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¹⁸O proteomics reveal increased Human Apolipoprotein CIII in Hispanic HIV-1 positive women with HAART that use cocaine

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Abstract

Purpose—Drug abuse is a major risk factor in the development and progression of HIV-1. This study defines the alterations in the plasma proteome of HIV-1 infected women that use cocaine.

Experimental Design—Plasma samples from 12 HIV-seropositive Hispanic women under antiretroviral therapy were selected for this study. Six sample pairs were matched between non-drug users and cocaine users. After IgG and albumin depletion, SDS-PAGE, and in-gel digestion, peptides from non-drug users and cocaine users were labeled with ¹⁶O and ¹⁸O respectively and subjected to LC-MS/MS and quantitation using Proteome Discover and QuiXoT softwares and validated by ELISA.

Results—A total of 1,015 proteins were identified at 1% FDR. Statistical analyses revealed 13 proteins with significant changes between the two groups, cocaine and non-cocaine users ($p < 0.05$). The great majority pertained to protection defense function and the rest pertained to transport, homeostatic, regulation, and binding of ligands. Apolipoprotein CIII was increased in plasma of HIV+ Hispanic women positive for cocaine compared to HIV+ non-drug users ($p < 0.05$).

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Author contributions

LMM, HS, and AS designed the study. HS, FZ and AC prepared samples using ¹⁸O. IJ and JV sequenced and analyzed the Proteomics Data. FZ wrote the manuscript and most of the figures. AC performed some figures. EZ did statistical analyses on patient sample pairs. LMM and AS revised the manuscript. LMM and HS share the corresponding authorship.

Competing interests

The authors declare no competing interests.

Conclusions and clinical relevance—Increased human Apolipoprotein CIII warrants that these patients be carefully monitored to avoid the increased risk of cardiovascular events associated with HIV, HAART and cocaine use.

Keywords

¹⁸O Labeling; HIV; Cocaine; Quantitative Proteomics; Apolipoprotein CIII

Introduction

The National Institute of Drug Abuse (NIDA) and National Institutes of Health (NIH) state that drug abuse is a main risk factor in the transmission of HIV-1 [6]. The use of non-intravenous drugs of abuse, such as cocaine, is also associated with an increased risk of HIV transmission due to a higher incidence of risk-taking behaviors, like unprotected sex. Moreover, HIV-1 is widespread among the Latino community in the USA. In 2009, Latinos accounted for 20% (9,400) of new HIV-1 infections in the USA, although they represent only 16% of the total US population. This HIV infection rate was nearly three times as high as that of whites (26.4 vs 9.1 per 100,000 populations) (CDC Facts & Figures, 2011). Recent studies have also demonstrated that drug users in Puerto Rico became infected with HIV-1 at a rate almost four times higher than Puerto Rican IV drug users or crack smokers living in New York, and their mortality rate is three times higher than in NY [7]. Despite the use of highly active antiretroviral therapy (HAART) among HIV + patients, HIV-associated neurocognitive disorders shows a high prevalence among these patients [8]. This is further compounded by the fact that substance abuse contributes to additional HIV health complications [9,10]. *In vitro* studies show that opiates, methamphetamine, and cocaine can potentiate HIV-1 replication and enhance or synergize with viral proteins to cause glial cell activation, neurotoxicity, and breakdown of the blood–brain barrier. [11]. Recent quantitative proteomic studies examining cocaine-induced neurotoxicity, have been reported in mice [12], and monkeys [13]. To our knowledge there are only few proteomics studies in humans, and none of them address the role of plasma proteome in HIV-1 infection and cocaine use. [14–17].

