Improved cytotoxicity of antiretroviral drug Lopinavir in combination with flavonoid Apigenin in a manner similar to the antagonist of Aryl-hydrocarbon receptor

Dominic Sales[1], Edward Lin[1], Victoria Stoffel[1], Marzia Niazi[2], Tania Mulherkar[1], Joris Beld[1], Joseph Salvino[3], and Pooja Jain[1]

[1]Department of Microbiology and Immunology, Drexel University College of Medicine
[2]Sidney Kimmel Cancer Center at Thomas Jefferson University
[3]The Wistar Institute, University of Pennsylvania, Philadelphia, PA, USA

Background
HTLV-1 infection, while mostly resulting in asymptomatic infections, can induce the development of Adult T-cell Leukemia/lymphoma (ATLL). Currently, no effective treatment exists for either disease manifestation, and despite a vigorous immune response, the virus is never eliminated. In previous studies we observed that Apigenin, a flavonoid compound, can exert immunomodulatory effects to reduce neuroinflammation. In this study we assessed activity of Apigenin against HTLV-1 in combination with antiretroviral drug (ART), Lopinavir (LPN) via an underlying mechanism involving aryl-hydrocarbon receptor (AhR), which is a ligand-activated transcription factor. Flavonoids are natural ligands for AhR that is constitutively active in tumor cells including ATLL. Therefore, we hypothesized that Apigenin modulates AhR pathway to increase sensitivity of HTLV-1 infected cells to ART.

Methods
HTLV-1 infected cell lines and freshly isolated PBMCs were used in this study. RNA expression was measured with qRT-PCR. Protein expression was measured by western blotting. Ligand-receptor binding was quantitated by nano-isothermal calorimetry. Intracellular concentrations were determined by mass-spectrometry. Expression of AhR was analyzed by flow cytometry and cytotoxicity was assayed by the colorimetric MTT assay to calculate IC_{50} values.

Results
We observed that Apigenin and its prodrug derivative can enter normal PBMCs, and infected cell lines. We also established direct interaction (via Nano-ITC) of Apigenin with AhR. In normal PBMCs, Apigenin activates AhR signaling by suppressing the expression of its repressor (AHRR) and altering expression of downstream factors. This leads to an improvement in the viability of normal T cells. However, in HTLV-1-infected cell lines, both Apigenin and VY-3-068 cooperated with LPN to impart cytotoxicity by exhibiting a major shift in LPN's IC_{50} values, similar to what was seen with the AhR antagonist CH223191.

Conclusion
Taken together, these results suggest the potential combinatorial use of Apigenin derivatives with current first-line antiretrovirals for the benefit of patients affected by HTLV-1 infection.

Disclosure of Interest Statement:
Nothing to disclose.