REVIEW ARTICLE
TREATMENT AND VACCINATION FOR HEPATITIS C: PRESENT AND FUTURE

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Hepatitis C is caused by Hepatitis C Virus (HCV), detriments the quality of life of 170 million people around the globe. Although, much has been known about the biology of the virus in recent years, a complete cure of hepatitis C remains difficult in a large majority of patients. The current treatment regimen comprising pegylated interferon alpha and ribavirin has sub-optimal effectiveness especially in patients infected with HCV genotype 1. The development of an effective vaccine against the virus as well as a potent anti-viral therapy remains urgently needed. Herein, we give a brief overview of the molecular biology of hepatitis C and the postulated mechanisms of hepatitis C pathogenesis. The issues surrounding the current treatment of hepatic C, the promising new therapies on the horizon and the experimental strategies to develop a vaccine have also been discussed in a greater detail.
Keywords: Hepatitis C, treatment, vaccination

INTRODUCTION

Until the last decade of 20th century, health professionals round the globe were facing a mammoth problem regarding blood transfusions. The blood transfusions carried out universally were plagued by the potential transmission of a highly morbid liver pathology commonly regarded then as Transfusion Associated Hepatitis (TAH). As the screening tests for Hepatitis A Virus (HAV) and Hepatitis B Virus (HBV) became common, it was demonstrated that only approximately 25% of all TAH's resulted from HBV and that none were related to HAV. Consequently, approximately 75% of TAH became classified as non-A, non-B hepatitis (NANBH).[1] The enigma did not resolve until 1989 when HCV was first isolated.[2] Hepatitis C remains endemic in many countries of the world with Global Disease Burden rounding 170 million.[3] However, a substantial number of patients remain asymptomatic and are never clinically identified. Studies conducted on healthy general populations in Turkey, Zimbabwe and Pakistan revealed the Hepatitis C seroprevalence of 2.2%, 7.7% and 5.3% respectively.[4-6] Considering the fact that Hepatitis C commonly affects the disadvantaged populations which never present to health care facilities; these figure may just be an under-representation of the actual prevailing threat.

The disease follows a slow progressive course. Persistent infection is the hallmark of the disease and is present in 75% of the initially infected individuals. Prospective analyses on natural history of the disease state that almost 20% of the diseased progress to Cirrhosis over a span of 20 years. However, Hepatocellular Carcinoma (HCC) is relatively uncommon and develops in 20% of the Cirrhotic after an average of 40 years down the initial infection.[7]

These figures emphatically fail to highlight the actual threatening impact of the disease in terms of patient mortality, morbidity, economic saddle as well as social stigmatization. It has been reported that Hepatitis C results in 8000–12000 deaths in the US per year.[8] Besides being potentially fatal, the persistent disease is associated with detrimental effects on the patients' quality of life. In addition to a physical deterioration, the patients experience internalized shame; are often subjected to social isolation and report financial insecurity.[9] The obscure course of the disease persisting over decades makes it hard to visualize the injurious impact Hepatitis C could have on a country's economy. Wong *et al* gauged Hepatitis C related American national expenditure to around $ 10.7 billion for the years 2010 through 2019.[10] For a developing country, even a fraction of this disbursement will be enough to cause a huge set back to the national economy.

Molecular Biology of HCV

HCV comprises a single stranded RNA genome of positive polarity and about 9.6 kb in size. The genome consists of the Open Reading Frame (ORF) flanked on either side by Non-Translated regions (NTRs) which have been recently explicated to play an important role in translation of ORF. The ORF encodes a single polyprotein of approximately 3000 amino acids which is sliced into separate proteins by a host signal peptidase in the structural region and the HCV-encoded proteases in the nonstructural (NS) region. The structural region contains the core protein and two envelope proteins (E1 and E2). E2 serves as the binding site for CD81, the alleged HCV receptor expressed on the hepatocytes.The nonstructural proteins include NS2, NS3, NS4A & B and NS5A & B. The functional characterization of each of these individual genomic proteins based upon a

scrupulous review of literature was recently explained by Brass et al.[11] The established/postulated functions of each of these genomic proteins has been represented in Table-1.

Table: Functions of HCV Genomic Proteins.