The overall hypothesis of this study is that cocaine produces a synergistic effect with HIV-1 on specific viral-immune parameters and protein composition that could be reflected in the patient blood. The purpose of this study was to quantify proteins from plasma of HIV positive women that tested positive for cocaine and compare them to those of HIV infected women that tested negative for cocaine, marijuana, heroin and methadone. These proteins may assist in the search for potential plasma biomarkers for HIV progression associated with cocaine use that could be used to improve therapy in HIV positive patients. We have shown in the past that isotopic labeling with ¹⁸O/¹⁶O can elucidate expression changes in the plasma proteome [18–23,25,27,29,31]. In this study we applied this method to quantify changes in protein expression between HIV positive women that tested positive for cocaine and HIV positive women that tested negative for cocaine. Over 33 million people around the world are living with HIV/AIDS [24] and the incidence and severity of AIDS is exacerbated by the use of psychostimulant drugs such as methamphetamine and cocaine [26,28,30]. However, the mechanisms in which drug abuse enhance HIV-1 neuropathogenesis remained

unknown. In this study we demonstrated using $^{18}\text{O}/^{16}\text{O}$ labeling, bioinformatics, and protein validation by ELISA that Apolipoprotein CIII can be a potential biomarker in HIV-1 seropositive patients with HAART that use cocaine.

Methods

Subject selection and plasma collection

Plasma samples were obtained from a repository of samples of the Hispanic Women cohort followed at the Specialized NeuroAIDS Research Program of the University of Puerto Rico Medical Sciences Campus from 2001-2009 [32,33]. The use of these samples for the current study was approved by the Institutional Human Subject Review Board (IRB# A9680112). The inclusion criteria of the original studies (IRB #0720109, 0720102) were as follows: HIV-1 infected women using HAART, ages 26-48 years, and nondrug users defined by a urine toxicology screen. Non-drug users were negative for cocaine in all the visits of this longitudinal cohort. Samples that were positive for cocaine by urine toxicology were stored and selected for this study as specified in the original subjects consents.

The study involves analyses of 12 retrospective plasma samples collected in tubes containing acid dextrose anticoagulant and stored in 0.5ml aliquots at -80°C in presence of 0.5 mM protease inhibitors (Sigma, St Louis, Missouri, USA). The plasma samples were matched, for each HIV+ cocaine+ and HIV+ cocaine -. The matching process was controlled for demographics and clinical condition in each pair of samples.

In-gel digestion, peptide ^{18}O labeling and SCX fractionation

The two most highly-abundant proteins present in human plasma samples, IgG and Albumin were removed using a ProteoPrep® Immunoaffinity Albumin and IgG Depletion Kit (Sigma), according to the manufacturer's instructions. Sample processing is described in **Figure 1**. The remaining proteins in the flow-through fractions (depleted samples) were then analyzed by a conventional SDS-PAGE. Protein concentrations were measured by BCA method (Bio-Rad, USA). A total of 100 μg of proteins from depleted samples were suspended in loading buffer (5% (w/v) SDS, 10% (v/v) glycerol, 25 mM Tris-Cl, pH 6.8, 10 mM dithiotreitol, and 0.01% (w/v) bromophenol blue), and subjected to SDS-PAGE gel (1.5mm-thick, 4% stacking, 10% resolving). The run was stopped when proteins were concentrated in the stacking/resolving interface, as described by [18], with partial modifications. The gel was visualized by Coomassie Brilliant Blue R250 staining. Total proteome bands were cut into cubes (2×2 mm). Samples were reduced with 10 mM dithiotreitol for 1 h at room temperature and thiol groups were blocked by incubation with 50 mM iodoacetamide for 1 h at room temperature in the dark. Then, samples were subjected to a standard in-gel digestion overnight at 37°C with sequencing grade trypsin (Promega, Madison, WI USA) at 5:1 protein:trypsin (w/w) ratio in 50 mM ammonium bicarbonate, pH 8.8, 10% (v/v) ACN, and 0.01% (w/v) 5-cyclohexyl-1-pentyl- β -D-maltoside [34]. The resulting peptides were desalted onto C18 OASIS cartridges (Waters, Milford, MA) and dried. Peptides from paired samples were differentially labeled with either H_2^{16}O or H_2^{18}O (95%, Isotec, Miamisburg, OH) as previously described [19]. Peptides from HIV+ cocaine- were labeled with ^{16}O and peptides from HIV+ cocaine + were labeled

