T-Cell Marker Activation, and Mitochondrial Membrane Integrity after Exposure to Lopinavir, Ritonavir or Efavirenz on HIV-negative CD4+ Lymphocytes

5th IAS Conference on HIV Pathogenesis, Treatment and Prevention
Cape Town, South Africa
July 19-22, 2009

MOPEA018

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Abstract

Background: Recent data suggest that although efavirenz (EFV) has a better rate of virologic suppression compared to lopinavir/ritonavir (LPV/r), LPV/r has a better CD4+ count response. While an earlier study showed no immunologic difference with protease inhibitors (PIs), it did not look at markers of T-cell activation. This study assessed the effect of LPV, RTV, LPV/r, and EFV on immune function of activated human CD4+ lymphocytes.

Methods: 24cc of blood was drawn from 16 HIV- controls (sample lost for 1 subject). Peripheral Blood Lymphocytes were washed and sub-cultured, then activated with IL-2. Cells were then exposed to 1.0uM LPV, 1.0uM RTV, LPV and RTV together, and 1.0uM EFV for 72 hours, or unexposed for control. Cells were stained with fluorochrome-tagged monoclonal antibody to CD4, CD25, CD38, and HLD-DR. Analysis of surface marker intensity was performed by flow cytometry, gated against CD4. PBL apoptosis was assessed using a mitochondrial membrane integrity assay [MitoProbe JC-1 assay kit for flow cytometry (M34152) molecular probes]. A system hardware/software error resulted in loss of data from some samples.

Results: No significant change in CD25, CD38 or HLA-DR receptor integrity was seen between cells treated with drug and control. There was an ~40% increase in CD4+ receptor intensity with both LPV and EFV compared to control (n=7; p=0.02 and 0.03). Mitochondrial membrane integrity increased by more than 25% in LPV/r exposed cells (n=5; p=0.017), but not in other cells; EFV had no effect (n=13; p=0.2).

Conclusions: Both LPV and EFV improved CD4 receptor intensity. The combination of LPV/r improved apoptosis markers, which may explain the improved immunologic outcomes seen in ACTG 5142. The results of the T-cell activation markers do not explain the difference in CD4 response seen in that study. Further studies should assess other antiretroviral agents and the clinical impact of these findings.

Background

Recent studies, including the ACTG 5142, have shown that despite improved virologic suppression of the NNRTI efavirenz compared to lopinavir/ritonavir, the subjects treated with lopinavir/ritonavir had a better immunologic response (as measured by increases in CD4 cells). The question then is why is this difference present? Possible reasons for this include better tolerability with efavirenz, but better virologic suppression in those subjects who were able to remain on the lopinavir/ritonavir regimen, and immune stimulating effects from the protease inhibitor(s). The latter point can be measured in a research laboratory setting.

Study Objectives

- The primary objective of the study was to compare the ex-vivo effects of selected protease inhibitors (PI), lopinavir, ritonavir (at boosting dose concentrations), and a non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz, on the function of non-HIV infected t-cells.
- The secondary objectives of this study were to compare the above medications on T-cell growth.
- We hypothesized that the protease inhibitor would result in less apoptosis and improved growth compared to the NNRTI.

Methods

- Sixteen HIV negative subjects (9 males, 7 females; mean age 30.8yrs, range 26-40) were recruited from an HIV testing clinic in Los Angeles and from Western University of Health Sciences.
- All subjects signed an informed consent approved by the Western University IRB.
- 6 subjects were Latino/a, 4 Caucasian, 4 Asian, 2 African American.
- None had any self-reported immune-related diseases.

