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

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To clarify the influence that a recently identified SEN virus (SENV) has on hepatitis C virus (HCV) response to therapy with interferon plus ribavirin, 2 SENV variants, SENV-D and SENV-H, were studied in 100 patients with chronic hepatitis C; 57 of these patients were positive for SENV-D/H DNA, and there were no differences, in clinicopathological features, between patients with and without SENV coinfection. However, patients with SENV coinfection had a higher prevalence of HCV genotype 2a than did those without it. The sustained HCV response rate after combination therapy was comparable between patients with and without SENV coinfection. Of the 57 patients with SENV coinfection, 18 (32%) had a sustained SENV response to combination therapy, and SENV-D had a higher sustained response rate than did SENV-H. These results suggest that SENV has a specific link to HCV genotype 2a and that SENV infection has no apparent effect on coexisting chronic hepatitis C.

Chronic liver diseases are endemic in Taiwan, and most of them can be attributed to infection with hepatitis B virus (HBV) or hepatitis C virus (HCV). Although GB virus C and TT virus (TTV) have been claimed to be associated with chronic non-A–non-E hepatitis [1, 2], most studies have indicated that neither virus causes liver disease [3, 4]. Recently, a new family of single-stranded DNA viruses has been isolated and designated “SEN virus” (SENV) [5]. By phylogenetic analysis, 8 different isolates (A–H) have been identified, with varying prevalence in different populations [5]. Among these isolates, SENV-D and SENV-H have been extensively studied [5–7]. However, the association of SENV infection with liver cell damage remains controversial. Furthermore, one of our recent studies has shown that persons with SENV-D/H infection alone or coinfected with SENV-D/H and either HBV or HCV did not have increased evidence of liver disease [8]. These results therefore argued against the causative role of SENV-D/H in liver disease. Rigas et al. [9] recently have suggested that coinfection with SENV-D/H might adversely affect the outcome of treatment with interferon and ribavirin in patients with chronic hepatitis C; however, their findings have been challenged.

Taking advantage of the frequency of SENV-D/H coinfection in cases of chronic hepatitis C in Taiwan [8], we investigated (1) the influence that SENV-D/H infection has on the clinical, virologic, and histologic characteristics of chronic hepatitis C, (2) the effect that SENV-D/H coinfection has on HCV response to combination therapy with interferon plus ribavirin, and (3) the effect that combination therapy has on the clearance of SENV-D/H.

Patients, materials, and methods. We studied serum samples from 100 patients (64 men and 36 women [mean age ± SD, 46 ± 11 years]) with histologically verified chronic hepatitis C, at the gastroenterological clinics of the National Taiwan University Hospital. These patients had persistent elevation of serum alanine aminotransferase (ALT) levels and were positive for anti-HCV and HCV RNA for ≥6 months. All the enrolled patients were negative for hepatitis B surface antigen (HBsAg) and had no markers suggestive of autoimmune hepatitis. None had a history of alcoholism or hepatotoxic drug intake. Metabolic liver disease was excluded on the basis of clinical and laboratory data. The diagnosis of chronic hepatitis was based on clinical and pathological grounds, including chronic persistent hepatitis and chronic active hepatitis [10]. No case was at the cirrhosis stage. Serum samples were stored at −70°C until used.

The patients had received 3 MU of interferon α-2b (Intron A; Schering-Plough) thrice weekly, plus 1000–1200 mg of orally administered ribavirin (ICN Pharmaceuticals) daily for 24 weeks. The presence of HCV RNA and SENV DNA in the serum
was determined (1) before initiation of combination therapy, (2) at the end of therapy, and (3) 24 weeks after the therapy had been completed. The response to combination therapy was classified into 2 patterns, according to the positivity of serum viral genomes. Patients with a sustained HCV response were defined as those whose HCV RNA in serum was undetectable both at the end of therapy and 24 weeks after combination therapy had been completed. Unsustained HCV response was defined as serum HCV RNA that remained detectable either at the end of therapy or 24 weeks after combination therapy had been completed. Similarly, patients with a sustained SENV response were defined as those whose SENV DNA was undetectable both at the end of therapy and 24 weeks after combination therapy had been completed.

HBsAg and anti-HCV were tested with commercially available kits (Abbott Laboratories).

Serum HCV RNA was tested by reverse transcription–polymerase chain reaction with primers from the most conserved 5′ untranslated region of the viral genome, and HCV genotypes were identified by type-specific primers [11]. Serum HCV RNA level was determined by a second-generation branched-DNA signal-amplification assay (Quantiplex HCV RNA; Bayer Diagnostics) with a detection limit of 0.2 mEq/mL.

SENV-D DNA and SENV-H DNA were amplified by polymerase chain reaction using strain-specific primers, as described elsewhere [8]. The amplified products (231 bp for SENV-D and 230 bp for SENV-H) were separated by 3% agarose gel electrophoresis and were stained with ethidium bromide. The presence of TTV DNA was assayed by nested polymerase chain reaction with primer pairs from open reading frame 1 of the viral genome [4].

