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Full-length Article

Recent stimulant use and leukocyte gene expression in methamphetamine users with treated HIV infection

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ABSTRACT

Stimulant use may accelerate HIV disease progression through biological and behavioral pathways. However, scant research with treated HIV-positive persons has examined stimulant-associated alterations in pathophysiologic processes relevant to HIV pathogenesis. In a sample of 55 HIV-positive, methamphetamine-using sexual minority men with a viral load less than 200 copies/mL, we conducted RNA sequencing to examine patterns of leukocyte gene expression in participants who had a urine sample that was reactive for stimulants (n = 27) as compared to those who tested non-reactive (n = 28). Results indicated differential expression of 32 genes and perturbation of 168 pathways in recent stimulant users. We observed statistically significant differential expression of single genes previously associated with HIV latency, cell cycle regulation, and immune activation in recent stimulant users (false discovery rate $p < 0.10$). Pathway analyses indicated enrichment for genes associated with inflammation, innate immune activation, neuroendocrine hormone regulation, and neurotransmitter synthesis. Recent stimulant users displayed concurrent elevations in plasma levels of tumor necrosis factor – alpha (TNF-α) but not interleukin 6 (IL-6). Further research is needed to examine the bio-behavioral mechanisms whereby stimulant use may contribute to HIV persistence and disease progression.

1. Introduction

Stimulant use may accelerate HIV disease progression via behavioral and biological mechanisms (Carrico, 2011; Salamanca et al., 2014). HIV-positive stimulant users are more likely to have difficulties with HIV disease management that contribute to elevated HIV viral load and hastened disease progression (Carrico, 2011; Carrico et al., 2011; Ellis et al., 2003). At the same time, recent findings from a cohort of HIV-positive sexual minority men receiving anti-retroviral therapy (ART) indicate that those who engaged in more frequent stimulant use had 50% greater odds of clinical progression even after accounting for adherence and viral load (Carrico et al., 2014). Because most studies examining stimulant-associated immune dysregulation have relied on *in vitro* and animal models (Cabral, 2006), research with treated HIV-positive persons is needed to elucidate the biological pathways whereby stimulants may promote disease progression.

Sympathetic nervous system activation could partially mediate the immunomodulatory effects of stimulants in HIV, but findings from preclinical and human studies are mixed. Methamphetamine activates the sympathetic nervous system (Irwin et al., 2007; Makisumi et al., 1998) leading to elevated norepinephrine, which has been linked to greater HIV replication (Cole et al., 1998; Ironson et al., 2015). This is further supported by findings that sympathetic nervous activation and methamphetamine use may contribute to HIV persistence. For example, one prior study observed 3.9-fold greater rates of SIV replication at sites where lymph nodes juncture with catecholaminergic varicosities (Sloan et al., 2006). Among HIV-positive persons who were virally suppressed, greater sympathetic nervous system activation (measured by a shorter cardiac pre-ejection period) has also been associated with greater intracellular HIV RNA but not higher proviral HIV

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DNA (Hecht et al., 2017). Finally, recent findings with virally suppressed HIV-positive persons indicated that methamphetamine users had higher levels of proviral HIV DNA, but these results were not significant after adjusting for ART regimen (Massanella et al., 2015). Further research is needed to examine stimulant-associated alterations in biological processes relevant to HIV persistence.

Immune activation and inflammation have emerged as plausible, downstream mechanisms for the *in vivo* immunomodulatory effects of stimulants. In one laboratory-based study with HIV-negative men, cocaine infusion (versus placebo) was associated with decrements in monocyte expression of pro-inflammatory cytokine markers and decreased responsiveness of monocytes to lipopolysaccharide (Irwin et al., 2007). Cocaine-related reductions in monocyte expression of tumor necrosis factor – alpha (TNF-α) were partially attributable to greater increases in autonomic nervous system activation. Stimulant use could also contribute to immune dysregulation by upregulating indoleamine 2,3-dioxygenase (IDO). This is supported by a prior investigation that observed stimulant-associated elevations in neopterin and depleted tryptophan in HIV-positive persons after adjusting for ART adherence (Carrico et al., 2008). Studies with virally suppressed, HIV-positive methamphetamine users also indicate that recent stimulant use is associated greater monocyte activation (Carrico et al., In Press) as well as CD4+ and CD8+ T-cell proliferation, activation, and exhaustion at rest compared to those who did not use methamphetamine (Massanella et al., 2015). More research is clearly needed to elucidate the mechanisms of stimulant-associated immune dysregulation in treated HIV.

