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RESEARCH LETTER

Voclosporin Induces Systemic Lipidomic Alterations: Implications for Lupus Nephritis Remission

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INTRODUCTION

Lupus nephritis (LN) is a major cause of morbidity and mortality with a lifetime incidence of up to 60% in systemic lupus erythematosus.[1] Cardiovascular diseases (CVDs) are also the leading cause of death in patients with systemic lupus erythematosus.[2] LN is an independent risk factor for CVD, which increases the risk of CVD by 2 times as compared to systemic lupus erythematosus patients without LN.[3] Goals of treatment in LN include preserving kidney function and reducing mortality, while minimizing treatment-related adverse events and improving quality of life.[4] Voclosporin is a second generation, calcineurin inhibitor approved in the USA, European Union, Great Britain, and Switzerland in combination with a background immunosuppressive therapy regimen for the treatment of adult patients with active LN.[5] Although cyclosporine increases plasma lipoproteins, the AURORA 1 phase 3 trial provided evidence for favorable changes in traditional lipoproteins with voclosporin therapy, in addition to its superior effect on the primary and secondary renal outcomes,[6] highlighting potential for a better CVD risk modification compared to cyclosporine. Despite the favorable alterations of the traditional lipid panel by voclosporin, its effect on various lipid classes of the circulating lipidome is unclear. It is also unclear if the observed lipid alterations could partially explain the superior renal outcome by voclosporin. This study was aimed at defining circulating lipidomic alterations by voclosporin in the AURORA 1 trial and evaluate the indirect effect of voclosporin on the primary and secondary outcomes mediated by lipid changes. We hypothesize that voclosporin decreases specific lipid species in the circulating lipidome compared with placebo, and that the effect of voclosporin in decreased proteinuria is mediated by lipidomic alterations.

RESULTS

Patients

The baseline characteristics of the participants are shown in Table 1. Patients in the voclosporin arm had a significantly higher estimated glomerular filtration rate at baseline ($P = 0.004$). There were no other differences in the treatment arms. Of the 30 participants in the placebo arm, 5 had complete renal response and 15 had partial renal response; whereas in the voclosporin arm, of the 28 participants, 9 had complete renal response and 15 had partial renal response by the end of treatment.

Lipid Alterations

Measured lipids are shown in Supplementary Table S1. Overall, the mean level of free fatty acids (FFA), acylcarnitine (AC), and complex lipids, except phosphatidylinositol, significantly decreased in the voclosporin arm by the end of treatment ($P \leq 0.001$, Figure 1).

Table 1. Baseline characteristics of selected participants in the placebo and voclosporin arms of the study

Characteristics	Control (n = 30)	Voclosporin (n = 28)
Age (yr)	36 ± 10	35 ± 11
Sex		
Female (%)	27 (90.0)	23 (82.1)
Male (%)	3 (10.0)	28 (17.9)
Race		
White (%)	11 (36.7)	15 (53.6)
Black (%)	5 (16.7)	2 (7.1)
Asian (%)	10 (33.3)	7 (25.0)
Other (%)	4 (13.3)	4 (14.3)
Geographical Region		
Americas (%)	11 (36.7)	11 (39.3)
Asia, (%)	8 (26.7)	7 (25.0)
Europe, South Africa (%)	11 (36.7)	10 (35.7)
Weight (kg)	68 ± 14	72 ± 17
eGFR (ml/min)[a]	72 ± 24	90 ± 21
UPCR (g/g)	3.9 ± 2.5	4.3 ± 2.1
C3 (mg/dl)	89 ± 43	95 ± 30
C4 (mg/dl)	17 ± 11	17 ± 10
Anti ds-DNA Ab (IU/ml) median (IQR)	53 (5–184)	57 (13–160)

eGFR, estimated glomerular filtration rate; IQR, interquartile range; UPCR, urine protein-to-creatinine ratio.
[a]P = 0.004.
Values are mean ± SD, or frequency (%).

Participants with complete renal response also had a significant decline in AC, triacylglycerols, phosphatidylcholines, phosphatidylethanolamines (PE), and sphingomyelins (SMs), and a significant increase in PE-O by the end of treatment. No other lipids had a statistically significant change by complete renal response. Similarly, triacylglycerols and PEs had a significant decrease on average, but PE-O, and lysophosphatidylcholines, had a significant increase in participants with partial renal response as compared to those without (Figure 1). Long-chain to medium chain AC ratio was not different by study arm or clinical renal response (Supplementary Figure S1). Changes of each lipid molecule within different lipid classes in the study arms, stratified by clinical response are illustrated in Supplementary Figures S2 to S17.

