

Cyclophilin inhibition with rencofilstat shifts the liver transcriptome and lipidome in preclinical models toward resolution of nonalcoholic steatohepatitis

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INTRODUCTION

CYCLOPHILINS: multi-isoform isomerase enzymes that influence the function of numerous, diverse proteins through regulation of proline-bond *cis-trans* conformation

RENCOFILSTAT (formerly CRV431): a cyclophilin inhibitor currently in Phase 2 clinical trials for NASH and HCC. Key activities of rencofilstat include anti-fibrotic, antiinflammatory, and metabolic regulation.

RENCOFILSTAT (RCF)

inhibits multiple cyclophilin enzymes that contribute to NASH pathogenesis

_ CYCLOPHILIN A (inflammation) **CYCLOPHILIN D (metabolism) CYCLOPHILIN B** (fibrosis) multiple roles in fibrotic collagen production

CYCLOPHILINS E, G, H, L1, L3 (RNA splicing)

Rencofilstat (RCF) 50 mg/kg/day

Elafibranor (ELF) 10 mg/kg/day

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Obeticholic acid (OCA) 20 mg/kg/day

AIMS

Characterize rencofilstat's effects on the liver transcriptome and lipidome in three liver disease models.

METHODS

TRANSCRIPTOMIC and LIPIDOMIC ANALYSES IN 3 MODELS:

MODEL #1: Diamond Mice Western Diet (WD)



MODEL #2: C57BL/6 Mice Western Diet + CCl₄ (carbon tetrachloride)



MODEL #3: S.D. Rats Thioacetamide (TAA) 9 weeks thioacetamide 9 weeks vehicle or RCF 40 mg/kg/day



Rencofilstat Elafibranor OCA Rencofilstat (1576 DEGs) Elafibranor (4577 DEGs) 26% (1169/4577) 55% (2529/457 60% (2529/4216 OCA (4216 DEGs)



Predominance of lipid and bile pathways in common among RCF, ELF, and OCA DEGs



Reactome Pathways, p<0.05



KEGG Pathways with DEG associations (P<0.01) HUMAN PCLS Stim vs Nonstin Western Diet + CCl₄ RCF vs VEHICL STAM Fatty liver: RCF vs vehi

> STAM Tumors: RCF vs vehic **DIAMOND ELF vs VEHICLE**

DIAMOND OCA vs VEHICL

LIVER TRANSCRIPTOMICS: Differentially Expressed Genes (DEGs)

DIAMOND MICE – Western Diet (WD)

Rencofilstat DEGs significantly overlapped with elafibranor and obeticholic acid DEGs

% OVERLAP OF DEGs (DEGs vs Vehicle)

Reactome Pathway Enrichment of DEGs OCA vs Veh 121 pathways ELF vs Veh 126 pathways

Veh = vehicle, RCF = rencofilstat, ELF = elafibranor, OCA = obeticholic acid

34 Reactome Pathways Shared by All Three Drugs
Attendation phase
Rile acid and hile salt metabolism
Carboxyterminal nost-translational modifications of tubulin
Common Pathway of Fibrin Clot Formation
Slycerophospholipid biosynthesis
HSP90 chaperone cycle for steroid hormone recentors (SHR)
GE1R signaling cascade
RS-related events triggered by IGE1R
Metabolism of folate and pterines
Metabolism of lipids
Metabolism of steroids
Metabolism of vitamins and cofactors
Metabolism of water-soluble vitamins and cofactors
Metallothioneins bind metals
Nuclear Receptor transcription pathway
Phospholipid metabolism
Platelet Aggregation (Plug Formation)
Platelet degranulation
Regulation of glycolysis by fructose 2,6-bisphosphate metabolism
Regulation of TLR by endogenous ligand
Response to elevated platelet cytosolic Ca2+
Response to metal ions
Signaling by Retinoic Acid
Signaling by Type 1 Insulin-like Growth Factor 1 Receptor (IGF1R)
SLC-mediated transmembrane transport
Synthesis of bile acids and bile salts
Synthesis of bile acids and bile salts via 27-hydroxycholesterol
Synthesis of bile acids and bile salts via 7alpha-hydroxycholesterol
Synthesis of PE
Ihreonine catabolism
ransport of bile saits and organic acids, metal ions and amine compounds
i rigiyceride biosynthesis

