Ribavirin in the Treatment of Hepatitis C

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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 ribavarin. 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,

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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-ribofuronosyl-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 inludes 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, glomerulo-nephritis, 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.

Figure 1. *Changes in the efficacy of therapy in chronic hepatitis C in Hungary.*
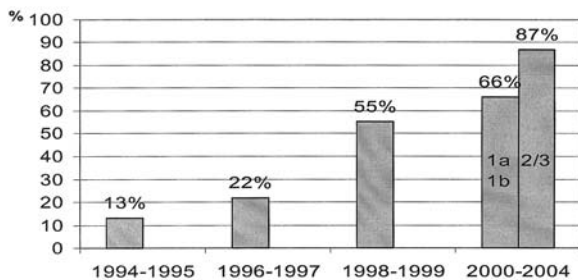
years	n	SVR: number of patients (%)
1994-95	43	6 (13%)
1996-97	188	41 (22%)
1998-99	282	155 (55%)
2000-04	122 (1a/1b)	81 (66%)
	15 (2, 3)	13 (87%)
Total	650	

SVR= sustained viral response. Genotypes: 1a/1b, 2 and 3
Differences between the four periods are significant (χ^2: $p<0.05$ between each group, 2000-2004 genotypes 2, 3 group were excluded from the statistical analysis)

In all patients the diagnosis of chronic hepatitis C was based on the consistent elevation of ALT levels and detection of HCV RNA (≥ 6 months), positive anti-HCV antibodies by third generation enzyme-immunoassay, histological examination, grading and staging according to Knodell (1). Indication for treatment is summarized in Table I. Patients were negative for hepatitis B surface antigen and antibodies to human immunodeficiency virus types 1 and 2. Patients with alcohol consumption of more than 20 g/day, *i.v.* drug abuse within 1 year, organ transplant, decompensated liver disease (Child C), neoplastic disease, severe cardiac or chronic pulmonary disease, autoimmune disease, not well-controlled thyroid disease, depression or other psychiatric disorders, seizure disorders, severe or not well-treated diabetes mellitus, severe retinopathy, unwillingness to use contraception or cytopenia (leukopenia <3000 /μl , platelets < 65,000 /μl) were excluded (18, 19, 25). The treatment protocols were approved by the local Ethics Committee and performed according to the Declaration of Helsinki. All patients gave written informed consent before enrollment on the study.

Detection and quantification of HCV RNA. HCV RNA was determined using quantitative and qualitative assay reverse-transcriptase-polymerase chain reaction assay (COBAS Amplicor HCV Monitor 2.0 Roche Diagnostic Systems, with a lower detection limit of 50-600 IU/ml). Serum samples were tested before treatment, at the end of therapy and at the end of the follow-up period (24 weeks after the end of therapy). End of treatment virological response and sustained virological response (SVR) were defined as undetectable HCV RNA for both assays at the end of treatment and 24 weeks after discontinuation of therapy.

