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SEN Virus Infection in HIV/HCV Coinfected Patients

A. Sagir[1], O. Adams[2], M. Oette[1], M. Vogel[3], B. Kupfer[4], J. Emmelkamp[3], J. Rockstroh[3], D. Häussinger[1]

[1]Klinik für Gastroenterologie, Hepatologie und Infektiologie der Universitätsklinik Düsseldorf,
[2]Institut für Virologie der Universität Düsseldorf,
[3]Medizinische Klinik der Universitätsklinik Bonn,
[4]Institut für Virologie der Universität Bonn, Germany

Abstract
Background: Chronic Hepatitis C Virus (HCV) infection is currently one of the most relevant coinfections in HIV positive patients. The influence of SEN Virus (SENV) on the outcome of HCV therapy in HIV/HCV coinfected patients who underwent combination therapy with pegylated interferon (PEG-IFN) and ribavirin is unclear.
Methods: SENV DNA was determined by polymerase chain reaction in 67 HIV/HCV coinfected patients, 77 HIV monoinfected patients, 95 treatment naïve HCV monoinfetcted patients, and 122 healthy blood donors. Quantitative analysis was done for SENV H DNA.
Results: SENV DNA was detected in 8 of 67 (12%) HIV/HCV coinfected patients, in 9 of 77 (11.7%) HIV monoinfected patients, in 21 of 95 (22%) HCV monoinfected patients, and 12 of 122 (9.8%) healthy blood donors. HIV monoinfected patients showed the highest mean SENV H DNA level. The mean SENV H DNA was significantly lower in HIV/HCV coinfected patients compared to all other groups. The sustained virological response rates to combination therapy of HCV in HIV/HCV coinfected patients did not differ between patients with detectable SENV 5/8 (62.5%) and without SENV 28/59 (47.5%; $p = 0.47$). We found no significant difference in SENV H DNA pretreatment levels between nonresponders and responders to combination therapy (112 ± 144 copies vs. 8 ± 7 copies/ml; $p = 0.27$).
Conclusion: Coinfection with HCV may reduce SENV H replication in HIV positive patients and results in significantly lower SENV H DNA levels in HIV/HCV coinfected patients. SENV infection has no influence on the outcome of HCV combination therapy in HIV/HCV coinfected patients.

Key words: HIV, SEN Virus, HCV

Introduction

Patients infected with human immunodeficiency virus (HIV) are often coinfected with other viruses [1- 3]. Prevalence of hepatitis C virus (HCV) infection among HIV positive patients with a history of intravenous drug use (IDU) or transfusion is high. The extensive use of highly active antiretroviral therapy (HAART) has dramatically improved the prognosis of HIV infection, prolonging and improving life of HIV positive patients [4-5]. On the other hand, mortality and morbidity due to liver disease have increased significantly [2, 6]. However, treatment of HCV infection in coinfected patients has become successful. Pegylated interferon a (PEG-IFN) in combination with ribavirin is the current gold standard for the treatment of chronic HCV infection [7 - 10]. In 1999, a new virus was isolated from the serum of a HIV positive i.v. drug-using patient who presented with post-transfusion hepatitis of unknown aetiology [11 - 13]. Preliminary data showed that SEN-Virus (SENV) is a single-stranded circular, non-enveloped DNA virus of ~3600 to ~3800 nucleotides with at least three open reading frames (ORFs) [12]. Eight different strains of SENV, named SENV-A, SENV-B, SENV-C, SENV-D, SENV-E, SENV-F, SENV-G, and SENV-H, were identified and provisionally classified as members of the Circoviridae family, a group of small, single-stranded, non-enveloped circular DNA viruses that includes TT virus (TTV), TUS01, SANBAN, PMV, and YONBAN. Only SENV-D and SENV-H seem to cause post-transfusion-hepatitis [14]. The high rate of SENV-D and SENV-H infections in transfusion-associated non A - E hepatitis compared to controls and the temporal association between viremia and ALT elevation suggests that SEN-V might be a causative agent of post-transfusion hepatitis [13].