C57BL/6 MICE – Western Diet (WD) + CCl₄

Rencofilstat opposed many significant DEGs associated with Western diet + CCl₄



KEGG pathways active in diseased livers were commonly opposed by rencofilstat (RCF)

Advaita iPathwayGuide VEH = Vehicle vs Normal livers RCF = Rencofilstat vs Vehicle livers





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Model	Vehicle n	RCF n	P-value	# DEGs	J,203 genes allered by rend	
HUMAN PCLS (stim)	3	3	P<0.05	250		
HUMAN PCLS (nonstim)	3	3	P<0.05	408		
DIAMOND Mice + WD	8	9	Padj<0.05	1650		
C57BL/6 Mice + WD + CCl_4	5	4	P<0.05	511		
STAM Mice + WD	9	8	Padj<0.05	1754		
RAT TAA	10	10	P<0.01	690		

80% of rencofilstat's DEGs are present in only one model, indicating a major role of disease-specific processes in transcriptional responses to rencofilstat

KEGG Pathways: Human F4 NASH vs Disease Models

	HUMAN NASH F4	NASH MODELS	NASH MODELS	NASH MODELS
	vs NAFLD F0/1	VEHICLE	RENCOFILSTAT	ELF + OCA
KEGG Pathway	(Govaere 2020)	Average	Average	Average
Metabolic pathways	-5.11	-5.11	-5.11	-5.11
Pathways in cancer	-5.56	-5.53	-4.63	-5.11
Human papillomavirus infection	-5.51	-5.42	-3.94	-4.71
PI3K-Akt signaling pathway	-4.89	-6.11	-3.93	-5.25
Human cytomegalovirus infection	-5.73	-5.18	-3.70	
Alcoholic liver disease	-4.20	-4.01	-4.19	-4.73
Pathways of neurodegeneration - multiple diseases	-3.71	-4.25	-3.98	-3.12
Alzheimer disease		-4.38	-3.80	-3.59
Rap1 signaling path way	-4.90	-5.45	-3.77	-4.94
Herpes simplex virus 1 infection		-4.00	-3.72	-2.00
FoxO signaling pathway	-2.37	-4.26	-3.65	-5.11
Coronavirus disease - COVID-19	-2.03	-5.99	-3.46	-3.17
Human T-cell leukemia virus 1 infection	-5.30	-5.29	-3.28	-2.33
Cushing syndrome	-6.01	-4.29	-2.95	-2.76
Kaposi sarcoma-associated herpesvirus infection	-5.76	-5.02	-2.88	
MAPK signaling pathway	-5.01	-5.22	-5.40	-6.74
Protein processing in endoplasmic reticulum	-2.90	-4.95	-4.59	

The top KEGG pathways associated with rencofilstat transcriptional effects in preclinical models overlap significantly with human F4 NASH (data from Govaere et al 2020; log10 P-values describing DEG association with KEGG pathways)

RAT TAA RCF vs VEHIC

e.g. Fasn, Scd1, Acot1, SerpinA4-ps1, Oncecut1, HK2, Elvovl5, Sox5











LIVER LIPIDOMICS

• sphingolipids, lysophospholipids, triglycerides (especially shorter chain), oxidized fatty acids

DIAMOND MICE - WD



CONCLUSIONS

- Rencofilstat exerted liver antifibrotic effects in all disease models
- distinctive to each model
- Rencofilstat affected KEGG pathways that also occur in advanced human NASH



Gene Ontologies

Rencofilstat exerted normalizing effects on several lipid classes in diseased livers, including lipids with known toxicities:

• Rencofilstat consistently attenuated disease-associated changes in gene expression and lipids, but the specific alterations were