Data were analyzed by Fisher’s exact test, χ² test with Yates’s correction, or Student’s t test, and variables that achieved statistical significance in univariate analysis were subjected to multivariate Cox regression analysis, to determine the statistically significant differences too were not statistically significant.

Results. Of the 100 patients with chronic hepatitis C, 57 (57%) were positive for serum SENV DNA. Of the 57 patients with SENV coinfection, 41 (72%) were infected with SENV-H alone, 8 (14%) with SENV-D alone, and 8 (14%) with both SENV-D and SENV-H. In addition, of the 57 patients with SENV coinfection, 27 (47%) also were positive for serum TTV DNA.

Of the 100 patients receiving combination therapy, 40 (40%) were sustained responders, and the remaining 60 (60%) were nonresponders. There was no significant difference, in terms of sex, age, or mean serum ALT level at onset of therapy, between responders and nonresponders. Although the baseline mean ± SD serum HCV RNA level in the responders was lower than that in the nonresponders (1.3 ± 3.1 vs. 2.0 ± 4.3 mEq/mL), the difference was not statistically significant. However, sustained HCV response rate in patients with either genotype 2a or genotype 2b was higher than that in patients with genotype 1b (57% vs. 22% [P < .001]).

There was no significant difference, in the clinicopathological features, between patients with chronic hepatitis C with and without SENV coinfection (table 1). However, by univariate analysis, the prevalence of HCV genotype 2a in patients with SENV coinfection was significantly higher than that in those without it (37% vs. 16% [P = .03]); by multivariate analysis, HCV genotype 2a was independently associated with SENV coinfection (P < .05). In contrast, the sustained HCV response rate after combination therapy was comparable in patients with and without SENV coinfection (44% vs. 35%; see table 1). Further analysis indicated that the sustained HCV response rate in patients with genotype 1b with and without SENV coinfection was 26% and 15%, respectively, whereas the sustained HCV response rate in patients with genotype 2a with and without SENV coinfection was 50% and 38%, respectively. These differences too were not statistically significant.

Of the 57 patients with SENV coinfection before initiation of combination therapy, 21 (37%) lost serum SENV DNA at the end of therapy, and 20 (35%) remained negative for serum SENV DNA 6 months after therapy had been completed (table 2). Accordingly, the sustained response rate of SENV was com-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Serum SENV DNA</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Patients, no.</td>
<td>57</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>34/23</td>
</tr>
<tr>
<td>Age, mean ± SD, years</td>
<td>47 ± 11</td>
</tr>
<tr>
<td>Transfusion history</td>
<td>20 (35)</td>
</tr>
<tr>
<td>Peak serum ALT, mean ± SD, IU/L</td>
<td>122 ± 68</td>
</tr>
<tr>
<td>Hepatitis histology</td>
<td></td>
</tr>
<tr>
<td>Chronic persistent</td>
<td>42 (74)</td>
</tr>
<tr>
<td>Chronic active</td>
<td>15 (26)</td>
</tr>
<tr>
<td>HCV genotype</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>22 (38)</td>
</tr>
<tr>
<td>2a</td>
<td>21 (37)*</td>
</tr>
<tr>
<td>2b</td>
<td>9 (16)</td>
</tr>
<tr>
<td>Mixed</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Serum HCV RNA, mean ± SD, mEq/mL</td>
<td>1.4 ± 4.0</td>
</tr>
<tr>
<td>Therapeutic HCV response</td>
<td></td>
</tr>
<tr>
<td>Sustained</td>
<td>25 (44)</td>
</tr>
<tr>
<td>Unsustained</td>
<td>32 (56)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, unless indicated otherwise. ALT, alanine aminotransferase; HCV, hepatitis C virus.

* P = .03, vs. patients without SENV coinfection.
Viral response rates of SENV-D were significantly higher than those seen. However, both the end-of-therapy and the sustained response rates of SENV-D were significantly higher than those of SENV-H (88% vs. 34% [P = .02] and 88% vs. 27% [P = .004], respectively).

**Discussion.** Of the 8 SENV isolates, only SENV-D and SENV-H have higher prevalence ratios. In Taiwan, our recent study had indicated that SENV-D/H infections occur more often in high-risk groups (54%–90%), patients with chronic hepatitis B (41%), HBV-related hepatocellular carcinoma (HCC) (54%), chronic hepatitis C (15%), and HCV-related HCC (76%) than in healthy adults (15%) [8]. However, the prevalence of SENV-D/H DNA in patients with non-A–non-E fulminant hepatitis (30%) was comparable to that in healthy adults (15%). In addition, most subjects with SENV-D/H infection alone had either no hepatitis or mild hepatitis. Thus, although an association of SENV-D/H with transfusion-associated hepatitis has been reported [7], whether SENV-D/H serve as causative agents of non-A–non-E hepatitis remains controversial [6–8].

