INTERFERON ALPHA (IFN-A) AND LAMDA (IFN-Λ) ROLES ON HCV AND THE THERAPEUTICS OPTION

Ilham T Qattan
College of Medicine and Applied Medical Sciences, Medical Laboratories Technology Department, Taibah University, AlMadenah AlMonwara, KSA

Abstract

Since the discovery of the hepatitis C virus, the goal of all treatment is to clear the virus and normalise the liver function, stopping the progression of the disease and thus reducing long-term complications of cirrhosis and hepatocellular carcinoma. The therapy for CHC used to consists of pegylated interferon alpha in combination with ribavirin and others new approved protease inhibitors, but still few of those treated can not achieved a compleat SVR. The reasons for failure are unknown, but may result from viral and host factors combined. The review here is to highlights and compare of what has been published previously.

Keywords: HCV,CHC, SVR, RBV, IFNs

Interferon alpha (IFN-α)

Interferon alpha is a member of the Interferon family, comprising a large group of multifunctional secreted proteins which have anti-viral, immunomodulatory and anti-proliferative activities. The Interferons can be classified into three types: Type I IFNs consist of IFN-alpha (IFN-α), IFN-beta (IFN-β) and IFN-omega (IFN-ω). They are produced in direct response to the virus infection and induce intracellular signalling pathways to activate transcription factors, such as the Interferon regulatory factors (IRF)-3, IRF-5, IRF-7 and NF-κB, which in turn initiates the transcription of interferon sensitive genes. IFN-α is a multi-gene family comprising more than 20 types which are synthesised by leukocytes, whereas IFN-β synthesis is seen in most cell types, in particular fibroblasts. The type II IFNs consist of INF-gamma (IFN-γ), which is synthesised by activated T lymphocytes and NK cells, in response to cytokines such as IL-12 and IL-18 or via the stimulation of T-cell or NK cell antigen receptors (Lemon et al, 2010). The third type is IFN–lamda (IFN-λ) or interleukin 28/29 - a class of cytokine with IFN-like activity.

Alpha (α) and Beta (β) IFNs bind to cell surface receptors which comprise two major sub-units, IFNαR1 and IFNαR2, whereas gamma interferon binds to IFNγR1 and IFNγR2. They then act through two distinct but related pathways. The pathway for IFN-α will be discussed in some detail. The binding of IFN-α to its receptor initiates the Janus Tyrosine kinase JAK–signal transducer and activator of the transcription STAT pathway. In humans, four JAKs (JAK 1-3 and Tyk2) and seven STATs (STAT 1-7) have so far been identified.

The ligand-receptor binding causes a conformational change in the cytoplasmic part of the receptor, which in turn activates the receptor associated kinases Tyk 2 and JAK1. Tyk2 then phosphorylates the tyrosine at amino acid 466 on the IFNαR1 to create a docking site for STAT2. Next, Tyk phosphorylates STAT2 at tyrosine 690, serving as a platform for the recruitment of the STAT1, which also becomes phosphorylated, at tyrosine 701. STAT-1 homodimerises and then translocates to the nucleus and binds to gamma-activated sequence GAS enhancer elements with the sequence TTC(N)2-4GAA, which drives the expression of nearby target genes. STAT-1 dimers and STAT 1 and 2 hetero-dimers also bind to IRF-9 p48, a member of the previously mentioned interferon regulatory factor family, to form a trimeric complex. In the case of STAT1/2, this is termed the IFN stimulated gene factor-3 ISGF-3, which then goes on to bind to IFN-stimulated regulatory elements ISREs with the sequence AGTTT(N)3TTTC found within the promoter region of IFN sensitive genes. ISREs drive the expression of most IFNα regulated genes.

Following HCV infection, the engagement of the cell receptors, such as the pathogen-associated molecular pattern (PAMP) or toll-like receptors (TLRs) and retinoic acid-inducible gene-1 (RIG-1), can initiate the signalling pathways to lead to production of IFN-α and other cytokines. Up to 150 genes which are stimulated by IFNα have been identified, including double stranded RNA protein kinase R, which inhibits protein synthesis, 2', 5'-oligoadenylate synthetase, which leads to RNA degradation and the Mx GTPase protein, which inhibits viral replication (Lemon et al, 2010).

Regions of the HCV genome can participate in the disarming of host antiviral defences (Taylor et al, 1999). HCV core and envelope E2 proteins have been shown to impair IFN-α signal transduction. In transient and stably transfected Huh7 cell lines, HCV core protein can lead to diminished STAT1 accumulation and promote its proteasome-dependent degradation; it can also impair the IFN-α induced STAT1 activation and reduce the binding of ISGF3 to the nuclear IFN-α ISRE (Lin et al, 2005). However, structural and functional studies have shown that the N-terminus of the HCV core region directly binds to STAT1 at its SH2 domain, which can impair subsequent IFN signalling and can in turn suggest a model to direct the interaction of the

HCV core with STAT1 to block its recruitment and phosphorylation by Jak1 (Lin et al, 2006).

