

Antiretroviral Effects on Host Lipoproteins Are Associated With Changes in Hepatitis C Virus (HCV) RNA Levels in Human Immunodeficiency Virus/HCV Coinfected Individuals

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We evaluated the impact of antiretroviral-induced dyslipidemia on hepatitis C virus (HCV) biogenesis in human immunodeficiency virus (HIV)/HCV coinfecting patients. This study used serum samples from antiretroviral-naïve HIV/HCV patients initiating their first regimen as part of AIDS Clinical Trials Group study protocols (A5142, A5202). Initiation of antiretrovirals increased most lipoproteins and apolipoproteins. In the multivariable model, changes in apolipoproteins were associated with changes in log₁₀ HCV RNA from baseline to week-24 of therapy. Off-target lipogenic changes need to be considered in the context of liver and other metabolic disease in HIV/HCV patients.

Keywords. apolipoprotein CIII; apolipoproteins; HCV; HCV RNA; HIV; lipoproteins.

Liver disease remains a leading cause of morbidity and mortality among people living with human immunodeficiency virus (HIV). In the HIV D:A:D study, liver disease accounted for

13% of deaths, and hepatitis C virus (HCV) coinfection was associated with a higher all-cause mortality [1]. Compared with persons with HCV mono-infection, patients with HIV/HCV have higher rates of cirrhosis, hepatocellular carcinoma, and hepatic decompensation [2,3]. It is likely that multiple factors play a role in liver pathogenesis in these complex hosts.

One possible mechanism of a pathogenic interaction between HIV and HCV is the effect of an induced atherogenic lipid profile by some antiretrovirals (ARVs) on HCV biogenesis. Hepatitis C virus circulates in the serum as very low-density lipo-virions, highly infectious viral structures composed of triglyceride (TG)-rich lipoproteins containing apolipoproteins (Apo) B and E [4]. Antiretrovirals have been reported to induce dyslipidemia; in fact, HIV protease inhibitors (PIs) have been reported to increase ApoCIII and ApoE-containing lipoparticles [5].

Increases in HCV RNA after initiation of ARVs have been reported in HIV/HCV patients, but the etiology of this observation remains unexplained [6]. In a pilot study, we observed an association between changes in host lipoprotein profiles and HCV RNA levels in HIV/HCV treatment-naïve patients initiating ARVs [7]. Our aims were to further validate these preliminary findings.

METHODS

Study Design

Antiretroviral-naïve HIV/HCV patients initiating their first ARV regimen as part of 2 AIDS Clinical Trials Group (ACTG) study protocols (A5142 or A5202) [8,9] for whom fasting serum samples were available at baseline and at weeks 24 and 48 postinitiation of therapy were considered for inclusion. Participants must have had a positive anti-HCV antibody at screening for the parent ACTG trial. All patients previously consented to use of clinical data and samples for future research purposes.

A5142 was a phase III, multicenter, open-label trial in which eligible HIV-1-infected treatment-naïve patients were randomized to either efavirenz plus 2 nucleoside reverse-transcriptase inhibitors (NRTIs); a fixed-dose combination of lopinavir/ritonavir plus 2 NRTIs; or lopinavir/ritonavir plus efavirenz [8]. A5202 was a phase III, multicenter, partially blinded trial in which eligible HIV-1-infected treatment-naïve patients were randomized to the following: efavirenz or atazanavir/ritonavir plus either abacavir/lamivudine or tenofovir/emtricitabine, both given once daily [9].

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Patients were excluded if there was evidence of active hepatitis B virus (HBV) infection (positive HBV surface antigen), treatment of HCV during the study period, use of a concomitant lipid-lowering agent (statin, fibrate, niacin, ezetimibe, omega-3-fatty acid ethyl ester) or an oral hypoglycemic (sulfonylurea, thiazolidinediones, alpha-glucosidase inhibitor, biguanide), or if there was a change in the initial regimen from either a PI- or efavirenz-based regimen (switches in nucleosides for toxicity were not excluded) during the 48-week period of observation. Of 757 patients enrolled in A5142, 42 met the study inclusion criteria and all were included. Of 1857 patients enrolled in A5202, 79 met the study inclusion criteria and the first 50 randomized into the parent study were included to fulfill the planned sample size. This research was conducted in accordance with the human experimentation guidelines of the United States Department of Health and Human Services and was approved by the Duke University Institutional Review Board.

