Eradicating Chronic Hepatitis C Viraemia
Lessons learnt during the last decade

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Abstract

Over the last decade a better understanding of the mechanism for immune disposal of hepatocytes infected with the Hepatitis C virus enabled the design of antiviral regimens with increasing efficacy. The poor rates of sustained viral clearance initially obtained with recombinant interferon (12%) have now improved to over 70 % with the use of a combination of pegylated interferon and the guanosine analogue ribavarine. The relatively poor response of genotype 1 strains to this regimen continues to challenge scientists and clinicians. This article reviews the theoretical and practical aspects of selection of patients for viral eradication, their management during treatment and subsequent follow-up

Introduction

Hepatitis C Virus (HCV) is a highly evolved hepatotropic virus that is capable of changing its genetic sequence during its sojourn in the liver of an infected patient to form different quasispecies. This reduces recognition and clearance by the host of virally infected cells leading to chronicity and eventual liver fibrosis or cirrhosis. The understanding of host-virus interactions provided scientists with a framework for the design of viral eradication regimens which have increased markedly in efficacy during the last decade. The physician needs to combine this basic scientific knowledge with an understanding of the armamentarium of drugs available for viral eradication.

Keywords

Hepatitis C, quasispecies, genotypes, viraemia, cirrhosis, fibrosis, transaminase

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The philosophy behind anti-viral treatment: know your aims

The ultimate objective of treatment is to reverse liver fibrosis or halt its progression so as to minimise the risk of future liver decompensation. The standard aim of anti viral treatment is absence of detectable HCV RNA by qualitative reverse transcriptase polymerase chain reaction (PCR) at the end of treatment and also at 24 weeks after the end of treatment i.e. a sustained viral response (SVR). The second NIH consensus conference on the management of Hepatitis C[1] reported that in patients with SVR, liver biopsies taken 1-11 years after treatment showed clear histological improvement in 89-100 per cent of cases (3 studies) and a late recurrence of viraemia (detected by qualitative PCR) in serum of only 0-4%, while HCV was undetectable by PCR in 400 liver biopsies 24 weeks after the end of treatment in another study.

The antiviral armamentarium: know how your weapons work

Interferons (alpha, beta and gamma) are glycoproteins produced respectively by leukocytes, fibroblasts and lymphoid cells. They are classified as negative growth factors since they inhibit growth of a variety of cell types including those infected by virus. Recombinant human interferon gamma had already been used to treat chronic non-A, non-B hepatitis (NANB) a few years before the discovery of HCV in 1989.[2] Interferons prevent viral entry and subsequent uncoating in the hepatocyte. They activate ribonucleases and deactivate protein elongation factors, degrading viral RNA and preventing its translation in the cell. They also amplify the response of natural killer cells (NK cells) and cytotoxic T lymphocytes (CTL) to viral protein. The relative contribution of these modes of action to viral eradication are not known. The ability of interferon to prevent viral entry into healthy uninfected hepatocytes has been shown to reduce the pool of circulating virus. However a greater and more sustained contribution to viral eradication stems from the ability of interferon to accelerate immune detection and removal of virally damaged hepatocytes. The liver is probably the sole site of replication of HCV as shown by the unique presence there of messenger RNA and high hepatic levels of virus as compared to serum.

In viraemic patients, typically less that one fifth of hepatocytes contain HCV RNA and many of these display no hepatocellular damage. This would suggest that HCV, though

highly hepatotropic, shows minimal cytopathic activity in the cells it infects. Incomplete clearance of virally infected cells is thought to result from an insufficient T cell response during the acute phase or one that is ill-sustained. This sets the stage for chronic inflammation and consequent fibrosis.

A poor initial immune response may be due to the fact that hepatocytes are not professional antigen presenting cells. Sustained viral clearance depends on presentation of adequate amount of antigen to CD4+ (T-helper) lymphocytes in lymph nodes as well as a Th1(T-helper l) cytokine response which promotes continuous and sustained activity of (CD8+) cytotoxic lymphocytes in the liver. Sufficient and continuous production of these cytokines is necessary to sustain the CTL response. The periodic administration of recombinant interferon boosts the response and sustains removal of virally infected hepatocytes.

The kinetics of the viral pool has been extensively studied during interferon therapy by serial measurements of circulating HCV RNA concentrations. The liver of viraemic patients contains a large viral pool ($10^8 - 10^{11}$ RNA copies/gm liver).[3] Viral production (approximately 10^{12} virions per day) is balanced by elimination into a virtual disposal compartment consisting mostly of apoptotic or dead cells so that plasma levels are kept relatively low (10^7 copies/ml plasma) which minimizes antigenic stimulation. Sustained clearance of viraemia (the previously defined SVR) is typically associated with a greater than 2 log drop in circulating HCV RNA by 12 weeks of treatment and an earlier normalisation of serum alanine aminotransferase (ALT) whereas smaller or slower drops in HCV RNA predict non-response or a relapse within 24 weeks of stopping treatment. Other predictors of a likely sustained response to interferon treatment are genotype non-1, younger age, lower baseline body weight or surface area and absence of bridging fibrosis on baseline liver biopsy. Further studies will clarify whether the poor response of genotype 1 HCV to interferon relates to greater replicative capacity or resistance to Interferon.[4] The original regimen of standard interferon (IFN) 3MU thrice weekly (TIW) for 24 weeks yielded SVRs of approximately 12% (2% in genotype 1 and 16% in genotype non-1). Higher doses of IFN or the use of daily "induction" dose IFN regimens unfortunately produced a high drop out rate due to side effects. Prolonging treatment to 48 weeks as recommended by the 1997 NIH Consensus Conference on the management of hepatitis C raised the overall SVR to 15-20%.

