PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(7):968-971
World Journal of Gastroenterology
 ISSN 1007-9327

• VIRAL HEPATITIS •

Effect of SEN virus coinfection on outcome of lamivudine therapy in patients with hepatitis B

Dong Xu, De-Ying Tian, Zhen-Gang Zhang, Hong-Yun Chen, Pei-Hui Song

Dong Xu, De-Ying Tian, Zhen-Gang Zhang, Hong-Yun Chen, Pei-Hui Song, Department of Infectious Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China
Supported by the Science and Technology Commission Foundation of Hubei Province, No. 2002AA301C32
Correspondence to: Dr. De-Ying Tian, Department of Infectious Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Jiefang Street 1095, Wuhan 430030, Hubei Province, China. xudongtj@tom.com
Telephone: +86-27-83662815 **Fax:** +86-27-83613408
Received: 2003-06-06 **Accepted:** 2003-07-30

Abstract

AIM: Interactions between hepatitis B virus (HBV) and other viral hepatitis infections are well known, whether the newly discovered SEN virus (SENV) has any effect on lamivudine antiHBV activity is unclear. Our aim was to clarify the effect on treatment outcome of coinfection with SEN virus in patients with hepatitis B during lamivudine therapy.

METHODS: Nested polymerase chain reaction (PCR) amplification was used to detect SENV-D and SENV-H strains in serum from 45 patients with chronic hepatitis B treated with lamivudine 100 mg daily for 12 mo. HBV DNA load was detected with fluorescence quantitative PCR (FQ-PCR) and YMDD (tyrosine, methionine, aspartate, aspartate) motif mutation of HBV DNA was investigated with cDNA microarray.

RESULTS: SENV DNA was detected in 5 of 45(11.1%) cases after 12 mo they received lamivudine treatment. SENV-D and SENV-H were 4.4% and 6.7% respectively. HBV DNA failed to respond to lamivudine therapy in 4 of 5 SENV coinfected patients while only 10 of 40 patients became SENV positive and the difference was statistically significant. Response of ALT and HBeAg to lamivudine had no significant difference between coinfection patients and single HBV infection ones.

CONCLUSION: Coinfection with SEN virus in chronic hepatitis B patients may adversely affect the outcome of lamivudine treatment.

Xu D, Tian DY, Zhang ZG, Chen HY, Song PH. Effect of SEN virus coinfection on outcome of lamivudine therapy in patients with hepatitis B. *World J Gastroenterol* 2004; 10(7): 968-971
http://www.wjgnet.com/1007-9327/10/968.asp

INTRODUCTION

A new DNA virus with approximately 3 800 nucleotides, referred to as SEN virus (SENV), has been isolated in blood of a human immunodeficiency virus (HIV)-infected injection drug user (IDU)[1,2]. Phylogenetic analysis showed that 8 strains of SENV were members of the circoviridae family, a group of small, single-strand, nonenveloped circular DNA virus that includes TT virus (TTV), TUS01, SANBAN and YONBAN[3-6]. Although structurally similar to TTV, SENV has less than 55% sequence homology and less than 37% amino acid homology with the TTV prototype[2]. A strong association between two SENV variants (SENV-D and SENV-H) infections and transfusion-associated non-A to E hepatitis has been reported[7]. SENV-D and SENV-H have been extensively studied, they were found to be present in approximately 2% of Americans and 20% of Japanese blood donors, and could be readily transmitted by blood transfusion and other common parenteral routes[8]. However, the association of SENV infection with liver cell damage remains controversial[9]. Furthermore, several recent studies have shown that persons with SENV-D/H infection alone or coinfected with HBV or HCV had no evidence of liver disease[10-14].

Chronic liver diseases are common in China, most of them can be attributed to infection with hepatitis B virus (HBV) or hepatitis C virus (HCV)[15-21]. Although GB virus and TT virus (TTV) have been claimed to be prevalent in chronic liver disease patients in our previous study and other researches, most studies have indicated that neither virus causes liver diseases[22-25]. Taking advantage of the frequency of SENV-D/H coinfection in cases of chronic hepatitis and transient elevations of alanine aminotransferase observed in babies following transmission of SENV from mothers[8,26], the preliminary observation of clinical relevance of SENV infection alone or in combination with HBV or HCV infection needs to be independently confirmed in larger numbers of patients and in different areas.