with ^{18}O . After labeling, trypsin activity was stopped as described [18]. The paired labeled samples were mixed and added to C18 OASIS cartridges using as elution solution 50% (v/v) ACN in ammonium formate pH 3.0 (FA3), and dried. Labelling efficiencies as a function of $\log_2$ -ratios (X_s - X) in all comparisons experiments between cocaine and non-cocaine users were calculated according to previous research [35,36] and is shown in **Supplementary Figure 1** Clean peptide pools were separated into 6 fractions by MCX Oasis cartridges (Waters, Milford, MA), eluted with 250 μl 25% (v/v) ACN in 0.5 M FA3, 25% (v/v) ACN in 1 M FA3, 25% (v/v) ACN in 1.5 M FA3, 25% (v/v) ACN in 0.5 M FA3 and 1.5 M KCl, 37.5 % (v/v) ACN in 1.25 M FA3, and 50% (v/v) ACN in 1M FA3. Each fraction was desalted and dried prior to LC-MS/MS analysis.

LC-MS/MS analysis and peptide identification

Samples were analyzed by LC-MS/MS using a nano-LC system (UltiMate 3000, LC Packings, Dionex Product, ThermoScientific, USA) coupled to an LTQ-Orbitrap XL mass spectrometer (Thermo, San Jose, CA). Peptides were loaded onto a nano-precolumn (PepMapTM C18, 5 μm , 100 $\text{\AA}$, 300 μm I.D. $\times$ 1mm, 5pcs; LC Packings) and eluted on line with an analytical column (C18, 2.6 μm , 35 cm, ref. MPL-ES-2.6C18, Teknokroma, Barcelona, Spain), at 430 nl/min and using the continuous acetonitrile gradient consisting of 0-12% B in 1 min, 12-45% B in 120 min, 45-90% B in 2 min, 90% B in 7 min (solvent A: 0.1% (v/v) formic acid; solvent B: 90% (v/v) acetonitrile, 0.1% (v/v) formic acid). LTQ-Orbitrap XL was operated in a data dependent mode with a normal FT-resolution spectrum (resolution=100000) followed by the MS/MS spectra from most intense ten parent ions, using CID normalized collision energy (35 eV), 2 microscans, and 90 s dynamic exclusion time.

Protein identification was performed using Proteome Discover 1.4.0.288 package (Thermo), allowing optional (methionine oxidation, lysine and arginine modification of +4 Da) and fixed modifications (cysteine carboxamidomethylation), 2 missed cleavages, 20 ppm tolerance for precursor and 1.2 atomic mass units mass tolerance for fragment ions. The MS/MS raw files were searched against the Human database supplemented with HIV database and porcine trypsin (Uniprot release December 2012). The same collections of MS/MS spectra were also searched against inverted databases constructed from the same target database. SEQUEST results were analyzed using the Probability ratio method [37] and false discovery rates (FDR 1%) of peptide identifications were calculated from the search results against the inverted databases using a modified method [38]. The protein identification was analyzed using 1% FDR.

Peptide quantification and analysis

Peptide quantification from FullScan spectra and calculation of labeling efficiencies of all the identified peptides with a FDR lower than 5% were performed as described [35,36] using QuiXoT software platform, a program written in C#. The quantification of the proteomics data was done on the basis of a random-effects model that includes four different sources of variance: at the spectrum-fitting, scan, peptide, and protein levels [19]. The $\log_2$ -ratio of peptide concentration in samples A (nonlabeled) and B (labeled) determined by scan s coming from peptide p derived from protein q is expressed as $X_{qps} = \log_2 (A/B)$. The

statistical model used to analyze the quantitative data is described in detail previously [39] For each experiment (one pair of samples) in each protein, we computed the Z-score as follows:

$$Z - score = \frac{\bar{X}_{wqp}}{\sqrt{O_s^2}} \sim N(0, 1)$$

Protein Validation by ELISA

Sandwich ELISA was used for the quantification of expression of human Apolipoprotein CIII in plasma samples (Abcam®, Cambridge, MA, USA) 1:4,000 dilution), Human Vitamin D Binding protein ELISA (R & D Systems, Minnesota, USA 1:2,000 dilution), Human Prothrombin (Abcam® Cambridge, MA) and Human Complement C7 (Abcam® Cambridge, MA 1:5000 dilution) following the manufacturers' instructions. Briefly, 50µl of each diluted plasma sample was incubated in a plate with anti-human protein antibody for 2 h at room temperature. After incubation, and five washes, 50µl of biotinylated antibody was added for 1 hour and washed three times. 50µl of conjugate was added and incubated for 30 min. Finally, 50µl of substrate was then added and incubated 10 min in the dark. 50µl of stop solution was added and absorbance read on a ELISA microplate reader (Dynex Technologies, VA, USA) at a wavelength of 450nm.

Data mining and statistical analyses

To select the most significant proteins that were over or under expressed for validation studies, we established the following operational criteria: (i) for every protein, the average Z-scores of the experimental pairs of samples ($\bar{Z}$) was determined; the maximum number of proteins identified was 6 if the protein was expressed in each one of the paired samples; (ii) the two-sided p-value with the t-distribution with degrees of freedom (n -1), equivalent procedure was determined using a paired t-test, as follows:

$$t_i = \frac{\bar{Z}_i}{\frac{1}{\sqrt{n_i}}} = \bar{Z}_i * \sqrt{n_i} \sim t_{(n_i-1)}$$

where n_i indicates the number experiments where the protein “i” was expressed. and (iii) the proteins with $p < 0.05$ and z-scores with the same sign in all pairs were identified. Proteins that met these three criteria were recommended for validation. Since we observed differences in age within a pairs of experimental and control subjects, this statistical analysis was also performed in two strata based on the age and CD4 count differences between these subjects (high vs low differences). The magnitude of the abundance change for each protein was expressed by ratio HIV+cocaine+/HIV+cocaine- and calculated as $2^{(X_q - X)}$.

Results

Patient plasma samples

Viral/ immune parameters and therapy of HIV positive patients selected for this study are described in Table 1. We did not detect differences in plasma viral load or antiretroviral therapy between the pairs of HIV patients and HIV/cocaine patients. Changes in the CD4 count were inversely proportional to changes in age. No differences were observed in CD4 counts and viral load between cocaine users and non-drug users

Proteomics analyses of plasma samples

Following sample processing for depletion of IgG and albumin, an acrylamide gel 10% was run with depleted and non-depleted plasma samples and stained with coomassie blue (**Figure 2**). Results show a significant decrease of albumin and IgG in depleted samples compared with non-depleted samples (**Figure 2**). However after depletion, these two highly abundant plasma proteins still remained in the samples.

Quantitative analysis of plasma protein from HIV-infected cocaine users compared to non-drug users

Differential protein expression analysis was performed by comparing 6 sample pairs of HIV positive cocaine users with HIV positive non-drug users. The mass spectrometry data were analyzed by QuiXot, a specialized software for quantitative proteomics. Using this method, a total of 1,015 proteins were quantified based on degrees of freedom and z-scores of all the 6 experimental sample pairs (**Supplementary Table 1**). Statistical analyses at 1% FDR revealed 119 quantified proteins (**Supplementary Table 2**). From these 13 proteins had significant changes between cocaine and non-cocaine users ($p < 0.05$). These proteins were quantified at 5% FDR and selected based on identification criteria of two or more different peptides present in two or more individual experiments (**Table 2**). The distribution of protein function in plasma samples from HIV seropositive women quantified by Gene Ontology is shown in figure 3A. The great majority (62%) pertained to protection defense function and the rest pertained to transport, homeostatic, regulation, and binding of ligands. The downregulated proteins included the same proportion of transport (40%) and protection/defense (40%) proteins with a small percentage of regulatory proteins (20%) (**Figure 3B**). Interestingly, the protein functions found as increased with cocaine use were: protection / defense (75%), homeostasis (13%), and binding of ligands (13%) (**Figure 3C**). The upregulated proteins after cocaine use included: Apolipoprotein CIII (ApoCIII), Hemoglobin subunit alpha and beta Human Ig delta chain C region, Human complement C7, while proteins downregulated included: Prothrombin, Histidine rich-glycoprotein, Human Ig heavy chain V-III region, and Human CD5 antigen-like. ApoCIII was identified with two or more peptides in three of the sample pairs (S2-S8, S3-S9, and S4-S7) by mass spectrometry in three of the six experimental replicates. Since this protein has been associated with hypercholesterolemia in patients receiving HAART [40], it was considered for further validation by ELISA.