Therapeutic efficacy remained limited until the approval in December 1998 of the therapeutic combination of subcutaneous interferon and oral ribavarine (RBV) a guanosine (nucleoside) analogue which is active against a number of viruses but which was found to be ineffective when used as monotherapy against HCV. FDA approval came shortly after McHutchinson *et al* reported an SVR of 38% in patients treated with subcutaneous IFN 3MU TIW and oral RBV 1000 or 1200mg daily for 48 weeks compared to an SVR of 13% in patients who received IFN 3MU TIW alone.[5] Again, Genotype 1 patients had a lower SVR, particularly when pre-treatment serum HCV RNA levels exceeded $2x10^6$ copies/ml. Subsequent trials [6] showed that prolonging treatment with combination therapy beyond 24 weeks was unnecessary except in genotype 1 patients with serum RNA > $2x10^6$ copies/ml in whom prolonging treatment to 48 weeks improved SVR from 17% to 29%. Genotype non-1 patients had an SVR of 62% during 24 weeks of combination treatment and the measurement of pre-treatment viral levels was not found to be of practical importance in this group.

The slow decline of Genotype 1 viral levels in serum during thrice weekly dosing with standard interferon may be due to unduly low virocidal levels at the end of the dose interval. The half-life of standard interferon was lengthened from 6 hours to 90 hours by combination with a 40 kD branched molecule of polyethylene glycol (PEG). Pegylated Interferon (PEGIFN) with an improved subcutaneous absorption and a reduced renal clearance was licensed by the FDA in January 2001. Fried *et al*[7] compared a new combination of PEGIFN with Ribavarine (PEGIFN+RBV) to PEGIFN alone and to the old IFN+RBV combination. The results are shown in Table 1.

The data in genotype 1 patients show that PEGIFN alone was found to be superior to the old IFN+RBV combinations (SVR 21%) and further efficacy was obtained by combining PEGIFN with RBV (SVR 46%) in 48 week regimens. Side effects necessitated dose reduction in some of the patients. Therefore it is likely that lower doses may be equally efficacious. It is also likely that some may be treated successfully with a shorter period of PEGIFN+RBV. Both these aspects are being studied further. A number of putative mechanisms for synergy between interferons and RBV are also being looked into.

Pre treatment protocol: prepare with care

Viraemic patients require a brief hospital admission for a detailed history, examination, blood tests and a liver biopsy during abdominal ultrasonography. Liver histology, genotype analysis and baseline HCV RNA levels will allow a prediction of sustained viral response to treatment which will guide the

Table 1: *Sustained viral response (%) to three 48 week regimens*

Treatment Regimen	Overall	Genotype 1*	Genotype non 1
PEGIFN 180μg once weekly + Ribavarine 1000 or 1200mg daily x 48 weeks	56	46 (41*)	76
PEG IFN 180μg once weekly + placebo x 48 weeks	29	21	45
STD IFN 3MU thrice weekly + Ribavarine for 48 weeks	44	25 (33*)	61

* Genotype 1 patients with pre treatment HCV RNA > 10^6 copies/ml serum

patient and physician to take a joint decision as to whether to proceed with treatment. Co-infection (e.g. CMV, HBV, HIV) and co-existent autoimmune disease in the liver or other organs should also be excluded. Baseline full blood count, thyroid function tests and blood glucose are also relevant (see below)

Selection of patients: some hosts fight the virus better better than others

Current minimal criteria for treatment include presence of HCV RNA in serum, some degree of fibrosis or moderate necroinflammation on liver biopsy, compensated liver disease, abstinence from alcohol and intravenous drugs and acceptance of treatment. Patients with genotypes 2 or 3 (especially those with mild to moderate liver fibrosis on liver biopsy) should be considered for immediate treatment with PEGIFN+RBV because they are more likely to respond to treatment. In patients with genotypes I SVR is 40% or lower (see below). In view of the rapid rise in efficacy of anti viral regimens over the last four years, genotype 1 patients may opt to wait for further developments.[8]

