Original Article

DOI: 10.5582/ddt.2023.01069

Elevated indoleamine 2,3-dioxygenase activity is associated with endothelial dysfunction in people living with HIV and ROS production in human aortic endothelial cells *in vitro*

Junyang Yang[1,§], Rentian Cai[2,§], Jingna Xun[1], Renfang Zhang[1], Li Liu[1], Yinzhong Shen[1], Tangkai Qi[1], Zhenyan Wang[1], Wei Song[1], Yang Tang[1], Jianjun Sun[1], Shuibao Xu[1], Bihe Zhao[1], Hongzhou Lu[3,*], Jun Chen[1,*]

[1] Department of Infectious and Immune Diseases, Shanghai Public Health Clinical Center, Fudan University, Shanghai, China;
[2] Department of Infectious Disease, The Second Hospital of Nanjing, Affiliated to Nanjing University of Chinese Medicine, Nanjing, China;
[3] Department of Infectious Diseases and Nursing Research Institution, National Clinical Research Center for Infectious Diseases, The Third People's Hospital of Shenzhen, Shenzhen, China.

SUMMARY The precise role of indoleamine 2,3-dioxygenase (IDO) in cardiovascular diseases (CVD) among people living with HIV (PLWH) is still under debate, despite recognized links. This study aimed to investigate the impact of elevated IDO activity on endothelial dysfunction in PLWH. A total of 38 PLWH, who had not previously received anti-retroviral therapy (ART), were enrolled in the study. These participants were monitored for 36 months following the initiation of ART. Measurements including plasma levels of IDO activity, markers of endothelial dysfunction, inflammatory factors, and lipids. *In vitro*, human aortic endothelial cells (HAEC) were exposed to interferon-γ, an IDO inhibitor, a kynurenine 3-hydroxylase (KMO) inhibitor, as well as different concentrations of kynurenine. Pre-ART, PLWH demonstrated notably elevated plasma concentrations of soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1(sVCAM-1), and IDO activity in comparison to healthy controls. Post-ART, both IDO activity and sICAM-1 levels experienced a significant decrease, with IDO activity reaching levels comparable to those observed in healthy controls. Furthermore, a positive correlation was observed between IDO activity and sICAM-1 ($p = 0.0002$), as well as sVCAM-1 ($p < 0.0001$) before ART. *In vitro*, the augmentation of kynurenine concentration in the medium and the induction of IDO expression in HAEC resulted in increased production of reactive oxygen species (ROS), with minimal impact on endothelial dysfunction. From these findings, it can be concluded that long-term ART has the potential to restore the heightened IDO activity observed in PLWH. The overexpression of IDO primarily influences the expression of ROS in HAEC.

Keywords Tryptophan metabolism, indoleamine 2,3-dioxygenase, endothelial dysfunction, human immunodeficiency virus (HIV)

1. Introduction

Cardiovascular disease (CVD) is a commonly occurring chronic complication in people living with HIV (PLWH) (*1,2*). The introduction of widespread antiretroviral therapy (ART) has yielded a significant enhancement in the life expectancy of PLWH, which may potentially contribute to an increased prevalence of CVD among this demographic (*3*). Several factors have been associated with the increased occurrence of CVD (*4,5*), encompassing hypertension, hyperlipidemia, smoking, alcohol abuse, diabetes, physical inactivity, overweightness, unhealthy lifestyle behaviors, HIV infection, and the use of ART medications, among others.

Tryptophan (Trp), an indispensable amino acid, plays a crucial role in protein synthesis, as well as the production of 5-hydroxytryptamine and melatonin in the human body. The kynurenine (Kyn) synthesis pathway (KP) is responsible for the metabolism of approximately 90% of Trp (*6*). Indoleamine 2-3 oxygenase (IDO) serves as the catalyst for the initial and rate-limiting step in the degradation of Trp through the KP, resulting in the generation of immunomodulatory tryptophan metabolites and subsequent suppression of immune function.

Recent research indicates that IDO may play a role in the development of atherosclerosis through multiple mechanisms (*7,8*). In non-HIV populations, there have been reports of decreased levels of serum Trp and increased Kyn/Trp ratios (K/T ratios) in those with coronary heart disease (*9*). Additionally, elevated plasma Kyn levels have been linked to acute myocardial infarction in patients with stable angina pectoris (*10,11*).

Overexpression of IDO activity is frequently observed in PLWH (*12*), with the HIV-1 Tat protein directly stimulating IDO production in human monocyte-derived dendritic cells (*13*). The potential correlation between this modification and the increased occurrence of CVD in PLWH is still a matter of discussion, necessitating additional research into the underlying mechanisms. Therefore, this study was conducted to investigate the association between the KP and endothelial dysfunction, a crucial initial step in CVD development.

2. Materials and Methods

2.1. Study population

In this study, a total of 50 PLWH and 18 HIV-negative healthy individuals as controls were recruited. The inclusion criteria required a positive HIV test, age above 16 years, and no prior ART exposure. Exclusion criteria included a history of opportunistic infections, cancer, pregnancy, hypertension, diabetes, or known CVD. At the time of enrollment, whole blood samples (10 mL) were obtained from both healthy controls and PLWH. After a three-year period of ART, 38 PLWH were effectively monitored and subsequently provided with 10 mL whole blood samples. These samples were subsequently employed to evaluate lipid profiles, HIV RNA, inflammatory markers, tryptophan, and tryptophan metabolites. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Shanghai Public Health Clinical Center (2016-S028-01).

