Early Markers of Renal Dysfunction Among Cocaine Users With HIV and HCV Infection

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ABSTRACT

Background: Cocaine use is associated with increased kidney and cardiovascular disease in the general population, but its effects on these comorbidities in people with HIV and HCV infection are not well characterized.

Methods: Case-control study to examine effects of cocaine use on plasma metabolite profiles in relation to comorbidities in 104 subjects in 3 cohorts; cocaine users (mainly crack cocaine) matched to controls for demographics, HIV serostatus, ART use, CD4 counts, plasma HIV viral load (VL) (n=38 HIV-negative from ALIVE and Bioreclamation, n=36 HIV+ IVDU from ALIVE [77% on ART], n=30 HIV+ from NNTC and CHARTER [100% on ART]; 52% cocaine users, 60% HCV+ (ALIVE subjects were HCV aviremic), 76% with VL <400 cps/ml. Metabolomics was performed by LC/MS/MS and GC/MS. Integrative analysis of metabolite, laboratory, and clinical data used Metaboanalyst and R. Multivariate logistic regression was done in SAS.

Results: Participants were predominantly male, black, with high prevalence of HCV (60%, 75%, and 60%, respectively) and renal dysfunction (40% had estimated GFR (eGFR) <90). Of >300 metabolites detected, 15 distinguished cocaine users from controls in HIV- and HIV+ cohorts, mapping to oxidative stress, altered tryptophan catabolism, phenylalanine/tyrosine/dopamine, and linoleic acid metabolism (p<0.05, FDR<0.10). Metabolites altered in cocaine users included uremic solutes indicative of early renal dysfunction, which correlated inversely with eGFR (c-glycosyl-tryptophan [c-glyTrp], pseudouridine, N6-carbamoyl-threonine, kynurenine, N-acetylated amino acids; p<0.05). Heme, a pro-oxidant with inflammatory and nephrotoxic activity, and kynurenine:tryptophan ratio (marker of tryptophan catabolism associated with immune activation) were also elevated. AUROC identified c-glyTrp and pseudouridine as uremic solutes with good classification power for eGFR <90 vs >90 (AUROC c-glyTrp single marker 83%; c-glyTrp combined with serum creatinine 95% vs 92% for creatinine alone; n=95). Cocaine use was associated with eGFR <90 in logistic models adjusted for older age, race, HIV, and HCV (OR 3.4, CI 1.3-8.7; p=0.01) and HCV status modified this association (OR 6.1, CI 1.6-23.6; p<0.01).

Conclusions: Cocaine use is associated with early markers of renal dysfunction in HIV and HCV infection. Tryptophan catabolism, oxidative stress, and pro-oxidant and nephrotoxic effects of circulating heme may contribute to mechanisms involved in cocaine-associated comorbidities that are augmented by these viral infections.

METHODS

Study participants: Subjects in the HIV-negative and HIV-positive test cohorts were from the AIDS Linked to the Intravenous Experience (ALIVE) Study, a longitudinal cohort of current or former injection drug users. Subjects in the HIV-positive validation cohort were from the National NeuroAIDS Tissue Consortium (NNTC) (Manhattan HIV Brain Bank, National Neurological AIDS Bank, California NeuroAIDS Tissue Network, Texas NeuroAIDS Research Center), and CHARTER study. All ALIVE, NNTC, and CHARTER subjects were enrolled with written informed consent and IRB approval at each study site. Additional HIV- and HCV-negative samples were obtained from Bioreclamation (Westbury, New York) with informed consent and IRB approval from Dana-Farber Cancer Institute. Inclusion criteria for the HIV-positive test cohort were between ages 30-60, HCV aviremic (no detectable HCV RNA), and HIV plasma viral load <10,000 copies/ml. Inclusion criteria for the HIV-positive validation cohort were between ages 30-60, HIV plasma viral load <5000 copies/ml, and >1 year on ART. The HIV-negative cohort was between ages 30-60. Matching for age, gender, race, and HCV serostatus was performed to achieve similar distributions of these covariates in groups defined by cocaine use. HIV-positive cohorts were also matched for stage of disease (current and nadir CD4 counts), plasma HIV viral load (VL), and ART use. Estimated GFR (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKI-EPI) equation. Cocaine use was assessed by self-reporting, urine toxicology and/or detection of cocaine metabolites (benzoylecgonine and norbenzoylecgonine) in plasma samples. All plasma samples were collected between 2002 and 2012.

