MANAGEMENT OF HEPATITIS C VIRUS INFECTION

CURRENT STATUS AND PERSPECTIVES

Review for partial fulfillment of Master degree in pharmacology

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Abstract

Background: Hepatitis C virus (HCV) infection is a major and growing global health problem and is a leading cause of liver cirrhosis and hepatocellular carcinoma. Currently, treatment is restricted to pegylated interferon alfa (Peg IFNα) and ribavirin (RBV), which leads to a successful outcome in only about 50% of individuals. New effective treatments with tolerable side-effect profiles are needed.

Method: The HCV clinical trials (including the ongoing ones) are assessed as regards the theoretical base for each treatment, its effectiveness and the potential adverse effects. Special attention is paid to trials on genotype 4. The details of each trial are obtained through searching of the MEDLINE database. Summary of the outcome of each trial is prepared including a criticism for each trial and comparison of the trials in each clinical phase is made.

Findings: Sustained virologic response (SVR) rates are significantly high among genotype 4 patients receiving Peg IFNα-2a (180 μg/week) or Peg IFNα-2b (1.5 μg/kg/week) plus RBV(800-1200 mg/day) according to body weight for 48 weeks**.** Peg IFNα-2a produces a significantly greater SVR rate than peg IFNα-2b but with similar safety profile in patients with chronic HCV infection**.** Virologic response rates do not differ between the two doses of Peg IFNα-2b (1.0 μg/kg and 1.5 μg/kg) with same adverse events. Viramidine is safer than RBV, even in higher exposures. The Clinical trials report that, there is no significant difference between the 36-week and 48-week treatment regimens.

Antiviral agents specifically targeting either the HCV protease or polymerase as TMC435 are now in clinical development and can result in rapid decline in serum HCV RNA levels. Also, Recent trial reports the

safety and efficacy of Nitazoxanide in combination with peginterferon, with or without ribavirin in chronic hepatitis C. Addition of amantadine to Peg IFNα-2a plus RBV, is an effective therapeutic option for patients nonresponsive to previous current treatment, but this is needed to be confirmed in large controlled clinical trial.

Conclusion: Although the combination therapy with Peg-IFN and RBV significantly improves SVR rates, many patients do not respond to this regimen. Thus, new therapeutic options are of great interest to the HCV community. In the last few years, the development of in vitro replication systems for HCV has led to development of many promising anti-viral agents and new hope that more effective therapies will soon emerge.

INTRODUCTION AND AIM OF THE WORK

Hepatitis C virus (HCV) is a major cause of liver diseases, affecting about 175 million people, with 3–4 million individuals newly infected each year ***(Koziel and Peters , 2007).*** The patients of hepatitis C virus are at risk for cirrhosis, liver failure, and hepatocellular carcinoma ***(Dienstag and McHutchison, 2006).***

Hepatitis C is caused by a small, single-stranded RNA virus ***(Robertson et al., 1998).*** There are six genotypes of HCV ***(Simmonds, 1999),*** of which genotype 4 in present in over 90% of cases in Egypt ***(Doss et al., 2008)***. The virus replicates in the liver at a high rate, resulting in average serum HCV RNA levels of 1 to 2 million genome equivalents per milliliter ***(Neumann et al., 1998)***. Although some patients with acute HCV infection have an immune response sufficient to clear the virus, chronic infection develops in 55 to 85% of patients. Among these individuals, 5% to 20% develop cirrhosis which can culminate in end-stage liver disease as well as hepato-cellular carcinoma ***(Thomas and Seeff, 2005).***

