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 animal or cell culture model of HCV infection, *in vitro* translation screening systems to identify inhibitors 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 proteinases, Hepatitis C virus RNA polymerase, Interferon

Tendances futures du traitement des patients atteints d'une infection chronique au virus de l'hépatite C

RÉSUMÉ : Le virus de l'hépatite C (HCV) aurait déjà contaminé plus de 170 millions de personnes dans le monde. Si l'interféron est à l'heure actuelle la monothérapie la plus utilisée, ce médicament pourrait n'entraîner l'élimination du virus que chez 15 % des patients. Il nous faut sans contredit de nouvelles options thérapeutiques. Avec le lancement de la ribavirine en Amérique du Nord et en Europe, on peut noter un taux d'élimination virale ou de guérison chez jusqu'à 40 % des patients. La mise au point d'un traitement antiviral efficace qui prévient ou retarde le développement de la cirrhose, de l'insuffisance hépatique et du cancer du foie, tout en diminuant les demandes de transplantation hépatique sont les objectifs clairement identifiés. Malheureusement, il n'existe aucun modèle *in vitro* complet de la réplication ou de la translation du HCV. Étant donné l'absence de modèles d'infection au HCV chez l'animal ou par la mise en culture de cellules, beaucoup de compagnies de biotechnologie sont à évaluer des systèmes de dépistage de translation *in vitro* afin d'identifier les inhibiteurs de la translation de la protéine du HCV. Grâce aux progrès des technologies informatisées, des procédés de dépistage de fort volume sont maintenant possibles et peuvent être adjoints à des systèmes de modèles *in vitro* de dépistage. La présente synthèse se penche sur les renseignements acquis sur le traitement du HCV et sur son génome, mentionne les cibles potentielles des nouvelles thérapeutiques et identifie les agents qui sont sur le point d'être appliqués en clinique.

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-Con₁, Amgen, California). These interferons as well as interferon alpha-1n (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-

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feron, allows interferon to persist in the circulation and to affect continuously the HCV virus for three to five days. Preliminary data suggest that this pegylated interferon produces an equal or better response with fewer side effects at a once or twice weekly dosing.

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 (Zadaxin, SciClone, California) are cytokines derived from immunologically 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-stimulating 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 statistically 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 treatment of patients with hepatitis B infection.

Results of a recently completed Italian study combining thymosin alpha-1 with interferon indicated that approximately 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 efficacious than interferon alone.

NEW ANTIVIRAL THERAPY

Ribavirin: Ribavirin (1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) (Virazole, Viratek, ICN Pharmaceuticals, California and Rebetol, Schering Plough), a nucleoside analogue with a structure similar to that of azathioprine, is effective in the treatment of respiratory syncytial virus and Hanta virus infections. The mechanism of action of ribavirin against HCV has not been defined. Postulated 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 immunomodulation effects of preserving T helper-1 and reducing T helper-2 effects (19,20). Recent studies evaluated 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 system and act as an immune stimulant, there is a theoretical advantage to using this medication in combination with interferon 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 sustained response rates that ranged from 30% to 70%) in clinically 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 response rate obtained compared with that for interferon therapy 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 genomic site and its translation product is a potential target for antiviral therapy (Figure 1). The first step in the development of therapeutic agents is to identify antiviral compounds 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, thereby, resulting in a much greater 'cure' rate. The lessons learned from the treatment of human immunodeficiency virus (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 estimated five to six million patients in Canada and the United States, as well as the 170 million HCV-infected persons worldwide. However, an important difference between HIV and HCV infection is that interferon is believed to result in complete HCV eradication in a subset of individuals.

The HCV RNA-dependent RNA polymerase is responsible for the replication of the entire HCV genome (41). Ideally, drugs targeting this enzyme need to be nucleotide or nucleoside analogues that act at the enzyme's active site. To

Figure 1) Hepatitis C genome. Hepatitis C genomic sites and their translation products are potential targets (proteinase cleavage sites) for anti-hepatitis C virus drug therapy (lightening bolts). The internal ribosomal entry site at the 5' noncoding region stimulates polyprotein translation and is a good target because of its three-dimensional structure because it is highly conserved across all genotypes. At the capsid area, increased inhibition of tumour necrosis factor dimer stops apoptosis. Host-derived proteinases start hepatitis C virus (HCV) protein cleavage from core-envelope 1 (E1) to NS2-3. Zinc metalloproteinase acts at the NS2 site. Drugs may be developed to inhibit the multiple HCV serine proteinases acting along segments NS3 to NS5b. At NS3, the potential targets are the RNA binding site and helicase binding site. Stabilizing proteinases and proteinase cofactors are potential targets in the NS4a regions. The proposed interferon 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

this end, x-ray crystallographic data have identified the three-dimensional structure of the RNA polymerase, allowing drug design to move forward (42). By blocking the replication of the complete viral genome, mutation rates may also be decreased, and the 'escape' of the mutant virus from immune surveillance may occur less often. In addition, the production of proteinases that are required for the generation of smaller viral protein products will be decreased, resulting in a fall in the production of complete viral particles.

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 intensive study aimed at drug development.

The structure, function and products of the HCV proteinases have been defined by a number of investigators (41,50-54). Accordingly, proteolytic processing begins with the host-derived proteinases cleaving the HCV proteins between the core-envelope 1 (E-1) regions, the E-1 and E-2 region, 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

Figure 2) The three-dimensional structure of hepatitis C virus helicase with unfolding of an RNA molecule

and serine proteinase 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 proteinases, great care is needed during drug design and development to ensure that drugs designed to inhibit HCV proteinases do not induce hyper- and hypocoagulable states. In addition, leukocyte elastase is another natural proteinase that may interact with HCV proteinase inhibitors, possibly obviating drug function and/or interfering with normal leukocyte activity. New methods for screening potential proteinase inhibitors are also being developed (55), and it is important to note that experience with the development of proteinase inhibitors has resulted in the availability of HIV proteinase inhibitors (aspartate proteinase), 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 potentially therapeutic sites, 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 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 or active immune response, but, most commonly, they appear to be a paraphenomenon indicating active infection in most instances or occasional passive exposure to antibodies in other cases (67,73-78). Supporting the concept that immune globulins modulate HCV infection, recent information suggests that there is an increase in the transmission of HCV when immune globulin preparations are administered after screening for the presence of HCV antibody (78). Immune globulin therapy is not recommended as part of postexposure prophylaxis. The development of monoclonal antibodies directed at HCV may be useful as part of combination antiviral therapy or may be useful in acute exposure, but conserved viral proteins across genotypes and quasispecies must be identified. Alternatively, a mixture of monoclonal antibodies may be useful to modulate disease and to act as an adjunct to direct antiviral therapy.

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.

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