A research letter appeared in Lancet by Basil Rigas and colleagues suggests that coinfection with SEN virus in hepatitis C patients (HC) may adversely affect the outcome of antiviral therapy with interferon and ribavirin[27]. On the contrary, another result indicated coinfection with SENV did not affect the clinicalpathological features of chronic hepatitis C and response to combination therapy[28]. These conflicting interpretations may reflect the difference in patient selection and sample size. Since coinfection of hepatitis B virus (HBV) and SEN virus is common[10,26], the precise role of SEN virus in chronic hepatitis B patients remains to be determined. Our aim was to provide the initial the evidence whether SENV coinfection affected the outcome of lamivudine therapy in patients with hepatitis B.

MATERIALS AND METHODS

Subjects

From Sept 2001 to July 2002, serum samples were obtained from 45 patients treated with lamivudine 100 mg daily in Hubei province. All patients were excluded infection of hepatitis viruses A, C, D, E, and TTV, HGV, HIV. These serum samples were stored at -70 ℃. Hepatitis B virus infection was confirmed by enzyme-linked immunosorbent assay (ELISA, second-generation). Commercially available ELISAs were used for immunoglobulin M (IgM) antibodies to hepatitis A virus, hepatitis B surface antigen (HBsAg) and e antigen (HBeAg), antibodies to hepatitis B core antigen (HBcAb) and e antigen

(HBeAb), hepatitis D virus, hepatitis E virus TTV, HGV and HIV. Serum HBV DNA levels was quantified using the hepatitis virus B nucleic acid amplification fluorescence Kit according to manufactures instructions (DA AN Gene Co. Ltd, Zhongshan University). The detection limit of this assay is 10^3 copies/ml[29].

Detection of SEN-V DNA by polymerase chain reaction

DNA was extracted from 50 μL of serum using Acupure DNA/RNA kit (Inc Biotronics, USA). PCR amplification was performed using primers specific for ORF1 region. Two common external primers were used. They were sense primer: 5' -TACCCCAACGACCAACTACGC-3' , antisense primer: 5' -GTTTGTGGTGAGCAGAACGGAA-3' . Inner primers for SENV-D were sense primer: 5' -TAAGCAGCCCTAACAC TCATCCA-3' , antisense primer: 5' -CAGTTGACCGCAAAG TTACAAG-3' . Inner primers for SENV-H were sense primer: 5' -ATACTTTGGCTGCACCTTCTG-3' , antisense primer: 5' -CCAACTGACTAGGGGAACCTTA-3' . The first- round PCR amplification was carried out in a volume of 30 μL including 3 μL of DNA extraction product, 1×PCR buffer (Promega), 1.5 mmol/L $MgCl_2$, 100 pmoles of each sense and antisense external primers, 20 mmol/L each dNTP and 1U Taq DNA polymerase (Promega). PCR was performed for 30 cycles at 94 ℃ for 45 s, at 55 ℃ for 45 s, at 72 ℃ for 50 s. Two microliter of the first PCR product was subjected to a second amplification for 30 cycles under the same condition as for the first PCR, using sense and antisense inner primers. The amplified products were visualized by 20 g/L agarose gel electrophoresis and ethidium bromide stained. The amplified DNA was directly sequenced by the BigDye terminator kit (Bioasia Biotehnology Ltd) using the ABI 377 sequencer.

DNA microarray analysis

HBV YMDD mutation chip was provided by Shanghai Institute of Microsystem and Information Technology and Ruixin Biotehnology Ltd. The probes on the chip were labeled with digoxingenin-dUTP for color detection with NBT/BCIP. HBV DNA extracted from 50 μL of serum was amplified with PCR. The PCR products were denatured respectively in a 95 ℃ bath for 5 min, then added on the chip. They were hybridized in a sealed chamber at 42 ℃ for 30 min and washed in turn with solutions of 2×SSC + 2 g/L SDS, 0.1×SSC + 2 g/L SDS and 1 g/L SSC for 10 min each, then dried at room temperature. The hybridization was detected with anti-digoxingenin-AP Fab fragments and visualized with the colorimetric substrate NBT/BCIP.

Data statistics

Data were analyzed by Fisher' s exact test, χ^2 test with Yate' s correction, or Student' s *t* test. A *P* value <0.05 was considered statistically significant.

RESULTS

Prevalence of SENV-DNA

SENV DNA was detected in 5 of 45 patients (11.1%) with chronic hepatitis B after 12 mo they received lamivudine treatment. Of the 5 patients with SENV coinfection, 2 (4.4%) were infected with SENV-D and 3 (6.7%) with SENV-H.

