Brief Report

Influence of Antiretroviral Therapy on Oxidative Stress and Cardiovascular Risk: A Prospective Cross-Sectional Study in HIV-Infected Patients

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ABSTRACT

Background: Oxidative stress (OS) results from excessive free radical production, exceeding endogenous antioxidant defense mechanisms, which can damage a wide variety of cellular components. One of the main consequences is the attack of free radicals on polyunsaturated fatty acids contained in low-density lipoprotein (LDL) lipids, causing lipid peroxidation and subsequent elevated concentrations of lipid peroxides and their metabolites, which are strongly suggestive of oxidative damage. OS is increased among HIV-infected patients, but whether it implicates a higher risk for cardiovascular disease or the influence of antiretroviral therapy (ART) on OS remains unknown.

Objective: The aim of this study was to assess the relationship of OS with established cardiovascular risk factors and with ART as measured by total peroxide concentration.

Methods: A prospective cross-sectional study was conducted in 245 consecutive HIV-infected patients during a 2-month period (September 15, 2003–November 15, 2003) at the HIV clinic of the Infectious Disease Unit, Hospital General Universitario de Elche, Universidad Miguel Hernández, Elche, Spain. Laboratory measurements included total peroxide concentrations, C-reactive protein (CRP) levels, fasting lipid levels, white blood cell type CD4+ T-lymphocyte counts, plasma HIV RNA, and routine blood tests. To measure OS, total peroxide concentration was determined quantitatively with a colorimetric assay. The association of peroxide concentrations with HIV-related variables and cardiovascular risk factors was examined using univariate and multivariate analyses.

Results: Two hundred forty-five patients were screened and enrolled in the study; no patients refused enrollment. Median (interquartile range [IQR]) age of the patients was 40.2 (35.4–46.2) years; 194 (79.2%) were male, and 238 (97.1%) white. Median (IQR) weight was 67.5 (60.4–76.0). Ninety-five (38.8%) patients were receiving a non-nucleoside reverse-transcriptase inhibitor (NNRTI)-based regimen at the time of enrollment; 52 (21.2%) were on a protease inhibitor (PI)-based regimen. Peroxide concentrations were above reference values (<400 μmol/L) in 121 (49.4%) patients. Peroxide levels correlated positively with CRP ($P < 0.001$) and LDL-cholesterol (LDL-C) ($P = 0.003$), and negatively with age ($P = 0.002$) and body mass index ($P < 0.001$). Among patients on ART, peroxide concentrations were significantly lower in those treated with NNRTI-based regimens than in those receiving PIs (median [IQR], 331.2 [196.2–495.7] vs 472.8 [302.5–586.5] μmol/L; $P = 0.003$). In multivariate analysis, when peroxide concentration was dichotomized according to reference values (<400 μmol/L), age (odds ratio [OR], 0.96; 95% CI, 0.93–0.99; $P = 0.007$) and ART including NNRTI (OR, 0.52; 95% CI, 0.28–0.95; $P = 0.03$) were associated with low peroxide concentrations, while LDL-C

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(OR, 1.01; 95% CI, 1.00–1.02; $P = 0.03$) predicted the highest values.

Conclusions: The results from this study suggest that, among this cohort of HIV-infected patients, peroxide concentration used as a marker of OS was associated with other established cardiovascular risk factors. Antiretroviral regimens based on NNRTIs were associated with low peroxide concentrations. In contrast, high peroxide levels were found in patients receiving PI-based regimens. (*Clin Ther*. 2007;29:1448–1455)

Key words: oxidative stress, cardiovascular risk, antiretroviral therapy, HIV infection, peroxides.

INTRODUCTION

There is an increasing awareness that the prevalence of cardiovascular disease (CVD) is augmented in HIV-infected patients compared with the general population.[1] Factors associated with HIV infection and antiretroviral therapy (ART) have been implicated in the premature development of atherosclerosis and coronary heart disease. ART may increase lipids and impair glucose metabolism, but classic risk factors do not fully account for the association between ART and an increased risk for CVD,[2] suggesting that additional mechanisms might be involved.