The geographic distribution of different SENV variants remains unclear. Previous studies have shown that SENV-D is the predominant strain in Japan [6, 12], whereas SENV-H is predominant in the United States and Taiwan [7, 8]. The findings of the present study consistently indicate that 72% of Taiwanese patients with chronic hepatitis C with SENV coinfection were infected with SENV-H, 14% with SENV-D, and 4% with both SENV-D and SENV-H. Whether differences in SENV variants affect the heterogeneity in clinical outcome and response to antiviral therapy in patients with chronic SENV infection in different parts of the world awaits further studies.

Coinfection with SENV has been frequently observed in 20%–76% of patients with chronic hepatitis C [6–9, 12]. The results of the present study showed that the prevalence of SENV-D/H infection in patients with chronic hepatitis C was 57%, implying that HCV and SENV may share common modes of transmission. Nonetheless, the mean serum HCV RNA level did not differ significantly between patients with HCV and SENV coinfection and those with HCV infection alone (table 1). Accordingly, our data consistently showed that SENV might not interfere with the replication of HCV [12].

The clinical relevance of SENV infection in combination with HCV infection remains controversial [6, 8, 9, 12]. Our data showed no significant difference, in terms of demographic features, peak serum ALT level, histological severity, and serum HCV RNA level, between patients with HCV and SENV coinfection and those with HCV infection alone (table 1). This fact confirms that coinfection with SENV-D/H in patients with chronic hepatitis C is not associated with increased biochemical or histological evidence of liver disease. However, HCV genotype 2a was more often found among patients with HCV and SENV coinfection than among those with HCV infection alone (37% vs. 16% [P = .03]), suggesting a specific link between SENV and HCV genotype 2a.

Combination therapy with interferon and ribavirin is the standard of therapy for naïve patients with chronic hepatitis C and has an overall sustained viral response rate of 40%–50% [13–15]. Both HCV genotype and pretreatment serum HCV RNA level are known predictors of sustained viral response to combination therapy [14, 15]. The present study consistently showed that 40% of the patients receiving therapy with interferon plus ribavirin had a sustained viral response and that patients infected with either genotype 2a or genotype 2b were more likely to have a sustained response than were those infected with genotype 1b (57% vs. 22% [P < .001]). In addition, there was no significant difference, in sustained HCV response, between those with HCV and SENV coinfection and those with HCV infection alone (44% vs. 35%; see table 1). Although patients with either genotype 1b or genotype 2a and SENV coinfection had a higher sustained HCV response rate than did those without it, the difference was not statistically significant. Our results contrast with those of a recent report indicating that HCV with SENV coinfection adversely affect sustained HCV response to combination therapy with interferon plus ribavirin [9]. Differences in patient selection, sample size, and/or distribution of HCV and SENV genotypes may explain this discrepancy. Thus, further studies are needed to address this important and interesting issue.

Umemura et al. [12] have recently reported that the sustained response rate of SENV to interferon therapy is significantly higher than that of HCV (69% vs. 37% [P = .035]) and that the sustained response rate of SENV-D was higher than that of SENV-H (73% vs. 33%), suggesting that SENV-D is highly susceptible to interferon. In our study, 21 (37%) of 57 patients with chronic hepatitis C with SENV coinfection lost serum SENV DNA at the end of therapy, and 20 (35%) remained negative for serum SENV DNA 6 months after therapy had been completed (table 2). Particularly noteworthy is that the

### Table 2. Response of SEN virus (SENV) variants to combination therapy with interferon plus ribavirin, in 57 patients with chronic hepatitis C with SENV coinfection.

<table>
<thead>
<tr>
<th>SENV variant(s)</th>
<th>Total no. of patients</th>
<th>SENV response, no. (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Only at end of therapy</td>
</tr>
<tr>
<td>H</td>
<td>41</td>
<td>14 (34)</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>7 (88)</td>
</tr>
<tr>
<td>H and D</td>
<td>8</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

a P = .02, vs. H variant of SENV.
b P = .004, vs. H variant of SENV.
end-of-therapy and sustained viral response of SENV-D were significantly higher than those of SENV-H (88% vs. 34% \( P = .02 \) and 88% vs. 27% \( P = .004 \), respectively), confirming that SENV-D is more sensitive to antiviral treatment than is SENV-H. Thus, the lower sustained SENV response rate in our study, compared with that in a Japanese report [12], may reflect merely the more prevalent SENV-H strain in Taiwan.

In summary, we found that coinfection with SENV is frequent in chronic hepatitis C in Taiwan and that it has a specific link to HCV genotype 2a. However, coinfection with SENV does not affect the clinicopathological features of chronic hepatitis C and the response to combination therapy. In addition, SENV-D is more susceptible to combination therapy than is SENV-H.

References