Interferon treatment of chronic hepatitis C. Basic imunobiology and imunopathology of the liver

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ABSTRACT

This article reviews the main aspects of chronic hepatitis C (epidemiological, genomic related to enhanced molecular understanding of the life cycle of the hepatitis C virus, HCV) especially standard therapy with pegylated interferon a and ribavirin. Emphasis is also placed on the immune response of the liver to HCV and the assessment of liver fibrosis via transient elastography.

Key words: Hepatitis C, Chronic ; Interferon-alpha; Ribavirin; Liver; Liver Cirrhosis; Spain; Prisons; Hepacivirus

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HCV VIRAL HEPATITIS: EPIDEMIOLOGICAL ASPECTS AND GENETIC SUSCEPTIBILITY FOR HEPATITIS C INFECTION

Viral hepatitis still are a first order public health issue worldwide[1], where the prevalence ranges between 1.5% and 3% among the population and are currently responsible for 90% of chronic hepatitis cases. As for chronic hepatitis C (CHC) it is considered the leading cause of post-transfusion hepatitis, terminal hepatic disease, indication for liver transplantation (35%) and hepatocellular carcinoma (HCC). It has been estimated that about 170 million people suffer from HCV induced chronic hepatitis C, of whom about 20% will develop cirrhosis and between 1% and 4% HCC every year. Standard treatment of CHC with Pegylated Interferon (IFN) and Ribavirin (RBV) entail a sustained virologic response (SVR) of about 50% in those infected by genotype 1 HCV (which is responsible for CHC in 75% of those infected by the virus). SVR is defined as undetectable HCV RNA levels 24 weeks after the treatment ends. In the case of HCV the cure is achieved in between 50% and 80% of cases. The percentage distribution of HCV genotypes among the Spanish population depicts a significant predominance of genotype 1 —most specifically 1b— which would account for over 70% of cases. Genotype 3 would be the second most common genotype, accounting for between 12% and 17% of all cases. Genotypes 2 and 4 are much less common: genotype 2 represents between 1 and 3% of all cases, and genotype 4 between 2% and 7%.

Currently there are effective treatments available to eradicate the causative hepatotropic virus. Several clinical assays are actively researching some very promising drugs derived from a better understanding of the HCV life cycle-whose RNA codifies over 10 different proteins[3]- its genetics and genomics, individual susceptibility to specific antiviral drugs and the management of the viral resistance to such drugs[4]. Allelic polymorphisms regarding the gene that codifies interleukin 28 (IL28) modify the kinetics of virologic response[5] but rapid virologic response (RVR) is actually the decisive factor in the prediction of the SVR (sustained virologic response). RVR is established when undetectable HCV levels are found 4 weeks after the initiation of treatment and sustained until the end of therapy. Genome-wide association studies (GWAS) through single-nucleotide polymorphisms (SNP) have shown the presence of a C-allele in a SNP near the ilyrtlrukin-28B gene in chromosome 19 which is associated to SVR[6], so that different responses are described according to different racial groups (see Figure 1).

INTERFERON: A PLEIOTROPIC CYTOKIN WITH ANTIVIRAL, ANTITUMORAL AND IMMUNOMODULATORY PROPERTIES

In 1957 IFN was discovered as a viral inhibition protein in chicken embryo cells exposed to the inactivated influenza virus[7]. The late 1960s saw renewed interest in IFN when Ion Gresser (1928-) an American researcher in Paris, discovered that the protein also slowed down the growth of tumors in mice and also stimulated the production of tumor-killing lymphocytes. Gresser and Finnish virologist Karl Cantell both developed a way to make IFN α in useful amounts from human blood cells. Monoclonal antibodies, first produced in 1975, made large-scale purification of interferon possible. In Christmas 1979 the Swiss scientist Charles Weissmann produced recombinant IFN from *E.coli*. In 2005 a group of researchers from Hoffman-La Roche detailed the structural and biophysical characterization of PEG-interferon alpha (2α) using NMR spectroscopy, analytical ultracentrifugation, circular dichroism, fluorescence spectroscopy, and differential scanning calorimetry[8].

