REVIEW

HEPATITIS C VIRUS AND HEPATITIS C-INFECTION

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Abstract

Hepatitis C virus (HCV) belongs to the Hepacivirus genus, Flaviviridae family, has six major genotypes and more than 70 subtypes. HCV has a major impact on public health, because it infects around 3% worldwide population, with an estimated global incidence of three to four million new infections per year. HCV infection was first suspected in the 1970s, when this new type of hepatitis transmitted by blood was then called "non-A, non-B" hepatitis. HCV is an enveloped, positive-stranded RNA virus, and its genome was identified in 1989. It's transmitted primarily via the blood route through of injection drug use, sharing syringes, and blood transfusion. Anti-HCV antibody and HCV RNA testing are used to diagnose acute and chronic hepatitis C, and HCV genotype should be systematically determined for indication and duration of treatment. After acute infection, 15%-25% of persons appear to resolve their infection without sequelae, whereas 75%-85% evolve to chronicity, which may exhibit various complications with the evolution of the infection. The standard treatment for chronic infection with HCV in the last decade has been the combination therapy of pegylated interferon alpha plus ribavirin. However, this therapy is associated with significant adverse effects, but recent developments of new drugs' combinations are changing the treatment paradigm in HCV infection. In this article, we review the information on HCV, how the HCV biology, diagnosis, management, preventive modalities, and therapeutics.

Keywords: Hepatitis C virus; Hepatitis C. Diagnosis; Prevention; Treatment.

INTRODUCTION

Hepatitis C virus (HCV) belongs to the Hepacivirus genus, Flaviviridae family, has six major genotypes, more than 70 subtypes. HCV has a major impact on public health because it infects around 3% worldwide population, and more than 350.000 people die from HCV-related liver diseases every year.[1,2] HCV infection was first suspected in the 1970s, when this new type of hepatitis transmitted by blood was then called "non-A, non-B" hepatitis. HCV is an enveloped, positive-stranded RNA virus, and its genome was identified in 1989.[3] The major routes of

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transmission of HCV are injection drug use, blood transfusion, and sharing syringes. Transmission can also occur by healthcare related procedures, tattooing, vertical transmission, and sexual transmission, but are less frequent as compared to transmission initially cited. Anti-HCV antibody and HCV RNA testing are keys to diagnosing correctly acute and chronic hepatitis C, and to determine the indication and duration of treatment.[4] Besides hepatocytes, HCV infects different cells.

The The standard treatment for HCV infection with the combination of pegylated interferon alpha (Peg-IFNα) with ribavirin (RIB) is associated with significant adverse effects. Recent developments of new drugs' combinations are changing the treatment paradigm.[5] In this article, we review the information on HCV infection, considering its biology, diagnosis, therapeutics modalities, and prevention.

THE HEPATITIS C VIRUS

HCV infection was first suspected in the 1970s, when this new type of hepatitis transmitted by blood was then called "non-A, non-B" hepatitis. In 1989, the HCV was first described by Choo et al. from the cloning of nucleic acids, derived from infectious chimpanzee plasma, and screening of clones produced with a human chronic non-A, non-B hepatitis serum.[3] HCV is shown to be responsible for most cases of post-transfusion hepatitis and it is a major cause of liver disease worldwide with potential morbidity. After cloning in 1989 described by Choo,[3] knowledge of clinical and virological aspects of HCV has grown substantially.