The currently recommended regimen for the treatment of chronic hepatitis C is the combination of weekly subcutaneous injections of peg-interferon and twice daily oral doses of ribavirin for 48 weeks in HCV genotype 4. This treatment has many limitations including the partial efficacy, the serious side effects as well as the high cost ***(Hoofnagle and Seeff., 2006).*** Several factors influence treatment response, including baseline viral load, age, body weight and sex; optimizing drug dosing and treatment duration is essential to maximizing response ***(McHutchison et al., 2002).*** There is an ongoing need for more effective treatment especially in patients fail to achieve a sustained virological response to antiviral therapy**.** Recently, there are many drugs in various phases of preclinical and clinical trials that target the replicative cycle of HCV e.g. those targeting HCV proteases, helicase, polymerase, the internal ribosomal entry site ... etc ***(Webster et al., 2009).***

The aim of present review is to assess the HCV clinical trials as regards the theoretical base for each treatment, its effectiveness and the

potential adverse effects. Special attention will be paid to trials on genotype 4. Summary of each trial will be prepared including assessment for each trial and Comparison of the trials in each clinical phase will be made.

BACKGROUND

Hepatitis C virus infection is major global health problem, affecting about 170 million people worldwide ***(WHO, 1999)***. HCV is a leading cause of liver cirrhosis and hepatocellular carcinoma. Liver related death from the virus are expected to nearly triple by the year 2020 ***(Alter et al, 1999)***. Egypt has the highest prevalence of infection, with estimates ranging from 6% to 28% ***(Doss et al., 2008)***.

Hepatitis C was first discovered in post-transfusion hepatitis patients and known as non-A, non-B hepatitis (NANBH) in 1975 ***(Feinstone et al., 1975).*** The identification of the etiological agent of NANBH took more than a decade, mainly because of the inability of the virus to propagate efficiently in cell culture. HCV was identified in 1989 by Michael Houghton's group ***(Choo et al., 1989)*** and this permitted identification of molecular characterizations of HCV.

Studies of HCV replication received a major boost with the development the sub-genomic replicon in 1999 ***(Lohmann et al., 1999).*** With the advent of a robust cell culture infection system (HCV cell culture) based on a unique isolate from a Japanese fulminant hepatitis patient in 2005, the complete life cycle of HCV infection could be studied in cell culture for first time ***(Wakita et al., 2005)***. The subsequent development of cell culture infectious clones of additional genotypes should facilitate the study of genotype-specific deference in the viral life cycle and pathogenesis ***(Gottwein et al., 2009).***

Hepatitis C Virus (HCV) Description:

HCV is a member of the genus hepacivirus in the family flaviviridae ***(Robertson et al., 1998)***. HCV is a positive-sense single stranded RNA virus of 9600 nucleotides, consists of a single open reading frame that is flanked by 5′- and 3′- untranslated regions (UTRs). It encodes a polyprotein of approximately 3000 amino acids. HCV polyprotein is co-translationally and post-translationally processed by cellular and viral proteases to form three structural proteins (core, E1, and E2), an ion channel protein (P7) and six non-structural Proteins (NS2, NS3A, NS4A, NS4B, NS5A and NS5B). The structural proteins are located at the N-terminus, whereas the non-structural proteins are situated at the C-terminus. The 5′- UTR contains six secondary structure domains termed stem loops (SLs) I-VI. SLII, III and IV form an internal ribosome entry site (IRES) that facilitates the translation of HCV RNA ***(Tang and Grise, 2009)***.

The IRES is a structurally complex region consisting of four domains which are critical for the regulation of the polyprotein translation and viral replication. The IRES binds sequentially the 40S ribosomal subunit, the eukaryotic initiation factors 3 and 2 (eIF), and the 60S subunit via a GTP-dependent reaction. The domain I of IRES is capable of binding a liver specific microRNA (miR-122) that appears to enhance HCV RNA replication rate in a cell specific manner ***(Jopling et al., 2006).*** Lately, miR-199a* has been reported to target domain II of the IRES region of HCV and to strongly inhibit viral replication in cell culture derived HCV (HCVcc) replicon systems of genotype 1b and 2a ***(Murakami et al., 2009)***. It is worthy to mention that ***Sarasin and his colleagues (2009),*** in their clinical study, found an inverse correlation between the level of miR-122 pretreatment and the sensitivity to interferon (IFN) therapy, suggesting a potential application of this miR-122 as a biomarker for IFN therapy.

