PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(27):4241-4245
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Co-infection of SENV-D among chronic hepatitis C patients treated with combination therapy with high-dose interferon-alfa and ribavirin

Chia-Yen Dai, Wan-Long Chuang, Wen-Yu Chang, Shinn-Cherng Chen, Li-Po Lee, Ming-Yen Hsieh, Nei-Jen Hou, Zu-Yau Lin, Ming-Yuh Hsieh, Liang-Yen Wang, Ming-Lung Yu

Chia-Yen Dai, Wan-Long Chuang, Wen-Yu Chang, Shinn-Cherng Chen, Li-Po Lee, Ming-Yen Hsieh, Nei-Jen Hou, Zu-Yau Lin, Ming-Yuh Hsieh, Liang-Yen Wang, Ming-Lung Yu, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, China
Chia-Yen Dai, Nei-Jen Hou, Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan, China
Chia-Yen Dai, Department of Occupational Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan, China
Supported by the National Science Council Grant, No. NSC-91-2314-B037-344
Correspondence to: Ming-Lung Yu, MD, PhD, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, No. 100, Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, China. d780178@kmu.edu.tw
Telephone: +886-7-3121101-7475 Fax: +886-7-3123955
Received: 2004-11-08 Accepted: 2004-12-20

Abstract

AIM: The clinical significance of co-infection of SENV-D among patients with chronic hepatitis C (CHC) and response of both viruses to combination therapy with high-dose interferon-alfa (IFN) plus ribavirin remain uncertain and are being investigated.

METHODS: Total 164 (97 males and 67 females, the mean age 48.1±11.4 years, range: 20-73 years, 128 histologically proved) naive CHC patients were enrolled in this study. SENV-D DNA was tested by PCR method. Detection of serum HCV RNA was performed using a standardized automated qualitative RT-PCR assay (COBAS AMPLICOR HCV Test, version 2.0). HCV genotypes 1a, 1b, 2a, 2b, and 3a were determined by using genotype-specific primers. Pretreatment HCV RNA levels were determined by using the branched DNA assay (Quantiplex HCV RNA 3.0). There are 156 patients receiving combination therapy with IFN 6 MU plus ribavirin for 24 wk and the response to therapy is determined.

RESULTS: Sixty-one (37.2%) patients were positive for SENV-D DNA and had higher mean age than those who were negative (50.7±10.6 years *vs* 46.6±11.6 years, $P = 0.026$). The rate of sustained viral response (SVR) for HCV and SENV-D were 67.3% (105/156) and 56.3% (27/48), respectively. By univariate analysis, the higher rate of SVR was significantly related to HCV genotype non-1b ($P<0.001$), younger ages ($P = 0.014$), lower pretreatment levels of HCV RNA ($P = 0.019$) and higher histological activity index (HAI) score for intralobular regeneration and focal necrosis ($P = 0.037$). By multivariate analyses, HCV genotype non-1b, younger age and lower pretreatment HCV RNA levels were significantly associated with HCV SVR (odds ratio (OR)/95% confidence interval (CI): 12.098/0.02-0.19, 0.936/0.890-0.998, and 3.131/1.080-9.077, respectively). The SVR of SENV-D was higher among patients clearing SENV-D than those who had viremia at the end of therapy ($P = 0.04$).

CONCLUSION: Coexistent SENV-D infection, apparently associated with higher ages, is found in more than one-third Taiwanese CHC patients. Both HCV and SENV-D are highly susceptible to combination therapy with high-dose IFN and ribavirin and SENV-D co-infection does not affect the HCV response. HCV genotype, pretreatment HCV RNA levels and age are predictive factors for HCV SVR.

Key words: Chronic hepatitis C; Combination therapy; Interferon; Ribavirin; SENV-D

Dai CY, Chuang WL, Chang WY, Chen SC, Lee LP, Hsieh MY, Hou NJ, Lin ZY, Hsieh MY, Wang LY, Yu ML. Co-infection of SENV-D among chronic hepatitis C patients treated with combination therapy with high-dose interferon-alfa and ribavirin. *World J Gastroenterol* 2005; 11(27): 4241-4245
http://www.wjgnet.com/1007-9327/11/4241.asp

INTRODUCTION

A new family of DNA viruses was recently isolated and designated as SEN virus (SENV)[1,2]. SENV is a single-stranded circular DNA virus distantly related to the large TT virus family with eight different variants (A-H) shown by phylogenetic analysis[3]. Two strains of SENV (SENV-D and SENV-H) are more prevalent among patients with transfusion-associated non-AE hepatitis than in healthy blood donors that suggested the significant associations between SENV-D/H and transfusion-associated hepatitis[2,4]. Nevertheless, the clinical implication and etiological importance in association with liver diseases of SENV infection still remain undetermined.