HCV BIOLOGY

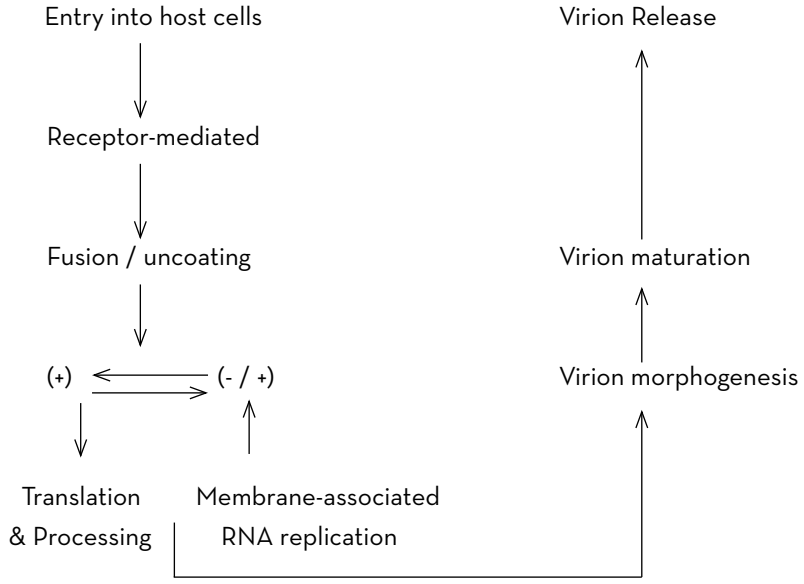
HCV is a hepatotropic RNA virus, enveloped, positive-stranded RNA virus that belongs to the genus of Hepacivirus in the Flaviviridae family, along with Pestiviruses and Flaviviruses.[2] The HCV has 50-80 nm in diameter, with a genome of positive-sense, single-stranded linear RNA with approximately 9600 nucleotides, containing a translational large open reading frame that encodes a polypeptide of 3010–3030 amino acid flanked by untranslated regions at both the ends.[6] The HCV genome is divided into two parts: a structural and other non-structural. The precursor is cleaved into at least 10 different proteins named of structural proteins that include the core protein followed by envelope glycosylated proteins: E1, E2, and p7, as well as the nonstructural domain encoding six proteins: NS2, NS3, NS4A, NS4B, NS5A, and NS5B. This genomic structure has a high degree of genetic variability.[7] HCV is classified into six major genotypes (numbered 1-6) that vary by over 30% in its nucleotide sequence, and more than 70 subtypes that differ in their nucleotide sequence by 20%-25% .[8,9] Viral quasispecies of mixed virus populations provides a survival advantage to the virus to create multiple variant genomes and a high rate of generation of variants to allow rapid selection of mutants to new environmental conditions. The genotypes 1, 2 and 3 are globally distributed while others are limited to specific regions of the world. The genotypes 1a and 1b are the most prevalent genotypes in the United States and western of Europe. The genotype 4 is restricted to the Middle East and Central Africa, the genotype 5 is prevalent in South Africa and the genotype 6, in South East Asia.[10] The genotyping is a primary tool for assessing the evolution of infection and determining treatment response and your continuance.[11] A Brazilian nationwide HCV seroprevalence study between in 2005-2009 in the state capitals of the five Brazilian regions showed a weighted prevalence of HCV antibodies in 1.38%. Sero-positivity varied from 0.7% in the northeastern region to 2.1% in the northern region. Based on this population-based survey and according to WHO criteria, Brazil can be classified as a country with low HCV endemicity (prevalence below the 2.5%). In a population-based study conducted in 1998 in Salvador, the capital of the northeastern State of Bahia, the HCV prevalence was 1.5%.[12] Several studies in Brazil have reported that HCV genotype 1 is more prevalent than genotype 3 among patients who had received a blood transfusion.[13] The first report on the HCV genotype distribution in Salvador - Bahia in intravenous drug users (IDU´s)

showed a high prevalence in genotype 1, followed by genotype 3.[14]

