

Impact of virus genotype on interferon treatment of patients with chronic hepatitis C: a multicenter controlled study

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BACKGROUND: Some factors have been reported to be associated with a greater likelihood of sustained viral response (SVR) in the interferon (IFN) treatment of chronic hepatitis C. The factors include HCV genotype, HCV RNA level in serum, state of liver disease, baseline body weight, age, sex, and race. The aim of this trial was to investigate the influence of HCV genotype on the IFN treatment of patients with chronic hepatitis C.

METHODS: The genotypes of HCV virus were determined in the patients with chronic hepatitis C from several hospitals of China enrolled into the randomized, opened and controlled trial of Peg-IFN alpha-2a (pegasys) treatment, controlled with IFN- α -2a (roferon-A). The serum ALT levels and HCV RNA concentrations of the patients were detected before and at the end of treatment and during the follow-up. The influence of HCV genotype on the IFN treatment of patients with chronic hepatitis C was analyzed in intention-to-treat (ITT) population.

RESULTS: The HCV genotypes of 202 patients were determined. Of these patients, 158 (78.22%) were infected with genotype 1 HCV and 44 (21.78%) with genotype non-1. The viral response at the end of treatment (ETVR) and sustained viral response (SVR) rates were 53.80% and 25.32% respectively in patients with genotype 1 HCV, but they were 61.36% and 43.18% in patients with genotype non-1. The difference of SVR between patients with geno-

type 1 HCV and those with genotype non-1 was significant ($P=0.021$). After being grouped by the used drugs, the ETVR rates of patients infected with genotype 1 and non-1 HCV were 76.83% and 80.95% in the patients treated with pegasys ($P=0.686$); but their SVR rates were 35.37% and 66.67% ($P=0.01$). The viral relapse rate of genotype 1 HCV (55.56%) was significantly higher than that of genotype non-1 HCV (23.53%) ($P=0.02$). In roferon-A group, the ETVR and SVR rates of patients with genotype 1 HCV were 28.95% and 14.47% respectively, which were lower but not more significant than those of patients with genotype non-1 HCV (43.48% and 21.74%). Moreover, the viral relapse rate of genotype 1 HCV (72.73%) was higher but not more significant than that of genotype non-1 HCV (50.00%) ($P=0.21$).

CONCLUSION: HCV genotype could affect the efficacies, mainly sustained responses, of IFN treatment in patients with chronic hepatitis C, and the effects of IFN are related to drugs and therapeutic course.

(*Hepatobiliary Pancreat Dis Int* 2004; 3: 369-374)

KEY WORDS: hepatitis C virus; genotype; chronic hepatitis C; interferon; PEG-IFN

Introduction

Because of high variation of genome, the isolates of HCV could be genotyped according to the nucleotide homology of the virus. The most common genotype for HCV was that developed by Simmonds, in which HCV could be divided into six genotypes.^[1] Reports showed that the HCV genotype was related to HCV RNA load in serum,^[2,3] the stage of liver disease and cirrhosis, and the risk of hepatocarcinoma happening;^[3-6] but some studies could not find this relationship. In interferon (IFN) treatment of chronic hepatitis C, some factors were associated with a greater likelihood of sustained viral response (SVR), including HCV genotype, HCV RNA level in serum, state of liver disease, baseline body weight, age, sex, and race.^[7-11] The main predictive factors of sustained response, however, were low serum HCV RNA and genotype other

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than genotype 1.^[12-19] Most studies indicated that HCV genotype could affect the efficacy of IFN in the treatment of chronic hepatitis C, and that the efficacy of IFN on genotype 2 or 3 was better than that on genotype 1 or 4.^[17-19] It was reported the genotype of HCV was the most important predictor to obtain the response to IFN treatment, and the isolate of genotype 1 showed a higher level of HCV RNA in serum, and poor response to IFV therapy compared to those genotype 2 and 3.^[20] The cause of unresponsiveness of HCV to IFN has not yet been elucidated at present. Some evidence has indicated that there are interferences between viral sequences and IFN-signal pathways.^[21-23] Additional potential causes of IFN resistance are the presence of quasispecies with various degrees of IFN-sensitivity.^[24,25] In the present study, the effect of HCV genotype on the efficacy of pegasys and roferon-A treatment in chronic hepatitis C was investigated.