There is no evidence that SENV infection affects the progression of HCV infection, but the influence of SENV on HCV response to PEG-IFN in patients infected with HIV is unknown. Till now, the effect of SEN V on HCV response to IFN combination therapy was only studied in HIV negative patients [15 - 17]. The aim of this study was to determine the prevalence of SENV infection in HIV positive patients coinfected with HCV and the effect of SENV coinfection on HCV response to combination therapy including PEG-IFN and ribavirin.

Subjects and Methods

Subjects

Sixty-seven HIV positive patients with chronic hepatitis C virus infection were seen at the outpatient department of the University of Düsseldorf and at the

outpatient department of the University of Bonn. 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 pegylated-interferon α (PEG-IFN) plus ribavirin, and were treated for 24 or 48 weeks with PEG-IFN a 2a (180 mg once weekly) or 2b (1.5 mg/kg/week), and oral ribavirin (Rebetol, Schering Plough). Patients infected with HCV genotype 1 or 4 were treated for 48 weeks, ribavirin doses were 1000 mg (weight < 75 kg) or 1200 mg (weight > 75 kg) daily. Patients with HCV genotype 2 or 3 were treated for 24 weeks and received 800 mg ribavirin daily. 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 weeks after treatment.

Responders were defined as patients who had undetectable levels of HCV RNA in serum and normal ALT 6 months after the end treatment. No patient developed decompensated cirrhosis or HCC before or during the study period.

A total of 67 HIV positive patients (57 men and 10 women, mean age 37 ± 7 years) were included into the study between June 2000 and April 2005. Diagnosis of HIV infection was confirmed by detection of HIV-antibodies in western blot. The clinical stage of HIV infection was classified according to the revised CDC staging system. Baseline characteristics of the patients are shown in Table 1.

Control Groups

HIV monoinfected patients: This group of controls consisted of 77 HIV-infected patients without evidence of HCV infection. Chronic hepatitis was excluded by using the above-mentioned serological tests. Characteristics of the control group are shown in Table 1.

HCV monoinfected patients: Ninety-five treatment naïve patients with chronic hepatitis C virus infection were used as HCV monoinfected control group (59 men and 36 women, mean age 42 ± 12 years).

Healthy control individuals: The group of healthy controls consisted 122 healthy blood donors (90 men and 32 women, mean age 37 ± 13 years).

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 resuspended in 200 µl of elution buffer. The oligonucleotide primers were synthesized on the basis of published SENV sequences. The selection of the real-time PCR primers for SEN-H-virus and SEN-D-virus was done with the support of the Primer Express Software (PE Applied Biosystems, Weiterstadt, Germany). The sequences of the primers for the SEN-H-virus were SEN-H-F1 (GGTTAACCKSAGC TGACTTCA (K = G/T; S = G/C)), SEN-H-R1 (GGAAGGTGTAGCAAGGGTTGTC) and for the fluorogenic TaqMan® probe (5' FAM TTTCCGTT CTGCTCACCACAAA 3'TAMRA). A 69-base pair amplicon in a conserved region of the ORF 1 gene was amplified and detected. The sequences of the primers for the SEN-D-virus were SEN-D-F1 (CCA GACTTRTGCAAAGTTCCTCTTG (R = A/G)), SEN-D-R1 (GTGGTGAGCAGAACGGATGTT) and for the fluorogenic TaqMan® probe (5' *FAM* AACTTTGCGGTCAACTGCCGCTG 3'*TAMRA*). A 76-base pair amplicon in a conserved region of the ORF 1 gene was amplified and detected. Each PCR contained 5 µl sample DNA, 300 nM forward and reverse primer, 200 nM fluorogenic Taq-man probe, 200 µM (each) dATP, dCTP, and dGTP, 400 µM dUTP, 10 mM Tris-HCl (pH 8.3), 5mM MgCl2, 0.5 U uracyl-N-glycosylase (UNG) and 1.25 U Taq Gold polymerase in a final volume of 50 µl. Following inactivation of

Table 1. Baseline characteristics of HCV/HIV coinfected and HIV monoinfected patients.

