SAT117

Combination of an Acetyl-CoA Carboxylase Inhibitor and Obeticholic Acid Reduced Lipids and Bile Acids, and Altered Lipid and Amino-Acid Metabolism in the Liver of Humanized Mice

David Hollenback,[1]* Wen-Wei Tsai,[1]* Andrew Schwab,[2] Megan Showalter,[2] James L. Trevaskis[1]

[1]Gilead Sciences, Inc., Foster City, California, USA; [2]Metabolon, Inc., Morrisville, North Carolina, USA; *Contributed equally to this poster.

Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, California, USA 94404
800-445-3235

Introduction

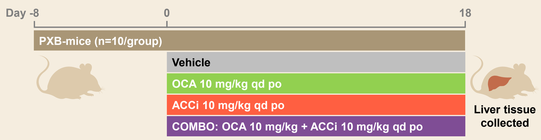
- Inhibition of the acetyl-coenzyme A (CoA) carboxylase (ACC) isoforms ACC1 and ACC2 reduces lipogenesis, stimulates lipid oxidation, and improves histologic features and fibrosis-related biomarkers in nonalcoholic steatohepatitis (NASH)
- Obeticholic acid (OCA), a farnesoid X receptor agonist, reduces bile-acid synthesis and regulates lipid metabolism in the liver

Objective

- To assess the effects of an ACCi and OCA in combination (COMBO) on lipids and amino-acid metabolism in the humanized liver of the chimeric PXB mouse® (PhoenixBio, Higashi-Hiroshima City, Japan)

Methods

Study Design



- Adult male PXB-mice (n=10; randomized on Day -8 by blood human albumin concentration) were administered ACCi (an analog of firsocostat) and/or OCA, and livers were collected 2 h after the last dose
- Untargeted metabolomic profiling of liver tissue was performed at Metabolon, Inc. using a combination of liquid chromatography–mass spectrometry methods[1]
- Structurally named metabolites were mapped onto their biochemical pathways

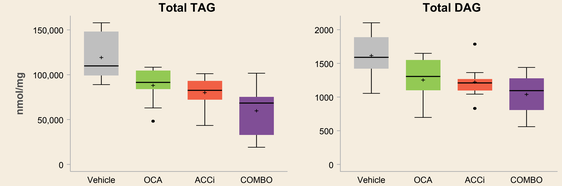
Results

- Global untargeted panel and complex lipid panel analyses of mouse liver tissue samples identified a total of 1698 metabolites (696 named, 95 unnamed, and 907 complex lipids)
- Unbiased metabolomic analysis revealed changes in multiple pathways, with acylcarnitine, lipid, bile-acid, and amino-acid metabolism being most altered with COMBO treatment

COMBO Showed Additive Effects to Reduce Neutral Lipids

Subpathway	Biochemical Name	OCA	ACCi	COMBO
TAG	Total TAG	0.74	0.67	0.50
DAG	Total DAG	0.78	0.76	0.64
MAG	Total MAG	0.61	0.74	0.69

Decrease ps0.05 0.05<p<0.10

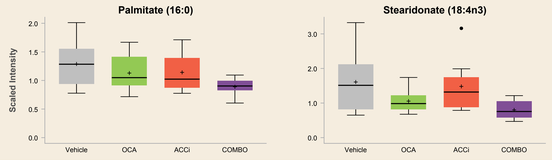


DAG, diacylglycerols; MAG, monoacylglycerols; TAG, triacylglycerols.