On the other hand the 3′- UTR which is essential for HCV replication ***(Ji et al., 2004),*** starts with a short variable region, followed by a polypyrimidine tract (poly U/UC) and a highly conserved 3′-x tail ***(Kolykhalov et al., 1996).*** Different genotypes differ in the sequence of

the variable region, but in general isolates of the same genotype it is fairly well conserved. The poly(U/UC) tract, along with the 5′ triphosphate of the genomic RNA, constitutes the major HCV-associated molecular pattern that is recognized by the innate immunity receptor RIG-I (retinoic-acid-inducible gene-I) ***(Saito et al., 2008).*** The 3′ UTR can increase HCV internal ribosome entry site (IRES)-mediated translation in the proper genomic context ***(Song et al., 2006)***, Figure (1).

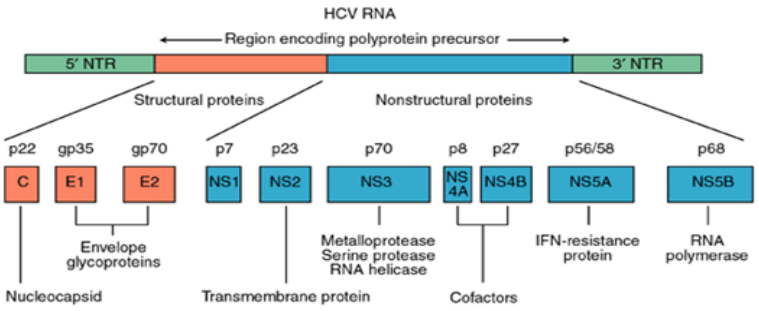

Fig. (1): *Schematic diagram of the hepatitis C virus genome. (NSR= non structural regions). Cited in* ***Myrmel et al., (2009).***

HCV Structural Proteins:

The structural proteins are released from the polyprotein by host signal peptidases that cleave signal peptides located between core/E1, E1/E2, E2/P7 and P7/NS2 ***(Lin et al., 1994).***

1-*Core protein*: It is processed further by a signal peptide peptidase into a mature protein. This cleavage/maturation process promotes the transport of core from the endoplasmic reticulum (ER) membrane to the surface of the lipid droplets ***(McLauchlan et al., 2002),*** the site of HCV particle assembly ***(Miyanari et al., 2007).*** The signal peptide peptidase cleavage of core is important for the production of both virus-like particles and infectious virions ***(Grakoui et al., 1993).*** It is highly basic, RNA-binding protein which forms viral nucleocapsid. It interacts with numerous cellular proteins and affects host cell functions such as gene transcription, lipid metabolism and apoptosis ***(Tellinghuisen and Rice, 2002).***

2-*Envelope glycoproteins*: HCV is enveloped by a lipid bilayer containing two glycoproteins (E1 and E2) for receptor binding and

entry of HCV into target cells. Two regions in E2 called hypervariable regions 1 and 2 (HVR 1 and HVR 2), show extreme sequence variability. E2 also contains the binding site for CD81 (a cell surface protein) and scavenger receptor class B type I (SR-B1), two of the several putative HCV receptor which is essential for HCV entry ***(Pileri et al., 1998 & Scarselli et al., 2002).***

3-*P7*: is a short peptide located at the junction between the structural and nonstructural region. It is thought to be a viroporin (an ion channel that mediates membrane permeability and secretion) ***(Griffin et al., 2003)***. P7 is essential for the production of infectious viruses both in vivo and in vitro, indicating a potential role of this protein in viral assembly and release ***(Steinmann et al., 2007),*** figure (2).