Core Protein	Nucleopcapsid, Suppression of Host immune response, Carcinogenesis
E1 & E2	Envelope Glycoproteins
p7	Polyprotein processing, Viral assembly
NS2/ NS3	NS2/NS3 Protease
NS3	Helicase
NS3/NS4A	Serine Protease
NS4B	Formation of Replication Complex
NS5B	Formation of Replication Complex

Functions for which the existing evidence is inconclusive or debatable have been written in Italics.

Mechanism of Pathogenesis

It has been long established that mechanism of hepatocyte damage in hepatitis C is immune mediated. Now a number of mechanisms associated with escape of the pathogen from the host's immune response, hepatocyte damage and molecular oncogenesis of hepatocellular carcinoma have been elucidated. The virus is able to evade the neutralizing antibodies in the blood through hypervariability in its envelope proteins which result in inefficient clearance of the viral dose.[12] Once the virus enters the hepatocytes through receptor mediated endocytosis and starts replicating, hepatocyte damage starts, the major component of which is through the host's own immune response.[13] Interferons make the most potent natural weapon of the host against intra-cellular viral infection. HCV, however, owing to intricate actions of its genomic proteins is equipped with ability to evade the natural interferon-mediated clearance. HCV core protein has been implicated to decrease the robustness of the host's immune response by decreasing transcription of interferon induced anti-viral genes.[14] HCV NS3/4A protease similarly has been implicated in inhibiting the interferon amplification loop which otherwise results in suppression of HCV replication. Pharmacologic active site inhibition of HCV protease has been shown to reverse the effects which make protease inhibitors one of the most noteworthy potential therapeutic agents for HCV.[15]

An explanation of HCV induced Liver Cirrhosis was lately proposed through in-vitro co-culture studies on mice using liver stellate cells expressing HCV core protein. The studies showed that expression of HCV core protein is associated with an elevation in expression of Transforming Growth Factor- B (TGF-B) and Connective Tissue Growth Factor (CTGF) both of which are known to induce Fibrin proliferation.[16] A mechanistic explanation for HCV induced oncogenesis has also been put forth. It has been found out that HCV

through its core protein and NS3 activate the gene for the enzyme iNOS (Nitrogen Oxide Synthase). The resulting NO causes DNA breaks and enhances DNA mutations which serve as the molecular trigger of oncogenesis in HCC.[17] It was proposed based upon experimentation on transgenic mice that HCV core protein is also incriminated in down regulation of SOCS-1 (suppressor of cytokine signaling) gene which has a potential tumor suppressor activity. HCV core transgenic mice differentially showed silencing of SOCS-1 gene and developed HCC in later life.[18]

CURRENT TREATMENT OPTIONS

The current standard treatment regimen used for Hepatitis C comprises of pegylated Interferon alpha (peg IFN-α) along with potent anti-viral Ribavirin. However, the success of the combination therapy has been plagued by the fact that it can not elicit long term response in all the patients and response is characteristically poor in patients seropositive for genotype 1. The efficacy of anti-virals in hepatitis C infection has conventionally been gauged by the presence/absence of sustained virologic response, i.e., persistence of viral clearance 24 weeks after therapy. Studies combining peg IFN-α 2b with Ribavarin showed a sustained virologic response of 54% irrespective of the genotype of HCV. For HCV genotype 1 infected patients , the sustained virologic response achieved with the combination therapy was a mere 40%.[19,20] Though this response rate is not very discouraging, highly morbid side effects of the combination therapy can not be ignored.

Multiple studies had earlier shown the association of psychiatric morbidities, primarily depression, with long term Interferon therapy. Now a role of Ribavirin has also been implicated.[21] Ever since the beginning of its therapeutic application in HCV treatment, Ribavarin has been shown to induce hematological dyscrasias most noteworthy being hemolytic anemia.[22] Although the anemia can be reversed by dose reduction of Ribavarin, it seems logical to believe that it must have a negative impact on the sustained anti-viral response. Another significant side effect remains IFN-α induced Thyroid Dysfunction usually secondary to development of anti-thyroid auto anti-bodies. Females and patients infected with multiple genotypes of the virus are found to be more susceptible. Thyroid dysfunction is however mostly transient and not an indication for discontinuation of the treatment. A periodic screening and treatment on clinical manifestation of hypothyroidism or hyperthyroidism (which is less common) is recommended.[23]

