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Authors

Carrico, Adam W

Cherenack, Emily M

Roach, Margaret E

et al.

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Substance-Associated Elevations in Monocyte Activation Among Methamphetamine Users with Treated HIV Infection

Adam W. CARRICO, Ph.D.[1], **Emily M. CHERENACK, BA**[2], **Margaret E. ROACH, Ph.D.**[1], **Elise D. RILEY, Ph.D.**[3], **Olorunleke ONI, MD**[1], **Samantha E. DILWORTH, M.S.**[3], **Steven SHOPTAW, Ph.D.**[4], **Peter HUNT, M.D.**[3], **Sabita ROY, Ph.D.**[1], **Suresh PALLIKKUTH, Ph.D.**[1], and **Savita PAHWA, M.D.**[1]

[1]University of Miami School of Medicine

[2]Duke University Department of Psychology and Neuroscience

[3]University of California, San Francisco School of Medicine

[4]University of California, Los Angeles School of Medicine

Abstract

Objective—Microbial translocation and monocyte activation predict mortality in treated HIV. We examined whether substance use independently contributes to these pathophysiologic processes.

Design—Cross-sectional study at baseline for a randomized controlled trial.

Methods—HIV-positive, methamphetamine-using men who have sex with men (MSM) with undetectable HIV viral load (< 40 copies/mL) were enrolled. We examined if plasma biomarkers of monocyte activation and intestinal barrier integrity were associated with the following: 1) reactive urine toxicology results (Tox+) for stimulants (i.e., methamphetamine or cocaine); and 2) substance use severity measured by the Addiction Severity Index. Multiple linear regression models adjusted for age, antiretroviral therapy regimen, CD4+ T-cell count, interleukin-6, and alcohol use severity.

Results—The sample of 84 virally suppressed MSM had a median CD4+ T-cell count of 645 cells/mm^3. Those who were Tox+ for stimulants displayed higher soluble CD14 (sCD14) levels (2,087 versus 1,801 ng/ml; p = .009), and this difference remained significant after adjusting for covariates (standardized Beta = 0.23; p = 0.026). Greater substance use severity was also independently associated with higher sCD14 after adjusting for covariates (standardized Beta = 0.29, p = 0.013). Being Tox+ for stimulants and substance use severity were not associated with soluble CD163 (sCD163) or intestinal fatty acid binding protein (iFABP) levels (p's > 0.05).

Conclusions—Monocyte activation is one plausible mechanism by which stimulant use may increase clinical HIV progression.

Corresponding Author: Adam W. Carrico, Ph.D., University of Miami Department of Public Health Sciences, 1120 NW 14th St., Office 1005, Miami, FL 33136, Tel: 305-243-6947, a.carrico@miami.edu.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Keywords

Alcohol; Cocaine; HIV; Immune Activation; Methamphetamine; Microbial Translocation

Introduction

Among HIV-positive persons receiving effective anti-retroviral therapy (ART), microbial translocation, immune activation, and inflammation are interacting pathophysiologic processes that drive morbidity and mortality [1–3]. Microbial translocation is partially attributable to intestinal damage by HIV early in infection that potentiates greater immune activation and inflammation despite effective ART [4,5]. The role of microbial translocation in HIV is evidenced by elevated lipopolysaccharide (LPS) levels in HIV-positive persons receiving ART [4,6,7]. Greater soluble CD14 (sCD14), a marker that partially reflects LPS-induced monocyte activation, is also elevated in treated HIV infection and independently predicts hastened disease progression [4,6,8,9].

The use of stimulants (i.e., methamphetamine, powder cocaine, and crack-cocaine) could independently promote HIV disease progression by dysregulating the autonomic nervous system [10]. In fact, more frequent stimulant use among HIV-positive men who have sex with men (MSM) receiving ART predicts faster progression to AIDS or mortality, even after adjusting for adherence and viral load [11]. Although stimulant users experience difficulties with HIV disease management and display a substantially elevated HIV viral load [12,13], many are achieving viral suppression with contemporary ART regimens. Research is needed to examine the biological pathways that could explain faster HIV disease progression among stimulant users receiving effective ART.