Studies in the chimpanzee model of HCV infection have shown that IFN-α response are detectable within the liver during the first week of acute HCV infection. The expression of IFN-α can alter the composition and proteolytic function of the immunoproteasome, the major antigen-processing enzyme complex which generates the major histocompatibility complex class-I bound peptides recognized by virus-specific CD8-T cells. This effect is observed not only in primary human hepatocytes and hepatoma cell lines but also in vivo in an HCV-infected chimpanzee model, where the expression kinetics of IFN-α in liver correlated closely with changes in proteasome composition (Shen et al, 2006). Furthermore, in acute HCV infection, IFN-α encourages recruitment of virus specific T cells in the liver, whereas, during antiviral therapy in chronic infection T cell responsiveness reduce and exogenous IFN-α acts as an antiviral agent (Rahman et al, 2004).

Interferon Lamda (IFN-λ):

It has been found that in the interferon (IFN) cytokine family a new was known as IFN III or as – Lamda (IFN-λ) with three types: IFN-λ1 (IL-29), -λ2 (IL-28A) and -λ3 (IL-28B) were interfering with the responsivity to the HCV treatment and were encoded by 3 different genes which are located on chromosome 19 (Kotenko et al., 2003; Sheppard et al., 2003). At the amino acid level IFN-λ2 and -λ3 are closely similar, having a 96% sequence identity while IFN-λ1 shares approximately 81% sequence identity with IFN-λ2 and IFN-λ3. The sequence of IFN-λ3 was shown to have two polymorphisms; G and C at the start codon upstream of the transition nucleotides 37. These two polymorphism residues are located at the AB loop of the IFN-λ structure in a variable position flanked by the three isoforms (S in λ1, R in λ2 and K in λ3). A study by Thomas in 2008/09 determined that these polymorphisms were involved in HCV's type of response to therapy (Thomas et al, 2008 and 2009).

Type III IFN or IFNλ3 were known also as a polymorphism of IL28B was associated with a better SVR in patients receiving peg-IFN-α and RBV. The virus is influenced by human genetics, as was identified by the Genome-wide association study (GWAS) in 2009, Ge in the same year identified an SNP (rs12979860) located in chromosome 19,3kb and then several publication of GWAS indicated that the genetic polymorphisms near the IL28B gene on chromosome 19 were associated with the improvement of treatment, with a direct correlation to the SVR in chronic HCV patients (Ge et al, 2009).

When HCV infected the hepatocytes it can induce the expression of IFN-α/β and IFN-λ genes, in order to lead to the phosphorylation of each STAT1 and STAT2, thence forming STAT1-STAT2 heterodimers. The

dimers then bind to IRF9 and form the ISGF3 complex, whereupon they migrate to the nucleus to bind to the ISRE elements in order to facilitate the transcription of ISGs.

The binding receptors of IFN-λ can form the complex which is needed to activate the JAK1 and TYK2. It also consists of an intracellular domain of 270 aa (Hamming et al, 2009). The two kinases of the IFN-λ cross-phosphorylate to activate one another for the phosphorylation of the three tyrosine residues on the intracellular part of IFNλ- R1: Tyr343, Tyr406 and Tyr517. Then the Tyr343 and Tyr517 create a docking site for the Src Homology 2 (SH2) domain of the transcription factor STAT2 (Hamming et al, 2010). When STAT binds to IFN-λR1 it activates JAK1 and TYK2 and allows for the phosphorylation to take part of the tyrosine residue towards the C-terminal end of the STAT proteins. A docking site then serves for the SH2 domains. The fact that the STATs 1 and 2 activation allows the joining of IRF9 to form the ISGF3 complex is considered the main gate for IFN-λ activation, which in turn activates the STATs 3 and 5 (Kelly et al 2011). The ISGF3 complex induces the transcription of the interferon stimulated genes (ISGs) by its translocation into the nucleus and interacts with a specific DNA sequence designated the IFN stimulated response element (ISRE), in order to bind to the gamma activated sequence (GAS) and induce expression of the gene (Dumoutier et al, 2004).

IFN-α/β and IFN-λ were reported to activate the MAP kinase pathway through JAKs and p38 phosphorylation, as mentioned in the first chapter, above. IFN III can also raise the levels of MHC Classes I and II, as well as the chemokine receptor CCR7, to stimulate the migration of DCs to the lymph nodes and the spleen, so as to induce immunity and display its antiviral effects (Walter et al, 2004; Lasfar et al, 2006; Ank et al, 2006); it is antiproliferative and acts with type I IFN as being immunomodulatory for the Th1/Th2 balance in the immune responses (Bartlett et al, 2005; Ank et al, 2009; Dellgren et al, 2009; Parlato et al, 2010; Lasfar and Cohen-Solal, 2011).