Furthermore, the high cost of the therapy continues to hurdle its common clinical usage. Armstrong *et al*

from USA reported that median Hepatitis C related health care expenditure of patients receiving Interferon therapy exceeds US Dollars 2470.[24]

POTENTIAL THERAPIES AGAINST HCV

The presumed HCV life cycle includes (1) binding to the cell surface receptors and internalization into the host (2) cytoplasmic release and uncoating of the viral genome (3) IRES mediated translation of the Open Reading Frame into a single large polyprotein (4) polyprotein processing by cellular and viral proteases (5) RNA replication (6) packaging and assembly (7) Virion maturation and (8) Release from the host cell. In principle each of these steps represents a target for antiviral intervention.[25]

A potential drug target against HCV is the enzyme RNA helicase located on the HCV non-structural protein NS3. The protein NS3 has a dual functional characterization with a Helicase domain at its carboxy terminal and a Protease domain at its amino end. RNA aptamers have been developed which have shown efficient inhibition of both the domains and resultant reduction in HCV replication in vitro.[26] Neutralization of Helicase by human recombinant antibodies has also been attempted. In 2003, it was established that Human Recombinant antibodies have an efficient inhibitory action on HCV helicase. A novel strategy of "Intracellular immunization" was crafted using them. Genome encoding an anti-helicase Human Recombinant Anti-body was introduced into HCV infected cells through expression vectors and it was proven that the intracellular expression of the anti-body resulted in significant shutting down of HCV negative strand synthesis.[27] Apart from gene therapeutics, more conventional small molecule inhibitors of HCV helicase have been developed and have showed promising site-specific inhibition of the enzyme.[28] Their overall impact on reduction of viral load and the sustainability of the response need a further exploration.

The viral serine protease remains one of the best studied drug targets for the development of novel therapeutic agents. This enzyme has been extensively characterized at biochemical and structural levels and has resulted in the discovery of several classes of compounds with potential antiviral activity.[29] A few years ago, macrycylcic beta-strand scaffolds were designed which evolved into first orally available site-specific inhibitors of HCV NS3/4A serine protease. Their further optimization led to the discovery of BLIN-2061, a highly effective HCV protease inhibitor, oral administration of which results in considerable reduction of viral load within 2 days of starting the drug irrespective of the clinical staging of the chronic infection with Hepatitis C genotype 1.[30] The results have not been as encouraging for HCV genotype 2 and 3 but this has

been attributed to decreased binding efficiency of the drug to Genotype 2 and 3 proteases. A modification in the formulation of BLIN-2061 can possibly combat this problem.[31] A complete safety profile, interaction dynamics and sustained anti-viral response of BLIN-2061 are yet to be explored into but its prospects as a potential future therapy for HCV remain very bright.

Vertex pharmaceuticals have come up with a promising small molecule inhibitor of HCV NS3/4A protease; VX-950. VX-950 achieved a remarkable decrease in HCV RNAs down to undetectable levels in patients chronically infected with Hepatitis C at the end of its 14 day phase 1b trail. The studies have been conducted in patients infected with HCV genotype 1 for which the standard treatment regimen (peg IFN-a + Ribavarin) has proven to be less effective which makes the results all the more significant. In the studies conducted so far, no major hazardous side effects have been reported and the most common noteworthy complaint has been of headache.[32]

Although resistance against both BLIN-2061 and VX-950 has been reported owing to mutations in the HCV protease, encouragingly different mechanisms of resistance have been thought to exist in case of each drug and cross-resistance does not exist in most of the cases i.e., the viral cluster in which the mutation confers resistance to BLIN-2061 remains susceptible to VX-950 and vice versa.[33]

Some drugs which are primarily non-viral and effect different human body systems have also been shown to hold anti-viral activity against HCV. Cyclosporin, the widely used immunosuppressant was shown to inhibit HCV replication in replicon cell-culture studies as well as HCV infected human hepatocyte cell lines. Clinical trials of Cyclosporin A combined with IFN-α showed better sustained anti-viral response than Interferon monotherapy especially in patients infected with HCV genotype 1 and those with high viral loads.[34] This anti-viral property is unique for Cyclosporin and is not shown by other immunosuppressive drugs. A further characterization of the anti-HCV activity of Cyclosporin could not only serve a treatment option for HCV but also might establish a new molecular target for drug and vaccine development.[34]

