GEMCABENE ATTENUATES THE NAFLD ACTIVITY AND FIBROSIS SCORES AND DOWNREGULATES HEPATIC INFLAMMATORY GENES IN THE STAM™ MURINE MODEL OF NASH-HCC

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ABSTRACT

Background and Aims: Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) advance, if untreated, to liver cirrhosis, fibrosis, hepatocellular carcinoma, liver failure and liver-related death. In the United States, NASH affects approximately 2-5% of the population, and an additional 10-30% have NAFLD. The number of drugs in development for NASH is growing steadily, along with nonclinical models to support prediction of clinical success. Here we are evaluating gemcabene, a first-in-class clinical candidate for dyslipidemia, for its potential utility, based on its robust and combined lipid-lowering and anti-inflammatory efficacy in clinical trials, in a preclinical model of NASH.

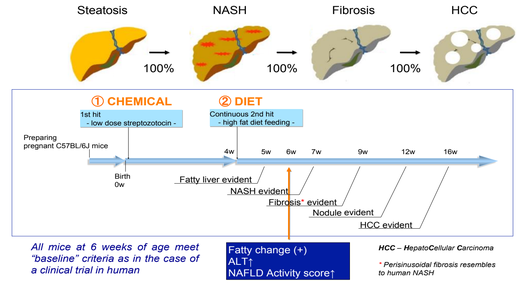
Methods: Gemcabene was evaluated in the STAM™ murine model of NASH. Gemcabene intervention in mice made diabetic with streptozotocin (STZ) and fed a high-fat caloric diet was assessed for changes in plasma, hepatic histological changes and hepatic mRNA markers of lipid metabolism and inflammation.

Results: Gemcabene significantly downregulated hepatic mRNA markers of inflammation (TNF-α, MCP-1, MIP-1β, CCR5, CCR2, NF-κB), lipogenesis and lipid modulation (ApoC-III, ACC1, ADH-4, sulf-2), fibrosis (TIMP-1), and hepatic carcinogenesis (MMP-2). These effects are important for the prevention of steatosis, inflammation, and hepatocyte ballooning (i.e., NAS score reduction), and inhibition of fibrosis progression, and were observed with gemcabene treatment.

Conclusions: These non-clinical findings corroborate with existing clinical data to support the clinical evaluation of gemcabene as a new treatment for NASH.

INTRODUCTION

IN VIVO PREDICTIVE PHARMACOLOGY MODEL OF NASH AND HCC



Recent clinical trials have shown that improvements in NASH or fibrosis are dependent on combined hepatic lipid-lowering and anti-inflammatory properties of a drug candidate. Prior research on gemcabene showed that such effects were translated from rodents to humans (1, 2, 3), and therefore a murine model of NASH-HCC may be adequate for a proof-of-concept experiment on the effect of gemcabene on NASH disease progression.

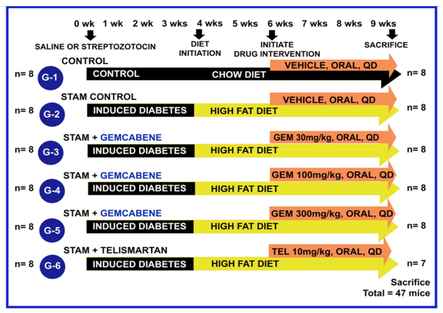
No NAFLD model is perfect (4). For instance, the methionine/choline deficient diet (MCD) fed mouse (5) develops hepatic steatosis and body weight loss without developing insulin resistance. The high-fat/high-calorie diet (HFC)-fed mouse features obesity, hepatic steatosis with mild injury, and insulin resistance (6). Genetic deficient ob/ob (leptin deficient), db/db mice (leptin-receptor defective) or *Zucker rats* (leptin-receptor deficient), do not develop steatosis implicitly, and often need to be fed an MCD or an HFC diet (5).

The STAM™ model of NASH-HCC is an HFC-fed mouse model, in which the NASH pathological progression is very similar to that in humans, as mice develop liver steatosis, inflammation, and partial fibrosis (7). Therefore, we selected this model for our proof-of-concept experiment to determine the effect of gemcabene on the inhibition of the NAFLD/ NASH progression.

