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A review on an update of NS5B polymerase hepatitis C virus inhibitors

J. VARSHNEY, PK. SHARMA, A. SHARMA

Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Baghpat Bypass Crossing, Delhi-Haridwar Highway, NH-58, Meerut, UP (India)

Abstract. – *Background:* **Hepatitis C virus (HCV) infection is widespread, abhorrently under-diagnosed, and radically under-treated. Globally, infection with HCV is a major cause of acute hepatitis and chronic liver disease. Therefore, novel HCV inhibitors are required for the treatment of the HCV infected patients.**

Objective and Perspectives: **This review gives the detailed knowledge of upcoming therapy such as NS5B polymerase inhibitors that are urgently needed.**

Conclusion: **In the past decade, intensive hard work has focused on the discovery of both structural and nonstructural inhibitors of the HCV NS5B polymerase. These demanding efforts have resulted in various promising agents advancing in clinical development with emphasis on clinical efficacy and impact for future combination studies.**

Key Words:

Hepatitis, NS5B, NS3, Interferon.

Introduction

Chronic infection with hepatitis virus is an emerging global epidemic. Viral hepatitis, caused by any of the six hepatotropic viruses viz hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV) and hepatitis G virus (HGV), represents a major health problem worldwide. HCV is a single positive stranded RNA virus that belongs to *Flaviviridae* family. Its genome encodes for a polyprotein consisting of structural core and envelope proteins, as well as non-structural (NS) proteins[1]. Approximately 170 million people (3% of the world population) are chronically infected by hepatitis C virus (HCV), a major cause of acute hepatitis and chronic liver disease, as well as liver failure, is the most common cause for the need of liver transplantation[2]. About 80% of the acute infection will become chronic, leading to liver cirrhosis and hepatocellular carcinoma[3]. Existing therapies, based on combinations of pegylated interferon's and ribavirin, provide a sustained response in only a fraction of the treated patients and side effects can be quite brutal[4]. Since there is still no effective, well-tolerated treatment for HCV infection, alternative novel therapies are needed[5]. The last years have witnessed extensive efforts from the pharmaceutical industry, for the development of specific antiviral agents for the treatment of HCV infection that lead to improved efficacy, tolerability, and compliance in addressing the unnecessary medical need[6,7].

Genome Organization

Hepatitis C virus is a positive strand RNA virus and the genome consists of a large open reading frame of approximately 9600 base pairs or approximately 3010 to 3033 amino acids that encode several structural and non-structural proteins[8-12]. It is just about 9.5 kb in length and comprised of a long open reading frame, which is flanked by highly preserved untranslated regions at both the 5' and 3' ends. The 5' untranslated RNA Sequence (UTR) is comprised of 341 nucleotides and contain an internal ribosome entry site that recruits host ribosome to translate the viral genome while the 3' UTR is responsible for RNA replication and packaging. The 5' UTR, 3' UTR, and the protein coding region have important cis-acting replication elements to regulate genomic RNA synthesis[13]. The key enzyme for HCV RNA synthesis is NS5B, the RNA-dependent RNA polymerase that replicates the viral genome. NS5B works in a membrane-associated complex that also contains NS4A, NS4B, NS3 protease-helicase and NS5A[14, 15]. These subunits

Corresponding Author: Jonish Varshney, MD; e-mail: jonishvarshney@gmail.com

can recognize cis-acting regulatory sequences in the HCV genome. These proteins also have some additional roles during the infection process that are independent of RNA synthesis. Therefore, targeting the viral replication enzymes could prevent the virus from affecting normal cellular processes as well as inhibiting HCV RNA synthesis[16-19]. HCV structural proteins are positioned in the amino terminal end of the polyprotein and comprised of Core, E1, E2 and p7. An extra protein of unknown function called as F or ARFP or core+1 was recently identified. It results from a frame shift in the core coding region. The HCV core is a highly basic protein and forms the structural element of the virus particle[20]. The E1 and E2 are the enveloped glycoproteins. The non-structural proteins NS2 cysteine protease, NS3 serine protease and helicase, NS4A serine protease cofactor, NS4B anchoring protein, NS5B RNA-binding protein and NS5B RNA dependent RNA polymerase are involved in viral replication. The non-structural NS3 protein is a tri-functional protein with a serine protease, an RNA helicase and NTPase activities. Its serine protease activity abides in the N-terminal (one-third) portion while the C-terminal portion possesses the NTPase and helicase activity. NS4A is a cofactor for NS3. The N-terminal portion of NS4A is responsible for membrane association of the NS3-NS4A complex. The NS3 serine protease is conscientious for cleavage at the NS3/4A, NS4A/4B and NS4B/5A and NS5A/5B junctions[21].