Oxidative stress (OS) results from an imbalance between formation and neutralization of pro-oxidants and nitrogen molecules, resulting in oxidative damage to proteins, lipids, and nucleic acids. One of the main consequences is reactive oxygen species that attack polyunsaturated fatty acids contained in low-density lipoprotein (LDL) lipids. This leads to lipid peroxidation and increased levels of lipid peroxides and their metabolites that constitute a hallmark of OS.[3] There is substantial evidence that OS plays a key role in the pathogenesis of atherosclerosis in the general population.[4–9] Reactive oxygen species and oxidatively modified LDL have been implicated in the initiation, progression, and final rupture of the atherosclerotic lesion. Studies have reported an enhanced OS in HIV-infected patients.[10,11] The long-term clinical consequences of such enhanced OS, including its role in the premature development of atherosclerosis, remain largely unknown. Additionally, the influence of ART on OS is controversial. Some studies have described a protective effect of ART against the oxidative damage,[10,12] but others have found that ART and therapeutic control of HIV replication induces a pro-oxidative state.[11,13,14] A search of English-language peer-reviewed literature using the MEDLINE database from 1990 to 2007, with the search terms *oxidative stress*, *cardiovascular*, and *heart disease*, did not identify any studies addressing the relationship of OS with CVD in HIV-infected patients. The association of OS with lipoproteins had neither been previously explored.

To investigate the role of the pro-oxidative state associated with HIV infection in the development of CVD and to determine the influence of ART on OS, we assessed peroxide concentrations in a clinic-based cohort of HIV patients, including ART-naive subjects, patients who had discontinued ART, and individuals receiving treatment with a broad spectrum of antiretroviral drugs. We aimed to investigate the relationship of OS to traditional cardiovascular risk factors and factors linked with HIV infection, including ART.

MATERIAL AND METHODS

Study Population

A prospective cross-sectional study was conducted at the HIV clinic of the Infectious Disease Unit, Hospital General Universitario de Elche, Universidad Miguel Hernández, Elche, Spain. All consecutive HIV-infected patients aged ≥18 years who attended the clinic during a 2-month period (September 15, 2003–November 15, 2003) were included. No other inclusion-exclusion criteria were used. The study was approved by the local hospital ethics committee. All subjects were informed about the study and gave their written consent before participation. No patient refused to participate in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki.[15]

Clinical and Laboratory Evaluations

Clinical data including cardiovascular risk factors and current ART were obtained at the visit by one of the investigators (M.M.) using a semistructured questionnaire. Data on previous ART were extracted from the patient medical records. Anthropometric measurements were performed by a research nurse according to a standardized protocol. Laboratory measurements included total peroxide concentration, C-reactive protein (CRP) levels, fasting lipid levels, white blood cell type CD4+ T-lymphocyte counts, plasma HIV RNA, and routine blood tests.

To determine total peroxide concentration and CRP levels, blood samples were collected in ethylene-

diamine tetra-acetic acid (EDTA) tubes, and plasma was isolated by centrifugation at 3000 rpm for 10 minutes on the same day and stored immediately at –70°C until analyzed. Quantitative determination of peroxides was performed using a commercially available colorimetric assay (OxyStat®, Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria; measurement range, 7–600 µmol/L; detection limit, 7 µmol/L; reference values from apparently healthy persons with no documented disease and medication provided by the manufacturer [EDTA-plasma], <400 µmol/L; intraassay CV, 3.1%; and interassay CV, 5.1%) in accordance with the instructions of the manufacturer. Reproducibility was determined using human samples in an internal protocol. CRP levels were measured using an ultrasensitive immunoturbidimetric assay (Tinaquant CRP detection method, Roche Diagnostics, Mannheim, Germany; detection limit, 0.003 mg/dL; interassay CV, 3.62%; intraassay variation, 1.09%) on an automated modular P analyzer (Roche Diagnostics).[16] A lyophilized control serum (Precinorm®, Roche Diagnostics) was used as quality control for monitoring accuracy and precision of the quantitative methods. Total and high-density lipoprotein cholesterol were measured by spectrophotometry (assays kits OSR 6216 and OSR 6187, respectively; Olympus Diagnostic Ireland, Palex Medical, Barcelona, Spain).[17] LDL-cholesterol (LDL-C) was measured directly (Roche Diagnostics). The rest of the laboratory evaluations were performed using standard techniques.

