Mitochondrial Toxicity Is Associated with Virological Response in Patients with HIV and Hepatitis C Virus Coinfection Treated with Ribavirin and Highly Active Antiretroviral Therapy

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The combination of highly active antiretroviral therapy (HAART) plus ribavirin (RBV) in patients with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) coinfection has been reported to cause mitochondrial toxicity (MT). Sixty-four patients with HIV-HCV coinfection who were receiving antiviral therapy were evaluated for MT. Patients with concomitant HAART showed greater increases in lactate levels than did patients without HAART, and this difference was more pronounced in patients who received higher dosages of RBV. The incidence of pancreatic enzyme elevations and symptomatic pancreatitis was higher among patients who received HAART and high-dose RBV. Hepatic steatosis increased in patients who received HAART and high-dose RBV. Patients who showed signs of MT achieved higher rates of sustained virologic response than did patients without MT (73% vs 44%).

Treatment of chronic hepatitis C virus (HCV) infection is of major importance for patients with human immunodeficiency virus (HIV) and HCV coinfection, who experience more-rapid progression of fibrosis and a higher risk of developing portal hypertension and other complications of cirrhosis [1, 2]. Based on large randomized trials, current guidelines recommend the combination of pegylated interferon alpha (PEG-IFN) and weight-based ribavirin (RBV) as standard therapy for chronic hepatitis C in HIV-infected patients [3]. Physicians have to address the issue of interactions between and the hepatotoxicity of HAART and anti-HCV therapy before initiation of HAART and of PEG-IFN plus RBV in HIV-HCV–coinfected patients. RBV is a synthetic guanosine analogue, which has some inherent antiviral activity against HCV, but its ability to enhance viral mutagenesis during IFN therapy appears to be more important [4]. However, RBV significantly adds to the adverse event profile of HCV antiviral regimens, particularly by causing hemolytic anemia and by drug interactions with HAART. Nucleotide reverse-transcriptase inhibitors (NRTIs) can cause mitochondrial toxicity (MT), as has been shown in vitro for (in descending order of toxicity) zalcitabine (ddC), didanosine (ddI), stavudine (d4T), zidovudine (AZT), lamivudine (3TC), abacavir (ABC), and tenofovir (TDF) [5]. The mechanism of MT of nucleoside drugs is thought to be inhibition of the mitochondrion-specific DNA replicase (polymerase γ) [6, 7]. Some NRTI combinations may cause additive or synergistic long-term MT, indicated by increased lactate production, decreased cell growth, and increased intracellular lipids [8]. Clinical data indicate that the risk of MT is higher in the setting of concomitant use of RBV with NRTIs, especially with ddI, d4T, and AZT [9]. Consequences of MT may manifest symptomatically with lactic acidosis, lipodystrophy, pancreatitis, hepatic steatosis, or even hepatic decompensation [10]. Concomitant use of AZT increases the risk of anemia, which may be related to AZT-induced reduction of globin messenger RNA synthesis or AZT-induced increases in plasma levels of RBV [11]. Thus, evidence exists that certain NRTIs should be avoided during concomitant anti-HCV treatment with PEG-IFN plus RBV, but to our knowledge systematic data about the time course of MT and about the incidence of hemolytic anemia during therapy with the combination of NRTIs and PEG-IFN and RBV do not exist. The aim of our study was to report the incidence of MT and of hemolytic anemia during concomitant HAART among HIV-HCV–coinfected patients undergoing antiviral therapy with PEG-IFN and RBV.

