Endothelial Microvesicles: Circulating Biomarker and Mediator of Vascular Dysfunction With HIV-1

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ABSTRACT

We have previously reported that circulating endothelial cell derived extracellular microvesicles (EMVs) are elevated in adults living with HIV (ALWH) receiving antiretroviral therapy (ART) contributing to endothelial vasodilator dysfunction by negatively affecting endothelial nitric oxide synthase (eNOS) and, in turn, impairing NO production. However, whether elevations in circulating EMVs and their pathologic effects on endothelial NO production was a direct effect of HIV-1 or a consequence of ART is unknown. The experimental aims of this study were to determine: 1) whether circulating EMVs are elevated in treatment-naïve ALWH and associated with HIV-1-related endothelial vasodilator dysfunction; and 2) the effects of EMVs isolated from treatment-naïve ALWH on endothelial cell NO production, *in vitro*. Twenty-four sedentary, adults were studied: 12 healthy (10M/2F; age 35±2 yr) and 12 treatment naïve ALWH (10M/2F; 35±2 yr; viral load: 7342 copies/mL). All subjects were non-obese, normotensive, normolipidemic and free of overt cardiometabolic disease. Circulating EMV (CD144-PE) number was determined by flow cytometry. Forearm blood flow (FBF: via plethysmography) was assessed by intra-arterial infusion of acetylcholine and sodium nitroprusside. Human coronary artery endothelial cells were cultured and treated with EMVs (CD144-PE) isolated from the healthy and treatment naïve ALWH. Circulating EMVs were ~75% higher (P<0.05) in the treatment naïve ALWH (234±20 EMV/μL) vs healthy (135±13 EMV/μL) adults. FBF responses to acetylcholine were significantly lower (~20%) in the treatment naïve ALWH adults (from 5.6±0.3 to 12.6±1.1 mL/100 mL tissue/min vs. 4.9±0.2 to 15.8±1.1 mL/100 mL tissue/min). There were no significant differences between the groups in FBF response to sodium nitroprusside. EMVs were strongly and inversely associated with the vasodilator response to acetylcholine (r=-0.49; P=0.01). Moreover, expression of phosphorylated eNOS (78.5±6.1 vs 102.9±7.9 AU) and NO production (6.0±0.4 vs 7.3±0.4 μmol/L) was significantly lower in cells treated with EMVs from treatment naïve ALWH vs. healthy adults. HIV-1 is associated with elevated circulating EMVs. Moreover, circulating EMVs are not only a systemic biomarker, but appear to be a mechanistic factor underlying endothelial dysfunction with HIV-1. Circulating EMVs represent a novel causative factor underlying vascular abnormalities and the increased CVD risk associated with HIV-1.

BACKGROUND

- Adults living with HIV (ALWH) have nearly twice the risk for atherosclerosis and myocardial infarction than uninfected adults.
- Vascular endothelial dysfunction underlies the initiation and progression of atherosclerotic vascular disease and has been linked to HIV-1.
- Extracellular vesicles, particularly circulating endothelial cell-derived microvesicles (EMVs) function as both a biomarker and a mediator of vascular health and disease.
- We have previously reported that circulating EMVs are elevated in ALWH receiving antiretroviral therapy (ART), contributing to endothelial vasodilator dysfunction by negatively affecting endothelial nitric oxide synthase (eNOS) and, in turn, impairing NO production.
- However, whether elevations in circulating EMVs and their pathologic effects on endothelial NO production is a direct effect of HIV-1 or a consequence of ART is unknown.

EXPERIMENTAL AIMS

The experimental aims of this study were to determine: 1) whether circulating EMVs are elevated in treatment-naïve ALWH and associated with HIV-1-related endothelial vasodilator dysfunction; and 2) the effects of EMVs isolated from treatment-naïve ALWH on endothelial cell NO production, *in vitro*.