HCV is the major etiologic agent of post-transfusion hepatitis and leads to chronic liver disease and primary

hepatocellular carcinoma[5,6]. With the prevalence rate of chronic hepatitis C (CHC) ranging from 0.95% to 2.6% among the general population[7,8], we had previously reported a positive rate of anti-HCV up to 57.9% in some communities in southern Taiwan[9]. For CHC, the combination therapy with interferon-alfa (IFN) and ribavirin has been considered as first-line therapy. Previous reports have demonstrated the increased rate of sustained viral response (SVR) to 31-43% after combination therapy for 24 or 48 wk than 6-19% of IFN monotherapy[10,11]. Lai *et al*., reported a SVR rate of 43% after combination therapy with standard IFN dose in Taiwan[12]. In our previous study, the rate of HCV SVR with a high-dose IFN monotherapy achieved 41.2% among Taiwanese CHC patients[13] and the tailored-dose IFN monotherapy according to the virological characteristics of CHC patients yielded a better efficacy[14]. The benefits of high-dose IFN may be gained in the combination therapy for CHC.

The clinical significance of SENV infection in combination with HCV infection remains controversial. For patients with CHC, Rigas *et al*., reported that co-infection with SENV might adversely affect the outcome of treatment with combination therapy[15]. Another study, however, did not support their findings[16]. The aims of the present study are to survey the prevalence and clinical implications of SENV-D co-infection on biochemical, pathological, and virological profiles among CHC patients. The response of HCV and SENV-D to combination therapy with high-dose IFN and ribavirin are also investigated. Furthermore, we elucidate the predictive factors for HCV SVR and the influence of concurrent SENV-D infection on HCV response to combination therapy.

MATERIALS AND METHODS

Patients

Between May 1998 and May 2001, a total of 164 Taiwanese CHC patients in the clinics of hepatological division of the Kaohsiung Medical University Hospital, 97 men and 67 women, aged between 20 and 73 years (mean 48.1±11.4 years) were enrolled in the study. All patients were diagnosed with chronic HCV infection based on continuous positivity for second-generation antibody to HCV (anti-HCV) in serum for more than 6 mo and positive for HCV RNA. Liver biopsies were carried out in 128 patients and the disease activity grade and fibrosis stage were quantitatively scored according to the histological activity index (HAI) scoring system[17]. Patients who were positive for hepatitis B surface antigen (HBsAg) had human immunodeficiency virus type I infection, autoimmune liver disease, metabolic liver diseases including α-1 anti-trypsin deficiency hemochromatosis or Wilson's disease, alcoholic liver disease or intravenous drug abuse were excluded. All the serum samples, when collected from patients at the time of their evaluation, were stored at -70 ℃ before testing. The study had been approved by the Ethics Committee of Kaohsiung Medical University Hospital and all patients had given their informed consent.

Methods

Laboratory tests Serum HBsAg was assayed using commercially available kits (General Biological HBsAg radio-immunoassay; General Biological Cooperation, Taiwan) and second-generation HCV antibody (anti-HCV) was detected with commercially available ELISA kits (Abbott, North Chicago, IL, USA). Alanine aminotransferase (ALT, normal upper limit of serum ALT = 34 IU/L) was measured on a multichannel autoanalyzer.

Detection of SENV-D DNA and detection/quantification/genotyping of serum HCV RNA The presence of SENV-D DNA was determined by PCR as described previously[18]. Detection of serum HCV RNA was performed using a standardized automated qualitative RT-PCR assay (COBAS AMPLICOR HCV Test, version 2.0; Roche, Branchburg, NJ, USA). The detection limit was 50 IU/mL. HCV genotypes 1a, 1b, 2a, 2b, and 3a were determined by amplification of the core region using genotype-specific primers described by Okamoto *et al*.[19]. Pretreatment HCV RNA levels were determined by using the branched DNA assay (Quantiplex HCV RNA 3.0, Bayer, Emeryville, CA, USA), performed strictly in accordance with the manufacturer's instructions. The quantification limit was 615 IU of HCV RNA per milliliter.

