467: EXTRACELLULAR VESICLES IN ALWH PROMOTE CEREBRAL ENDOTHELIAL PROFILES LINKED TO STROKE

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ABSTRACT

Background: The incidence of ischemic stroke in adults living with HIV (ALWH) is three times higher than uninfected adults. Mechanisms underlying this heightened rate of ischemic stroke are poorly understood. Circulating endothelial cell-derived microvesicles (EMVs) have been linked to cerebrovascular events. We have previously reported that EMVs are elevated in ALWH; whether HIV associated EMVs promote endothelial abnormalities known to increase stroke risk is unknown. The experimental aim of this study was to determine the effect of EMVs isolated from ALWH on brain endothelial cell inflammation, nitric oxide (NO) and endothelin (ET)-1 production and fibrinolytic capacity.
Methods: Circulating EMVs (CD 144-PE) were isolated (flow cytometry) from 18 adults: 9 healthy (6M/3F; age 55±1 yr; BMI: 24.2±0.5 kg/m^2) and 9 ALWH on stable antiretroviral therapy (6M/3F; 53±2 yr; 26.1±0.8 kg/m^2). Human cerebral microvascular endothelial cells (hCMECs) were cultured and separately treated with EMVs from each subject.
Results: EMVs from ALWH induced significantly greater release of interleukin (IL)-6 (32.2±1.6 vs 23.4±1.2 pg/mL) and IL-8 (792.8±26.7 vs 661.3±36.4 pg/mL) vs EMVs from healthy adults. Concordantly, HIV-1-related EMVs induced higher (P<0.05) expression of phosphorylated-NF-κB p65 (Ser536; active NF-κB) (14.9±0.6 vs 10.8±0.7 AU). Although total endothelial nitric oxide synthase (eNOS) expression was not significantly altered; active eNOS (pSer1177) (46.7±2.8 vs 57.2±2.1 AU) and, in turn, NO production (6.0±0.3 vs 7.7±0.3 mmol/L) was lower (P<0.05) in cells treated with EMVs from ALWH. HIV-associated EMVs also significantly increased the expression of endothelin converting enzyme (39.2±1.3 vs 33.4±1.4 AU) and ET-1 production (35.6±0.9 vs 30.6±0.8 pg/mL), reduced tissue plasminogen activator (t-PA) (8.7±0.4 vs 10.8±0.6 AU) and increased plasminogen activator inhibitor (PAI)-1 (25.4±1.5 vs 18.1±1.0 AU) protein expression in hCMECs. The tPA:PAI-1 protein ratio (2.9±0.1 vs 1.8±0.1 AU; P<0.05) was higher in HIV-1 EMV treated cells, indicative of decreased fibrinolytic capacity.
Conclusion: EMV-induced increase in inflammation, reduction in NO bioavailability, increased ET-1 production and impairment in fibrinolytic capacity in brain endothelial cells may contribute to the heightened risk and accelerated rate of ischemic stroke with HIV-1. EMVs represent a potential therapeutic target for reducing cerebrovascular risk in ALWH.

BACKGROUND

- Adults living with HIV-1 (ALWH) are at three times increased risk of ischemic stroke compared with non-infected adults. However, the mechanisms underlying this increased risk are not completely understood.
- Circulating endothelial cell-derived microvesicles (EMVs) are associated with increased risk and severity of ischemic stroke as well as outcome.
- EMVs are anucleoid extracellular vesicles, 0.1-1μm in diameter, released from endothelial cells in response to stimuli that promote activation and/or apoptosis.
- We have previously reported that EMVs are elevated in ALWH; whether HIV associated EMVs promote endothelial abnormalities known to increase stroke risk is unknown.

EXPERIMENTAL AIM

The experimental aim of this study was to determine the effect of EMVs isolated from ALWH on brain endothelial cell inflammation, nitric oxide (NO) and endothelin (ET)-1 production and fibrinolytic capacity.

