Circulating Endothelial Microvesicles with HIV-1 Promote Cerebral Endothelial Cell Stroke Profile

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ABSTRACT

The incidence of ischemic stroke in adults living with HIV (ALWH) is three times higher than healthy adults. Circulating endothelial cell-derived microvesicles (EMVs) have been linked to cerebrovascular events. We have previously reported that EMVs isolated from ALWH receiving antiretroviral therapy (ART) impair brain endothelial cell nitric oxide (NO) production and fibrinolytic capacity, central etiologic mechanisms in the pathogenesis of ischemic stroke. However, it is unknown whether the pathologic EMV phenotype is a consequence of HIV-1 per se or ART. The experimental aim of this study was to determine the effect of EMVs isolated from treatment naïve ALWH on brain endothelial cell nitric oxide NO production and fibrinolytic capacity. Circulating EMVs (CD 144-PE) were isolated (flow cytometry) from 16 young and middle-aged men (age range: 21-43 yr): 8 healthy (age 33±3 yr; BMI: 26.0±1.2 kg/m 2 ; BP: 113/72±2/2 mmHg) and 8 treatment naïve ALWH (8M; 36±2 yr; 25.3±1.5 kg/m 2 ; BP: 117/76±3/3 mmHg; viral load: 7200 copies/mL). All men were free of overt cardiometabolic disease and not taking any medication. Human cerebral microvascular endothelial cells (hCMECs) were cultured and separately treated with EMVs from each subject. Circulating EMVs were significantly higher in the treatment naïve ALWH (229±23 EMV/μL) compared with healthy adults (133±3 EMV/μL). Although total endothelial nitric oxide synthase (eNOS) expression was not significantly altered (60.1±2.3 vs 65.3±2.1 AU); active eNOS (Ser1177) (19.0±0.8 vs 26.6±1.5 AU) and, in turn, NO production (5.7±0.2 vs 6.7±0.3 μmol/L) was lower (P<0.05) in cells treated with EMVs from treatment naïve ALWH vs EMVs from healthy adults. HIV-associated EMVs also significantly reduced tissue-type plasminogen activator (t-PA) (25.5±1.2 vs 34.7±1.7 AU) and increased plasminogen activator inhibitor (PAI)-1 (146.0±4.5 vs 110.0±3.8 AU) protein expression in hCMECs. The t-PA:PAI-1 intracellular protein ratio (5.9±0.3 vs 3.3±0.1 AU; P<0.05) was higher in HIV-1 EMV treated cells, indicative of decreased fibrinolytic capacity. HIV-1, independent of traditional risk factors and ART, is associated with a pathologic circulating EMV phenotype. Reduced NO bioavailability and impaired fibrinolytic capacity in brain endothelial cells heighten the risk and accelerated rate of ischemic stroke. EMVs represent a mechanistic factor underlying HIV-1-related cerebrovascular risk.

BACKGROUND

- The incidence of ischemic stroke in adults living with HIV-1 (ALWH) is three times higher than healthy adults.
- Endothelial dysfunction, specifically reduced nitric oxide (NO) production and impaired endothelial tissue-type plasminogen activator (t-PA) release is a central etiologic feature in the development of ischemic stroke and associated thrombotic events.
- Circulating endothelial cell-derived microvesicles (EMVs) have been linked to cerebrovascular events.
- We have previously reported that EMVs isolated from ALWH receiving antiretroviral therapy (ART) impair brain endothelial cell NO production and t-PA release.
- However, it is unknown whether the pathologic EMV phenotype is a consequence of HIV-1 per se or ART.

AIM

The experimental aim of this study was to determine the effect of EMVs isolated from treatment naïve ALWH on brain endothelial cell NO production and fibrinolytic capacity.