***Ex-Vivo* Testing**

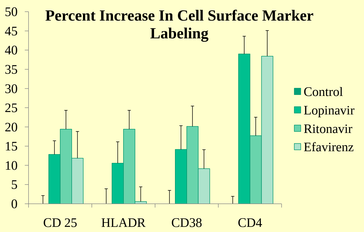
- PBL isolation and culturing: Human PBL from healthy donors are isolated from freshly heparinized (citrate?) blood using a one step FICOLL™ HYPAQUE™ cell separation system (BD 362753 Vacutainer® CPT™ Cell Preparation Tube with Sodium Heparin or BD 362761 Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate) and washed three times in PBS. PBL are subcultured in 25- or 75-cm^2 Falcon plastic flasks (BD 353110 and BD at a density of 1 x 106 cells/ml in RPMI 1640 with L-glutamine (Invitrogen 11875-119) with 10% FBS (Invitrogen 10437-077), 1% nonessential amino acids (Invitrogen 11140-050), penicillin (100 IU/ml), and streptomycin (100mg/ml) (Invitrogen 10378-016) at 37oC in a humidified 5% CO2 atmosphere.
- T cell activation: T cells are activated for 72 h with Phytohemagglutinin (PHA 2ug/ml; Invitrogen 10576-015) and IL-2 (60 IU/ml; Invitrogen PHC0023).
- Drugs and chemicals: For in vitro treatments, pure lyophilized powders of lopinavir, ritonavir, and efavirenz were kindly provided by the manufacturers. For cell surface expression measurements, fluorochrome conjugated anti-CD4, anti-CD69, anti-CD38, and anti-HLA-DR antibodies, along with their appropriate fluorochrome-conjugated Igs as negative controls were used (All from Invitrogen/Caltag).
- Evaluation of Cell surface receptors: Cell surface receptor CD4 was used to establish the T cell population and only gated CD4+ lymphocytes were considered in further analysis. T cell activation was determined by cell surface marker expression (CD69, CD38, HLA-DR) by flow cytometry (Cytomics FC500; Beckman Coulter) on resting and activated human lymphocytes.
- Apoptosis evaluation: Quantitative analysis of apoptosis was performed on CD4+ gated lymphocytes by flow cytometry using the following methods: 1) DNA fragmentation by TUNNEL; 2) apoptosis cascade activation by Caspase 3, 7, and 8; 3) and mitochondrial membrane integrity by penetration with the cationic dye JC-1.

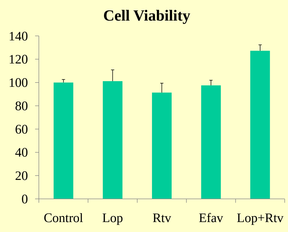
Statistics

Differences in CD marker activation and apoptosis were compared to control and study medications using t-tests and ANOVA.

Results

- Cells for one subject were lost in transport.
- Mechanical error with the flow cytometer resulted in the loss of some data points from the different assays, and all data points from Lopinavir + Ritonavir.
- Lopinavir and efavirenz had similar increases in the CD4 cell marker (39% vs. 38.4%, respectively), which were significantly greater than control or ritonavir (p<0.05).
- The combination of Lopinavir + Ritonavir resulted in a 25% increase in cell viability compared to control (p=0.017); there was not change with any single agent.





Discussion

- All ARV's studied increased T-cell marker intensities, with the effects of lopinavir and efavirenz on CD4 being most pronounced.
- There was no difference in the intensity of CD4 activation between lopinavir and efavirenz.
- While exposure to the antiretrovirals studied one at a time were not significantly different from control on effects on apoptosis and cell viability, the combination of lopinavir and ritonavir (at therapeutic concentrations) resulted in improved cell viability.
- It is unclear at this time of the effects seen with efavirenz on cell viability account for the differences seen in lipoatrophy between study arms in ACTG 5142, but it may explain the improved CD4 cell response seen with lopinavir/ritonavir in this trial.

Conclusions

- This data helps support improvements in CD4 counts seen in clinical trials.
- This data is consistent with data from ACTG 5142, but may not reflect a causal relationship.
- This study involved ex-vivo cells, and a limited number of antiretrovirals. This data should be repeated with more antiretrovirals and more cell-types.
- Unintentional loss of cells and data decreased the number of subjects.

Acknowledgements

- The Authors would like to thank the following people/organizations for their assistance.
  - Patricia F. Kao, MS, PhD Candidate, Boston University School of Medicine for performing the lab assays
  - Abbott Labs for supporting this research grant
  - Robert K. Bolan, MD, Jason Bethel, and The Jeffrey Goodman Clinic for assisting in recruitment of research subjects
  - All of the research subjects, whether they were employees, students or friends