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Investigating endothelial dysfunction as a pathophysiological consequence of HIV-infection and anti-retroviral treatment.

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Introduction: Endothelial dysfunction (ED) is characterized by decreased nitric oxide (NO) bioavailability and increased oxidative stress creating a pro-inflammatory environment. Increased ED and cardiovascular risk have been observed in HIV-1 infected patients. With cardiovascular disease identified as an important comorbidity with HIV-infection, elucidating the link between HIV and antiretroviral treatment (ART) by studies such as this one will aid decision-making about HIV-treatment for patients who exhibit cardiovascular risk.

Purpose: The main aim of the study was to investigate ED as a pathophysiological consequence of HIV-infection and ART. Ex vivo study: determining the effects of a fixed-dose combination of nucleoside and non-nucleoside reverse-transcriptase inhibitors (NRTI/NNRTIs) (efavirenz, emtricitabine and tenofovir) (ART1) on the vascular function of aortas from rats and the signalling proteins associated with ED in aortic tissue. In vitro study: determining the effects of ART1 and protease inhibitors (PIs) (lopinavir and ritonavir) (ART2) on aortic endothelial cells (AECs) (co-treated with a cocktail of recombinant HIV-1 proteins: Nef, Tat and Gp160) (HIV-1) on cell viability, oxidative stress and NO production.

Methods: Ex vivo: Rats received ART1 for 6 weeks (once, daily) before being sacrificed and thoracic aortas excised. Aortic ring function was evaluated. Aortic tissue was snap-frozen / stored for western blot analysis of signalling of NO (eNOS and PKB/Akt), NFkB (total IKBa) and oxidative stress (nitrotyrosine and p22-phox). In vitro: Firstly, AECs were treated with HIV-1 proteins (100ng/ml / 24 hours) to simulate an HIV-1 infection microenvironment. Secondly, AECs were co-treated with HIV-1 and three dosages of ART1 or ART2. Cell viability, oxidative stress and NO production were assessed by fluorescence.

Results: Ex vivo: ART1 showed increased aortic ring relaxation vs. untreated controls (p<0.0001). Phosphorylated eNOS was upregulated in ART1 vs. untreated controls (2.33±0.35 vs. 1±0.11, p < 0.05). Total PKB/Akt was downregulated in ART1 vs. untreated controls (0.26±0.04 vs. 1±0.12, p < 0.05), while phosphorylated PKB/Akt (as a ratio of total) was upregulated in ART1 vs. untreated controls (3.36±0.19 vs. 1±0.13, p < 0.05). In vitro: HIV-1 decreased NO production (100±1.25% vs. 76.00±3.67%; p<0.05), with no effect on cell viability and oxidative stress. ART2+HIV-1 decreased NO production (control: 100±0.72% vs. medium: 94.47±1.91%; high: 84.67±1.41%; p<0.05).

Discussion / Conclusion: In our ex vivo model, NRTI/NNRTIs seemed to be beneficial to vascular function (increased relaxation). This might be due to the upregulated PKB/Akt-eNOS signalling. In our in vitro model, HIV-proteins decreased NO production in AECs. Co-treatment with NRTI/NNRTIs seemed to ameliorate the NO-lowering effect of treatment with HIV-1 proteins only, while co-treatment with PIs seemed to be harmful.
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