Validation of proteomics results by ELISA

Changes in protein expression for ApoCIII, Prothrombin, Complement C7, and Vitamin D Binding Protein were compared by ELISA. Results confirmed a significant increase in ApoCIII concentration in plasma of HIV-infected women that use cocaine compared with plasma from non-drug users (**Figure 4A**). Although not significant, a trend towards decreased Human Prothrombin ($p=0.07$) (**Figure 4B**) was found in plasma of 6 HIV-infected women that use cocaine compared with plasma from 6 non-drug users. No significant differences were found in Complement C7 (**Figure 4C**) and Vitamin D Binding Protein (**Figure 4D**) by ELISA in the samples tested. Since ELISA is dependent of recognition of specific epitopes by an antibody, it is possible that mass spectrometry data identified different epitopes than those detected by the available antibody.

Discussion

The current study used proteomics to determine possible protein markers in HIV- infected drug users with antiviral therapy. We found 7 proteins differentially expressed between the HIV positive cocaine users and non-drug users. Most of these were abundant proteins in plasma. Apolipoprotein CIII was found significantly increased in HIV-1 patients that use cocaine. This is a promising candidate for further studies of disease progression. However given the small number of samples tested, Human Vitamin D binding protein, Human Prothrombin and Human Complement C7 also deserve further studies.

ApoCIII is a small protein of 79 amino acids and a surface component of the very low density and high density lipoproteins [41] as well as the most abundant C-apolipoprotein in humans [42]. One of its principal functions, is the inhibition of lipoprotein lipase activity by displacement of the enzyme from lipid droplets [43]. Immunological studies show that this protein is associated with hyperactivation of voltage-gated Ca^{2+} (CaV) channels in the pancreatic β cell, participating in diabetes-related pathological events.[44]. Recent studies show that ApoCIII is associated with hypercholesterolemia in HIV infected children [40], women [45,46] and men [47] receiving combined antiretroviral therapy and/or HAART [48]. Moreover, levels of ApoCIII are linked to an increased incidence of ischemic stroke in patients with HIV-1 [49] and has been proposed as a possible biomarker of hemorrhagic stroke [50]. Our results suggest that the increased expression of ApoCIII observed in HIV positive patients undergoing HAART and cocaine use may be related to an increased risk of stroke as reported in the literature [49–51]. However, one limitation of this study is that APOCIII was found elevated in three of the six sample pairs and we could not determine if these differences between the pairs were influenced by the concentration or frequency of cocaine use.

Among the other potential candidates found by proteomics in our study is Complement component C7. This protein is a single chain glycoprotein of 821 amino acids with a molecular weight of about 97kDa. This protein is an important component of innate immune defense system against invading microorganisms. However uncontrolled activation of complement system has been shown to be detrimental to HIV-1 disease [52]. Prothrombin, also known as Factor II, is a protein with a molecular weight of 72 kDa. Expression of this protein was significantly decreased in five of the six experiments and has also been

associated with HIV infection [53]. A correlation of CD4 count with how long it takes the blood to clot (prothrombin time) exists among the HIV patients in Nigeria [54]. Furthermore, the activation of coagulation biomarkers are independent predictors of opportunistic diseases in HIV patients [55] and is also a possible potential marker for the identification of disease status in Brugada Syndrome; a genetic disease that is characterized by abnormal electrocardiogram findings and an increased risk of sudden cardiac death. [56]. Histidine rich-glycoprotein (HRG) is a 75-kDa single polypeptide chain protein [57] isolated and characterized from human serum in 1972 [58]. This protein was significantly decreased in HIV cocaine users. This is an abundant plasma protein also known as histidine-proline-rich glycoprotein. Although its function has not been well characterized, various coagulation and fibrinolytic abnormalities associated with decreased levels of histidine-rich glycoprotein has been reported in HIV infected patients[59]. Also, it has been established that HRG plays an important role in immunity such as in the modulation of the immune complexes [60], facilitating the removal of dying or dead cells [61,62], regulation of angiogenesis [63–66], coagulation and fibrinolysis [67–70] and some studies have established a role of HRG in cancer progression [71,72].