Mediation Analysis

Using a mediation analysis (Supplementary Methods), we tested the indirect effect of voclosporin on decreased proteinuria mediated by change in lipids. We identified that the effect of voclosporin on decreased proteinuria was mediated in part by change in PE-P, SM, diacylglycerols (DAGs), cholesteryl esters (CE), lactosylceramides, FFA, dihydroceramides, ceramides, and hexosylceramides with indirect effects ranging from 10.1% to 41.3% of the total effect, respectively (Supplementary Table S2). To test the hypothesis that lipid changes are mediated by change in proteinuria, in the next step we tested the indirect effect of voclosporin on lipid levels mediated by decrease in proteinuria. Accordingly, we noted that the indirect effect of voclosporin mediated by decreased proteinuria was significant only for lactosylceramides, dihydroceramides, FFA, hexosylceramides, and ceramides with a mediation effect ranging from 17.4% to 32.5% of the total effect, respectively (Supplementary Table S3).

DISCUSSION

Voclosporin has several advantages compared with traditional calcineurin inhibitors, including a more favorable effect on lipids. In this study, participants who received voclosporin had a significant decline in the abundance of FFA, AC, and complex lipids by the

Lipids	Mean lipid class by study arms			Mean lipid class by presence of complete response				Mean lipid class by presence of partial response			
	Placebo	Voclosporin	P value	No	Yes	P value	P interaction	No	Yes	P value	P interaction
FFA	0.128	-0.132	<0.0001	0.002	-0.006	0.092	0.043	-0.005	0.005	0.873	0.002
AC	0.087	-0.089	0.0003	0.04	-0.136	0.0004	0.08	0.038	-0.036	0.13	0.789
TAG	0.169	-0.181	<0.0001	0.047	-0.147	<0.0001	<0.0001	0.094	-0.088	<0.0001	<0.0001
DAG	0.184	-0.197	<0.0001	0.002	-0.007	0.181	0.010	0.018	-0.016	0.624	0.0009
MAG	0.14	-0.15	0.0010	-0.02	0.064	0.067	0.0006	0.034	-0.031	0.315	0.021
PC	0.239	-0.256	<0.0001	0.078	-0.246	<0.0001	0.017	-0.01	0.01	0.209	0.159
PE	0.156	-0.167	<0.0001	0.083	-0.26	<0.0001	0.888	0.07	-0.065	0.001	0.475
PE-O	0.204	-0.219	<0.0001	-0.102	0.322	<0.0001	<0.0001	-0.116	0.109	<0.0001	<0.0001
PE-P	0.259	-0.277	<0.0001	-0.004	0.014	0.749	0.152	-0.034	0.032	0.070	0.104
LPC	0.282	-0.302	<0.0001	0.025	-0.079	0.485	0.024	-0.1	0.093	0.0005	<0.0001
LPE	0.128	-0.137	0.0040	0.045	-0.141	0.124	0.948	-0.001	0.001	0.980	0.0001
CE	0.186	-0.199	<0.0001	0.038	-0.119	0.351	0.060	0.039	-0.037	0.201	0.272
CER	0.12	-0.128	0.0009	0.001	-0.003	0.462	0.351	0.091	-0.088	0.031	0.024
DCER	0.136	-0.145	<0.0001	-0.012	0.038	0.120	0.188	0.098	-0.091	0.016	0.017
HCER	0.173	-0.185	<0.0001	-0.001	0.004	0.235	0.188	0.037	-0.035	0.471	0.029
LCER	0.138	-0.148	0.0001	-0.0002	0.001	0.331	0.158	-0.038	0.036	0.264	0.676
SM	0.172	-0.184	<0.0001	0.074	-0.234	0.011	0.361	0.039	-0.036	0.412	0.689
PI	0.011	-0.011	0.9410	0.055	-0.173	0.018	0.685	-0.098	0.091	0.014	0.0357

Figure 1. Comparing the change of standardized mean levels of lipid class by study arms and clinical response. P interaction is the P-value of the interaction between the main effects of study arm and complete response, or study arm and partial response. Values are z-score standardized mean level per lipid class. Red bars represent increase and blue bars represent decrease in the corresponding lipid.

end of treatment. Abundance of triacylglycerols, phosphatidylcholines, PE, and SM were decreased in participants with a complete renal response, whereas abundance of PE-O increased in this group. Mediation analysis revealed that the indirect effect of voclosporin on complete renal response was mediated by change in PE-P, SM, DAG, CE, lactosylceramides, FFA, dihydroceramides, ceramides, and hexosylceramides, along with no mediation effect of proteinuria on CE, DAG, SM, and PE-P levels.