Association of SENV with severity of liver disease

Of the 45 patients received lamivudine treatment 100 mg daily, 1 had abnormal serum alanine transaminase (ALT) (>45 IU/L) among 5 cases of SENV infection and 8 had no SENV infection among 40 cases of SENV infection. The ALT level between patients with and without SENV coinfection was not statistically significant (42.0±19.9 U/L *vs* 39.2±35.8 U/L, t=0.174, P=0.863) (Table 1).

Table 1 Association of SENV with severity of liver disease

	Normal ALT (*n*)	Abnormal ALT (*n*)	Total
SENV DNA P	4	1	5
SENV DNA N	32	8	40

Abbreviations: *P*, positive; *N*, negative.

Association of SENV with HBeAg response to lamivudine treatment

None of the 5 patients coinfected with SENV had HBeAg seroconversion or HBeAg loss, and 4 of 40 patients without SENV coinfection had HBeAg seroconversion or HbeAg loss. There was no significant difference between patients with SENV infection and those without SENV coinfection in HBeAg seroconversion (χ^2=0.549, P=0.459).

Table 2 Association of SENV with HBeAg response to lamivudine treatment

	HBeAg *P* (*n*)	HBeAg *N* (*n*)	Total
SENV-DNA P	5	0	5
SENV-DNA N	36	4	40

Abbreviations: *P*, positive; *N*, negative.

Association of SENV with HBV DNA response to lamivudine treatment

Only one of the 5 patients coinfected with SENV responded to lamivudine treatment in terms of HBV DNA, while 30 of the 40 patients infected with HBV alone responded to the treatment of lamivudine. The difference was statistically significant (χ^2=3.97, P=0.046). Although the baseline mean of serum HBV DNA level in the patients coinfected with SENV was higher than that in those without SENV infection (5.50±0.47 *vs* 4.98±0.75), the difference was not statistically significant(t=1.246, P=0.236).

Table 3 Effect of lamivudine treatment on patients with SENV coinfection

	HBV DNA positive patients (*n*)	Mean Log_{10} HBV DNA (copies / mL)	HBV DNA negative patients(*n*)
SENV DNA Positive	4	5.50±0.47	1
SENV DNA Negative	10	4.98±0.75	30

Abbreviations: *P*, positive; *N*, negative.

Table 4 Clinical features of 5 patients with SENV infection

Patient	Sex (M / F)	Age (yr)	ALT (IU / L)	HBeAg	HBV DNA	YMDD motif
SENV-D1	M	24	25	P	P	YMDD
SENV-D2	M	34	96	P	P	YIDD
SENV-H1	M	27	38	P	P	YMDD
SENV-H2	M	29	17	P	N	
SENV-H3	M	19	29	P	P	YVDD

Abbreviations: *P*, positive; *N*, negative; *M*, male.

Clinical features of 5 patients with SENV infection

Of the 14 HBV DNA positive patients, 2 had YMDD mutation in 4 patients with SENV coinfection and 5 had YMDD

mutation in 10 patients with HBV infection alone. One was YIDD mutant and the other was YVDD mutant in 4 coinfected patients, 3 were YVDD mutants and 2 were YIDD mutants in 10 HBV infected alone patients. Lamivudine resistant mutation (YMDD mutate to YIDD or YVDD) had no significant difference in SENV coinfected group and HBV infected alone group (χ^2=0.35, P=0.72).

DISCUSSION

Recent studies indicated that SENV-D/H infections occurred more often in high-risk groups (54-90 %), patients with chronic hepatitis B (41%), HBV-related hepatocellular carcinoma (HCC) (54%), chronic hepatitis C (67%), and HCV-related HCC (76%) than in healthy adults (15%)[10]. Although most subjects with SENV-D/H infection alone had no hepatitis or mild hepatitis[13,30-32], an association of SENV-D/H with transfusion-associated hepatitis has been reported[7]. Whether SENV-D/H serves as causative agents of non-A and non-E hepatitis remains controversial[7,12,30].