2.2. Quantification of lipid profiles, HIV RNA, and CD4 and CD8 T-cell counts

Total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined using the ultracentrifugation Abell-Levy-Brodie-Kendall method. Triglyceride (TG) levels were measured using the glycerol lipase oxidase method. HIV-1 RNA loads were quantified using polymerase chain reaction (PCR) with the Cobas Amplicor system (Roche, Basel, Switzerland). CD4 and CD8 T-cell counts were evaluated using flow cytometry (BD Biosciences, Franklin Lakes, New Jersey).

2.3. Quantification of tryptophan and kynurenine levels

Plasma levels of tryptophan and kynurenine were quantified by high performance liquid chromatography as previously described (*14*). IDO activity was calculated using the plasma K/T ratio.

2.4. Quantification of plasma markers of immune activation and endothelial dysfunction

Plasma levels of markers including angiopoietin-2 (R&D Systems, Minneapolis, Minnesota), sCD14 (R&D Systems, Minneapolis, Minnesota), sCD163 (R&D Systems, Minneapolis, Minnesota), endogenous endotoxin-core antibody (EndoCAb) (Hycult Biotech, Uden, the Netherlands), LPS-binding protein (LBP) (Hycult Biotech, Uden, the Netherlands), IL-6 (Raybiotech, Norcross, America), sICAM-1/ sCD54 (R&D Systems, Minneapolis, Minnesota) and sVCAM-1/sCD106 (R&D Systems, Minneapolis, Minnesota), were determined using enzyme-linked immunosorbent assays in accordance with the manufacturers' instructions.

2.5. Cell Culture

Human aortic endothelial cells (HAECs) (Sciencell, Carlsbad, USA) were cultured in Endothelial Cell Medium (ScienCell, Carlsbad, USA) supplemented with 5% FBS (ScienCell, Carlsbad, USA), 1% Endothelial Cell Growth Supplement (ScienCell, Carlsbad, USA), and 1% penicillin/ streptomycin (ScienCell, Carlsbad, USA) as per the manufacturer's instructions. Cells within passages 3 to 8 were utilized and allowed to reach 70-80% confluence before exposure to various agents.

HAECs were subjected to pretreatment with 0.2% dimethyl sulfoxide (DMSO), IDO inhibitor (1-methyl-DL-tryptophan (1-MT) (Sigma-Aldrich, Stockholm, Sweden)), or KMO inhibitor (Ro61-8048) (MedChem Express, Monmouth Junction, USA), for a duration of one hour. Subsequently, the cells were stimulated with Kyn (10 μM or 100 μM) (Sigma-Aldrich, Stockholm, Sweden) or IFN-γ (100 ng/ml) (PEPROTECH, Rocky Hill, USA) for 48 hours. All experiments were conducted in triplicate.

The levels of sICAM-1/sCD54 (R&D Systems, Minneapolis, Minnesota), sVCAM-1/sCD106 (R&D Systems, Minneapolis, Minnesota), Tryptophan (MyBioSource, California, USA), and Kynurenine (MyBioSource, California, USA) were quantified in the cell supernatant using ELISA. Additionally, the levels of caspase-3 and reactive oxygen species (ROS) were quantified in the cell suspension using the PE Active Caspase-3 Apoptosis Kit (BD Biosciences, Franklin Lakes, New Jersey) and the Reactive Oxygen Species Assay Kit (Yisheng, Shanghai, China) respectively, through flow cytometry.

2.6. Data analysis

The data analysis was conducted using IBM SPSS version 19.0 (IBM SPSS, Inc., Armonk, NY, USA), while the figures were generated using GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego, CA, USA). Categorical variables were presented as n (%). For continuous data with a normal distribution, means ± SD were presented, whereas non-normally distributed data were described using the median with interquartile range (IQR). The statistical tests employed in this study included the *t*-test and Mann-Whitney rank-sum test to identify significant differences between pre- and post-ART conditions, as well as healthy controls. Additionally, Pearson correlation and Spearman rank correlation tests were utilized to assess the relationship between IDO activity and other variables. Statistical significance was determined by *p*-values less than 0.05.

3. Results

3.1. Demographic and clinical characteristics of the study participants

The demographic and clinical characteristics of PLWH who have not yet received ART and healthy controls are presented in Table 1. Out of the 38 PLWH included in the study, a substantial majority of 89.5% (34/38) were male, with a median age of 32. The majority of PLWH were prescribed a regimen consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) in combination with one nonnucleoside reverse transcriptase inhibitor (NNRTI).

3.2. Characteristics changes in PLWH at pre- and on-ART

After ART, all PLWH achieved viral suppression (< 50 copies/μL), accompanied by a significant increase in the CD4/CD8 ratio (0.42 (0.32-0.51) *vs.* 0.74 (0.51-0.97), $p < 0.001$), indicating an improvement in immune function. However, there was no significant change observed in the CD4 cell count (357 (264-527) cells/μL *vs.* 400 (307-530) cells/μL, $p = 0.117$). Prior to initiating ART, PLWH exhibited higher plasma Kyn concentrations and IDO activity compared to healthy control. However, after three years of ART, these levels were reduced to a similar extent as observed in healthy controls (Figure 1). Additional information pertaining to changes in lipid profiles, markers of endothelial dysfunction, microbial translocation, and chronic inflammation following ART in PLWH can be found in Table 2.

