**VIRAL HEPATITIS**

SEN virus does not affect treatment response in hepatitis C virus coinfected patients but SEN virus response depends on SEN virus DNA concentration

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**Abstract**

**AIM:** To clarify the effect of SEN virus (SENV) infection on a combination therapy including interferon alfa (IFN-α) or pegylated-IFN with ribavirin in patients with chronic hepatitis C and the effect of a combination therapy on SENV.

**METHODS:** SENV DNA was determined by polymerase chain reaction in serum samples from 95 patients with chronic hepatitis C. Quantitative analysis was done for SENV H DNA.

**RESULTS:** Twenty-one (22%) of 95 patients were positive for SENV DNA. There was no difference in clinical and biochemical parameters between patients with HCV infection alone and coinfected patients. The sustained response rate for HCV clearance after combination therapy did not differ between patients with SENV (52%) and without SENV (50%, n.s.). SENV DNA was undetectable in 76% of the initially SENV positive patients at the end of follow-up. SENV H response to combination therapy was significantly correlated with SENV DNA level (P=0.05).

**CONCLUSION:** SENV infection had no influence on the HCV sustained response rate to the combination therapy. Response rate of SENV to the combination therapy depends on SENV DNA level.

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**INTRODUCTION**

Five hepatitis viruses (A-E) are responsible for more than 80% of cases of viral hepatitis. Recently, a new family of DNA viruses was discovered and designated as “SEN virus” (SENV)[1]. SENV is a single-stranded circular virus of approximately 3 800 nucleotides. By phylogenetic analysis, 8 different strains of SENV have been identified. SENV is a member of the Circoviridae family, a group of small, single-stranded, nonenveloped circular DNA virus that includes TT virus, TUS01, SANBAN, and YONBAN[2].

Two strains of SENV (SENV D and SENV H) have been extensively studied and have been shown to be present in approximately 2% of blood donors in the United States, 2% in Italy and 10% in Japan and to be readily transmitted by blood transfusion and other common parenteral routes[3]. Although SENV infection is cleared spontaneously in the majority of patients, approximately 45% develop persistent infection that exceeds 1 year and has been documented as long as 12 years. Hypervariable regions with mutation rates of 7.32×10^−4 per site per year may be involved in the persistence[4]. Although SENV D and SENV H infections were strongly associated with transfusion-associated non-A to non-E hepatitis in one study, this association does not establish causality and the vast majority of patients infected with SENV did not develop hepatitis at the time of transfusion[5]. However, the clinical role of SENV infection is not yet clear.

HCV is a major cause of post-transfusion hepatitis and chronic liver disease[5]. More than half of patients with HCV infection develop chronic hepatitis that leads to liver cirrhosis, and hepatocellular carcinoma (HCC)[6]. The prevalence of SENV in patients with chronic hepatitis was reported to be between 24% and 40%[3,7]. There is no evidence that SENV infection affects the progression of HCV infection, but the influence of SENV on HCV response to interferon alfa (IFN-α) is not clear[8]. Three studies investigated the influence of SENV on the response to interferon therapy in HCV infected patients[9-11]. Rigas et al. and Kao et al. investigated the patients with chronic hepatitis treated for 6 mo with 3 million units interferon (IFN) 2 alfa 3 times per week plus 1 000-1 200 mg ribavirin daily. The morning dose was reduced to 400 mg for those weighing less than 72 kg. Rigas et al. could demonstrate a negative effect of SENV coinfection on the outcome of therapy[9]. Kao et al. showed that SENV infection has no effect on response to combination therapy[10]. The third study investigated 104 patients who were treated with IFN at a dosage of 9 million units (MU) daily for 2 wk, followed by 9 MU 3 times a week for 22 wk (total dose, 720 MU). There was no effect of SENV coinfection on interferon response in this study[9].