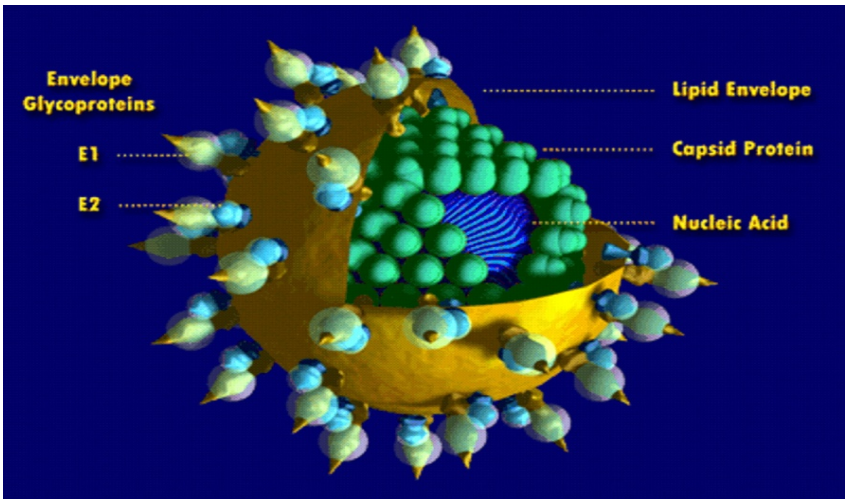

Fig. (2): Model structure of HCV. Image at www.bbm1.ucm.es/public_html/res/prot/virprot.html

HCV Nonstructural Proteins:

1-*NS2-NS3 proteinase:* The C-terminal half of NS2 contains a protease domain that, together with the N-terminal protease domain of the NS3, forms a catalytically active protease. It is a zinc-dependent metalloproteinase that cleaves the site between NS2 and NS3 in the polyprotein ***(Grakoui et al., 1993).*** This proteinase is required for the production of infectious viruses in vitro ***(Jones et al., 2007).***

2- *NS3-NS4A*: The N-terminal third of NS3 is a serine protease that cleaves all other downstream NS junctions (NS3/NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B) with assistance of the cofactor NS4A, NS3-NS4A protease is an attractive target for STAT-C (specifically targeted antiviral therapy for HCV). The two-thirds of NS3 also have helicase and NTPase activities that unwinds duplex RNA structures ***(Tomei et al., 1993).*** Both the protease and The helicase activities are important for HCV replication ***(Kolykhalov et al., 2000 & Lam et al., 2006).*** The NS3 helicase has been reported lately to be involved in an early step of viral assembly ***(Ma et al., 2008).***

3-*NS4A*: serves as an essential co-factor for the NS3 protease and it plays an important role in HCV replication by regulating NS5A phosphorylation ***(Lindenbach et al., 2007).***

4-*NS4B*: is known that expression of NS4B induces a specific membrane alteration, designated as membranous web that serves as a scaffold for formation of the viral replication complex ***(Egger et al., 2002).***

5-*NS5A*: is a phosphorylated zinc metalloprotein. The phosphorylation status of NS5A has important role in viral replication and assembly of infectious viral particles in cell culture ***(Tellinghuisen et al., 2008 & Appel et al., 2008)***. It consists of three domains, domain II and domain III exhibit more sequence diversity among the different genotypes, although domain III is important for virus production, it is dispensable for RNA replication ***(Tellinghuisen et al., 2008).*** NS5A also interacts NS5A also interacts with many cellular proteins that are implicated in HCV replication, including the geranylgeranylated F-box protein FBL2 (F-box and leucine-rich repeat protein 2) ***(Wang et al., 2005).***