Quantification of soluble markers in plasma: Heme oxygenase [HO-1] (Enzo Life Sciences Inc.) and soluble CD163 [sCD163] (Trillium Diagnostics) levels were measures by ELISA. Hemopexin, ferritin, and haptoglobin levels were measured using the Bio-Plex Pro™ Human Acute Phase Multiplex Assay (Bio-Plex System, Bio-Rad Laboratories).

Metabolite profiling: Metabolite profiling was performed by Metabolon (Durham, NC) using ultra high performance liquid chromatography and tandem mass spectrometry (UHLC/MS/MS2) and gas chromatography (GC)/MS. Plasma samples were extracted using the MicroLab STAR® system (Hamilton Company, Salt Lake City, Utah). LC/MS extracts were reconstituted in acidic or basic conditions and run on a Waters ACQUITY UPLC and a Thermo-Finnigan LTQ mass spectrometer, which consisted of an electrospray ionization (ESI) source and linear ion-trap (LIT) mass analyzer. GC/MS samples were run on 5% phenyl column and analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer using electron impact ionization. Data files were loaded into a relational database, and compounds were identified using Metabolon's proprietary peak integration software, which compares chromatographic and mass spectra properties to those from a library of purified standards or recurrent unknown entities (>1000 compounds).

Data processing, bioinformatics, and statistical analysis: Metabolite data was normalized by median centering. Missing values were imputed with the lower limit of detection for a given metabolite. Significantly altered metabolites were defined by a fold change (FC) >1.2, p-value <0.05, and false discovery rate (FDR) ≤0.1. Multiple hypothesis testing corrections were performed by calculating the local false discovery rates using fdrtool in R. Advanced analyses including unsupervised hierarchical clustering and partial least squares discriminant analysis (PLS-DA), metabolite set enrichment analysis (MSEA), and ROC analysis was performed in the Metaboanalyst web portal (http://www.metaboanalyst.ca). Correlation analysis was performed on log-transformed data in R. Metabolic pathways and biological processes were extracted from the Kyoto Encyclopedia of Genes and Genomes (KEGG), Small Molecule Pathways Database (SMPDB), and PubChem. Biofunctions were extracted from Human Metabolome Database (HMDB) (www.hmdb.ca). Visualization of pathway mapping was performed in Cytoscape. Multivariate logistic regression was performed in SAS.

RESULTS

Table 1. Clinical and demographic characterisitics of study cohorts

	HIV negative cohort (n=38)	HIV positive test cohort (n=36)	HIV positive validation cohort (n=30)
Age (years)*	49 (36-60)	48 (35-56)	45 (33-59)
Gender (male)	53%	61%	70%
Race (African American)	82%	78%	63%
BMI*	26 (20-39)	25 (21-35)	24 (22-32)
Smoking	68%	64%	NA
Alcohol use	37%	44%	NA
Cocaine use	47%	53%	40%
Crack Use (% total cocaine users)	94%	74%	NA
Injection Use (% total cocaine users)	5%	26%	NA
HCV seropositive	58%	61%	60%
HCV RNA positive	0%	0%	NA
% on ART	NA	77%	100%
PI (% total on ART)	NA	78%	80%
Current CD4 (cells/ul)*	NA	347 (61-992)	191 (16-709)
Nadir CD4 (cells/ul)*	NA	208 (6-856)	53 (2 - 536)
Plasma HIV viral load (copies/ml)*	NA	144 (50-9900)	88 (25-2349)
Serum Creatinine	0.97 (0.7-1.4)	0.91 (0.48-1.4)	0.85 (0.6-1.4)
eGFR	91 (63-126)	98 (67-155)	103 (65-130)
% with eGFR ≤90	45%	34%	32%

Abbreviations: BMI, body mass index; ART, anti-retroviral therapy; eGFR, estimated glomerular filtration rate.

