Methamphetamine (MA) Use in People with HIV (PWH) on Antiretroviral Therapy (ART) Is Associated With Increased Inflammation and Residual HIV Transcription

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BACKGROUND

- While antiretroviral therapy (ART) is able to suppress virus to undetectable levels, virus rapidly rebounds from latently-infected cells ("the HIV reservoir") within weeks of ART interruption.[1,2]
- A major goal of HIV cure is to reduce the HIV reservoir size to induce ART-free remission in people with HIV (PWH).[3]
- Methamphetamine (MA) use is highly prevalent among PWH and is associated with ART non-adherence and low level viremia.[4]
- Among viremic PWH, MA has been associated with elevated inflammatory markers from blood[4] and rectal mucosa,[5] and preclinical studies in mice[6] and non-human primates[7] have shown that MA can directly induce HIV/SIV transcription.
- No study to date has determined the impact of long-term MA use on PWH during continuous ART suppression.
- Here, we performed a cross-sectional study among PWH virally suppressed on ART to determine the impact of chronic MA use on residual HIV-1 transcription and plasma cytokine expression.

METHODS

- Participants were enrolled from the EMRLHD (Effect of Methamphetamine on Residual Latent HIV Disease) study.
- Inclusion criteria were laboratory confirmed HIV, >12 months of undetectable plasma HIV RNA, and no current use of other illicit substances (e.g., cocaine, fentanyl, heroin).
- PWH on ART reporting ongoing MA use were sampled at two timepoints, approximately 3 months apart, and underwent inguinal lymph node fine needle aspiration, whole blood collection, and pharmacokinetic sample collection (plasma, serum, urine).
- PWH on ART reporting no prior or current MA use (or other illicit substances) were included as controls.
- Pharmacokinetic (PK) data were collected for MA and its metabolite, amphetamine (AMP), and were quantified using liquid chromatography-mass spectrometry (LC- MS/MS).[8]
- Plasma cytokine levels were measured using a high-sensitivity multi-plex chemiluminescence immunoassay (Mesoscale Diagnostics).
- HIV reservoir transcription was measured from cryopreserved PBMCs (10^6 cells) using a reverse transcription (RT)-ddPCR assays and quantified on a Bio-Rad QX200 Droplet Digital qPCR System reflecting transcription initiation (TAR), elongation ("Long LTR"), mid-transcription ("Pol"), distal transcription (Nef), completion ("PolyA"), and multiple splicing (Tat-Rev) events.[9]
- Univariate comparisons were performed using Wilcoxon rank sum (MA vs. no MA) and signed rank (within MA group comparison) tests.
- Multivariate logistic models were fit to compare MA vs. no MA use groups, as well as to analyze differences within the MA use group (by PK concentrations) using the log transformed lm function in R (version 4.3.1). Potential clinical covariates tested for inclusion in multivariate models included age, sex, race/ethnicity, nadir CD4+ T cell count, CD4:CD8 ratio.

- **No study to date has determined the impact of long-term MA use on PWH during continuous ART suppression.**
- **Our findings suggest that PWH on ART who use MA have increased proinflammatory responses and enhanced HIV transcription.**

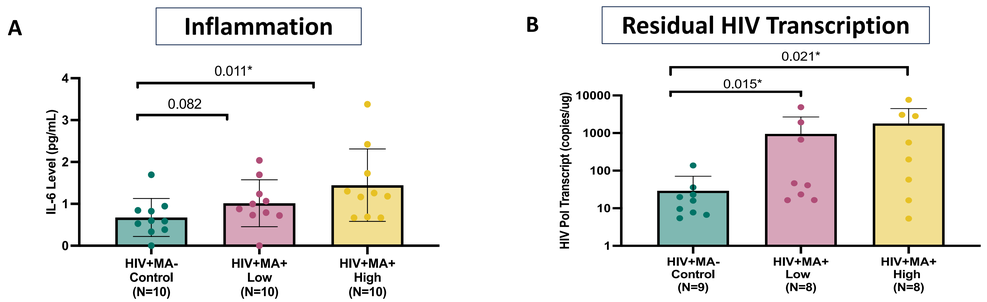
RESULTS

Table 1. Study participants characteristics

Characteristic	HIV+MA+ (N=10)	HIV+MA- (N=10)	Total (N=20)
Age (years)	54 (50-60)	44.5 (36.5-50.8)	50.5 (44.8-56.8)
Sex at birth (% male)	9 (90%)	10 (100%)	19 (95%)
Race/Ethnicity (self-reported)			
African-American (%)	0 (0%)	1 (10%)	1 (5%)
Asian (%)	1 (10%)	1 (10%)	2 (10%)
White/Hispanic/Latino (%)	1 (10%)	3 (30%)	4 (20%)
White/European American (%)	8 (80%)	5 (50%)	13 (65%)
Nadir CD4+ T cell count (cells/mm^3)	449 (113.8-583.8)	252 (199-370)	370 (131.5-520.0)
Study baseline visit CD4+ T cell count (cells/mm^3)	631.5 (321.8-837.8)	665.5 (517.3-688.8)	665.5 (496.8-773.5)
Study baseline visit CD4:CD8 ratio	0.8 (0.5-1.3)	1.2 (1.1-1.2)	1.1 (0.6-1.3)

