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Microbe-Drug Interactions Between Antiretrovirals and the Gut Microbiome in HIV Infection

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Surgery

INTRODUCTION

For people with HIV (PWH), antiretroviral therapy (ART) typically controls HIV replication and greatly reduces mortality, however, does not cure HIV and PWH must maintain ART treatment indefinitely. During passage through the GI tract, antiretrovirals (ARVs) encounter the gut microbiome, where they may affect the growth of microbes or potentially be metabolically transformed by bacteria into less active or toxic forms. Here, we investigated the *in vitro* ability of several ARVs to alter microbial compositions and growth to better understand how ART may impact the gut microbiome in PWH. Furthermore, we investigated the ability of gut bacteria to metabolize ARVs to understand if the gut microbiome may play a role in bioavailability of ARVs.

METHODS

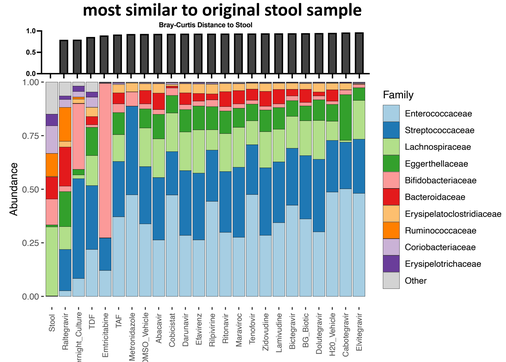
Stool samples from HIV+ and HIV- individuals were resuspended in pre-reduced PBS and used to make glycerol stocks of polymicrobial communities. A HIV- glycerol stock was grown overnight in BG media and diluted to an OD of 0.01 in a 96-well plate containing fresh media with 50 uM of one of the tested ARVs. This plate was grown for 24 hours anaerobically at 37Cº and then spun down to collect the cell pellets which underwent DNA extraction and 16S V4 rRNA sequencing on an Illumina MiSeq. Microbiome analysis was done in R using a variety of packages including Dada2, phyloseq, microViz, and MaAsLin2. Antimicrobial effects of ARVs were tested by growing individual bacterial strains in anaerobic media for 48 hrs containing 50 uM of each ARV. The area under the growth curve was determined using the growthcurver R package and used to compare to control wells. Depletion of ARVs in stool communities was determined by culturing glycerol stocks of stool samples overnight and then distributing this culture into a 96-well plate containing 50 uM of each ARV of interest. This 96-well plate was then incubated for 24 hours with samples collected at 0, 4, and 24 hours for LC-MS/MS prep. The area ratio of each ARV in each timepoint was determined using an in-house LC-MS/MS assay, and foldchanges at either 4 or 24 hours were compared to abiotic wells containing no bacteria.

HYPOTHESIS

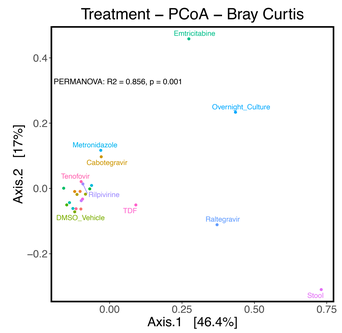
1. ARV drugs can alter the composition of polymicrobial cultures isolated from stool *in vitro*
2. Gut bacteria can metabolize and deplete ARV drugs *in vitro*

Effects of ARVs on the composition of *in vitro* bacterial communities from stool

A. Raltegravir treated *in vitro* stool cultures are most similar to original stool sample



B. Microbial compositions shift overtime in culture and with ARV treatment



C. Alpha diversity of *in vitro* microbial cultures change depending on ARV treatment

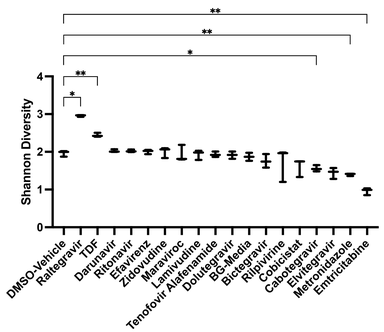
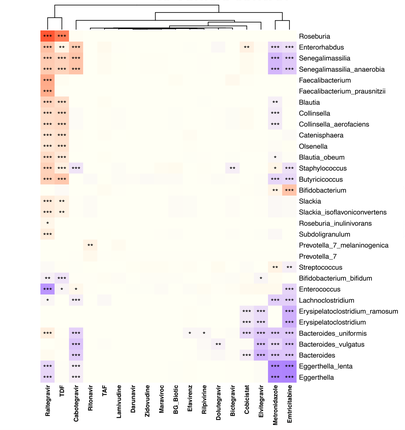


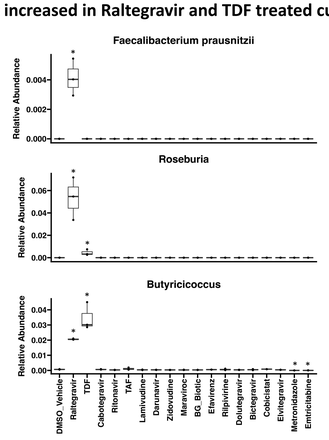
Figure 1. Top 10 family level taxa of the *in vitro* stool communities after exposure to ARVs for 24 hours. Treatment groups are ordered from left to right based on the Bray-Curtis distance from the original stool sample (A). PCoA plot depicting the Bray-Curtis distance of each sample calculated from the ASV level relative abundance table (B). Shannon diversity of each ARV treated culture with pairwise comparisons between treatment and vehicle control performed using Dunnett's T3 multiple comparisons test, * = p < 0.05, ** = p < 0.01. TDF = Tenofovir Disoproxil Fumarate.