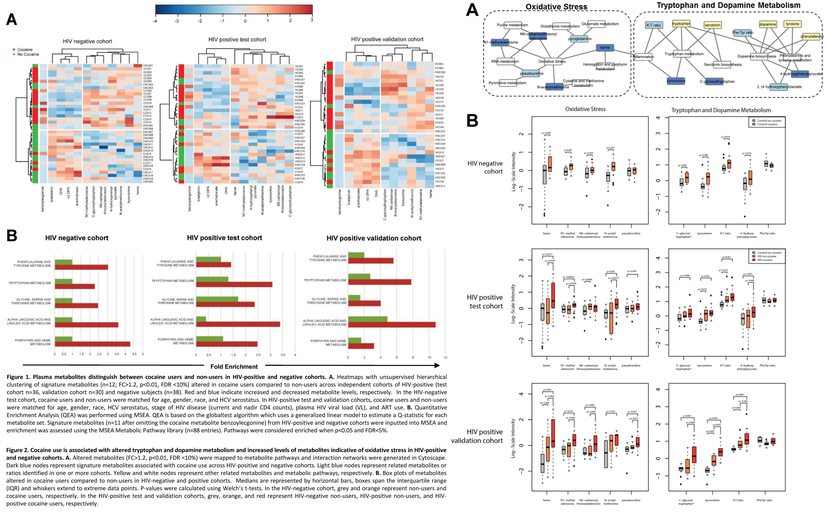
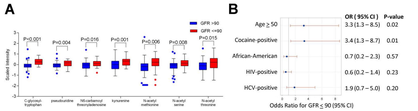
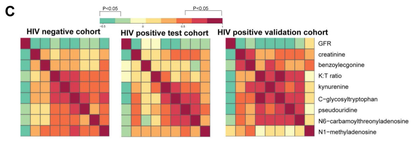
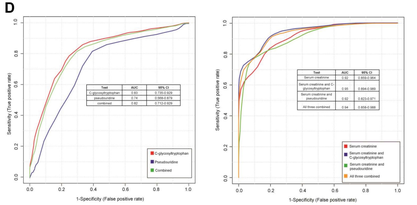


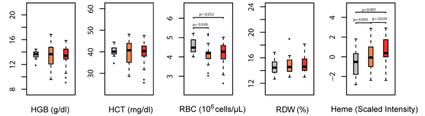
Figure 1. Plasma metabolites distinguish between cocaine users and non-users in HIV-positive and negative cohorts. A. Heatmaps with unsupervised hierarchical clustering of signature metabolites (n=12; FC>1.2, p<0.01, FDR <10%) altered in cocaine users compared to non-users across independent cohorts of HIV-positive (test cohort n=36, validation cohort n=30) and negative subjects (n=38). Red and blue indicate increased and decreased metabolite levels, respectively. In the HIV-negative test cohort, cocaine users and non-users were matched for age, gender, race, and HCV serostatus. In HIV-positive test and validation cohorts, cocaine users and non-users were matched for age, gender, race, HCV serostatus, stage of HIV disease (current and nadir CD4 counts), plasma HIV viral load (VL), and ART use. **B.** Quantitative Enrichment Analysis (QEA) was performed using MSEA. QEA is based on the globaltest algorithm which uses a generalized linear model to estimate a Q-statistic for each metabolite set. Signature metabolites (n=15 after omitting the cocaine metabolite benzoylecgonine) from HIV-positive and negative cohorts were inputted into MSEA and enrichment was assessed using the MSEA Metabolic Pathway library (n=88 entries). Pathways were considered enriched when p<0.05 and FDR<5%.

Figure 2. Cocaine use is associated with altered tryptophan and dopamine metabolism and increased levels of metabolites indicative of oxidative stress in HIV-positive and negative cohorts. A. Altered metabolites (FC>1.2, p<0.01, FDR <10%) were mapped to metabolite pathways and interaction networks were generated in Cytoscape. Dark blue nodes represent signature metabolites associated with cocaine use across HIV-positive and negative cohorts. Light blue nodes represent related metabolites or ratios identified in one or more cohorts. Yellow and white nodes represent other related metabolites and metabolic pathways, respectively. **B.** Box plots of metabolites altered in cocaine users compared to non-users in HIV-negative and positive cohorts. Medians are represented by horizontal bars, boxes span the interquartile range (IQR) and whiskers extend to extreme data points. P-values were calculated using Welch's t-tests. In the HIV-negative test and validation cohorts, grey, orange, and red represent HIV-negative non-users, HIV-positive non-users, and HIV-positive cocaine users, respectively.