Moreover based on the concept that quantitatively and qualitatively insufficient CD4+ and CD8+ T cell response may contribute to viral persistence, immunotherapeutic strategies, aimed at enhancing the cellular immune response against HCV are currently being investigated. A possible role of IFN-γ in clearing up the infection has been put worth based on the observation that IFN-γ was detected from liver of the chimpanzees which effectively suppressed the infection.[35]

Traditionally HCV has been difficult to study because of the lack of an efficient *in vitro* replication system. A milestone was recently achieved when Heller *et al* developed a robust in vitro model of HCV virion production. They were able to translate the ORF and express all the viral proteins which could be detected by immunofloresence and western blot. Detection of both the positive and negative RNA strands also established that HCV is efficiently replicated within the model system. This system supports the production and secretion of high level of HCV virions and extends the gamut of tools available for the study of HCV biology.[36]

VACCINATION STRATEGIES

The development of a vaccine against HCV remains a failure owing to unique characteristics of the virus. It is attributed to the high replication rate of the virus and the error-prone polymerase. The extent of HCV replication provides ample opportunity for the introduction of mutations into the viral population within an infected individual. Viral production has been estimated at 10^{12} (one trillion) new HCV virions per day.[37] The mutation rate of HCV has been around 0.001substitutions per genomic site per year, based on studies of chronically infected patients. Considering the 9.6 kb size of the genome such high mutation rate would culminate into 8-18 mutations in the genomic RNA per year. Also, it has been determined that E2 with its hypervariable region HVR1 is the highly mutated site. The hypervariablity in the envelope proteins results in immuneescape of the virus from the neutralizing antibodies and hence explains the persistent viremia. Besides for E2 an increased variability has been mainly detected in p7, the genome fragment extending from approximately the middle of NS3 to NS4B, and the segment corresponding to the C-terminus of the NS5A protein.[38] Such high variability in different parts of the genome hurdles the development of genomic vaccines.

Encouragingly, however, a number of conserved regions have been proven to exist and play vital roles in the viral replication cycle. On either side of the 9.6 kb Open Reading Frame (ORF), flanking non translated regions (NTRs) exist. 5' NTR is long and highly conserved among different quasispecies. A part of it is believed to function as Internal Ribosomal Entry Site (IRES) and considered indispensable for translation of ORF. The high conservativity of IRES and its significance for the initiation of translation makes it an ideal target for interference by small nuclear RNAs A further exploration showed that out of the 4 domains of IRES, it is the stem loop III which is the most suitable therapeutic target as its RNA interference strongly inhibits IRES-mediated initiation of

translation.[39] Similarly, the core protein is implicated to be one of the most conserved regions of HCV genome. The initial attempts to use DNA vaccines containing HCV core protein failed to induce a successful cellular and humoral response in mice models. Recent results, however, have been very encouraging. HCV core vaccine was shown to mount both humoral and cellular responses against the virus in primate studies.[40] Core Vaccines employing the core protein have demonstrated specific CD4+ T-cell response against the core protein in recent experiments.[41] Vaccination potential of core protein has also been tested through the novel strategy of Dendritic Cell vaccination.[42] The core protein hence should be one of the prime targets for intervention.

From an optimistic point of view, merely watchful observation might prove to be a successful 'strategy'. Since the HCV polymerase is error prone, a sound possibility exists that it may induce a mutation somewhere in the genome which would be lethal for the virus itself. That genomic region would thus come into sight as being indispensable for virus survival and could be further targeted for therapeutic intervention.

A cure for Hepatitis C can not be predicted at this point in time. However, the exploration of the pathogen down to the genomic levels which has been possible in the last few years is not only intellectually fascinating but also stands as a hope for millions of the diseased round the globe. It might be sanguine but certainly not imprudent to state that a cure for Hepatitis C is not only possible but is something which could be anticipated in the next few years.

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