
Hepatitis C and Ozone Therapy

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Abstract

Hepatitis C (HCV) is a global disease with an 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 host make it a formidable enemy and therapeutic interventions need considerable honing to counter its progress. Ozone, because of its special biological properties, has theoretical and practical attributes to make it a potent HCV inactivator.

History of the virus A form of hepatitis became recognized in the 1970's that resembled hepatitis B, serum hepatitis, and to a lesser extent hepatitis A, infectious hepatitis. It had, however, novel features, amongst them, a distinctive serological profile. In 1989, the genome of hepatitis C (HCV) 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 expanded its territories, geographically, and demographically. There is every indication that the evolution of this virus, in all its forms, is currently manifesting 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. The nucleocapsid is surrounded by an envelope which allows attachment and penetration into host cells. The genome encodes structural proteins designated as core (C), envelope 1 (E1), envelope 2 (E2), and P7 (unknown 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 release 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 which makes for sophisticated evasiveness to host defenses.

The nucleocapsid is surrounded by an envelope, a lipid bilayer associated with a union of carbohydrates and proteins, glycoproteins. Up to 60% of the lipid component of the envelope is phospholipid and the remainder is mostly cholesterol. It possesses projections called peplomers which facilitate attachment to host cells. One protein on peplomers of the HCV particle which is 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 led to a system of classification of the HCV family into 6 genotypes (1 to 6), and approximately 100 subtypes (designated a, b, c, ect.). Genotypes vary from each other by a factor of 30% over the entire genome. Subtypes vary by about 20%. Genotypes 1 to 3 have global distribution, while genotype 4 and 5 are found mainly in Africa, and 6 is distributed in Asia. Importantly, genotype and subtype differences have shown varying susceptibility to antiviral therapy.

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 the important armaments of HCV. Continuously generated genetic diversity gives it great advantage in negotiating and conquering immune defense and therapeutic strategies. Furthermore, the antigenic differences between genotypes may have implications regarding the proper evaluation and the therapeutic regimen of patients.

Viral life cycle A freely circulating virion enters a host cell by binding to a cell surface receptor. 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 to commence its replication. 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 endoplasmic reticulum. 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, re-infect already diseased cells, or a new host, mainly through bodily fluid transmission pathways. HCV RNA, as measured by polymerase chain reaction (PCR) may show 10 million or more virions per ml. 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 presence and timely detection of these viremic waves may offer novel therapeutic considerations.

Clinical and laboratory manifestations Hepatitis, from anyone of the several viruses capable of inducing liver inflammation, produce a spectrum of clinical and laboratory manifestations. Hepatitis C distinguishes itself by the low incidence of acute phases and by the high incidence of progression to chronicity. Acute hepatitis C progresses from exposure, to incubation, to pre-icteric, icteric, and convalescent phases. With an incubation period of about 6 weeks, the first and sometimes only symptoms include weakness, fatigue, indolence, headache, nausea, poor appetite, 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 the gradual disappearance of symptoms.

Chronic hepatitis C is characterized by the presence of HCV RNA and the elevation of liver enzymes for 6 months or longer. Patients may be asymptomatic, or at times suffer an acute exacerbation with a return of symptoms. Approximately 75% of acutely ill patients continue into a chronic phase evidenced by parameters of viral presence.

Hepatitis C can only be 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, a diffuse disruption of liver tissue architecture with regenerative nodules surrounded by fibrosis, is an important sequel to hepatitis C. Within 20 years post HCV infection 20 to 25% of patients will develop cirrhosis. Hepatic decompensation ensues with ascites as the salient marker.

Hepatocellular carcinoma, another notable outcome of HCV infection is present in approximately 5% of patients post infection. The presence

of cirrhosis is central to its genesis. Although the mechanisms by which cirrhosis ushers carcinoma are unknown, it is likely that chronic inflammation and the sustained pressure of cellular regeneration play important roles.

Up to 10% of patients appear to have fully conquered the disease. HCV antibodies are undetectable, as is HCV RNA. Liver enzymes are fully normalized, but liver biopsy may show lingering areas of stagnant inflammation and spotty necrosis. It is thus possible for host immunocompetence to vanquish HCV infection and therapeutic strategies aim to assist the host immune system to achieve this goal.

Immunological response to the virus HCV particles are detected early in the infection, usually 1 to 2 weeks following exposure. Antibodies to HCV core, nonstructural, and envelope elements appear about 6 weeks after exposure. A broad 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 interferon and ribavirin. Interferons are natural cellular products which 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 the replication of many DNA and RNA viruses. It is, however, mutagenic to mammalian cells. Ribavirin and interferon have significant medical and psychiatric side effects.

