Rifaximin Use Does Not Alter Soluble CD14 Levels in HIV-Infected Persons on Suppressive ART

POSTER 313

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Abstract

Background: Immune activation (IA) plays a central role in the pathogenesis of HIV infection. A potential driver of IA is the translocation of gut microbial products. Elevated levels of soluble CD14 (sCD14) are associated with all-cause mortality in HIV-infected persons. One source of elevated sCD14 is lipopolysaccharide (LPS) exposure from gut bacteria. Rifaximin (RFX) is an oral, non-absorbable systemic antibiotic that reduces serum LPS levels. Previously, RFX had a modest effect on cellular IA markers in HIV-infected immune non-responders (Tenorio, 2015). We sought to study the effects of RFX on sCD14 levels in chronically suppressed HIV-infected persons.

Methods: In this double blind, randomized, crossover clinical trial, HIV-infected persons on suppressive ART (< 50 cps/mL, ≥3 years), were randomized to receive either RFX 550 mg or placebo twice daily for 28 days. After a 4-6 week wash-out, participants switched treatment arms completing an additional 28 days of treatment. The study had 95% power to detect a 0.20 mcg/mL difference in the primary outcome measure, sCD14 levels by Luminex assay, between weeks 0 and 4 for each arm. Expression of CD38 and HLA-DR on T cells was assessed by flow cytometry. To assess treatment effects, we calculated the difference in changes, for all parameters, for the participants. Using a paired Wilcoxon test, we tested the hypothesis that the median differences of these changes were zero.

Results: Of 42 randomized participants, 37 had evaluable results (median age 47y, 92% male, 32% African-American, median baseline CD4 count 646 cells/uL; 2 participants were non-responders). The median baseline sCD14 level was 1.45 mcg/ml (range 0.86-3.0). The median change in plasma sCD14 levels during the RFX and placebo phases of the study were 0.07 (IQR -0.10, 0.22) mcg/mL and 0.00 (-0.19, 0.15) mcg/mL, respectively, with no significant change in plasma sCD14 levels (p=0.51). We found no change in the frequency of CD4+ or CD8+ T cells expressing HLA-DR, CD38, or both with the use of RFX. An additional statistical test to assess drug carryover revealed no differential effect by period, (p=0.661).

Conclusion: Short term RFX in HIV- infected persons taking ART with a median CD4 count >500 cells/ul did not reduce sCD14 levels or result in changes in cellular IA; reductions in IA noted previously in immune non-responders were not present in this broader cohort of HIV- infected individuals. Earlier intervention or longer duration of RFX therapy may be necessary to affect IA.

Introduction

- In HIV-infected persons with suppressed viremia on antiretroviral therapy (ART), chronic persistent immune activation is associated with deleterious consequences.[1]
- Drivers of IA include systemic translocation of gut microbial products.[2] Virally-mediated damage to the gut epithelium and gut associated lymphoid tissue occurs during HIV infection, allowing for the systemic translocation of gut microbial products.[3]
- Intestinal dysbiosis has been observed in numerous diseases including HIV infection.[4,5] Given the intertwined relationship between the human GIT and HIV infection, strategies that target the gut microbial flora could modulate the persistent IA observed in this disease.
- Rifaximin is a poorly absorbed antibiotic (<0.4% absorbed systemically) that achieves high fecal concentrations and has broad spectrum activity against Gram positive and Gram negative bacteria. Unlike other antibiotics, rifaximin use appears to have beneficial effects on the gut microbiome, with demonstrable increases in bacteria such as *Bifidobacterium*, *Faecalibacterium prausnitz*ii, and *Lactobacillus*; reduction in bacterial virulence and gut microbial translocation; and downregulation of the inflammatory response triggered by gut microbes.[6]
- Rifaximin is approved for use in several disease in which gut dysbiosis plays a dominant role such as hepatic encephalopathy.[7]
- Its use in individuals with hepatic encephalopathy has demonstrated declines in systemic markers of gut microbial translocation.[8]
- An earlier study of rifaximin in virally-suppressed immune non-progressors was associated with modest declines in markers of IA.[9]
- We sought to examine the effects of oral rifaximin on systemic markers of microbial translocation and inflammation and on cellular markers of immune activation in persons with chronic HIV infection on virally suppressive ART.