Transmission

HCV is blood borne pathogen and high-risk have been observed in the recipients of multiple or repeated blood transfusions or blood products, intravenous drug abusers, prisoners, organ donors, hemodialysis patients, healthcare workers exposed to needle stick and sharps injuries. Factors responsible for HCV disease evolution include age (growing age is associated with more rapid progression), gender (males have more rapid rate of progression of this disease than females), alcohol consumption (associated with an amplified rate of disease progression), HIV co-infection and fatty liver[22,23].

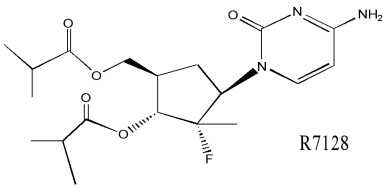
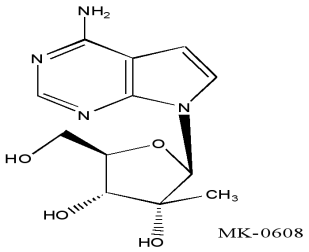
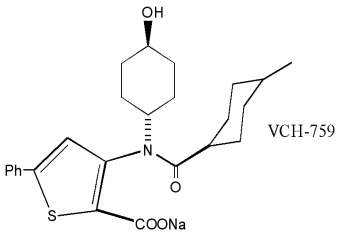
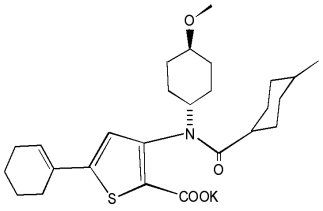
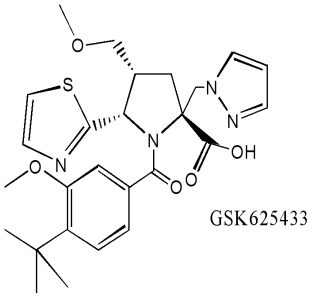
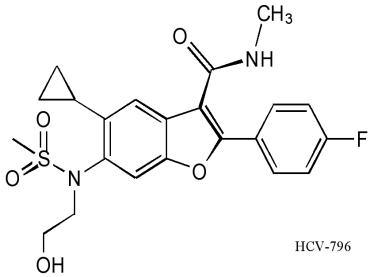
Diagnosis

Diagnosis of hepatitis C is based on serological assays which detect HCV-specific antibodies (anti-HCV) and on molecular assays which detect HCV RNA. The molecular assays currently available are reverse transcriptase (RT)-PCR and the branched DNA (bDNA) signal amplification assay[24,25]. By RT-PCR the viral RNA is reverse transcribed into complementary DNA (cDNA) that is then amplified by PCR. In the bDNA assay the signal resulting from a specific hybridization of capture and detection probes with the viral RNA are amplified. Third-generation anti-HCV enzyme-linked immunosorbent assays (ELISAs) are highly sensitive as well as specific and represent the primary diagnostic assay while the recombinant immunoblot assay (RIBA) is a supplemental assay that can be used to confirm a positive ELISA, seen in low-risk populations. But no routine serological test for a viral antigen is available as yet. However, immunoassays based on the use of high-affinity monoclonal antibodies against core protein to detect and quantitate HCV in serum are currently being evaluated.

Function of viral proteins.