Table 1. Study participants

Characteristics	PLWH ($n = 38$)	HIV-negative controls ($n = 18$)
Age, year, median (year)	32 (26-50)	31 (26-41)
Male sex, No. (%)	34 (89.47)	12 (66.67)
CD4 T-cell count (cells/μL)	357 (264-527)	N/A
CD4/CD8 ratio	0.42 (0.32-0.51)	N/A
ART regimen (No, %)		
TDF+3TC+EFV	32 (84.2)	N/A
TDF+3TC+LPV/r	2 (5.3)	N/A
AZT+3TC+EFV	3 (7.9)	N/A
TDF+3TC+RAL	1 (2.6)	N/A

Abbreviations: PLWH, people living with HIV; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine; EFV, efavirenz; LPV/r, lopinavir/ritonavir; RAL, raltegravir.

3.3. Plasma Kyn concentration and IDO activity was associated with endothelial dysfunction in PLWH

Prior to ART, PLWH displayed notably higher plasma concentration of sICAM-1 and sVCAM-1 compared to the control group of healthy individuals (217.00 ng/mL (156.80-241.80 ng/mL) *vs.* 187.90 ng/mL (117.70-209.40 ng/mL), $p = 0.0317$; 712.40 ng/mL (416.70-1168.00 ng/mL) *vs.* 467.80 ng/mL (284.40-522.70 ng/mL), $p = 0.0052$; for sICAM-1 and sVCAM-1 respectively). Post-ART, a significant decrease was observed in plasma sICAM-1 concentration, bringing it in line with the level observed in the healthy control group, while the sVCAM-1 level remained unchanged (Figure 2A). The plasma level of ANG-II in PLWH, both before and after ART, was found to be similar to that of healthy controls. However, a decrease in plasma ANG-II concentration was observed after ART (255.90 pg/mL (206.80-454.60 pg/mL) *vs.* 241.40 pg/mL (166.60-289.70 pg/mL), $p = 0.0075$) (Figure 2A).

Prior to ART, IDO activities and plasma Kyn concentration were positively correlated with plasma sVCAM-1 and sICAM-1 concentration in PLWH. However, the correlation between IDO activities and sICAM-1 lost significance after ART. Neither plasma Kyn concentration nor IDO activities showed any correlation with Ang-II in PLWH (Figures 2B and 2C). Furthermore, the changes in IDO activities and plasma Kyn concentration were positively correlated with the concentrations of sVCAM-1, sICAM-1, and Ang-II (Figure S1, *http://www.ddtjournal.com/action/getSupplementalData.php?ID=172*).

3.4. Kyn and IDO activity was not associated with lipids, microbial translocation, and chronic inflammation markers

A significant increase in plasma HDL levels was observed after ART (0.94 mmol/L (0.80-1.08 mmol/L) pre-ART *vs.* 1.08 mmol/L (0.90-1.30mmol/L) post-ART; $p=0.0082$). However, these levels remained lower than those observed in healthy controls (1.08 mmol/L (0.90-1.30 mmol/L) *vs.* 1.24 mmol/L (1.11-1.57 mmol/L); $p=0.0148$). Plasma levels of TG, TCH, and LDL were comparable to those of the healthy controls, both before and after ART. No significant correlations were found

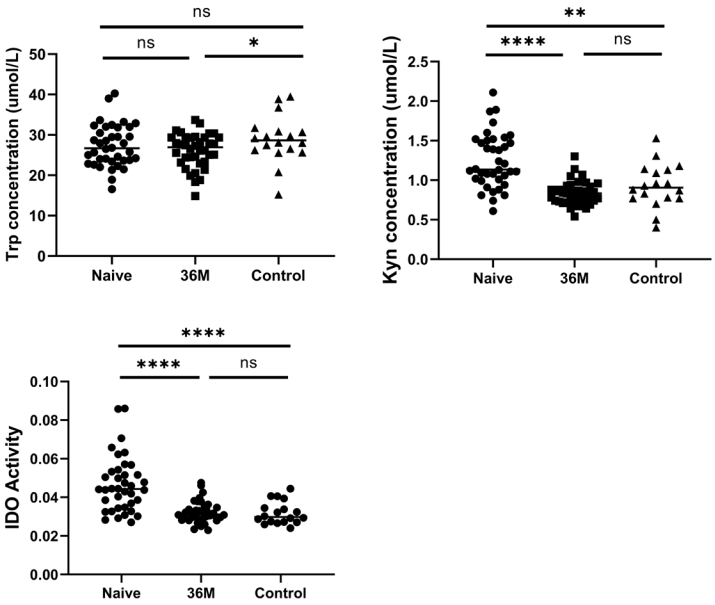

Figure 1. The impact of ART on plasma concentrations of Trp (A), Kyn (B), and IDO activity (C) in PLWH. ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Abbreviations: Trp, Tryptophan; Kyn, Kynurenine; IDO activity, Kyn/Trp ratio