Methods and Materials

Study Participants

- Eligible participants were HIV-infected adults on suppressive ART-with HIV RNA levels <50 copies/mL for three or more years.
- Exclusion criteria: pregnancy, chronic kidney disease, significant liver disease, active hepatitis B and C, concurrent use of immunomodulatory agents, probiotics, receipt of recent or ongoing antibiotics, history of *C. difficile* colitis.
- Participants were recruited at 3 sites using an IRB-approved protocol and all participants provided written informed consent before partaking in study procedures.

Study Design

- Using a crossover design, HIV-1 infected adults were enrolled and randomized in a double-blind fashion to receive either 4 weeks of rifaximin 550 mg administered twice a day or a placebo twice a day (phase A). Upon completion of phase A, participants underwent a 4–6 week washout phase, subsequently switching treatment assignments to complete an additional 4 weeks (phase B) in the opposite assignment.

Laboratory Methods

- We used HLA-DR, CD38, and co-expression of CD38 and HLA-DR to identify activated CD4+ and CD8+ T cells. Immunophenotyping of peripheral blood drawn into ethylenediaminetetracetate was performed according to the manufacturer's instruction in a CLIA-certified laboratory.
- Soluble CD14 , a marker of monocyte activation by LPS and other stimuli, was measured in batch using a magnetic Luminex assay (R&D Systems) on plasma diluted 1:200 as per manufacturer's instructions.

Statistical Methods

- The *a priori* calculated sample size for this study (n=40) had a 95% power (with type I error of 0.05 based on a one-sample paired difference T test) for detecting a 0.20 difference in sCD14 levels between the placebo and rifaximin phases of the study.
- To assess the effects of treatment, we calculated the difference in changes, for all parameters, for each person using the formula (XFR – XIR) – (XFP –XIP), where X is the measure of the outcome (eg, sCD14 level cell at either the initial visit (I)(week 0) or the final visit (F)(week 4), while the participant was receiving either rifaximin (R) or placebo (P). We tested the hypothesis that the median difference of these changes were zero using a paired Wilcoxon test. Two-sided p-values less than 0.05 were considered significant. No adjustment for multiplicity was made for the secondary endpoints.

Results

Participant Characteristics (N=37)	
Age yrs (median/IQR)	47 (41,52)
Gender (% Male)	92%
Ethnicity	
AA (%)	30%
Caucasian (%)	49%
Hispanic (%)	19%
Asian	2%
CD4+ (median/IQR)	670 (514,819)

Participant Characteristics (N=37)	Median (IQR)
sCD14 level, mcg/mL (mcg/mL)	1.45 (1.2-1.6)
CD38⁺HLA-DR⁺ CD4+ T cells, %	4(3 - 6)
CD38⁺HLA-DR⁺ CD8+ T cells, %	15(11 - 22)
CD38 expression on CD4+ T cells	53(47 - 62)
CD38 expression on CD8+ T cells	43(36 - 50)
HLA-DR expression on CD4+ T cells	11(8 - 14)
HLA-DR expression on CD8+ T cells	31(26 - 43)

Table 1. Baseline Characteristics of Study Participants
The right panel depicts the demographic/clinical characteristics and the left panel baseline laboratory values

Results Continued

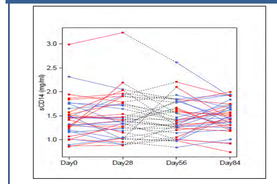


Figure 1 A: Graphical representation of changes observed in sCD14 levels during the rifaximin and placebo phases of the study. Depicted in red are changes during the rifaximin phase and in blue the changes in the placebo phase of the study

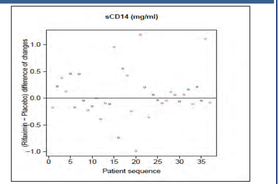


Figure 1 A: Graphical representation of the difference in sCD14 levels between the rifaximin and placebo phases of the study. Depicted in red are changes during the rifaximin phase and in blue the changes in the placebo phase of the study

