Future directions in the treatment of patients with chronic hepatitis C virus infection

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Robert G Gish. Future directions in the treatment of patients with chronic hepatitis C virus infection. Can J Gastroenterol 1999;13(1):57-62. Hepatitis C virus (HCV) infects over 170 million people worldwide. While interferon is currently the most used single agent therapy, this drug may result in a sustained loss of virus from the blood in only up to 15% of patients; new options for treatment are needed. With the release of ribavirin in North America and Europe, a viral clearance rate or 'cure' may be attained in up to 40% of patients. Developing successful antiviral therapy that prevents or delays the development of cirrhosis, liver failure and liver cancer as well as decreasing the demand for liver transplantation are clearly identified goals. Unfortunately, there is no complete in vitro model of HCV replication or translation. Due to the lack of an an in vitro or cell culture model of HCV infection, in vitro translation screening systems to identify in vitro inhibited of HCV protein translation are being evaluated by a large number of biotechnology companies. With advancing computer technology, high throughput screening processes are now possible and can be joined to specific in vitro model testing systems. Along with examining some of the information known about HCV therapy and the HCV genome, the present review discusses potential targets for new therapies and identifies therapeutic agents that are nearing clinical application.

Key Words: Antiviral therapy, Hepatitis C, Hepatitis C virus helicase, Hepatitis C virus proteases, Hepatitis C virus RNA polymerase, Interferon

INTERFERON

Interferon therapy forms the basis for the management of acute and chronic hepatitis C virus (HCV) infection. The use of interferon has been reviewed in detail in several reports (1-3). Major advances for the treatment of HCV infection include higher dose interferon therapy, daily therapy and combination therapy. The three interferons that are presently approved in the United States are interferon alfa-2b (Intron A, Schering Plough, New Jersey), interferon alfa-2a (Roferon-A, Hoffman La Roche Laboratories, Basle, Switzerland) and consensus interferon (r-metIFN-Con, Angen, California). These interferons as well as interferon alpha-1b (Wellferon, Burroughs Wellcome, North Carolina) are approved in Canada and appear to be clinically equivalent.

Emerging data about HCV viral kinetics indicate that the viral replication half-time is approximately 3 h, leading to studies that use dosing intervals of 24 h or less (4). Therapy with pegylated interferon, a new long acting form of inter-
nucleoside analogue with a structure similar to that of aza-
ceuticals, California and Rebetol, Schering Plough), a
seraHCV RNA levels (22,23,25-31).
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hibition of 5\textsuperscript{-}12).
other TheraPeuTic Cytokines
Thymus-derived products: Crude thymus extracts (Thymic
Fractions 1402 A and Immunoplex 402A, Biotherapeutics,
Wisconsin), thymosin fraction 5 and thymosin alpha-1 (Za-
daxin, SciClone, California) are cytokines derived from im-
umonologically active proteins found in the thymus gland.
Notably, these thymus-derived proteins appear to modulate
the progression and stabilization of chronic viral hepatitis
(5-12).
Thymosin alpha-1 is a naturally occurring immune-stimu-
ulating protein reportedly present in decreased amounts in
the serum of patients chronically infected with hepatitis B
virus (HBV) (13). Results of initial studies in animals and
humans infected with HBV suggested that thymosin alpha-1
and thymosin factor 5 increased the rate of clearance of HBV
DNA and the subsequent seroconversion from positive to
negative for hepatitis B surface antigen (13-17). Results of
a subsequent controlled trial did not demonstrate any statisti-
cally significant difference between thymosin alpha-1 and
placebo at short term follow-up (18). Thymosin alpha-1 has
been approved in Singapore, China and Italy for the treat-
ment of patients with hepatitis B infection.
Results of a recently completed Italian study combining
thymosin alpha-1 with interferon indicated that approxi-
ately 60% of patients lost HBV DNA and HBV e antigen
(17). Trials combining these agents are currently underway
in patients with chronic HCV infection to determine
whether this combination of immune stimulation is more ef-
facious than interferon alone.