6-*NS5B*: is RNA-dependent RNA polymerase (RdRp) that catalyses replication of the viral genome**.** HCV is characterized by significant genetic heterogeneity and of the accumulation of mutations, which is the consequence of the high level of viral replication during its life cycle (10^{12} new HCV particles/day) ***(Neumann et al., 1998)***. This is attributed to the error-prone replication system because the viral RNA-dependent RNA polymerase enzyme Like other viral RNA

polymerases, lacks proofreading activity, so that mutations accumulate in newly generated HCV genomes (quasispecies) ***(Farci et al., 2006).***

Quasispecies are composed of a complex mixture of different, but closely related genomes whose shape is subjected to continuous changes ***(Martell et al., 1992).*** This viral heterogeneity leads to prevention of development of conventional vaccines, allows the virus to escape eradication by the host's immune system, and affects the completeness of the response to antiviral therapies such as interferon ***(Farci et al., 1992).***

HCV Genotypes:

On the basis of variations in the nucleotide sequence of HCV, there are six recognized genotypes and more than 50 subtypes which are distributed worldwide. Genotypes 1a, 1b, 2a, 2b, 2c, and 3a account for more than 90% of the HCV infections in North and South America, Europe, Russia, China, Japan, Australia, and New Zealand. Genotype 3a is predominant in Pakistan, other subtypes of genotype 3 are highly prevalent in Nepal, Bangladesh, India, and Indonesia. Most infections in Egypt are genotype 4a and other subtypes of genotype 4 are found in central Africa. Genotype 5 is found in South Africa**.** Genotype 6 is found in Southeast Asia ***(Maertens and Stuyver, 1997)***. HCV genotype is important factor influencing the severity of liver disease and response to the treatment ***(Poynard et al., 1998),*** Figure (3).

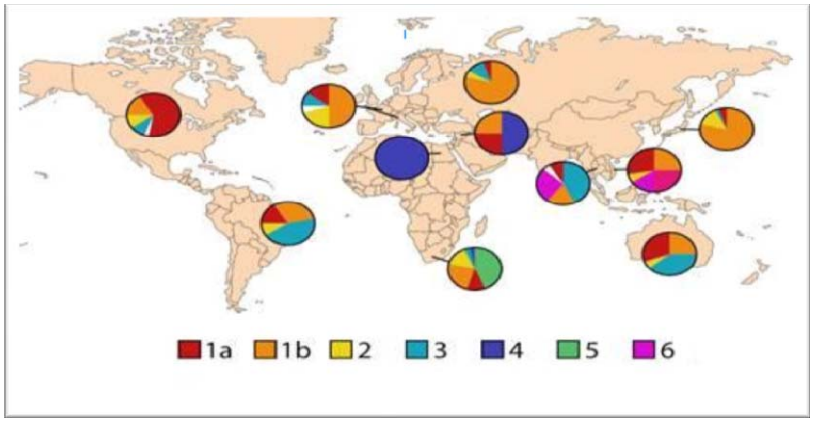

Fig. (3): Worldwide distribution of genotypes of HCV. Cited in ***Forns and Bukh, (1999).***

HCV Life cycle

• HCV Entry:

The first step of HCV infection is the attachment of the virus to the host cell surface by means of week interactions with glycosylamino-glycans ***(Barth et al., 2003)*** and/or the low –density lipoprotein receptor (LDLR) ***(Molina et al., 2007)***. Once bound to cell surface, viral envelope glycoproteins bind to entry receptors such as CD81 and SR-BI with high affinity. CD81 has a critical role in HCV entry ***(Helle and Dubuisson, 2008).*** A clinical study showed that the pretreatment with anti-CD81 antibodies completely blocked HCV infection ***(Meuleman et al., 2008).***

The virus–receptor complex then translocates to the tight junctions where claudin-1(CLDN1) ***(Evans et al., 2007)*** and occludin (OCLN) act as cofactors and induce receptor-mediated endocytosis ***(Liu et al., 2009)***. CLDN1 is a tight junction protein (TJ) that is required for HCV entry into cells expressing CD81 and SR-BI and may facilitate direct cell to cell virus spread during infection ***(Timpe et al., 2008)***. Lately another TJ protein, human occludin, has identified as an HCV entry factor and may play a role in restricting species specificity of HCV infection ***(Ploss et al., 2009).*** SRBI, CD81 and CLDN1 appear to be expressed in the basolateral–sinusoidal membrane of hepatocytes in man, consistent with the fact that HCV enters the liver via the sinusoidal blood ***(Reynolds et al., 2008)***, Figure (4).