COMBO Reduced Long-Chain Fatty-Acid Levels

Decrease ps0.05 0.05<p<0.10

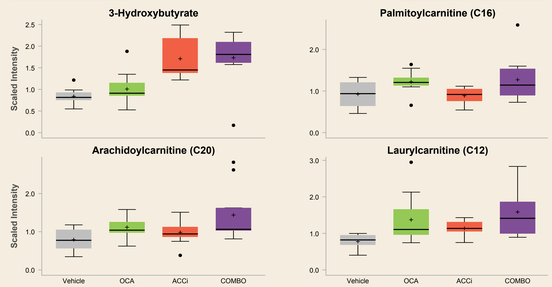
Subpathway	Biochemical Name	OCA	ACCi	COMBO
Fatty-acid synthesis	Malonylcarnitine	0.99	0.99	0.88
Fatty-acid metabolism	Acetyl CoA	1.01	1.19	0.96
Long-chain saturated fatty acid	Myristate (14:0)	0.71	0.81	0.51
	Pentadecanoate (15:0)	0.89	0.95	0.76
	Palmitate (16:0)	0.88	0.89	0.69
	Margarate (17:0)	0.89	0.83	0.68
	Stearate (18:0)	0.86	0.86	0.72
	Arachidate (20:0)	0.89	0.83	0.70
Long-chain mono-unsaturated fatty acid	Palmitoleate (16:1n7)	0.74	0.79	0.50
	10-heptadecenoate (17:1n7)	0.77	0.79	0.57
	Oleate/vaccenate (18:1)	0.81	0.80	0.64
	10-nonadecenoate (19:1n9)	0.83	0.81	0.65
Long-chain polyunsaturated fatty acid (n3 and n6)	Stearidonate (18:4n3)	0.66	0.92	0.50
	Heneicosapentaenoate (21:5n3)	0.77	0.81	0.53
	Docosapentaenoate (n3 DPA; 22:5n3)	0.87	0.99	0.71
	Nisinate (24:6n3)	0.80	0.72	0.52
	Hexadecadienoate (16:2n6)	0.74	0.74	0.54
	Linoleate (18:2n6)	0.82	0.80	0.64
	Linolenate [α or γ; (18:3n3 or 6)]	0.72	0.81	0.51
	Dihomo-linoleate (20:2n6)	0.86	0.78	0.68
	Dihomo-linolenate (20:3n3 or n6)	0.85	0.98	0.72
	Docosadienoate (22:2n6)	0.71	0.75	0.66



ACCi, OCA, and COMBO Increased 3-Hydroxybutyrate and Acylcarnitines

Increase ps0.05 0.05<p<0.10

Subpathway	Biochemical Name	OCA	ACCi	COMBO
Fatty-acid metabolism (acylcarnitine, medium chain)	Hexanoylcarnitine (C6)	1.36	1.35	2.17
	Octanoylcarnitine (C8)	1.29	1.29	1.72
	Cis-3,4-methyleneheptanoylcarnitine	1.74	1.03	1.74
	Decanoylcarnitine (C10)	1.53	1.48	2.17
	Laurylcarnitine (C12)	1.77	1.46	2.04
Fatty-acid metabolism (acylcarnitine, long chain saturated)	Myristoylcarnitine (C14)	1.63	1.20	1.79
	Pentadecanoylcarnitine (C15)	1.91	1.47	2.10
	Palmitoylcarnitine (C16)	1.32	0.96	1.38
	Margaroylcarnitine (C17)	1.35	1.12	1.62
	Stearoylcarnitine (C18)	1.25	1.02	1.21
	Arachidoylcarnitine (C20)	1.41	1.24	1.81
	Behenoylcarnitine (C22)	1.42	1.15	1.72
	Lignoceroylcarnitine (C24)	1.13	1.17	1.37
Fatty-acid metabolism (acylcarnitine, mono-unsaturated)	5-dodecenoylcarnitine (C12:1)	1.59	1.34	1.74
	Myristoleoylcarnitine (C14:1)	2.00	1.45	2.27
	Palmitoleoylcarnitine (C16:1)	1.50	1.05	1.70
	Oleoylcarnitine (C18:1)	1.20	0.91	1.34
	Eicosenoylcarnitine (C20:1)	1.38	1.52	2.29
	Erucoylcarnitine (C22:1)	1.48	1.37	2.05
	Nervonoylcarnitine (C24:1)	1.35	1.15	1.68
	Ximenoylcarnitine (C26:1)	1.16	0.95	1.07
Fatty-acid metabolism (acylcarnitine, polyunsaturated)	Linoleoylcarnitine (C18:2)	1.42	0.83	1.47
	Linolenoylcarnitine (C18:3)	1.45	0.98	1.58
	Arachidonoylcarnitine (C20:4)	1.23	1.08	1.45
	Dihomo-linolenoylcarnitine (C20:3n3 or 6)	1.35	1.00	1.35
	Docosapentaenoylcarnitine (C22:5n3)	1.60	1.07	2.48
	Docosahexaenoylcarnitine (C22:6)	1.49	0.99	1.86
Ketone bodies	3-hydroxybutyrate	1.20	2.03	2.06

