Ribavirin in the Treatment of Hepatitis C

MARGIT E. ABONYI and PETER L. LAKATOS

1st Department of Medicine, Semmelweis University, Budapest, Hungary

Abstract. The hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide, with approximately 170-200 million people infected. The HCV virus is transmitted by blood and blood products and such transmission occurs primarily through drug use by injection, sex with an infected partner and occupational exposure. The severity of the disease varies widely from mild chronic hepatitis to cirrhosis and hepatocellular carcinoma (HCC). Nowadays, the reference treatment is combination therapy of pegylated interferon and ribavirin, which is an inosine monophosphate dehydrogenase inhibitor and immunomodulator. Efficacy of treatment in our clinical trials is 87% in patients infected by HCV genotypes 2 or 3, whereas in patients infected by HCV genotype 1 response to treatment is 66%. The current combination treatment has significant side-effects and sometimes is poorly tolerated. HCV genotypes 2 or 3 can be treated with a lower dose of ribavirin and a shorter course of therapy, 24 weeks vs 48 weeks for patients with genotype 1. There is a growing consensus that acute control of HCV infection is associated with a vigorous intrahepatic antiviral CD4+ and CD8+ T-cell response, enhanced Th1 and natural killer activity. Pretreatment genotype and response to therapy measured at weeks 12 and 24 of treatment have been identified as key determinants in decisions about continuing treatment. Elevated serum ferritin levels and hepatic iron deposition as well as hepatic steatosis and high ALT levels with chronic hepatitis C are risk factors for HCC development. Heterozygosity for the C282Y mutation in HFE contributes to iron accumulation and fibrosis progression in chronic hepatitis C. Ribavirin could cause dose-dependent reversible haemolytic anaemia, which can be managed with dose reductions or with administration of epoetin alpha at 40,000 IU once weekly without sacrificing the optimal dosing of ribavirin. Among patients who received ribavirin alone, serum ALT levels and necroinflammatory features of liver histology were improved, whereas symptoms, HCV RNA levels and hepatic fibrosis scores were not changed significantly from baseline. For HCV-HIV co-infected patients, treatment is given when blood CD4 counts are above 350/ml and before antiretroviral (ART) treatment is needed.

The early diagnosis and treatment of hepatitis C infection is still a great healthcare problem worldwide. The prevalence of chronic hepatitis C ranges from 0.1-6% in different countries, being 0.7% in Hungary. There are an estimated 5 million chronic HCV carriers in Western Europe and 4 million in the United States (8, 27). The incidence of new symptomatic infection has been estimated to be 1-3 cases/1,000,000 annually, with the actual incidence of new infections clearly being much higher, because the majority of cases are asymptomatic. The incidence is declining for 2 reasons: (a) transmission by blood products has been reduced to near zero; (b) universal precautions have markedly reduced transmission in medical settings.

At present, intravenous drug use remains the main mode of transmission, along with piercing and tattooing, haemodialysis and healthcare accidents. The natural history of hepatitis C infection is different from hepatitis B. The progression of fibrosis determines the ultimate prognosis and, thus, the need for therapy. Fibrogenesis is a complex dynamic process, which is mediated by necroinflammation and activation of stellate cells. Liver biopsy remains the gold standard to assess fibrosis and cirrhosis. The major factors known to be associated with fibrosis progression are older age at infection, male gender, excessive alcohol consumption, high serum iron and ferritin levels, obesity, higher BMI index, diabetes mellitus and NASH. In Hungary the most frequent genotype in 86% of cases is 1b. Hepatitis C directly damages the hepatocytes, as well as by immunomodulant routes (17, 20).

In chronic hepatitis C infections there is diminished cytotoxic T-cell and natural killer (NK) activity. There is also a Th2 overwhelming elevated B-cell activity, IgH gene changes, hypergammaglobulinaemia, immunecomplexes and autoimmune-disorders as well as B-cell lymphomas (3, 4, 15, 27).
Table I. Criteria for treatment of chronic HCV infection.

- Persistent elevation of ALT for >6 months (normal ALT?)
- Positive anti-HCV by EIA-2
- Positive qualitative and quantitative HCV RNA (Roche TaqMan)
- Liver biopsy: with chronic hepatitis or well-compensated liver disease (HAI)
- And/or extrahepatic HCV manifestations

Table II. Mechanism of action of ribavirin, 1-b-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide.

