Poster #647

Tocilizumab alters lipids in HIV+ individuals in a randomized, double-blind study

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ABSTRACT

Background: Cardiovascular disease (CVD) risk is increased in HIV infection, despite suppressive antiretroviral therapy (ART). Increased IL-6 levels are linked to CVD, and are predictive of morbidity and mortality in HIV infection. Tocilizumab (TCZ), a monoclonal antibody that inhibits IL-6 activity, can reduce inflammation and improve disease outcomes in individuals with rheumatoid arthritis (RA). Increased plasma lipids (total cholesterol, HDL, LDL) were observed following TCZ treatment, but were not significantly linked to CVD risk in the RA population. The effects of TCZ on inflammation, lipid profiles, and clinical outcomes in HIV+ individuals is not known.

Methods: This was a phase I/II double-blind, placebo controlled, crossover trial of TCZ administered intravenously (IV) every 4 weeks for 3 doses. Male and female ART-treated HIV+ study participants were randomized to receive either TCZ or placebo followed by a 12 week washout period and treatment crossover. At each study visit, lipid panels and detailed lipidomics analyses, measuring ~1100 lipid species across 13 classes, were performed by mass spectrometry. Paired t tests were used for statistical analyses.

Results: Traditional lipid measurements for total cholesterol, LDL, and VLDL levels were increased following TCZ treatment ($p<0.01$ for all). Plasma concentrations of total lipids ($p=0.0001$), and lipid classes, CE, CER, DAG, FFA, HCER, LPC, LPE, PC, PE, SM, TAG, were increased following TCZ treatment compared to baseline and placebo ($p<0.05$ for all). We also measured significant changes in concentrations of 129 individual lipid species ($p<0.05$). Additionally, fatty acid composition was altered among lipid species; TCZ treatment reduced the proportion of free saturated fatty acids (SaFAs) (47% vs 43%, $p=0.05$), and increased the proportion of free monounsaturated fatty acids (MUFAs) (32% vs. 35%, $p=0.06$) and polyunsaturated fatty acids (PUFAs) (21% vs 22%). *In vitro* exposure of PBMCs to SaFAs induced inflammatory cytokine production and monocyte activation.

Conclusions: TCZ therapy alters lipid profiles in HIV+ individuals on ART. The concentrations of multiple lipid classes increased during TCZ treatment, however, the SaFA/UFA ratio was improved for some classes. IL-6 blockade may reduce some indices of inflammation in HIV+ individuals, but also exacerbates lipid levels, potentially limiting benefits in this population. Further study is needed to determine the consequences of TCZ-mediated lipidome alterations on CVD risk.

Lipid Analysis

Lipid Class	Abbreviation	Species #
Cholesterol Ester	CE	26
Ceramide	CER	12
Diacylglycerol	DAG	60
Dihydroceramide	DCER	12
Free Fatty Acid	FFA	26
Hexosylceramide	HCER	12
Lactosylceramide	LCER	12
Lysophosphatidylcholine	LPC	26
Lysophosphatidylethanolamine	LPE	26
Phosphatidylcholine	PC	150
Phosphatidylethanolamine	PE	220
Sphingomyelin	SM	12
Triacylglycerol	TAG	520

Table 1. Plasma lipids were analyzed using the direct infusion-tandem mass spectrometry (DI-MS/MS) Lipidyzer platform. Results provided the concentration (uM) and fatty acid composition (mol%) of 13 lipid classes and ~1100 individual lipid species.

RESULTS

Study Design

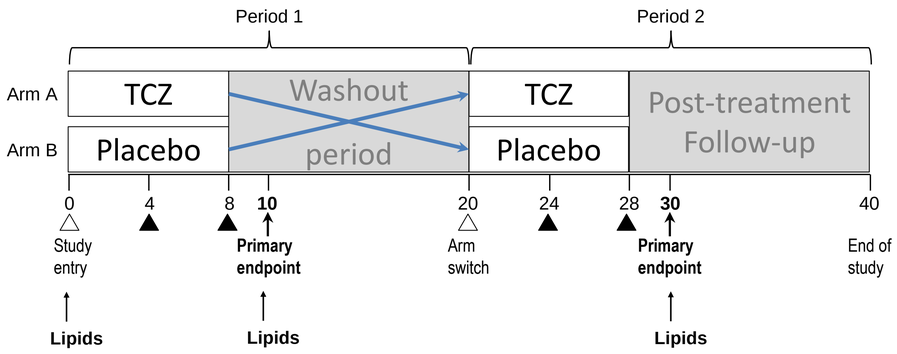

Figure 1. Triangles indicate the dates of study agent administration. Empty symbols = 4 mg/Kg dose; solid symbols = 8 mg/Kg dose. Plasma lipid measurements were collected at indicated timepoints. Arm A: N=15; Arm B: N=16

The concentration of total plasma lipids is increased following TCZ administration

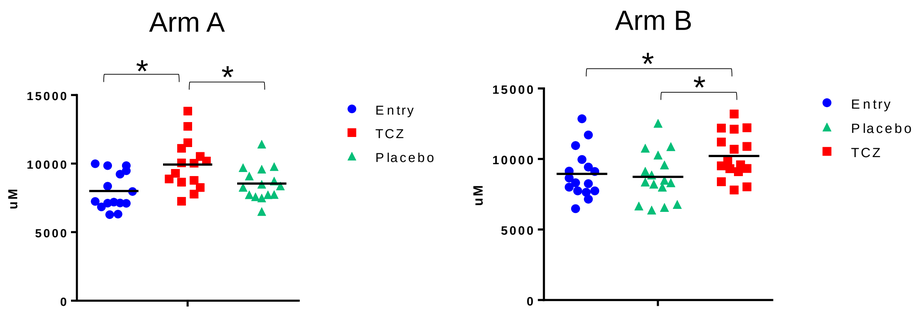

Figure 2. Total plasma lipid concentration (uM) across all classes measured was performed by mass spectrometry. *p<0.05

