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Pre-exposure Prophylaxis Therapy and the Blood-brain Barrier: Is PrEP Neuroprotective?

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To the Editor;

Pre-exposure prophylaxis (PrEP)[combination emtricitabine (FTC, 200mg) and tenofovir disoproxil fumarate (TDF, 300mg)] prevent human immunodeficiency virus type 1 (HIV)-infection with 99% effectiveness when taken daily(Division of HIV/AIDS Prevention, 2018). However, little is known about its effects on the blood-brain barrier (BBB). The vast area of the BBB(~12m²) is thought to be the main route of HIV-seeding in the brain through infected blood monocytes(D'Antoni et al., 2018; Sweeney et al., 2018). HIV crosses the BBB and replicates, causing inflammation and BBB dysregulation, leading to HIV-associated neurocognitive disorders (HAND) affecting 20–50% of persons living with HIV (PLWH)(Gates et al., 2016; D'Antoni et al., 2018). Since HAND is a major cause of morbidity for PLWH and HIV enters the central nervous system as early as eight days post-infection, it is important to determine the impact of PrEP on the BBB and PrEP strategies that may be neuroprotective (Valcour et al., 2012).

Efficacy of PrEP drops significantly with non-adherence (44–75%) and due to concerns of drug resistance, additional strategies have been proposed, such as replacement with or inclusion of a CCR5-inhibitor such as Maraviroc (MVC) (Neff et al., 2010; Veselinovic et al., 2014; Gulick et al., 2017; Division of HIV/AIDS Prevention, 2018). Data suggests MVC reduces HIV-infected monocyte transmigration across the BBB and improves cognition in

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Conflict of Interest

No conflicts are reported.

PLWH(Ndhlovu et al., 2014; Gates et al., 2016). Although MVC alone is insufficient as PrEP, MVC in combination with TDF/FTC appears to be safe and potentially effective in preventing HIV infection in a phase II clinical trial, meriting further exploration as a neuroprotective addition to current PrEP(Neff et al., 2010; Massud et al., 2013; Gulick et al., 2017).

We present a brief assessment of PrEP with and without MVC on human adult primary BMVEC and on HIV-negative monocyte transmigration across an *in vitro* bilayer BBB model.

Indirect Enzyme-linked immunosorbent assays (ELISAs) were completed to assess occludin, zonula occludens (ZO-1), platelet endothelial cell adhesion molecule 1 (PECAM-1), and intercellular adhesion molecule 1 (ICAM-1). Human adult primary BMVEC (Angio-Proteomie, MA) were grown to confluence on rat-tail collagen type I-coated (50 µg/mL) flat-bottomed 96-well plates with or without PrEP (0.1 µM) and/or MVC (0.2 µM). Cells were fixed and blocked using 5% serum. Primary antibodies were used at dilutions: occludin (1:500), ZO-1 (1:50), PECAM-1 (1:1,000), and ICAM-1(1:200) followed by biotinylated secondary antibody. Avidin DH and Biotinylated Alkaline Phosphatase H (Vector Laboratories, CA) was used and the reaction developed with p-nitrophenyl-phosphate.

Nitrophenol was quantified spectrophotometrically at 405 nm. Results were adjusted to controls containing secondary antibody only. Bradford assays were used to normalize to total protein concentration and analyzed using a 10-parameter logistical standard curve with GraphPad Prism Software (GraphPad Software, CA). Mann-Whitney tests compared expression between conditions: no drug, PrEP, MVC, and PrEP+MVC.

Occludin, ZO-1, PECAM-1, and ICAM-1 expression were visualized via immunofluorescence after primary BMVEC were grown to confluence on a rat-tail collagen type I-coated (50 µg/mL) glass coverslips with or without PrEP (0.1 µM) and/or MVC (0.2 µM). Cells were fixed and blocked using 5% serum. Primary antibodies were used at dilutions: occludin (1:166), ZO-1 (1:100), PECAM-1 (1:250), and ICAM-1 (1:1,000) followed by fluorophore-conjugated secondary antibody. Mounted coverslips were imaged using a fluorescent microscope at 20X (Leica, IL) and analyzed using ImageJ Software (National Institutes of Health, USA).

HIV-negative subjects initiating PrEP and at 12 weeks post-PrEP were recruited to assess the impact of PrEP with and without MVC on monocyte transmigration across an *in vitro* bilayer BBB model. Peripheral blood mononuclear cells (PBMCs) were isolated and resuspended at 1×10^6 cells/mL. Attune NxT Flow Cytometry Software (ThermoFisher, USA) was used to analyze CD3-CD14+ cells (monocytes) in PBMC samples at entry and post-PrEP treatment prior to transigrations.

In vitro bilayer BBB models were constructed using primary BMVEC (2×10^4) and primary adult astrocytes (10×10^4) (Angio-Proteomie, MA) cultured on opposite sides of 24-well polyethylene terephthalate inserts containing 3µm pores coated with rat-tail collagen type I (50 µg/mL) and grown to confluence over six days with trans-endothelial electrical

resistance confirmed $>160 \text{ ohm/cm}^2$. BBBs were switched to medium without growth factors 12–16 hours prior to experiments. 0.5×10^6 PBMC were added to the apical side of each BBB. Transmigrations were 24 hours at 37°C , 5% CO_2 with 100 ng/mL MCP-1 as a chemoattractant for monocytes. To assess the effect of MVC on monocyte transmigration, $0.2 \mu\text{M}$ MVC was added *in vitro* to a subset of BBBs at 12 weeks post-PrEP treatment. Transmigrated cells were harvested and stained with CD3 and CD14 and analyzed using Attune NxT Flow Cytometry Software and FlowJo Software (FlowJo, USA). Post-transmigration BBB integrity was determined using 0.45% Evans Blue dye-conjugated bovine serum albumin. Mann-Whitney tests assessed differences between groups: no drug, PrEP, PrEP+MVC.

PrEP/MVC/PrEP+MVC treatment on primary BMVEC via ELISA (Fig 1a) and immunofluorescence (Fig 1b) showed no changes in BBB protein expression except for an increase of the tight junction protein occludin ($p < 0.01$) with PrEP/MVC/PrEP+MVC treatment.

Experiments involving subjects instituting PrEP are limited by the small number of subjects and adherence via plasma concentration was not determined. However, two of three subjects, subjects B ($p = 0.016$) and C ($p = 0.0952$) showed reductions in the percentage of monocytes transmigrated across an *in vitro* bilayer BBB model after 12 weeks with PrEP and PrEP+MVC treatment. In combination with ELISA analysis showing increased expression of tight junction protein occludin ($p < 0.01$) with PrEP/PrEP+MVC and corresponding immunofluorescence, these results suggest that current PrEP, both with and without MVC, may be neuroprotective. To our knowledge, this has not been previously reported. Thus, further studies are needed to confirm the results. The study was supported in part by U54MD007584, U54MD007601, R01MH102196, P30GM114737.

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Figure.
a) ELISA analysis of PrEP, MVC, PrEP+MVC on primary BMVEC characteristics (passage 9, n=3–5). **b)** Immunofluorescence analysis of PrEP, MVC, PrEP+MVC on primary BMVEC characteristics (passage 9). **c)** Percentage monocytes transmigrated in a subset of three subjects pre- and post- 12-weeks of a PrEP regimen with and without an *in vitro* addition of 0.2μM MVC.