Among the systems which have enabled the discovery and implementation of new drugs based on genetic and biochemical analysis we have HCV RNA replicons —pseudoparticles to study HCV cell entry mechanisms— and animal models mostly immunodeficient strains in which chimeras have been induced[9]. Another exciting research field is that of microRNA antagonists (antagomirs) which contain modified oligonucleotides of about 20 units or ribonucleotides conjugated with lipophilic compounds such as cholesterol which stimulate transport through the Robertsonian unitary lipid bilayer membrane in eukaryotic cells. From an informational point of view algorithms have been designed to predict antagomir attack target sequences from databases. MiR-122 is specifically expressed in hepatocytes and is crucial to the replication cycle of HCV replicons. Therapies with antagomir have proven efficient in the treatment of chronic hepatitis C in experimental models[10].

Pegylation is a chemical process of covalent attachment of polyethylene glycol (PEG) polymer chains to another molecule (normally a drug, peptide or protein) therefore increasing its therapeutic effect, prolonging its circulatory time, providing water solubility to hydrophobic drugs and reducing its toxicity. Ribavirin, also known as Virazole, is a synthetic nucleoside in which the nitrogenous base is thiazole-carboxamide with antiviral properties. Ribavirin may be administered orally, topically and on inhalation therapy. Ribavirin has proven to inhibit in vitro both DNA and RNA viruses such as myxovirus, paramyxovirus, arenavirus, bunyavirus, herpesvirus, adenovirus and poxvirus. RBV suffers a phosphorylation process in infected cells by means of tissue enzymes such as adenosine kinase. RBV monophosphate inhibits the synthesis of guanosine monophosphate, thereby reducing its intracellular levels. RBV triphosphate inhibits the enzyme guanylyltransferase thereby repressing the synthesis of viral mRNA and RNA polymerase. In vitro on high concentration it has also proven to inhibit HIV reverse transcriptase.

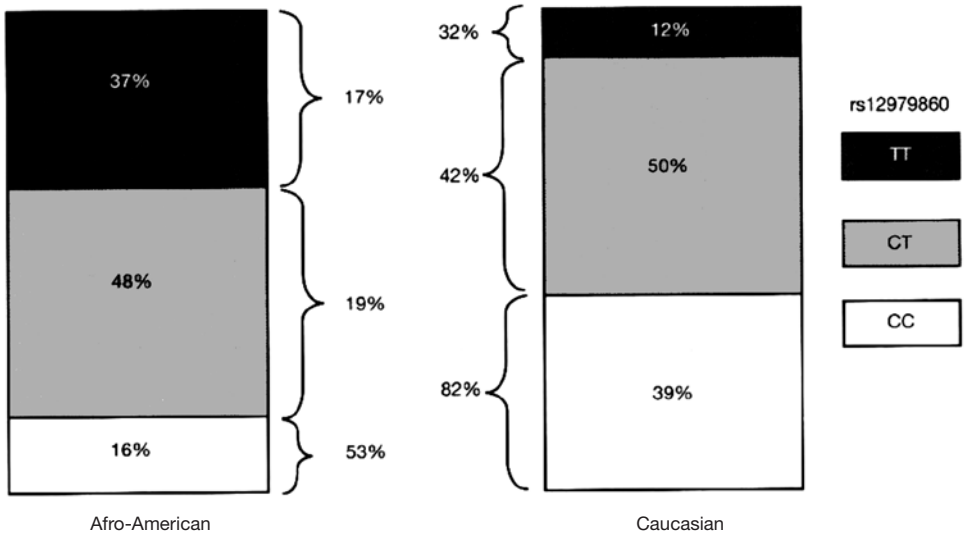

Figure 1: Sustained virologic response (SVR) according to ethnic group and host genotype.

