Effects of Integrase-Inhibitor-Based ART on the NLRP3 Inflammasome Among ART-Naïve People With HIV

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Abstract
The NLRP3 inflammasome, activated in the setting of HIV, contributes to pro-atherogenic inflammation. Among ART-naïve PWH (vs. controls), levels of caspase-1 – a key component of the NLRP3 inflammasome – were significantly increased. Six months of elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate significantly decreased caspase-1 levels in association with CD4+/CD8+ ratio recovery.

ClinicalTrials.gov Registration: NCT 01766726
Introduction

Among people with HIV (PWH), heightened systemic immune activation and inflammation contribute to an increased risk for atherosclerotic cardiovascular disease (ASCVD).\(^1\)\(^2\)\(^3\) Regulation of the immune/inflammatory milieu occurs in part through intracellular multi-protein signaling complexes known as inflammasomes – particularly the NOD-like receptor protein family pyrin domain containing 3 (NLRP3) inflammasome. General-population studies suggest that NLRP3 inflammasome activation fuels atherogenesis (Figure 1).\(^4\)

Moreover, administration of a monoclonal antibody therapy targeting an NLRP3 inflammasome-modulated immune pathway was shown in the general population to prevent ASCVD events.\(^5\) Importantly, the HIV virus promotes NLRP3 inflammasome activation within monocytes.\(^6\)\(^7\) Thus, understanding how HIV infection and integrase inhibitor-(INSTI)-based anti-retroviral therapy (ART) affects the NLRP3 inflammasome may elucidate possible mechanisms for heightened ASCVD risk among PWH on ART.

Activation of the NLRP3 inflammasome results in the generation of caspase-1, a proteolytic enzyme that generates the pro-inflammatory cytokines interleukin-18 (IL-18) and interleukin-1β (1L-1β).\(^8\)\(^9\)\(^10\) Thus, caspase-1 plays a central role in the inflammatory response generated by NLRP3 inflammasome activation. Recent studies have shown that caspase-1 activation also triggers a highly inflammatory form of programmed cell death, pyroptosis, contributing to CD4+ T cell depletion in HIV infection.\(^11\) Given the influence of HIV on the NLRP3 inflammasome,\(^6\)\(^7\)\(^12\) we hypothesized that suppression of HIV viremia with INSTI-based ART among ART-naïve PWH would reduce NLRP3 inflammasome activation, reflected in reduced circulating levels of caspase-1. Moreover, given the role of caspase-1 in mediating CD4+ T cell depletion in HIV, we further hypothesized that ART-induced changes in caspase-1 levels may relate to concomitant changes reflecting immune recovery.
Methods

Study design

Twelve ART-naïve individuals who were newly diagnosed with HIV and who were being started on elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (E/C/F/TDF) by their infectious disease provider were recruited and enrolled. These participants underwent study assessments at baseline and 6 months after E/C/F/TDF. Twelve participants without HIV-matched based upon CVD risk scores were recruited via flyer and online advertisements and also underwent study assessments at baseline. Eligibility criteria were similar for participants in both groups and included no history of coronary artery disease or diabetes. We previously reported the effects of E/C/F/TDF on arterial inflammation and high density lipoprotein (HDL) cholesterol efflux capacity, but effects of E/C/F/TDF on NLRP3 inflammasome activation have not been previously published.

Patient Consent Statement

The Partners Institutional Review Board approved this study, and written informed consent was obtained from study participants.

Laboratory assessments

Plasma caspase-1 levels were assessed using an ELISA immunoassay (R&D Systems; Minneapolis, MN, mean minimum detectable limit of 0.68 pg/mL, intra-assay coefficient of variability of 4.9 to 6.1%, and the inter-assay coefficient of variability of 8.3 to 9.4%). HIV viral load was assessed using ultrasensitive reverse transcription polymerase chain reaction (Cobas Ampliprep; Roche Molecular Diagnostics, lower limit of detection 20 copies/mL) and CD4+ and CD8+ T cell counts were assessed using standard techniques.

Statistical analysis

The primary endpoint for this analysis was the change in caspase-1 levels among participants with HIV. Between-group comparisons were performed using student’s t-test, Wilcoxon rank sum, or Fisher exact test, as appropriate. The change in caspase-1 in
response to INSTI-based ART among participants with HIV was assessed using a Wilcoxon signed rank test. Bivariate analyses were performed using the Spearman correlation coefficient. All statistical analyses were performed using SAS JMP software, version 12.0 (SAS Institute).

**Results**

**Baseline characteristics**

Comparing ART-naïve PWH and participants without HIV, the median age tended to be higher among participants without HIV (median [IQR]: 29 [26, 44] vs. 48 [27, 51] years, P=0.31; Supplemental Table 1). Baseline 10-Year ASCVD Risk Score was similar between groups (median [IQR]: 2.1 [0.7, 3.6] vs. 1.8 [0.7, 5.3], P=0.91). Among PWH, the median duration of HIV infection was 0.9 [0.2, 1.7] years, baseline CD4+ T cell count was 483 ± 166 cells/mm³, and baseline log viral load was 4.3 ± 0.6 copies/ml. Baseline plasma caspase-1 was significantly higher among PWH compared to participants without HIV (104.7 [77.2, 159.3] vs. 45.2 [39.0, 60.3] pg/mL, P<0.0001; Figure 2).