METHODS

Subjects

- 24 sedentary adults (35±2 yr.) were studied:
  - 12 healthy (10M/2F)
  - 12 treatment naïve ALWH (10M/2F; viral load: 7342 copies/mL; CD4 count: 630 cells/μL)

Inclusion Criteria

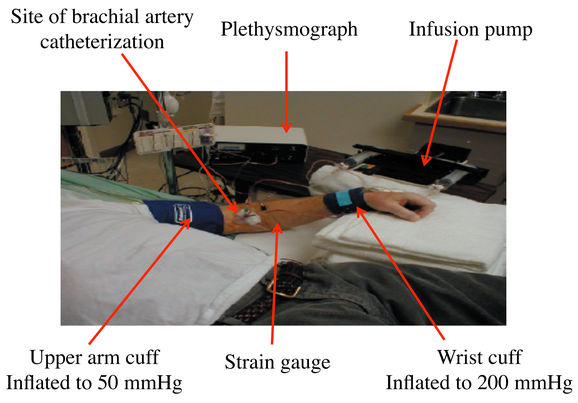
- Non-obese
- Normotensive
- Normolipidemic
- Free of overt cardiovascular disease

EMV Identification and Enumeration

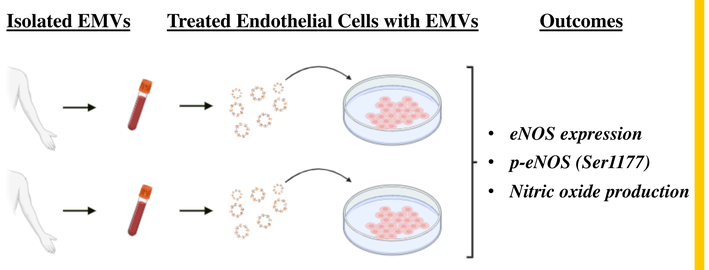
- EMV lineage was determined using fluorescent-conjugated antibody ($CD144^+$) and enumerated by flow cytometry.

Assessment of Endothelial Vasodilator Function

- The brachial artery of the non-dominant arm was catheterized.
- Forearm blood flow (FBF) was assessed by strain gauge venous occlusion plethysmography in response to intra-arterial:
  1) Acetylcholine (ACh; 4-16 μg/100 mL tissue/min)
  2) Sodium Nitroprusside (SNP; 1-4 μg/100 mL tissue/min)



Human Coronary Artery Endothelial Cells (HCAECs) Culture



STATISTICAL ANALYSIS

Group differences in baseline characteristics and EMV levels were determined by analysis of variance (ANOVA). Group differences in FBF responses to ACh and SNP were determined by repeated measures ANOVA. Relations between variables of interest were determined by using linear regression. Statistical significance was set *a priori* at P<0.05. Values are mean±SEM.

RESULTS

I. Selected Physical Characteristics

Variable	Healthy (n=12)	Treatment naïve ALWH (n=12)
Age, yrs,	35±2	35±2
Body mass, kg	81.7±8.1	78.2±3.8
BMI, kg/m^2	26.7±1.0	26.9±1.3
Body fat, %	23.6±2.5	29.7±3.7
Systolic BP, mmHg	116±2	117±3
Diastolic BP, mmHg	72±2	76±2

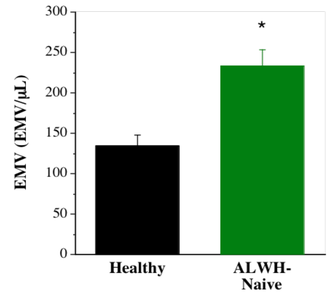
BMI, body mass index; BP, blood pressure.
Values are mean±SEM.

