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

Department of Infectious Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China

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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

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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 activity is unclear. Our aim was to clarify the effect on treatment outcome of coinfeciton 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 coinfected patients and single HBV infection ones.

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

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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[8,9]. However, the association of SENV infection with liver cell damage remains controversial[9,10]. 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[9,10].

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[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 clinical pathological 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 °C. 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
DNA extracted from 50 µL of serum using Acupure DNA/RNA kit (Inc Biotronics, USA). PCR amplification was performed using primers specific for ORF1 region.

Detection of SENV-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: were sense primer: 5'-TACCCCAAGCACTACGCG-3', antisense primer: 5'-TTACAAG-3'. Inner primers for SENV-D were sense primer: 5'-TAACGCAGCCCTAACAC TCATCCA-3', antisense primer: 5'-CAGTGGACCGAAG TTACAAG-3'. Inner primers for SENV-H were sense primer: 5'-ATACCTTTGCTGCACTTCTG-3', antisense primer: 5'- CCAACTGAATGGGAACCTTAT-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 MgCl₂, 100 pmol of sense and antisense external primers, 20 mmol/L each dNTP and 1U Taq DNA polymerase (Promega). PCR was performed for 30 cycles at 94 °C for 45 s, at 55 °C for 45 s, at 72 °C 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 Biotechnology 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 Ltd) using the ABI 377 sequencer.

Data statistics
Data were analyzed by Fisher’s exact test, \( \chi^2 \) 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)).

| SENV DNA | Normal ALT (n) | Abnormal ALT (n) | Total |
|----------|----------------|------------------|-------|
| P        | 4              | 1                | 5     |
| 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 (\( \chi^2 = 0.549, P = 0.459 \)).

| SENV-DNA | Positive | Negative |
|----------|----------|----------|
| P        | 4        | 5        |
| N        | 10       | 30       |

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 (\( \chi^2 = 3.97, P = 0.046 \)). 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 (\( t = 1.246, P = 0.236 \)).

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                     |
| SENV DNA Negative             | 10                               | 4.98±0.75                     |

Abbreviations: P, positive; N, negative.

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 ($\chi^2=0.35, P=0.72$).

**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%)[30]. Although most subjects with SENV-D/H infection alone had no hepatitis or mild hepatitis[13,30,31], an association of SENV-D/H with transfusion-associated hepatitis has been reported[37]. Whether SENV-D/H serves as causative agents of non-A and non-E hepatitis remains controversial[12,30].

Coinfection with HBV and hepatitis D virus has been reported to be associated with severe and rapidly progressive liver diseases[13]. 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[44]. 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[13]. 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[46]. 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 consistent with the previous observation that the vast majority of SENV-infected hemodialysis patients did not develop hepatitis[35].

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 SENV 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[38].

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 superinfection 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 predict 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.

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