Treatment regimen for CHC

Genotypes 1-4

Pegylated IFN α2a (180 µg once a week)
Ribavirin (200mg tablets):

- If <75 kg: 5 tablets per day (2-0-3)
- If >75kg: 6 tablets per day (3-0-3)

48 weeks

Genotypes 2-3

Pegylated IFN α2a (180 µg once a week)
Ribavirin (200mg tablets):

- 4 tablets per day (2-0-2)

24 weeks

Current CHC treatment guidelines recommend that patients with genotypes 2 and 3 be submitted to PEG-IFN α2a or 2b in combination with a basal dose of 800 mg of RBV for 24 weeks. Thin patients with normal platelet counts and undetectable HCV RNA levels for 4 weeks present low relapse risk rates after a brief treatment period; however genotypes 2 and 3 patients who relapse after a short period of treatment have over 70% of probabilities to achieve SVR after a 24week retreatment period.

In the *7th Conference of the Spanish Society of Prison Health* held in Seville from 11th to 13th November 2010 the results concluded by two Spanish studies (RibaDOT and EPIBAND) which have analyzed hepatitis C treatment in the imprisoned population were reported. In the *15th Meeting of the Spanish Society of Prison Health* held in Orense in the 21st-22nd October 2011 the joint analysis of the studies RibaDOT and EPIBAND were presented thereby pointing out the high cure rate achieved among patients in the imprisoned population[11-12]. The prevalence of HCV in the imprisoned population is the highest of all chronic infectious diseases. As far as genotypes are regarded genotype 1 is the most common and accounts for 65% of cases, genotype 3 for between 20% and 22% of cases although it is more prevalent among IDUs, genotype 4 accounts for between 8% and 12% of cases, and genotype 2 for between 2% and 8%, and is more prevalent among HIV coinfected patients.

TRANSIENT ELASTOGRAPHY AND FIBROSCAN® IN CHRONIC HEPATITIS C (CHC)

In the evolution of chronic forms of hepatitis C progress towards liver fibrosis is a very relevant factor, especially in those patients coinfected with HIV and HCV, in whom the progression is assessed by Fibroscan®-transient elastography-[13] (see Figure 2). Towards 2004 Dr. Xavier Forns from the Hepatology Service in *Hospital Clinic* in Barcelona got acquainted with his technique in an American Liver Conference and contacted the company Echosense, in Paris, which counted with two or three members at the time. They had a small office in the centre of Paris and after visiting they agreed to cede a machine to *Hospital Clinic* to initiate the studies which would later be spread across Spain (Xavier Forns, personal communication). Echosense® achieved the approval by the European Community for FibroScan®502 in 2003. Currently there are over 1000 FibroScan units worldwide (Aurélie Houet, Marketing & Communication Manager, Echosense). The performance of vibration-controlled transient elastography has been long proved by clinical essays in many etiologies. There is a series of indirect serum indexes used for non-invasive assessment of fibrosis in chronic viral hepatitis which include the combination of two or more of the following parameters: platelet count, hyaluronate, α2 macroglobulin, cholesterol, prothrombin index, AST, albumin, apolipoprotein A1, haptoglobin, total bilirubin (Fibrotest, Forns Index, FibroSpect, Hepasocre, etc.). The AST/ALT ratio provides inconsistent results, AST to platelet ratio index (APRI) does not surpass the information value provided by liver biopsy, Fibrometer (based on platelet count, prothrombin index, AST levels, α2 macroglobulin, hyaluronate, blood urea nitrogen and age) provides an acceptable predictive capability of fibrosis in chronic viral hepatitis, ActiTest incorporates ALT levels to FibroTest parameters as to depict liver fibrosis and necroinflammatory activity.

ANALYTICAL CONTROL OF CHRONIC HCV INFECTION

Approximately one third of patients infected by HIV in the United States are coinfected by hepatitis C (HCV). HCV liver disease has become the leading cause of mortality among HIV infected patients, partly due to longer survival times because of the availability of highly active antiretroviral therapies (HAART). Standard treatment of chronic HCV infection for those patients without HIV infection consists of a combination of pegylated interferon (PEG-IFN) and Ribavirin (RBV). This regimen has proven superior to the "standard" therapy with IFN and Ribavirin. PEG-IFN/Ribavirin entails sustained