**Effects of integrase inhibitor-based ART on plasma caspase-1 levels and HIV-specific parameters**

Among PWH, levels of plasma caspase-1 were significantly reduced after 6 months of E/C/F/TDF (104.7 [77.2, 159.3] to 57.9 [43.7, 88.1] pg/mL, P=0.003; Figure 2). Caspase-1 levels among PWH treated with E/C/F/TDF tended to remain higher than levels among participants without HIV, but this difference was not statistically significant (57.9 [43.7, 88.1] pg/mL vs. 45.2 [39.0, 60.3] pg/mL, P=0.19). As expected, among PWH, 6 months of E/C/F/TDF resulted in a reduction in log viral load (4.3 ± 0.6 to 1.3 ± 0.03 copies/ml, P<0.0001), an increase in CD4+ T cell count (483 ± 166 to 698 ± 197 cells/mm³, P=0.0004), and an increase in the CD4+/CD8+ ratio (0.6 ± 0.3 to 0.9 ± 0.4, P=0.0003).
Relationship between ART-induced changes in plasma caspase-1 levels and ART-induced changes in HIV-specific parameters

Among PWH, following 6 months of E/C/F/TDF, change in plasma caspase-1 level was inversely related to change in CD4+/CD8+ ratio ($\rho = -0.66$, $P=0.02$). The change in plasma caspase-1 levels did not relate to changes in log viral load, CD4+ T cell count, or CD8+ T cell count (data not shown).

Discussion

In our study, ART-naïve individuals newly diagnosed with HIV (vs. matched controls) had significantly higher systemic levels of caspase-1, reflective of increased NLRP3 inflammasome activation. Among ART-naïve PWH, INSTI-based ART with E/C/F/TDF resulted in a significant reduction in caspase-1 levels. However, post-ART levels of caspase-1 still tended to be higher than those among matched participants without HIV. We also found a significant inverse relationship between the change in caspase-1 and the change in a key marker of immune recovery in HIV – CD4+/CD8+ ratio. These novel findings demonstrate a possible difference in an important inflammatory pathway in atherogenesis—the NLRP3 inflammasome – among participants with vs without HIV.

In our study, we demonstrated that 6 months of an INSTI-based ART regimen significantly lowers plasma caspase-1 levels among ART-naïve PWH. In a prior study, Cai et al. demonstrated that newly-initiated ART reduced the percentage of circulating CD4+ T-cells expressing caspase-1. This study differs from our study in several key ways. First, our study assessed ART effects on systemic levels of plasma caspase-1. Second, 95% of the participants in the prior study were initiated on a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART regimen. Of note, INSTI-based ART regimens have been shown to more potently reduce systemic markers of immune activation, such as soluble CD14 and high sensitivity C-reactive protein, compared to NNRTI regimens. Such studies
highlight the importance of evaluating regimen-specific effects on inflammation and immune activation in HIV. Our study is the first to assess the effects of an INSTI-based ART regimen on NLRP3 inflammasome activation among PWH.

Our study is also the first to show that among newly-diagnosed PWH, initiation of an INSTI-based regimen reduces plasma caspase-1 levels in association with bolstering a measure of immune recovery—the CD4+/CD8+ratio. In a study by Serrano-Villar et al., INSTI-based ART regimens (as compared to protease inhibitor- and NNRTI-based regimens) resulted in greater percentage of PWH achieving CD4+/CD8+ normalization. The effect of INSTI-based ART regimens to improve CD4+/CD8+ ratios may be physiologically related to the role of caspase-1 in CD4+ T cell depletion in the setting of HIV infection. Caspase-1 has been shown to trigger a highly inflammatory form of programmed cell death, pyroptosis, contributing to CD4+ T cell depletion among PWH. The destruction of a CD4+ T cell through pyroptosis, in turn, results in the release of pro-inflammatory cytokines that trigger pyroptosis of other nearby CD4+ T cells, resulting in self-propagating cycle of CD4+ T cell depletion. Thus, caspase-1-mediated pyroptosis contributes to a hallmark of HIV infection—concomitant immunodeficiency and immune activation. Future studies aimed at investigating how INSTI-based regimens affect caspase-1-mediated pyroptosis may inform our understanding of immune recovery among PWH treated with ART.

Although this study had a small sample size, it focused on an ideal population—ART-naïve PWH—to investigate the effects of ART on an important inflammatory pathway in atherogenesis. The results of this study may not be broadly generalizable to other regimens, and additional studies comparing regimen-specific effects of newly-initiated ART on NLRP3 inflammasome activation are needed. Moreover, as this study included only male participants, the findings will need to be replicated among women with HIV. Lastly, additional
studies among PWH are needed to determine how cell-specific inflammasome activation contributes to increased circulating levels of inflammasome proteins and how both processes contribute to atherogenesis – both before and after ART initiation.