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 weelky 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 Pegintron 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. χ^2-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 43% 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
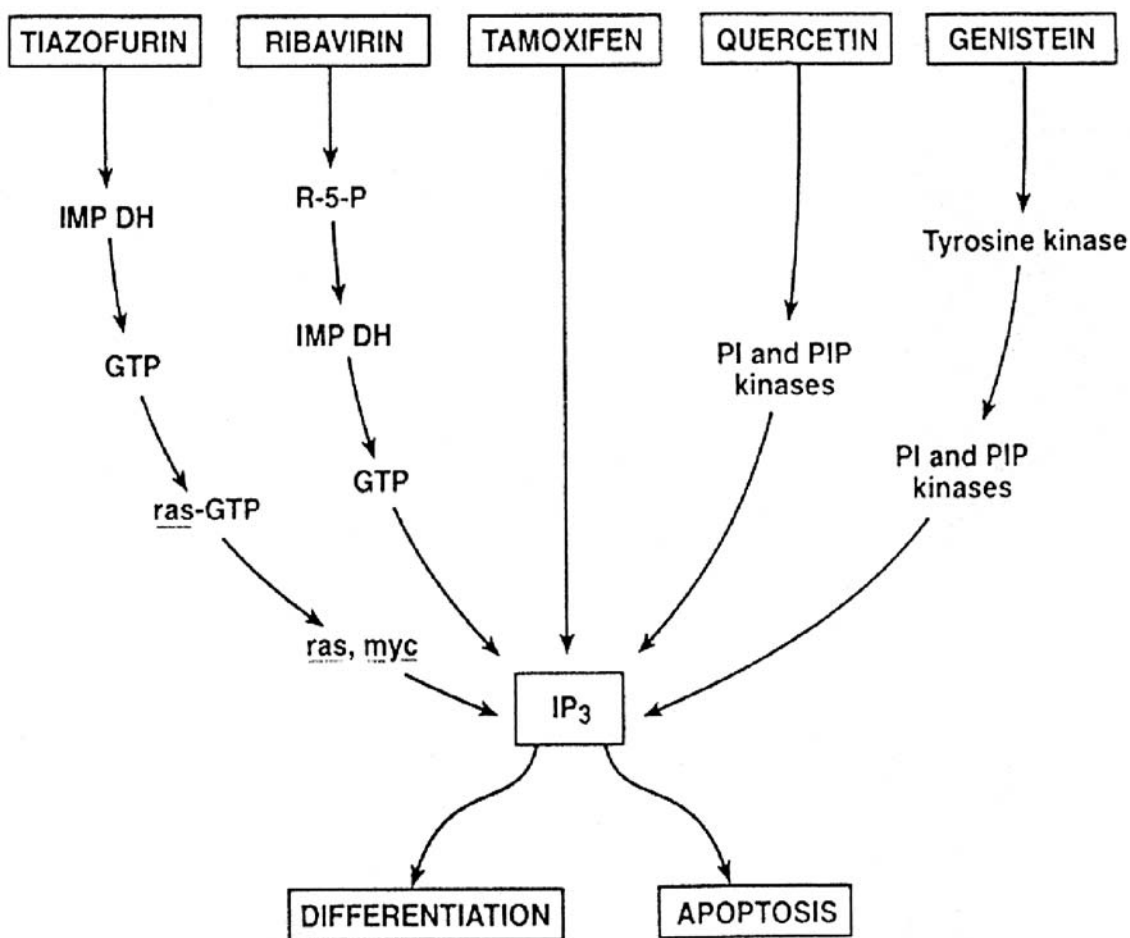
Figure 2. *The final common pathway in the induction of apoptosis and differentiation. The biological impact depends on the reduction of IP3 concentration by these drugs.*

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 (personnal communication) measured the genotypes in Hungary, determing 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. 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 ribozyme 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 mininal 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.

Acknowledgements

References

1 Dancygier H: Hepatitis C. *In*: Lebererkrangungen 1 x 1 der Therapy (ed: Dancygier H). Berlin, Springer Verlag, pp. 49-60, 2004.

2 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J and Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C-virus infection. N Eng J Med *347*: 975-982, 2002.

3 Gasztonyi B, Par A, Szomor A, Battyany I, Nagy A, Kereskai L, Losonczy H and Mozsik G: Hepatitis C virus infection associated with B cell non-Hodgkin lymphoma in Hungarian patients. Br J Hematology *110*: 497-498, 2000.

4 Gervain J, Simon G Jr, Papp I and Szabone BK: Determination of the type and subtype of the hepatitis C virus in chronic viral hepatitis patients in Hungary. Orv Hetil *142*: 1315-1319, 2001.

5 Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, Balart L, Reindollar R, Reddy RK, Wright TL, Lin A, Hoffman J and De Pamphilis J: Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Eng J Med *343*: 1673-1680, 2000.

6 Jaeckel E, Cornberg M, Wedemeyer H, Santantonio T, Mayer J, Zankel M, Pastore G, Dietrich M, Trautwein C, Manns MP and German Acute Hepatitis C Therapy Group: Treatment of acute hepatitis C with interferon alfa-2b. N Eng J Med *345*: 1452-1457, 2001.

7 Brau N, Rodriguez-Torres M, Prokupek D, Bonacini M, Giffen CA, Smith JJ, Frost KR and Kostman JR: Treatment of chronic hepatitis C in HIV/HCV-coinfection with interferon alpha-2b+ full-course *vs*. 16-week delayed ribavirin. Hepatology *39*: 989-998, 2004.

8 Lauer GM and Walker BD: Hepatitis C virus infection. N Eng J Med *345*: 41-52, 2001.

9 Lindsay KL, Trepo C, Heintges T, Shiffman ML, Gordon SC, Hoefs JC, Schiff ER, Goodman ZD, Laughlin M, Yao R, Albrecht JK and Hepatitis Interventional Therapy Group: a randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C Hepatology *34*: 395-403, 2001.

10 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M and Albrecht JK: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 358: 958-965, 2001.

ll McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S and Albrecht JK: Interferon alfa-2b alone or in combination with ribavirin as initial treatment for hepatitis C. N Eng J Med 339: 1485-1489, 1998.

12 NIH Consensus Statement on Management of Hepatitis C: 2002. NIH Consens State Sci Statements 19: 1-46, 2002.

13 Par A: Hepatitis C virus infection-after 12 years. Advances in the management of chronic hepatitis C. Orv Hetil 143: 2667-2674, 2002.

