Development of novel anti-HCV therapies : HCV protease, helicase, and polymerase as therapeutic targets

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Keywords : HCV, anti-HCV, IFN, ribavirin, protease, helicase, polymerase.

"If I have seen further it is by standing on the shoulders of giants".

Sir Issac Newton 1642-1727 B.C.
In *Correspondence* Vol 1 :
Letter to Robert Hooke, 1676

With the discovery of HCV by Dr. Choo and his colleagues (1), the development of assays by Dr. Kuo and his colleagues (2), a large body of knowledge on the natural history, clinical features, disease pathogenesis, and virology has been generated. This rapid advancement was achieved through the hard work of the many clinicians and scientists. Therapies have already been developed which help a proportion of patients infected with this viral infection. It is the knowledge generated by the scientific community that forms the basis for the development of future therapeutic strategies.

Up to May 1998, interferon-a was the only approved therapy for treating patients with chronic HCV infection. Unfortunately, only a small proportion of patients (<10%) responded virologically to interferon-α monotherapy in a complete and sustained fashion (3). In June and December 1998, interferon-α-2b/ribavirin combination therapy (Rebetron™, Schering-Plough, Kenilworth, NJ) was approved by the Food and Drug Administration of the United States for the treatment of patients with chronic HCV infection who relapsed after previous interferon-a therapy and for the treatment of naive patients, respectively (4,5). This combination therapy was also approved by the European Union Health Authority in February, 1999. This treatment regimen improves the complete and sustained virological response rate to 49% for patients who relapsed after a previous course of interferon-α therapy, and 41% for treatment of naive patients (6)

Despite the improved efficacy of the combination therapy, 50-60% of the patients failed to respond in a sustained fashion. The current combination therapy also requires a treatment period of 6-12 months. Hence, newer and better anti-HCV therapies are still needed. Knowledge on the function of the viral genome and its encoded viral proteins is essential in determining the validity of these targets for anti-HCV drug discovery and development (Table I). A general strategy is outlined to evaluate these targets as follows : (i) generate and characterize recombinant HCV proteins ; (ii) develop *in vitro* screening for anti-HCV compounds ; and (iii) optimize anti-HCV drugs through structure-activity relationship studies. A few criteria on the development of direct antivirals against HCV protease, helicase, and polymerase are given below : (1) essentiality of the viral target for viral replication (e.g. protease) ; (2) viral specificity ; (3) validation as an antiviral target in the history of drug development ; (4) extensive biochemical and biophysical characterization which aids rational drug design.

HCV Protease : The serine protease activity encoded by the amino (N)-terminal 181 amino acids of NS3 is responsible for proteolytic processing of four downstream sites in the viral polyprotein, (NS3/NS4A, NS4A/4B, NS4B/SA, and NSSA/SB) (7-9). The enzymatic activity of HCV NS3 protease is well characterized. The NS3 protein contains three highly conserved residues, His^{57}, Asp^{81}, and Ser^{139}, which represent the catalytic triad of the serine proteinase family. Substitutions of any of these residues abolish proteolytic processing. The 54 amino acid NS4A protein functions as cofactor for NS3 protease activity. NS4A is essential for cleavages at the NS3/4A, NS4A/4B, and NS4B/5A junctions and enhances cleavage at the NS5A/SB site. Mutagenesis experiments have shown that the N-terminal region of NS3 between amino acids 15-22 interacts with amino acids 21-34 of NS4A. The X-ray crystal structure of the HCV NS3 serine protease domain revealed that it is structurally similar to previously characterized proteases (10). The molecular fold consists of two ß-barrels and resembles that of the chymotrypsin-like serine proteases. The catalytic residues, His^{57}, Asp^{81}, and Ser^{139} participate in a hydrogen-bond network which is a signature of serine proteases. The catalytic triad spans the ß-barrels, as does the proposed substrate binding site. There is a structural zinc atom in the enzyme

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Presented at the International Symposium on Viral Hepatitis beyond the Millennium Session of December 10, 1999.

