In Vitro Models Concur with Clinical Results to Confirm Pleiotropic Mechanisms of Action for Gemcabene

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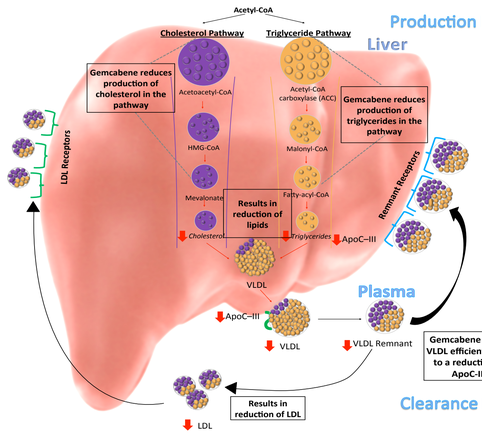
ABSTRACT

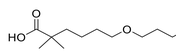
Introduction. Gemcabene, a fraudulent fatty acid, induces biological properties in nonclinical models, of which many translate to clinical findings in dyslipidemia patients. Its known mechanism of action includes reduction of the overall hepatic *de novo* triglyceride and cholesterol synthesis, inhibition of apolipoprotein C-III (ApoC-III), and increase in VLDL clearance. Clinical benefits in various human populations infer that other mechanisms are involved that warrant evaluation.
Methods and Results. We assessed the gemcabene effects in other molecular mechanistic models, particularly to study inhibition of human ATP Citrate Lyase (hACL), apolipoprotein gene expression in Sandwich-Cultured Transporter Certified™ Human Hepatocytes (SCHH), and expression of genes related to inflammation and cell signaling.
Gemcabene, MEDICA-16, palmitic acid and their CoA thioesters were studied in a recombinant hACL in vitro assay system. Gemcabene-CoA thioester, but not the parent compound, inhibited recombinant hACL activity in vitro in a dose-dependent manner, in agreement with results obtained with palmitoyl-CoA and palmitic acid. Unlike palmitic acid, MEDICA-16 and bempedoic acid, gemcabene does not form a CoA ester when incubated in human hepatocytes: radioisotope studies show 98.8% of the parent gemcabene remained.
Further, the gemcabene potential on the gene expression of lipogenesis and inflammation markers was assessed in SCHH. Gemcabene showed significant regulation of HMG-CoA synthase 2 (HMGCS2) and CRP mRNAs. A marked induction response of the HMGCS2 mRNA content was observed, ranging from 2.26 - to 2.73-fold ($p \leq 0.05$) over control in SCHH treated with all concentrations of gemcabene (500, 1000 and 1500 µM). Also, a clear and statistically significant (p-value ≤ 0.05) concentration-related suppression response of CRP mRNA content was observed, ranging from (-2.58) to (-2.65)-fold below control in SCHH treated with 1000 and 1500 µM gemcabene.
Finally, RT-PCR analysis of liver samples from STAM™ mice, a rodent model of nonalcoholic steatohepatitis (NASH), treated with gemcabene revealed its downregulating effect on inflammatory, lipogenic, lipoprotein metabolism, and cell signaling genes, among which TNF-α, MCP-1, NF-κB, ApoC-III, and ACC1.
Conclusion. Gemcabene manifests its pharmacological profile in lipid management and inflammation by multiple mechanisms of action.

INTRODUCTION

GEMCABENE HAS MULTIPLE MECHANISMS OF ACTION



The Gemcabene Molecule



Blocks de novo production of fatty acids and cholesterol in liver and enhances clearance of VLDL (LDL) in plasma

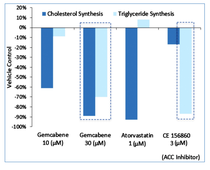
- Plasma half-life of 32 to 41 hrs
- Liver is the target organ
- Gemcabene is the active compound
- Renal elimination

PRECLINICAL EVIDENCE OF MECHANISMS

1. Inhibition of Cholesterol and Triglyceride Synthesis in Primary Rat Hepatocytes
2. Reductions in LDL-cholesterol (LDL-C) and Hepatic Triglycerides (TG) in Rodent Models