Patients and methods. Data for HIV-HCV–coinfected patients who were treated in a prospective multicenter trial (HIV-HCV Coinfection Study: Pegylated Interferon Alfa and Ribavirin in Patients with Chronic Hepatitis C and HIV Coinfection...
Venous lactate levels were measured before (screening and baseline), during (weeks 4, 12, 24, 36, and 48), and after an-tiviral therapy (months 1, 3, and 6 of follow-up). Serum levels of α-amylase, pancreas-iso-amylase and lipase, hemoglobin, indirect bilirubin, lactate dehydrogenase, and aspartate transaminase were recorded at the same time points. Clinical symptoms of MT and pancreatitis (eg, pain, steatorrhea, and diabetes) were documented. Asymptomatic hyperlactatemia and lactic acidosis were defined as venous lactate level >2 mmol/L and >5 mmol/L, respectively [12]. Patients underwent liver biopsy at baseline and 6 months after cessation of therapy. MT in liver specimens was evaluated by recording the presence or absence of hepatic steatosis (micro- or macrosvesicular lipid accumulation).

**Results.** Baseline parameters of the 64 HIV-HCV coinfected patients who were included in the study were similar between patients who received HAART and those who did not receive HAART. During antiviral therapy with PEG-IFN plus RBV, both patients with and patients without HAART showed increases in venous lactate levels, which continuously decreased again after cessation of PEG-IFN plus RBV at month 1, month 3, and month 6 of follow-up, respectively. Mean venous lactate levels of all patients with HAART were higher, compared with those of patients without HAART during the first 24 weeks of treatment, with statistically significant differences at week 4 (mean percentage of baseline [± standard deviation], 149 ± 12 vs 120 ± 9; *P* = .05) and week 12 (mean percentage of baseline [± standard deviation], 166 ± 16 vs 132 ± 9; *P* = .04). After treatment week 24 and during follow-up, venous lactate levels were similar for patients with and patients without HAART (Figure 1A). Patients with HCV-GT 1 and 4 who were treated with higher dosages of ribavirin (1000–1200 mg/day) showed greater increases in venous lactate levels, compared with increases for patients with HCV-GT 2 and 3 who received lower dosages of ribavirin (800 mg/day), with statistically significant differences at week 4 (mean percentage of baseline [± standard deviation], 146 ± 8 vs 122 ± 13; *P* = .05), week 12 (mean percentage of baseline [± standard deviation], 162 ± 9 vs 121 ± 16; *P* = .02) and week 24 (mean percentage of baseline [± standard deviation], 155 ± 13 vs 124 ± 14; *P* = .04). This difference disappeared after treatment week 24, at which point a dosage reduction of RBV to 800 mg for patients with HCV-GT 1 and 4 was scheduled in the study protocol (Figure 1B). When looking at the subgroup of patients who received high ribavirin dosages (ie, those with HCV genotypes 1 and 4), statistically significant differences in venous lactate levels were noted during the period from treatment week 4 through treatment week 24 between patients who received HAART (*n* = 34) and patients not treated with HAART (*n* = 12); mean percentage of baseline [± standard deviation], 158 ± 13 vs 124 ± 8 for week 4 (*P* = .037), 160 ± 12 vs 134 ± 10 for week 8 (*P* = .042), 177 ± 16 vs 136 ± 9 for week 12 (*P* = .031), and 165 ± 14 vs 126 ± 13 for week 24 (*P* = .02; Figure 1C). No difference in venous lactate levels during and after antiviral treatment was noted for patients who received low RBV dosages (HCV genotypes 2 and 3) when comparing patients with HAART (*n* = 14) and those without HAART (*n* = 4); *P* values were not significant for all time points (Figure 1D). For patients without HAART, no statistically significant difference between lactate levels was noted between patients with HCV-GT 1 and 4 who were treated with high dosages of RBV and patients with HCV-GT 2 and 3 who were treated with low dosages of RBV (*P* values were not significant for all time points).

No statistically significant differences were observed in serum levels of amylase, pancreas iso-amylase, and lipase for patients with or patients without HAART. HCV-HIV–coinfected patients who were treated with high and low dosages of RBV showed similar levels of amylase, pancreas iso-amylase, and lipase during PEG-IFN plus RBV treatment. Two peaks of amylase and lipase levels were observed at treatment weeks 4 and 24 for patients who received HAART and for patients who received high dosages of RBV, reflecting pancreatitis in 6 patients.