Methods

Patients and treatment

The trial was designed according to the randomized, opened and multicenter controlled principles. The patients were enrolled according to the following criteria: age was from 18 to 65 years, with chronic hepatitis C except other liver diseases, anti-HCV positive and serum HCV RNA level above 600 IU/ml during the screening course, ALT value between 41 U/L and 400 U/L, being relapse after IFN therapy or never treated with IFN before. After enrollment, 208 patients were randomly divided into two groups: pegasys and roferon-A. Patients in the pegasys group were injected with 180 µg of pegasys subcutaneously every week and those in the roferon-A group were injected with roferon-A 3 MU three times a week for 24 weeks, and were followed up for another 24 weeks.

Parameter examination

The ALT level in serum was detected by an automatic biochemical analyzer.

The HCV RNA level in serum was detected by the Cobas Amplicor Monitor Test, version 2.0 according to the instructions of the manufacturer.

The genotype of HCV RNA was determined by restriction enzyme analysis of PCR product in the region of 5'-NCR of the HCV genome.^[26] In brief, the HCV RNA was extracted from the serum of the patient and standard serum containing genotype 1a, 1b, 2a, 2b, 3a, 4a and 6a HCV isolates. Subsequently, reverse-transcription was followed by 35 cycles of amplification, each consisting of 60 seconds at 94 °C, 60 seconds at 55 °C, and 90 seconds at 72 °C, and then 5 µl product of the first PCR was taken for the second round PCR amplification. The product of second round PCR amplification

was purified and digested by restriction enzyme, in which the group 1 used HaeIII (1 µl), RsaI (1 µl) and the group 2 used HinFI (1 µl), MvaI (1 µl) at 37 °C for 4-16 hours. According the results of groups 1 and 2, restriction enzyme digestions of the groups 3 and 4 were performed using BstUI (1 µl) at 60 °C for 4-16 hours and ScrFI (1 µl) at 37 °C for 4-16 hours respectively. The digestive products were analyzed by electrophoresis, and the genotype of sample was confirmed by the standard serum.

Before treatment of the patients, their HCV RNA level in serum, genotype of HCV isolate, and ALT level were determined, and at the end of treatment the levels of HCV RNA and ALT in serum were detected also. The viral response was defined negative when the HCV RNA level in serum was below 600 IU/ml, and biochemical response defined when the level of ALT was normal. Negative HCV RNA combined with normal ALT showed complete response. Sustained viral response was defined as HCV RNA in serum keeping negative during the follow-up. The efficacy of the treatment was valued when sustained viral response was obtained.

Statistical analysis

The response rate was analyzed in ITT people through the Cochrane Mantel Haenszel test using the SAS statistical software.

Results

HCV isolates genotypes were determined in 202 patients. If one patient infected with mixed genotypes containing genotype 1 isolates, he or she was included in genotype 1 group. Of the 202 patients, 158 (78.22%) were infected with genotype 1 HCV isolates and 44 (21.78%) with genotype non-1 isolates.

In the pegasys group, 3 of 106 patients were not determined for HCV genotypes. In the genotyped patients, 82 (79.61%) infected with genotype 1 and 21 (20.39%) with genotype non-1 HCV isolates. In the roferon-A group, the HCV genotypes of 3 patients could not be determined, and 76 (76.77%) patients infected with genotype 1 and 23 (23.23%) with genotype non-1 HCV isolates. The composition of general data including age, sex, HCV genotype, and HCV RNA load was not different between the two groups.

Overall, the end-treatment viral response (ETVR) rate of the patients infected with genotype non-1 was higher but not more significant than that of the patients infected with genotype 1; but the biochemical response rate in the genotype non-1 patients was more significant than that in the genotype 1 patients at the end of treatment. The sustained viral response (SVR) and biochemical response rates of genotype non-1 were more significant than those of genotype 1 (Table 1).