Table I. — **Potential Antiviral Targets Present in the HCV Genome**

Target	Biological Role	Other Properties	Enzymatic or Functional Activity	*In vitro* assay	Interest level In Antiviral Development
5' UTR	translational control	unique RNA structure	IRES	Yes	Moderate-High
Core (C)	capsid protein	membrane association	Homotypic Multimerization	Yes	Moderate
E1	envelope protein	membrane association	homo- and heterotypic interactions	No	Low
E2	envelope protein	membrane association	homo- and heterotypic interactions	No	Low
p7	Unknown	unknown	Unknown	No	None
NS2/3	Metalloprotease	unknown	NS2/3 cleavage	Yes	Low
NS3	protease	structure known	releases NS proteins	Yes	High
NS3	helicase	structure known	unwinds viral nucleic acid	Yes	High
NS4A	cofactor for NS3 protease	membrane association	essential for cleavage at 4A/4B and 4B/5A sites	Yes	Moderate/ Low
NS4B	unknown	membrane	unknown association	No	None
NS5A	Viral replication ?	confers resistance to interferon ?	unknown	Yes (for PKR interaction)	Low
NS5B	polymerase	membrane association	replicates RNA	Yes	High
3' UTR	regulates replication	Unique RNA structure		No	Further information needed

complex. The substrate binding site is a shallow, hydrophobic cleft rather than the deeper pocket lined with some charged residues that characterizes elastase.

Our understanding of the biochemical behavior and crystal structure shed light on anti-HCV protease drug design. The substrate binding site, the zinc atom site, and the interaction between NS3 and NSS4A are possible targets for anti-HCV protease drug development. However, the shallow substrate binding site suggests that targeting this pocket is inherently difficult. Inhibitors that eject the zinc atom are likely to be reactive and non-specific. The tight binding between NS3 and NS4A also cast doubt on the potential of developing inhibitors to interfere this interaction.

HCV Helicase : Viral replication, like other cellular processes such as DNA replication and mRNA transcription and translation, typically requires a separation of duplex nucleic acids. This reaction is mediated by a class of RNA/DNA unwinding enzymes called helicases, which use energy derived from coupled NTP hydrolysis. The exact mechanism of unwinding is not clear, but it is believed to involve recognition of an unpaired nucleic acid followed by a processive energy-dependent translocation of the helicase along the nucleic acid or a cooperative binding of the protein-nucleic acid complex. The biochemical characteristics of HCV NS3-4A helicase activity have been determined (11). The enzyme can utilize all four ribonucleotides (NTPs) as the energy source for nucleic acid unwinding. This hydrolysis is stimulated by the presence of nucleic acid polymers. The enzyme unwinds all homo- and hetero- duplexes of DNA and RNA, provided that a 3' overhang is present in one of the strands. The crystal structure of HCV helicase has been resolved (12). The structure reveals an HCV helicase divided into three domains. Our understanding of the crystal structure of this enzyme provides us an excellent opportunity for structure-based drug design.

NS5B RNA-dependent RNA Polymerase : HCV NSSB RdRp is the key enzyme that drives the HCV genome replication and thus is essential for viral growth. HCV replication can be divided into two steps, (1) synthesis of the (-)- stranded RNA replication intermediate from the 3' end of the input (+)-stranded RNA genome, and (2) synthesis of progeny (+)-stranded RNA genome from the (-)- stranded RNA replication intermediate. As HCV RdRp is likely to be the viral enzyme that drives both steps of replication, HCV RdRp is an attractive therapeutic target (13-15). It should be noted that viral polymerases have proved be good targets for drug discovery and development.

Biochemical characterization of this viral enzyme revealed that its polymerization activity requires an RNA template and primer. By analogy with other polymerases that lack a proofreading capability, the misincorporation rate of the NSSB is presumed to be high, which contributes to the genetic heterogeneous nature of HCV. As NSSB is a virus-specific enzyme with no functional homologs in the host, inhibitors may have

less toxicity/side effects. Sequence analysis has demonstrated that NSSB is highly conserved among all six clades of HCV. Thus, it is possible to develop anti- HCV polymerase inhibitors with broad-spectrum antiviral activities. This is further aided by the resolution of X-ray crystal structure of HCV RdRp (16). These features provide additional enthusiasm for developing inhibitors against this viral target.

The challenges to the development of HCV polymerase inhibitors are conventional. With nucleoside analogue- based inhibitors, attention to non-specific activity against host polymerases should be carefully evaluated.