In conclusion, we demonstrate for the first time that among newly diagnosed PWH, initiation of an INSTI-based ART regimen significantly reduced NLRP3 inflammasome activation in association with a marker of immune recovery. Given the key role of the NLRP3 inflammasome in atherogenesis, NLRP3 inflammasome activation may serve as a future target to mitigate CVD risk among PWH. One potential immunomodulatory CVD-preventive strategy being explored involves IL-1β antagonism. Statin therapy may represent a second potential immunomodulatory CVD-preventive strategy. Select statins, such as atorvastatin, have been shown to reduce NLRP3 inflammasome activation in the general population and thus may serve to reduce residual NLRP3 inflammasome activation among PWH on ART. The ongoing 7,700-participant Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE) will demonstrate whether statin therapy affords ART-treated PWH cardio-protection via effects to dampen key indices of systemic immune activation – including those triggered by NLRP3 inflammasome activation.
Figure Legend

Figure 1: Role of NLRP3 Inflammasome Activation in Atherogenesis.

NLRP3 inflammasome activation within macrophages requires two distinct signals. *In vitro* studies have demonstrated that HIV infection can serve as the first signal for inflammasome activation by stimulating nuclear factor-κB (NF-κB) intracellular signaling within monocytes. NF-κB intracellular signaling, in turn, promotes the expression of the protein, NLRP3, and the formation of the NLRP3 inflammasome. A distinct secondary signal, such as cholesterol crystals, is then required for inflammasome activation. After inflammasome activation, pro-caspase-1 is cleaved to generate caspase-1, which in turn mediates the generation of pro-inflammatory and pro-atherogenic cytokines, such as interleukin (IL)-18 and IL-1β.

Abbreviations: ATP, adenosine triphosphate; interleukin-18, IL-18; interleukin-1 beta, IL-1β; LDL, low density lipoprotein; LPS, lipopolysaccharide; NLRP3, NOD-like receptor protein family pyrin domain containing 3; NF-κB, nuclear factor-κB; TLR4-MD2, toll-like receptor 4-myeloid differentiation factor 2

Figure 2. Plasma Caspase-1 Levels Among ART-naïve PWH, PWH Treated With INSTI-based ART, And Matched Control Participants

Box-plots are used to demonstrate non-normally distributed data. Whiskers represent minimum and maximum data values.

Newly-diagnosed ART-naïve PWH demonstrated significantly higher plasma caspase-1 levels at baseline as compared to matched participants without HIV. Among PWH, 6 months of treatment with INSTI-based ART (E/C/F/TDF) resulted in a significant reduction in plasma caspase-1 levels. However, among PWH, post-treatment plasma caspase-1 levels tended to remain higher than levels observed among matched participants without HIV.

Abbreviations: E/C/F/TDF, elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate; PWH, people with HIV
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**Author contributions:** MT was involved in study concept, study design, data acquisition, data analysis, data interpretation, table and figure preparation, and manuscript writing and revision. THB was involved in study concept, data acquisition, data interpretation, and manuscript revision. ESF and MC were involved in study concept, table and figure preparation and manuscript revision. SMC was involved in study concept and manuscript revision. MNF was involved in study concept, study recruitment, performing study visits, data acquisition, and manuscript revision. GKR was involved in study concept, study recruitment and manuscript revision. TGN and KM were involved in study concept and manuscript revision. SKG was involved in study concept, study design, and manuscript revision. MVZ was involved in study concept, study design, study recruitment, performing study visits, data acquisition, data interpretation and manuscript writing and revision.

**Conflicts of Interest:** MT, ESF, MA, SMC, MNF, and GKR have no disclosures to report. THB has equity in Excision BioTherapeutics, unrelated to the present project. TGN has been a consultant to and received fees from Parexel Imaging, Bristol Myers Squibb, AbbVie, Intrinsic Imaging, and H3-Biomedicine, unrelated to the present project. KM is an employee of Gilead Sciences, Inc and owns stock at Gilead Sciences, Inc. SKG has received research support to his institution from KOWA Pharmaceuticals, Inc, Gilead Sciences, Inc and ViViV Healthcare and consulting fees from Theratechnologies and ViViV Healthcare, unrelated to the present project. MVZ is Principal Investigator of an Industry-Sponsored Research Grant from Gilead Sciences, Inc to her institution, unrelated to the present project.
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Figure 1

Role of NLRP3 Inflammasome Activation in Atherogenesis
Figure 2

Plasma Caspase-1 Levels Among ART-naïve PWH, PWH Treated With INSTI-based ART, and Matched Control Participants

P<0.0001

P=0.003

P=0.19

Plasma Caspase-1 (pg/mL)

PWH Pre-ART

PWH Post-ART

Participants without HIV