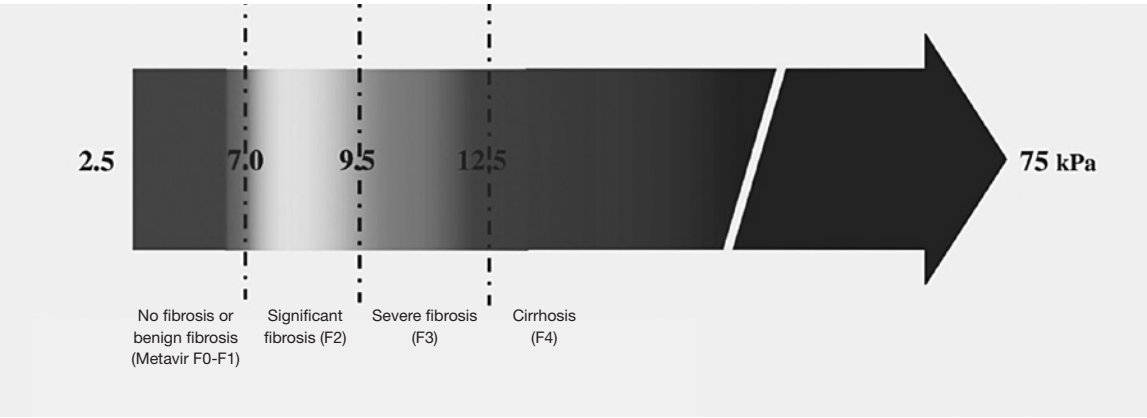

Figure 2: Values of transient elastography and correlation with degree of hepatic cirrhosis.

virologic responses of about 50-60% of HCV patients without HIV. Nevertheless efficacy and safety have not been assessed in patients with HIV, and the first studies (mostly observational) with PEG-IFN/RBV in coinfected patients were not very encouraging. Due to the promising results achieved with PEG-IFN/RBV in HCV patients without HIV and the need to better manage the infection by HCV in coinfected patients, several groups have thoroughly assessed the treatment with PEG-IFN/RBV in randomized trials. Two studies on this issue recently published in the New England Journal of Medicine proved that PEG-IFN/RBV entailed a significant sustained virologic response and was relatively well tolerated. In both studies, antiviral therapy against HCV did not seem to have any adverse effects on the control of the HIV disease.

The largest of studies corresponds to 2004, when Torriani and colleagues, assessed 868 patients coinfected with HCV and HIV from 19 countries. Among other inclusion criteria, patients presented HCV basal viral loads of > 600 IU/ml and liver biopsy results were compatible with HCV chronic infection in the last 15 months. Patients were treated with one of the three HCV treatment regimens for 48 weeks and were monitored for another 24 weeks. The main objective was to achieved sustained virologic response, defined as serum HCV RNA levels of <50 IU/ml by the end of the monitoring period. Patients were assigned one of the three HCV treatment groups: PEG-IFN α2a (180 µg once a week) plus Ribavirin (800 mg per day); PEG-IFN α2a plus placebo or IFN α2a (3 million IU 3 times per week) plus Ribavirin.

The global rate of sustained virologic response (SVR) was of 40% among patients treated with PEG-IFN α2a plus RBV against 12% among those who received IFN α2a plus RBV (p<0.001). PEG-IFN α2a plus placebo also was superior to the treatment with IFN α2a plus RBV (40% against 20%, p<0.001). In the group of those infected by genotype 1 HCV, SVR rates were 29% with PEG-IFN α2a plus RBV, 14% with PEG-IFN α2a plus placebo and 7% with IFN α2a plus RBV. Corresponding rates among those infected by genotypes 2 or 3 were 62%, 36% and 20% respectively. The authors point out that the spectrum and frequency of adverse effects were similar to those published regarding the treatment of HCV infection in the absence of HIV infection. While neutropenia and thrombocytopenia were more common among those patients treated with regimens which included pegylated interferon, anemia was most frequently found among those treated with ribavirin. Researchers found out that those patients who do not achieve early virologic response (EVR) (HCV RNA levels <50 IU/ml or a reduction of 2log or more on week 12, which means that RNA levels are detectable on week 4 but undetectable on week 12) will rarely achieve sustained virologic response. In another study published in the same edition of the New England Journal of Medicine, Chung and collaborators (2004) included 133 individuals coinfected with HCV and HIV and were randomly assigned to receive either 180 mg of PEG- IFN α2a every week during 48 weeks or 6 million IU of IFN α2a twice or three times every week during 12 weeks followed by 3 million IU three times a week during another 36 weeks. Both groups received Ribavirin according to schedule of increasing doses. Treatment with PEG-IFN/RBV associated a remarkably higher rate of sustained virologic response- HCV RNA levels of <60 UI/ml on week 24 after the end of the treatment- in comparison with IFN/ribavirin treatment (27% against 12%, p=0.03). In the group treated with PEG-IFN/RBV only 14% of patients infected by genotype 1 HCV achieved sustained virologic response while 73% of patients with other genotypes achieved so (p<0.001).