Combination therapy with high-dose IFN and ribavirin Total 156 CHC-naive patients (92 males, 64 females, mean age: 48.0±11.5 years, 122 patients with liver biopsies) received combination therapy for 24 wk using IFN subcutaneously at a dosage of 6 MU thrice a week and ribavirin by mouth at a dosage of 1 000-1 200 mg daily. After the cessation of therapy, all of them received 24 wk of follow up for evaluation of the response. A SVR for HCV was defined as clearance of serum HCV RNA at the end of the therapy and 24 wk after the cessation of combination therapy. All other patients were defined as non-responders (NR). To evaluate the response of SENV-D, the presence of SENV-D DNA was determined at wk 24 and 48. An end-of-treatment viral response (ETVR) and a SVR for SENV-D were indicated by negative PCR results at wk 24 and 48, respectively.

Statistical analysis

Serum HCV RNA levels were expressed as the mean±SD after logarithmic transformation of original values. Frequency was compared between groups using the χ^2 test or Fisher's exact test, and group means were compared using the *t*-test. For all tests a *P* value lesser than 0.05 was considered to be significant. Stepwise logistic regression was used to analyze factors associated with response to combination therapy in CHC patients. Odds ratios (ORs) and their associated 95% confidence intervals (CIs) were used to quantify the magnitude of their associations.

RESULTS

Study population

The mean pretreatment ALT and HCV RNA levels of the 164 CHC patients was 136.9±168.9 IU/L and 5.79±0.67 log IU/mL, respectively. There were 140 (85.4%) patients with abnormal ALT levels. The HCV genotype distribution was as follows: 1b in 80 (48.8%) patients, 2a in 48 (29.3%) patients, 2b in 18 (11.0%) patients, mixed in 13 (7.9%)

patients and unclassified in 5 (3.0%) patients. Of 128 patients undergoing liver biopsies, the mean scores for peri-portal necrosis, intralobular necrosis, portal inflammation (grading) and fibrosis were 1.11±1.34, 0.52±0.88, 1.91±1.24, 3.51±2.55, and 1.25±1.36, respectively.

SENV-D viremia in chronic hepatitis C patients

Of 164 CHC patients, 61 patients were positive for SENV-D DNA showing a prevalence of 37.2%. The comparison of clinical characteristics between patients with and without SENV-D co-infection was shown in Table 1. The mean age was higher among patients with positive SENV-D DNA than those who were negative for SENV-D DNA (50.7±10.6 years *vs* 46.6±11.6 years, $P = 0.026$). No other clinical and virological factor was related to positive SENV-D DNA. Among 128 patients that underwent liver biopsies, all the mean scores were similar between SENV-D DNA-positive and -negative patients.

Table 1 Comparison of clinical characteristics between individuals with and without SENV-D viremia in 164 CHC patients

	SENV-D viremia		*P*
	Positive (*n* = 61)	Negative (*n* = 103)	
Sex (male) (%)	36 (59.0)	61 (59.2)	NS
Age (yr)	50.7±10.6	46.6±11.6	0.026
Serum ALT (IU / L)	144.0±164.3	125.8±164.2)	NS
Normal (≤34 IU / L) (%)	8 (33.3)	16 (66.7)	NS
Abnormal (>34 IU / L) (%)	53 (37.9)	87 (62.1	
HCV RNA levels (log IU / mL)	5.72±0.70	5.85±0.63	NS
HCV genotype 1b (%)	26 (42.6)	54 (52.4)	NS
Histology (HAI scores)	46	82	
Peri-portal necrosis	1.11±1.34	1.11±1.35	NS
Intralobular necrosis	0.70±1.00	0.41±0.78	NS
Portal inflammation	1.80±1.31	1.98±1.21	NS
Total score (grading)	3.54±2.65	3.49±2.52	NS
Fibrosis	1.39±1.50	1.16±1.28	NS

Results are expressed as mean±SD. HAI: histological activity index, NS: no significance.