Kurosaki in 2011 conducted a study to investigate the possibility of developing a model for the pre-treatment prediction of response using host and viral factors. The researchers found that the IL28B polymorphism correlated with early virological response and predicted null virological response (NVR) (odds ratio = 20.83, $p < 0.0001$) and sustained virological response (SVR) (odds ratio = 7.41, $p < 0.0001$). Furthermore, it was revealed that mutations in the ISDR were able to predict relapse and SVR independent of IL28B. Moreover, the using of this model showed that patients with the minor IL28B allele and low platelet counts had the highest NVR (84%) and lowest SVR (7%). On the other hand, those with the major IL28B allele and

mutations in the ISDR or high platelet counts had the lowest NVR (0–17%) and highest SVR (61–90%) (Kurosaki et al, 2011).

In another study, the effects of IL-28B polymorphisms on response to treatment with peg interferon and ribavirin were investigated in a well-characterized cohort of genotype 2/3 patients. The results showed that an IL-28B polymorphism was associated with an SVR in patients infected with genotype 2/3 HCV who did not achieve a RVR. They conclude that analysis of IL-28B genotype might benefit the guiding treatment for these patients (Mangia, 2010). Such findings were similar to results reported by (Rauch et al, 2010) in which they found genetic variation in interleukin 28B (IL28B) gene as the strongest predictor for the control of HCV infection. Furthermore, it was a significant finding that single nucleotide polymorphisms (SNPs) in IL28B gene were strongly able to predict both spontaneous and treatment-induced HCV recovery.

A study by (Diegelmann et al, 2010) showed that IL-28A and IL-29 gene transcription was up-regulated in HCV patients and showed similar effective pattern in inducing antiviral genes and inhibiting HCV replication. Thus both IFN-λs may have therapeutic potential in the treatment of chronic HCV.

Clark in 2011 conducted a review of the output and possible future implications of the recent discoveries of Genome-wide association studies (GWAS). These studies have recently identified the critical role of host genetic variation in predicting treatment response and spontaneous clearance in patients infected with hepatitis C virus (HCV). Studies revealed that SNPs in the region of the IL28B gene are strongly associated with treatment response to pegylated IFN and ribavirin in patients infected with genotype 1 HCV. It has been found that the good response variant to be associated with a twofold increase in the rate of cure. Because the allele frequencies vary between ethnic groups, it can be largely explain the observed differences in response rates between Caucasians, African Americans and Asians. IL28B polymorphism has also been found to correlate well with spontaneous clearance of HCV (Clark, 2011). The general background of information in the role of interferon in HCV treatment implies that biological mechanisms responsible for these genetic associations remain unknown and are the focus of ongoing research. As previously stated, knowing a patient's IL28B genotype will help in clinical decision making with standard of care regimens. It is thought that in future, clinical studies will focus on the potential use of IL28B type for individualizing treatment duration and novel regimens (Clark, 2011).

IFN Phosphorylation

The different phosphorylates occurs at the tyrosine to form STAT dimers which translocate into the nucleus to bind to the protein and form a

hetero-tri-meric complex ISGF3 that binds directly to the IFN sensitivity responsive gene ISRE on the DNA to induce the transcription (Tibotec, 2009).

Therapeutic approaches to control HCV infection

Interferon Alpha (IFN-α)

Treatment of the chronic hepatitis C virus (CHCV) with interferon-based therapy has evolved dramatically since the discovery of the hepatitis C virus in 1989. The therapy for chronic HCV infection has evolved from interferon IFN monotherapy,(Di Bisceglie et al, 1990) through to combination treatment of pegylated interferon α and ribavirin as the standard therapy for patients with chronic hepatitis C infection (CHC) (table 1) (Chang et al, 2011).

Two types of recombinant interferon are widely used; pegylated interferon alpha 2a (peg-IFN-α-2a) (Pegasys®, Hoffmann La-Roche) and pegylated interferon alpha 2b (peg-IFN-α-2b) (Peg-Intron, Schering-Plough-Viraferon®). These two forms of IFN-α are modified with a polyethylene glycol chain (40kD) covalently bound via a stable amide bond at a lysine residue (Anonymous peg-IFN-α, 2002). PEG is usually non-toxic and greatly alters the pharmacokinetic properties of the proteins to which it is attached (Fried, 2001; Nieforth et al, 1996).

Pharmacokinetic studies have demonstrated that plasma concentrations of IFN-α can fluctuate greatly. Conjugation with PEG increases the half-life of IFN-α to 168 hours allowing a course of peg-IFN-α to be administered as a once-weekly injection of a fixed dose minimising peak/trough variation (Xu et al, 1998; Perry and Jarvis, 2001). The metabolic products in peg-IFN are excreted by the kidney, with 15% of the total dose being recovered from the urine after 14 days, compared with standard IFN (Perry and Jarvis, 2001).

Ribavirin, a nucleoside analogue has a half-life of approximately 140-160 hours and can accumulate after multiple dosing, increasing the concentration level up to four-fold (Anonymous ribavirin COPEGUS®, 2003). Both forms of peg IFN-α are given by subcutaneous injection once a week, while ribavirin is administered orally once daily. HCV genotypes 2 and 3 require only six months' treatment, unlike genotype 1, which requires 48 weeks. Significant side effects are occasionally seen during treatment, including 'flu-like symptoms, depression and thrombocytopenia associated with interferon and hemolyticanemia with ribavirin. Side effects are sometimes serious enough to warrant stopping treatment or reducing the dose (Chany et al, 2009).