Statistical Analysis

Descriptive statistics were computed by standard methods. To assess factors associated with peroxide concentrations, demographic, clinical, and laboratory variables, and treatment status were analyzed. To investigate the association of peroxide levels with current ART, it was prospectively defined that only patients receiving the same ART regimen during the previous 3 months were candidates to be included. Of the 245 patients, 181 of them were on ART. Based on this amount, the study had a power of 83% to detect an association, or an odds ratio (OR), of 0.40 assuming an α error of 0.05 and a prevalence of elevated peroxide levels among those not on treatment of 70%. For detecting an OR of 0.50, the power was 60%. The Spearman correlation coefficient was used to determine the association between peroxide concentrations and continuous variables. To compare median peroxide concentrations according to categorical variables, we applied the Mann-Whitney test. Multivariate analyses were carried out using forward logistic regression models to obtain an adjusted measure of the effect of cardiovascular-related variables and ART treatment on the risk for high peroxide concentrations. A P value of <0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, Illinois).

RESULTS

Patient Characteristics

Demographic and clinical characteristics and cardiovascular risk factors for the 245 patients included in the study are shown in **Table I**. Median (interquartile range [IQR]) age of the patients was 40.2 (35.4–46.2) years; median (IQR) weight was 67.5 (60.4–76.0); 194 (79.2%) were male, and 238 (97.1%) white. One hundred eighty-one (73.9%) were receiving ART. Median lifetime exposure to ART was 4.0 years. Ninety-five (38.8%) patients were receiving nonnucleoside reverse-transcriptase inhibitor (NNRTI)-based regimens, and 52 (21.2%) patients were receiving protease inhibitor (PI)-based regimens.

Peroxide Concentrations

Median (IQR) total peroxide concentration among the 245 patients was 394 (243.5–566.7) µmol/L. Levels of peroxides were above reference values (<400 µmol/L) in 121 (49.4%) patients. Concentrations were not different between patients who were or were not receiving ART (median [IQR], 370.9 [217.5–550.9] µmol/L vs 454.4 [263.7–598.7] µmol/L; $P = 0.23$). Concentrations were significantly lower in patients receiving NNRTI-based regimens than in those treated with PI (median [IQR], 331.2 [196.2–495.7] vs 472.8 [302.5–586.5] µmol/L; $P = 0.003$). Patients on NNRTI-based regimens had significantly lower peroxide concentrations than patients not receiving ART ($P = 0.03$). The peroxide levels were not significantly different between patients with ART interruptions and ART-naive patients (median [IQR], 557.0 [195.0–630.0] vs 437.6 [288.0–528.1] µmol/L, respectively).

To assess the relationship between OS and cardiovascular risk factors, demographic, clinical, and laboratory variables, including lipid levels, were analyzed (**Table II**). Total peroxide concentration correlated positively with CRP ($r = 0.30$; $P < 0.001$) and with LDL-C serum levels ($r = 0.20$; $P = 0.003$), and negatively with

Table I. Demographic and clinical characteristics of 245 HIV-infected patients. All values are median (interquartile range) unless otherwise specified.