Treatment response is defined as undetectable viral load 6 months following therapy. Contemporary detection methods of quantitative HCV RNA determinations are capable of detecting approximately 1000 viral copies per serum ml.

Resistance to antiviral therapies is a particularly vexing problem in anti HCV treatment. Novel and 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 in this condition implies a dauntingly diverse and variable array of viral antigenic components. It is estimated, for example, that HCV mutates significantly in its own host approximately a thousand times a year. This implies that within any one afflicted individual there exists an awesomely large array of viral quasispecies, which in turn creates commensurate difficulties in 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 forms. At room temperature, ozone has a half life of about one hour, reverting to oxygen. A powerful oxidant, ozone has unique biological properties which are being investigated for applications in various medical fields. 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, lipoproteins, lipids, carbohydrates, electrolytes). Administering increasing dosages of 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 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 which protect them from oxidative stress. These enzymes include superoxide dismutase, glutathione, and catalase. Research has shown that 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 (pO_2) in erythrocytes and enhances their pliability so that capillary circulation is facilitated.

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 such dosages designed to respect the integrity of blood's cellular and constituent elements, is capable of inactivating a spectrum of viral families.

Some viruses are much more susceptible to ozone's action than others. It has been found that lipid-enveloped viruses are the most sensitive. This group includes, amongst others, HCV, Herpes 1 and 2, Cytomegalus, HIV1 and 2.

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. They can be disrupted by ozone and its by-products.

In HCV, viral load appears to be a major factor in the invasiveness and virulence of the disease process. Preliminary research has shown that reduction of viral load in Hepatitis C by means of ozone therapy can significantly normalize hepatic enzymes and improve measures of global patient health. Volunteers administered ozone therapy according to the method outlined below achieved a viral load reduction in the order of 5 log, or 99.9%, along with a normalization of liver enzyme levels.

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 major autohemotherapy for hepatitis C, an aliquot of blood is withdrawn from a virally-afflicted patient, anticoagulated, interfaced with an ozone/oxygen mixture, then re-infused. This process is repeated serially until viral load reduction is documented.

The aliquots of blood range from 50 ml. to 300 ml. Ozone dosages and treatment frequency vary according to treatment protocols. The reason aliquots of blood are treated and not, as one would propose, the entire blood volume, is that in the latter case the total ozone dosage administered would exceed toxic limits.

The average adult has 4 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?

Ozone: Possible mechanisms of anti-viral action

The viral culling effects of ozone in infected blood may recruit the following mechanisms:

Denaturation of virions through direct contact with ozone. Ozone, via this mechanism, disrupts viral envelope proteins, lipoproteins, lipids, and glycoproteins. The presence of numerous 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 these redox reactions. Double bonds are thus reconfigured, molecular architecture is disrupted and widespread breakage of the envelope ensues. Deprived of an envelope, virions cannot sustain nor replicate themselves.

Ozone proper, and the peroxide compounds it creates, may directly alter structures on the viral envelope which are necessary for attachment to host cells. Peplomers, the viral glycoproteins protuberances which 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.

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 which serve to further reduce viral load. This factor may explain in part the reason for the fact that ozonated blood in the amount processed in usual treatment protocols is able to reduce viral load values in the total blood volume.

Immunological effects of ozone have been documented. Cytokines are proteins manufactured by several different types of cells which regulate the functions of other cells. Mostly released by leucocytes, they are important in mobilizing the immune response. It has been found that ozone induces the release of cytokines which in turn activate a spectrum of immune cells. This is likely to constitute a significant avenue for the reduction of circulating virions.

Ozone action 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, 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.

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, survival, and propagation mechanisms. They have adapted, modified their biological strategies, and evolved impressive genetic diversity and mutational capacity to cope with the changing ecology of planetary life.

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. Their astounding numbers stress the immune system relentlessly and produce an inexorable compromise in all

parameters of its functioning.

Viral load reduction by means of ozone blood treatment 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 pan-humoral activation, and host-specific autovaccine creation are suggested mechanisms. Due to the excess energy contained within the ozone molecule, it is theoretically likely that ozone, unlike antiviral options available today, will show effectiveness across the entire genotype and subtype spectrum.

Ozone embodies unique physico-chemical and biological properties which suggest an important role in the therapy of hepatitis C, either as a monotherapy, or as an adjunct to standard treatment regimens.

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