Histological response was observed in 35% of those without virologic response, and therefore authors point out that such regimens would provide clinical benefits even in the absence of viral elimination. Alike the study by *Torriani and collaborators*, the absence of predictors of uniform early virologic response would entail the uselessness of HCV treatment, so that *Chung and collaborators* recommend to consider the interruption of treatment in week 12 for patients with minimal fibrosis. However they recommend that therapy be continued in patients with advanced disease and a similar response to HCV treatment since the main objective is to avoid the progression of the disease in the liver instead of eradicating the virus. Despite the encouraging results achieved in both studies, successful treatment rates in patients coinfected by HCV and HIV were lower than those observed in patients without HIV. Moreover, strategies are needed to improve the results in patients infected with treatment-resistant HCV genotypes. In patients with chronic hepatitis C and null response to standard treatment regimens there are direct antiviral alternatives such as Boceprevir and Telaprevir available for triple therapy retreatment[14] along with other antiviral drugs in phase of R&D[15] which have achieved SVR rates of over 60% in clinical trials. Some of the experimental data suggest a synergic mechanism in the coinfection by HCV and HIV between the regulation of the expression of Fas in CD4+ T cells induced by HIV and high levels of FasL both soluble and in cells. Both effects jointly contribute to the apoptosis of CD4+ T cells in coinfected patients. Although the mechanism of action remains unclear in experimentation animals, the proteolytic excision of protein NS2/3 in HCV is crucial for the persistence of the viral infection. This is a singular protein since it lacks sequence homology in the phylogenetic scale and therefore constitutes a very promising target for the pharmaceutical industry as a powerful antiviral therapy.

Virologic response during HCV treatment has also associated the presence of high concentrations of sTNF-RI (soluble TNF receptor type I). In other cases, the apoptosis of hepatocytes implies other biochemical signalization pathways. As for the life cycle of the hepatocyte it has been published that the arrest of the cell cycle takes place at G2 interphase therefore blocking the initiation of mitosis [16]. It looks like the apoptosis in chronic HCV infection is mediated by a group of enzymes called caspases and whose functional blockage may entail the slowdown of the progression towards liver fibrosis. In the liver IFN-γ is able to induce the apoptosis of hepatocytes or to inhibit the progression of their cell cycle. Associations of the NK (natural killer) phenotype with the genotype IL28B and gene expression patterns have also been described, as well as the role of NK cells in the elimination of the virus in IFN-induced chronic hepatitis C. Recent publications have proven mitochondrial dysfunction in chronic hepatitis C therefore implying an immunologic pathogenesis through a redox mechanism.

BIOLOGICAL RESPONSE OF THE LIVER AGAINST THE VIRAL AGGRESSION

Normal adult livers contain 10^{10} lymphoid cells located in portal tracts or scattered in the parenchyma. Lymphoid cells in the human blood and liver are distributed as Figure 3 depicts. One of the main functions of this organ may be related to the extrathymic generation and maturation of lymphocytes, a much debated issue with important implications in the maintenance of a functional immune system through life, especially after the involution or deterioration of the structures in charge of such function, mainly the bone marrow and the thymus. The role of the liver during the embryogenesis of the immune system is known with some of its peculiarities: the presence of lymphoid progenitor stem cells which can also lead to myeloid cells, the absence or T cell receptor (TCR) gene rearrangement, the activity of the enzyme terminal deoxynucleotidyl transferase (TdT) and the expression of Major Histocompatibility Complex (MHC) class II molecules. In adults such generation function could be preserved.