In our study, we could observe several types of Immunoglobulins that were significantly altered in HIV seropositive women after cocaine use despite depletion. We recognize that working with clinical samples has some limitations such as the variability in Immunoglobulin concentration, especially in HIV-infected patients with frequent hemolysis. However, the presence of immunoglobulin G also suggest their important role in cocaine abuse. The over expression of this protein cannot be ignored and may have to be considered for future studies.

Finally, one protein that decreased with cocaine use even though it was not significant ($p=0.10$) in our data is Vitamin D binding protein (VDBP). This protein is a plasma globulin of 56 kDa synthesized by parenchymal cells originally named “group specific component” (Gc) [73]. Characterization studies have revealed that it may serve as the main serum transporter for vitamin D and its metabolites [74]. VDBP also has other physiological properties not restricted to vitamin D transportation, such as a high-affinity to form complexes with globular (G)-actin monomers [75]. Moreover, reduced levels of VDBP was found in cerebrospinal fluid of HIV-infected patients with cognitive impairment [76]. However, although there was a tendency toward decreased expression in HIV patients that use cocaine, we did not detect significant differences in VDBP ELISA.

In this study changes in proteins levels in the plasma proteome of these cocaine users reveal potential candidate biomarkers for HIV-infected patients that are drug users while in HAART. Our results suggest that ApoCIII could be a candidate surrogate marker for cardiovascular complications in HIV infected patients that use cocaine and are taking antiviral therapy. Additional studies need to be performed in order to validate the specific role of these proteins in HIV-1 disease progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of abbreviations

AIDS	Acquired Immunodeficiency Syndrome
APOC3	Apolipoprotein CIII gene
ApoCIII	Apolipoprotein CIII protein
HAND	HIV-associated neurocognitive Disorders
HAART	Highly Active Antiretroviral Therapy

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Clinical Relevance

Drug abuse is a principal risk factor for the development of HIV-1, especially among the Latino community that comprises approximately 16% of the total USA population. Drugs of abuse such as crack/cocaine, increase the incidence of risk-taking behaviors, like unprotected sex, that in turn contributes to HIV-1 transmission [1,2]. Studies of HIV patients have reported that cocaine, more than other drugs, increases the incidence and the progression to AIDS[3,4]. Persistent crack users are over three times as likely as nonusers to die from AIDS-related causes and show greater CD4 cell loss and higher HIV-1 RNA levels [5]. The objective of this study was to determine if there are differences in the plasma proteome between HIV-1 seropositive patients that test positive or negative for cocaine using ^{18}O quantitative proteomics. Identifying changes in protein expression activated by HIV and/or cocaine can provide novel diagnostic and/or therapeutic targets that may assist to diminish the detrimental health sequelae of HIV infection in drug abusers.

Figure 1. Schematic representation of proteomics processing of study samples

Total protein concentrations of plasma samples from Hispanic women cohort were determined using the DC (detergent compatible) protein assay (BioRad, Hercules, CA) followed by immunodepletion to eliminate abundant proteins using the Proteoprep® Immunoaffinity Albumin and IgG Depletion Kit. Immunodepleted samples were run in a SDS PAGE, protein reduced, alkylated, and trypsin-digested, peptide desalted, and $^{16}\text{O}/^{18}\text{O}$ labeled. Samples were analyzed by mass spectrometry based in differential mass to charge ratios). The oxygen isotope labeling allows us to quantify the abundance of proteins identified by mass spectrometry and determined differences in population of proteins identified by mass spectrometry.