HCV virological response to combination therapy

Of all 156 naive CHC patients receiving combination therapy, the HCV genotype distribution was as follows: 1b in 76 (48.7%) patients, 2a in 47 (30.1%) patients, 2b in 16 (10.3%) patients, mixed in 12 (7.7%) patients, and unclassified in 5 (3.2%) patients. The mean pretreatment ALT and HCV RNA levels were 134.8±166.8 IU/L and 5.79±0.66 log IU/mL, respectively with 133 (85.3%) and 99 (63.5%) patients having abnormal ALT levels and high serum HCV levels (≥200 000 IU/mL). Of 122 patients undergoing liver biopsies, the mean scores for peri-portal necrosis, intralobular necrosis, portal inflammation (grading), and fibrosis were 1.09±1.31, 0.52±0.89, 1.93±1.22, 3.52±2.48, and 1.23±1.33, respectively. After combination therapy with high dose IFN and ribavirin for 24 wk, 105 (67.3%) of 156 patients achieved SVR. The clinical and virological features between CHC patients with HCV SVR and those with NR are shown in Table 2. In comparison between these two groups by univariate analysis, the higher rate of SVR was significantly related to younger ages ($P = 0.014$), lower pretreatment levels of HCV RNA (<200 000 IU/mL, $P = 0.019$), HCV genotype non-1b ($P<0.001$) and higher HAI score for intralobular regeneration and focal necrosis ($P = 0.037$). No significant association between other clinical and virological factors and HCV response of combination therapy was observed. Based on multivariate regression analyses, the significant factors associated with HCV SVR after combination therapy were HCV genotype non-1b, younger age and pretreatment HCV RNA levels less than 200 000 IU/mL with the OR and 95%CI of these factors summarized in Table 3.

Table 2 Comparison of clinical and virological features between sustained viral responders (SVR) and non-responders (NR) of CHC patients after combination therapy

	HCV response		*P*
	NR (*n* = 51)	SVR (*n* = 105)	
Sex (male) (%)	31 (60.8)	61 (58.1)	NS
Age (yr)	51.24±11.3	46.4±11.3	0.014
Serum ALT (IU / L)	122.2±105.0	140.9±189.9	NS
Normal (≤34 IU / L) (%)	4 (18.2)	18 (81.8)	NS
Abnormal (>34 IU / L) (%)	47 (34.6)	87 (65.4)	
HCV RNA levels (log IU / mL)	5.90±0.62	5.73±0.68	NS
High level (≥200 000 IU / mL) (%)	39 (39.4)	60 (60.6)	0.019
Low level (<200 000 IU / mL) (%)	12 (21.1)	45 (78.9)	
HCV genotype 1b (%)	44 (86.3)	32 (30.5)	<0.0001
Positive SENV-D DNA (%)	16 (31.4)	41 (39.0)	NS
Histology (HAI scores)			
Patients no.	37	85	
Peri-portal necrosis	1.08±1.30	1.091±1.323	NS
Intralobular necrosis	0.278±0.61	0.64±0.97	0.037
Portal inflammation	2.03±1.17	1.89±1.25	NS
Total score (grading)	3.32±2.22	3.60±2.59	NS
Fibrosis	1.41±1.38	1.157±1.31	NS

Results are expressed as mean±SD. HAI: histological activity index, NS: no significance.

Table 3 Stepwise logistic regression analysis of factors significantly associated with HCV sustained virologic response (SVR) after combination therapy in 156 CHC patients

Dependent variable	Independent variable	Comparison	OR (95%CI[1])	*P*
HCV SVR				
	HCV genotypes	1b = 0	12.098 (0.02–0.19)	<0.001
		Non-1b = 1		
	Age	Per year increased	0.936 (0.890–0.998)	0.011
	HCV RNA level	High (≥200 000 IU / mL) = 0	3.131 (1.080–9.077)	0.036
	Low (<200 000 IU / mL) = 1			

[1]CI: Confidence interval.

Clearance of SENV-D DNA after combination therapy

SENV-D DNA was followed in 48 CHC patients (28 males, 20 females, mean age: 50.2±10.5 years) concomitant with SENV-D viremia before combination therapy. Their mean ALT level was 154.6±179.8 IU/L (range: 16-1 112 years) and 41 patients were abnormal. The HCV genotype distribution is as follows: 1b in 19 patients, 2a in 19 patients, 2b in 4 patients, mixed in 5 patients, and unclassified in 1 patient. The clinical characteristics and virological features between individuals with and without SENV-D DNA after combination therapy were analyzed and shown in Table 4. At the end of treatment, SENV-D DNA was negative in 37 patients (77.1%). Thirteen of thirty-seven patients (35.1%) had reappearance of serum SENV-D DNA when followed 24 wk after the cessation of therapy. Three of eleven patients (27.3%) with positive SENV-D DNA at the end of treatment were cleared of SENV-D DNA 24 wk after the cessation of therapy. The rate of SVR of SENV-D DNA after combination therapy was 56.3% (27/48). As shown in Table 4, the SVR of SENV-D was higher among patients with ETVR than those who were SENV-D viremia at the end of treatment (88.9% *vs* 61.9%, $P = 0.04$). No other clinical and virological factor was related to SVR for SENV-D.