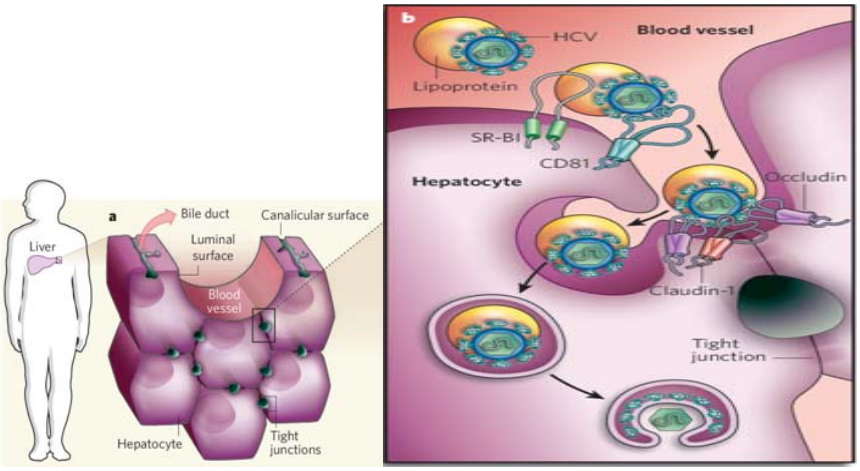

Fig. (4): Entry pathway of hepatitis C virus (HCV). a, Tight junctions between hepatocytes liver cells establish functionally different cell-surface domains, with the luminal surface

facing the bloodstream and the canalicular surface in contact with the bile-duct system. b, HCV travels through the bloodstream in association with lipoproteins. Its initial adhesion to hepatocytes may be mediated by direct interaction with SR-BI and CD81proteins. On transfer to a tight-junction complex, the virus may interact directly with claudin-1 and occludin, allowing viral uptake into the cell. Cited in ***Pietschmann T, (2009).***

- **HCV Replication:**

The combination between receptor binding, post-binding association with TJ proteins, internalization by endocytosis and followed by a PH-dependent step by acidification of virus containing endosome in the cytoplasm, results in the fusion of the virus membrane with the endosome membrane ***(Hsu et al., 2003;Tscherne et al., 2006)***. Initiation of translation of viral RNA is mediated by an internal ribosomal entry site in 5′-untranslated region of the genome ***(Otto and Puglisi, 2004)***. The polyprotein precursor generated by translation is cleaved by host and viral proteases ***(Suzuki et al., 2007).*** Host proteases generate the structural proteins while the viral proteasesNS2/NS3 andNS3/4 generate respectively the non-structural proteins ***(Bartenschlager et al., 1994)*** The building-up of the replication complexes is assumed to be initiated at ER membranes by NS4B, an integral membrane protein ***(Egger et al., 2002 & Gao et al., 2004)***.

Analysis of replication complexes showed that the replicating HCV RNA and the NS proteins colocalize on cytoplasmic membrane structures. It is now admitted that NS proteins form complexes in both ER and Golgi membranes, but that HCV RNA replication takes part in the Golgi membranes ***(Aizaki et al., 2004).*** HCV genome replication begins with the RdRp (NS5B)-mediated synthesis of a complementary replicative (negative) strand. The latter is then used as a template to synthesize the genomic (positive) strand by the same enzyme ***(Bartenschlager et al., 2004)*** These RNA strands are either encapsulated to form new viruses or used as mRNA for viral protein synthesis ***(Penin et al., 2004)***.