The aim of therapy is to a achieve a sustained virological response which is the loss of detectable HCV RNA during treatment and its continued absence for at least 6 months after stopping therapy. About 60% of patients

subjecting to treatment with an initial 48-week regimen of pegylated-interferon and ribavirin, do not reach SVR(McHutchison et al, 2009; Ghany et al, 2009). There is a remaining risk for progressive liver disease if treatment is not efficient and a person does not achieve a SVR (Morgan , 2008). Genotypes 2 and 3 tend to have better response rates (78% - 84%) than genotype-1 (42% - 52%) (Fried et al, 2002; Hadziyannis et al, 2004; Manns et al, 2001). Long-term follow-up of SVR patients has demonstrated that the response is durable in around 95% of patients when there is an end of treatment response (ETR). Both ETR and SVR depend greatly on the early response to treatment (Sarasin-Fillipowicz et al, 2008).

Patients who do not show an early virological response (EVR), defined as a decline of the viral load by > 2 log10 after 12 weeks of therapy, are unlikely to develop an SVR, whereas those patients who develop the EVR (around 65%) have a good chance of being cured, (Fried et al, 2002; Davis et al, 2003). In fact, the prognosis is even better in patients who have a rapid virological response (RVR), which is defined as serum HCV RNA being undetectable after 4 weeks of treatment. Approximately 85% of these can achieve SVR (Ferenci et al, 2005). Unfortunately, approximately 20% of patients with genotype 1 and around 60% of patients with genotypes 2 or 3 show an RVR (Yu et al, 2007). The early prediction of virologic response to interferon-based therapy can help identify patients who are unlikely to have a sustained response and allow clinicians the option to discontinue treatment, saving patients the side effects and healthcare systems the cost of additional therapy.

In patients with both HCV genotype 1 and high base-line viral loads, a substantially higher proportion obtained a SVR when treated with peg-IFN-α-2a and ribavirin, compared to those treated with peg-IFN-α-2b and ribavirin. As usual with interferon-based therapy, reductions can occur in neutrophils and platelet counts, however these reductions were greater in patients treated with peg-IFN-α-2a and ribavirin than in those treated with peg-IFN-α -2b and ribavirin. These phenomena generally were not serious and were effectively managed by dose modifications. Interestingly, in patients treated with peg-IFN-α-2a and ribavirin who had an EVR, the completion of therapy with dose reduction was not associated with a substantial decrease in efficacy and peg-IFN-α-2a was able to significantly enhance the SVR in CHC patients, regardless of HCV genotype and viral load (Fried et al, 2002).

Despite the improvements in treatment, many patients do not respond completely. Patients who have relapsed are more likely to be successfully re-treated than non-responders. Approximately 48% of relapsed patients who were treated with IFN monotherapy can achieve an undetectable viral load during treatment. In contrast, in 82% of initial relapses, re-treatment with a

combination therapy of standard interferon and ribavirin can reduce to HCV RNA below detectable levels whereas 47% can achieve SVR (Davis et al, 1998). Among non-responders (NR) to IFN monotherapy 13% - 15% of patients achieve a SVR when re-treated with standard IFN and ribavirin, while 25% - 40% achieve a SVR when re-treated with peg-IFN-α and ribavirin. In non-responding patients, the viral load increases, although some have no decrease in viral load during therapy, or experience only a modest decrease, from 1-2 logs. Others have a reduction of at least 2 logs, but their viral load remains detectable during therapy; this is known as a partial or transient response. Patients who experience a significant reduction in viral load may demonstrate a reduction of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) levels and reduced liver tissue damage, even if they do not achieve an undetectable viral load (Gow and Mutimer, 2001).

ALT and AST belong to a family called transaminase enzymes. Alternatively, ALT and AST are called glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT), respectively (Tolman et al, 1999).

ALT is mainly produced in the liver and work to catalyze the transfer of amino groups between L-alanine and glutamate to meet physiological needs. AST works to catalyze the transfer of amino and keto groups between alpha-amino acids and alpha-keto acids (Tolman et al, 1999). In case of having injured or inflamed liver, this is usually accompanied by increased levels of ALT in the blood; therefore, this test is important to assess liver disease. AST is found in liver as well as several tissues including the heart, muscle, kidney, brain, and lung. If damage occurred in AST containing tissues, AST is released into the bloodstream (Tolman, 1999; Pratt, 2000).