Table 4 Comparison of clinical characteristics and virological features between 48 CHC patients with and without sustained clearance of SENV-D after combination therapy

	SENV-D response		*P*
	NR (*n* = 21)	SVR (*n* = 27)	
Sex (male) (%)	15 (71.4)	13 (48.2)	NS
Age (yr)	48.3±10.6	51.7±10.3	NS
Serum ALT (IU/L)	157.6±122.9	152.4±216.3	NS
High HCV RNA level (≥200 000 IU/mL) (%)	9 (42.9)	18 (66.7)	NS
HCV genotype 1b (%)	7 (33.3)	12 (44.4)	NS
SENV-D ETVR (%)	13 (61.9)	24 (88.9)	0.04
HCV SVR (%)	17 (81.0)	18 (66.7)	NS

Results are expressed as mean±SD. ETVR: end-of-treatment viral response, SVR: sustained viral responder, NR: non-responders, NS: no significance.

DISCUSSION

The geographic distribution of different SENV variants has been noted. In Japan, SENV-D is more prevalent than SENV-H[20-23], but the predominant strain of SENV-H has been reported in the USA[4]. Kao *et al*., reported that the prevalence of SENV-H was 2-7 times higher than that of SENV-D in different northern Taiwanese individuals[16,24]. The findings of the present study revealed that 37.1% of Taiwanese patients with CHC were co-infected with SENV-D which is higher by 28% than reports by Kao *et al*.[16]. Our previous studies have shown that the prevalence of SENV-D was also higher than SENV-H not only among southern Taiwan blood donors (19.7% and 5.8%)[18] but also among patients on maintenance hemodialysis (46.5% and 27.3%)[25]. Furthermore, we found the prevalence of SENV-H was 19.2% among Taiwanese CHC patients (unpublished data) which is lower than that of SENV-D. It is interesting that a marked difference of genotypic distribution of SENV between southern and northern Taiwan exists.

There was a higher mean age among CHC patients who were SENV-D viremic than non-viremic. We found similar results among blood donors too[18]. However, we did not observe significant correlation between age and the prevalence of SENV-H among blood donors[18] and CHC patients (unpublished data). The cause of discrepancy between trends of change in the prevalence of SENV-D and -H was not clear. Whether the possible assumptions such as the different exposure rate or routes of infection or different rates of spontaneous clearance between these two strains can clarify the issues needs further large-scale and longitudinal studies.

The clinical significance of SENV infection in combination with HCV infection remains unclear[15,16]. Kao *et al*., reported the relevance between HCV genotype 2a and SENV co-infection[16]. The present study revealed that the pretreatment mean ALT levels and HCV RNA levels, and the histological scores between CHC patients with and without SENV-D co-infection were compatible and failed to show association between SENV-H and HCV genotype. Nevertheless, we have found a correlation between SENV-H co-infection and HCV genotype 1b among CHC patients (unpublished data). The discrepancy needs further studies. The biochemical and histological characteristics of CHC patients were not influenced by SENV-D co-infection that indicated the irrelevance between severity of liver disease and SENV-D co-infection.

As previous reports from researchers in Taiwan, the SVR rate of CHC patients was high (40-43%) after combination therapy with IFN 3 MU and ribavirin for 24 wk[12,16]. In the present study, the HCV SVR rate was 67.3% after combination therapy with 6 MU IFN and ribavirin for 24 wk, which may further indicate the favorable results of combination therapy for Taiwanese CHC patients. The positive predictors of SVR to combination therapy with high-dose IFN were elucidated as HCV genotype non-1b, lower pretreatment HCV RNA levels and younger age. HCV genotype non-1b, having 12 times of SVR rate than genotype 1b in the present study, is the most important factor predicting SVR. Previous reports have demonstrated that HCV with SENV co-infection affected HCV response to combination therapy with IFN plus ribavirin adversely[15] but the other report denied the relevance between HCV response and SENV co-infection[16,22]. Our data here indicates that SENV-D co-infection does not affect the HCV response in the combination therapy with high dose IFN and ribavirin. In addition to co-infection with GB virus C/hepatitis G virus or TT virus that had no impact on the response to IFN monotherapy in CHC patients in our previous studies[13,26,27], it seems unnecessary to determine whether CHC patients coinfected these viruses or not before they received combination therapy.