METHODS

Subjects

- 16 young, midlife men were studied:
  - 8 healthy (8M: age: 33±3 yr)
  - 8 treatment naïve ALWH (8M; 36±2 yr; viral load: 7200 copies/ml; CD4 count: 650 cells/μL)

Inclusion Criteria

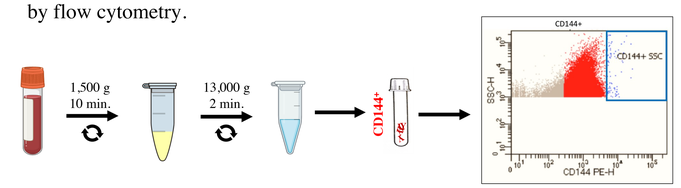
- Sedentary
- Non-smoker
- Normotensive
- Normoglycemic
- Normolipidemic
- Free of overt cardiovascular disease

Screening and Testing Procedures

- Medical history and physical examination
- Fasting blood chemistries
- Body composition (dual energy X-ray absorptiometry)

EMV Identification and Enumeration

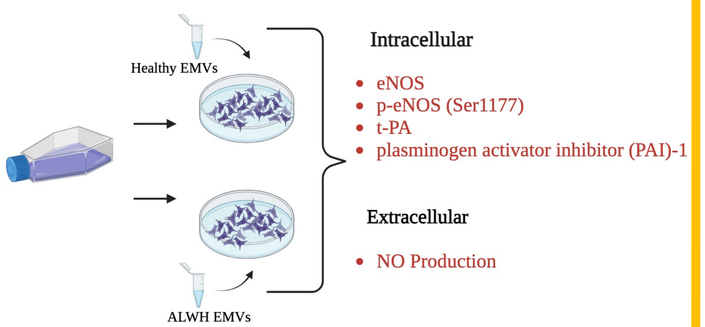
- EMV phenotype was determined using fluorescent-conjugated antibodies specific to markers indicative of endothelial lineage ($CD144^+$) and enumerated by flow cytometry.



$$\frac{\text{\# OF EVENTS IN REGION CONTAINING EMVs}}{\text{\# OF ABSOLUTE COUNT REGION}} \times \frac{\text{TOTAL \# OF BEADS PER TUBE}}{\text{TOTAL SAMPLE VOLUME}} = [\text{EMV}/\mu\text{L}]$$

hCMEC Culture

- Cells were seeded into flasks and grown to 100% confluence. After the third passage, cells were seeded into six well-plates. Thereafter, hCMECs were treated with the isolated EMVs from healthy adults and treatment naïve ALWH.



ELISA

- Enzyme-linked immunosorbent assay (ELISA) was used to detect and measure NO production in the media.

STATISTICAL ANALYSIS

Differences between groups protein values were determined by one-way analysis of variance (ANOVA). Statistical significance was set *a priori* at P<0.05. Values are mean±SEM.

RESULTS

I. Selected Physical Characteristics

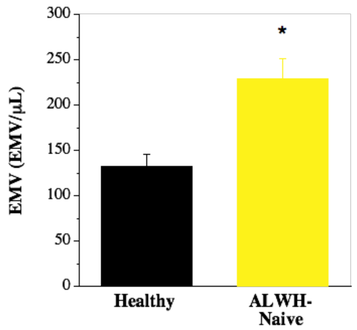
Variable	Healthy (n=8)	Naïve ALWH (n=8)
Age, yrs,	33±3	36±2
Body mass, kg	76.8±11.6	73.9±4.6
BMI, kg/m^2	26.0±1.2	25.3±1.5
Body fat %	22.3±2.5	23.5±3.4
Systolic BP, mmHg	113±2	116±4
Diastolic BP, mmHg	72±2	76±3