Concentrations of lipid classes increase during TCZ treatment

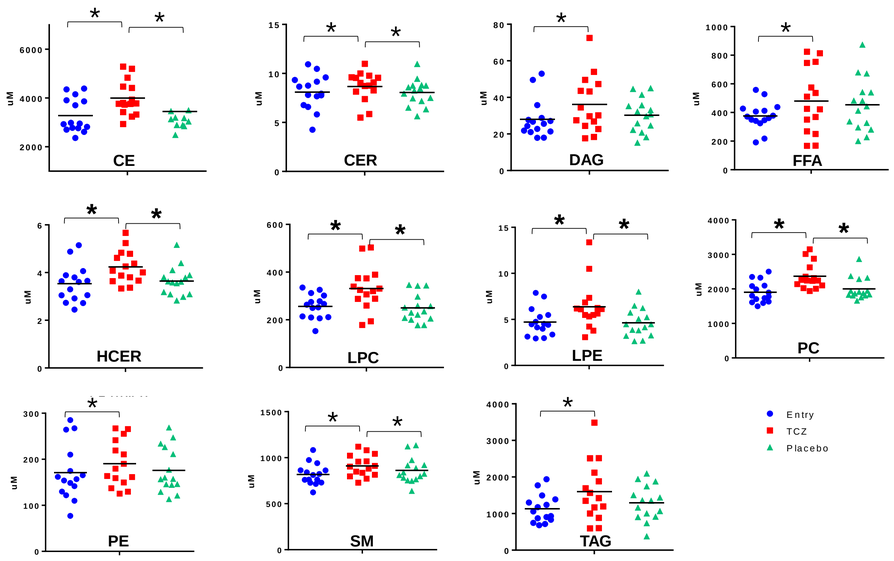

Figure 3. Plasma lipid concentration (uM) by class. TCZ administration resulted in significant increases in levels of 11 of 13 lipid classes tested. Data shown are from Arm A *p<0.05

TCZ administration alters traditional lipid panel measurements

	Entry	TCZ	Placebo
TC (mg/dL)	166	192 *	172 #
LDL (mg/dL)	96	111 *	101 #
VLDL (mg/dL)	21	28 *	24
TG (mg/dL)	106	140 *	119

Table 2. Conventional lipid measurements. * p<0.05 Entry v TCZ; # p<0.05 TCZ v Placebo

TCZ altered proportions of saturated and unsaturated fatty acids

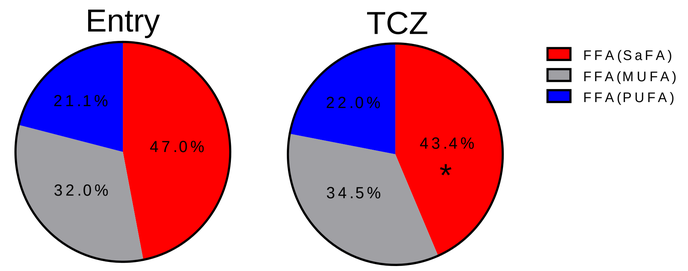

Figure 4. Fatty acid composition (mol%) is altered during TCZ treatment. TCZ administration resulted in reduced proportional representation of saturated fatty acids and increased proportions of unsaturated fatty acids. Data shown are from Arm A.

In vitro exposure of PBMCs to saturated fatty acids induces inflammatory cytokine production and monocyte activation

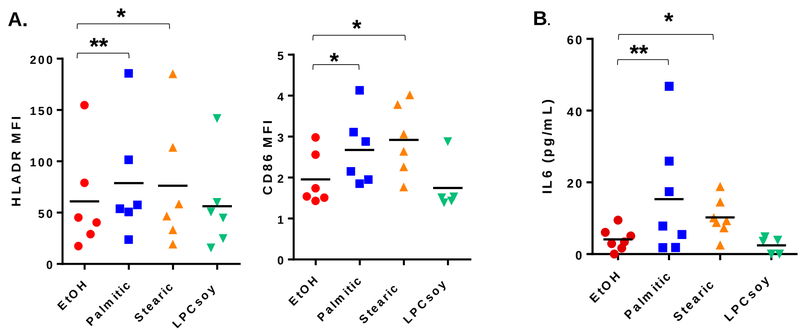

Figure 5. PBMCs were exposed to saturated fatty acids (palmitic acid (16:0) and stearic acid (18:0), 10 uM) or PUFA-enriched LPC (LPCsoy, 10 uM) for 24 h, and monocyte activation markers were measured by A) flow cytometry and supernatant expression of IL-6 was measured by (B) ELISA.

CONCLUSIONS

- TCZ complexes with IL-6R and results in increased plasma IL-6 levels. Indeed, IL-6 levels rose during TCZ exposure and returned to baseline within 10 weeks after exposure. (Data not shown)
- TCZ administration increases total plasma lipids across multiple lipid classes, and alters the fatty acid composition of the lipidome.
- *In vitro* exposure of PBMCs to SaFAs induced inflammatory cytokine production and monocyte activation.
- IL-6 blockade reduces indices of inflammation in HIV+ individuals, but the consequences of TCZ-mediated alteration of the lipidome and CVD risk require further study.