Gemcabene inhibits *de novo* synthesis of both cholesterol and triglycerides

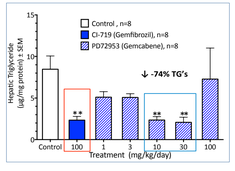
(from [^{14}C]-Acetate in Primary Rat Hepatocytes)



***In vitro* Rat Liver POC Chol and TG**

Gemcabene reduces hepatic *de novo* cholesterol and TG synthesis from acetate similar to atorvastatin and ACC inhibitors

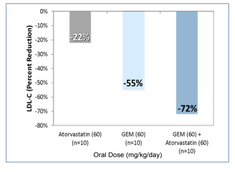
Two-week gemcabene treatment reduces hepatic triglycerides in the chow-fed Sprague Dawley rat model



***In vivo* Rat Liver POC TG**

Gemcabene significantly reduces hepatic triglycerides in a rat model

Two-week gemcabene treatment reduces LDL-C in LDL-Receptor Deficient Mice, an HoFH rodent model



***In vivo* Mouse Liver POC Chol**

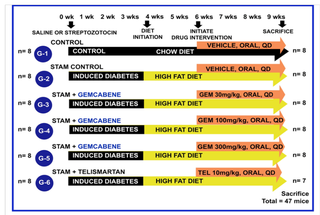
Gemcabene significantly reduces LDL- C alone and in combination with atorvastatin in a mouse model of homozygous familial hypercholesterolemia (HoFH)

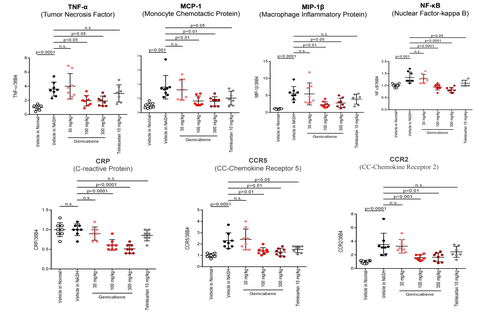
RESULTS

EFFECT OF GEMCABENE ON MARKERS OF INFLAMMATION IN STAM™ MICE

Hepatic gene expression indicative of inflammation are significantly reduced by gemcabene

PROTOCOL



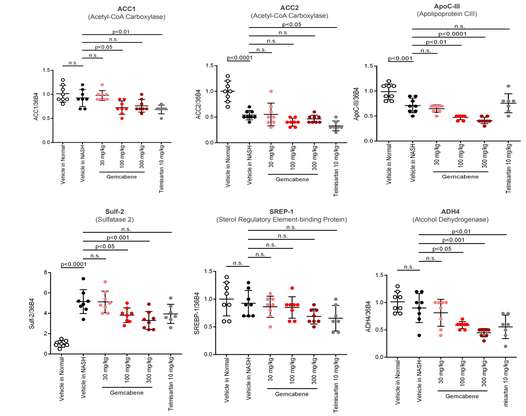


Two-day old neonatal C57BL/6 male mice were administered low-dose streptozotocin (STZ), and were subsequently fed a HFC diet from 4 weeks of age. mice were administered daily oral gemcabene starting at week 6 of age and were sacrificed at week 9. Telmisartan (with anti-steatotic, anti-inflammatory and anti-fibrotic effects in STAM™ mice) was used as a positive comparator. Various gene expression markers of liver metabolism were evaluated by Real-Time PCR (RT-PCR) in all groups. In order to calculate the relative mRNA expression level, the expression of each gene was normalized to that of the reference gene 36B4 (gene symbol: Rplp0).