In treated HIV infection, stimulant-associated alterations in microbial translocation and residual immune dysregulation could contribute to elevated risk of disease progression despite effective ART. Even after adjusting for ART adherence, more frequent stimulant use is associated with lower tryptophan and higher neopterin, both of which reflect greater innate immune activation [14]. Another cross-sectional study with virally suppressed, HIV-positive MSM observed that methamphetamine users displayed greater CD4+ and CD8+ T-cell spontaneous proliferation, activation, and exhaustion compared to those who did not use methamphetamine [15]. Further research is clearly needed to examine the associations of susbstance use with immune activation in treated HIV infection.

This cross-sectional study examined if there are substance-associated elevations in markers of monocyte activation and intestinal damage in virally suppressed, methamphetamine-using MSM. In order to provide multiple indices of substance use, we included two measures: 1) Recent stimulant use - urine toxicology screening for methamphetamine and cocaine, which measures any use in the prior 72 hours; and 2) Substance use severity - the Addiction Severity Index (ASI) is a self-report measure of the extent to which individuals engage in more problematic, chronic patterns of substance use. We hypothesized that biologically confirmed, recent stimulant use and greater self-reported severity of all substances used would be associated with elevations in two markers of monocyte activation that predict HIV disease progression: higher sCD14 and soluble CD163 (sCD163) levels [9,16,17]. We also

proposed that substance-associated elevations in these markers of monocyte activation would be mediated by greater intestinal damage as measured by higher intestinal fatty acid binding protein (iFABP) levels [18].

Methods

HIV-positive, methamphetamine-using MSM were recruited from substance abuse treatment programs, HIV medical clinics, AIDS service organizations, the community, and referrals from active participants for a randomized controlled trial [19]. At an in-person screening visit, participants completed a signed informed consent that included consent for specimen banking. All enrolled participants met the following inclusion criteria: 1) 18 years of age or older; 2) be a man who has sex with men; 3) have documentation of HIV-positive serostatus (i.e., letter of diagnosis or ART medications other than Truvada matched to photo identification); and 4) provide a urine or hair sample that was confirmed to be reactive for methamphetamine.

Enrolled participants completed a separate baseline assessment approximately one week later that included a detailed battery of psychosocial measures, a second urine sample for on-site toxicology testing, and peripheral venous blood sample to measure HIV disease markers. Participants also provided an additional two 10 mL EDTA tubes for specimen banking. Only those with undetectable HIV viral load (< 40 copies/mL) were included in the present study. This study was approved by the Institutional Review Boards for the University of California, San Francisco as well as the University of Miami and Northwestern University.

Measures

Demographics and ART measures—Participants completed a demographic questionnaire assessing age and race/ethnicity. Participants also reported their current ART regimen, time since starting ART, and ART adherence during the past 30 days using the visual analogue scale [20].

Severity of alcohol and substance use—The ASI was administered to assess the severity of alcohol and other substance use [21]. This measure includes the self-reported number of days using multiple illicit substances during the past 30 days, perceived impairment related to substance use, and perceived need for substance abuse treatment.

On-site urine screening—Urine samples were collected for on-site toxicology screening using the iCup at both the screening and baseline visits (Redwood Biotech, Inc.; Santa Rosa, CA). Results were used to identify participants who tested reactive (Tox+) for recent use of stimulants (i.e., methamphetamine or cocaine) at either the screening or baseline visits (1) compared to those who were non-reactive (Tox−) at both visits (0). The iCup is capable of detecting stimulant use within the past 72 hours.

HIV disease markers—HIV viral load testing was performed to detect plasma HIV RNA using the Abbott Real Time HIV-1 assay (Abbott Molecular, Inc.; Des Plaines, IL). This

assay has a lower limit of detection of 40 copies/mL. CD4+ T-cell count was measured with whole blood using flow cytometry, and assays were performed by Quest Diagnostics.