14 Fried MW: Viral factors affecting the outcome of therapy for chronic hepatitis C. Rev Gastroenterol Disord 4(suppl1):S8-S13, 2004.

15 Gasztonyi B, Par A, Kiss K, Kereskai L, Szomor A, Szeberenyi J, Pajor L and Mozsik G: Activation of the nuclear factor kappa B--key role in oncogenesis? Chronic hepatitis C virus infection and lymphomagenesis. Orv Hetil 144: 863-868, 2003.

16 Par G, Rukavina D, Podack ER, Horanyi M, Szekeres-Bartho J, Hegedus G, Paal M, Szereday L, Mozsik G and Par A: Decrease in CD3-negative CD8dim + and V2/V9 TcR + peripheral blood lymphocyte counts, low perforin expression and the impairment of natural killer cell activity is associated with chronic hepatitis C virus infection. J Hepatol 37: 514-522, 2002.

17 Perlemuter G, Letteron P, Carnot F, Zavala F, Pessayre D, Nalpas B and Brechot C: Alcohol and hepatitis C virus core protein additively increase lipid peroxidation and synergistically trigger hepatic cytokine expression in a transgenic mouse model. J Hepatol 39: 1020-1027, 2003.

18 Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C and Albrecht J: Randomized trial of interferon alfa-2b plus ribavirin for 48 weeks versus interferon alfa-2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 352: 1426-1432, 1998.

19 Laguno M, Blanch J, Murillas J, Blanco JL, Leon A, Lonca M, Larrousse M, Biglia A, Martinez E, Garcia F, Miro JM, de Pablo J, Gatell JM and Mallolas J: Depressive symptoms after initiation of interferon therapy in human immunodeficiency virus-infected patients with chronic hepatitis C. Antivir Ther 9: 905-909, 2004.

20 Sherman KE, Rouster SD, Chung RT and Rajicic N: Hepatitis C virus prevalence among patients infected with human immunodeficiency virus: a cross-sectional analysis of The US Adult AIDS Clinical Trials Group. Clin Infect Dis 34: 831-837, 2002.

21 Shiffman ML: Retreatment of HCV non-responders with peginterferon and ribavirin: results from the lead-in phase of the hepatitis C antiviral longterm treatment against cirrhosis (HALT-C) trial. (Abstract) Hepatology 36: 295A, 2002.

22 Silvestri F, Pipan C, Barillari G, Zaja F, Fanin R, Infanti L, Russo D, Falasca E, Botta GA and Baccarani M: Prevalence of hepatitis C virus infection in patients with lymphoproliferative disorders. Blood 87: 4296-4301, 1996.

23 Soriano V, Maida I, Nunez M, Garcia-Samaniego J, Barreiro P, Martin-Carbonero L and Gonzalez-Lahoz J: Long-term follow-up of HIV-infected patients with chronic hepatitis C virus infection treated with interferon-based therapies. Antivir Ther 9: 987-992, 2004.

24 Telegdy L, Gorog D, Horanyi M and Schaff Z: Severe relapse of hepatitis C following liver transplantation. Successful treatment with a combination of interferon-alpha and ribavirin. Orv Hetil 140: 1891-1893, 1999.

25 Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, O'Grady J, Reichen J, Diago M, Lin A, Hoffman J and Brunda MJ: Peginterferon alfa-2a in patients with chronic hepatitis C. N Eng J Med 343: 1666-1672, 2000.

26 Vo NV, Young KC and Lai MMC: Mutagenic and inhibitory effects of ribavirin on hepatitis C virus RNA polymerase, Biochemistry 42: 10462–10471, 2003.

27 Poynard T, Yuen MF, Ratziu V and Lai CL: Viral hepatitis C. Lancet 362: 2095-2100, 2003.

28 Patterson JL and Fernandez-Larsson R: Molecular mechanisms of action of ribavirin. Rev Infect Dis 12: 1139–1146, 1990.

29 Weber G, Shen F, Prajda N and Abonyi M: Down-regulation by drugs of the increased signal transduction in cancer cells. Advan Enzyme Regul 40:19-34, 2000.

30 Abonyi M, Nemeth ZK, Toth T, Tordai A and Prajda N: Hemochromatosis gene mutations, HCV infection, alcohol intake and hepatocellular carcinoma with liver cirrhosis (Abstract). J Hepatol 32(Suppl.2.): 165, 2000.

31 Tam RC, Lau JYN and Hong Z: Mechanisms of action of ribavirin in antiviral therapies. Antiviral Chem Chemother 12: 261–272, 2002.