METHODS

Subjects

- Eighteen middle-aged adults were studied:
  - 9 healthy adults (6M/3F)
    - Age range: 47-62 yrs
    - BMI: 25.8±3.2 kg/m^2
  - 9 ALWH (6M/3F)
    - Age range: 44-61 yrs
    - BMI: 26.1±0.8 kg/m^2
    - HIV-1-seropositive > 12 months
    - Mean duration of antiretroviral therapy: 62±11 months
    - CD4$^+$ T cell count: 539.1±82.2 cells/μL
    - Virologic suppression < 50 copies/mL

Inclusion Criteria

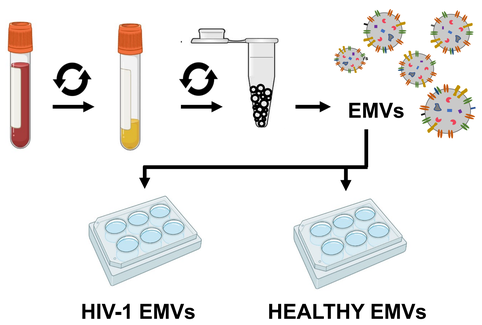
- All subjects were sedentary, normotensive, normolipidemic, normoglycemic and free of overt cardiometabolic disease.

Screening and Testing Procedures

- Medical history with physical examination
- Graded exercise test
- Fasting blood chemistries
- Body composition (DXA scan)

EMV Isolation

- EMVs (CD144+) were isolated from plasma by ultracentrifugation, identified and collected by fluorescence activated cell sorting.



Cell Culture

- Human cerebral microvascular endothelial cells (hCMEC/D3) were cultured and treated (2:1) with EMVs isolated from either healthy adults or ALWH, taking combination antiretroviral therapy, for 24hr.

Analysis

- Expression of specific proteins were analyzed by capillary electrophoresis immunoassay.
- NO and ET-1 production were determined in cell supernatant by enzyme-linked immunoassay.

STATISTICAL ANALYSIS

Distribution of data was assessed by the Shapiro-Wilk test. Differences in subject characteristics, cellular expression of proteins, NO and ET-1 production were determined by analysis of variance. All data are presented as mean±SEM. Statistical significance was set a priori at P<0.05.

RESULTS

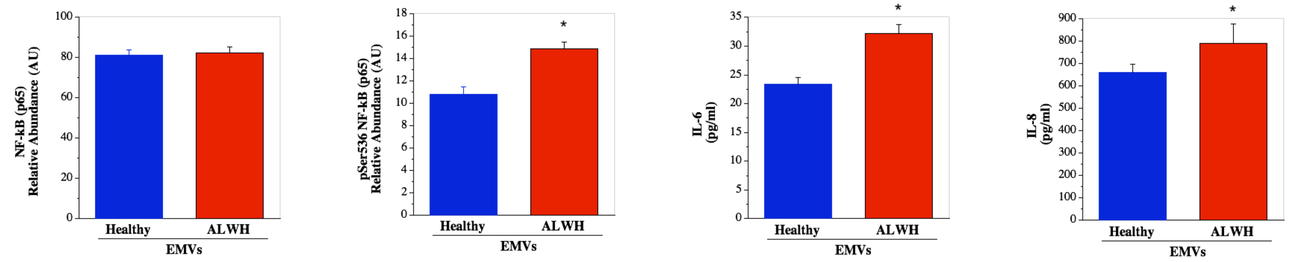


Figure 1. There was no difference in the total expression of nuclear factor kappa B (NF-κB) in cells treated with EMVs isolated from ALWH compared with EMVs from healthy adults. However, EMVs isolated from ALWH induced significantly greater expression of phospho-NF-κB (Ser536) (~40%), and release of IL-6 (~40%) and IL-8 (~20%) compared with EMVs from healthy adults. * P<0.05.