Table 1. The overall viral response and biochemical response rates in genotype 1 and non-1 patients (%)

	n	Viral response			Biochemical response				
		ETVR	χ^2/P value	SVR	χ^2/P value	24-week	χ^2/P value	48-week	χ^2/P value
Genotype 1	158	53.80	0.798/0.372	25.32	5.313/0.021	41.14	4.480/0.034	37.97	7.678/0.006
Genotype non-1	44	61.36		43.18		59.09		61.36	

Table 2. The biochemical and viral response of genotype 1 and non-1 from pegasys and roferon-A groups at the end of treatment

	Biochemical-response (%)	χ^2/P value	ETVR (%)	χ^2/P value	Complete response (%)	χ^2/P value
Pegasys						
Genotype 1	31(37.80)	3.968/0.046	63(76.83)	0.164/0.686	25(30.49)	3.525/0.061
Genotype non-1	13(61.90)		17(80.95)		11(52.38)	
Roferon-A						
Genotype 1	34(44.74)	0.984/0.321	22(28.95)	1.704/0.192	19(25.00)	0.011/0.916
Genotype non-1	13(56.52)		10(43.48)		6(26.09)	

Table 3. The biochemical and viral response of genotype 1 and non-1 from pegasys and roferon-A groups at the end of follow-up

	Biochemical-response (%)	χ^2/P value	SVR (%)	χ^2/P value	Complete response (%)	χ^2/P value
Pegasys						
Genotype 1	36(43.90)	9.188/0.002	29(35.37)	6.735/0.01	23(28.05)	8.429/0.004
Genotype non-1	17(80.95)		14(66.67)		13(61.90)	
Roferon-A						
Genotype 1	24(31.58)	1.109/0.292	11(14.47)	0.688/0.407	10(13.16)	0.001/0.989
Genotype non-1	10(43.48)		5(21.74)		3(13.04)	

Table 4. The relapse rates of genotype 1 and non-1 patients in pegasys and roferon-A groups

	Pegasys	χ^2/P value	Roferon-A	χ^2/P value	Overall relapse (%)	χ^2/P value
Genotype 1	55.56% (35/63)	5.4959/0.02	72.73% (16/22)	1.5742/0.21	60.00%	5.8585/0.016
Genotype non-1	23.53% (4/17)		50.00% (5/10)		33.33%	

After being layered by the drugs used, in the pegasys group, the biochemical response rate of genotype non-1 was significantly higher than that of genotype 1; but the ETVR and complete response rates were not of significant difference between the genotype 1 and non-1 patients at the end of treatment. In the roferon-A group, however, the biochemical, ETVR and complete response rates were not significantly different between the genotype 1 and non-1 patients (Table 2).

At the end of follow-up in the pegasys group, the SVR and complete response rates of the genotype non-1 patients were significantly higher than those of the genotype 1 patients; but in the roferon-A group, the biochemical, SVR and complete response rates were still not different between the genotype 1 and non-1 patients (Table 3).

Overall, 85 patients with genotype 1 HCV isolates showed ETVR, but 60% of them relapsed at the end of follow-up. The percentage was significantly higher than that of the genotype non-1 patients. In the pegasys group, the relapse rates of genotype 1 and non-1 were

55.56% and 23.53% respectively, and the difference was significant; but in the roferon-A group they were 72.73% and 50.00%, and the difference was not significant. The relapse rate in the roferon-A group, either genotype 1 or non-1 patients, was higher than that in the pegasys group (Table 4).

