Leucine synergizes with metformin and sildenafil to treat Non-Alcoholic Fatty Liver Disease (NAFLD) and Steatohepatitis (NASH)

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Introduction

High-fat diets and diabetes downregulate the Sirt1/AMPK axis, resulting in hepatic steatosis and inflammation, while Sirt1 overexpression or activation protects against NASH (1-3) and represents a therapeutic target for NASH (3).

We have found leucine to allosterically activate Sirt1 (4), and to synergize with metformin. This enables a 65-80% dose reduction with no loss of anti-diabetic efficacy and reverses NAFLD in mice despite the lack of efficacy of full-dose metformin (5-7). PDE5 inhibitors also activate the Sirt1/AMPK axis via eNOS/NO signaling, and leucine also synergizes with sildenafil via eNOS to activate Sirt1 and inhibit steatosis and inflammation (8). Accordingly, we tested the efficacy of a multi-component activation of this pathway in a more severe preclinical model of NASH.

Materials & Methods

animals:
C57BL/6 mice (n=10/group) fed either a low fat (LF) or a high fat/atherogenic diet (HF/HC) containing 1.25% cholesterol (w/w) and 60% calories as saturated fat (lard) for 8 weeks to induce the development of NASH and insulin resistance. Then they were randomized to one of the treatment groups of study 1 or study 2 for additional 8 weeks.

Treatment groups (Study 1):
Control LF = 10% calories from fat, Control HF/HC = 1.25% cholesterol, 0.5% cholate, 60% calories from fat, HF/HC + leucine (24 g/kg diet: a two-fold increase over control levels) + metformin (0.25 g/kg diet, <20% of therapeutic dose), HF/HC + sildenafil (25 mg/kg/diet, <10% of therapeutic dose), HF/HC + leucine + sildenafil, HF/HC + metformin + sildenafil, HF/HC + leucine + metformin + sildenafil

Results: Interactive effects of leucine, metformin and sildenafil

Figure 1: Liver weight, liver triglycerides and Alanine-Amino-Transferase (ALT)

Figure 2: Sirius Red (Collagen) and CD68 staining

Figure 3: Liver Histology

Figure 4: Gene expression of fibrogenetic, inflammatory, and oxidative genes in liver

Figure 5: Inflammation and steatosis score in dosing efficacy study (Study 2)

Conclusions

Leu-Met-Sil feeding resulted in significant reductions of liver weight, liver triglycerides and ALT, and reversed the diet-induced steatohepatitis.

Leu-Met-Sil combination markedly reduced fibrogenesis and inflammation

Leu-Met-Sil shows promise for the treatment of NAFLD and NASH

References

2. Liu Y, Wan Q, Guan Q, Gao L, Zhao J. High-fat diet feeding impairs both the expression and activity of AMPKs in rat’s skeletal muscle. Biochem Biophys Res Commun 2006;339:701-7

Disclosure information: Authors Zemel and Bruckbauer are employees & Stockholders of NuSirt Biopharma.
Authors Xue and Shi have no conflicts to disclose.