	HIV/HCV coinfected patients (n = 67)	HIV monoinfected patients (n = 77)	P
Male (%)	57 (85%)	56 (73%)	0.103[1]
Mean age (years)	37 ± 7	42 ± 11	0.004[2]
Mean CD 4 cells/mm^3	526 ± 273	457 ± 210	0.076[2]
Mean CD 8 cells/mm^3	1065 ± 529	980 ± 549	0.208[2]
Mean ALT (IU/l)	84 ± 82	16 ± 14	< 0.001[2]
Mean AST (IU/l)	51 ± 40	14 ± 6	< 0.001[2]
Mean GGT (IU/l)	89 ± 103	35 ± 47	< 0.001[2]
CDC stage C (%)	7 (10.4%)	18 (23.4%)	0.076[1]
HCV genotype n (%)			
1, 4	40 (59.7%)		
2, 3	26 (38.8%)		
unknown	1 (1.5%)		
HCV RNA (copies/ml)	6 863 724 ± 8 678 622		

[1] χ^2-test; [2] Mann-Whitney-U-test

the UNG (2 min, 50 °C) and activation of the AmpliTaq Gold for 10 min at 95 °C, 40 cycles (15 sec at 95°C and 1 min at 60 °C) were performed with an thermocycler5700 system (PE Applied Biosystems). As a DNA-standard for the SEN-H-PCR, a SEN-H-Virus -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 CT values obtained from serial dilutions of the standard was constructed by the software and the CT values of the unknown samples were plotted on the standard curves and finally, the number of SENV-H genomes was calculated. For the SEN-D-PCR the results were determined qualitatively only.

Serologic Testing of Hepatitis B and C

The qualitative analysis of HCV-RNA was tested by a commercial PCR assay (Amplicor 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 (Amplicor HCV Amplification 2.0, Roche Diagnostics, Indianapolis, IN).

Statistical Analysis

Data were entered in SPSS (version 11.0, Inc., Munich, Germany). A c2 or Fisher´s exact test (F-test) was used for the comparison of categorical variables, and a Mann-Whitney-U-test was used for the comparison of continuous variables. The significance level was set at 0.05, and all p values were two tailed.

Results

SENV D and -H were detected in 8 of 67 (12%) HIV/HCV coinfected patients, in 9 of 77 (11.7%) HIV monoinfected patients, in 21 of 95 (22%) HCV monoinfected patients, and 12 of 122 (9.8%) healthy blood donors. There was no significant difference in the prevalence of SENV between the HIV monoinfected, HCV monoinfected and HIV/HCV coinfected group. Two HIV/HCV coinfected patients were infected with SENV-D (3%), 5 with SENV-H (7.5%) and one with both strains (1.5%) compared to one (1.3%), 6 (7.8%) and 2 (2.6%) in the HIV monoinfected cohort respectively. Of the 21 HCV monoinfected patients 16 were infected with SENV H, 4 with SENV D and one with both strains.

The lowest mean SENV H-DNA level was detected in the HCV/HIV coinfected group (43 ± 84 copies/ml), followed by the HCV monoinfected group (461 + 381 copies/ml), the healthy control group (530 + 962 copies/ml), and the HIV monoinfected patients (7153 ± 10 855 copies/ml). The difference in the SENV H-DNA levels between the HIV monoinfected patients and all other groups were significant (HIV moninfected vs. healthy controls, p = 0.009; HIV monoinfected group vs. HCV monoinfected group, p = 0.007; HIV monoinfected vs. HIV/HCV cooinfected group, p<0.001). In addition to these differences

Table 2. Baseline characteristics and therapy outcome of HIV/HCV coinfected patients after dividing concerning their SENV status.