Discussion

Many studies have indicated that the viral factors affecting the efficacy of IFN on chronic hepatitis C include HCV genotype, baseline HCV RNA level in serum and the viral dynamics of hepatitis C virus after initiation of IFN treatment.^[27-30] HCV genotype is considered not only associated with HCV RNA level but also with the viral dynamics of hepatitis C virus during the therapy.^[27,31] It was reported that HCV genotype was one of the most important factors affecting the efficacy of IFN on chronic hepatitis C and that the effect of IFN on genotype 1 was lower than that on genotypes 2 and 3 regardless whether or not it was combined with ribavirin.^[32-36]

In this study, 158 (78.22%) of the genotyped patients were infected with genotype 1 HCV isolates and 44 (21.78%) infected with genotype non-1 HCV isolates. At the end of treatment, the biochemical, not ETVR, response of patients with genotype non-1 was significantly higher than that of those with genotype 1. This result indicated that at the end of the treatment HCV genotype mainly affected biochemical response to IFN treatment of patients with chronic hepatitis C. At the end of treatment, although, the ETVR of genotype 1 was not strikingly different from that of genotype non-1, at the end of follow-up the biochemical response and SVR rates of genotype 1 were significantly lower than those of genotype non-1. This result could be explained by the immunostimulatory activity of pegasys. Kamal^[37] reported that pegasys alone or combined with ribavirin treatment could induce significant increase in the frequency, strength, and breadth of HCV-specific CD4+ T-cell responses with type 1 predominance; whereas interferon alpha-2a monotherapy was associated with lower, fluctuating, short-lived responses. The HCV special cellular immunity elevated by pegasys might clean virus by killing the infected cells continually and keep virus load at a low level. These results indicated that HCV genotype mainly affected the SVR to IFN treatment of patients with chronic hepatitis C.

After being layered by the drugs used in the pegasys group, the biochemical response in patients with genotype non-1 HCV isolates was significantly higher than that in patients with genotype 1 HCV isolates at the end of the treatment, but the difference of ETVR was not significant. At the end of follow-up, however, the biochemical response and SVR rates of genotype non-1 HCV isolates were significantly higher than those of genotype 1. The results indicated at the early stage of pegasys treatment for patients with chronic hepatitis C, the genotype of HCV isolates mainly affected biochemical response, but after 6-month treatment it affected not only biochemical response but also SVR rates. Portal and his colleagues^[38] retreated relapsed patients with chronic hepatitis C using IFN or IFN + ribavirin. It was found that in combined treatment group, the ETVR rates between genotypes 1 and 2 or 3 were not significantly different, but the SVR rates in genotypes 2 or 3 were always higher than those of genotype 1.

In the roferon-A treated patients, neither biochemical response and ETVR rates at the end of treatment nor biochemical response and SVR rates at the end of follow-up were significantly different between the genotype 1 and non-1 HCV infected patients. The difference of SVR between the genotype 1 and non-1 patients was smaller than that of ETVR, indicating that some patients relapsed during the course of follow-up. In the present study, patients included the relapsed after naive treatment of IFN. In the relapsed patients, the treatment course should be extended to elevate the SVR rate. The

results of Sievert^[39] study showed that 65% patients with chronic hepatitis infected with genotypes 2 and 3 HCV could obtain SVR after 6-month IFN treatment, but in the relapsed patients the time of treatment with IFN + ribavirin was longer than 6 months to get SVR again. In the present study, why no difference of SVR was observed between the genotype 1 and non-1 patients in the roferon-A treated patients might be in adequate therapeutic time. It was reported that prolonged therapeutic course could elevate the SVR rate.^[40-43] The results in Bellobuono's study suggested that HCV genotype and therapeutic duration were independent predictors assessing SVR in the IFN treatment of patients with chronic hepatitis C.^[44] Moreover Saracco's study concluded that the duration of therapy rather than the dosage of IFN is more important in increasing SVR in HCV-positive patients with genotypes 1 and 4 who relapsed after IFN monotherapy. Patients with genotypes 2 and 3 can be effectively retreated with a 6-month course of combination therapy, avoiding possible side-effects and waste of resources.^[45] A small number of patients infected with genotype non-1 in this study might be due to another possibility that there was not difference of SVR between the genotype 1 and non-1 patients in the roferon-A treatment group.