- non-interferon-inducing purine nucleoside analog
- main effect: inhibition of the activity of de novo inosinmonophosphate - dehydrogenase (IMP DH) and the salvage guanosyl - phosphoribosil-transferase (GPRT) enzymes
- inhibits the replication of a wide range of RNA and DNA viruses
- antiviral effects by altering intracellular nucleotide pools and messenger RNA formation
- inhibition of viral encoded polymerase,
- inhibition of genomic RNA capping,
- promotes T helper-cells (CD4+) and Th1 cytokine and immune response “in vivo”.
- induces IL-12 level (contribute to the Th1 enhancing effect)
- IL-12 promotes the differentiation of CD4+ naive T-cells into Th1-cells and thereby plays a central role in the regulation of the Th1/Th2 balance,
- promotes C190-specific immune responses towards Th1-like pattern by inducing the antigen-presenting cells to produce a higher IL-12 level (IFN-gamma and IL-2).

16, 22, 24). HCV accounts for 20% of acute hepatitis, for 80% of chronic hepatitis, for 40% of end-stage cirrhosis, for 60% of hepatocellular carcinoma (HCC) and for 30-50% of liver transplants. The current system of nomenclature includes 6 major genotypes and more than 80 different subtypes. An inherently greater pathogenicity of type 1 hepatitis C virus (HCV) has been implied in patients with cirrhosis and HCC. Within an individual patient, HCV may exist as quasispecies.

Different co-factors influence the rate of progression to cirrhosis in chronic hepatitis C. Young age at diagnosis and female gender has better prognosis, while excessive alcohol intake, male gender, immunocompromised situation, iron overload, liver steatosis, HBV or HAV or HIV co-infections and AIDS, obesity, diabetes mellitus, genotype 1b and higher viral load have more rapid development to fibrosis, cirrhosis and HCC in 20 years (8, 27). Our knowledge about the oxidative stress in liver disease is also important. We know that there is an important role of oxidative stress on living cells and cell responses e.g. apoptosis and necrosis, which leads to cell death. At the same time, mild oxidative stress can modulate signal transduction cascades and redirect gene expression and influence many cellular responses, e.g. proliferation, differentiation, reproduction. Regulations of the cell cycle depend on the intracellular redox state. Critical steps in the signal transduction cascade are sensitive to oxidative stress and antioxidants. Heavy metal accumulation may inhibit enzyme activities, influence the acute phase protein synthesis and gene expression, as well as the pro-oxidant and anti-oxidant forms of scavenger molecules. Polyphenols and flavonoid type anti-oxidants may influence the signal transduction routes as well (1, 30).

In 80% of patients suffering from hepatitis C, the diagnosis was made accidentally by measuring the ALT, AST or other liver laboratory tests. In some other patients, there is fatigue, anorexia, loss of weight and hepatomegaly. There are also elevated liver enzyme tests, ALT 2-3 times higher upper normal limit, anti-HCV Ig-positive and HCV RNA PCR-positive. The liver biopsy shows a higher activity rate (HAI≥7), bridging necrosis and piecemeal necrosis and fibrosis (1). There are extrahepatic manifestations associated with chronic HCV infection: cryoglobulinaemia, glomerulonephritis, lichen planus, sicca syndrome, porphyria cutanea tarda, autoimmune thyreoiditis, idiopathic pulmonary fibrosis, focal lymphocytic sialadenitis (8). In chronic hepatitis C patients, there is an elevated incidence rate of B-cell lymphoma and non-Hodgkin lymphoma (23, 27). The genotypes 1b is the most therapy resistant, therefore the prognosis is much better with genotypes 2 or 3. Progression of fibrosis is more rapid in immunocompromised patients. There are no tests that reliably predict the rate of progression of fibrosis in an individual patient, but high ALT levels are associated with higher risk of fibrosis progression. Liver biopsy provides the most accurate information on the stage of fibrosis and grade of necroinflammation, both having prognostic significance.

Patients and Methods

Patients. Between January 1994 and October 2004, a total of 650 patients with chronic hepatitis C were treated, following worldwide accepted standards: 426 males and 224 females (mean age: 45 SD±14 years). Treatment withdrawal due to side-effects or lack of compliance occurred in 15 patients.