One important consideration is that stimulant use may influence multiple biological pathways and trigger opposing biological processes initiated to maintain homeostasis, obscuring the association of stimulant use with plasma immune markers. Gene expression is a transcriptomic approach that allows for exploration of multiple stimulant-associated alterations in leukocyte signaling, potentially providing greater clarity regarding the mechanisms of stimulant-associated biological alterations in treated HIV. For example, one *in vitro* study observed treatment of dendritic cells with methamphetamine led to altered gene expression for pathways governing chemokine regulation, cytokine production, signal transduction mechanisms, apoptosis, and cell cycle regulation (Mahajan et al., 2006).

The present study examined the association of recent stimulant use with leukocyte gene expression in a sample of HIV-positive, methamphetamine-using sexual minority men who were receiving effective ART. We hypothesized that participants who provided a urine sample that was reactive for stimulants (i.e., Stimulant Tox+) would display differential gene expression across multiple biological pathways relevant to HIV pathogenesis as compared to those who tested non-reactive for recent stimulant use (i.e., Stimulant Tox−). We also hypothesized that recent stimulant users would display elevated plasma levels of TNF-α and interleukin 6 (IL-6).

2. Methods

HIV-positive, methamphetamine-using sexual minority men were recruited for a randomized controlled trial from substance abuse treatment programs, HIV medical clinics, AIDS service organizations, the community, and referrals from active participants (Carrico et al., 2016). At an in-person screening visit, participants completed 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) a sexual minority man; 3) 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 at the screening visit that was reactive for methamphetamine. For the purposes of this study, participants were restricted to individuals who had an HIV viral load < 200 copies/mL.

Enrolled participants completed a separate baseline assessment approximately one week later that included a detailed battery of psychosocial measures, a urine sample for on-site toxicology testing, and peripheral venous blood sample to measure HIV disease markers. In addition, participants also provided two × 10 mL EDTA and two × 2.5 mL PAXgene® Blood RNA tubes (Qiagen, Inc.) for specimen banking. 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. A certificate of confidentiality was obtained from the National Institute on Drug Abuse.

2.1. Measures

2.1.1. Demographics and health status

Participants completed a demographic questionnaire assessing age, race, ethnicity, and health-related factors such as current ART regimen.

2.1.2. ART adherence and persistence

Participants rated their adherence to each ART medication during the past 30 days using the visual analogue scale (Walsh et al., 2002), which was averaged to calculate the percentage of medications taken. Participants also indicated if they have experienced a treatment interruption, a period of two days or more in the past six months where all HIV medications were stopped without guidance from an HIV primary care provider. Participants without a treatment interruption (1) were compared to those with a treatment interruption (0) as a measure of ART persistence.

2.1.3. Psychiatric comorbidities

The Addiction Severity Index (ASI) was administered to assess the severity of alcohol and other substance use (McLellan et al., 1992). Depressive symptom severity was assessed using the 20-item Centers for the Epidemiologic Study – Depression (CES-D) scale (Radloff, 1977). Post-Traumatic Stress Disorder (PTSD) symptoms were measured with the PTSD Checklist – Civilian (PCL-C) version (Wilkins et al., 2011). Because depression and PTSD may have immunomodulatory effects, we examined whether they were potential confounders by comparing the Stimulant Tox+ and Stimulant Tox− groups.

2.1.4. 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.

2.1.5. On-site urine screening

Urine samples were collected for on-site toxicology screening using the iCup (Redwood Biotech, Inc.; Santa Rosa, CA), which is capable of detecting stimulant use within the past 72 h. Results were used to identify participants who tested reactive for recent methamphetamine or cocaine use (i.e., Stimulant Tox+) versus those who were non-reactive for both (i.e., Stimulant Tox−). Among those who were Stimulant Tox+, all but one were reactive for methamphetamine. Given the relatively rapid temporal dynamics of leukocyte gene expression, we examined recent stimulant use as the primary predictor.

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