Asymptomatic hyperlactatemia occurred in 14 patients (Table 1). Lactic acidosis was noted in 1 patient who was treated with HAART and high-dose RBV (1200 mg/day) during the first 12 weeks. Nine patients had severe weight loss (>10% of body weight), all of whom had received high-dose RBV. Twenty (31%) of 64 patients experienced a transient elevation of pancreas enzymes, and 6 (9%) of 64 developed clinical signs of pancreatitis.

Hepatic steatosis was present in 45% of HIV-HCV coinfect ed patients at baseline and in 45% after cessation of therapy. The
Figure 1. Venous lactate levels during antiviral therapy with pegylated interferon plus ribavirin (RBV). Values are expressed relative to baseline (BL) venous lactate levels in percentages. A. Patients concomitantly treated with highly active antiretroviral therapy (HAART) had greater increases in venous lactate levels from BL to treatment week 24, compared with patients without HAART. Significance levels for differences were \( P = .05 \) for week 4, \( P = .09 \) for week 8, \( P = .04 \) for week 12, and \( P = .06 \) for week 24. \( P \) values were not significant for week 36 through follow-up month 6. B. Comparison of venous lactate levels between patients with hepatitis C virus (HCV) genotype (GT) 1 or 4 infections (treated with higher RBV dosages) and patients with HCV-GT 2 or 3 infections (treated with lower RBV dosages). Significance levels for differences were \( P = .05 \) for week 4, \( P = .08 \) for week 8, \( P = .022 \) for week 12, and \( P = .04 \) for week 24. \( P \) values were not significant for week 36 through follow-up month 6. C. Changes in venous lactate levels in patients with HCV-GT 1 or 4 infection. Those patients without concomitant HAART showed significantly smaller increases in venous lactate levels than did patients concomitantly treated with HAART. Significance levels for differences were \( P = .037 \) for week 4, \( P = .042 \) for week 8, \( P = .031 \) for week 12, and \( P = .02 \) for week 24. \( P \) values were not significant for week 36 through follow-up month 6. D. No differences between patients with HAART and patients without HAART were observed among patients who received lower doses of RBV (those with HCV-GT 2 or 3 infections). Significance levels for differences were \( P = .087 \) for week 4, \( P = .33 \) for week 8, \( P = .66 \) for week 12, and \( P = .057 \) for week 24. \( P \) values were not significant for week 36 through follow-up month 6.

The highest prevalence of hepatic steatosis at baseline was found among patients with HCV-GT 3 (71%). Patients who were receiving HAART and low-dose ribavirin (most of whom had infection due to HCV GT-3) showed a trend towards a decrease in hepatic steatosis, from 67% at baseline to 40% after treatment (\( P = .09 \)). Among patients who received high-dose RBV and concomitant HAART, 36% had hepatic steatosis at baseline; this increased to 56% after cessation of antiviral therapy, without reaching statistical significance (\( P = .09 \)).

Lactate dehydrogenase elevations were recorded for 15 patients (23%), with 13 of them receiving HAART and 11 of them receiving HAART plus high-dose RBV. Elevations of lactate dehydrogenase levels were more common for patients who were treated with higher RBV dosages (26%, compared with 16% for those with low RBV dosages; \( P = .079 \)) and for patients who were receiving HAART (28%, compared with 12% for those without HAART; \( P = .10 \)) without reaching statistical significance. Of those patients who received HAART and high-dose RBV, 32% showed lactate dehydrogenase elevations during antiviral therapy. The overall incidence of hemolytic anemia was 9%. No patient without concomitant HAART presented with hemolytic anemia, whereas 15% of patients with HAART plus high-dose RBV developed hemolytic anemia (\( P = .05 \) for receipt of HAART vs no HAART).