None of the studies represent the current therapy scheme. Therapy duration depends on HCV genotype. Patients with HCV genotype 2 or 3 are treated for 24 wk with IFN 3 MU 3 times a week plus ribavirin at a dosage of 1 200 mg or 1 000 mg daily. Patients with genotypes other than 2 or 3 are treated for 48 wk[11,12].

The aim of this study was to determine the prevalence of SENV infection in chronic HCV infected patients, the influence on clinical and virological characteristics and the effect of SENV coinfection on HCV response to combination therapy including IFN and ribavirin.

**MATERIALS AND METHODS**

**Subjects**

Ninety-five patients with chronic hepatitis C virus infection
were seen at the outpatient department of the University of Düsseldorf. Diagnosis of chronic hepatitis C was based on the following criteria: (1) detectable HCV-RNA; (2) absence of detectable hepatitis B surface antigen; (3) exclusion of other liver diseases (autoimmune hepatitis, hemochromatosis, Wilson disease). All patients received combination therapy with IFNα or pegylated-interferon (PEG-IFNα plus ribavirin, and treated for 24 or 48 wk with IFNα 2a (Intron) or 2b (Roferon)- or PEG-IFNα 2a (PEG-Intron) or 2b (Pegasys), and oral ribavirin (Rebetol, Schering Plough) at a dose of 1 000 mg (weight<75 kg) or 1 200 mg (weight>75 kg) daily. Patients infected with HCV genotype 1 or 4 were treated for 48 wk. Patients with HCV genotype 2 or 3 were treated for 24 wk. Blood samples were taken at baseline, at the end of treatment and six months after the treatment. HCV RNA was detected at baseline, 24, 48 and 24 wk after treatment.

Responders were defined as patients who had undetectable levels of HCV RNA in serum and normal ALT 6 mo after the treatment. Patients, who were positive for HCV-RNA at wk 24 discontinued the treatment and were defined as nonresponders. Patients who relapsed after end of treatment were defined as nonresponders. No patient had a history of or developed decompensated cirrhosis or HCC during the study period and all were negative for the antibody to human immunodeficiency virus.

Ninety-five patients entered the study between 1999 and 2002. Forty-nine patients were treated in combination with IFN and 46 patients in combination with PEG-IFN. Fifty-nine of them were men and 36 women with mean age of 42±12 years. Sixty-eight (71%) of them were infected with HCV genotype 1 or 4 and 26 (27%) with genotype 2/3 and HCV genotype of 2(2%) patients were undetermined. Mean ALT was 72±73 U/l.

**SENV-detection and quantification**

The presence of SENV D and SENV H DNA was determined by PCR. Total DNA was extracted from 200 µL serum with the QIAamp blood kit (QIAGEN) and resuspend in 200 µL of elution buffer. The oligonucleotide primers were synthesized according to the published SENV sequences. The selection of the real-time PCR primers for SENV H-virus and SENV D was done with the support of the Primer Express Software (PE Applied Biosystems, Weiterstadt, Germany).

The primer for the SENV H were designated SENV H-F1 (GGTTAACCKSAGCTGACTTCA (K=G/T; S=G/C)) and SENV H-R1 (GGAAGGTGTAGCAAGGGTTGTC), the fluorogenic Taq-man probe, 200 µmol/L (each) dATP, dCTP, and dGTP, 300 nmol/L forward and reverse primer, 200 nmol/L fluorogenic Taq-man probe, 200 µmol/L (each) dATP, dCTP, and dGTP, 400 µmol/L dUTP, 10 nmol/L Tris-HCl (pH 8.3), 5 mmol/L MgCl2, 0.5 µ uracil-N-glycosylase (UNG) and 1.25 U Taq Gold polymerase in a final volume of 50 µL. Following inactivation of the UNG (2 min, 50 °C) and activation of the AmpliTaq Gold for 10 min at 95 °C, 40 cycles (15 s at 95 °C and 1 min at 60 °C) were performed with an thermocycler 5 700 system (PE Applied Biosystems). As a DNA standard for the SENV H-PCR, a SENV H-coding plasmid (pSGSEN-H), encompassing the amplified region of the TaqMan®-PCR, was created by PCR-cloning and serially diluted. The sensitivity of the TaqMan PCR was determined as <5 copies/assay. A standard graph of the C_T values obtained from serial dilutions of the standard was constructed by the software and the C_T values of the unknown samples were plotted on the standard curves and the number of SENV H genomes were calculated. For the SENV D-PCR no standard-plasmid was created and the results were only determined qualitatively.