Table 2. Characteristics changes in PLWH pre- and on-ART

Characteristics	Pre-ART	On-ART	*p* value
CD4 T-cell count (cell/μL)	357.50 (264.80-527.50)	399.50 (307.80-530.80)	0.117
CD4/CD8 rate	0.42 (0.32-0.51)	0.74 (0.51-0.97)	< 0.001
sICAM-1 (ng/mL)	217.00 (156.80-241.80)	172.00 (135.60-231.10)	0.008
sVCAM-1 (ng/mL)	712.40 (416.70-1168.00)	668.50 (578.90-778.50)	0.278
ANG-II(pg/mL)	255.90 (206.80-454.60)	241.40 (166.60-289.70)	0.008
TG (mmol/L)	1.15 (0.88-1.55)	1.51 (0.90-2.07)	0.086
TCH (mmol/L)	4.21 (3.35-5.11)	4.12 (3.47-4.98)	0.468
LDL (mmol/L)	2.40 (1.77-3.2)	2.73 (2.26-3.45)	0.086
HDL (mmol/L)	0.94 (0.80-1.08)	1.08 (0.90-1.30)	0.009
Tryptophan (μmol/L)	23.68 (23.57-31.87)	26.96 (23.31-29.35)	0.145
Kynurenine (μmol/L)	1.14 (1.01-1.51)	0.84 (0.73-0.92)	< 0.001
IDO Activity (nM/μM)	44.32 (34.72-53.56)	31.10 (28.76-34.81)	< 0.001
Endocab (MMu/mL)	72.30 (34.88-111.50)	169.50 (89.80-293.70)	0.001
LBP (ng/mL)	5623.00 (3362.00-7936.00)	5896.00 (5047.00-7289.00)	0.24
sCD14 (ng/mL)	1450.00 (490.20-2111.00)	1514.00 (1218.00-1829.00)	0.396
sCD163 (ng/mL)	774.30 (578.30-1081.00)	712.10 (579.20-895.90)	0.236
IL-6 (pg/mL)	77.39 (23.93-124.10)	27.67 (14.33-47.08)	0.005

Abbreviations: ART, anti-retroviral therapy; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; ANG-II, angiopoietin-2; TG, triglyceride; TCH, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IDO, indoleamine 2,3-dioxygenase; EndoCAb, endogenous endotoxin-core antibody; LBP, LPS-binding protein; IL-6, interleukin-6.

between TG, TCH, HDL, LDL, and plasma Kyn levels or IDO activity.

Plasma concentration of sCD14 and sCD163 were found to be significantly elevated, while plasma IL-6 levels were comparable, and plasma EndoCAb and LBP level were lower among untreated PLWH compared to healthy controls. However, apart from sCD14, which demonstrated a significant correlation with IDO activity prior to the initiation of ART ($R^2 = 0.154$, $p = 0.01$), there is no observed correlation between plasma Kyn concentration or IDO activity and plasma levels of LBP, sCD163, EndoCAb, and IL-6, both before and after ART in PLWH.

3.5. Activation of KP induces ROS production in HAEC *in vitro*

To further elucidate the impact of IDO activity on endothelial cells, HAECs were pretreated with 1-MT or Ro61-8048 for 1 hour prior to stimulation with 100 ng/mL IFN-γ for 48 hours. Our observations revealed that IFN-γ effectively induced KP activation in HAECs, an effect that could be mitigated by the administration of 1-MT and Ro61-8048 (Figure 3A). Furthermore, these interventions yielded comparable results in terms of ROS production and IDO activity, while exerting minimal impact on sICAM-1, sVCAM-1, and caspase3

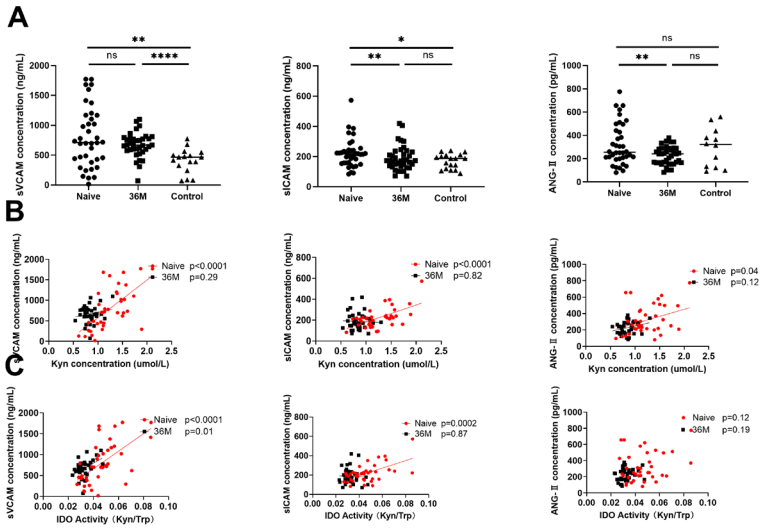

Figure 2. The impact of ART on endothelial dysfunction and its correlation with the metabolites of tryptophan. A tripartite panel representation delineating the impact of ART on plasma concentrations of sVCAM-1, sICAM-1, and ANG-II, sequentially from left to right (**A**). A series of three scatter plots demonstrating the relationships between plasma KYN levels and the respective plasma concentrations of sVCAM-1, sICAM-1, and ANG-II, arranged from left to right (**B**). A series of three scatter plots demonstrating the associations between IDO activity and the corresponding plasma concentrations of sVCAM-1, sICAM-1, and ANG-II, sequentially from left to right (**C**). ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Abbreviations: sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; ANG-II, Angiopoietin-2; Kyn, Kynurenine; IDO, indoleamine 2,3-dioxygenase.