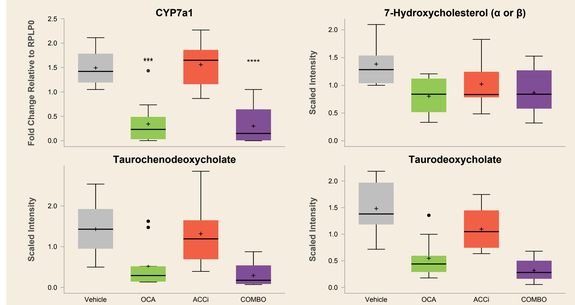


- Decreases in long-chain fatty acids, and increases in 3-hydroxybutyrate and acylcarnitines may indicate reduced fatty-acid biosynthesis and increased β-oxidation

Expected OCA-Dependent Decreases in Bile Acids

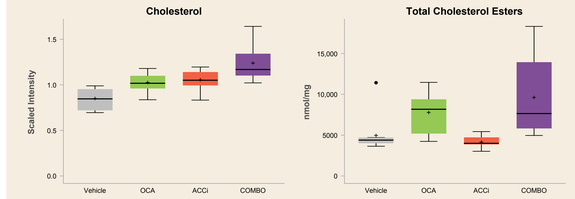
Decrease ps0.05 0.05<p<0.10
Increase ps0.05 0.05<p<0.10

Subpathway	Biochemical Name	OCA	ACCi	COMBO
Primary bile-acid metabolism	Glycocholate	0.62	1.39	0.30
	Taurocholate	1.02	0.54	1.13
	Chenodeoxycholate	0.27	1.86	0.29
	Glycochenodeoxycholate	0.57	1.87	0.13
	Taurochenodeoxycholate	0.36	0.92	0.20
	α-muricholate	0.31	1.39	0.23
	Tauro-α-muricholate	0.43	0.65	0.35
	Tauro-β-muricholate	0.74	0.49	0.79
	Glyco-α-muricholate	0.60	1.87	0.32
	Glyco-β-muricholate	0.49	1.57	0.45
Secondary bile-acid metabolism	Deoxycholate	0.25	1.43	0.17
	Glycodeoxycholate	0.47	1.97	0.14
	Taurodeoxycholate	0.37	0.74	0.22
	Taurodeoxycholic acid 3-sulfate	0.28	0.62	0.20
	6-β-hydroxylithocholate	0.34	1.52	0.33
	Lithocholate sulfate (1)	0.33	1.60	0.31
	Lithocholic acid sulfate (2)	0.32	2.98	0.29
	Glycolithocholate	0.41	2.02	0.16
	Glycolithocholate sulfate	0.28	2.53	0.12
	Taurolithocholate	0.21	1.10	0.19
	Taurolithocholate 3-sulfate	0.19	1.24	0.21
	Glycoursodeoxycholate	0.56	1.70	0.18
	Tauroursodeoxycholate	0.53	0.68	0.40
	Tauroursodeoxycholic acid sulfate (1)	0.61	0.56	0.43
	Taurohyocholate	0.75	0.59	0.46
	Taurohyodeoxycholic acid	0.55	0.76	0.36
	Glycocholenate sulfate	0.68	1.52	0.30
	Glycodeoxycholate 3-sulfate	0.32	1.96	0.15
	Taurochenodeoxycholic acid 3-sulfate	0.27	0.97	0.26
	Ursocholate	0.48	0.90	0.55