Coinfection with HBV and hepatitis D virus has been reported to be associated with severe and rapidly progressive liver diseases[33]. The clinical manifestations of patients with HBV/HCV coinfection seemed mild and occult. Additionally, it was shown that HBV could inhibit HCV replication, but no evidence that HCV could suppress HBV replication was found in the data[34]. In contrast, another study showed that acute superinfection in patients with chronic hepatitis might increase the risk of severe hepatitis, suggesting that HBV as a newcomer might suppress pre-existing HCV[35]. Together with the earlier observation that acute HCV superinfection suppressed pre-existing HBV, it seemed that the time or sequence of infection was a factor influencing the outcome of viral interactions. Newly published issue indicated that infections with HCV plus other hepatitis viruses might exacerbate the pathological lesion of the liver[36]. Interactions between two specific viruses need to be determined. Our data showed the ALT level (42.0±19.9 U/L *vs* 39.2±35.8 U/L) between patients with or without SENV coinfection was not statistically significant, so did HBeAg response to lamivudine antiviral treatment. These data suggested that SENV had limited or no hepatic pathogenicity, which was consistenting with the previous observation that the vast majority of SENV-infected hemodialysis patients did not develop hepatitis[37].

The clinical relevance of SENV infection alone or in combination with HBV remains controversial. Whether SENV affects other virus replication needs to be determined. In our study, the baseline mean of serum HBV DNA level in the patients coinfected with SENV was higher than that in those without SENV infection (5.50±0.47 *vs* 4.98±0.75), the difference was not statistically significant. However, the lamivudine antiviral response rate of HBV DNA was lower in patients coinfected with SENV infection than those without (20% *vs* 75%), and the result indicated that coinfection with SEN virus in chronic hepatitis B patients might adversely affect the outcome of lamivudine treatment. In patients with chronic hepatitis C infection after interferon plus ribavirin therapy in another study, serum HCV level in patients coinfected with SENV was not significantly lower than that in patients without SENV infection, but HCV genotype 2a was more often found among patients with HCV and SENV coinfection than among those with HCV infection alone, suggesting that there was a specific link between SENV and HCV genotype 2a[28].

Recent registration of lamivudine, a dideoxycytidine analogue that inhibits HBV reverse transcriptases, has provided new perspectives for the treatment of chronic HBV infection[38,39]. Mutation of methionine to valine or isoleucine at the YMDD motif of HBV reverse transcriptase has been shown to be responsible for lamivudine resistance in HBV[40,41]. HBV precore stop mutant was increased in the first stage following acute superinfeciton of HCV and then decreased in the later stage[42]. The observation raised the possibility of a limit relation of HBV mutant to coinfected virus in different stages of coinfection. Whether YMDD mutant has an association with SENV coinfection is unknown. No significant association was observed between SENV and HBV YMDD mutations during lamivudine treatment in our present study.

Coinfection with SENV might adversely affect the outcome of lamivudine treatment. It is not surprising that infection of the liver with more than one virus might render it resistant to antiviral therapy. Indeed, coinfection with HBV, either HDV or HIV, could predicts the unfavourable outcome of antiviral treatment compared with those infected with HBV only[43], but TTV coinfection did not influence the outcome of long- term lamivudine therapy on hepatitis B[44], so did interferon therapy on chronic hepatitis B or C[45]. Thus, further studies need to address this important and interesting issue.

In summary, coinfection with SENV in chronic hepatitis B in Wuhan area might adversely affect the outcome of lamivudine treatment. SENV should be detected when HBV DNA fails to respond to lamivudine treatment for HBV infected patients.

ACKNOWLEDGEMENTS

We thank Dr. Chen-Hui Huang, Third Hospital, Zhongshan University for providing SEN virus primers, Drs. Yu-Hu Song and Hao-Yi Yang, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

REFERENCES

1 **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

2 **Fiordalisi G**, Bonelli M, Olivero P, Primi D, Vaglini L, Mattioli S, Bonelli F, Dal CA, Mantero GL, Sottini A. Identification of SENV genotypes. International patent number WO0028039 (international application published under the patent cooperation treaty). Internet address: http: / / epcespacenet.com /

3 **Nishizawa T**, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem Biophys Res Commun* 1997; **241**: 92-97

4 **Okamoto H**, Takahashi M, Nishizawa T, Ukita M, Fukuda M, Tsuda F, Miyakawa Y, Mayumi M. Marked genomic heterogeneity and frequent mixed infection of TT virus demonstrated by PCR with primers from coding and noncoding regions. *Virology* 1999; **259**: 428-436

5 **Hijikata M**, Takahashi K, Mishiro S. Complete circular DNA genome of a TT virus variant (isolate name SANBAN) and 44 partial ORF2 sequences implicating a great degree of diversity beyond genotypes. *Virology* 1999; **260**: 17-22