**Serologic testing of hepatitis B and C**

The qualitative analysis of HCV-RNA was tested by a commercial PCR assay (Amplificor HCV Amplification 2.0, Roche Diagnostics, Indianapolis, IN). The quantitative analysis of HBV-DNA was made by a commercial assay (Digene Hybrid Capture System HBV DNA Assay). HBs-Ag as a serologic marker of HBV-infection was detected by a commercial immunoassay (Axsym HBs-Ag, Abbott Laboratories, North Chicago, IL).

**Genotyping of HCV**

The genotype analysis of HCV was performed by a commercial hybridization assay (Inno-Lipa HCV II, Innogenetics, Ghent, Belgium) using HCV-positive amplification products from the PCR assay (Amplificor HCV Amplification 2.0, Roche Diagnostics, Indianapolis, IN).

**Statistical analysis**

Data were entered in SPSS (version 11.0, Inc., Munich, Germany). A χ² or Fisher´s exact test (F-test) was used for the comparison of categorical variables, and a Mann-Whitney test was used for the comparison of continuous variables. The significance level was set at 0.05, and all P values were two tailed. All statistical analyses were performed using SPSS.

**ULTS**

SENV was detected in 21 (22%) of 95 patients. Of the 21 patients, 16 were infected with SENV H, 4 with SENV D, and one with both strains. The mean SENV H DNA level in serum was 461±381 copies/mL. The mean age and the proportion of men and women were similar in the SENV positive and SENV negative group. There was no significant difference between both groups concerning serum levels of ALT, HCV genotype, number of patients who received PEG-IFN, and the proportion of pretreated patients (Table 1). Therefore, many confounding variables known to influence the outcome of the therapy were excluded. Sustained response rates did not differ significantly between the 21 SENV positive patients and 74 SENV negative patients. Eleven of the 21 SENV positive and 37 of the 74 SENV negative patients were responders (52% vs 50%, P=1.0).

| Table 1 Characteristics of SENV positive and SENV negative patients |
|----------------------------------|-------------------|-----------------|-----------------|
| SENV positive (n=21)         | SENV negative (n=74) | P value |
| Median age (yr)         | 42±12             | 42±12          | 0.99*           |
| Male, m (%)            | 13 (62%)          | 46 (62%)       | 1.02            |
| Median baseline ALT (IU/L) | 81±109           | 69±60          | 0.56*           |
| Pretreated, n (%)       | 3 (14%)           | 10 (14%)       | 1.02            |
| Hepatitis C genotype, n (%) | 1.4               | 16 (76%)       | 0.62            |
| Undetermined            | 2.3               | 5 (24%)        | 0.79*           |
| PEG-IFN                 | 11 (52%)          | 35 (47%)       | 0.81*           |
| Sustained HCV response to therapy | 11 (52%) | 37 (50%) | 1.02 |

*1Mann-Whitney test; *2Fisher’s exact test.
Of the 95 patients receiving combination therapy, 48 were sustained responders, who lost detectable HCV RNA for more than 24 wk after completing the therapy (51%). Of these 48 patients, 30 were infected with HCV genotype 1 or 4 and 16 with HCV genotype 2 or 3 and in 2 patients HCV genotypes were undetermined. Sustained responders were found to be independent in relation to gender, ALT before treatment, HCV genotype, and SENV DNA positivity. Sustained HCV response rate to the combination therapy was correlated inversely with age of the patients ($P<0.01$). A higher mean SENV H DNA level was observed in the nonresponder group, but the difference was not statistically significant (393±297 vs 523±452, $P=0.48$) (Table 2).