History and physical examination, full blood testing and biochemical liver function tests (LFT), namely serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl-transferase (GGT), as well as albumin level, prothrombin, protein electrophoresis, electrolytes, serum ferritin, iron, ceruloplasmin, copper and alpha-fetoprotein (AFP) levels were determined by means of standard automated techniques (Olympus AU600, Olympus Co. Ltd, Shizuoka, Japan). All patients underwent X-ray examination, ECG and abdominal ultrasound. The upper limit of normal (ULN) for ALT was 42 IU/l, and for γ-GT was 52 IU/l. Patients with chronic hepatitis C and elevated ALT values had to have 2 or 3 or more times higher ALT values above ULN.
HCV genotyping was performed by reverse hybridization assay (Inno-LIPA HCV II; Innogenetics). Liver biopsy was performed in all patients showing chronic hepatitis C with or without fibrosis or cirrhosis Child A.

Treatment regimens. i. First period (1994-95): s.c. injections of 3x3 MU interferon-alpha 2a or 2b (IFN), Intron-A (Schering-Plough, Kenilworth, USA) or Roferon A (Roche Pharma, Basel, Switzerland) were given for 6 months. A total of 43 patients were treated and followed-up. ii. Second period (1996-97): IFN alpha was given 3-5 MU s.c. 3 times weekly for 12 months. A total of 188 patients were treated and followed-up. iii. Third period (1998-99): IFN-alpha were given 3-5 MU s.c. 3 times weekly together with ribavirin daily dose of 1000-1200 mg depending on body weight (< or > 70 kg). A total of 282 patients were treated and followed-up. iv. Fourth period (2000-2004): peginterferon was given (Pegasys 180 µg s.c., Roche, weekly or Peginteron A 1.5 µg/ kg, Schering-Plough) together with ribavirin daily dose of 1000-1200 mg depending on body weight (< or > 70 kg). A total of 137 patients were treated and followed-up.

Response definition. End of treatment response (ETR): Normalization of ALT and disappearance of HCV RNA at the end of therapy. Sustained viral response (SVR): Normalization of ALT and HCV RNA disappearance during the therapy and maintained at 24 weeks after the end of therapy (follow-up period). Partial response (PR): A significant decrease in ALT and HCV RNA levels compared to the initial values (>50%). Non-responder (NR): NO reduction of ALT and HCV RNA levels compared to initials after 3-6 months of therapy. Relapser rate (RR): Re-elevation of ALT and re-appearance of HCV RNA after remission, following treatment withdrawal (18, 25).

Statistical analysis. χ²-square test and t-test for separate variance estimates were used. For the statistical analysis, Statistica for Windows 6.1 (StatSoft Inc., OK, USA) was used. A p value <0.05 was considered significant.

Results

The route of HCV transmission could not be identified in 45% of the patients. Twenty-one % had transfusion in the medical history, 5% were former i.v. drug users, 4% had mother-child or sexual transmission possibilities and 27% of the patients had surgery or dental surgery as the risk factor.

We have discussed only the SVR rates, the results of which are presented in Figure 1. Altogether, 15 patients dropped out, 7 because of lack of compliance, while the others had severe side-effects: 3 patients had significant decrease of haemoglobin, haematocrit levels (>30%), 1 patient had severe leukopenia (<1000/µl), 1 had thrombocytopenia (<45,000/µl), 2 patients developed severe depression and anxiety and 1 patient had active thyroid disorders.

Discussion

The therapy of chronic hepatitis C has changed in the last 10 years, in Hungary. Before 1992, the main risk factors
were blood transfusion or blood products; nowadays, the main transmission routes are i.v. drug use, tattooing and piercing, and, to a lesser extent, vertical or sexual transmission or medical procedures (2). The main problem is that we do not know about the cause and infection route of about 43% of patients with acute or chronic hepatitis C.

Gervain et al. (4) and Horanyi (personal communication) measured the genotypes in Hungary, determining 86-90% of the chronic hepatitis C patients to be genotype 1b or 1a and 10-14% of patients types 2 or 3. Our early data were mixed results for all genotypes, since regular genotyping before therapy has only been undertaken in the last 4 years. We got better results with genotypes 2 or 3 than genotype 1b or 1a (7, 8, 12), and the results improved with longer therapy, but the real change was when the patients could get the combination therapy of IFN-alpha together with ribavirin. Among the first, McHutchison et al. (11) achieved a SVR of 41% with a 48-week combination therapy versus 16% with IFN-alpha monotherapy in naive patients (26, 28, 31).