6 **Takahashi K**, Hijikata M, Samokhvalov EI, Mishiro S. Full or near full length nucleotide sequences of TT virus variants (Types SANBAN and YONBAN) and the TT virus-like mini virus. *Intervirology* 2000; **43**: 119-123

7 **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

8 **Pirovano S**, Bellinzoni M, Ballerini C, Cariani E, Duse M, Albertini A, Imberti L. Transmission of SEN virus from mothers to their babies. *J Med Virol* 2002; **66**: 421-427

9 **Wilson LE**, Umemura T, Astemborski J, Ray SC, Alter HJ, Strathdee SA, Vlahov D, Thomas DL. Dynamics of SEN virus infection among injection drug users. *J Infect Dis* 2001; **184**: 1315-1319

10 **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

11 **Yoshida EM**, Buczkowski AK, Giulivi A, Zou S, Forrester LA. A cross-sectional study of SEN virus in liver transplant recipients. *Liver Transpl* 2001; **7**: 521-525

12 **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

13 **Mikuni M**, Moriyama M, Tanaka N, Abe K, Arakawa Y. SEN virus infection does not affect the progression of non-A to -E liver disease. *J Med Virol* 2002; **67**: 624-629

14 **Yoshida H**, Kato N, Shiratori Y, Shao R, Wang Y, Shiina S, Omata M. Weak association between SEN virus viremia and liver disease. *J Clin Microbiol* 2002; **40:** 3140-3145

15 **Fan CL**, Wei L, Jiang D, Chen HS, Gao Y, Li RB, Wang Y. Spontaneous viral clearance after 6-21 years of hepatitis B and C viruses coinfection in high HBV endemic area. *World J Gastroenterol* 2003; **9**: 2012-2016

16 **Wang FS**. Current status and prospects of studies on human genetic alleles associated with hepatitis B virus infection. *World J Gastroenterol* 2003; **9**: 641-644

17 **Hou CS**, Wang GQ, Lu SL, Yue B, Li MR, Wang XY, Yu JW. Role of activation-induced cell death in pathogenesis of patients with chronic hepatitis B. *World J Gastroenterol* 2003; **9**: 2356-2358

18 **Qin LX**, Tang ZY. The prognostic significance of clinical and pathological features in hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 193-199

19 **He QQ**, Cheng RX, Sun Y, Feng DY, Chen ZC, Zheng H. Hepatocyte transformation and tumor development induced by hepatitis C virus NS3 c-terminal deleted protein. *World J Gastroenterol* 2003; **9**: 474-478

20 **Li K**, Wang L, Cheng J, Lu YY, Zhang LX, Mu JS, Hong Y, Liu Y, Duan HJ, Wang G, Li L, Chen JM. Interaction between hepatitis C virus core protein and translin protein–a possible molecular mechanism for hepatocellular carcinoma and lymphoma caused by hepatitis C virus. *World J Gastroenterol* 2003; **9**: 300-303

21 **Wu CH**, Ouyang EC, Walton C, Promrat K, Forouhar F, Wu GY. Hepatitis B virus infection of transplanted human hepatocytes causes a biochemical and histological hepatitis in immunocopetentent rats. *World J Gastroenterol* 2003; **9**: 978-983

22 **Tian DY**, Yang DF, Xia NS, Zhang ZG, Lei HB, Huang YC. The serological prevalence and risk factor analysis of hepatitis G virus infection in Hubei Province of China. *World J Gastroenterol* 2000; **6**: 585-587

23 **Jeon MJ**, Shin JH, Suh SP, Lim YC, Ryang DW. TT virus and hepatitis G virus infections in Korean blood donors and patients with chronic liver disease. *World J Gastroenterol* 2003; **9**: 741-744

24 **Hu ZJ**, Lang ZW, Zhou YS, Yan HP, Huang DZ, Chen WR, Luo ZX. Clinicopathological study on TTV infection in hepatitis of unknown etiology. *World J Gastroenterol* 2002; **8**: 288-293

25 **Zhu WF**, Yin LM, Li P, Huang J, Zhuang H. Pathogenicity of GB virus C on virus hepatitis and hemodialysis patients. *World J Gastroenterol* 2003; **9**: 1739-1742

26 **Chemin I**, Parana R, Trepo C. A new viral agent, SEN virus (SENV), has been detected in patients from several countries: the pathogenic role of SENV in coinfections with hepatitis B virus or hepatitis C virus should be investigated. *J Infect Dis* 2002; **185**:710