ARVs have differential effects on the relative abundance of individual taxa *in vitro*

A. Differentially abundant taxa compared to vehicle control



B. Relative abundance of SCFA producing bacteria is increased in Raltegravir and TDF treated cultures



C. Relative abundance of opportunistic pathogens is reduced in several ARV treated cultures

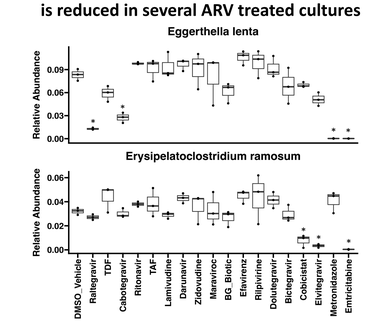
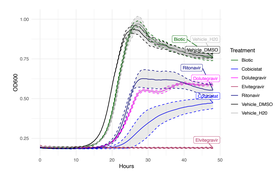


Figure 2. Differentially abundant taxa in the ARV treated cultures compared to a DMSO vehicle control using MaAsLin2, * = q < 0.05, ** = q < 0.01, *** = q < 0.001 (A). Representative boxplots of SCFA producing bacteria, * = q < 0.05 (B). Representative boxplots of possible opportunistic pathogens, * = q < 0.05 (C). TDF = Tenofovir Disoproxil Fumarate.

ARVs have *in vitro* antimicrobial effects

A. *P. stercorea* inhibition by ARVs



B. Elvitegravir and Dolutegravir exhibit increased antimicrobial activity

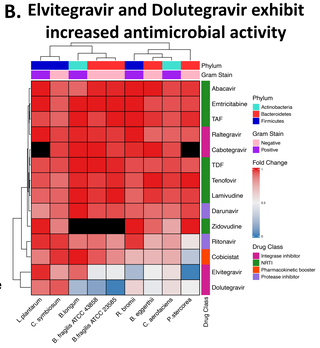
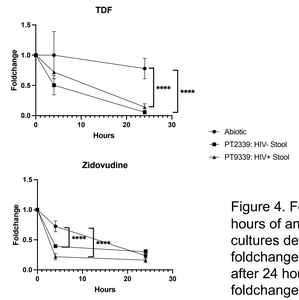


Figure 3. *P. stercorea* growth curves with ARVs resulting in significantly lower area under the curve (Dunn's test, p < 0.05) (A). Heatmap depicting the area under the curve foldchanges for the ARV treated cultures compared to vehicle controls (B). TDF = Tenofovir Disoproxil Fumarate.

Depletion of ARVs in bacterial stool cultures *in vitro*

A. Significant depletion of TDF and Zidovudine in stool cultures



B. Depletion of ARVs *in vitro* after incubation with stool cultures

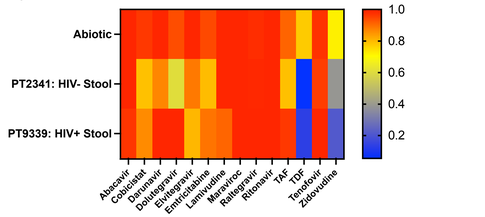


Figure 4. Foldchanges of Tenofovir Diproxyl Fumarate (TDF) and Zidovudine after 4 and 24 hours of anaerobic incubation. Significant differences between abiotic cultures and stool cultures determined by one-sided Welch t-test, **** = p <0.0001 (A). Heatmap of the average foldchange of ARV LC-MS/MS area ratios in stool cultures or abiotic (no-bacteria) samples after 24 hours of anaerobic incubation. Foldchanges greater than 1.0 are shown as 1.0, foldchange for Zidovudine is shown after 4 hours of incubation.

CONCLUSIONS

- Culturing polymicrobial communities from stool in ARVs resulted in differential effects on alpha diversity and the abundance of individual taxa.
- Raltegravir treated cultures had the highest alpha diversity and were most similar to the original stool sample
- Raltegravir and TDF treated cultures were the only ones that had increased relative abundance of many commensal SCFA producing bacteria such as *F. prausnitsii*, *Roseburia*, and *Butyricicoccus.*
- Treatment with other ARVs such as emtricitabine resulted in decreased relative abundance of possible opportunistic pathogens including *E. lenta*
- ARVs had antimicrobial effects on individual strains of gut bacteria. Dolutegravir and elvitegravir exhibited the most inhibition in the tested strains
- TDF and zidovudine were significantly depleted when cultured with polymicrobial communities from stool samples, indicating a possible drug metabolism mechanism by bacteria

SUMMARY

- This work highlights a bidirectional relationship between ARVs and the gut microbiome. ARVs have antimicrobial activity and can alter the composition of polymicrobial communities, and bacteria may have metabolic activity which can deplete ARVs
- Several studies have shown that PWH on raltegravir based ART have increased gut diversity and reduced microbial translocation[1,2,3]. Our work supports a possible beneficial effect of raltegravir on the gut microbiome by showing that *in vitro* treatment with raltegravir resulted in increased alpha diversity and abundance of beneficial microbes in stool bacterial communities.

ACKNOWLEDGEMENTS

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