After combination therapy with 6 MU IFN and ribavirin for 24 wk among 48 CHC patients concomitant with SENV-D viremia, the rates of viral clearance at the end of follow-up achieved 56.3%. In an earlier study on the response of SENV-D among CHC patients, the sustained response rate of SENV-D has recently been reported by Umemura

et al., (73.3%) after high-dose IFN monotherapy[22] and by Kao *et al*., (87.5%) after combination therapy with 3 MU IFN and ribavirin for 24 wk[16]. The lower SVR rate of SENV-D than SENV-H (78.3%, unpublished data) in our study from southern Taiwan was different from results from northern Taiwan that showed higher SENV-D SVR rate (87.5%) than SENV-H (26.8%)[16]. The causes of different response to combination therapy, in addition to the different prevalence, of these two strains in southern and northern Taiwan need further research.

In conclusion, we find that more than one-third Taiwanese patients with CHC are coinfected with SENV-D and coexistent SENV-D infection is apparently associated with higher ages but does not have an influence on the clinico-pathological characteristics of HCV infection. SENV-D is highly susceptible and does not affect the HCV response to combination therapy. After combination therapy with high dose IFN and ribavirin, two-thirds of Taiwanese CHC patients achieve SVR and HCV genotype non-1b, lower pretreatment HCV RNA levels and younger age are predictive factors for SVR.

REFERENCES

1 **Mushahwar IK.** Recently discovered blood-borne viruses: are they hepatitis viruses or merely endosymbionts? *J Med Virol* 2000; **62**: 399-404

2 **Bowden S.** New hepatitis viruses: contenders and pretenders. *J Gastroenterol Hepatol* 2001; **16**: 124-131

3 **Tanaka Y,** Tanaka Y, Primi D, Wang RY, Umemura T, Yeo AE, Mizokami M, Alter HJ, Shih JW. Genomic and molecular evolutionary analysis of a newly identified infectious agent (SEN virus) and its relationship to the TT virus family. *J Infect Dis* 2001; **183**: 359-367

4 **Umemura T,** Yeo AE, Sottini A, Moratto D, Tanaka Y, Wang RY, Shih JW, Donahue P, Primi D, Alter HJ. SEN virus infection and its relationship to transfusion-associated hepatitis. *Hepatology* 2001; **33**: 1303-1311

5 **Alter MJ,** Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE. The natural history of community-acquired hepatitis C in the United States. *New Engl J Med* 1992; **327**: 1899-1905

6 **Lauer GM,** Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52

7 **Chuang WL,** Chang WY, Lu SN, Lin ZY, Chen SC, Hsieh MY, Wang LY, You SL, Chen CJ. The role of hepatitis C virus in chronic hepatitis B virus infection. *Gastroenterol Jpn* 1993; **28** (Suppl 5): 23-27

8 **Chen DS,** Chen DS, Wang JT, Chen PJ, Wang TH, Sung JL. Hepatitis C virus infection in Taiwan. *Gastroenterol Jpn* 1991; **26**(Suppl 3): 164-166

9 **Wang JH,** Lu SN, Wu JC, Huang JF, Yu ML, Chen SC, Chuang WL. A hyperendemic community of hepatitis B virus and hepatitis C virus infection in Taiwan. *Trans R Soc Trop Med Hyg* 1999; **93**: 253-254

10 **McHutchison JG,** Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S, Albrecht JK. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med* 1998; **339**: 1485-1492

11 **Poynard T,** Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J. Randomised trial of interferon alpha2b plus ribavirin for 48 wk or for 24 wk versus interferon alpha2b plus placebo for 48 wk for treatment of chronic infection with hepatitis C virus. *Lancet* 1998; **352**: 1426-1432

12 **Lai MY,** Kao JH, Yang PM, Wang JT, Chen PJ, Chan KW, Chu JS, Chen DS. Long-term efficacy of ribavirin plus interferon alfa in the treatment of chronic hepatitis C. *Gastroenterology* 1996; **111**: 1307-1312