Lymphocyte type	Peripheral blood (%)	Liver (%)
T αβ	72.0	37.0
NK	13.0	31.0
B	10.0	6.0
Tγδ	3.0	14.0
NKT	2.0	12.0

Figure 3: Distribution of lymphoid cells in the human blood and liver

T cells respond to pro-inflammatory cytokines synthesized by hepatocytes and Kupffer cells, by releasing interferon γ (IFNγ), tumoral necrosis factor α (TNF α) and IL-2. TNF α or cachectin acts as an autocrine regulation factor in the synthesis of IL-

2, through the activation of nuclear transcription factor NF-κβ [17-18]. 5% of T cells produce Th2 profile cytokines and IL-4 but not IL-5; other produce IL-4 and IFNγ at the same time, and are therefore classified as Th0. Once stimulated with cytokines, T cells are able to generate Lymphokine-Activated Killer (LAK) cells, with powerful cytolytic activity. Between 15% and 35% of T lymphocytes are T γδ which means that the liver is one of the richest sources of this subpopulation in the body. Among all the functions attributed to these lymphocytes apart from the elimination of antigens, the reparation and regeneration of liver tissue and the regulation of T αβ lymphocytes, it has been postulated that they play a regulatory role in the differentiation of Th1/Th2 on account of their capability to secrete Il-4 and IFNγ. This last aspect is crucial in the control of viral hepatitis. A third population includes natural killer T cells which simultaneously express CD3 and CD56 markers. All these three populations infiltrate and reject tumors. The first description of NK cells was precisely made in the liver, although in another species, the rat. They represented between 30% and 50% of all hepatic lymphocytes. If we take into account that hepatocytes express low levels of MHC class II, NK would be of paramount importance in the control of viral hepatotropic infections. There is a dynamic balance in the immunological response due to the existence of suppressor T cells discovered by the early-deceased Richard ("Dick") K. Gershon (1932-1983). Experimentally peptides which inhibit IL-10 have been designed so that the dendritic cell-mediated response against HCV is stimulated[19].

The risk of chronic infection by HCV is high. Between 80% and 100% of patients still have detectable HCV RNA levels after acute hepatitis. In most of them there is also persistent elevation of liver enzymes. By definition, hepatitis C is considered chronic when it lasts for over 6 months. Once the chronic infection has been established there is a minimum rate of spontaneous elimination. There are still poorly known genetic factors which could explain the chronicity of this viral infection. The diversity of HCV and its ability to generate mutations allow it to "escape" immunological recognition molecular systems. Individual host factors could play a role in the spontaneous clearance of the virus. The eradication of HCV is carried out by HCV specific CD4+T cells, having observed high levels of neutralizing antibodies against structural proteins of HCV and restricted to MHC-DRB1 and DQB1.

The infection by HCV during childhood seems to associate a lower risk of chronic infection, approximately between 50% and 60%. Certain ethnic groups show a lower risk of chronicity without us knowing why. Phase I and II clinical trials are being implemented in healthy volunteers and infected patients regarding a vaccine for HCV which includes peptides, DNA recombinant proteins and other vaccines based on viral vectors[20]. In a chimpanzee model prophylactic vaccines including recombinant envelope glycoproteins gpE1/E2 have been used as adjuvant therapies or immunization regimens with defective adenovirus and DNA plasmids which express non-structural genes[21].

Currently a Joint European Project is being run to develop a vaccine against HCV (HCVAX) based on nanotechnology. The transnational consortium which includes a group of German, French and Swiss researchers is trialing innovative biocompatible nanogels which allow that the viral genetic information be transported to the organism by means of the so called "RNA replicons". Synthetic nanogels have a diameter of barely a few nanometers and are composed of a bio-polymeric matrix. Immune cells will be in charge of the nanogels which contain the genetic information and will develop harmless HCV compounds. Therefore immune cells will respond against these strange structures and will produce a pool of memory cells. If so, the vaccine would be effective against a potential infection by the pathogen HCV.

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