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Microbial Translocation and Inflammation Responses to Lubiprostone in HIV Infection

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Background

- Incomplete immune recovery in HIV is associated with impaired immune response to antigens, opportunistic infections (OIs), cardiovascular disease and malignancies, and increased mortality
- Increased microbial translocation and immune activation may play a causative role in these patients with limited CD4 recovery with ART
- The gut mucosa of HIV+ individuals sustains a rapid and profound depletion of gut mucosal CD4+ cells after infection, and these changes lead to defects in mucosal immune and epithelial barrier function that allows the translocation of gut microbial products
- Adjunctive therapies aimed at reducing microbial translocation and/or its inflammatory consequences could improve the long-term prognosis of HIV-infected individuals
- An intervention that decreases levels of translocated gut microbial products using modulators that act at the mucosal tight junction barrier is a strategy that has not been studied in HIV
- Lubiprostone, a bicyclic fatty acid with no drug-drug interactions that specifically activates ClC-2, a selective chloride channel constituent of the apical membrane of the human intestine, is indicated for treatment of chronic idiopathic constipation
- Lubiprostone has been shown to stimulate recovery of mucosal barrier function in animal models via the restoration of tight junction protein complexes through intracellular trafficking of occludins

Materials and Methods

Location: The CORE Center, Chicago, IL Study Period: June 2013 - April 2014

Study Population:

- HIV-1 infected adults aged >18 years with CD4 <350 cells/ul within 120 day prior to study after >72 weeks of continuous ART and >48 weeks of virologic suppression (N=20)
- In order to preclude potential confounding effects of different ARVs on MT, only patients with continuous tenofovir/emtricitabine/efavirenz were eligible

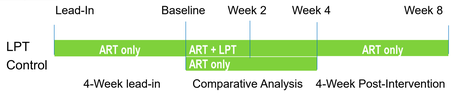
Exclusion Criteria:

1. Active diarrhea (≥3 unformed stools per day) within 28 days prior to study
2. History of chronic constipation or active inflammatory bowel disease.
3. Receipt of antimicrobial therapy within 30 days prior to study entry.
   NOTE: prophylaxis for OIs, e.g., azithromycin or TMP-SMX were allowed

Randomization

- 4:1 to 4 weeks of once-daily LPT (24 µg)(N=16) vs control (no drug)(N=4)

Timepoints



Data collected:

- Demographic, clinical (Body Mass Index, CD4 and nadir CD4, HIV RNA), and behavior characteristics
- MT markers: intestinal fatty acid binding protein (iFAPB)(pg/mL), zonulin (ng/mL), sCD14 (pg/mL), sCD163 (pg/mL) by EIA
- Inflammation markers: IL-6 (pg/mL), hsCRP (pg/mL)
- Adherence and side effects

Statistical analysis: All analyses were performed using SAS Version 9.3 (Cary, NC)