Figure 2. Depletion gel

Plasma from 6 HIV infected and 6 HIV infected- cocaine users were depleted for the most abundant plasma proteins using the ProteoPrep[®] Immunoaffinity Albumin & IgG Depletion Kit (SIGMA). Plasma proteins (depleted and non-depleted) were separated by SDS-PAGE in a 10% polyacrylamide gel at 40V for 2 hours. A significantly decrease of abundant proteins was observed in depleted plasma samples compared with non-depleted plasma samples.

A

B

C

Figure 3. Biological function of differentially changed proteins detected in plasma samples

Biological function of the 13 proteins associated with HIV and cocaine (A). Most of them were clustered in regulation and protection/defense pathways. Downregulated proteins showed similar proportions of transport and defense proteins (B) while the majority of upregulated proteins belonged to the defense proteins (C). Protein data was analyzed using Gene Ontology (GO).

Figure 4. Protein validation

Plasma samples from 12 Hispanic women cohort were collected; 6 HIV-infected cocaine users and 6 HIV-infected non-drug users. ELISA was performed for Human Apolipoprotein C III (A) Human Vitamin D binding Protein (B) Human Prothrombin (C) and Human Complement C7 protein (D). A significant increase in Apolipoprotein CIII was observed in plasma samples of HIV-infected women cocaine users compared with HIV-infected non-cocaine users ($***p < 0.001$). Changes in Human Vitamin D binding Protein ($p = 0.1050$), human Prothrombin ($p = 0.07$) and human complement C7 ($p = 0.13$) were not significant.

Table 1

Demographics, treatment, viral, and immune parameters of Hispanic women

PT ¹	Age	CD4 ²	CD4 NADIR ³	PLASMA VL ⁴	CSF VL ⁵	Cocaine Use ⁶	Cigarette Smoke	Combined antiretroviral Therapy
1	39	455	111	1517	50	Yes	ND*	AZT-3TC, Nelfinavir, MTV, Folic Acid
2	35	564	418	50	50	Yes	Yes	Nelfinavir (Viracept)
3*	33	275	275	1530	50	Yes	ND	Trizivir (AZT-3TC-ABC)
4	43	21	236	50	50	Yes	Yes	d4T (Zerit, Stavudine), ddI (Videx), Kaletra (Lopinavir-Ritonavir), Proventil
5	43	163	42	933	50	Yes	ND	AZT-3TC (Combivir), Nelfinavir (Viracept), Folic Acid, MTV
6	41	342	N/A	1372	50	Yes ⁷	Yes	Ritonavir (Norvir), Lexiva, Tuvada, MTV, Folic Acid, Zantac
7	41	111	78	111.00	482	No	Yes	AZT-3TC (Combivir), Septra, Lexiva, Fuzeon (Enfuvirtide)
8	42	235	235.00	1,580	50	No	Yes	AZT-3TC (Combivir), Efavirenz (Sustiva)
9	35	456	239.00	1,245	50	No	Yes	d4T(Zerit, Stavudine, 3TC (Epivir, Lamivudine), AZT-3TC (Combivir),
10	48	732	350	50	50	No	Yes	Abacavir(Ziagen,ABC), Ritonavir (Norvir), Viread, Reyataz, MTV, Vit C
11	37	1,258	477	50	50	No	Yes	3TC (Epivir, Lamivudine), Efavirenz (Sustiva), Viread
12	26	589	461	290	50	No	Yes	AZT-3TC (Combivir), Viread

4) Plasma viral load or number of HIV RNA copies per mL of blood

¹ Patient ID number² CD4 lymphocyte count/mL of blood³ CD4 nadir is the lowest CD4 count in patient clinical history⁵ Cerebrospinal viral load or number of HIV RNA copies per mL of CSF. 5) Cocaine use determined by toxicology⁶ Combined antiretroviral therapy against HIV-1⁷ Marijuana user.

