CONCISE COMMUNICATIONS

Increase in Hepatitis C Virus Load in Hemophiliacs during Treatment with Highly Active Antiretroviral Therapy

Margaret V. Ragni1,2,3 and Franklin A. Bontempo1,2,3

1Department of Medicine, University of Pittsburgh School of Medicine, 2The Hemophilia Center of Western Pennsylvania, and 3The Institute for Transfusion Medicine, Pittsburgh, Pennsylvania

The effect of highly active antiretroviral therapy (HAART) on liver function and viral load of hepatitis C virus (HCV) was studied in 21 hemophiliac men coinfected with HCV and human immunodeficiency virus (HIV). HCV RNA polymerase chain reaction was measured by branched DNA Quantiplex assay on frozen plasma samples obtained at baseline and at 24, 48, and 96 weeks after initiation of HAART. HCV RNA increased at 48 and 96 weeks after initiation of HAART therapy (198 × 10^6 Eq/mL [P = .03] and 227 × 10^7 Eq/mL [P < .0001], respectively, compared with baseline [141 × 10^6 Eq/mL]). This increase was associated with an increase in CD4 cell count and reduction in HIV viral load but no change in hepatic transaminases. With discontinuation of HAART, HCV RNA decreased as HIV RNA rebounded. Further study is required to clarify the histopathologic significance of this finding.

Hepatitis C virus (HCV) infection is the leading cause of chronic hepatitis in the United States [1]. Liver disease is the third leading cause of death among hemophilic men, of whom >90% are HCV-infected, 60% have alanine aminotransferase (ALT) elevation, and 20% have cirrhosis [2–4]. In contrast to other risk groups, 40% of HCV-infected hemophiliacs are coinfected with human immunodeficiency virus (HIV) [5], and HCV/HIV coinfection is the major comorbid condition in hemophilia, leading to persistence of HCV replication, higher HCV RNA levels, more rapid progression of liver disease, and a higher liver-related mortality rate than in those with HCV infection alone [6, 7].

The mechanism by which HIV hastens HCV-associated liver disease is unknown, although HIV-associated immune dysfunction and cytokine upregulation [8] may worsen the inadequate HCV-specific cytotoxic T-cell response [9], leading to accelerated HCV disease progression [10]. Among coinfected hemophiliacs, in whom highly active antiretroviral therapy (HAART) may increase HCV viral load [6], there is concern that HAART may accelerate the progression of liver disease.

We therefore prospectively studied HCV RNA and liver function in a cohort of coinfected hemophiliacs treated with HAART.

Methods

Study subjects. Between 1996 and 1998, 21 HCV/HIV coinfected hemophilic men cared for at the Hemophilia Center of Western Pennsylvania were prospectively enrolled in the study at the initiation of HAART. They represented all HCV/HIV coinfected hemophiliacs treated with HAART with available blood samples, or 72.4% of the 29 coinfected patients at this center. Among the 8 patients excluded from the study were 3 patients who refused HAART, 3 patients who discontinued HAART within 2 months, and 2 patients who had no blood samples. Five patients who were not receiving HAART served as control subjects. The study subjects and control subjects did not differ in the proportion with hemophilia A (19 patients [90.5%]), the median age at seroconversion (20 years), the median year of seroconversion (1982), the median follow-up (29 months [range, 7–36 months]), and the proportion with AIDS (61.9%). All patients were hepatitis B antibody positive and hepatitis B surface antigen negative. Initial antiretroviral therapy included protease inhibitor indinavir in 18 patients (85.7%) and nelfinavir in 3 patients (14.3%), combined with nucleoside analogues zidovudine, lamivudine, stavudine, and didanosine.

Blood samples were obtained at baseline and at 24, 48, and 96 weeks after initiating HAART. Baseline samples for HCV RNA polymerase chain reaction (PCR) were obtained within 2 months of initiation of HAART in all but 4 patients, in whom samples were obtained between 5 and 11 months before HAART. The results remained unchanged when these 4 patients were excluded from analysis (see next section). Laboratory tests included complete blood counts, chemistries, CD4 cell count, ALT, aspartate aminotransferase (AST), and PCR for HIV RNA and HCV RNA.

Laboratory methods. Quantiplex branched DNA HCV-RNA
2.0 (Chiron, Emeryville, CA) and Amplicor HIV RNA PCR Monitor test (Roche, Branchburg, NJ) [11] were performed in duplicate on fresh or frozen (−80°C) blood samples, within 3 h of drawing the blood samples, and within 24 months of freezing the samples.

Statistical analysis. HCV RNA results were analyzed by χ² analysis, Fisher’s exact test, and Wilcoxon rank sum tests (Mann-Whitney U test). Continuous data were evaluated by Student’s t test.

Results

With the median increase in CD4 cell count, from 152/μL to 303/μL, and the median fall in HIV RNA from 47,000 to 400 copies/mL in the 21 HCV/HIV co-infected subjects over a period of 96 weeks, a significant increase in HCV RNA occurred (table 1). Median HCV RNA increased from 141 × 10³ Eq/mL at baseline to 198 × 10³ Eq/mL at 48 weeks (P = .03) and 227 × 10³ Eq/mL at 96 weeks (P < .0001). The increase at 24 weeks, 120 × 10³ Eq/mL, was not significant (Wilcoxon rank sum). No change occurred in either ALT or AST.

The proportion of subjects with HCV RNA levels >400 × 10⁴ copies/mL at 96 weeks, 7 (41.2%) of 17, was significantly higher than the proportion with that level at baseline, 1 (4.8%) of 21 (P = .009; table 1). By comparison, the control subjects (1 of whom was an alcoholic) showed no change in HCV RNA during the study, but 4 did so after initiating HAART after the study: 220 × 10³ (baseline) versus 201 × 10⁴ (before HAART) versus 324 × 10³ Eq/mL (after HAART). Data from the alcoholic patient were excluded because his HCV viral load, 3- to 8-fold greater at all time points, skewed results.