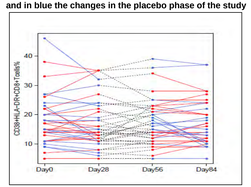


Figure 1 B: Graphical representation of changes in CD8+ T cell activation during the rifaximin and placebo phases of the study. Depicted in red are changes during the rifaximin phase and in blue the changes in the placebo phase of the study

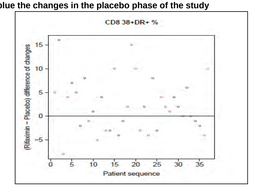


Figure 1 B: Graphical representation of the differences in the proportion of activated CD8+ T cell during the rifaximin and placebo phases of the study. Depicted in red are changes during the rifaximin phase and in blue the changes in the placebo phase of the study

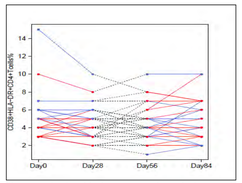


Figure 1 C: Graphical representation of changes in CD4+ T cell activation during the rifaximin and placebo phases of the study. Depicted in red are changes during the rifaximin phase and in blue the changes in the placebo phase of the study

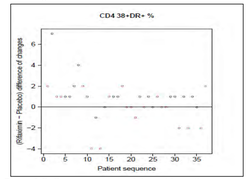


Figure 1 C: Graphical representation of the differences in the proportion of activated CD4+ T cells during the rifaximin and placebo phases of the study. Depicted in red are changes during the rifaximin phase and in blue the changes in the placebo phase of the study

Biomarker	Changes (Final-Baseline) Rifaximin Phase median (IQR)	Changes (Final-Baseline) Placebo Phase median (IQR)	Treatment effect (difference of changes) median (IQR)	P value
sCD14 (mcg/mL)	0.067(-0.097,0.22)	0.0035(-0.195,0.154)	-0.032(-0.011, 0.21)	0.51
CD38⁺HLA-DR⁺ CD4+ T cells (%)	0(0 ,1)	0(-1, 0)	1(0, 1)	0.07
CD38⁺HLA-DR⁺ CD8+ T cells (%)	0(-1, 2)	-1(-3, 1)	2(-2, 5)	0.04
CD4+ HLA-DR+ CD4+ T cells (%)	0(-1, 1)	0(-2 ,1)	0(-1, 2)	0.51
CD8 +HLA-DR+ CD8+ T cells (%)	-1(-3,3)	0(-3, 2)	0(-6, 6)	0.83
CD38+ CD4 +T cells (%)	0(-4 , 3)	-1(-2, 1)	0(-5, 3)	0.51
CD38 +CD8+ T cells (%)	1(-2, 4)	0(-2, 2)	1(-4, 6)	0.38

Table 2. The Effect of Rifaximin and Placebo on Markers of Microbial translocation and T cell activation

Discussion

Our study results suggest short term rifaximin use, in immune reconstituted HIV-infected individuals on virally suppressive ART, is not associated with declines in levels of the immune activation marker, sCD14.

We observed a modest increase in the proportion of activated CD8+ T cells (as assessed by HLA DR and CD38 expression) during the rifaximin phase of the study, but our results are not adjusted for multiple comparisons.

Our results are different from the results reported by Tenorio et al.[9] In their study virally suppressed immune non-responders receiving rifaximin exhibited modest declines in sCD14 levels and T cell activation compared to individuals receiving placebo.[9]

Differences in study population could account for these differences. Further, in the study by Tenorio et al. the observed declines in T cell activation appear to be driven by increase in individuals receiving placebo, rather than declines in individuals receiving rifaximin.

Even in individuals with significant liver disease, where there is a demonstrable clinical benefit for rifaximin use, the effects of rifaximin on gut microbial translocation are inconsistent.[6,7,10]

The strength of the study include the case cross-over study design which accounts for intra-individual variation; limitations include the small sample size, short dosing interval, and relatively healthy population.

In conclusion, in a well-controlled study in a chronically HIV-infected population, rifaximin use for 4 weeks did not decrease sCD14 or T cell activation. The effects of rifaximin in sub-groups with significant baseline elevations in markers of microbial translocation, i.e. those with uncontrolled viremia or in acute HIV infection, are unknown and warrant further study.

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