- **HCV Formation and Release:**

Human apolipoproteins (Apos) are detected in HCV virions. ***Hung and his colleagues (2007a)*** demonstrated that HCV assembles in ApoB

and MTTP (microsomal triglyceride transfer protein) enriched vesicles and that the viral secretion is dependent on both ApoB expression and VLDL assembly from subdomains of the ER. This suggests that HCV uses the lipoprotein/cholesterol export system at its own benefit to escape from hepatocytes, and These results were confirmed lately ***(Nahmias et al., 2008).***

infectivity and production, suggesting that HCV virions are assembled as apoE-enriched lipoprotein particles. ApoE-specific monoclonal antibodies as well as ApoE-specific siRNA strongly decreased the release of infectious virions ***(Owen et al., 2009)***. HCV also induces the accumulation of lipid droplets (LDs) on which the HCV core protein is known to reside ***(Boulant et al., 2008).***

The density of HCV particles is determined by its association with lipoproteins and it is inversely related to the level of viral infectivity ***(Lindenbach et al., 2006).*** HCV has been suggested to complex with low density lipoprotein in chronically infected patients ***(Nielsen et al., 2006).*** Human Apos are detected in HCV virions and both genetic and pharmacological inhibtion of ApoE significantly reduces HCV production ***(Chang et al., 2007)***. HCV assembly appears to occur on lipid droplets and the core protein coats the surface of this organelle ***(Miyanari et al., 2007),*** Figure (5 and 6).

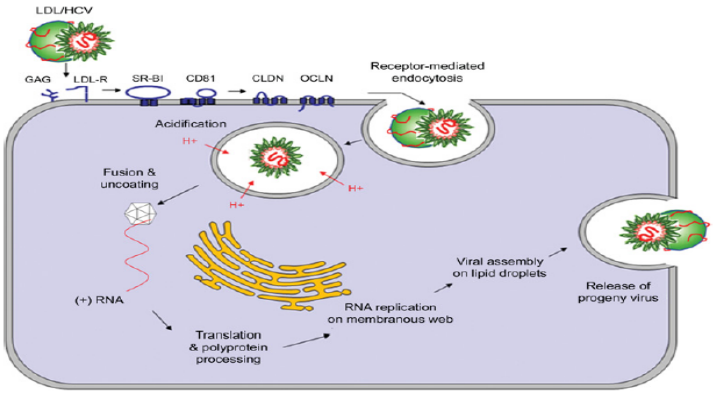

Fig. (5): Entry into target cells initiates HCV infection. This Figure is available at www.ClinSci.org/cs/117/0049/cs1170049add.htm,16/1/2010.

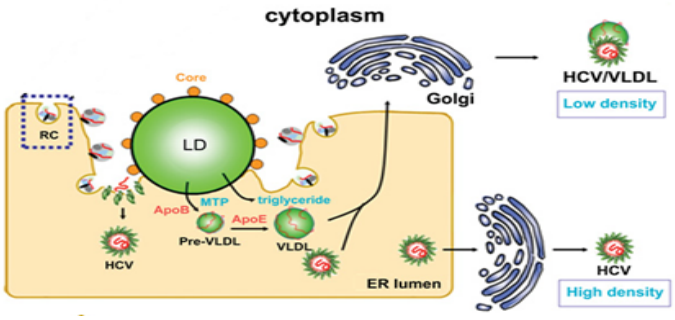

Fig. (6): Model for HCV assembly and release. The lipid droplet, with core and NS complex (NPC) interacting on its surface not only serves as a site for viral assembly, but also supplies lipoproteins that complex with HCV particles. The density of HCV particles is determined by its association with lipoproteins. MTP, microsomal triacylglycerol transfer protein; triglyceride, triacylglycerol; VLDL, very-LDL; NPC, non-structural protein complex. Cited in ***Shimotohno, (2008).***