Monocyte activation and intestinal barrier integrity—Plasma levels of sCD14, sCD163, and iFABP were determined by the use of Human Quantikine Immunoassay (R&D Systems, Minneapolis, MN) following the manufacturer's instructions. For sCD14 measurement, samples were diluted 400-fold and results were expressed in ng/ml. For sCD163 measurement, samples were diluted 30-fold and results were expressed in ng/ml. For iFABP measurement, samples were diluted 15-fold and results were expressed in ng/ml.

Inflammation—Because monocyte activation may also be induced by peripheral inflammation [22], plasma levels of interleukin-6 (IL-6) were determined by the use of Human Quantikine Immunoassay using undiluted samples (R&D Systems, Minneapolis, MN) following the manufacturer's instructions.

Statistical Analyses

We conducted independent samples t-tests to examine mean differences in SCD14, sCD163, and iFABP as a function whether participants were Tox+ for stimulants in urine. Multiple linear regression analyses examined the independent associations of being Tox+ for stimulants in urine and self-reported substance use severity with these outcomes after adjusting for age, ART regimen characteristics, CD4+ T-cell count, and IL-6. We also chose to control for self-reported alcohol use severity, based on prior research demonstrating alcohol-associated elevations in microbial translocation and sCD14 [23–25].

Results

Participant age ranged from 24 to 59 years, with a mean of 43.3 (*SD* = 8.7). Half of participants were Caucasian (49%), 29% were Hispanic/Latino, 11% were African American, and 11% were other ethnic minorities or multicultural. The median CD4+ T-cell count was 645 (Interquartile Range = 449–829) cells/mm,^{3} and all participants had an HIV viral load less than 40 copies/mL.

Those who were Tox+ for stimulants in urine displayed significantly higher sCD14 (2,087 versus 1,800 ng/ml; t (82) = −2.68; p = .009) mean levels (see Table 1). There were no differences in sCD163 or iFABP as a function of whether participants were Tox+ for stimulants. As shown in Table 2, being Tox+ for stimulants in urine continued to be significantly associated with higher sCD14 after adjusting for covariates (standardized Beta = 0.23; p = 0.026). Similarly, greater substance use severity was independently associated with higher sCD14 after adjusting for covariates (standardized Beta = 0.29, p = 0.013). Neither time on ART (r = −0.01, p = 0.92) nor self-reported ART adherence (r = −0.14, p = 0.22) were significantly associated with sCD14. No significant associations of recent stimulant use and substance use severity with iFABP or sCD163 levels were observed (see Table 2).

Discussion

Most HIV-positive persons display residual immune dysregulation despite effective ART [4,26]. This study is among the first to demonstrate that recent, biologically confirmed stimulant use and greater self-reported substance use severity are independently associated with higher sCD14 in methamphetamine-using MSM with treated HIV. Results are consistent with a prior study in which self-reported stimulant use was associated with higher plasma neopterin after adjusting for ART adherence [14] and subsequent hypotheses indicating that morbidity and mortality are partially attributiable to ongoing substance use and not solely to HIV [27]. Taken together, results suggest that monocyte activation is one mechanism by which substance use may increase clinical HIV progression.

Results presented here stand in contrast to another recent cross-sectional study with virally suppressed persons where there were no significant methamphetamine-associated elevations in sCD14, but methamphetamine users did display greater T-cell dysfunction compared to non-users [15]. There are multiple reasons for the discrepancy in sCD14 findings. Prior research focused on comparing those who reported lifetime methamphetamine use to non-users [15]. The present study examined the dose-response associations of recent stimulant use and substance use severity among biologically confirmed methamphetamine users. Taken together, these studies suggest that effects of stimulant use on sCD14 may be more time-dependent or only observed among more frequent, active stimulant users.

We hypothesized that the associations of substance use with greater monocyte activation would be partially attributable to intestinal damage. However, there were no elevations in iFABP levels as a function of recent, biologically confirmed stimulant use or self-reported substance use severity. Further research is needed to characterize the biological pathways whereby stimulant use may increase monocyte activation in HIV. In particular, research should examine whether and how intestinal dysbiosis and higher LPS levels may account for stimulant-associated increases in monocyte activation in treated HIV [28].