The long-chain to medium-chain AC ratio, a metric of mitochondrial β-oxidation,[7,8] was not significantly different in study arms or when stratified by clinical renal response, suggesting that defect in mitochondrial lipid oxidation is not the underlying cause of the observed changes. Therefore, a lower level of FFA along with other complex lipids suggests that voclosporin decreases renal and systemic *de novo* lipogenesis, synthesis of complex lipids and their downstream lipolysis (Supplementary Figure S18). In the mediation analysis, ceramides and FFAs mediated the mitigation of proteinuria. Further mediation analysis showed that alterations in ceramides and FFA may also be mediated by decreased proteinuria (Supplementary Table S3), and therefore it is unclear whether changes in FFA or ceramides contributed to decreased proteinuria or decreased proteinuria contributed to their changes. Strikingly, the mediation analysis further showed that the effect of voclosporin on decreased proteinuria was indirectly mediated by changes in CE, DAG, SM, and PE-P; changes not mediated by decreased proteinuria. These findings suggest that alterations in CE, DAG, SM, and PE-P may be an integral part of voclosporin-induced metabolic alterations required for remission of LN. The renal clinical response by lipid changes may also include amelioration of immune cell activities mediated by decreased oxidative phosphorylation, reduced mitochondrial reactive oxygen specious, and mitigation of glycolysis, glutaminolysis, and mTORC1 defects characteristics of lupus flare,[9] with salutary effects on podocytes and/or glomerular endothelial cells evidenced by diminished glomerular proteinuria. Future studies will need to address the mechanisms underlying reduction in proteinuria, and its generalizability.

These findings have important clinical implications. In addition to a favorable renal outcome, voclosporin decreases multiple lipid classes in circulating lipidome. The reduction of the circulating lipidome may provide further CVD benefits which need to be evaluated in future clinical studies. The mediation effect of CE, DAG, SM, and PE-P on clinical remission also highlights potentially novel pathways involved in renal remission.

This study has limitations. Despite the use of data from a randomized controlled clinical trial, the observational nature of the analytical approach does not allow inference of causality. The sample size of this study is also relatively low. However, to overcome this limitation, we have applied mixed general linear models for each lipid class to reduce dimensionality and false discovery, and to further power the analysis at the lipid class level.

In summary this study shows that voclosporin treatment decreased multiple lipid classes in circulating human lipidome. It also showed an indirect effect of voclosporin on decreased proteinuria in part mediated by changes of CE, DAG, SM, and PE-P. The decreased lipids may contribute to modification of CVD risk. These data form a strong preliminary evidence for future clinical studies designed to demonstrate CVD risk modification by voclosporin.

DISCLOSURE

LMR, and JLC are employees and shareholders of Aurinia Pharmaceuticals Inc. RBH is a former employee of Aurinia Pharmaceuticals Inc. and is a current consultant and shareholder of Aurinia Pharmaceuticals Inc. All the other authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary Reference.

Figure S1. Comparing long-chain to medium-chain acylcarnitine ratio reveals no significant differences by treatment arms or clinical response. NS, Not significant.

Figure S2. Comparing the standardized mean levels of free fatty acids (FFA) and acylcarnitines (AC) in voclosporin versus placebo arms, stratified by complete and partial renal response.

Figure S3. Comparing the standardized mean levels of free triacylglycerols in voclosporin versus placebo arms, stratified by complete and partial renal response.

Figure S4. Comparing change of diacylglycerols by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).

Figure S5. Comparing change of monoacylglycerols by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).

Figure S6. Comparing change of cholesteryl esters by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S7. Comparing change of phosphatidylcholines by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S8. Comparing change of PE-Os by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S9. Comparing change of PE-Ps by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S10. Comparing change of PEs by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S11. Comparing change of lysophosphatidylethanolamines by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S12. Comparing change of lysophosphatidylcholines by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S13. Comparing change of dihydroceramides by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S14. Comparing change of hexosylceramides by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S15. Comparing change of lactosylceramides by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S16. Comparing change of ceramides by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S17. Comparing change of sphingomyelins by study arm, clinical response (+response vs. no response), and renal outcome (complete or partial).
Figure S18. Summary findings of the study.
Table S1. List of quantified lipids.
Table S2. The indirect treatment effect of voclosporin mediated by lipids on change of urine protein-to-creatinine ratio (UPCR).
Table S3: The indirect treatment effect of voclosporin mediated by change of urine protein-to-creatinine ratio (UPCR) on lipids.
STROBE Checklist.

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