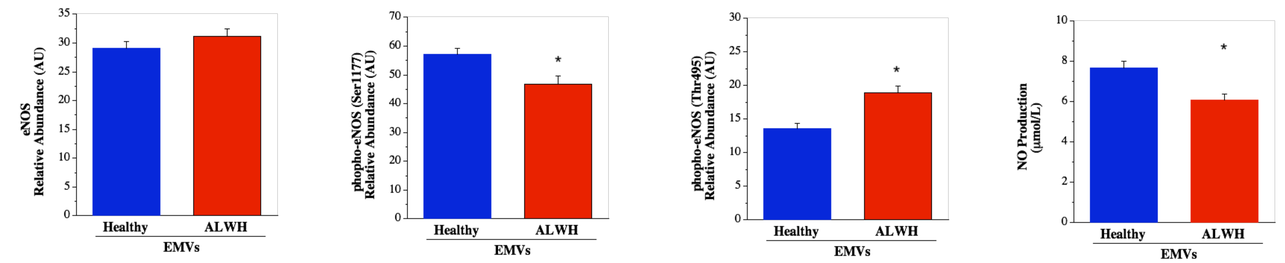


Figure 2. There was no difference in the total expression of endothelial nitric oxide synthase (eNOS) in cells treated with EMVs isolated from ALWH compared with EMVs from healthy adults. However, EMVs isolated from ALWH significantly reduced expression of activated, or phospho-eNOS (Ser1177) (~20%), increased inhibitory site phosphorylation, phospho-eNOS (Thr495) (~40%) and concomitantly decreased NO production (~25) compared with EMVs from healthy adults. * P<0.05.

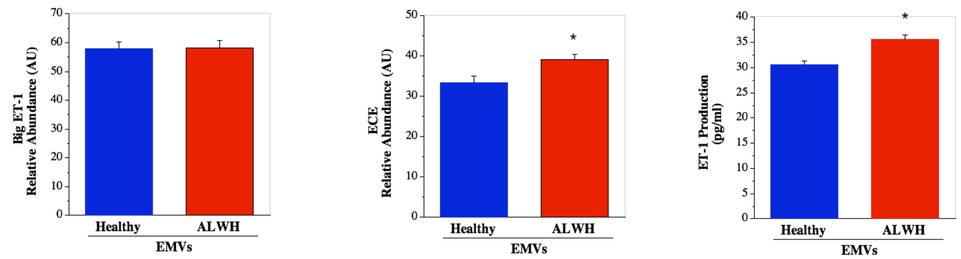


Figure 3. There was no difference in the total expression of big ET-1 in cells treated with EMVs isolated from ALWH compared with EMVs from healthy adults. However, EMVs from ALWH significantly increased expression of endothelin converting enzyme (ECE) (~20%) as well as ET-1 production (~20%). * P<0.05.

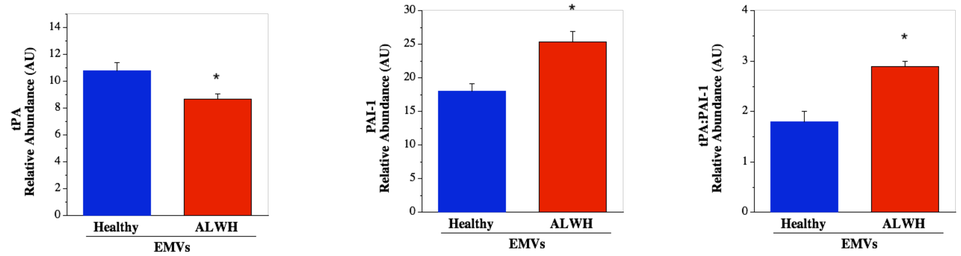


Figure 4. EMVs isolated from ALWH markedly reduced expression of tissue plasminogen activator (tPA) (~20%) and increased expression of plasminogen activator inhibitor-1 (PAI-1) (~40%) compared with EMVs from healthy adults. The tPA:PAI-1 protein ratio was significantly increased (~60%) in cells treated with EMVs from ALWH. * P<0.05.

CONCLUSIONS

- EMV-induced increase in inflammation, reduction in NO bioavailability, increased ET-1 production and impairment in fibrinolytic capacity in brain endothelial cells may contribute to the heightened risk and accelerated rate of ischemic stroke with HIV-1.
- EMVs represent a potential therapeutic target for reducing cerebrovascular risk in ALWH.