**Table 2** Clinical, biochemical and viral characteristics in relation to HCV response to combination therapy

|                         | Sustained response (n=48) | No sustained response (n=47) | $P$ value |
|-------------------------|--------------------------|-------------------------------|-----------|
| Male, n (%)             | 30 (63%)                 | 29 (62%)                      | 0.34$^a$  |
| Age (yr)                | 39±11                    | 45±11                         | $<0.01^a$ |
| ALT                     | 70±98                    | 74±68                         | 0.54$^a$  |
| HCV genotype, n (%)     |                          |                               |           |
| 1 or 4                  | 30 (63%)                 | 37 (79%)                      |           |
| 2 or 3                  | 16 (33%)                 | 10 (21%)                      | 0.16$^a$  |
| Undetermined            | 2 (4%)                   |                               |           |
| SENV positive, n (%)    | 10 (21%)                 | 11 (23%)                      | 0.81$^a$  |
| SENV H-DNA (copies/mL)  | 393±297                  | 523±452                       | 0.48$^a$  |

$^a$Fisher’s exact test; $^b$Mann-Whitney test.

We analysed the response of SENV to the combination therapy in 20 HCV/SENV-coinfected patients. The clinical and virological characteristics are shown in Table 3. Of the 21 patients with SENV DNA, 16 were infected with SENV-H, 3 with SENV-D, and 1 with both. Fifteen (75%) of 20 patients had no detectable SENV DNA after treatment. Two (66%) of the 3 patients infected with SENV D responded to the combination therapy, 12 (75%) of the 16 patients infected with SENV H, and the patient who was infected with both strains responded to combination therapy. SENV response to the combination therapy did not correlate with sex, age, or ALT level (Table 4). SENV H response rates correlated with the SENV-DNA titer before treatment. SENV H-DNA level was significantly lower in patients who responded to the combination therapy (371±276 copies/mL vs 820±497 copies/mL, $P=0.05$).

Of the five patients who failed to eradicate SENV, two lost HCV-RNA, one of these two patients did not have ALT normalized.

**Table 4** Clinical and virological parameter of SENV response to combination therapy in 20 HCV/SENV-coinfected patients

|                          | SENV response (15) | SENV nonresponse (5) | $P$       |
|--------------------------|--------------------|----------------------|-----------|
| Age (yr,mean)            | 41±12              | 48±13                | 0.23$^b$  |
| Male                     | 10 (63%)           | 3 (60%)              | 1.0$^b$   |
| ALT                      | 85±61              | 80±123               | 0.35$^b$  |
| SENV H-DNA (copies/mL)   | 371±276 (n=13)     | 820±497 (n=4)        | 0.05$^b$  |

$^b$Mann-Whitney test; $^c$Fisher’s exact test.

**DISCUSSION**

SENV D or SENV H-DNA was detectable in 22% of the patients, which is comparable to recent reports$^{[13]}$. The observed frequency of HCV and SENV infection is not surprising because these agents are transmitted by similar routes. SENV has been shown to be associated with blood transfusion and intravenous drug abuse$^{[3,14]}$. Dual infection with HCV and other
hepatitis virus (HAV, HBV) has been reported to be associated with a more severe and rapidly progressing liver disease\(^\text{15-17}\). In our study no clinical or biochemical difference was found between patients with HCV infection alone and those coinfected with HCV and SENV. In this and previous studies, there was no evidence to suggest that SENV coinfection leads to increased severity or persistence of chronic hepatitis C\(^\text{17}\). It was demonstrated that infection with SENV in patients with non-A to non-E liver disease has no effect on disease progression\(^\text{18}\).