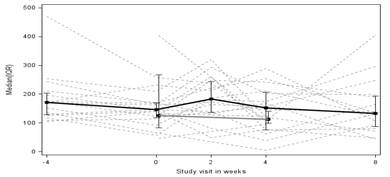
Table 1. Baseline Characteristics

	Total (N=20) N (%) or Median (Q1, Q3)	LPT (N=16) N (%) or Median (Q1, Q3)	Control (N=4) N (%) or Median (Q1, Q3)	P-value [a]
Age in years	49.5 (45.5-54.5)	49.5 (46-54.5)	49 (40.5-55)	0.766
Gender				
Male	15 (75.0)	13 (81.3)	2 (50.0)	0.249
Female	5 (25.0)	3 (18.7)	2 (50.0)	
Race/Ethnicity				
Black	10 (50.0)	6 (37.5)	4 (100.0)	0.128
Hispanic	5 (25.0)	5 (31.3)	0 (0.0)	
White	5 (25.0)	5 (31.3)	0 (0.0)	
Alcohol use, current				
Yes	4 (20.0)	4 (25.0)	0 (0.0)	0.538
No	16 (80.0)	12 (75.0)	4 (100.0)	
Alcohol use, past				
Yes	6 (30.0)	6 (37.5)	0 (0.0)	0.268
No	14 (70.0)	10 (62.5)	4 (100.0)	
Drug use, current				
Yes	4 (20.0)	1 (6.3)	3 (75.0)	0.013
No	16 (80.0)	15 (93.7)	1 (25.0)	
Drug use, past				
Yes	7 (35.0)	4 (25.0)	3 (75.0)	0.101
No	13 (65.0)	12 (75.0)	1 (25.0)	
Smoking, current				
Yes	5 (25.0)	3 (18.8)	2 (50.0)	0.249
No	15 (75.0)	13 (81.2)	2 (50.0)	
Bactrim				
Yes	5 (25.0)	4 (25.0)	1 (25.0)	1.000
No	15 (75.0)	12 (75.0)	3 (75.0)	
CD4+ screening	271.5 (182.5-310.0)	274.0 (228.0-320.5)	146.0 (108.0-229.0)	0.052
CD4+ baseline	333.0 (275.0-384.5)	333.0 (275.0-384.5)	329.5 (229.5-379.5)	0.820
CD4+ nadir	129.0 (60.5-206.5)	129.0 (40.0-206.5)	117.5 (83.0-235.5)	0.807
Body Mass Index	27.5 (25.0-31.0)	26.5 (24.5-28.0)	31.0 (30.0-31.5)	0.048

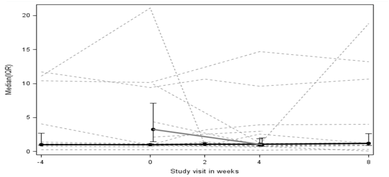
a. Fisher's exact test for categorical variables and exact Wilcoxon rank-sum test for continuous variables

Table 2. Changes Over Time in Biomarkers (LPT —— Control ——)

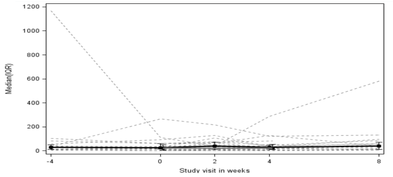
iFABP	LPT (N=16) Median (Q1, Q3)	Control (N=4) Median (Q1, Q3)	P-value
Lead-in	171.02 (127.99, 203.61)	--	
Baseline	145.76 (118.14, 169.35)	125.36 (82.43, 267.76)	
Week 2	183.27 (135.47, 243.02)	--	
Week 4	151.50 (75.86, 206.63)	111.73 (98.44, 139.84)	
Week 8	133.11 (86.57, 193.51)	--	
Δ baseline to 4 weeks	-9.34 (-74.32, 71.74)	14.47 (-155.83, 43.93)	0.751



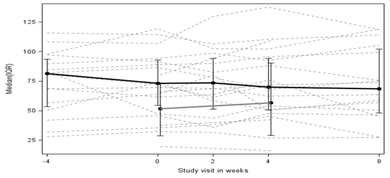
Zonulin	LPT (N=16) Median (Q1, Q3)	Control (N=4) Median (Q1, Q3)	P-value
Lead-in	1.01 (0.96, 2.70)	--	
Baseline	1.00 (0.95, 1.11)	3.24 (1.46, 7.14)	
Week 2	1.09 (0.92, 1.31)	--	
Week 4	1.06 (0.83, 1.88)	0.98 (0.77, 2.04)	
Week 8	1.17 (0.90, 2.64)	--	
Δ baseline to 4 weeks	0.05 (-0.08, 0.15)	-1.80 (-6.16, 0.37)	0.211



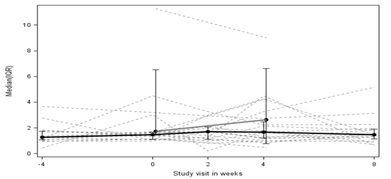
hsCRP	LPT (N=16) Median (Q1, Q3)	Control (N=4) Median (Q1, Q3)	P-value
Lead-in	29.06 (12.62, 49.05)	--	
Baseline	25.99 (13.97, 60.57)	21.48 (4.73, 48.47)	
Week 2	37.98 (10.43, 72.22)	--	
Week 4	27.72 (14.76, 47.94)	22.97 (11.53, 54.39)	
Week 8	38.28 (11.74, 73.09)	--	
Δ baseline to 4 weeks	0.51 (-4.98, 6.17)	6.80 (-1.47, 14.19)	0.437