	SENV positive (n = 8)	SENV negative (n = 59)	p value
Mean age (years)	34 ± 11	37 ± 6	0.091
Male, m (%)	8 (100%)	42 (49%)	0.342
Mean baseline ALT (IU/l)	88 ± 105	84 ± 80	0.611
Mean baseline AST (IU/l)	51 ± 37	52 ± 60	0.491
Mean baseline GGT (IU/l)	99 ± 177	88 ± 90	0.231
CD 4 cells/mm^3	494 ± 262	530 ± 276	0.551
CD 8 cells/mm^3	950 ± 529	1082 ± 531	0.361
CDC stage C (%)	2 (25%)	6 (10%)	0.212
HIV RNA (copies/ml)	16 430 ± 33 850	14 822 ± 36 005	0.791
Hepatitis C genotype			
1,4 (%)	5 (62.5%)	35 (59.3%)	
1.02			
2,3 (%)	3 (37.5%)	23 (39%)	
Unknown (%)	0 (0%)	1 (1.7%)	
HCV RNA (copies/ml)	9 550 835 ± 7 903 198	6 479 851 ± 8 782 031	0.131
End of treatment HCV response	6 (75%)	38 (64%)	0,712
Sustained HCV response	5 (62.5%)	28 (47.5%)	0.472

[1] Mann-Whitney-U-test; [2] χ^2-test

the following SENV H-DNA levels were significantly different: HIV/HCV coinfected patients vs. healthy controls, p = 0.012; HIV/HCV coinfected group vs. HCV monoinfected group, p<0.001. SENV H-DNA levels were higher in healthy controls compared to HCV monoinfected patients (530 + 962 vs. 461 + 381 copies/ml; p = 0.18), but the difference did not reach the level of significance.

Of the 67 patients receiving combination therapy, 44 (66%) were negative for HCV RNA at the end of treatment. HCV RNA was undetectable 24 weeks after completing the therapy in 33 patients (49%). Of these 33 patients, 16 were infected with HCV genotype 1 or 4 and 17 patients with HCV genotype 2 or 3.

In the next step we studied the influence of SENV on the PEG-IFN combination therapy outcome. SENV was detected in 8 (12%) of the 67 HIV/HCV coinfected patients. There was no significant difference between both the SENV positive and SENV negative groups concerning their baseline characteristics (sex, age, ALT, AST, GGT, CDC stage, HCV genotype, HCV RNA, HIV RNA, CD4 cells, and CD8; Table 2). Therefore, many confounding variables known to influence the HCV therapy outcome were excluded. At the end of treatment six of the 8 SENV positive patients were negative for HCV RNA compared to 38 of the 59 SENV negative patients (75% vs. 64%; p = 0.71). Five of the eight SENV positive patients (62.5%) were sustained responders compared to 28 of the 59 SENV negative patients (47.5%; p = 0.47).

In order to study the influence of SENV H DNA levels on the therapy outcome, all SENV H positive patients were divided concerning their therapy outcome. The mean pretreatment SENV H DNA was 112 ± 144 copies/ml in non-responders compared to 8 ± 7 copies/ml in responders. This difference did not reach the level of significance (p = 0.27).

Discussion

HIV positive patients are often coinfected with other viruses. Chronic HCV infection is currently one of the clinically most relevant comorbidities in the HIV population. Progression to end-stage liver disease occurs faster in coinfected patients and decompensated cirrhosis is one of the main causes of hospitalisation and death in this population (6, 18). The current treatment of chronic HCV infection in HIV positive patients is the combination therapy including PEG-IFN and ribavirin (10, 19, 20). The influence of SENV on the therapy response to combination therapy in HIV negative patients with chronic HCV infection is well studied. SENV seems not to influence therapy response to combination therapy. Here we studied the prevalence of SENV in HIV/HCV coinfected patients and the influence of SENV on the therapy outcome in HIV/HCV coinfected who underwent combination therapy including PEG-IFN and ribavirin.

