and evolved impressive mutational capacity to keep pace with our changing planetary ecology.

HCV has an extremely high rate of mutation and within any one individual there may exist millions of antigenic quasispecies. The disease process is marked by periods of viral quiescence alternating with viremic waves whereby billions of virions are poured into the blood and lymphatic reservoirs.

Viral load reduction alleviates immune system fatigue. Ozone-mediated viral culling may be achieved by anyone of a number of possible mechanisms. Direct virion denaturation, peplomer alteration, lipid and protein peroxide formation, cytokine induction, host humoral activation, and host-specific autovaccine creation are suggested mechanisms. It is also suggested that, in the management of hepatitis C, close monitoring of viral load measures be performed to identify the onset of viremic episodes. Indeed, it is during HCV viremic episodes that ozone blood ozonation may be most helpful.

Research is needed to determine the indications for ozone administration relative to the HCV life cycle and to standardize ozonation treatment protocols, whether for ozone serial hemotherapy or for extracorporeal total blood and lymphatic system ozonation.

Due to the excess energy contained within the ozone molecule, it is theoretically likely that ozone, unlike antiviral drug options available today, will show effectiveness across the entire hepatitis C genotype and subtype spectrum.

The recent discovery that ozone is generated at the molecular level in antigen-antibody reactions, thus giving it a central role in the body's natural anti-viral defense may greatly privilege the clinical use of blood ozonation therapies such as autohemotherapy and extracorporeal total blood volume ozonation in the management of hepatitis C infection.

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