New Antiviral Drugs for HCV

Due to limitations of current treatment for viral hepatitis C, there is a need to develop a new generation of therapies. Telaprevir and boceprevir (NS3/4A serine-protease inhibitors), and Filibuvir (a non-nucleoside NS5B RNA polymerase inhibitor also known as RG-7128, RG-7227 and ANA-598) are three such compounds that have either been licensed of undergoing advanced clinical evaluation (Kronenberger and Zeuzem, 2012). It is likely that these new drugs will be used in combination with the current standard treatment of pegylated interferon plus ribavirin. (Fernández-Montero et al, 2011). According to the results published by Vermehren and Sarrazin in 2011, monotherapy with protease inhibitors has shown high antiviral activity, but there is a problem associated with the frequent selection of resistant HCV variants.

The antiviral activity, pharmacokinetics, safety and tolerability of multiple doses of Filibuvir in treatment-naïve and treatment-experienced

patients chronically infected with HCV genotype 1 has been investigated in two Phase 1b clinical studies (Study 1 was a randomized, placebo-controlled dose escalation study and Study 2 was a non-randomised, study) . Filibuvir was given at doses ranging from 200 mg to 1400 mg daily over a period of 3 to 10 days.

The results showed that Filibuvir was a potent dose-dependent inhibitor of HCV replication with a mean maximum HCV RNA change from baseline ranging from -0.97 log(10) IU/ml with Filibuvir 100 mg BID (twice daily) to -2.30 log(10) IU/ml with Filibuvir 700 mg BID in treatment-naïve patients. In treatment-experienced patients, an HCV RNA reduction of 2.20 log (10) IU/ml was achieved with Filibuvir dose of 450 mg BID. The results showed that Filibuvir was well tolerated in both studies. Mutation at amino acid 423 of the HCV NS5B protein was the predominant site of mutation following Filibuvir treatment. There are current trials to use Filibuvir in combination with peg IFN-α 2a plus ribavirin in treatment-naïve patients (Fernández-Montero et al, 2011).

It has been shown that there is a 66 amino acid sequence within NS5A which is capable of binding to PKR and inhibiting protein synthesis (Gale et al, 1998). It is within this region that a smaller (40 amino acid) domain residing between amino acids 2209 and 2248 has been defined as the Interferon Sensitivity Determining Region (ISDR). (Enomoto et al, 1995 and 1996) were the first to describe an association between mutations in the ISDR and the response rate; they found that patients infected with a virus with more than 4 mutations in the ISDR, compared to the HCV1b prototype HCV-J, were more likely to respond to interferon alpha therapy. These findings were confirmed, mainly in Japanese studies (Komatsu et al, 1997; Chayama et al, 1997). Other studies in the USA and Europe have failed to find such an association in patients infected with the HCV genotype 1b (Squadrito et al, 1997; Chung et al, 1999; McKechnie et al, 2000) and indeed only a small number of mutations in the ISDR region have generally been seen in isolates from these countries.

Studies have shown that HCV NS5A is able to reduce ISG expression and the Jak-STAT signaling downstream of the IFN-α receptor is inhibited by the HCV core protein. IFN production, as a result of dsRNA binding by TLR3, is blocked by the HCV proteases NS3 and NS4A, which cleave the TLR3 adaptor TRIF between amino acids 372 and 373 (Li et al, 2005). However, it is now known that HCV suppresses responses from intracellular dsRNA, since NS3 and NS4A cleaves MAVS at Cys-508. After cleavage, the MAVS diffuse away from the mitochondria and cannot activate IRF3 and NFκB (Li et al, 2006; Meylan et al, 2005).

Replication of HCV can also interfere with IFN-α signaling through activating and/or inhibiting the other pathways. One such inhibitor is the

protein phosphatase-2A (PP2A). The hypomethylation of STAT1 promotes the binding to the protein inhibitor of the STAT-1, in order to combat the inability of the phosphorylated STAT1 to bind to the DNA (Duong et al, 2005). Interestingly, in vitro, S-adenosyl methionine (SAMe; the principal methyl donor for STAT1) combined with betaine and was found to reverse the HCV blockade of IFN-α signaling. This finding suggested that the addition of SAMe and betaine to peg-interferon could improve the responses in patients with chronic HCV who are not responding to therapy (Duong et al, 2006).

Protein Kinase Receptor (PKR) Stimulation

The inducible components of the antiviral response with multifunction activities to control the transcription and the translation. Two domains are well known: the regulatory N-terminus domain which contains the ds-RNA binding site and the catalytic domain which contains the entire conserved motif for the protein kinase activities. The ds-RNA binds to PKR and activates its function by forming a dimer of two molecules which then trans-phosphorylate to regulate the cell function. HCV can interfere and block the activity of the eIF2 by binding through NS5A and E2.

Ribavirin (RBV)

In 1991 a pilot study addressed the utility of treatment with ribavirin (RBV, 1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1H-1,2,4-triazole-3-carboxamide) for CHC patients. This was followed by several controlled trials showing that ribavirin monotherapy (Copegus®) can lead to a decline of aminotransferase levels in a significant proportion of CHC patients (Richard et al, 1991; DiBisceglie et al, 1991 & 1995; Hoofnagle et al,1996; Dusheiko et al, 1996).