A milestone in chronic hepatitis C therapy was the introduction of ribavirin as part of the combination therapy. Ribavirin is a guanosine-analogue which has a broad antiviral spectrum, not yet fully elucidated, though some mechanisms of action are known (Table II). Ribavirin can cause a decrease of ALT levels, but it has little effect on the HCV RNA levels. Ribavirin is known as an immunomodulator, inhibiting the viral RNA-polymerase, balancing Th1 and Th2 cell responses and acting by direct cytoprotection. We know from the data of George Weber et al. (29) that ribavirin acts via the signal transduction pathway, too, like other cytostatic agents. The final common pathway in the actions of tiazofurin, ribavirin, tamoxifen, quercetin and genistein in the induction of apoptosis and differentiation is summarized on Figure 2. It is postulated that the biological impact depends on the reduction of IP3 concentration by these drugs.
concentration by these drugs. Contraindications to ribavirin include end-stage renal failure, anaemia, severe heart disease, pregnancy and inadequate contraception. The major side-effect of ribavirin is haemolytic anaemia, which can be severe. Cardiovascular disease should be carefully excluded in patients considered for combination therapy, as anaemia may lead to angina or heart failure in these patients.

Short disease history, female sex, low serum ferritin level and low viral concentration are predictors for a higher SVR rate, while co-infection with HBV or HIV, and existing fibrosis are associated with a low response rate (14). We analyzed only the SVR patients, all of whom had lower pretreatment viral load and 67% of the treated patients had no fibrosis in the pretreatment period. In the last 4 years, we achieved a higher SVR rate. Many patients, who had not achieved SVR or relapsed, could participate for a second course of therapy with pegylated interferon plus ribavirin together. In patients who had more than one negative predictor, the treatment duration was longer (52 weeks) or the start was with high-dose induction for the first 6 weeks, 5-6 MU IFN-alpha plus 1000-1200 mg ribavirin / day, followed by combination therapy (1, 2, 5, 6, 10). Nowadays, we also treat more intensively Child A and B cirrhotic patients. We have the best results with the pegylated interferons plus ribavirin combination. The conjugation of IFN with polyethylene glycol increases the half-life of the molecule from 4 h to 40-80 h and improves the pharmacodynamics of IFN (9, 13, 21).

Although the results are much better than 10 years ago, emerging therapies for hepatitis C are still needed, such as inhibitors of viral enzymes (protease, helicase or polymerase), antisense oligonucleotides (inhibits HCV replication), or ribosome therapy. According to previous data, interleukins (IL-2, IL-10, IL-12) showed poor antiviral efficacy with limitations related to their toxicity. Viramidine is a carboxamidine version of ribavirin, which is a carboxamide. Viramidine could replace ribavirin in the future, because the side-effect profile is more favourable, the haemolytic anaemia being less prevalent with the new drug. A 28-day toxicology study in monkeys showed that viramidine induced minimal anaemia at 600 mg/kg, whereas ribavirin at 300 mg/kg induced hemolytic anaemia.

In conclusion, the combined therapy, ribavirin (more anti-inflammatory, immunomodulant, immunosuppressive) plus PEG-interferon (more antiviral) is the recommended choice for patients with chronic hepatitis C and/or extrahepatic manifestations. Our results are better than 10 years ago, but we need more effective antiviral modalities with multiple drug combinations for the treatment of hepatitis C. Ribavirin is the first milestone along the road. Successful antiviral treatment has important results as it reduces the number of infected patients, thus diminishing the spread of the pathogen, also inhibiting hepatitis C progression to cirrhosis and perhaps lowering the risk of hepatocellular carcinoma. Inositol hexaphosphate (IP6), a dietary constituent, has shown promising efficacy against various cancers in experimental oncology, although only limited studies have been done with IP6 against HCC or cirrhosis. Controlled clinical studies are needed to evaluate the effect of IP6 + inositol in the treatment of hepatocellular lesions caused by HCV.

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