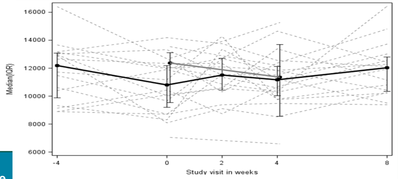
sCD163	LPT (N=16) Median (Q1, Q3)	Control (N=4) Median (Q1, Q3)	P-value
Lead-in	81.42 (53.60, 93.45)	--	
Baseline	73.09 (54.57, 92.88)	51.75 (28.68, 73.13)	
Week 2	73.49 (51.28, 94.17)	--	
Week 4	69.96 (50.61, 94.16)	56.74 (29.21, 90.24)	
Week 8	68.66 (48.12, 102.34)	--	
Δ baseline to 4 weeks	-2.56 (-10.39, 6.81)	4.99 (0.54, 17.12)	0.290



IL-6	LPT (N=16) Median (Q1, Q3)	Control (N=4) Median (Q1, Q3)	P-value
Lead-in	1.25 (1.07, 1.75)	--	
Baseline	1.46 (1.11, 1.66)	1.72 (1.44, 6.51)	
Week 2	1.70 (1.10, 2.07)	--	
Week 4	1.67 (1.19, 2.48)	2.65 (0.75, 6.64)	
Week 8	1.47 (1.12, 1.91)	--	
Δ baseline to 4 weeks	-0.12 (-0.49, 0.38)	-0.68 (-1.48, 0.93)	0.211



sCD14	LPT (N=16) Median (Q1, Q3)	Control (N=4) Median (Q1, Q3)	P-value
Lead-in	12184.27 (9863.94, 13088.19)	--	
Baseline	10807.06 (9198.00, 12183.11)	12370.30 (9548.08, 13117.20)	
Week 2	11517.87 (10442.71, 12692.60)	--	
Week 4	11177.92 (10025.86, 12108.48)	11345.27 (8565.97, 13704.10)	
Week 8	12039.20 (10340.08, 12794.22)	--	
Δ baseline to 4 weeks	247.20 (-1499.26, 2414.21)	-930.48 (-1448.47, 1053.26)	0.617



Exact Wilcoxon tests were used to compare median change in biomarkers from baseline to 4 weeks between groups

Table 3. Trends Over Time Biomarkers in the LPT Group N=16

	Change Relative to Baseline Median (Q1, Q3)	P-value [a]
iFABP		
Lead-in vs. Baseline	42.22 (-9.81, 60.05)	0.288
Week 2 vs. Baseline	30.23 (-25.96, 115.22)	0.481
Week 4 vs. Baseline	-9.34 (-74.32, 71.74)	0.222
Week 8 vs. Baseline	2.57 (-77.12, 49.87)	0.717
Zonulin [b]		
Lead-in vs. Baseline	0.05 (-0.02, 0.18)	0.675
Week 2 vs. Baseline	0.05 (-0.05, 0.48)	0.781
Week 4 vs. Baseline	0.05 (-0.08, 0.15)	0.743
Week 8 vs. Baseline	0.11 (-0.05, 0.22)	0.997
IL-6		
Lead-in vs. Baseline	-0.01 (-0.36, 0.30)	0.321
Week 2 vs. Baseline	-0.02 (-0.38, 0.39)	0.507
Week 4 vs. Baseline	-0.12 (-0.49, 0.38)	0.582
Week 8 vs. Baseline	0.05 (-0.40, 0.32)	0.724
sCD163		
Lead-in vs. Baseline	-3.77 (-4.52, 3.63)	0.657
Week 2 vs. Baseline	-2.04 (-9.40, 2.08)	0.425
Week 4 vs. Baseline	-2.57 (-10.39, 6.81)	0.558
Week 8 vs. Baseline	-2.62 (-9.58, 2.23)	0.151
sCD14		
Lead-in vs. Baseline	784.83 (-656.80, 186.3.08)	0.099
Week 2 vs. Baseline	523.25 (-737.40, 1900.43)	0.210
Week 4 vs. Baseline	247.20 (-1499.26, 2414.21)	0.441
Week 8 vs. Baseline	900.66 (-25.97, 2125.88)	0.046
hsCRP		
Lead-in vs. Baseline	5.24 (-8.45, 12.57)	0.722
Week 2 vs. Baseline	0.05 (-8.77, 15.53)	0.553
Week 4 vs. Baseline	0.51 (-4.98, 6.17)	0.579
Week 8 vs. Baseline	1.46 (-10.53, 25.37)	0.507