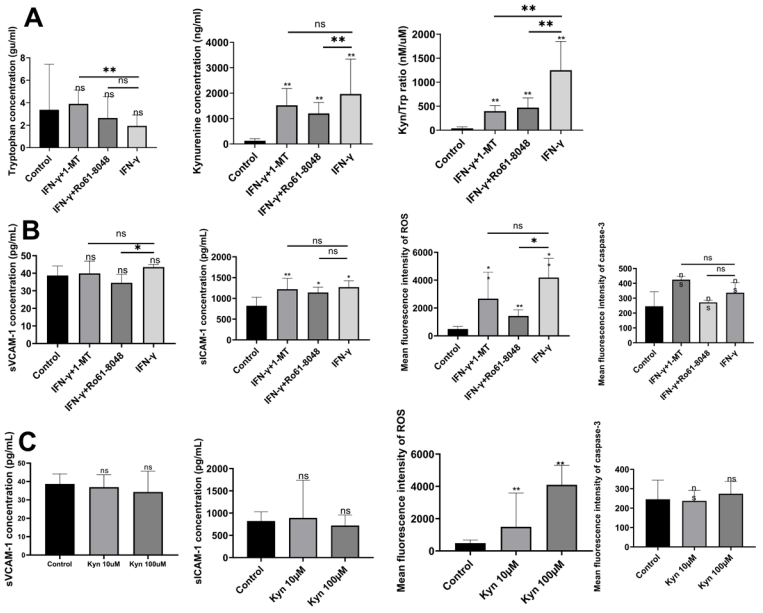

Figure 3. The impact of tryptophan and its metabolic products on HAEC in vitro. A set of three bar graphs, arranged from left to right, depicting the effects of IFN-γ, 1-MT, and Ro61-8048 on tryptophan concentration, kynurenine concentration, and Kyn/Trp ratio in HAEC *in vitro*, respectively (**A**). A set of four bar graphs, arranged from left to right, depicting the effects of IFN-γ, 1-MT, and Ro61-8048 on sVCAM concentration, sICAM concentration, ROS production, and caspase-3 activity in HAEC in vitro, respectively (**B**). A set of four bar graphs, arranged from left to right, depicting the effects of 10 μM and 100 μM KYN on sVCAM concentration, sICAM concentration, ROS production, and caspase-3 activity in HAEC *in vitro*, respectively (**C**). ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Abbreviations: Kyn/Trp ratio, kynurenine/tryptophan ratio; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1.

(Figure 3B).

In order to provide additional evidence for the hypothesis that KP activation may initiate the generation of ROS, we exposed HAECs to exogenous Kyn at concentrations of 10 μM or 100 μM for a duration of 48 hours. It was observed that Kyn had the ability to induce the production of ROS, with the intensity of ROS production increasing proportionally with higher concentrations of Kyn. However, it is worth noting that both interventions had minimal impact on the production of sICAM-1 and sVCAM-1, as well as HAEC apoptosis, as depicted in Figure 3C.

4. Discussion

Endothelial dysfunction plays a crucial role in the development of atherosclerosis and other vascular disorders (*15*). Understanding the factors involved in HIV-related endothelial dysfunction is vital for the effective long-term management of AIDS. Our study revealed a significant association between IDO activity, plasma Kyn concentration and endothelial dysfunction in PLWH. Additionally, we confirmed that activation of KP in increased production of ROS in HAECs *in vitro*. These findings suggest that targeting this pathway could potentially reduce the risk of CVD in individuals with HIV.

In our study, we noted a notable increase in markers of endothelial dysfunction in PLWH who had not received ART, as compared to healthy controls. After ART, only levels of sVCAM-1 remained higher in PLWH compared to the healthy controls. Similarly, prior to ART, there was a significant elevation in IDO activity and plasma KYN concentration, which returned to normal levels after treatment. Consistent with our previous findings and those of Routy *et al*., it was observed that IDO activity did not fully recover even after antiviral therapy (*12,16*). The observed disparity between our present findings and prior research can be ascribed to variances in the demographics of the study cohorts. Specifically, the individuals included in this investigation displayed comparatively elevated CD4 levels, which previous studies have established to have an inverse association with IDO activity (*12*). Interestingly, a previous study has documented that PLWH who exhibited persistently low CD4 levels after receiving treatment exhibited a notably heightened susceptibility to cardiovascular events (*17*). This finding may partially account for the heightened association observed between pre-treatment IDO activity and plasma Kyn levels, as well as endothelial damage biomarkers in our investigation, which diminished following treatment. Subsequent research endeavors should prioritize the inclusion of populations with lower CD4 levels to gain further insight into these associations.