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

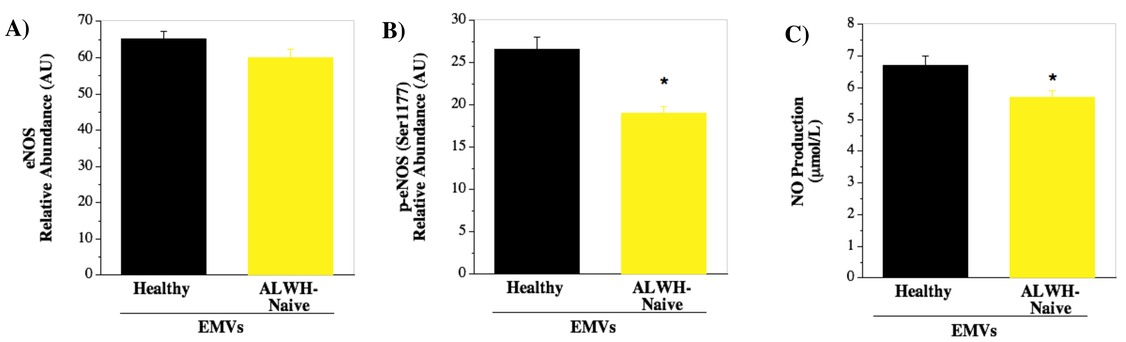
II. Metabolic Characteristics

Variable	Healthy (n=8)	Naïve ALWH (n=8)
Total cholesterol, mg/dL	176±9	170.2±17
HDL-cholesterol, mg/dL	43±4	37±3
LDL-cholesterol, mg/dL	110±6	105±14
Triglycerides, mg/dL	118±21	140±22
Glucose, mg/dL	88±2	90±4
Insulin, μU/mL	7.7±1.0	8.8±2.2

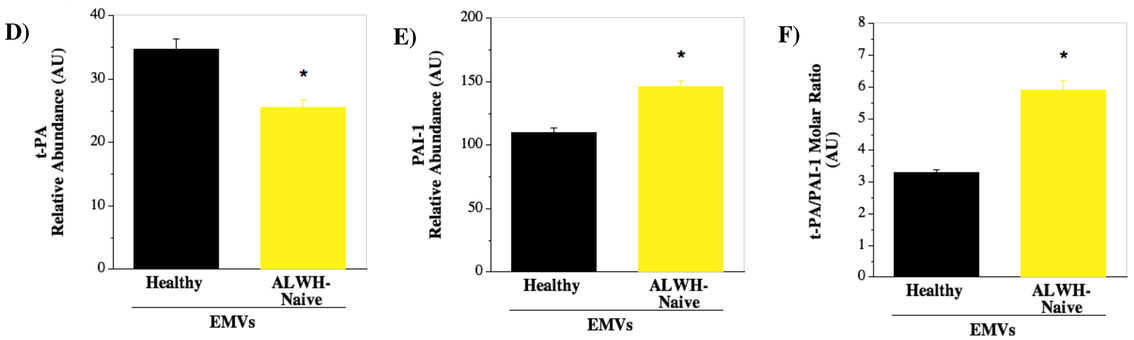
HDL, high-density lipoprotein; LDL, low-density lipoprotein. Values are mean±SEM.



III. Circulating EMVs were significantly higher (~50%; *P<0.05) in the treatment naïve ALWH compared with healthy adults.



IV. Total endothelial nitric oxide synthase (eNOS) expression (**A**) was not significantly different in hCMECs treated with EMVs from healthy adults compared with treatment naïve ALWH; however, p-eNOS (Ser1177) (**B**) and NO production (**C**) were significantly lower (~25%; *P<0.05) in hCMECs treated with EMVs from treatment naïve ALWH.



V. t-PA expression was significantly lower (~35%; *P<0.05) in hCMECs treated with EMVs from treatment naïve ALWH compared with healthy adults (**D**). Expression of PAI-1 was significantly higher in the hCMECs treated with EMVS from treatment naïve ALWH (**E**). Consequently, expression of t-PA/PAI-1 protein ratio (**F**) were significantly higher (~35%; *P<0.05) in hCMECs treated with EMVs from treatment naïve ALWH.

CONCLUSIONS

- EMVs from treatment naïve ALWH reduced brain endothelial cell NO production and impaired endothelial fibrinolytic capacity.
- Circulating EMVs may contribute to the increased risk of cerebrovascular events associated with HIV-1.