II. Metabolic Characteristics

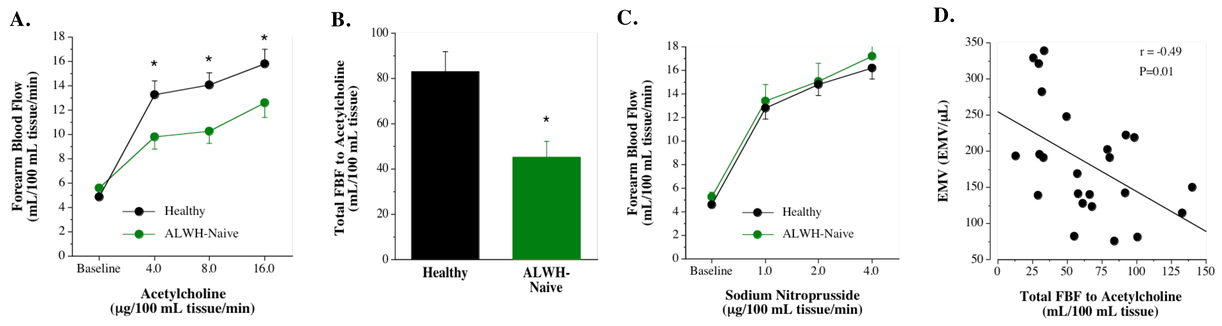
Variable	Healthy (n=12)	Treatment naïve ALWH (n=12)
Total cholesterol, mg/dL	177±9	174±12
HDL-cholesterol, mg/dL	44±3	37±2
LDL-cholesterol, mg/dL	109±6	108±10
Triglycerides, mg/dL	111±15	148±22
Glucose, mg/dL	87±2	90±3
Insulin, μU/mL	7.0±0.8	9.8±1.7

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Values are mean±SEM.

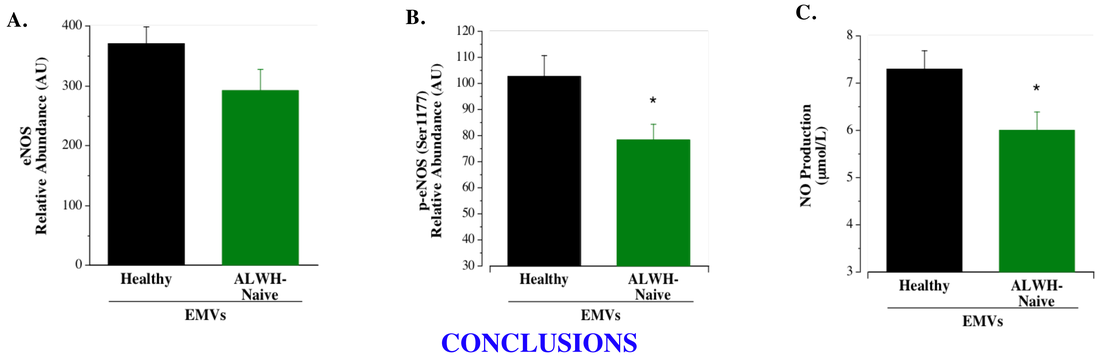
III. Circulating EMV levels were significantly (~75%) higher in treatment-naïve ALWH compared with healthy adults. *P<0.05 vs. Healthy.



IV. FBF response to acetylcholine (panel A) and the total FBF response to acetylcholine (panel B) were significantly lower (~20%; P<0.001) in ALWH vs. healthy adults. There was no significant difference in FBF responses to sodium nitroprusside between the groups (panel C). EMVs were strongly and inversely associated with the vasodilator response to acetylcholine (r=-0.49; P=0.01) (panel D). *P<0.05 vs. Healthy.



V. The expression of eNOS was not significantly different in HCAECs treated with EMVs from ALWH vs. healthy adults (panel A). Expression of p-eNOS (Ser1177) (panel B) and NO production (panel C) were significantly lower in HCAECs treated with EMVs from treatment naïve ALWH. *P<0.05 vs. Healthy.



CONCLUSIONS

- HIV-1 is associated with elevated circulating EMVs.
- Circulating EMVs are not only a systemic biomarker but appear to be a mechanistic factor underlying endothelial dysfunction with HIV-1.
- EMVs represent a novel systemic, causative factor underlying vascular abnormalities and the increased CVD risk associated with HIV-1.