Both the beneficial and detrimental effects of CVD on Trp and its metabolites have been documented (*18*). Zhang *et al.* (*8*) determined the atheroprotective effects of 3-hydroxybutyrate anthranilic acid, while Kynurenic acid and aryl hydrocarbon receptor were found to inhibit the immune response, thereby enhancing the stability of carotid plaques (*19*). Conversely, Wang *et al.* argued that 3-hydroxyanthranilic acid activated the nuclear factor-kappa B transcription factor, leading to increased expression of matrix metallopeptidase 2 in vascular smooth muscle cells and contributing to AngII-induced abdominal aortic aneurysm *in vivo* (*20*). K/ ratio demonstrated a positive association with BMI, LDL, TG, carotid intima-media thickness, and abdominal circumference, while exhibiting a negative association with HDL (*10,21,22*). Furthermore, increased plasma Kyn concentrations were found to be positively associated with the likelihood of experiencing an acute coronary event among older individuals (*23*). In the context of this study, IDO activity exhibited a positive correlation with plasma sICAM-1 and sVCAM-1 levels in ART-naïve PLWH. These findings are consistent with previous research conducted on PLWH (*11,24*).

Based on the aforementioned studies, it appears that the overexpression of IDO may contribute to endothelial dysfunction and have detrimental effects on CVD. However, an inexplicable phenomenon was observed *in vitro*, where the activation of IDO and the introduction of exogenous Kyn had minimal impact on endothelial dysfunction and apoptosis in HAEC. Interestingly, in hypercholesterolemic $ApoE^{-/-}$ mice, the deficiency of IDO resulted in a significant increase in lesion size and a decrease in blood levels of IL10 (*25*). Furthermore, the inhibition of IDO led to heightened vascular inflammation and an upregulation of monocyte chemoattractant protein-1 and sVCAM-1 expression in $ApoE^{-/-}$ mice (*26*). Regulatory T cells and KP exhibit reciprocal regulation within the vessel wall, thereby facilitating vascular tolerance mechanisms and mitigating inflammation and atherosclerosis (*27*).

Our investigation further revealed that the activation of IDO and elevation of Kyn concentration resulted in the generation of ROS in HAEC *in vitro*, without causing any endothelial dysfunction. Furthermore, the treatment of mouse and human myoblasts with Kyn led to a two-fold increase in ROS levels (*28*). ROS, functioning as a subcellular messenger in signal transduction pathways, has been found to exert both advantageous and detrimental effects on the cardiovascular system (*29*). Under normal physiological conditions, the production of ROS at low levels has been observed to play a regulatory role in gene expression, excitation-contraction coupling, cell growth, migration, differentiation, and programmed cell death. However, in pathological scenarios, ROS can lead to oxidative modifications of crucial cellular macromolecules (*29*). *Lubrano's* research (*30*) has demonstrated that elevated levels of ROS can directly enhance the production of lectin-like oxidized low-density lipoprotein. This increase in LOX-1 has been linked to apoptosis induction in various cell types when present in high concentrations, while low concentrations have been associated with cell proliferation.

Our previous research has demonstrated the prevalence of dyslipidemia, microbial translocation, and chronic inflammation in PLWH (*31,32*), which have been considered to be associated with endothelial dysfunction (*15, 33*). In $Apuet^{-/-}$ mice, inhibition of IDO resulted in an increase in the level of TCH and the ratio of VLDL/HDL (*26*). The involvement of Kyn metabolites in regulating lipid metabolism, thermogenic

gene expression, and anti-inflammatory gene expression in adipose tissue has been observed through the activation of G protein-coupled receptor Gpr35 or aryl hydrocarbon receptor/signal transducer or transcription 3/interleukin-6 signaling (*34,35*). Catabolism of tryptophan (Trp) plays a significant role in the precise regulation of intestinal physiology (*36*), and it has also been linked to CVD (*37*). Numerous studies have reported the involvement of IDO in the pathophysiology of various inflammatory conditions, such as infection, allergy, autoimmunity, chronic inflammation, and inflammatory neurological diseases (*38,39*). Based on the aforementioned evidence, it is justifiable to postulate that KP might potentially contribute to the development of endothelial dysfunction through its influence on lipid metabolism, microbial translocation, or chronic inflammatory response. However, this conjecture was promptly refuted as our study revealed minimal association between IDO activity and plasma lipid levels, as well as plasma markers for inflammation or microbial translocation.

Our study is subject to several limitations and deficiencies. Firstly, the majority of participants were young and middle-aged men, thus limiting the generalizability of our findings to women and the elderly. Secondly, the assessment of atherosclerosis was incomplete as we did not measure carotid artery intima-media thickness, a crucial parameter for evaluating this condition. Thirdly, the utilization of the K/T ratio as a representative measure of IDO activity may lead to erroneous conclusions, as it does not fully capture the complexity of IDO activity. Additionally, the verification of our results through *in vitro* experiments using HAEC may be deemed less persuasive compared to *in vivo* studies.

In summary, long-term ART has the potential to restore normal levels of IDO activity that is elevated due to HIV infection. The activity of IDO is found to be positively associated with endothelial dysfunction in PLWH. The excessive expression of IDO primarily impacts the production of ROS, while its direct influence on endothelial dysfunction in HAEC is limited.

Acknowledgements

We thank all workers for their diagnosis, nursing and treatment of HIV/AIDS patients at Shanghai Public Health Clinical Center.