On the other hand patients aged over 40 and patients with bridging fibrosis and/or high levels of serum HCV RNA (all predictors of a less favorable response to treatment) should be advised that sustained response to currently available antiviral treatment will drop as they grow older. Patients should also be made aware that re-treatment with alternative regimens after failure of regimens that contain interferon to eradicate the virus

may be less efficacious in interferon-failure patients than in interferon-naïve patients.[9] Patients with established cirrhosis may be considered for treatment with PEGIFN+RBV unless there are contraindications such as previous decompensation, Childs-Pugh score >10 or cytopenias.[10] Although some cirrhotic patients will achieve sustained viral clearance, for the majority, the secondary end point (currently being studied in the HALT-C trial)[11] is a significant reduction in liver inflammation and fibrosis that may be maintained by long-term treatment with PEGIFN alone. Patients with normal or near-normal ALT levels respond to PEGIFN+RBV as well as patients with raised ALT.[12] Selection of such patients for treatment should be based on other factors such as genotypes, age and degree of liver fibrosis rather than on ALT levels. Patients who did not respond during, or relapsed after, IFN or IFN+RBV treatment should be considered for PEGIFN+RBV treatment especially if there was previous poor compliance to treatment which may be improved by counseling and closer support.[14]

Counselling pre-treatment: preparing the host for attack

Patients who manage to take more than 80% of the total dose of PEG IFN + RBV have improved SVR to treatment.[14] Adherence can be maximised by adequate counselling and follow up. Female patients and female sexual partners of male patients should not become pregnant during and for six months after the end of PEGIFN+RBV treatment. Continued

Table 2: *Side effects during PEG IFN + RBV treatment*

Side effects	Likely Cause	Monitoring	Action
Headaches, lassitude	IFN		
Fever, flu like symptoms	IFN	CBC	Exclude cytopenia, infections
Mood changes	IFN	Psychiatric assessment	Consider withdrawal of IFN + RBV
Transient ALT rise	IFN	Serial liver function tests	Observe closely
Progressive ALT rise	IFN	INR, Bilirubin	Discontinue treatment
Neutrophils < 750/mm³	IFN	CBC	Reduce IFN to 135µg/week
Neutrophils < 500/mm³	IFN	CBC	Suspend IFN until neutrophil count exceeds 1000/cu mm then restart at 90 µg/week
Platelet < 50,000/mm³	IFN	CBC	Reduce IFN to 90µg/week
Progressive thyroid dysfunction	IFN	TFT	Reduce/suspend IFN
Anaemia, no cardiac disease	RBV	CBC	Hb < 10g/dl, reduce RBV to 600mg/daily Hb < 8.5g/dl, suspend RBV
Anaemia, stable cardiac disease	RBV	CBC	Hb drop > 2g/dl reduce RBV to 600mg/day. Hb < 12g/dl despite 4 weeks at reduced dose: discontinue RBV
Pulmonary Infiltrates	IFN	Chest X-Ray	Discontinue IFN
Visual problems	IFN	Fundoscopy, check visual activity	Ischaemic retinal lesions, macular oedema, papilloedema: stop IFN

intravenous drug abuse, depression, cytopenias, certain anaemias (e.g. sickle cell anaemia), autoimmune disease (e.g. of liver, thyroid and colon), uncontrolled diabetes and decompensated liver disease are contraindications to treatment. Coronary or cranial arterial diseases, renal impairment and retinopathy and relative contraindications. The manufacturer's recommendations for dosing and dose reduction should be observed e.g. genotypes 1,4: Pegasys® (Peg interferon alpha 2a) 180µgms s/c weekly with Copegus® (Ribavarine) 1000mg for BW < 75kg, 1200 for BW > 75kg orally daily x 48 weeks; for genotypes 2,3: Pegasys^R 180 µg s/c weekly with Ribavarine 800mg orally daily x 24 weeks.

Treatment supervision: maximising the offensive safely

Patients should be aware that certain complications may necessitate dose reduction or withdrawal of treatment. Besides attending regular follow up and having blood tests, they should be able to report significant side effects immediately (see Table 2).

A favorable response to treatment is usually indicated by an early fail in serum ALT. Serum HCV RNA levels should be checked after 12 weeks of treatment and compared to pre-treatment levels. A drop of less then 2 log in viral load (copies per ml) by quantitative PCR is highly predictive of failure to achieve sustained viral clearance and is usually an indication to stop treatment so as to limit costs and side effects. Patients who complete their treatment (24 or 48 weeks) should have their serum re-tested by qualitative PCR in order to ensure that there has been an end-of-treatment response. HCV RNA is then again checked 24 weeks after stopping treatment to ensure that viral clearance is sustained. Periodic follow up will be necessary to ensure continued absence of HCV RNA in the serum.

Conclusion: watch that space

During the last decade we have witnessed an impressive improvement in the efficacy of anti-viral regimens for the eradication of HCV. Research into antiretroviral therapy has focused the search for more efficacious, better- tolerated drugs. Novel subcutaneous albumin-linked and even oral polyaminoacid-based interferons are on the horizon. Non-haemolysing isomers or prodrug forms of ribavarine, inhibitors of the NS3 protease component of the HCV polyprotein, antisense molecules and ribozymes hold promise for increased viral clearance especially in the difficult-to- treat genotype 1 disease.

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