Characteristic	Value
Age, y	40.2 (35.4–46.2)
Sex, female, no. (%)	51 (20.8)
Race, no. (%)	
White	238 (97.1)
Hispanic	3 (1.2)
Black	1 (0.4)
Other	3 (1.2)
HIV transmission category, no. (%)	
IV drug user	135 (55.1)
Men who have sex with men	44 (18.0)
Heterosexual contact	43 (17.6)
Other/unknown*	23 (9.4)
CD4+ T-lymphocyte cell count, cells/μL	500.5 (325.5–696.5)
Plasma HIV viral load, copies/mL	<50 (<50–4750)
HIV viral load <50 copies/mL, no. (%)	133 (54.3)
Time of exposure to antiretroviral therapy, y	4.0 (2–6.7)
Current antiretroviral therapy, no. (%)	
None	46 (18.8)
Naive	30 (12.2)
NNRTI-based regimen	95 (38.8)
Efavirenz	64 (26.1)
Nevirapine	31 (12.7)
PI-based regimen	52 (21.2)
Lopinavir/ritonavir	41 (16.7)
Other	11 (4.5)
NRTI-based regimen	24 (9.8)
NNRTI + PI-based regimen	10 (4.1)
Missing	18 (7.3)
Cardiovascular risk factors, no. (%)	
Current smoker	157 (64.1)
Hypertension†	21 (8.6)
Type 2 diabetes mellitus	15 (6.1)
Serum total cholesterol level >200 mg/dL	56 (22.9)
Serum triglyceride level >200 mg/dL	54 (22.0)
Weight, kg	67.5 (60.4–76.0)
Body mass index, kg/m^2	23.9 (21.6–26.5)
Total peroxide concentration, μmol/L	394 (243.5–566.7)
C-reactive protein concentration, mg/dL	0.24 (0.08–0.49)

NNRTI = non–nucleoside reverse-transcriptase inhibitor; PI = protease inhibitor; NRTI = nucleoside reverse-transcriptase inhibitor.
*Four patients were hemophilic, and 1 patient was an IV drug user and homosexual.
†Defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg.

age (r = −0.21; P = 0.002) and body mass index (r = −0.24; P < 0.001). Female sex was significantly associated with higher peroxide levels (P = 0.001).

Forward logistic regression analysis was used to identify significant predictors of elevated peroxide levels. When peroxide concentration was dichotomized according to reference values (<400 μmol/L), age (OR, 0.96; 95% CI, 0.93–0.99; P = 0.007) and ART including NNRTIs (OR, 0.52; 95% CI, 0.28–0.95; P = 0.03) were associated with low peroxide concentrations, while LDL-C (OR, 1.01; 95% CI, 1.00–1.02; P = 0.03) predicted the highest values. One hundred ninety pa-

Table II. Univariate analysis of clinical variables and plasma peroxide concentrations in HIV-infected patients (N = 245).

Variable	Median Total Peroxide Concentration, μmol/L (IQR)	Spearman Correlation Coefficient	P
Age		−0.21	0.002
Serum LDL-C level		0.20	0.003
Serum HDL-C level		−0.06	0.3
Serum triglycerides		−0.003	0.9
C-reactive protein		0.30	<0.001
Body mass index		−0.24	<0.001
CD4+ T-lymphocyte cell count		−0.03	0.6
Plasma viral load			
≥200 Copies/mL	426.1 (259.3–645.4)		0.06
<200 Copies/mL	368.6 (215.3–520.2)		
ART			
None	454.4 (263.7–598.7)		0.03*
Naive (n = 30)	437.6 (288.0–528.1)		0.07*
ART interruption (n = 16)	557.0 (195.0–630.0)		0.17*
ART including protease inhibitors†	472.8 (302.5–586.5)		0.003*
ART including NNRTI†	331.2 (196.2–495.7)		–
Sex			0.001
Female	484.2 (368.4–658.5)		
Male	361.2 (205.7–543.7)		
Current smoker			0.16
Yes	410.5 (243.5–585.7)		
No	347.2 (199.0–520.0)		
Hypertension			0.7
Yes	408.0 (265.9–605.2)		
No	390.5 (217.5–566.9)		
Type 2 diabetes mellitus			0.8
Yes	370.9 (268.4–533.7)		
No	394.0 (217.5–571.2)		

IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; ART = antiretroviral therapy; NNRTI = non–nucleoside reverse-transcriptase inhibitors.

*Versus patients receiving NNRTI-based regimens.