In our study, the overall relapse rate of genotype 1 was 60.00% and that of genotype non-1 was 33.33%; the difference was significant. It was indicated that HCV genotype could affect the relapse rate. In the pegasys group of this study, the relapse rate of genotype 1 was significantly higher than that of genotype non-1. This could be postulated that after a 6-month pegasys treatment, most genotype non-1 HCV infected patients could clean the virus, but a 6-month therapy is not enough for patients with genotype 1 HCV, and prolonged therapeutic time or its combined use with ribavirin may help to enhance the SVR rate in the relapsed patients.

In the roferon-A group of this study, the relapse rate of genotype 1 was up to 72.73% and that of genotype non-1 was 50.00%. It was indicated that either for genotype 1 or non-1 HCV infected patients the relapse rate was higher after a 6-month roferon-A treatment. It was reported that in the patients with genotype 1 HCV, prolonged time of treatment to one year or combined treatment of roferon with ribavirin could significantly enhance the SVR rate.^[46]

In conclusion, among the factors affecting the efficacy of IFN treatment of patients with chronic hepatitis C, the genotype of HCV isolates mainly affect the SVR rate, but the effects of IFN treatment is also related to the drugs used as well as the therapeutic course.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

- 1 Simmonds P, Holmes EC, Cha TA, Chan SW, McOmishi F, Irvine B, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* 1993;74:2391-2399.
- 2 Bukh J, Miller RH, Purcell RH. Genetic heterogeneity of hepatitis C virus: Quasispecies and genotypes. *Semin Liver Dis* 1995;15:41-63.
- 3 Anne G, Martinot M, Boyer N, Auperin A, Breton VL, Degott C, et al. Quantitation of hepatitis C virus RNA in patients with chronic hepatitis C. Relationship with severity of disease, viral genotype and response to treatment. *J Hepatol* 2001;35:399-405.
- 4 Pozzato G, Kaneko S, Moretti M, Croce LS, Franzin F, Unoura M, et al. Different genotype hepatitis C virus are associated with different severity of chronic liver disease. *J Med Virol* 1994;43:291-296.
- 5 Qu D, Li JS, Vitvitski L, Mechai S, Berby F, Tong SP, et al. Hepatitis C virus genotype in France: comparison of clinical features of patients infected with HCV type I and type II. *J Hepatol* 1994;21:70-75.
- 6 Zein NN, Poterucha JJ, Gross JB Jr, Wiesner RH, Thorneau TM, Gossard AA, et al. Increases risk of hepatocellular carcinoma in patients infected with hepatitis C genotype 1b. *Am J Gastroenterol* 1996;91:2560-2562.
- 7 Lindsay KL. Introduction to therapy of hepatitis C. *Hepatology* 2002;36:S114-120.
- 8 McHutchison JG, Manns MP, Ling MH, Koury K, Albrecht JK. Peginterferon alpha-2b plus ribavirin for treatment of chronic hepatitis C: is patient gender a confounding factors for sustained virologic response when ribavirin dose is expressed as mg/kg of body weight? *Hepatology* 2001;34:329A.
- 9 Reddy KR, Hoofnagle JH, Tong MJ, Lee WM, Pockros P, Heathcote EJ, et al. Racial differences in responses to therapy with interferon in chronic hepatitis C. *Hepatology* 1999;30:779-787.
- 10 Medina J, Garcia-Buey L, Moreno-Monteagudo JA, Trapero-Marugan M, Moreno-Otero R. Combined antiviral options for the treatment of chronic hepatitis C. *Antiviral Res* 2003;60:135-143.
- 11 Fukutomi T, Fukutomi M, Iwao M, Watanabe H, Tanabe Y, Hiroshige K, et al. Predictors of the efficacy of intravenous natural interferon-beta treatment in chronic hepatitis C. *Med Sci Monit* 2000;6:692-698.
- 12 Martinot-Peignoux M, Marcellin P, Pouteau M, Castelnau C, Boyer N, Poliquin M, et al. Pretreatment serum hepatitis C virus RNA levels and hepatitis virus genotype are the main and independent prognostic factors of sustained response to interferon alpha therapy in chronic hepatitis C. *Hepatology* 1995;22:1050-1056.