HCV LIFE CYCLE

Significant advances in understanding the mechanisms of HCV infection have been reported. HCV infection is a complex process requiring the involvement of viral envelope proteins and multiple host proteins (Figure 1). The entry of the virus into the cell is the first step in viral infection, and replication is a process that involves multiple cell surface molecules in sequential steps.[15] As soon as HCV crosses the endothelium, the virus potentially concentrates the on the basal surface of the hepatocyte. Posteriorly, the virus internalization occurs by pH--dependent and clathrin-mediated endocytosis, involving a series of interactions with viral receptors or entry factors such as the scavenger receptor class B type I, CD81, claudin-1, occludin, receptor tyrosine kinases, epidermal growth factor receptor, ephrin receptor A2 and Niemann-Pick C1-like 1 cholesterol absorption receptor.[19,20] The Trojan horse strategy help shields the virus from neutralization.[21] Within hepatocytes, highly sulfated glycosaminoglycans serve as the first attachment sites. While direct E2 binding to sulfated glycosaminoglycans is held, E1 and apolipoprotein E are also attached to sulfated glycosaminoglycans. Following hepatocyte entry, HCV particle penetrates in acidic endosomal compartment and release its RNA genome into the cytoplasm. The HCV polyprotein is then translated, producing a single polyprotein precursor that is processed, generating 10 individual viral proteins, including core and envelope glycoproteins E1,E2, p7, NS2, NS3, NS4A, NS4B and NS5A.[19] The HCV release process appears to be closely linked to lipid metabolism because the core association with lipid droplets appears to be critical in viral assembly.[20] The HCV replication is estimated in 10 trillion virion particles produced per day during the active phase of infection.[21]

Figura 1 - HCV life cycle.



HCV DIAGNOSIS

Nucleic acid test (NAT) for the detection of HCV RNA is the gold standard for diagnosing active HCV infections; however, novel technologies being developed. Serologic assays and molecular assays are useful because they may detect early infections before clinical signs of disease appear, differentiate acute from chronic infections, and detect persistence of the virus or verify development of

immunity. There are essentially two approaches to the diagnosis of HCV infection.

SEROLOGY AND VIROLOGY IN HCV

The serological tests and virological tests have become essential in the diagnose HCV infection, beyond of manage treatment, and evaluate the virological response to antiviral therapy. The serological assays in HCV include anti-HCV antibody detection, and serological determination of the HCV genotype.

Anti-HCV antibody detection: The "serologic window" between HCV infection and the detection of specific antibodies is variable from individual to individual. The tests are already in the third generation (Table 1).

Table 1 - Generations of immunoassays anti-HCV

	Developed year	Core	E1/E2/NS1	NS2	NS3	NS4	NS5
1st generation	1989	-	-	-	-	C100-3 (recombinant)	-
2nd generation	1992	c22-3	-	-	c33c	c200, HC-31	-
3rd generation	1993	C22p	-	-	C33c	C100-3, 5-1-1p	NS5

The first-generation test incorporates the c100-3 recombinant epitope from the NS4 region. The incorporation of epitopes c22-3 on HCV core and of c33c to NS3 regions, resulted in the second-generation test with sensibility of 60%, and the recombinant immunoblot assay II increased its sensitivity to 90%. The third-generation test contains a reconfigured core and NS3 antigens added to an antigen NS5 region, the sensitivity increased by over 99%.[22] The fourth-generationtest detects, simultaneously, the HCV capsid antigen as well as antibodies to the core, NS3, NS4, and NS5 regions of the virus, but its use is still limited. [23] The detection of anti-HCV antibodies in the blood is based on the use of third-generation enzyme immunoassay, which detect mixtures of antibodies directed against various HCV epitopes, and are used to diagnose acute and chronic hepatitis C. The method consists of the capture of circulating anti-HCV antibodies using recombinant antigens, that are demonstrated by anti-antibodies labeled with an enzyme that catalyzes the transformation of a substrate .[24] This technique led to the development of commercially available screening and supplemental assays for anti-HCV immunoglobulin G.