New Antiviral Therapy
Ribavirin: Ribavirin (1-beta-D-ribofuranosyl-1,2,4-tria-
zeole-3-carboxamide) (Virazole, Viratек, ICN Pharma-
caceuticals, California and Rebetol, Schering Plough), a
nucleoside analogue with a structure similar to that of aza-
thioprine, is effective in the treatment of respiratory syn-
cytial virus and Hanta virus infections. The mechanism of
action of ribavirin against HCV has not been defined. Post-
tulated antiviral effects based on events in other virus
models include depletion of intracellular phosphate pools,
inhibition of 5' cap structure of viral mRNA, inhibition of
viral-dependent RNA polymerases, and the possible im-
munomodulation effects of preserving T helper-1 and re-
ducing T helper-2 effects (19,20). Recent studies evalu-
ated the use of ribavirin as a single agent for the treatment
of chronic HCV infection (21-26). Among these studies,
decreases in inflammation in the liver as well as a decrease
in serum alanine aminotransferase levels were reported in
a portion of patients. However, most patients on single
agent therapy did not have a measurable decrease in their
serum HCV RNA levels (22,23,25-31).

Because ribavirin therapy may modulate the immune sys-
tem and act as an immune stimulant, there is a theoretical
advantage to using this medication in combination with in-
terferon and other cytokines (19,20). When ribavirin and
interferon alfa-2b were used together in one study, a 40%
sustained response, as defined by long term clearance of
HCV RNA, was reported, although the number of patients
studied was small (32). Other studies using larger patient
populations and including control groups in some trials have
shown a significant level of efficacy (as judged by the sus-
tained response rates that ranged from 30% to 70%) in clinici-
diverse patient populations (32-37). An extrapolation of the
data in the available literature implies that the use of
interferon alfa-2b with ribavirin (Rebetron, Schering
Plough) appears to double or quadruple the sustained re-
pose rate obtained compared with that for interferon ther-
aphy alone. This result was evident in both patients who had
never been treated with interferon as well as for patients who
had relapsed after stopping an initial interferon treatment
regimen (38,39). Moreover, separate data also indicate that
interferon therapy with ribavirin may be promising (24%
sustained response rate) for patients with recurrent HCV
disease after liver transplantation (40).

Targeting Functional Sites

IN The HCV Genome

The HCV genome has been studied extensively over the
past nine years (41). The data acquired have allowed the
identification of various HCV protein products involved in
viral replication, translation and packaging. Each active ge-
nomic site and its translation product is a potential target for
antiviral therapy (Figure 1). The first step in the develop-
ment of therapeutic agents is to identify antiviral com-
pounds that can be taken orally so that they are delivered in
an active state to hepatocytes, with the goal of remaining
nontoxic while inhibiting viral replication. The use of one
or more compounds now appears to be the likely means
through which more effective therapy can be developed.

Ultimately, these newly identified single agents may not
result in viral clearance or sustained response, that is, 'cure',
when used alone. If used with cytokines such as interferon,
these designed drugs may dramatically decrease the level of
viral replication, viral mutation and genetic diversity,
therby, resulting in a much greater 'cure' rate. The lessons
learned from the treatment of human immunodeficiency vi-
rus (HIV) infection with combination therapy will probably
be applicable to HCV therapy. It is likely that clinicians will
be using a combination of antiviral therapies to treat the es-
imated five to six million patients in Canada and the
United States, as well as the 170 million HCV-infected per-
sons worldwide. However, an important difference between
HIV and HCV infection is that interferon is believed to re-
sult in complete HCV eradication in a subset of individuals.

The HCV RNA-dependent RNA polymerase is respon-
sible for the replication of the entire HCV genome (41). Ide-
ally, drugs targeting this enzyme need to be nucleotide or
nucleoside analogues that act at the enzyme's active site. To
HCV helicase enzyme (Figure 2) is derived from the core-envelope 1 (E1) to NS2-3. Zinc metalloproteinase acts at the NS2 site. Drugs may be developed to inhibit the multiple HCV serine proteases acting along segments NS3 to NS5b. At NS3, the potential targets are the RNA binding site and helicase binding site. Stabilizing proteasome and proteasome cofactors are potential targets in the NS4a regions. The proteasome sensitivity-determining region described by Japanese research (56) but not confirmed elsewhere is within NS5a. This region may be involved in interferon resistance. For the NS5b region, RNA-dependent RNA polymerase inhibitors may provide another method of inhibiting viral replication.