Table 2

Quantified significant proteins by QuiXot using ^{18}O labeling in seropositive HIV women cocaine users

Uniprot ¹	Protein name ²	Patients = P		P. S1 vs S10		P. S2 vs S8		P. S3 vs S9		P. S4 vs S7		P. S5 vs S11		P. S6 vs S12		fold change ³	average Zq ⁵	count ⁷	pValue ⁸
		Xq-X ³	Zq ⁴	Xq-X	Zq	Xq-X	Zq	Xq-X	Zq	Xq-X	Zq	Xq-X	Zq	Xq-X	Zq				
P69905	HBA_HUMAN Hemoglobin subunit alpha	3.22	1.31	0.47	0.42	-4.72	-3.79	-2.79	-2.72							1.94	-1.20	4	0.024168
P02656	APOC3_HUMAN Apolipoprotein C-III			-2.45	-2.18	-0.02	-0.02	-0.88	-0.85							2.17	-1.02	3	0.055045
P01880	IGHD_HUMAN Ig delta chain C region	-8.11	-3.33	0.84	0.76	2.54	2.03	-2.07	-2.03	1.23	1.98	-3.75	-5.47			2.93	-1.01	6	0.013975
P68871	HBB_HUMAN Hemoglobin subunit beta	2.96	1.22	0.29	0.26	-3.94	-3.16	-2.59	-2.57	-0.06	-0.10	-0.68	-0.95			1.59	-0.88	6	0.020671
P10643	CO7_HUMAN Complement component C7			-0.76	-0.68	-0.49	-0.39	-0.79	-0.74	-0.54	-1.01	-0.53	-0.63			1.54	-0.69	5	0.049411
P00734	THRB_HUMAN Prothrombin	2.63	1.08	-0.95	-0.86	0.84	0.68	0.68	0.67	0.81	1.70	0.28	0.43			1.64	0.62	6	0.047822
P02768	ALBU_HUMAN Serum albumin	2.89	1.18	-0.84	-0.76	1.31	1.06	1.27	1.26	0.24	0.49	1.11	1.55			1.99	0.80	6	0.027098
P01781	HV320_HUMAN Ig heavy chain V-III region GAL	1.80	0.73	1.50	1.33	1.51	1.20			0.16	0.30					2.36	0.89	4	0.04309
P04196	HRG_HUMAN Histidine-rich glycoprotein	0.12	0.05	-0.66	-0.60	2.28	1.83	0.63	0.62	0.93	1.82	1.09	1.63			1.66	0.89	6	0.020123
P06312	KV401_HUMAN Ig kappa chain V-IV region (Fr.)			1.05	0.92	2.46	1.96	0.67	0.63			0.89	1.18			2.40	1.17	4	0.025172
P01857	IGHG1_HUMAN Ig gamma-1 chain C region			1.58	1.42	3.25	2.61	1.19	1.18	0.33	0.62					3.01	1.45	4	0.0155
P01859	IGHG2_HUMAN Ig gamma-2 chain C region			1.81	1.59	3.50	2.78									6.32	2.18	2	0.049886
P01860	IGHG3_HUMAN Ig gamma-3 chain C region			2.83	2.51	3.22	2.53									8.15	2.52	2	0.043554

Filter conditions

-proteins quantified with two or more peptides

-proteins quantified in two or more experiments

Color scale of the expression change magnitud

Xq-X:	-6	-3	-1	0	1	3	6
Zq:	-3	-2	-1	0	1	2	3

- 1) Uniprot accession number
- 2) Proteins quantified with two or more unique peptides and in two or more experiments
- 3) Log2-ratios corrected by subtracting the grans mean value obtained in each experiment.
- 4) Standardized normal values obtained by dividing protein log2-ratios by their variance.
- 5) $2^{(Xq-X)}$ The fold change and the FDRq (false discovery rate at the protein level) were calculated by analyzing as a whole all the results from the six experiments. The magnitudes of expression change and of the standardized variable are shown according to the color scales on the top.
- 6) Average of the standardized normal values. Red = down regulated and Green = up regulated.
- 7) Number of experimental pairs considered.
- 8) p-value based on the Student-t test distribution with the following characteristics: (i) degrees of freedom, equal number of z-scores where the protein was expressed (ii) test statistics was computed with square root(n) average z-scores, where n the number of protein expressed. The n value could change for every protein, depending on the number of proteins expressed in ech pair of subjects.