HCV genotyping: Recently, HCV genotyping assays evolved and ultrasensitive quantitative molecular assays were developed. The genotyping is fundamental to determine the therapy type and duration of treatment.[8] The HCV genotyping assay is performed by analyzing banding patterns that are indicative of the genotype. The HCV genotyping is determined by search for antibodies directed to genotype-specific HCV epitopes with a competitive enzyme immunoassay, where two regions of the genome are assessed, the 5' UTR and the core region. Thus, the reference method for HCV genotyping is genome sequencing of the core/E1 or the NS5B regions and subsequent phylogenetic analysis.[25,9] Less than 5% of genotyping feature indeterminate results because of the high genetic variability of HCV.[26]

MANAGEMENT OF HCV

Management of HCV objective is to block disease progression, cirrhosis prevent, reduce the risk of hepatocellular carcinoma, and treat extrahepatic complications. Thence, the management of HCV requires a multidisciplinary approach in health.

Management of treatment HCV: For several years the standard treatment for chronic infection with HCV was 24 or 48 weeks of therapy with PEG-IFNα and RIB. This changed since direct-acting antiviral agents (DAA) was added to treatment in 2011, increasing the likelihood of sustained virological response (SVR) to 67–75% in patients without previous treatment infected with genotype 1.[27,28]

The treatment remains open, since that PEG-IFNα, with several side effects, became harder add on a DAA, that can increasing toxicity potential.[29,30] A major obstacle to treatment of HCV, are adverse effects. Almost all patients treated with PEG-IFNα and RIB presents one or more adverse side effects during the course of treatment that includes influenza-like symptoms, neuropsychiatric effects, hematologic abnormalities, and induction of autoimmune disorders.[31] The clinical trials show that, approximately 10–15% of patients discontinued Peg-IFNα and RIB therapy due to adverse effects.[32] Changes in lifestyle are recommendations in managing chronic hepatitis C similar to those for obesity, diabetes and metabolic syndrome, confirming the role and injury caused by metabolic factors on the clinical course of HCV infection. Targeted physical activity is a well-understood behavioral modification that decreases metabolic disorders, and the molecular mechanism has been determined to involve exercise-stimulated glucose transport through AMP cyclic. In addition, this change can increase insulin sensitivity in HCV-infected patients, as well as improve early viral response to antiviral therapy and decrease serum α-fetoprotein levels.[33] Furthermore, several studies have established a strong link between HCV infection and extrahepatic manifestation, such as rheumatological, endocrine, hematological, dermatological, renal, neurological, autoimmune and systemic manifestations.[34] These data are important for that the physicians offer better management to patients.

HCV TREATMENT

Protect patients from HCV-related complications and permanent viral eradication is a necessity. The goal of treatment is to eradicate HCV or healing set with SVR, evidenced by undetectable serum HCV-RNA 4-24 weeks after the end of treatment.

PEG-IFNα: IFNα was the base of therapy for HCV infection in the 1990s.[35] Posteriorly, the conjugation of polyethylene glycol (PEG) conjugated to IFN showed enhanced solubility, reduced antigenicity, reduced sensitivity to proteolysis, and reduced rate of kidney clearance. The development of PEG-IFN has significantly improved the eradication rates of HCV infection and, adherence to treatment. Two forms of PEG-IFN have been developed, based on two pegylated chemistries: the 12-kDa linear PEG-IFNα 2b and the 40-kDa branchy PEG-IFN-α 2a.[36] Mathematical models have been described to infer the mechanisms of action of IFN. When following the initial dose, there is a rapid viral decline, between 0.5 to 2.0 log, during the next 24 to 48 hours due free virion clearance. The degree of antiviral effectiveness in blocking viral production, following viral declines, is more slow over the next 14 days. In this stage, the viral decline is primarily dependent on the blocking of new virions.[37,21] The standard therapy, for patients with HCV infection, is the administration of PEG-IFNα by period of 48 weeks in case of HCV genotypes 1, 4, 5, and 6 or 24 weeks for HCV genotypes 2 and 3.[38]