SENV D and SENV H were detectable in 8 of 67 (12%) HIV/HCV coinfected patients compared to 9 of 77 (11.7%) HIV monoinfected patients. The difference in the baseline ALT, AST, and GGT between these two groups is an effect caused by inflammation due to the hepatitis C virus. The highest SENV prevalence was detected in HCV monoinfected patients, but this difference did not reach significance. The observed comparable frequency of SENV in these three groups is not surprising, because all three infections are transmitted by similar routes. A significant difference was found in the SENV H-DNA levels between HIV monoinfected, HCV monoinfected patients, healthy controls, and HIV/HCV coinfected patients. SENV H-DNA was significantly lower in HIV/HCV coinfected patients (43 ± 84 copies/ml) than in HIV monoinfected patients (7153 ± 10 855 copies/ml; p< 0.001). In a previous study we showed that SENV H-DNA levels are significantly higher in HIV infected patients than in healthy controls (21). Viral interaction between HCV and SENV may influence the SENV H-DNA. A trend to lower SENV H DNA levels in HCV monoinfected patients compared to healthy controls may support this assumption. This phenomenon may be comparable with the reduction of HBV-DNA levels in HCV monoinfected infected patients (22, 23).

In the next step we studied the influence of SENV on therapy outcome in coinfected patients who underwent combination therapy including PEG-IFN and ribavirin. Several studies were published with different results in patients with HCV monoinfection. While the first published study from Rigas et al. found a significant influence of SENV on the therapy outcome, no other of the following studies could support this result [15 - 17]. Here we analysed 67 HIV/HCV coinfected patients who underwent the actual standard treatment of HCV infection in HIV positive patients. The overall response at the end of treatment was 66%. At the end of follow up 49% remained negative for HCV RNA. Of the 67 coinfected patients 8 were infected with SENV D or SENV H. We found no significant difference in the end of treatment response rates and in sustained response rates between HIV/HCV coinfected patients who were positive for SENV and those who were negative. Other factors like baseline ALT, AST, sex, HCV genotype, and HCV RNA, which are associated with response to combination therapy, did not differ between both groups. A significant difference was observed for the age at the time of combination therapy initiation. However, mean age in both groups was less than 40 years, so that this influence may be disregarded.

After dividing all SENV H positive patients according to their therapy result, we did not find a significant difference in the SENV H-DNA levels between non-responders and responders (112 ± 144 copies/ml vs. 8 ± 7 copies/ml; p = 0.27). Not reaching the level of significance may be due to the small number of SENV H positive patients. SENV H influence depending on the viral load could be similar to HBV. It is known that virulence of HBV depends on HBV load. Patients with a low HBV load show no or little elevated transaminases and inflammation.

In conclusion, there was no difference in the prevalence of SENV between HIV monoinfected, HCV monoinfected and HIV/HCV coinfected patients, however significantly higher SENV H DNA levels were observed in HIV monoinfected patients. Viral interaction between HCV and SENV could be a reason,

which is supported by the lower, but not significant, SENV H DNA levels in HCV monoinfected patients compared to the SENV H DNA levels in healthy controls. We found no differences in the SVR between SENV positive and negative patients. Higher SENV H DNA levels were observed in non-responders compared to responders, but limited by the small number of SENV H positive patients this difference was not significant.

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Address fore correspondence:
Dr. A. Sagir
Klinik für Gastroenterologie, Hepatologie und Infektiologie
Universitätsklinik Düsseldorf
Moorenstr. 5
40225 Düsseldorf
Germany
Tel.: +49-211-811 7820
Fax: +49-211-811 8752
E-mail: sagir@med.uni-duesseldorf.de