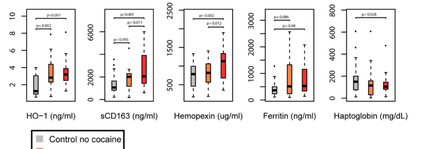


Figure 4. Cocaine use in HIV-positive subjects is associated with alterations in heme, a pro-oxidant with inflammatory and nephrotoxic activity, along with heme/hemoglobin scavenging pathways. Box plots of red blood cell cell counts and indices, hemoglobin (HGB) or hematocrit (HCT) levels, red blood cell count (RBC), red cell distribution width (RDW), free heme, heme oxygenase [HO-1] and proteins involved in heme/hemoglobin scavenging (soluble CD163, hemopexin, ferritin, and haptoglobin) in HIV-positive subjects with and without cocaine use (n=95). Medians are represented by horizontal bars, boxes span the interquartile range (IQR) and whiskers extend to extreme data points. P-values were calculated using Welch's t-tests. Grey, orange, and red represents HIV-negative non-users, HIV-positive non-users, and HIV-positive cocaine users, respectively.

Figure 3. Plasma metabolites altered in cocaine users are associated with kidney dysfunction. A. Box plots of metabolites associated with kidney dysfunction (eGFR<90, n=40; eGFR>=90, n=55) across all subjects (HIV-positive and HIV-negative) with available serum creatinine values. Medians are represented by horizontal bars and boxes span the interquartile range (IQR). P-values were calculated using Welch's t-tests. **B.** Adjusted odds ratios and Wald's 95% confidence intervals for eGFR <=90 vs. >90 (n=95 subjects; OR 3.4, 95% CI 1.3-8.7; p=.01 by multivariate logistic regression). HCV serostatus modified this association in similar logistic regression models testing interaction terms (OR 6.1, CI 1.6-23.6, p<0.01). **C.** Interrelationships between metabolite levels and kidney function in HIV-positive and negative cohorts. The correlation matrix was constructed in R using the heatmap.2 function. Pearson correlations were used to evaluate relationships between plasma metabolites and markers of kidney function (p<0.05, FDR<0.1). Significant correlations had a -0.35> r >0.35, p<0.05, and FDR<10%. False Discovery Rate was used to correct for multiple hypothesis testing. Red and green indicate positive and negative correlations, respectively (see Color Key). **D.** Receiver Operating Characteristic (ROC) curves of top-ranked metabolite predictors of kidney dysfunction in HIV-positive and negative subjects (GFR<90 vs. GFR<=90, n=95). ROC analysis was performed in Metaboanalyst with C-glycosyltryptophan and pseudouridine alone, together and in combination with serum creatinine. ROC curves and area under the curve (AUC) with 95% confidence intervals for each test identify c-glycosyltryptophan and pseudouridine as uremic solutes with good classification power for eGFR <=90 vs. > 90.

SUMMARY AND CONCLUSIONS

- Cocaine use was associated with altered tryptophan/kynurenine and tyrosine/dopamine metabolism and metabolites indicative of oxidative stress in HIV-positive and HIV-negative cohorts.
- Metabolites altered in cocaine users included uremic solutes indicative of early renal dysfunction. Among these uremic solutes, c-glycosyltryptophan and pseudouridine had the best classification power for eGFR <90 vs. >90 in ROC analyses.
- Heme, a pro-oxidant with inflammatory and nephrotoxic activity, and kynurenine:tryptophan ratio (marker of tryptophan catabolism associated with immune activation) were also elevated among cocaine users.
- Cocaine use is associated with increased odds of low eGFR and early markers of renal dysfunction in HIV and HCV infection. Tryptophan catabolism, oxidative stress, and pro-oxidant and nephrotoxic effects of circulating heme may contribute to mechanisms involved in cocaine-associated renal dysfunction that are augmented by these viral infections.