- 13 Serfaty L, Giral P, Loria A, Andreani T, Legendre C, Poupon R. Factors predictive of the response to interferon in patients with chronic hepatitis C. *J Hepatol* 1994;21:12-17.
- 14 Tsubota A, Chayama K, Ikeda Y, Yasuji A, Koida I, Saitoh S, et al. Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. *Hepatology* 1994;19:1088-1104.
- 15 Kondo M, Tanaka K, Ikeda M, Arata S, Saiton S, Sakaguchi T, et al. Hepatic HCV RNA as a predictor of outcome after interferon therapy in patients with chronic hepatitis C. *J Gastroenterol Hepatol* 1996;11:236-240.
- 16 Hino K, Sainokami S, Shimoda K, Lino S, Wang Y, Okamoto H, et al. Genotype and titers of hepatitis C virus for predicting response to interferon in patients with chronic hepatitis C. *J Med Virol* 1994;42:299-305.
- 17 Moreno L, Quereda C, Moreno A, Perez-Elias MJ, Antela A, Casado JL, et al. Pegylated interferon alpha2b plus ribavirin for the treatment of chronic hepatitis C in HIV-infected patients. *AIDS* 2004;18:67-73.
- 18 Perez-Olmeda M, Soriano V, Asensi V, Morales D, Romero M, Ochoa A, et al. Treatment of chronic hepatitis C in HIV-infected patients with interferon alpha-2b plus ribavirin. *AIDS Res Hum Retroviruses* 2003;19:1083-1089.
- 19 Jiao J, Wang JB. Effects of HCV genotypes and HLA-DRB alleles on the response of chronic hepatitis C patients to interferon alpha and libavilin. *Zhonghua Gan Zang Bing Za Zhi* 2003;11:620-622.
- 20 Orito E. HCV genotype as a predictor of response to interferon therapy in patients with chronic hepatitis C. *Nippon Rinsho* 2001;59:1356-1362.
- 21 Taylor DR, Shi ST, Romano PR, Barber GN, Lai MM. Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. *Science* 1999;285:107-110.
- 22 Chayama K, Suzuki F, Tsubota A, Kobayashi M, Arase Y, Saitoh S, et al. Association of amino acid sequence in the PKR-epsilon2 phosphorylation homology domain and response to interferon therapy. *Hepatology* 2000;32:1138-1144.
- 23 McKennie VM, Mills PR, McCrudden EA. The NS5A gene of hepatitis C in patients treated with IFN-alpha. *J Med Virol* 2000;60:367-378.
- 24 Pawlotsky JM. Hepatitis C virus resistance to antiviral therapy. *Hepatology* 2000;32:889-896.
- 25 Nousbaum J, Polyak SJ, Ray SC, Sullivan DG, Larson AM, Carithers RL Jr, et al. Prospective characterization of full-length hepatitis C virus NS5A quasispecies during induction and combination antiviral therapy. *J Virol* 2000;74:9028-9038.
- 26 Sun NX, Fan XF, Du SC. The study on hepatitis C genotype based on simmonds' classification by using restriction endonuclease method. *Jiangshu Med* 1999;25:481-483.
- 27 Karino Y, Toyota J, Sugawara M, Miyazaki K, Kuwata Y, Yamazaki K, et al. Hepatitis C virus genotypes and hepatic fibrosis regulate 24-h decline of serum hepatitis C virus RNA during interferon therapy in patients with chronic hepatitis C. *J Gastroenterol Hepatol* 2003;18:404-410.
- 28 Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R, et al. Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. *Hepatology* 2003;37:600-609.
- 29 Thelu MA, Baud M, Leroy V, Seigneurin JM, Zarski JP. Dynamics of viral quasispecies during interferon therapy in non responder chronic hepatitis C patients. *J Clin Virol* 2001;22:125-131.
- 30 Ferenci P. Predicting the therapeutic response in patients with chronic hepatitis C: the role of viral kinetic studies. *J Antimicrob Chemother* 2004;53:15-18.
- 31 Halfon P, Neumann AU, Bourliere M, Rieu A, Chadapaud S, Khiri H, et al. Slow viral dynamics of hepatitis C virus genotype 4. *J Viral Hepat* 2003;10:351-353.
- 32 Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alpha-2b alone or in combination