***p<0.001; ****p<0.0001: Wilcoxon unpaired t-test. CYP7a1, cytochrome P450 7a1.

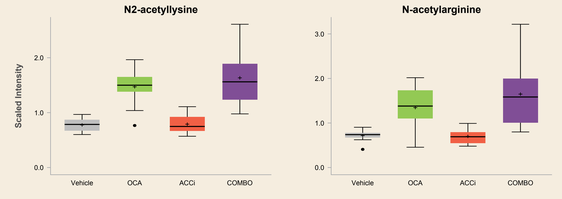
Expected OCA Increases of Liver Cholesterol and Cholesterol Esters



OCA and COMBO Increased N-Acetylated Amino Acids

Increase ps0.05 0.05<p<0.10

Subpathway	Biochemical Name	OCA	ACCi	COMBO
N-acetyl amino acids	N-acetylglycine	0.83	1.52	1.11
	N-acetylasparagine	1.56	0.98	1.74
	N-acetylhistidine	1.27	1.05	1.65
	N-acetyl-3-methylhistidine	2.02	1.13	3.74
	N-acetyl-1-methylhistidine	2.06	1.24	2.78
	N-acetyl-2-aminoadipate	1.67	1.03	1.54
	N-acetylphenylalanine	1.32	1.06	1.68
	N-acetyltyrosine	1.48	1.08	1.75
	N-acetyltryptophan	1.76	1.24	2.46
	N-acetylkynurenine (2)	2.16	1.17	2.43
	N-acetyltaurine	2.57	0.47	2.72
	N-acetylarginine	1.88	0.98	2.30
	N-acetylcitrulline	1.59	1.00	2.86
	N-acetylhomocitrulline	1.13	1.09	1.32
	N-acetyl-isoputreanine	1.33	1.33	1.71
	N2-acetyllysine	1.90	1.02	2.11
	N6-acetyllysine	1.01	1.04	1.62
	N2-acetyl,N6-methyllysine	1.88	0.97	2.43
	N2-acetyl,N6,N6-dimethyllysine	1.56	1.02	2.01

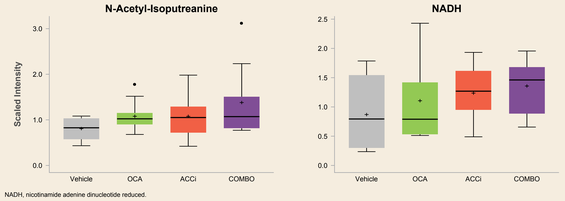


- Increases in N-acetylated amino acids may represent increased protein turnover in the liver or changes in energy homeostasis

COMBO Showed Additive Effects to Increase Polyamines and NADH

Subpathway	Biochemical Name	OCA	ACCi	COMBO
Polyamine metabolism	Putrescine	1.12	1.09	1.69
	N-acetylputrescine	1.17	1.14	2.52
	N-acetyl-isoputreanine	1.33	1.33	1.71
	Spermidine	1.18	1.08	1.35
	(N(1) + N(8))-acetylspermidine	1.25	1.44	1.63
	Spermine	1.20	1.12	1.30
Nicotinate and nicotinamide metabolism	NADH	1.27	1.42	1.56

Increase ps0.05 0.05<p<0.10



NADH, nicotinamide adenine dinucleotide reduced.

Conclusions

- Metabolomic analysis revealed that lipid metabolism and energetic profiles were rapidly changed by ACCi or OCA treatment, and COMBO further reduced lipid synthesis and promoted fatty-acid oxidation in the liver of humanized mice
- These changes are similar to the clinical findings in patients with NASH treated with an ACCi and farnesoid X receptor agonist combination,[2] and support the utility of the humanized mouse model to elucidate molecular pathways mediated by NASH therapies

References: **1.** Evans AM, et al. Metabolomics 2014;4:132; **2.** Alkhouri N, et al. J Hepatol 2022;S0168-8278(22)00235-5. **Acknowledgments:** This study was funded by Gilead Sciences, Inc. Editing and production assistance were provided by BioScience Communications, New York, New York, USA, funded by Gilead.

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