Findings from this cross-sectional study should be interpreted in context of some important limitations. This study relied on the biologically confirmed presence of any stimulant use, which limits the interpretation of results. Furture research should assess methamphetamine and cocaine concentrations through quantitative toxicology measures as well as include a comparison group of non-users. Findings should also be considered preliminary due to the small sample size and cross-sectional design. In addition, polysubstance use is common in this population and additional substances were not accounted for, which makes confounding by other substances possible. Despite the fact that we controlled for ART regimen, nadir CD4+ T-cell count was also not measured in this study as an important potential confounder. The observed dose-response associations between substance use and higher sCD14 could be attributable to impaired impaired microbial clearance by the liver. Future studies should include the FIB-4 score. Finally, it is important to note that recent receptive anal intercourse could potentiate microbial translocation in MSM and this should be more carefully examined in future research [29].

Despite these limitations, findings from this study provide preliminary evidence for the biological plausibility of substance-associated increases in monocyte activation in treated HIV. This supports the scientific premise for clinical research examining the mechanisms that underlie substance-associated increases in residual immune dysregulation to advance our understanding of HIV pathogenesis.

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All authors contributed to the refinement of hypotheses for this project. Management of plasma specimens and conduct of assays was led by Drs. Margaret Roach, Suresh Pallikkuth, and Savita Pahwa. Ms. Samantha Dilworth managed and coded data for this project as well as provided feedback on statistical analyses. Statistical analyses and manuscript preparation were led by Dr. Adam Carrico, Ms. Emily Cherenack, and Dr. Olorunleke Oni with input from the entire team. Dr. Elise Riley assisted with interpretation of statistical models and urine biomarker testing for stimulants as well as provided extensive comments during a revisions of this manuscript. Dr. Peter Hunt provided expertise in plasma markers of microbial translocation and monocyte activation as well as possible HIV medication regimen confounders. Dr. Steven Shoptaw provided expertise in substance use measurement. Dr. Sabita Roy provided expertise in interpreting the plausible role of intestinal dysbiosis.

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Table 1

Associations of recent stimulant use with intestinal barrier integrity and monocyte activation (N = 84).

	Stimulants Tox+ (n = 51)	Stimulants Tox− (n = 33)	Cohen's *d*	p-value
	M (SD)	M (SD)		
sCD14 (ng/ml)	2,087.28 (543.51)	1,800.73 (351.28)	0.63	0.009
sCD163 (Log_{10})	2.82 (0.19)	2.79 (0.20)	0.15	0.440
iFABP (Log_{10})	3.51 (0.25)	3.56 (0.17)	−0.23	0.343

sCD14 = soluble CD14; sCD163 = soluble CD163; iFABP = intestinal fatty acid binding protein; Tox+ = reactive urine toxicology results for stimulants at screening or baseline; Tox− = non-reactive urine toxicology results for stimlants at screening and baseline

Table 2

Associations of recent stimulant use and substance use severity with intestinal barrier integrity and monocyte activation (N = 84).

	sCD14	Log_{10} sCD163	Log_{10} iFABP
Model 1: Tox+ in Urine for Stimulants	**B**	**β**	**β**
Age	0.14	−0.07	0.09
Prescribed a Protease Inhibitor	0.27*	−0.02	−0.08
Prescribed Efavirenz	0.11	−0.14	−0.04
CD4+ Count	0.19	−0.09	−0.11
IL-6	0.11	0.35**	0.04
ASI Alcohol Composite Score	0.15	0.01	0.07
Tox+ in Urine for Stimulants	0.23*	0.06	−0.13
Model 2: Substance Use Severity	**β**	**β**	**β**
Age	0.11	−0.06	0.08
Prescribed a Protease Inhibitor	0.25*	−0.02	−0.07
Prescribed Efavirenz	0.11	−0.15	−0.04
CD4+ Count	0.20	−0.12	−0.08
IL-6	0.12	0.36**	0.02
ASI Alcohol Composite Score	0.08	0.08	0.04
ASI Drug Composite Score	0.29*	−0.14	−0.01

sCD14 = soluble CD14; sCD163 = soluble CD163; iFABP = intestinal fatty acid binding protein; IL-6 = Interleukin-6; Tox+ = reactive urine toxicology results for stimulants at screening or baseline