EFFECT OF GEMCABENE ON PLASMA AND LIVER MARKERS IN STAM™ MICE

Markers	Vehicle in NASH (vs. Vehicle in Normal)	Gemcabene 30 mg/kg	Gemcabene 100 mg/kg	Gemcabene 300 mg/kg	Telmisartan 10 mg/kg
		(vs. Vehicle in NASH)			
Plasma triglyceride	↑	↓	↓	↓	-
Plasma total cholesterol	↑	-	↑	↑	↑
Plasma leptin	-	-	-	-	-
Plasma CRP	↑	-	↓	↓	-
Plasma adiponectin	↓	-	-	-	↑
Liver triglyceride	↑	-	-	-	↓
Liver free fatty acid	↑	-	-	↑	↑
Liver hydroxyproline	-	-	-	-	-

EFFECT OF GEMCABENE ON HEPATIC MARKERS OF LIPID REGULATION IN STAM™ MICE



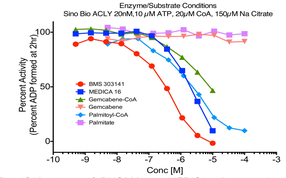
	Vehicle in NASH (vs. Vehicle in Normal)	Gemcabene 100mg/kg (vs. Vehicle in NASH)	
TNF-α	▲	▼	Inflammation
MCP-1	▲	▼	Inflammation
MIP-1β	▲	▼	Inflammation
CCR5	▲	▼	Inflammation
CCR2	▲	▼	Inflammation
NF-kB	▲	▼	Inflammation
CRP	-	▼	Inflammation
ACC1	-	▼	Lipid Metabolism
ApoC-III	▼	▼	Lipid Metabolism
Sulf-2	▲	▼	Lipid Metabolism
ADH4	-	▼	Alcohol Catabolism
	-	no significant difference	
	▲	significant increase	
	▼	significant decrease	

RESULTS (CONTINUED)

EFFECT OF GEMCABENE AND GEMCABENE-COENZYME-A ON RECOMBINANT HUMAN ATP CITRATE LYASE ACTIVITY

Gemcabene- CoA, but not Gemcabene, has an Effect on Recombinant Human ATP-citrate Lyase(hACL) *in vitro*

ATP Citrate Lyase Activity



The IC50 values of BMS303141, MEDICA 16, gemcabene CoA thioester, and palmitoyl-CoA thioesters against recombinant hACL in the ADP-Glo™ assay method were 0.392 µM, 17.9 µM, 66.6 µM, and 2.6 µM, respectively. These data suggest that gemcabene-CoA thioester, but not the free acid, is capable of inhibiting hACL activity *in vitro*.

Assessment of Gemcabene Glucuronide Formation in Human Hepatic Microsomes and Hepatocytes

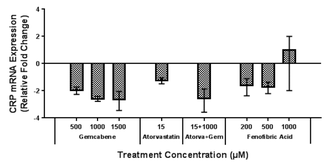
Gemcabene is essentially unchanged in human hepatocytes

Species	Extract	Time (minutes)	Percent Gemcabene Glucuronide	Percent Gemcabene (Parent Drug)
Mouse[a]	Microsomes	90	nd	100
Rat[a]	Microsomes	90	nd	100
Dog[a]	Microsomes	90	nd	100
Monkey[a]	Microsomes	90	nd	100
Human[a]	Microsomes	90	nd	100
Mouse[b]	Hepatocytes	180	8.30	90.7
Rat[a]	Hepatocytes	180	23.9	76.1
Dog[a]	Hepatocytes	180	20.4	77.8
Monkey[a]	Hepatocytes	180	15.5	84.5
Human[c]	Hepatocytes	180	0.513	98.8

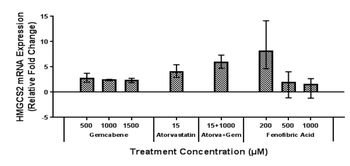
All of gemcabene remained in the parent form following incubation with liver microsomes from all species tested. Similarly in human hepatocytes, 98% gemcabene remained as parent. This suggests that there is essentially no gemcabene-CoA thioester formation. We did find the formation of gemcabene glucuronide in all species, albeit to a different degree. Taken together, these data rule out gemcabene free acid and gemcabene-CoA thioester-mediated inhibition of hACL *in vivo*.