Results from a Cochrane systematic review of a meta-analysis of 11 randomised trials with a total of 521 patients – showed that RBV monotherapy was associated with a biochemical response by the end of 8 - 48 weeks of treatment, which was not sustained (Brok et al, 2006) and also that long-term RBV monotherapy can lead to liver histology improvement in cirrhotic patients or in liver transplant recipients with recurrent hepatitis C. Furthermore, monotherapy with RBV does not lead to a virological end-of-treatment response or sustained virological response (SVR) and has no significant effect on liver-related morbidity or mortality (Brok et al, 2005). Five to six years later, results from a Cochrane systematic review of another meta-analysis of 72 clinical trials with a total of 9991 patients showed that treatment with a combination of RBV and IFN-α substantially reduced morbidity and mortality, with a significant improvement in sustaining viral clearance in naïve patients, virological relapses and non-responders, as well as improvement in the liver histological response (Brok et al, 2005). Recent pharmacokinetic studies suggest that high concentrations of RBV in blood as

soon as the first day of dosing, or, if measured, by week 4, are highly predictive for a subsequent favorable antiviral response (Maynard et al, 2007; Loustaud et al, 2008).

Factors affecting the therapeutic responses

In the earlier stages of HCV treatment, the HCV genotype and treatment duration were thought to be the most important factors in achieving SVR. Depending on the duration of treatment (24 vs. 48 weeks) and HCV genotype (HCV 1 vs. HCV 2,3), SVR rates ranged between 2% and 33% for patients treated with IFN-α. Meanwhile, the addition of RBV to IFN-α has improved treatment outcomes, ranging from 28% to 79%, following the development of peg-IFN, which improved the SVR rates from 42% to 82% (Yuan and Lee, 2008). As has been noted, the treatment for CHC, 24 or 48 weeks of Peg-IFN-α, combined with RBV and ERV, is considered a good indicator for the treatment. Nevertheless, ETR at the end of treatment shows a response of undetectable RNA; if this is sustained for six months after cessation of treatment, SVR appears.

Patients who fail to get an EVR are usually unable to achieve an SVR and for this reason the discontinuation of treatment after 12 weeks is recommended. Meanwhile, patients with an SVR greater than 95% will have undetectable HCV RNA indefinitely. However, there are considerable side effects associated with IFN treatment, which cause up to 15% of patients to stop therapy. Up to 40% may have dose reductions, which will also probably reduce efficacy (Chung et al, 2008).

The viral load and genotype can both influence the response. Genotype 1 is the most difficult to treat and has the lowest response rates, while patients with viral loads lower than 800000 IU/ml have a significantly better chance of having an SVR. Other factors are associated with SVR, such as lower age, shorter duration of infection, absence of cirrhosis, better liver function, female gender and lower hepatic iron.

The basis for the non-response of CHC to treatment has until recently remained doubtful. HCV has evolved extraordinary mechanisms to counteract both the innate and adaptive immune and pro-inflammatory responses which are likely to contribute to both persistence and limitations in responsiveness to antiviral therapy (Yuan and Lee, 2008). Some CHC patients may have a virus which is more resistant to interferon therapy, while others appear to have defective immune responses or poor tolerance to or compliance with interferon-based antiviral therapy. The possible strategies to improve antiviral efficacy in these non-responders are to increase the dosage, prolong the duration of treatment and improve the compliance of patients (Yuan and Lee, 2008).

It must be mentioned before going further that the analyses of HCV RNA levels during antiviral therapy has identified important factors

associated with and contributing to the response to treatment. These factors can be divided into three categories: host factors, virus factors and environmental factors. Host factors, such as age, sex, race, body weight, hepatic steatosis, insulin resistance, hepatic fibrosis and immunodeficiency. Viral factors such as HCV genotype and initial HCV RNA level; are all elements which are associated with the responses to treatment (Yuan and Lee, 2008).

It has been suggested that inherited variation could play a significant part in determining the host response to HCV infection (Price et al, 2006; Zeuzem et al, 2004). The differences of response due to ancestry, for example, African American and Caucasian American, have been constantly noted and these differences cannot be attributed to viral subtype (1a versus 1b), initial viral levels, gender, age, body weight or hepatic fibrosis. Thus, patterns of viral kinetics were similar in the responders to treatment, regardless of race; African Americans were merely more likely to have a non-response (Yuan et al, 2008). The overall SVR rates were 52% among Caucasians but only 28% among African Americans and when researchers controlled for differences in adherence, race was still a strong predictor of response (Conjeevaram et al, 2006).

More analysis of the relative effects of body weight, obesity, hepatic steatosis, diabetes and insulin resistance has suggested that insulin sensitivity has a major role in responses to therapy in CHC (Chung et al, 2008). In multivariate analysis, insulin resistance but not age, gender, body weight, body mass index or hepatic steatosis was associated with lack of SVR. The association of insulin resistance and non-response to IFN therapy is potentially important, because insulin sensitivity is modifiable by weight loss or drug therapy. Furthermore, these associations suggest that there may be important intracellular interactions between the signaling pathways of insulin and IFN-α (Chung et al, 2008).