METHODS

GEMCABENE ASSESSMENT IN A MURINE MODEL OF NASH

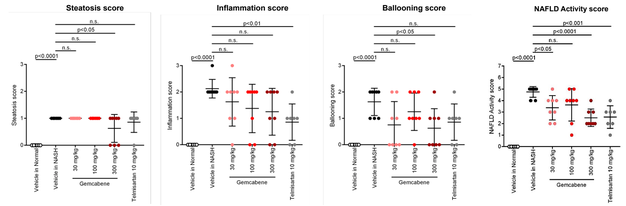
PREDICTIVE PHARMACOLOGY OF NASH



Forty-eight two-day old neonatal C57BL/6 male mice randomized to 6 groups of 8 animals were initially administered a single dose of vehicle (Group 1) or streptozotocin (STZ) (Groups 2-6). At four weeks of age animals were allowed chow *ad libitum* (Group 1) or a high-fat caloric diet (Groups 2-6) until completion of the experiment. Beginning at six weeks of age, mice were orally administered daily either water-vehicle (Groups 1 and 2), gemcabene at 30, 100 or 300 mg/kg (Groups 3-5), or telmisartan (Micardis®) 10 mg/kg (Group 6). All groups were sacrificed at week 9. Telmisartan (with antisteatotic, anti-inflammatory and antifibrotic effects in STAM™ mice) is generally used as a positive comparator.

RESULTS

GEMCABENE NAS COMPOSITE SCORE



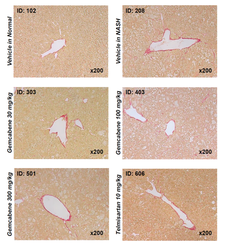
Group	n	NAS (mean ± SD)
Vehicle in Normal	8	0.0 ± 0.0
Vehicle in NASH	8	4.8 ± 0.5
Gemcabene 30 mg/kg	8	3.4 ± 1.1
Gemcabene 100 mg/kg	8	3.6 ± 1.4
Gemcabene 300 mg/kg	8	2.5 ± 0.8
Telmisartan 10 mg/kg	7	2.6 ± 1.0

Inflammation and Ballooning Scores Were Reduced Across All Doses of Gemcabene

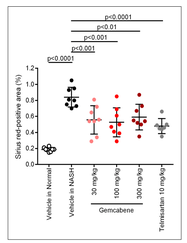
Gemcabene Reduced NAS Score by 25% to 48% (1.2 to 2.3 points) Compared with the Vehicle in NASH Group

FIBROSIS MEASURED BY SIRIUS RED-STAINED LIVER SECTIONS

Representative Photomicrographs of Sirius Red-Stained Liver Sections



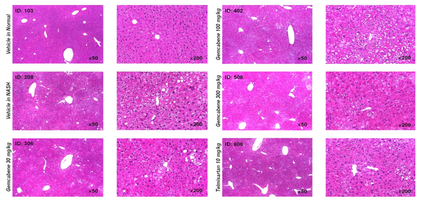
Fibrosis area



Parameter (mean ± SD)	Vehicle in Normal (n=8)	Vehicle in NASH (n=8)	Gemcabene 30 mg/kg (n=8)	Gemcabene 100 mg/kg (n=8)	Gemcabene 300 mg/kg (n=8)	Telmisartan 10 mg/kg (n=7)
Sirius red-positive area (%)	0.19 ± 0.03	0.84 ± 0.12	0.56 ± 0.18	0.53 ± 0.18	0.59 ± 0.16	0.48 ± 0.09

Sirius Red stained liver sections from the Vehicle in NASH group (top right) showed increased collagen deposition in the pericentral region of liver lobule compared with the Vehicle in Normal group (top left). The gemcabene 30mg, 100mg and 300mg groups showed decreases of 33%, 37% and 30% in fibrosis area compared with the Vehicle in the NASH groups, respectively.

REPRESENTATIVE PHOTOMICROGRAPHS OF HEMATOXYLIN AND EOSIN-STAINED LIVER SECTIONS



H&E stained liver sections from the Vehicle in NASH group exhibited micro- and macrovesicular fat deposition, hepatocellular ballooning and inflammatory cell infiltration compared with the Vehicle in Normal group. The gemcabene 30 and 300 mg/kg and Telmisartan groups showed less steatosis, and less lobular inflammation and degeneration of liver cells and nuclei (ballooning) than the Vehicle in NASH.