Serological and Clinical Assessments

Stored, fasting plasma samples from the baseline pretreatment visit and from treatment weeks 24 and 48 were assayed for ApoE, ApoB, ApoC-III, and ApoA-I (Clinical and Epidemiologic Research Laboratory, Children's Hospital, Boston, MA), and HCV RNA was quantified by polymerase chain reaction (Roche TaqMan HCV Test, version 1.0, University of Alabama). Other laboratories were collected from the parent study including the following: baseline and posttreatment week-24 and week-48 fasting lipid panels (low-density lipoprotein [LDL] cholesterol, high-density lipoprotein [HDL] cholesterol, total cholesterol, TG), body mass index (BMI), HIV RNA, CD4 cell count, fasting blood glucose, aminotransferases (aspartate aminotransferase, alanine aminotransferase), total bilirubin, alkaline phosphatase, platelet count; ARVs initiated; concomitant medications; comorbidities; and demographics. Aspartate aminotransferase-to-platelet ratio index (APRI) and FIB-4, noninvasive markers of fibrosis that have been previously validated in HIV/HCV patients [10, 11], were calculated.

Statistical Methods

Based on the pilot data, a study sample size of 69 study patients (2-sided test of 5% type I error with at least 85% power) was determined to detect a change in ApoC-III of at least 0.4 times the standard deviation (SD) of the change. This sample size also had similar power to detect a correlation of at least 0.35. The study sample size of 92 patients assumed that 20% of patients with a positive anti-HCV antibody would not have HCV viremia and up to 5% loss due to sample/assay problems. Patients with a positive anti-HCV antibody and an undetectable HCV RNA at baseline were excluded from the analysis (designated anti-HCV positive). Patients with a positive anti-HCV antibody and a detectable HCV RNA at baseline were designated chronically infected with HCV.

Demographics, baseline values, and changes from baseline values were described with means and SDs for continuous data, and numbers of patients and percentages were calculated for categorical data. Fisher's exact tests were used to evaluate the associations between the variables. Paired *t* tests were used for comparisons of changes in lipoprotein fractions from baseline to week 24 and week 48. Pearson correlation coefficients (r^2) were used to assess (1) how changes in \log_{10} HCV RNA levels were associated with lipoproteins and apolipoproteins and (2) associations between lipoproteins and apolipoproteins. Backward multivariable regression models were used in the analyses to control for key baseline covariates. Interaction terms were included for baseline and change based on the biologic association of specific Apo with lipid particles or with other apolipoproteins: LDL_ApoB, HDL_ApoA1, ApoCIII_ApoE. Two-sided tests at a type I error rate of 5% were considered.

RESULTS

The study cohort ($N = 92$) was young (mean age of 42), and almost half were African American (45.7%) and predominantly male (79.3%) (Table 1). The mean pretreatment baseline CD4 was low (185 cells/mm³). Forty-two (46%) patients were enrolled from A5142 and 50 (54%) patients were enrolled from A5202. The majority (69.6%) of patients were started on a PI-based regimen (Table 1).

Fifty-nine (64%) patients had detectable HCV RNA at baseline. Anti-HCV patients were younger than patients with chronic HCV infection and had higher mean pretreatment CD4 cell counts (Table 1). Anti-HCV only patients were less likely to be African American than the chronically infected patients (33.3% vs 52.5%) and had lower median index scores for noninvasive serum markers for fibrosis (APRI and FIB-4) than the patients with chronic HCV infection.

Most lipoprotein and Apo levels increased from baseline by week 24 of ARV therapy, with no further increases observed at week 48 (Supplementary Table 1). Although not statistically significant, changes in \log_{10} HCV RNA at weeks 24 and 48 were greatest for patients with pretreatment CD4 counts ≥ 200 (0.29 IU/mL and 0.32 IU/mL, respectively) and for those started on a lopinavir-containing PI-based regimen (0.54 IU/mL and 0.32 IU/mL, respectively). Changes in the lipoproteins/Apo correlated with increases in \log_{10} HCV RNA at week 24 (Supplementary Table 2). There were strong positive correlations between lipoproteins and the biologically corresponding Apo (data not shown).