Although HCV is a single (+) stranded virus, specific areas of the viral genome interact with each other to form a complex structure that requires denaturation for genetic replication and protein translation to take place (41). The HCV helicase enzyme (Figure 2) is derived from the carboxyl end of the NS3 region and is involved in the ‘unwinding’ or unfolding of the HCV RNA quaternary structure (40,42-48). The structure and active functional areas of the helicase enzyme have been described by researchers and are now the areas of intense study aimed at drug development.

The structure, function and products of the HCV proteases have been defined by a number of investigators (41,50-54). Accordingly, proteolytic processing begins with the host-derived proteases cleaving the HCV proteins between the core-envelope 1 (E1) regions, the E-1 and E-2 regions, and the E-2 and NS2 segments, with added autoproteolytic cleavage occurring between the NS2 and NS3 sequences. The subsequently produced NS3 protein is then complexed with protein product NS4a, and together they cleave at the NS3-4a, NS4a-4b, NS4b-5a and NS5a-5b junctions.

Because directed proteolytic processing is crucial for HCV production and maturation, zinc metalloproteinase and serine protease enzymes have been implicated among the most likely targets for immediate anti-HCV drug development. However, because multiple proteins in the coagulation pathway are serine proteases, great care is needed during drug design and development to ensure that drugs designed to inhibit HCV proteases do not induce hypercoagulable states. In addition, leukocyte elastase is another natural protease that may interact with HCV protease inhibitors, possibly obviating drug function and/or interfering with normal leukocyte activity. New methods for screening potential protease inhibitors are also being developed (55), and it is important to note that experience with the development of protease inhibitors has resulted in the availability of HIV protease inhibitors (aspartate protease), trypsin inhibitors, thrombin inhibitors and angiotensin-converting enzyme inhibitors.

The internal ribosomal entry site (IRES), located at the 5′ noncoding region of the HCV genome, is another potential site suitable for antiviral drug development. HCV translation involves the production of a single contiguous polyprotein product (Figure 1). The IRES region directs the polyprotein production, which is cap-independent, and requires complete ribosomal assembly. The blockage or inhibition of IRES-mediated translation would stop the production of all viral proteins because binding of the 40S ribosomal subunit is essential for the translation of the viral genome.

The interferon sensitivity-determining region is a postulated area in the NS5a region of the HCV genome that may ‘program’ interferon resistance when mutations occur (Figure 1) (56). These mutations appear to lead to the pro-
duction of a protein product that represses the action of the protein kinase RNA-activated (PKR) gene product. The proper function of this PKR protein is a key aspect of the therapeutic intracellular events initiated by interferon (57-60). Thus, agents that block the repression of the PKR protein appear to be potential candidates as new therapeutic agents to be used alone or with interferon, yet further study is warranted.

Despite the promise of these proteinase, helicase and RNA-dependent RNA polymerase inhibitors, development of these agents is slowed by the lack of a suitable cell culture or animal model. In addition, drug selection with high throughput methodologies is necessary and will undoubtedly be enhanced after one or two model inhibitors are developed. The large open cleft in the serine proteinase molecule also makes drug design difficult due to the poor binding characteristics of the active site to new medications. Can available drugs be used, or must a new class of medications be designed? Given the various genomic loci available as potential drug targets, current advances in biotechnology are pointing towards new ways to stop HCV replication. The following is a discussion of the most biologically plausible approaches designed to block viral replication.

**NEW METHODS OF INTERFERING WITH VIRAL REPLICATION**

**Antisense oligonucleotides:** Antisense molecules are targeted to a complementary sequence of viral RNA. The binding of an antisense RNA molecule blocks translation by hybrid arrest of the translational machinery or by the induction of ribonuclease (RNAase) activity that results in the cleavage of the double-stranded RNA portion of the hybrid. The attachment of these complementary RNA sequences blocks the binding of ribosomes to the viral RNA, inhibiting subsequent assembly of amino acids into viral proteins. These oligonucleotide sequences may be a powerful tool to stop viral replication without the use of potentially toxic nucleoside analogues used to block transcription of the HCV genome. An in vitro model of HCV translation has shown the ability of antisense oligonucleotides to inhibit HCV translation (61,62). Wakita et al (62) quantified the level of inhibition by using nucleotides complementary to a segment of the HCV genotype 1b genome that were attached to the firefly luciferase reporter gene. Drug delivery and the nonspecific nature of antisense strategies will limit the application of this technology for the near future.