All but 4 (80.9%) of the 21 patients experienced an increase in HCV RNA during HAART therapy. The median baseline HIV RNA in these 4 patients (11,200 copies/mL) was significantly lower than in that of the rest of the group (58,700 copies/mL, [P < .01, Wilcoxon rank sum]; table 2). Among those with baseline HIV RNA >50,000 copies/mL, the median increase in HCV RNA by week 96 (week 48 in patients who did not reach week 96) was 2.63-fold, compared with 1.77-fold among those with baseline HIV RNA <50,000 copies/mL (P = .022, Wilcoxon rank sum).

Among those with a baseline CD4 cell count <200/μL, the baseline HCV RNA (32.6 × 10⁴ Eq/mL) was significantly lower than in those with a baseline CD4 >200/μL (239.05 × 10³ Eq/mL; P = .0146, Wilcoxon rank sum). The median increase in HCV RNA by week 96 (or by week 48 in the 5 patients not yet reaching week 96) was significantly greater (5.2-fold vs. 1.35-fold, respectively [P < .001, Wilcoxon rank sum]).

During the first year of treatment, liver disease progression with tender hepatomegaly, ascites, and edema, occurred in one patient (4%) treated with HAART. Combination interferon and ribavirin treatment led to an unsustained reduction in HCV viral load. Three additional patients, discontinuing HAART before 24 weeks and thus ineligible for study, experienced a fall in HCV RNA levels as the HIV RNA rebounded from cessation of HAART.

Discussion

This study demonstrates that HCV/HIV coinfected hemophiliacs treated with HAART develop a persistent and significant increase in HCV viral load, which is temporally related to a persistent decrease in HIV viral load. In contrast to 5 previous studies of HAART therapy in HCV/HIV coinfected patients [12–16], which found no increase in HCV RNA, this study had longer (2 years) follow-up, compared with ≤24 weeks in the other studies. Moreover, at week 24, PCR showed no significant increase in HCV RNA, which was similar to the findings in the previous studies.

Previous studies have shown a significantly greater HCV viral load in HCV-positive/HIV-positive coinfected patients, compared with HCV-positive/HIV-negative patients [7], which has been attributed to the immunodeficiency in the former patients. Yet, because HAART improves immune function, the mechanism of the HCV RNA increase with HAART is unclear and

### Table 1. Distribution of HIV-infected hemophilic men according to HCV viral load at baseline and after initiation of HAART.

<table>
<thead>
<tr>
<th>HCV RNA</th>
<th>Baseline</th>
<th>Week 24</th>
<th>Week 48</th>
<th>Week 96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (× 10⁴ Eq/mL)</td>
<td>140.8</td>
<td>111.95</td>
<td>196.1*</td>
<td>227.2 b</td>
</tr>
<tr>
<td>Subgroups (× 10⁴ Eq/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>100-500</td>
<td>11</td>
<td>5</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>&gt;500</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Total patients</td>
<td>21</td>
<td>14</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

**NOTE.** Data are expressed as no. (%) of patients unless otherwise indicated.

- HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus.
- a for patients with increase in HCV RNA versus those with no increase in HCV RNA.
- P = .03; median HCV viral load vs. baseline HCV viral load.
- b P < .0001; median HCV viral load vs. baseline HCV viral load.

### Table 2. Relationship between baseline HIV RNA viral load and increase in HCV RNA in hemophiliacs coinfected with HCV/HIV and treated with HAART.

<table>
<thead>
<tr>
<th>Baseline HIV RNA</th>
<th>HCV RNA Response to HAARTa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (copies/mL)</td>
<td>Increase (%)</td>
</tr>
<tr>
<td>&lt;500</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>1000-9999</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>10,000-99,000</td>
<td>7 (50.0)</td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>4 (28.6)</td>
</tr>
<tr>
<td>Total patients (n)</td>
<td>14</td>
</tr>
</tbody>
</table>

**NOTE.** Data are expressed as no. (%) of patients unless otherwise indicated. HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

a P < .01 for patients with increase in HCV RNA versus those with no increase in HCV RNA.
is possibly unrelated to HIV-related immune dysfunction. The absence of hepatic transaminase increases suggests that the HCV RNA increase accompanying HAART does not lead to histopathologic worsening or progression of HCV disease, although liver biopsy will be necessary to confirm this. Whether HAART disrupts host immune response to HCV infection remains unclear. The suboptimal host cytotoxic T-cell response to HCV results in viral persistence [9], which in the presence of upregulated cytokine production with concomitant HIV infection—including interleukin-6 and transforming growth factor–β [8]—could potentially contribute to liver damage and liver disease progression [17]. Thus, it is possible that by reducing viral load from HIV, HAART may reverse cytokine upregulation and prevent liver damage. The latter would be consistent with the lack of worsening in liver function observed in this study despite the HAART-associated increase in viral load from HCV.

In summary, although the mechanism is unknown, there appears to be a dynamic reciprocal relationship between HIV RNA and HCV RNA, such that HCV RNA levels increase with HAART-induced HIV RNA reduction, and HCV RNA levels decrease with HAART discontinuation and HIV RNA levels rebound. This dynamic relationship could result from limited host immune system regulation of HIV and HCV infections, neither of which it is able to eradicate. It would seem possible, then, that any intervention reducing HIV RNA levels may increase HCV RNA levels, by virtue of this dynamic relationship, without necessarily inducing liver damage or liver disease progression.

References