IN VITRO ASSESSMENT OF GEMCABENE INDUCTION POTENTIAL IN SANDWICH-CULTURED TRANSPORTER CERTIFIED™ HUMAN HEPATOCYTES (HSCH)

Relative-fold changes in C-reactive Protein (CRP) mRNA following 72 hr exposure to fenofibric acid, gemcabene, and atorvastatin



Relative-fold changes in HMG-CoA Synthase (HMGCS2) mRNA following 72 hr exposure to fenofibric acid, gemcabene, and atorvastatin



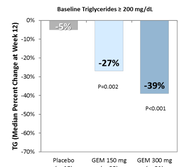
Cryopreserved hepatocytes were thawed and HSCH were prepared by plating cryopreserved hepatocytes were suspended in QTS propriety hepatocyte seeding medium (QualGro™ Seeding Medium) at a density of 0.8 million viable cells/mL onto BioCoat® 24-well cell culture plates. Following plating, cells were allowed to attach for 2-4 hours, rinsed and fed with warm (37°C) seeding medium. Eighteen to 24 hours later, cells were fed and overlaid with QTS propriety culture medium (QualGro™) supplemented with extracellular matrix (ECM), Matrigel® (0.25 mg/mL). Cells were maintained in QualGro™ Hepatocyte Culture Induction Medium until consumed in studies.

CLINICAL EVIDENCE OF MECHANISMS

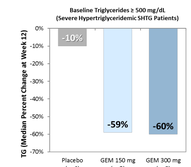
Evidence of Plasma Triglycerides, LDL-C and Anti-inflammatory Reduction by Gemcabene in Humans

Median Percent Change from Baseline in Hypertriglyceridemic Patients with TG ≥ 200 mg/dl at 12 weeks

Bays *et al* 2003

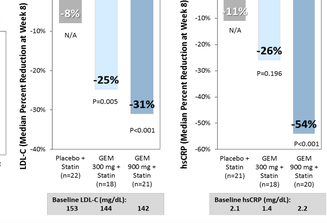


Post-Hoc Analysis



Median Percent Change from Baseline at Week 8 in Patients with Hypercholesterolemia on Background Stable Statin Therapy

Stein *et al* 2016



CONCLUSIONS AND REFERENCES

- Gemcabene has pleiotropic effects and multiple mechanisms of action.
- Gemcabene significantly inhibits hepatic triglyceride and cholesterol synthesis.
- Gemcabene significantly reduces triglyceride levels in the liver of chow-fed rats.
- Gemcabene significantly reduces LDL-C in LDL-receptor deficient mice alone and in combination with atovastatin.
- Gemcabene in humans significantly reduces plasma triglycerides, LDL-cholesterol and C-Reactive Protein.
- Gemcabene does not form a CoA thioester and does not inhibit recombinant human ATP Citrate Lyase (hACL).
- In sandwich-cultured transporter certified™ human hepatocytes (SCHH), gemcabene reduces C-Reactive Protein (CRP) and increases HMG-CoA Synthase (HMGCS2) mNRA levels.
- Diabetic mice fed a high-fat caloric diet develop Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatits (NAFLD/NASH), also known as the STAM™ mouse model. Gemcabene significantly attenuates a variety of hepatic inflammatory markers in the STAM™ mice.
- Gemcabene significantly decreases hepatic lipid regulating markers including Acetyl CoA Carboxylase 1 (ACC1), Apolipoprotein C-III (ApoC-III), Sulfatase 2 (Sulf2) and Alcohol Dehydrogenase (ADH4) in the STAM™ mice.
- Gemcabene significantly reduces plasma triglycerides and CRP in the STAM™ mice.

Bisgaier, C. L. et al. A novel compound that elevates high density lipoprotein and activates the peroxisome proliferator activated receptor. J Lipid Res 39, 17-30 (1998); US 2002/0103252 (2002)
Bays, H. E. et al. Effectiveness and tolerability of a new lipid-altering agent, gemcabene, in patients with low levels of high-density lipoprotein cholesterol. Am J Cardiol 92, 538-543 (2003)
Stein, E. et al. Efficacy and safety of gemcabene as add-on to stable statin therapy in hypercholesterolemic patients. J Clin Lipidol 10, 1212-1222 (2016)