Funding: This work was supported by a grant from the Shanghai Public Health Clinical Center (KY-GW-2022-10; KY-GW-2022-10); Shanghai Science and Technology Committee (20MC1920100); Shanghai Talent Development Fund (2020089).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Paisible AL, Chang CC, So-Armah KA, *et al*. HIV infection, cardiovascular disease risk factor profile, and risk for acute myocardial infarction. J Acquir Immune Defic Syndr. 2015; 68:209-216.
2. Freiberg MS, Chang CC, Kuller LH, *et al.* HIV infection and the risk of acute myocardial infarction. JAMA Intern Med. 2013; 173:614-622.
3. So-Armah K, Benjamin LA, Bloomfield GS, Feinstein MJ, Hsue P, Njuguna B, Freiberg MS. HIV and cardiovascular disease. Lancet HIV. 2020; 7:e279-e293.
4. Jokinen E. Obesity and cardiovascular disease. Minerva Pediatr. 2015; 67:25-32.
5. Leong DP, Joseph PG, McKee M, Anand SS, Teo KK, Schwalm JD, Yusuf S. Reducing the global burden of cardiovascular disease, Part 2: Prevention and treatment of cardiovascular disease. Cir Res. 2017; 121:695-710.
6. King NJ, Thomas SR. Molecules in focus: indoleamine 2,3-dioxygenase. Int J Biochem Cell Biol. 2007; 39:2167-2172.
7. Liu G, Chen S, Zhong J, Teng K, Yin Y. Crosstalk between tryptophan metabolism and cardiovascular disease, mechanisms, and therapeutic implications. Oxid Med Cell Longev. 2017; 2017:1602074.
8. Zhang L, Ovchinnikova O, Jönsson A, Lundberg AM, Berg M, Hansson GK, Ketelhuth DF. The tryptophan metabolite 3-hydroxyanthranilic acid lowers plasma lipids and decreases atherosclerosis in hypercholesterolaemic mice. Eur Heart J. 2012; 33:2025-2034.
9. Wirleitner B, Rudzite V, Neurauter G, Murr C, Kalnins U, Erglis A, Trusinskis K, Fuchs D. Immune activation and degradation of tryptophan in coronary heart disease. Eur J Clin Invest. 2003; 33:550-554.
10. Pertovaara M, Raitala A, Juonala M, Lehtimaki T, Huhtala H, Oja SS, Jokinen E, Viikari JS, Raitakari OT, Hurme M. Indoleamine 2,3-dioxygenase enzyme activity correlates with risk factors for atherosclerosis: the Cardiovascular Risk in Young Finns Study. Clin Exp Immunol. 2007; 148:106-111.
11. Pedersen ER, Tuseth N, Eussen SJ, Ueland PM, Strand E, Svingen GF, Midttun O, Meyer K, Mellgren G, Ulvik A, Nordrehaug JE, Nilsen DW, Nygard O. Associations of plasma kynurenines with risk of acute myocardial infarction in patients with stable angina pectoris. Arterioscler Thromb Vasc Biol. 2015; 35:455-462.
12. Chen J, Shao J, Cai R, Shen Y, Zhang R, Liu L, Qi T, Lu H. Anti-retroviral therapy decreases but does not normalize indoleamine 2,3-dioxygenase activity in HIV-infected patients. PloS one. 2014; 9:e100446.
13. Planès R, Bahraoui E. HIV-1 Tat protein induces the production of IDO in human monocyte derived-dendritic cells through a direct mechanism: Effect on T cells proliferation. PloS One. 2013; 8:e74551.
14. Chen J, Xun J, Yang J, Ji Y, Liu L, Qi T, Wang Z, Zhang R, Shen Y, Ponte R, Mehraj V, Routy JP, Lu H. Plasma indoleamine 2,3-dioxygenase activity is associated with the size of the human immunodeficiency virus reservoir in patients receiving antiretroviral therapy. Clin Infect Dis. 2019; 68:1274-1281.
15. Poredos P, Poredos AV, Gregoric I. Endothelial dysfunction and its clinical implications. Angiology. 2021; 72:604-615.
16. Jenabian MA, El-Far M, Vyboh K, *et al*.