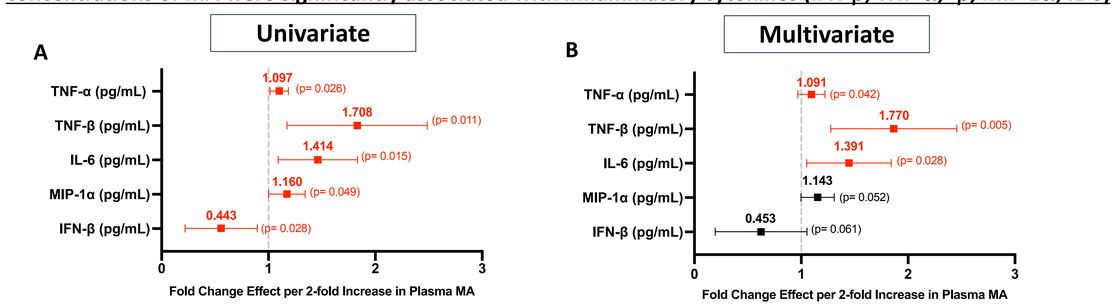
Medians with interquartile ranges or absolute numbers with % shown.

Figure 1. Univariate analyses demonstrated that long-term MA use was associated with increased inflammation (plasma IL-6) and residual HIV reservoir transcription (Pol)



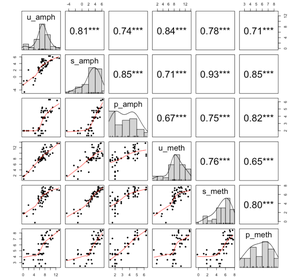
Comparisons between HIV+MA- vs. HIV+MA+ groups were performed using Wilcoxon rank sum tests while comparisons within the HIV+MA+ group were performed using Wilcoxon signed rank tests. N=1 HIV+MA- (control) and N=2 HIV+MA+ individuals were excluded from the HIV transcript analysis due to low transcript results for all 5 HIV-1 transcripts in the assay. The other HIV-1 transcripts from the ddPCR assay were analyzed but did not meet statistical significance of p<0.05. Figure visualized on $\log_{10}$ scale. Error bars show mean and standard deviation.

Figure 2. Multivariate modeling among PWH on ART with long-term MA use demonstrated that higher concentrations of MA were significantly associated with inflammatory cytokines (IFN-β, TNF-α, -β, MIP-1α, IL-6)



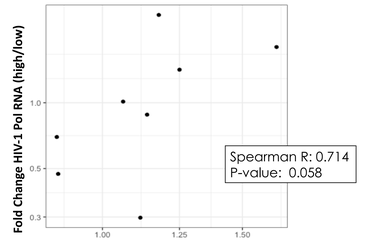
Fold-change effect estimates in plasma cytokine levels are shown per 2-fold increases in methamphetamine concentrations (composite data from multiple PK measures were used). Multivariate linear models were adjusted for nadir CD4+ T cell count and duration of ART suppression. Red indicates values that meet statistical significance of p<0.05.

Figure 3. Methamphetamine concentrations were highly correlated across sample types (plasma, serum, urine)



Spearman correlations between each type of PK sample (plasma, serum, urine) are shown with p-values shown in boxes. Multiple imputation, using all sample types and all participant data, was performed to estimate missing PK data for use in regression models.

Figure 4. Among the significantly associated plasma cytokines with MA concentrations, only TNF-α levels were associated with HIV Pol



Spearman correlations between HIV-1 Pol transcripts and the other plasma cytokines from multivariate modeling (IFN-β, TNF-β, MIP-1α, IL-6) showed similar positive trends but did not meet statistical significance at p<0.05.

CONCLUSIONS

- To our knowledge, this is the first study in humans evaluating the impact of MA use on circulating cytokine levels and residual HIV transcription during suppressive ART.
- Worldwide, MA is the second most commonly used illicit substance,[10] and in San Francisco, up to 39% of HIV+ individuals report recent MA use.[11]
- Our findings suggest that long-term MA exposure is associated increased host innate immune and proinflammatory response, as well as residual viral transcription, even during adequate ART-mediated viral suppression.
- These results highlight the importance of determining whether MA use itself may alter the host immune response and the nature of the reservoir, which may confer additional challenges in eliciting an HIV cure in this population.

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