Decreases in HCV RNA level after 4 and 12 weeks of PEG-IFN plus RBV combination therapy were greater for patients who received HAART, although differences did not attain statistical significance. Sustained virologic response (SVR) was achieved for 56% of patients who received HAART and 31%
of patients who did not receive HAART (P = .088). HIV-HCV–
coinfected patients who developed MT during treatment with
PEG-IFN plus RBV had significantly higher RVR rates than did
patients without MT-related adverse events (RVR, 51% vs 21%;
P = .015). SVR was achieved in 73% of patients with an MT
event, compared with 44% of patients without an MT event
(P = .031).

Discussion. This study provides detailed data on MT and its
consequences during PEG-IFN plus RBV therapy in combination
with currently recommended HAART regimens among patients
with HCV–HIV coinfection. The longitudinal evaluation of MT
allowed us to demonstrate that MT-associated laboratory ab-
normalities are frequent, especially when high dosages of RBV
are used. Although there was a high incidence of asymptomatic
hyperlactatemia, pancreas enzyme elevations, and hepatic stea-
tosis in paired liver biopsy samples, only a few clinical adverse
events (eg, pancreatitis and lactic acidosis) were documented.

During antiviral therapy with PEG-IFN plus RBV, venous
lactate levels were significantly higher for patients who received
concomitant HAART than they were for patients who did not
receive HAART, especially when higher dosages of RBV were
used. The overall incidence of asymptomatic hyperlactatemia
during antiviral therapy was 22%; this rate was 32% among
patients who were concomitantly treated with HAART and
high-dose RBV. Of note, 1 patient who received HAART and
1200 mg/day of RBV developed moderate lactic acidosis, which
fully resolved after RBV treatment was discontinued.

MT-related elevations of pancreas enzymes were frequent,
with an overall incidence of 31%. Pancreas enzyme elevations
were more common among patients with concomitant HAART
(32%) than they were among patients without concomitant
HAART (12%). Six patients developed pancreatitis during an-
tiviral therapy with PEG-IFN plus RBV; of these patients, 5
received HAART and 4 received high-dose RBV, again under-
lining the deleterious role of RBV in MT.

Patients treated with HAART and high-dose RBV also
showed an increase in hepatic steatosis, whereas patients treated
with HAART and low-dose RBV showed a slight decrease in
hepatic steatosis. Most of the patients who received low-dose
RBV were infected with HCV GT-3, which has already been
reported to be associated with hepatic steatosis, probably by
interaction of the HCV GT-3 core protein and lipid oxidation
[13]. The high rate of SVR among patients who received low-
dose RBV, who predominantly had HCV GT-3 infections, could
therefore account for the decrease in hepatic steatosis in this
patient group, because such patients lose a pro-steatotic path-
ogen. However, the number of patients with HCV GT-3 in-
fecction and available paired liver biopsy specimens is unfor-
nately not sufficient to provide a correct statistical subanalysis
of liver steatosis among those patients. On the other hand, the
increase in hepatic steatosis in patients treated with HAART
and high-dose RBV could be explained by MT causing hepa-
tocellular lipid accumulation. An additional role of NRTIs con-
tributing to the RBV-induced hemolytic anemia could be sug-
gested, because 13% of patients who received HAART de-
veloped hemolytic anemia, compared with no patients who did
not receive HAART.