In 2003, Bressler suggested that obesity, as defined by a BMI greater than 30 kg/m2, was an independent risk factor for non-response to antiviral therapy and can lower the chances of reaching an SVR (Bressler et al, 2003). BMI has been shown to correlate with the degree of liver steatosis seen in HCV (Bressler et al, 2003). Steatosis can also lead to an increase in lipid deposits within cells, which may cause a functional disturbance by reducing the contact area between the drugs and the hepatocytes containing the virus, thus causing a reduction in antiviral drug efficacy. Furthermore, the degree of steatosis has been shown to correlate with the severity of fibrosis (Lerat et al, 2002). Bressler's study did not show an association between the presences of greater than 5% steatosis with sinusoidal fibrosis; however, it did show that BMI and fibrosis were risk factors for non-response and that

the presence of hepatic steatosis does not influence patients' response to antiviral therapy when their BMI is taken into account (Bressler et al, 2003).

Those with a fatty liver and alcoholics can both show a poorer response to treatment and hasten the progression of fibrosis. A report from a national study in 2006 by Anand et al demonstrates that alcohol users have a comparable response to IFN-α plus RBV and have a lower rate of response than non-alcohol users, due to their lower adherence to the treatment (Anand et al, 2006). Recent data also show that the response rate can be affected by the degree of adherence to the treatment. When adherence diminishes for any reason, such as dose reduction, non-completion or even early withdrawal, the SVR can be reduced by up to 48% (Ferenci et al, 2001 and McHutchison et al, 2002). In 2007 Lecube found that glucose abnormalities are an independent predictor of poor virological response to combined therapy in hepatitis C virus-infected patients. Insulin resistance has also been found to impair virological response to combined therapy in chronic hepatitis C patients (Lecube et al, 2006).

Viral factors other than genotype, viral load and the chronology of serum HCV RNA clearance during treatment are also involved in the therapeutic responsiveness to treatment. However, most studies analysing the implications of HCV genotyping and quasispecies analysis in the response to antiviral treatment have been conducted in patients treated with IFN-α alone (Forns & Bukh, 1999, Farci& Purcell, 2000). In observations by Enomoto in 1996, Duverlie in 1998 and several others, no significant changes in the amino acid position residues or motifs were found to be associated with different responses to therapy in the NS5A region. Furthermore, phylogenetic analyses did not show any clustering of NS5A variants in relation to different responses to therapy (nor any in the results and conclusions of this study).

HCV has also been able to modulate antiviral responses in the host through interactions with elements of the inflammatory response. Among its many activities, it uses IRF-3 in order to regulate the expression of inflammatory chemokine which inhibits the antiviral actions of IFN-α (Imbert-Bismut et al, 2001). Moreover, inherent genetic variability which can lead to clinical consequences evolves from acute to chronic infection, as it can rely in part on mutations occurring within antigenic epitopes by which the virus escapes from immune surveillance. Mutations may also adversely affect viral fitness. Finally, genetic variability may account for differences in the rates of response to therapy. Resistance mutations are well known to occur in HCV (Alberti et al, 2005).

A report by Tavis in 2008 found a small number of amino acids in HCV which differed consistently by response and race and that the viral sequences were significantly more diverse in good responders than poor

responders, while those with an intermediate response were usually intermediate in diversity. The genetic diversity was not uniformly distributed across the HCV genome. The major regions having greater variability were the NS5A and the NS3 regions for genotype 1a and core and NS3 for genotype 1b (Tavis et al, 2008; Chung et al, 2008).

A separate study has shown variability in NS3 to be associated with a virological response while the variability was confined to the NS3 C-terminal helicase domain (Donlin et al, 2007). The viral kinetics during IFN-α therapy of CHC has also defined two phases of viral decline: a rapid, initial first phase, which is believed to reflect the antiviral efficacy of IFN-α and a more delayed and slower second phase, which is believed to reflect the eradication of virus-infected hepatocytes. In several studies, RBV has been found to have little or no effect on the first phase of viral decline but to enhance the second phase, in particular when IFN-α effectiveness is limited (Dixit et al, 2004).

In a study of viral kinetics and dynamics of HCV in chronically infected patients, the levels in the blood are often stable and a statistical approach is often used in determining the rate of changes in the levels of the virus; these are also used in the derivation of mathematical models. The mathematical model which was used by Neumann et al in 1998 assumes that there are a large number of viral free molecules circulating in the blood and that a number of liver cells are not infected with HCV. These cells then do become infected and in turn produce more viruses in the circulatory system. According to the mathematical model, both the virus and infected cells and the liver are at risk of death in chronic infections. Perhaps the levels of virus are relatively stable and thus the productivity of new strains of infected cells is steady as well. This is the case of course, if nothing intervenes to change this balance, such as the initiation of antiviral therapy which can make the viral load fluctuate (Neumann et al, 1998).