**Ribozymes and nonfunctioning ribozymes:** Ribozymes are catalytic RNA molecules that serve as enzymes able to break RNA molecules at specific sites (63-65). Ribozymes designed for specific sites in the HCV genome may lead to decreased viral protein production. Alternatively, false or nonfunctional ribozymes can be delivered or synthesized in hepatocytes. The presence of these nonfunctioning ribozymes results in the inhibition of the protein synthesis required for viral replication, packaging and release.

One ribozyme system named the ‘hammerhead’ system contains flanking nucleotides in the head region that attach to key sites in the HBV RNA replication intermediate and allow specific cleavage. A recent study using these hammerhead ribozymes directed at the 5’ noncoding region of HCV demonstrated direct inhibition of viral RNA translation (66). Further development in this area will require improved delivery techniques in humans to allow entry of these molecules into intact hepatocytes. However, drug delivery of any protein through other than parenteral routes remains a major clinical challenge.

**Vaccines:** Vaccine therapy for hepatitis viruses may be directed at stimulating a T cell response rather than at B cell antibody production as is used for most viral vaccines because antibodies appear not to be protective and are not able to prevent chronic infection. Chimpanzee models have not shown that vaccines can protect animals from subsequent inoculations of HCV virus of the same or similar genotypes (67). Yet, the need for effective vaccines is clear given the high infection rates among populations at risk of disease. Thus, a phase I clinical trial of an HCV vaccine has been completed in the United States (personal communication); the results of the trial and the initiation of a phase II trial are pending.

**DNA-based vaccines:** DNA-based vaccines are an innovative technique in the treatment of chronic viral infections (68,69). An example of a model HBV DNA-based vaccine includes a plasmid containing a designed DNA gene fragment attached to a cytomegalovirus promoter intended for injection into muscle tissue. Once in the muscle, transcription of the DNA fragment takes place and results in the translation of a specified viral protein. The viral protein is taken into antigen-presenting cells and is expressed, with class-I molecules, to lymphocytes, resulting in a cytotoxic T cell response. Class-II expression with viral protein also results in a B cell response, thereby producing antibody directed at the viral protein. To date, pre-S-1 and pre-S-2 sequences from HBV have been used in an in vivo model, with results suggesting an enhanced immune response directed at HBV. This technology may be applicable to the treatment of chronic or acute HCV infection as well.

**Dominant negative viral mutants:** Genetic suppresser elements are cDNA sequences that encode antisense RNA and may be applied to the treatment of hepatitis infections by targeting key genetic sequences essential for viral replication or packaging (70). Preliminary studies of retroviruses in vitro systems have been promising (71,72). Specially created viral mutants may be designed to interfere with the assembly of the HBV nucleocapsid, causing the termination of viral replication. Theoretically, the carboxyl terminus of a truncated core protein (required for DNA binding) could be fused with the surface (S)-gene of HBV and mixed with wild type virus. Treatment could be initiated by using adenovirus-mediated transfection of this construct. The resulting transfected material would putatively block further transcription by binding to the targeted gene sequence. The HCV genome is a likely target of such therapy.

**Immune globulin:** There is evidence that up to 15% of individuals who become infected with HCV clear the virus through internal immune processes. HCV has the ability to
undergo rapid genetic changes that aid HCV survival by allowing an ongoing escape from both B (immune globulin production) and T cell immune surveillance, and recognition. HCV antibodies have been suggested to form a passive production and to minimize viral replication. Within five to 10 years, a cure is conceivable in 50% to 80% of individuals infected with HCV, and vaccines may prevent or abrogate most infections.

CONCLUSIONS

The treatment of HCV infection is rapidly evolving. The addition of knowledge about the genetic structure and function of the HCV genome is allowing researchers to explore new possible targets for therapy. Moreover, rapidly advancing technologies are yielding more effective ways to develop new drugs and to minimize viral replication. Within five to 10 years, a cure is conceivable in 50% to 80% of individuals infected with HCV, and vaccines may prevent or abrogate most infections.

ACKNOWLEDGEMENTS: The authors thank Dr Nanhuu Yao at Schering Plough Research Institute, Kenilworth, New Jersey, for the use of the three-dimensional image of the hepatitis C virus helicase enzyme structure. Schering Plough provided an unrestricted educational grant for the Conference on Hepatitis C Virus Therapies as part of the Canadian Digestive Disease Week, February 1998 from which this article was derived.

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