a. P-values were generated from mixed effects modeling of the $\log_{10}$ value of the each marker as the outcome and categorical time as the explanatory variable
b. There were no significant trends when zonulin was stratified by <1.5 vs. ≥1.5 at baseline

Table 4. Trends Over Time in Biomarkers Relative to Baseline in the LPT Group, N=16

	Beta for time trend (95% CI)	p-value [a]
iFABP	-0.01 (-0.03, 0.01)	0.451
Zonulin, all	0.001 (-0.02, 0.03)	0.920
Zonulin, <1.5 at baseline [b]	-0.005 (-0.03, 0.02)	0.650
IL-6	-0.0005 (-0.02, 0.02)	0.960
sCD163	-0.003 (-0.01, 0.001)	0.164
sCD14	0.005 (-0.0004, 0.01)	0.070
hsCRP	0.02 (-0.02, 0.07)	0.351

a. P-values were generated from linear mixed effects model with random intercept with the log10 value of the each marker as the outcome and continuous time as the explanatory variable. Adjustment for baseline values of drug use, smoking, alcohol use, CD4 count, and BMI did not change the results
b. n=13 individuals

Table 5. Group differences in Biomarkers at 4 weeks, N=20

	Unadjusted group effect, beta (95% CI) [a]	p-value	Adjusted group effect, beta (95% CI) [b]	p-value
iFABP	-0.04 (-0.50, 0.42)	0.862	0.58 (-0.13, 1.29)	0.100
Zonulin, all	0.23 (-0.21, 0.67)	0.285	0.34 (-0.30, 0.99)	0.274
IL-6	0.05 (-0.29, 0.39)	0.769	0.33 (-0.15, 0.80)	0.162
sCD163	-0.06 (-0.16, 0.05)	0.287	0.01 (-0.17, 0.19)	0.935
sCD14	0.03 (-0.05, 0.10)	0.511	0.10 (-0.01, 0.21)	0.070
hsCRP	-0.36 (-0.95, 0.22)	0.208	0.17 (-0.69, 1.03)	0.671

a. Effect estimate represents the difference between groups in the log10 value of each marker at 4 weeks, adjusted only for the baseline value of the outcome. Adjustment was made for the baseline value of the biomarkers to control for residual confounding due to baseline differences; however, there were no differences with the fully unadjusted model
b Effect estimate represents the difference between groups in the log10 value of each marker at 4 weeks, adjusted for baseline value of outcome and current drug use, BMI, and CD4 at screening

CD4+	LPT (N=16) Median (Q1, Q3)	Control (N=4) Median (Q1, Q3)	P-value
Baseline	333 (275, 384.5)	329.5 (229.5, 379.5)	
Week 4	339 (269.5, 405.5)	230 (196, 336)	
Δ baseline to 4 weeks	5.5 (-27.05, 24.5)	-17.5 (-107, 30)	0.682

Conclusions

- In this pilot trial, there was no change in MT or inflammation markers in HIV-infected persons with delayed CD4 recovery on suppressive ART following 4 weeks of LPT, a duration of therapy that produces appropriate responses in CIC-2 activation for its indicated use in humans
- The most commonly reported side effect was soft stools, and there were no LPT discontinuations or virologic rebound events
- Further investigation with LPT is ongoing to characterize possible alterations of gut microbiota in stools samples to evaluate potential mechanisms of MT in HIV infection