Protein	Category	Function
Core	Structural	RNA binding; nucleocapsid
F-protein or ARF-protein	–	–
E1	Structural	Enveloped glycoprotein; associated with E2
E2	Structural	Enveloped glycoprotein; receptor binding; associated with E1
P7	Structural	Ion channel
NS2	Non-Structural	Component of NS2-3 proteinase
NS3	Non-Structural	N-terminal proteinase domain; C-terminal NTPase/helicase domain
NS4A	Non-Structural	NS3-4A proteinase cofactor
NS4B	Non-Structural	Induces membrane alterations
NS5A	Non-Structural	Phosphoprotein
NS5B	Non-Structural	RNA-dependent RNA polymerase

Table I. Polymerase inhibitors that have entered clinical development.

S. No	Polymerase inhibitor	Category	Company	Phase
1	 R7128	Nucleoside	Pharmasset/Roche	1
2	 MK-0608	Nucleoside	Isis/Meck Sharp & Dohme	1
3	 VCH-759	Non-Nucleoside	ViroChem Pharma	1
4	 VCH-916	Non-Nucleoside	ViroChem Pharma	1
5	 GSK625433	Non-Nucleoside	GlaxoSmithKline	1
6	 HCV-796	Non-Nucleoside	Wyeth/ViroPharma	2

HCV becomes affirmative by RT-PCR as early as 1-2 weeks after infection and 4-6 weeks prior to anti-HCV seroconversion. The determination of HCV RNA is important for the selection of patients for antiviral therapy and for the consideration of its efficacy. Optimal RT-PCR assays at nearby have a sensitivity of less than 100 copies of HCV RNA per ml of plasma or serum. Standardization of assays and proficiency testing of diagnostic laboratories has recently improved the previously high rate of false-positive and negative PCR results. In the case of a positive ELISA, RT-PCR allows to discriminate between patients with chronic hepatitis C and those with resolved HCV infection that can remain anti-HCV positive for years or decades. Recent studies have revealed that the antibody response to HCV fades over time in many patients after successful elimination of the virus. Discrimination of genotype 1 from genotypes 2 and 3 as well as quantitative determination of viremia levels has become important for the selection of the optimal treatment regimen. In general, however, genotyping and quantitative RT-PCR tests should be used only in the context of a defined therapy protocol and not for the initial diagnosis of HCV infection[26,27].

Inhibitors of Hepatitis C Virus

NS5B Polymerase

Inhibitors of HCV NS5B can be classified into four classes based on their chemical composition and/or mechanism of action. The first class consists of nucleoside or nucleotide analogs that work as competitors of NTPs during RNA synthesis; this class also includes the purine analog ribavirin. The second class consists of non-nucleoside inhibitors that allosterically aim the NS5B, and the majority of these inhibit the commencement stage of RNA synthesis. A third, small class has a discrete mechanism of inhibition in that its members covalently change the residues near the active site of NS5B and inhibit its activity and some act by chelating the divalent metal ions needed by NS5B. A fourth and hurriedly emerging class consist of compounds that target cellular proteins needed for HCV polymerase function. This class may have advantages in that the virus cannot rapidly evolve resistance to the inhibitors[28].

Conclusions and Future Prospects

Hepatitis C virus is a highly variable virus with a daily virion production rate higher than that of HIV. Because of the anticipated emergence of viral resistance, it is anticipated that immunomodulators such as IFN will remain a obligatory part of HCV therapy. Combination therapy with a Peg-IFN, NS3/NS4A protease inhibitor, Ribavirin, and NS5B polymerase inhibitor will be perhaps one of the most underlying principle options for the treatment of this disease but combination of specific HCV NS3/NS4A inhibitors with Peg-IFN or Peg-IFN + Ribavirin treatment therapy may be synergistic which will open the door to future combination therapies. A rationale for these synergistic possessions has been proposed, as inhibitors of the HCV NS3/4A and NS5B polymerase. On the other hand, viral resistance will be less expected to appear if two protease inhibitors are administered with drugs targeting other additional viral enzymes NS5B RNA-dependent RNA-polymerase therefore several HCV polymerase inhibitors as well as protease inhibitors are in clinical development and some encouraging results have been found.

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