Five independent factors could be identified which are significantly associated with response to antiviral therapy: genotype 2 or 3, viral load less than 2 million copies/mL, age 40 years or less, minimal fibrosis stage, and female sex\(^\text{11}\). Of these five factors, we studied sex, age, ALT, and HCV genotype and found a significant correlation for response of age (\(P=0.006\)) but not of sex, ALT, or HCV genotype. This result may reflect the patient selection. We observed on an average, 1.3 times higher SENV DNA level in patients who failed to the therapy compared with patients with sustained response. Nevertheless, this difference was not statistically significant (393±297 vs 523±452, \(P=0.48\)) and may be due to the small cohort studied.

Response rates to antiviral therapy in hepatitis C has increased after combination therapy consisting of IFN and ribavirin was used. Further increase was observed after introduction of PEG-IFN. Sustained response rates of up to 80% in HCV genotype 2/3 are described\(^\text{12}\). Patients coinfected with other hepatotropic viruses are still difficult to treat. Coinfection with hepatitis B virus impairs sustained rate\(^\text{13}\). On the other hand, it could be demonstrated that coinfection with TT virus has no influence on response rates to IFN therapy in patients with chronic hepatitis C. Rigas et al. reported that coinfection with HCV and SENV adversely affected the HCV response to combination therapy with interferon and ribavirin\(^\text{8}\). This cohort was treated for 6 mo with 3 million units IFN 2ab three times per week plus 1 000/1 200 mg ribavirin/day depending on body mass more or less than 72 kg. They did not take into account that antiviral therapy duration in hepatitis C depends on HCV genotype. In contrast, Umemura et al. found no influence of SENV coinfection on HCV therapy\(^\text{14}\). They investigated the patients with chronic hepatitis C virus infection, who underwent an IFN-monotherapy. Kao et al. studied 100 patients with chronic hepatitis C who were treated with IFN and ribavirin. These patients were treated for 24 wk independent of HCV genotype. They showed that coinfection with SENV has no effect on response to the therapy. However, none of the studies represented the current standard in the therapy of HCV infection.

In our study, patients received combination therapy for a duration depending on HCV genotype. We found no significant difference in sustained response rates between patients with HCV and SENV coinfection and those who had HCV infection alone (52% vs 50%, \(P=1.0\)). Therefore, our data are in concordance with the results from Umemura et al. and Kao et al. Overall sustained response rates were higher in our study as compared with the study by Umemura. This results from the higher efficiency of combination therapy compared with IFN-monotherapy.

Sixteen of 20 coinfected patients lost SENV DNA after treatment. There was no influence of the SENV strain on the response rates. SENV D and SENV H response to the combination therapy was not different (2/3 (66%) vs 12/16 (75%), \(P=1.0\)). This result is in contrast to the results published by Umemura et al. The authors reported a response rate of SENV D to a IFN-\(\alpha\) therapy of 73% (11/15 patients) and 33% (1/3 patients) for SENV H. This discrepancy may be a result of our cohort. In contrast to their studies we detected more often SENV H than SENV D.

It is well known that virus load is a predictive factor for response to treatment in different virus infections. For example, sustained response rate of HCV therapy correlates with the virus load at initiation of therapy\(^\text{20}\). Patients with high HBV-DNA levels fail more often to antiviral therapy than patients with low HBV-DNA levels\(^\text{22}\). Similarly, a significant difference in SENV DNA levels between patients who lost SENV DNA and whose who did not respond was found. SENV H-DNA levels were significantly lower in the group who lost SENV (\(P=0.05\)).

In conclusion, SENV infection is common in patients with HCV infection. SENV coinfection does not influence the clinical course of hepatitis C or the response rates of HCV to the combination therapy. SENV is sensitive to IFN-\(\alpha\), and additional medication with ribavirin does not improve response to IFN-\(\alpha\). The SENV response rate is correlated with the SENV DNA level prior to treatment. Higher levels influence response rate adversely.

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