References

1. CHOO Q.L., KUO G., WEINER A.J., OVERBY L.R., BRADLEY D.W., HOUGHTON M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*, 1989, **244** : 59-362.
2. KUO G., CHOO Q.L., ALTER H.J., GITNICK G.L., REDEKER A.G., PURCELL R.H., MIYAMURA T., DIENSTAG J.L., ALTER M.J., STEVENS C.E., TEGTMEIER G.E., BONINO F., COLOMBO M., LEE S., KUO C., BERGER K., SHUSTER J.R., OVERBY L.R., BRADLEY D.W., HOUGHTON M. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B-hepatitis. *Science*, 1989, **244** : 362-364.
3. DAVIS G.L., BALART L.A., SCHIFF E.R., LINDSAY K., BODENHEIMER H.C. JR, PERRILLO R.P., CAREY W., JACOBSON I.M., PAYNE J., DIENSTAG J.L. *et al.* Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. *N. Engl. J. Med.*, 1989, **321** : 1501-6.
4. MCHUTCHISON J.G., GORDON S.C., SCHIFF E.R., SHIFFMAN M.L., LEE W.M., RUSTGI V.K., GOODMAN Z.D., LING M.H., CORT S., ALBRECHT J.K. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N. Engl. J. Med.*, 1998, **339** : 1485-92.
5. POYNARD T., MARCELLIN P., LEE S.S., NIEDERAU C., MINUK G.S., IDEO G., BAIN V. HEATHCOTE J.,ZEUZEM S. TREPO C., ALBRECHT J. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet*, 1998, **352** : 1426-32.
6. DAVIS G.L., ESTEBAN-MUR R., RUSTGI V., HOEFS J., GORDON S.C., TREPO C., SHIFFMAN M.L., ZEUZEM S., CRAXI A., LING M.H. ALBRECHT J. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N. Engl. J. Med.*, 1998, **339** : 1493-9.
7. GRAKOUI A., MCCOURT D.W., WYCHOWSKI C., FEINSTONE S.M., RICÉ C.M. Characterization of the hepatitis C virus-encoded serine protease : determination of proteinase-dependent polyprotein cleavage. *J. Virol.*, 1993, **67** : 2832-2843.
8. BARTENSCHLAGER R., RHLBORN-LAAKE L., MOUS J., JACOBSON H. Kinetics and structural analysis of hepatitis C virus polyprotein processing. *J. Virol.*, 1994, 68 : 5045-5055.
9. SATOH S., TANJI Y., HIJIKATA M., KIMURA K., SHIMOTOHNO K. The N-terminal region of hepatitis C virus non-structural protein (NS3) is essential for stable complex formation with NS4A. *J. Virol.*, 1995, **69** : 4255-4260.
10. KIM J.L., MORGENSTERN K.A., LIN C., FOX T., DWYER M.D., LANDRO J.A., CHAMBERS S.P., MARKLAND W., LEPRE C.A., O'MALLEY E.T., HARBESON S.L., RICE C.M., MURCKO M.A., CARON P.R., THOMSON J.A. Cryystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide. *Cell*, 1996, **87** : 343-355.
11. TAI C.L., CHI W.K., CHEN D.S., HWANG L.H. The helicase acclivity associated with hepatitis C virus non-structural protein 3 (NS3). *J. Virol.*, 1996, **70** : 8477-8484.
12. YAO N., HESSON T., CABLE M., HONG Z., KWONG A.D., LE H.V., WEBER P.C. Structure of the hepatitis C virus RNA helicase domain. *Nat. Struct. Biol.*, 1997, **4** : 463-7.
13. BEHRENS S.E., TOMEI L., DE FRANCESCO R. Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus. *EMBO J.*, 1996, **15** : 12-22.
14. LOHMANN V., KORNER F., HERIAN U., BARTENSCHLAGER R. Biochemical properties of hepatitis C virus NS5B RNA- dependent RNA polymerase and identification of amino acid sequence motifs essential for enzymatic activity. *J. Virol.*, 1997, **71** : 8416-28.
15. FERRARI E., WRIGHT-MINOGUE J., FANG J.W., BAROUDY B.M., LAU J.Y., HONG Z. Characterization of soluble hepatitis C virus RNA-dependent RNA polymerase expressed in Escherichia coli. *J. Virol.*, 1999, **73** : 1649-54.
16. LEESBURG C.A., CABLE M.B., FERRARI E., HONG Z., MANNARION A.F., WEBER P.C. Crystal structure of the RNA- dependent RNA plymrease from hepatitis C virus reveals a fully encircled active site. *Nature Structural Biology*, 1999, **10** : 937-943.