27 **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

28 **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

29 **Li G**, Shu X, Ma HH, Chen W, Chen WS, Chen Q, Jiang YS, Yao JL. Detection of HBV, HCV and HBV YMDD mutants by DNA microarray. *Shijie Huaren Xiaohua Zazhi* 2003; **11**: 178-181

30 **Wong SG**, Primi D, Kojima H, Sottini A, Giulivi A, Zhang M, Uhanova J, Minuk GY. Insights into SEN virus prevalence, transmission, and treatment in community-based persons and patients with liver disease referred to a liver disease unit. *Clin Infect Dis* 2002; **35**: 789-795

31 **Tangkijvanich P**, Theamboonlers A, Sriponthong M, Thong-Ngam D, Kullavanijaya P, Poovorawan Y. SEN virus infection in patients with chronic liver disease and hepatocellular carcinoma in Thailand. *J Gastroenterol* 2003; **38**: 142-148

32 **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

33 **Jardi R**, Rodriguez F, Buti M, Costa X, Cotrina M, Galimany R, Esteban R, Guardia J. Role of hepatitis B, C, and D viruses in dual and triple infection: influence of viral genotypes and hepatitis B precore and basal core promoter mutations on viral replicative interference. *Hepatology* 2001; **34**: 404-410

34 **Fan CL**, Wei L, Jiang D, Chen HS, Gao Y, Li RB, Fei R, Ji Y, Zhu L, Wang Y. Clinical and virological course of dual infection by hepatitis B and C viruses in China. *Zhonghua Yixue Zazhi* 2003; **83**: 1214-1218

35 **Liaw YF**, Yeh CT, Tsai SL. Impact of acute hepatitis B virus superinfection on chronic hepatitis C virus infection. *Am J Gastroenterol* 2000; **95**: 2978-2980

36 **Chen YD**, Liu MY, Yu WL, Li JQ, Dai Q, Zhou ZQ, Tisminetzky SG. Mix-infections with different genotypes of HCV and with HCV plus other hepatitis viruses in patients with hepatitis C in China. *World J Gastroenterol* 2003; **9**: 984-992

37 **Schroter M**, Laufs R, Zollner B, Knodler B, Schafer P, Feucht HH. A novel DNA virus (SEN) among patients on maintenance hemodialysis: prevalence and clinical importance. *J Clin Virol* 2003; **27**: 69-73

38 **Chang CN**, Skalski V, Zhou JH, Cheng YC. Biochemical pharmacology of (+)- and (-)-2', 3'-dideoxy-3'-thiacytidine as antihepatitis B virus agents. *J Biol Chem* 1992; **267**: 22414-22420

39 **Jaboli MF**, Fabbri C, Liva S, Azzaroli F, Nigro G, Giovanelli S, Ferrara F, Miracolo A, Marchetto S, Montagnani M, Colecchia A, Festi D, Reggiani LB, Roda E, Mazzella G. Long-term alpha interferon and lamivudine combination therapy in non-responder patients with anti-HBe-positive chronic hepatitis B: Results of an open, controlled trial. *World J Gastroenterol* 2003; **9**: 1491-1495

40 **Tipples GA**, Ma MM, Fischer KP, Bain VG, Kneteman NM, Tyrrell DL. Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine *in vivo*. *Hepatology* 1996; **24**: 714-717

41 **Bai YJ**, Zhao JR, Lv GT, Zhang WH, Wang Y, Yan XJ. Rapid and high throughput detection of HBV YMDD mutants with fluorescence polarization. *World J Gastroenterol* 2003; **9**: 2344-2347

42 **Yeh CT**, Chu CM, Liaw YF. Progression of the proportion of hepatitis B virus precore stop mutant following acute superinfection of hepatitis C. *J Gastroenterol Hepatol* 1998; **13**: 131-136

43 **Torresi J**, Locarnini S. Antiviral chemotherapy for the treatment of hepatitis B virus infections. *Gastroenterology* 2000; **118**: S83-103

44 **Garcia JM**, Marugan RB, Garcia GM, Lindeman MLM, Abete JF, del Campo Terron S. TT virus infection in patients with chronic hepatitis B and response of TTV to lamivudine. *World J Gastroenterol* 2003; **9**: 1261-1264

45 **Lai YC**, Hu RT, Yang SS, Wu CH. Coinfection of TT virus and response to interferon therapy in patients with chronic hepatitis B or C. *World J Gastroenterol* 2002; **8**: 567-570

Edited by Wang XL and Xu FM