Multivariable models were developed to control for potential confounders and interactions, including baseline and change values for all lipoproteins and Apo, HIV viral load, CD4 cell count, BMI, and stage of fibrosis at baseline as assessed by the FIB-4 index. In the final 24-week HCV RNA model, an increase in ApoE and ApoC-III was associated with increases in \log_{10}

Table 1. Patient Characteristics

Characteristic	All (N = 92)	Chronic HCV Infection (N = 59)	Anti-HCV Positive (N = 33)	P Value
Age, mean (SD)	42 (8.2)	45 (5.6)	36.8 (9.3)	<.0001
Race, N (%)				.188
African American	42 (45.7)	31 (52.5)	11 (33.3)	
Caucasian	33 (35.9)	18 (30.5)	15 (45.5)	
Latino	15 (16.3)	9 (15.3)	6 (18.2)	
Other	2 (2.1)	1 (1)	1 (3)	
Male Gender, N (%)	73 (79.3)	44 (75.6)	29 (88)	.181
Baseline Markers				
Absolute CD4 cells/mm ³ , mean (SD)	185 (138)	164 (118)	224 (162)	.068
log HIV RNA copies/mL, mean(SD)	4.7 (0.6)	4.7 (0.5)	4.8 (0.8)	.284
log HCV RNA IU/mL, mean (SD)	...	6.2 (1.0)
APRI score, median (IQR)	0.60 (0.66)	0.71 (0.62)	0.40 (0.38)	.005
FIB-4 score, median (IQR)	1.20 (0.94)	1.29 (1.14)	1.05 (0.59)	.009
Body mass index, mean (SD)	19.6 (9.5)	20.2 (9.7)	18.4 (9.1)	.707
Primary antiretroviral initiated, N (%)				
Atazanavir/ritonavir	23 (25)			
Lopinavir/ritonavir	41 (44.6)			
Efavirenz	18 (19.6)			
Efavirenz + lopinavir/ritonavir	10 (10.9)			
Quantifiable HCV RNA, N (%)	59 (64)	59 (100)

Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; SD, standard deviation.

HCV RNA, whereas a decrease in TG, ApoA-I, and ApoB was associated with increases in log₁₀ HCV RNA (Table 2). Other variables associated with change in HCV RNA included a PI-containing regimen, pretreatment HIV viral load and baseline BMI, TG, and ApoB. In addition, the baseline lipoprotein-Apo interaction terms were all statistically significant: in the negative direction between LDL and ApoB, and positive for HDL and ApoA-I and ApoE and ApoC-III. For these interaction terms on week-24 changes, only the LDL_ApoB interaction was statistically significant, again in the negative direction. The associations between lipoprotein changes and HCV RNA were more pronounced at week 24; by week 48, the final model accounted for less variation in log₁₀ HCV RNA, and no independent variable remained significantly associated with the observed changes (data not shown).

DISCUSSION

The impact of HIV coinfection on the natural history of HCV infection has been well described, with increased rates of cirrhosis, end-stage liver disease-related and HCV-related liver mortality [1–3]. The use of ARV therapy in patients infected with HIV-infected has had a favorable impact on HCV natural history in this group, reducing rates of liver fibrosis and liver-related mortality. Despite these improved overall outcomes,

there remain concerning observations [2]. Increases in HCV viral load have been described in the setting of ARV initiation in HIV/HCV patients, and higher HCV viral loads are usually noted in HIV/HCV patients compared with persons with HCV mono-infection [6]. In this study, we report the association of ARV-induced lipoprotein changes with increased levels of HCV replication in HIV/HCV persons.

Metabolic perturbations associated with the use of ARVs have been extensively described, particularly PI-based regimens that have been reported to increase ApoE and ApoC-III containing lipoproteins by 2- to 3-fold [5]. Although the observed ARV-induced changes in lipoproteins in HIV/HCV patients are attenuated compared with HIV patients without HCV, we found that most lipoproteins and Apo increased after ARV initiation.