GENE BIOMARKERS

Gene Expression Analysis

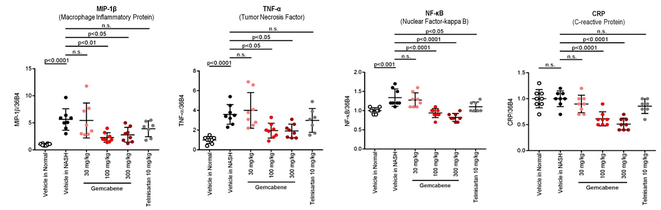
	Vehicle in NASH (vs Vehicle in Normal)	Gemcabene 100 mg/kg (vs Vehicle in NASH)	
CRP	-	▼	Inflammation
CCR2	▲	▼	Inflammation
CCR5	▲	▼	Inflammation
TNF-alpha	▲	▼	Inflammation
MCP-1	▲	▼	Inflammation
MIP-1Beta	▲	▼	Inflammation
NF-kB	▲	▼	Inflammation
TIMP-1	▲	▼	Fibrosis
MMP-2	▲	▼	Fibrosis
ACC1	-	▼	Lipid Metabolism
ApoC-III	▼	▼	Lipid Metabolism
Sulf-2	▲	▼	Lipid Metabolism
ADH4	-	▼	Alcohol Catabolism

\- no significant difference
▲ significant increase
▼ significant decrease

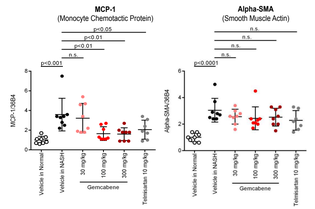
Hepatic gene expression and plasma markers indicative of inflammation, fibrosis, and lipid metabolism were significantly reduced by gemcabene.

RESULTS (CONTINUED)

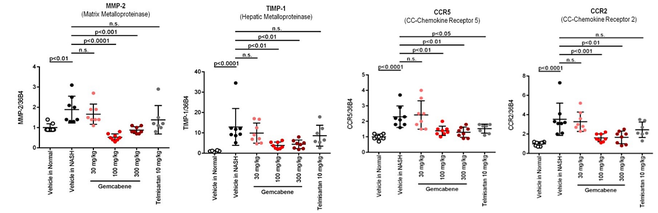
INFLAMMATORY MARKERS



HEPATIC INFLAMMATION AND STELLATE CELL ACTIVATION



COLLAGEN AND FIBROSIS MARKERS



At the highest dose level (300 mg/kg), gemcabene-treated STAM mice demonstrated a statistically significant histological reduction in NAS. Gemcabene showed significant decreases in the fibrosis area at all doses compared to the vehicle-treated NASH mice. Moreover, gemcabene significantly downregulated mRNA markers of inflammation (TNF-α, MCP-1, MIP-1β, CCR5, CCR2, NF-κB), fibrosis (TIMP-1), and hepatic carcinogenesis (MMP-2).

CONCLUSIONS

The effect of gemcabene on the liver histology and gene expression levels associated with inflammation are complementary to our prior findings in dyslipidemia patients, and supports the clinical evaluation of gemcabene as a potential treatment for NAFLD/NASH. In the current study, gemcabene-treated STAM™ mice demonstrated a significant histological reduction in both NAS score and fibrosis progression. Further, analysis of hepatic expression of inflammation related genes: TNF-α, MCP-1, MIP-1β, CCR5, CCR2, NF-κB, suggesting gemcabene hits multiple targets and has a hepatoprotective effect on liver pathology. Also, gemcabene reduced the mRNA expression levels of metabolism-related genes: ACC1, ApoC-III, SULF-2, ADH-4. Plasma CRP levels were also decreased by gemcabene treatment along with the down regulation of the CRP gene expression, which is in agreement with human clinical data (1). Data from previous non-clinical and clinical studies have shown that gemcabene reduces plasma TG, ApoC-III mRNA and plasma levels, and enhances VLDL clearance (2, 3).

The STAM™ model is induced with STZ, with near complete loss of pancreatic insulin production, and, therefore, translation effects of drugs on insulin sensitization are not expected. However, this model demonstrated that pleiotropic drugs, such as gemcabene, and/or multi-modal combination therapy approaches may effectively guide treatments for NASH. The current nonclinical data corroborated with earlier clinical findings support the evaluation of gemcabene in the resolution of NASH in humans.

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