Constructing kinetic models of these changes to gain insight into the dynamics of HCV replication has been extremely useful in understanding therapeutic responses; such analysis requires the deployment of sensitive and reproducible assays for viral load and can allow estimates of the cell death to produce sophisticated viral dynamic models (Schouten et al, 2000).

Through preliminary studies on the dynamics of HCV infection, a significant reduction was observed in the levels of HCV RNA when IFN was given to the patient (Layden et al, 2005) and a lag phase was apparent for 6-8 hours, this being the time taken by IFN to induce antiviral effects. After a physiological delay, there followed changes in the viral load with a rapid decline in the levels, often 1-2 log10. This was followed by a second slower phase, within 48 hours of treatment (Romeo et al, 1996; Benvegnú et al, 1997). Some patients have a slower response, with low levels of virus,

whereas in others the levels of the virus do not decline further or rise, during the second phase.

The theoretical studies on HCV viral dynamics during IFN treatment demonstrate its ability to reduce the levels of the virus or inhibit its replication (Layden-Almer et al, 2005). Studies indicate that the effectiveness of IFN and/or RBV is incomplete, because if the treatment was 100% effective it would lead to rapid and complete eradication of the virus in the blood rather than just diminishing it in the binary phase. It is interesting that the effectiveness of IFNα- inhibition on HCV replication can be measured by the viral decline during the first 24 hours of treatment (Takahashi et al, 1993; Kobayashi et al, 1996; Vogt et al, 1999). The addition of RBV leads to increased rates of decline of the virus in the blood and thus the rate of the patient's response to treatment.

Neumann et al in a study of 23 patients using a mathematical model showed that the main effect of initial IFN-α is to block the production of the virus by the hepatocyte. Usually in the first phase of antiviral activity there is a rapid decline in viral load within 48 hours of starting the treatment. After this, the second phase, the rate of viral decline can be significantly different between patients. There is also an inverse relation with the baseline viral load. The rapid first-phase drop in serum HCV RNA corresponds to the clearance of plasma and inhibition of replication, whereas the second phase represents net loss of HCV infected cells (Charuworn and Keeffe, 2009).

RVR becomes undetectable after 3 months of treatment. The target of treatment is to achieve sustained virological response (SVR). At (SVR) stage the virus cannot be detected in the blood for 24 weeks after treatment and there is a steady-state kinetic viral balance between the infection of new cells and the death of infected cells to ensure the size of a group of cells in the liver. From a clinical point of view, patients at a SVR stage are stable. On the other hand, each cell in the infected liver with a weak daily production rate should ideally be targeted at once, to eradicate the virus within a reasonable period of time (Wölk et al, 2008).

Moreover, the treatment of genotype 2 patients for 24 or 48 weeks results in the same long-term response rate, whereas for genotype 1 infected patients, the response rate increases with 48 weeks of treatment (Bartenschlager et al, 1994 and Pawlotsky et al, 1995). The reasons for these differences in response to combination IFN-α and ribavirin (RBV) therapy are thought to involve host and viral factors and other factors which have been associated with improved treatment response including age (Chamberlain et al, 1997) gender and extent of hepatic damage (Bortolotti et al, 1995 and 1996). As has already been mentioned the response to IFN in genotype 1b infection has been shown in some studies (Castillo et al, 1995; Chevaliez and Pawlotsky, 2007) to correlate with the number of amino acid

changes in codons 2209–2248, the interferon sensitivity determining region (ISDR) of the non-structural protein 5A (NS5A) and with specific mutations in the ISDR. In addition, it was recently shown that, in patients infected with HCV genotype 2a, a large number of mutations in the corresponding ISDR (codons 2l63–2228) is associated with a higher response rate to IFN treatment (Lan et al, 2007). Thus, the relation between mutations in the ISDR, genotype and the IFN effectiveness in blocking production of virus requires further study.

Another possible explanation for the differences in efficacy of IFN between the genotypes is related to the HCV second envelope glycoprotein (E2), which has significant sequence variations between virus strains. E2 has a relatively conserved 12 amino acid sequence from most virus isolates similar to the PKR phosphorylation site and the translation initiation factor eIF2a phosphorylation site (Guadagnino et al, 1997). It was found that the 12 amino acid sequence in genotype 1a and 1b virus more closely resembles the PKR- eIF2a homology domain than the less IFN-resistant virus strains, genotypes 2 and 3 (Murphy et al, 2002).

The clearance rate, which reflects the half-life of virions in the serum, may be enhanced by antibody-mediated viral clearance in addition to the intrinsic nonspecific clearance of virions in the body. Indeed, a number of studies have shown that antibody responses to the hypervariable region 1 (HVR-1) of the HCV envelope glycoprotein E2 were significantly more vigorous and frequent in HCV genotype 2 infected subjects than in genotype 1 infected patients. Furthermore, the more rapid variation in HVR-1 viral sequences from genotype 2 patients than in genotype 1, also suggests a stronger antibody mediated immune pressure on the genotype 2 virus (Rumin et al, 1999; Fried et al, 2002; Lin et al, 2006).

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