Immunosuppressive tryptophan catabolism and gut mucosal dysfunction following early HIV infection. J Infect Dis. 2015; 212:355-366.
17. Ho JE, Scherzer R, Hecht FM, Maka K, Selby V, Martin JN, Ganz P, Deeks SG, Hsue PY. The association of CD4+ T-cell counts and cardiovascular risk in treated HIV disease. Aids. 2012; 26:1115-1120.
18. Ala M, Eftekhar SP. The footprint of kynurenine pathway in cardiovascular diseases. Int J Tryptophan Res. 2022; 15:11786469221096643.
19. Baumgartner R, Berg M, Matic L, Polyzos KP, Forteza MJ, Hjorth SA, Schwartz TW, Paulsson-Berne G, Hansson GK, Hedin U, Ketelhuth DFJ. Evidence that a deviation in the kynurenine pathway aggravates atherosclerotic disease in humans. J Intern Med. 2021; 289:53-68.
20. Wang Q, Ding Y, Song P, Zhu H, Okon I, Ding YN, Chen HZ, Liu DP, Zou MH. Tryptophan-derived 3-hydroxyanthranilic acid contributes to angiotensin II-induced abdominal aortic aneurysm formation in mice *in vivo*. Circulation. 2017; 136:2271-2283.
21. Jeong YI, Kim SW, Jung ID, *et al.* Curcumin suppresses the induction of indoleamine 2,3-dioxygenase by blocking the Janus-activated kinase-protein kinase Cdelta-STAT1 signaling pathway in interferon-gamma-stimulated murine dendritic cells. J Biol Chem. 2009; 284:3700-3708.
22. Cuffy MC, Silverio AM, Qin L, Wang Y, Eid R, Brandacher G, Lakkis FG, Fuchs D, Pober JS, Tellides G. Induction of indoleamine 2,3-dioxygenase in vascular smooth muscle cells by interferon-gamma contributes to medial immunoprivilege. J Immunol. 2007; 179:5246-5254.
23. Eussen SJ, Ueland PM, Vollset SE, Nygard O, Midttun O, Sulo G, Ulvik A, Meyer K, Pedersen ER, Tell GS. Kynurenines as predictors of acute coronary events in the Hordaland Health Study. Int J Cardiol. 2015; 189:18-24.
24. Qi Q, Hua S, Clish CB, *et al*. Plasma tryptophan-kynurenine metabolites are altered in HIV infection and associated with progression of carotid artery atherosclerosis. Clin Infect Dis. 2018; 67:235-242.
25. Cole JE, Astola N, Cribbs AP, Goddard ME, Park I, Green P, Davies AH, Williams RO, Feldmann M, Monaco C. Indoleamine 2,3-dioxygenase-1 is protective in atherosclerosis and its metabolites provide new opportunities for drug development. Proc Natl Acad Sci U S A. 2015; 112:13033-13038.
26. Polyzos KA, Ovchinnikova O, Berg M, Baumgartner R, Agardh H, Pirault J, Gisterå A, Assinger A, Laguna-Fernandez A, Bäck M, Hansson GK, Ketelhuth DF. Inhibition of indoleamine 2,3-dioxygenase promotes vascular inflammation and increases atherosclerosis in Apoe-/- mice. Cardiovasc Res. 2015; 106:295-302.
27. Forteza MJ, Polyzos KA, Baumgartner R, Suur BE, Mussbacher M, Johansson DK, Hermansson A, Hansson GK, Ketelhuth DFJ. Activation of the regulatory T-cell/indoleamine 2,3-dioxygenase axis reduces vascular inflammation and atherosclerosis in hyperlipidemic mice. Front Immunol. 2018; 9:950.
28. Kaiser H, Yu K, Pandya C, Mendhe B, Isales CM, McGee-Lawrence ME, Johnson M, Fulzele S, Hamrick MW. Kynurenine, a tryptophan metabolite that increases with age, induces muscle atrophy and lipid peroxidation. Oxid Med Cell Longev. 2019; 2019:9894238.
29. Dubois-Deruy E, Peugnet V, Turkieh A, Pinet F. Oxidative stress in cardiovascular diseases. Antioxidants (Basel). 2020; 9:864.
30. Lubrano V, Balzan S. LOX-1 and ROS, inseparable factors in the process of endothelial damage. Free Radic Res. 2014; 48:841-848.
31. Yang J, Chen J, Ji Y, Tang Q, Zhang R, Liu L, Shen Y, Xun J, Song W, Tang Y, Wang Z, Qi T, Lu H. Lipid profile and renal safety of tenofovir disoproxil fumarate-based anti-retroviral therapy in HIV-infected Chinese patients. Int J Infect Dis. 2019; 83:64-71.
32. Ji Y, Zhang F, Zhang R, *et al*. Changes in intestinal microbiota in HIV-1-infected subjects following cART initiation: Influence of CD4+ T cell count. Emerg Microbes Infect. 2018; 7:113.
33. Karki P, Birukov KG. Lipid mediators in the regulation of endothelial barriers. Tissue barriers. 2018; 6:e1385573.
34. Dadvar S, Ferreira DMS, Cervenka I, Ruas JL. The weight of nutrients: kynurenine metabolites in obesity and exercise. Journal Intern Med. 2018; 284:519-533.
35. Huang T, Song J, Gao J, *et al*. Adipocyte-derived kynurenine promotes obesity and insulin resistance by activating the AhR/STAT3/IL-6 signaling. Nat Commun. 2022; 13:3489.
36. Borghi M, Puccetti M, Pariano M, Renga G, Stincardini C, Ricci M, Giovagnoli S, Costantini C, Romani L. Tryptophan as a central hub for host/microbial symbiosis. Int J Tryptophan Res. 2020; 13:1178646920919755.
37. Kazemian N, Mahmoudi M, Halperin F, Wu JC, Pakpour S. Gut microbiota and cardiovascular disease: Opportunities and challenges. Microbiome. 2020; 8:36.
38. Mellor AL, Munn DH. IDO expression by dendritic cells: Tolerance and tryptophan catabolism. Nat Rev Immunol. 2004; 4:762-774.
39. Melhem NJ, Taleb S. Tryptophan: From diet to cardiovascular diseases. Int J Mol Sci. 2021; 22:9904.

Received August 28, 2023; Revised October 13, 2023; Accepted October 21, 2023.

[§]These authors contributed equally to this work.
**Address correspondence to:*
Hongzhou Lu, Department of Infectious Diseases and Nursing Research Institution, The Third People's Hospital of Shenzhen, Shenzhen, China.
E-mail: luhongzhou@fudan.edu.cn

Dr. Jun Chen, Department of infection and immunology, Shanghai Public Health Clinical Center, No. 2901 Caolang Road, Shanghai, China.
E-mail: qtchenjun@163.com

Released online in J-STAGE as advance publication October 26, 2023.