RIB: The RIB is an inhibitor of some viral RNA guanylyl transferase and guanine-7N-methyl transferase enzymes that may contribute to a defective 5'-cap structure of viral mRNA transcripts and inefficient viral translation for certain DNA viruses. It has been suggested that incorporation of RIB into the 5'-end of mRNA transcripts would mimic the 7-methyl guanosine endcap of cellular mRNAs, causing poor cellular translation of these. This process could generate a cell-toxic effect, but it does not seem to be important at therapeutic RIB concentrations. Any difference between cellular and viral enzyme handling of RIB-containing mRNA transcripts is a potential mechanism of differential inhibition of RIB to translation of mRNAs from viruses.[39]. RIB is also incorporated into the viral genome causing lethal mutagenesis and a

subsequent decrease in specific viral infectivity.[38] The most common and significant side effect of RIB therapy is hemolytic anemia. Hemoglobin concentrations reach a nadir after 4–8 weeks after the start of treatment. The prevalence is 19–29 % with Peg-IFNα and RIB dual therapy and 37–49 % in triple therapy.[28,40] This adverse effect of RIB is reversible, and hemoglobin concentrations will return to pretreatment concentrations after termination of therapy.[41] Lindahl et al.[42] found that RIB-induced hemolytic anemia was dependent on RIB plasma concentrations rather than on dose per kilogram body weight. Other factors apart from RIB concentrations can play a role in developing or worsening anemia, for example, the bone marrow suppressive effect of Peg-IFN and suboptimal endogenous erythropoietin production. RIB-induced anemia is treated by dose reduction and/or treatment with erythropoietin or blood transfusions. Another serious adverse effect of RIB is that the drug are demonstrated to have significant teratogenic effects in all animal species exposed to RIB.[43-45] It is therefore contraindicated in pregnant women, and a negative pregnancy test is required before starting HCV therapy including RIB.

The standard therapy for patients with HCV infection has been the use of PEG-IFNα plus RIB.[38] After dual therapy with PEG-IFNα and RIB, treatment response varies according to HCV genotype. It ranges from 40–50 %, in patients with genotype 1 or 4, and from 70–80 %, in genotype 2 or 3.[46-49] When the DAAs, which will be mentioned later, boceprevir or telaprevir are added to PEG-IFNα and RIB for genotype 1infections, these drugs increase SVR rates by 25–31 %.[28,40]

DAA: The DAAs are assuming a more important role in HCV treatment. The DAAs are classified according to their action sites, such as a protease inhibitor, polymerase inhibitor, NS5B inhibitor, and NS5A inhibitor. The main mechanism of action is the inhibition of the enzyme, protease or polymerase. Another mechanism of action in HCV therapy is to target the host factors that the virus uses to its life cycle, such example, cyclophilin inhibitors or nitazoxanide.[50] Two first-generation telaprevir and boceprevir were licensed for use in conjunction with PEG-IFNα and RIB in adult patients chronically infected with HCV genotype 1. Telaprevir is an HCV protease inhibitors approved in U.S., in Canada, in European Union, and Japan. Telaprevir is a peptidomimetic inhibitor of the HCV non-structural 3-4A serine protease, not only interrupting the viral life-cycle, but would also restore the innate immune response.[51] Boceprevir is another HCV protease inhibitors approved in the U.S. and Europe for treating chronic genotype 1 HCV infection. Boceprevir is covalent linear inhibitors that act via formation of a reversible covalent interaction with serine-139, that if binds reversibly to the NS3 protease active site and shows potent activity in the HCV replicon system.[52]