- with ribavirin for the treatment of relapse of chronic hepatitis C. *N Eng J Med* 1998;339:1493-1499.
- 33 Davis GL, Lau JYN. Factors predictive of a beneficial response to therapy of hepatitis C. *Hepatology* 1997;26:122S-127S.
- 34 Tassopoulos NC, Ketikoglou I, Tsantoulas D, Raptopoulou M, Hatzis G, Vafiadis I, et al. A randomized trial to assess the efficacy of interferon-alpha daily in combination with ribavirin in the treatment of naive patients with chronic hepatitis C. *J Viral Hepat* 2003;10:383-389.
- 35 Mangia A, Minerva N, Annese M, Leandro G, Villani MR, Santoro R, et al. A randomized trial of amantadine and interferon versus interferon alone as initial treatment for chronic hepatitis C. *Hepatology* 2001;33:989-993.
- 36 Westin J, Lindh M, Nenonen N, Lagging LM, Norkrans G, Wejstal R. Monitoring virological responses to interferon-ribavirin and interferon monotherapy of chronic hepatitis C re-treated due to relapse or non-response. *Scand J Infect Dis* 2001;33:110-115.
- 37 Kamal SM, Fehr J, Roesler B, Peters T, Rasenack JW. Peginterferon alone or with ribavirin enhances HCV-specific CD4 T-helper 1 responses in patients with chronic hepatitis C. *Gastroenterology* 2002;123:1070-1083.
- 38 Portal I, Bourliere M, Halfon P, De Ledinghen V, Couzigou P, Bernard PH, et al. Retreatment with interferon and ribavirin vs interferon alone according to viraemia in interferon responder-relapser hepatitis C patient: a prospective multicenter randomized controlled study. *J Virol Hepatol* 2003;10:215-223.
- 39 Sievert W. Management issues in chronic viral hepatitis: hepatitis C. *J Gastroenterol Hepatol* 2002;17:415-422.
- 40 Thevenot T, Regimbeau C, Ratzu V, Leroy V, Opolon P, Poynard T. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C in naive patients: 1999 update. *J Viral Hepat* 2001;8:48-62.
- 41 Cheng SJ, Bonis PA, Lau J, Pham NQ, Wong JB. Interferon and ribavirin for patients with chronic hepatitis C who did not respond to previous interferon therapy: a meta-analysis of controlled and uncontrolled trials. *Hepatology* 2001;33:231-240.
- 42 Par A, Telegdy L, Gogl A, Muller E. Interferon therapy of chronic viral hepatitis in Hungary: 5-year experience. A multicenter study. *Orv Hetil* 1999 30;140:1227-1233.
- 43 O'Brien CB, Henzel BS, Moonka DK, Inverso J, Rook A. Extracorporeal photopheresis alone and with interferon-alpha2a in chronic hepatitis C patients who failed previous interferon therapy. *Dig Dis Sci* 1999;44:1020-1026.
- 44 Bellobuono A, Mondazzi L, Tempini S, Silini E, Ideo G. Prospective comparison of four lymphoblastoid interferon alpha schedules for chronic hepatitis C. A multivariate analysis of factors predictive of sustained response to treatment. *Eur J Gastroenterol Hepatol* 1997;9:1169-1177.
- 45 Saracco G, Olivero A, Ciancio A, Careni S, Smedile A, Cariti G, et al. A randomized 4-arm multicenter study of interferon alfa-2b plus ribavirin in the treatment of patients with chronic hepatitis C relapsing after interferon monotherapy. *Hepatology* 2002;36:959-966.
- 46 Saracco G, Olivero A, Ciancio A, Careni S, Rizzetto M. Therapy of chronic hepatitis C: a critical review. *Curr Drug Targets Infect Disord* 2003;3:25-32.

Received May 12, 2004

Accepted after revision June 16, 2004