The cardiovascular implications of altered lipoprotein profiles are well known, but the impact of such changes on liver disease among in HIV/HCV patients may also be clinically important, perhaps mediated by the complex interaction between the HCV life cycle and host lipid metabolism. The correlation between ARV-associated changes in ApoE and ApoC-III and increases in HCV RNA does not in itself prove causation, but it does provide a plausible pathophysiologic link between these 2 observations, supported by its significance in the multivariable model. The hypothesis that ARV-induced lipid changes

Table 2. Final Multivariable Model for Week-24 HCV RNA Log₁₀ Change*

Predictor	Week 24	
	Standardized Coefficients	P Value
Baseline Variables		
Primary ARV (PI, NNRTI, or combination)	-0.420	.014
Baseline BMI	0.279	.018
Baseline log ₁₀ HIV RNA	0.563	<.001
Baseline TG	-0.489	.009
Baseline ApoB	1.506	<.001
Change in Variables from Baseline		
Change log ₁₀ HIV RNA	0.602	.001
Change TG	-0.80	<.001
Change ApoA1	-0.497	.001
Change ApoB	-0.548	<.001
Change ApoCIII	0.895	<.001
Change ApoE	0.737	.001
Interaction Terms		
Baseline LDL_ApoB	-5.064	<.001
Baseline HDL_ApoA1	4.300	<.001
Baseline ApoCIII_ApoE	2.878	.008
Change LDL_ApoB	-2.221	.035
Model Summary: adjusted R square 0.666		

Abbreviations: Apo, apolipoprotein; BMI, body mass index; HCV, hepatitis C virus; HDL, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; NNRTI, nonnucleoside Reverse transcriptase inhibitor; PI, protease inhibitor; TC, total cholesterol; TG, triglyceride.

*Independent variables removed by backward selection: baseline CD4, change CD4, change BMI, baseline LDL, change LDL, baseline HDL, change HDL, baseline TC, change TC, baseline FIB-4, baseline ApoA1, baseline ApoB, baseline ApoCIII.

in HIV/HCV patients leads to increased HCV replication has potential therapeutic implications for the selection of ART in this group of patients.

Although this is the first report of an association between ARV-induced lipid changes and increased HCV replication, it is not the first report of an association between ApoC-III or ApoE and HCV RNA or liver fibrosis. In a cohort of patients with chronic HCV infection, Rowell et al [12] reported ApoC-III was the most significant baseline factor associated with advanced hepatic fibrosis. Sun et al [13] reported an association among VLDL-ApoC-III, lipoprotein lipase, and plasma HCV viral load. Furthermore, ApoE is enriched in infectious HCV particles, and neutralizing anti-ApoE antibodies have been reported to block HCV infectivity in vitro [14].

There are limitations to this study. The use of clinical trial patients may lead to selection bias and a nonrepresentative patient sample. For example, the proportion of HCV coinfection in the 2 parent studies was only 7.5% and 10%, lower than that observed in most HIV-infected populations. The study was powered on the primary objective to assess associations of change in

Apo and log HCV RNA, thus it was underpowered to show an overall change in log HCV RNA and any differences in log HCV RNA change by subpopulation. Lastly, this is an observational study and thus cannot prove causation. There remains a need for appropriately designed prospective studies to validate these observations.

CONCLUSIONS

In summary, this hypothesis-generating study reports an association between ARV-associated lipoprotein changes, in particular ApoE and ApoCIII, and increases in HCV viral load in HIV/HCV patients. Furthermore, change in lipoproteins after ARV initiation seems to account for two-thirds of the variation in HCV RNA noted in this study. Although the mechanism of this relationship is unproven, the effect of ARV therapy on ApoC-III and ApoE appears to influence HCV RNA levels at the site of the hepatocyte, potentially affecting release of virus into the circulation. These observations suggest that further research is needed to determine whether the use of lipogenic ART contributes to liver disease progression and/or response to HCV treatment. With an increasing range of options for ARV therapy now available, off-target lipogenic changes need to be considered in the context of liver and other metabolic diseases in HIV/HCV patients.

Supplementary Material

Supplementary material is available online at *Open Forum Infectious Diseases* (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

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