13 **Dai CY,** Yu ML, Lin ZY, Chen SC, Hsieh MY, Lee LP, Hou NJ, Hsieh MY, Wang LY, Tsai JF, Chuang WL, Chang WY. Clinical significance of TT Virus (TTV) infection in chronic hepatitis C patients with high dose interferon-alpha therapy in Taiwan: re-evaluated by using new set of TTV primers. *Hepatol Res Hepatol Res* 2003; **27**: 95-100

14 **Yu ML,** Dai CY, Chen SC, Lee LP, Huang JF, Lin ZY, Hsieh MY, Wang LY, Chuang WL, Chang WY. A prospective study on treatment of chronic hepatitis C with tailored and extended interferon-alpha regimens according to pretreatment virological factors. *Antiviral Res Antiviral Res* 2004; **63**: 25-32

15 **Rigas B,** Hasan I, Rehman R, Donahue P, Wittkowski KM, Lebovics E. Effect on treatment outcome of coinfection with SEN viruses in patients with hepatitis C. *Lancet* 2001; **358**: 1961-1962

16 **Kao JH,** Chen W, Chen PJ, Lai MY, Chen DS. SEN virus infection in patients with chronic hepatitis C: preferential coinfection with hepatitis C genotype 2a and no effect on response to therapy with interferon plus ribavirin. *J Infect Dis* 2003; **187**: 307-310

17 **Knodell RG,** Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; **1**: 431-435

18 **Dai CY,** Yu ML, Lin ZY, Chen SC, Hsieh MY, Wang LY, Tsai JF, Chuang WL, Chan WY. Prevalence and clinical significance of SEN virus infection among volunteer blood donors in southern Taiwan. *Dig Dis Sci* 2004; **49**: 1181-1185

19 **Okamoto H,** Tokita H, Sakamoto M, Horikita M, Kojima M, Iizuka H, Mishiro S. Characterization of the genomic sequence of type V (or 3a) hepatitis C virus isolates and PCR primers for specific detection. *J Gen Virol* 1993; **74**(Pt 11): 2385-2390

20 **Kobayashi N,** Tanaka E, Umemura T, Matsumoto A, Iijima T, Higuchi M, Hora K, Kiyosawa K. Clinical significance of SEN virus infection in patients on maintenance haemodialysis. *Nephrol Dial Transplant* 2003; **18**: 348-352

21 **Umemura T,** Alter HJ, Tanaka E, Yeo AE, Shih JW, Orii K, Matsumoto A, Yoshizawa K, Kiyosawa K. Association between SEN Virus Infection and Hepatitis C in Japan. *J Infect Dis* 2001; **184**: 1246-1251

22 **Umemura T**, Alter HJ, Tanaka E, Orii K, Yeo AE, Shih JW, Matsumoto A, Yoshizawa K, Kiyosawa K. SEN virus: response to interferon alfa and influence on the severity and treatment response of coexistent hepatitis C. *Hepatology* 2002; **35**: 953-959

23 **Shibata M,** Wang RY, Yoshiba M, Shih JW, Alter HJ, Mitamura K. The presence of a newly identified infectious agent (SEN virus) in patients with liver diseases and in blood donors in Japan. *J Infect Dis* 2001; **184**: 400-404

24 **Kao JH,** Chen W, Chen PJ, Lai MY, Chen DS. Prevalence and Implication of a Newly Identified Infectious Agent (SEN Virus) in Taiwan. *J Infect Dis* 2002; **185**: 389-392

25 **Dai CY,** Chuang WL, Chang WY, Chen SC, Sung MH, Hsieh MY, Lin ZY, Hsieh MY, Wang LY, Tsai JF, Yu ML. SEN virus infection among patients on maintenance hemodialysis in southern Taiwan. *J Infection* 2005: (in press)

26 **Yu ML,** Chuang WL, Dai CY, Chen SC, Lin ZY, Hsieh MY, Tsai JF, Wang LY, Chang WY. GB virus C/hepatitis G virus infection in chronic hepatitis C patients with and without interferon-alpha therapy. *Antiviral Res* 2001; **52**: 241-249

27 **Dai CY,** Yu ML, Chuang WL, Hou NJ, Hou C, Chen SC, Lin ZY, Hsieh MY, Wang LY, Chang WY. The response of hepatitis C virus and TT virus to high dose and long duration interferon-alpha therapy in naive chronic hepatitis C patients. *Antiviral Res* 2002; **53**: 9-18

Science Editor Guo SY **Language Editor** Elsevier HK