† Ten patients receiving dual therapy with protease inhibitors and NNRTIs were excluded.

tients were included in the model. When the model was constructed with the dependent variable peroxide concentration dichotomized according to the highest quartile, age (OR, 0.95; 95% CI, 0.92–0.99; $P = 0.02$) and NNRTI-based treatment (OR, 0.44; 95% CI, 0.21–0.89; $P = 0.02$) were inversely associated with the highest values.

DISCUSSION

We have observed an association between total peroxide concentrations and established risk factors for CVD, such as LDL and CRP. Age, however, correlated inversely with peroxide concentrations. Peroxide concentrations differed according to class of ART. Antiretroviral regimens based on NNRTIs were associated with low peroxide concentrations. In contrast, high peroxide levels were found in patients receiving PI-based regimens.

LDL-C is the major atherogenic lipoprotein and the primary target of cholesterol-lowering treatment.[18] CRP is considered an additional cardiovascular risk marker in the general population, and has also been implicated in the pathogenesis of atherosclerosis and CVD.[4] The association of OS with both LDL-C and CRP may advocate for a potential contribution of OS to the increased CVD risk in HIV-infected patients. The inverse relationship of OS with age in the present study does not constitute a novel issue, although it does go against that hypothesis. In a large study[19] including a community-based cohort of 2828 non–HIV-infected subjects from the Framingham study, there was also a negative correlation between age and OS. The association of female sex and OS has been described,[19] and a study[20] of 298 healthy adults determined sex to be the strongest predictor of lipid peroxidation ($P < 0.001$).

An important objective of the present study was the evaluation of the effect of ART on OS. In previous studies, both NRTI and PI have been associated with an increased OS state,[13,14] but limited data existed on the influence of NNRTI on OS.[11] Our study included a large cohort of patients in whom the influence of different antiretroviral regimens on OS markers has been analyzed. Of particular note in this study was that 7 of the 16 patients who had interrupted ART had very high peroxide concentrations, all of them within the upper quartile. Should this be confirmed, it might indicate that virologic rebound resulting from treatment interruption might induce a pro-oxidative state that could contribute to explain the recent unexpected findings of the Stategies for Management of Antiretroviral Therapy study,[21] in which an elevated risk for major CVD events was observed in patients who discontinued ART.

We observed a different influence of ART on OS according to regimen composition, with NNRTI-based regimens being associated with lower total peroxide concentrations, and regimens containing PI associated with the highest concentrations. Such differences are congruent with the proatherogenic metabolic disturbances associated with PI, and they are in accordance with the results of a recent report from the Data Collection on Adverse Events of Anti-HIV Drugs cohort.[2] In that study, while PI exposure was associated with a significant increase in the risk for myocardial infarction, investigators did not find the same evidence for NNRTI exposure.

Study Limitations

It should be acknowledged that our findings apply only to this small selected population, and generalization to other cohorts of HIV-infected patients might be premature in the absence of additional data to confirm our results. In addition, the cross-sectional design limits the conclusions with regard to the effect of the ART on peroxide levels because potential confounders such as duration of exposure to antiretroviral drugs or previous exposure to other antiretroviral families might not be controlled. Plasma drug concentrations were not measured in the present study. Therefore, noncompliance cannot be ruled out in some cases. Had drug levels been measured, improved associations between peroxide levels and ART exposure might have been observed. The actual accuracy of the biomarkers to quantify oxidation has not been well established, and there is currently no clear marker of choice.[5] In this study, the direct measurement of total lipid peroxide concentrations generated during the oxidation process was used. Future studies with a longitudinal design and including a larger number of patients should be desirable to corroborate our results, and to correlate OS with CVD events development in HIV-infected patients.

CONCLUSIONS

The results from this study suggest that, among this cohort of HIV-infected patients, peroxide concentration used as a marker of OS was associated with other

established cardiovascular risk factors. Antiretroviral regimens based on NNRTIs were associated with low peroxide concentrations. In contrast, high peroxide levels were found in patients receiving PI-based regimens.

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