Interestingly, HCV-HIV–coinfected patients who developed
clinical signs of MT achieved significantly higher rates of RVR
and SVR, compared with rates among patients without an event

<p>| Table 1. No. (%) of Patients with Adverse Events Associated with Mitochondrial Toxicity |
|---------------------------------|--------|--------|--------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Adverse event</th>
<th>All (n = 64)</th>
<th>No HAART (n = 16)</th>
<th>HAART (n = 48)</th>
<th>No HAART plus low-dose RBV (n = 4)</th>
<th>No HAART plus high-dose RBV (n = 12)</th>
<th>HAART plus low-dose RBV (n = 14)</th>
<th>HAART plus high-dose RBV (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic hyperlactemia</td>
<td>14 (22)</td>
<td>2 (12)</td>
<td>12 (25)</td>
<td>0 (0)</td>
<td>2 (16)</td>
<td>1 (7)</td>
<td>11 (32)</td>
</tr>
<tr>
<td>Lactate acidosis</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Weight loss (&gt;10% of BW)</td>
<td>9 (14)</td>
<td>2 (12)</td>
<td>7 (15)</td>
<td>0 (0)</td>
<td>2 (16)</td>
<td>0 (0)</td>
<td>7 (21)</td>
</tr>
<tr>
<td>Pancreas enzyme elevation</td>
<td>20 (31)</td>
<td>2 (12)</td>
<td>18 (38)</td>
<td>0 (0)</td>
<td>2 (16)</td>
<td>0 (0)</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>6 (9)</td>
<td>1 (6)</td>
<td>5 (10)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>1 (7)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Hepatic steatosis at baseline</td>
<td>24/53 (45)</td>
<td>5/13 (38)</td>
<td>18/40 (45)</td>
<td>2/4 (50)</td>
<td>3/9 (30)</td>
<td>8/12 (67)</td>
<td>10/28 (36)</td>
</tr>
<tr>
<td>Hepatic steatosis after therapy</td>
<td>13/29 (45)</td>
<td>1/6 (17)</td>
<td>12/32 (52)</td>
<td>0/3 (0)</td>
<td>1/3 (33)</td>
<td>2/5 (40)</td>
<td>10/18 (56)</td>
</tr>
<tr>
<td>LDH elevation</td>
<td>15 (23)</td>
<td>2 (12)</td>
<td>13 (27)</td>
<td>1 (25)</td>
<td>1 (8)</td>
<td>2 (14)</td>
<td>11 (32)</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>6 (9)</td>
<td>0 (0)</td>
<td>6 (13)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (7)</td>
<td>5 (15)</td>
</tr>
</tbody>
</table>

NOTE. BW, body weight; HAART, highly active anti-retroviral therapy; LDH, lactate dehydrogenase; RBV, ribavirin.

a P = .037 for comparison of the incidence of asymptomatic hyperlactatemia between patients with HAART plus low-dose RBV and patients with HAART plus high-dose RBV.

b P = .035 for comparison of the incidence of weight loss between patients with HAART plus low-dose RBV and patients with HAART plus high-dose RBV.

P = .029 for comparison of the incidence of pancreas enzyme elevations between patients with HAART and without HAART.

d P = .09 for comparison of the prevalence of hepatic steatosis before and after antiviral therapy in patients with HAART plus low-dose RBV.

e P = .09 for comparison of the prevalence of hepatic steatosis before and after antiviral therapy in patients with HAART plus high-dose RBV.

f P = .067 for comparison of the incidence of hemolytic anemia between patients with HAART and patients without HAART.
suggestive of MT (RVR, 51% vs 21%; SVR, 73% vs 44%). Because MT is thought to result from an inhibition of mitochondrial polymerase γ, it is likely that MT is a correlate of RBV exposure. The association of MT and SVR is probably not causative, but rather, reflects increased intracellular RBV levels, leading to a more sufficient inhibition of HCV-RNA replication.

We conclude, on the basis of our data, that physicians have to be aware of potential interactions between NRTIs and RBV in patients with HIV-HCV coinfection, because under certain circumstances such drug interactions can result in severe morbidity and mortality. Importantly, patients with signs of MT show faster decreases in HCV-RNA levels and achieve higher rates of SVR. This has the potential to become an interesting tool for tailoring RBV dosages. If proven prospectively to be a predictor of SVR, the increase in lactate levels could be used to adjust the RBV dosage to optimal efficacy.

Acknowledgments

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References