Actually, hepatitis C therapy is undergoing a revolution. Enormous research and development efforts have produced a large number of new antiviral drugs, including DAA and host-targeted agents. In 2013, the US FDA approved two more drugs for HCV treatment: simeprevir for genotype 1 infections (in combination with PEG-IFNα and RIB); and sofosbuvir for patients with genotype 1 or 4 (with PEG-IFNα and RIB) and for patients with genotype 2 or 3 (with RIB).[53-55] These two new targeted drugs have further improved SVR rates to 80–90 %. In addition, these new drugs are better tolerated, their treatment schedules are less complex and there are fewer drug–drug interactions. More than 90% of infections were reported to be cured in phase II and III trials, with or without PEG-IFNα and/or RIB. However, a number of unresolved issues remain. PEG-IFNα will remain the backbone of some HCV treatment strategies in 2014 and 2015, before the slowly disappearing from HCV treatment regimens, at least in areas of the world that will be able to afford the high cost of IFN-free combinations.RIB can be used to increase rates of SVR or to shorten treatment duration without altering the rates of SVR with both PEG-IFNα and IFN-free regimens because it prevents relapses through unknown mechanisms. For this reason could remain a useful adjunct in some IFN-free treatment strategies. Researchers aim to improve the currently available classes of HCV drugs. Second- and third-generation

NS3-4A protease inhibitors, nucleoside/nucleotide analogues, non-nucleoside inhibitors of HCV RNA-dependent RNA polymerase, and NS5A inhibitors that have increased potency, pan-genotypic antiviral activity, and high barriers to resistance likely will enter clinical development within the next 2–5 years. It is unlikely that further investment will be made beyond this point because there will be a sufficient number and range of drugs to fulfill clinical needs. In 2014 and 2015, new IFN-containing and IFN-free regimens will become available. Starting in 2015 and onward, IFN-containing regimens will be replaced by all-oral, IFN-free therapies, at least in areas of the world where these regimens are approved, and their cost is covered.[56]

MEASURES OF PREVENTION

The pathways of transmission include blood-blood link, such as occur during IDU´s, and sexual contact. An important question is co-infection with HIV that is very often. It has been estimated that 25% of people, infected with HIV, in the United States, are also infected with HCV. In South America, Brazil study, HCV/HIV co-infection rates vary from 4.4% to 36.2% .[57] Blood safety has been changing since HIV outbreak in 1980´s, and many parts of the world, the NAT became reliable way to decrease blood-borne infectious diseases.[58] The IDU´s are considered to be the main risk group for HCV infection acting as a source of this infection because the use of injecting drugs correlates strongly with HCV infection in the entire world. IDU's infected at an early age become an important reservoir beyond the higher risk of HIV co-infection or other parenterally transmitted viruses, which make HCV more difficult to eliminate.[59] Considering IDU, it is important to determine the route transmission and virus similarity. A study has showed the intra-familial transmission of HCV based on bioinformatics analyses. This work demonstrated that two relatives living in the same house were infected by less divergent strains than two subjects living in different houses or belonging to different families.[60] Another group that's exposed to HCV infection risk is the Healthcare workers. In the last 30 years, the transmission among this group decreased strongly, despite still needed control measures. Laboratory workers are at risk of a variety of hazards doing their work, such as collection, transport, processing, and analysis of patient specimens that represent risk for contamination.[61]

The prevention of HCV transmission is a challenge to public health authorities because it is very complicated follow a single strategy to control this disease. For IDU´s, for example, harm reduction programs have been tried in many countries with activities as condom distribution, serological screening, and disposable syringes exchange.[62] Healthcare workers integrated approach to prevention should be adopted. Education and training, elimination of unnecessary needles, adopting safe procedures for using and disposing of sharps, use of personal protection devices, and following appropriate surveillance and monitoring, response and follow-up of accidentally-exposed workers. , are all necessary elements to guarantee an appropriated working environment. .The development of an efficient vaccine to prevent HCV infection has been hampered because HCV is an RNA virus. In addition to the difficulty in raising protective immune responses in human beings using classic approaches, and also by the uncertain definition of the target populations.. These factors have caused many companies to withdraw from this field of investigation. The antiviral approach therefore probably will be the only option to control the HCV epidemic. This will be possible only by combining highly efficient and well-tolerated, affordable drug combinations, active screening strategies, and easy access to care.[63]

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