Leucine Synergizes With Phosphodiesterase 5 (PDE5) Inhibitors And Metformin To Reverse Hepatic Lipid Accumulation And Inflammation And Treat Non-alcoholic Fatty Liver Disease (NAFLD)

[1]Fu L., [1]Li F., [1]Cao Q., [1]Cui X., [1]Xue B., [1]Shi H., [2]Bruckbauer A., [2]Zemel M.B.

[1]Georgia State University, Atlanta, GA

[2]NuSirt BioPharma Inc., Knoxville TN

260-LB Abstract

Sirt1 and AMPK regulate hepatic lipid and energy metabolism and are therapeutic targets for NAFLD. We previously demonstrated that L-leucine (leu) has a unique role as an allosteric activator of Sirt1, enabling synergy with metformin (met) to increase insulin sensitivity and reverse NAFLD in mice. PDE5 inhibitors converge on the Sirt1/AMPK pathway via eNOS/NO signaling, and leu also synergizes with sildenafil(sild) to inhibit both steatosis and inflammation. Here we demonstrate the potential for multi-component activation of this pathway with leu (0.5 mM), met (10 M) and sild (1 nM) to yield greater therapeutic efficacy in NAFLD. Leu-met-sild activated Sirt1 to a greater degree than leu-met or leu-sild, resulting in markedly greater stimulation of hepatocyte fat oxidation (~60%, p<0.01) and inhibition of palmitate-induced triglyceride accumulation (~70%, p<0.001). To evaluate this synergy in vivo, non-alcoholic steatohepatitis (NASH) was induced in mice via high fat diet supplemented with cholesterol and cholate (HF/ATH diet); mice were then treated with combinations of leu (24 g/kg diet), met (0.25 g/kg diet; <20% of therapeutic dose) and sild (25 mg/kg diet; <10% therapeutic dose). The HF/ATH diet caused a ~6-fold increase in alanine aminotransferase (p<0.0001) which was modestly reduced by leu-met and leu-sild (30-40%, p<0.01) and further reduced by leu-met-sild (60%, p<0.0001). The HF/ATH diet also caused increases in liver mass and a marked increase in liver fat content (~7-fold, p<0.0001). Leu-met and leu-sild elicited modest reductions in both, while the leu-met-sild combination reduced liver mass (p<0.003) and liver fat to a significantly greater degree (38%, p=0.02). These data demonstrate preclinical therapeutic potential for leucine combined with sub-therapeutic levels of metformin and sildenafil in the treatment of NAFLD and NASH.

Introduction

Sirt1 and AMPK are key regulators of systemic and hepatic lipid and glucose metabolism and inflammatory pathways. High fat diets and diabetes downregulate the Sirt1/AMPK axis, resulting in hepatic steatosis and inflammation, similar to the effects of hepatic Sirt1 knockout, while Sirt1 overexpression or activation protects against non-alcoholic steatohepatitis (NASH) in preclinical models (1-3), thus representing theraupeutic targets for NAFLD and NASH (3).

We have demonstrated leucine to allosterically activate Sirt1 (4), and thereby serves as a partial mimetic of energy restriction. Leucine synergizes with metformin, enabling 65-80% dose reduction with no loss of antidiabetic efficacy and reverse 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 we have found that leucine also synergizes with sildenafil to inhibit steatosis and inflammation (8). **Accordingly, here we have sought to determine whether a multi-component activation of this pathway would result in greater therapeutic efficacy in treating a more severe preclinical model of NASH.**

Materials & Methods

animals: C57/BL6 mice (n=10/group) were fed either a low fat (LF) diet or a high fat/atherogenic diet (HC) containing 1.25% cholesterol by weight and 60% calories as saturated fat (lard) for 6-8 weeks to induce the development of NASH and insulin resistance. Then they were randomized to one of the treatment groups for additional 6-8 weeks.

Treatment groups:

1.Control LF = 10% calories from fat
2.Control HC = 1.25% cholesterol, 0.5% cholate, 60% calories from fat
3.HC + leucine (24 g/kg diet; a two-fold increase over control levels) + metformin (0.25 g/kg diet
4.HC+ sildenafil (25 mg/kg/diet)
5.HC + leucine (24 g/kg diet) + sildenafil (25 mg/kg/diet)
6.HC+ metformin (0.25 g/kg diet) + sildenafil (25 mg/kg/diet)
7.HC+ leucine (24 g/kg diet) + metformin (0.25 g/kg diet) + sildenafil (25 mg/kg/diet)

Results

Interactive Effects of leucine, metformin and sildenafil

in vivo (mice)

Figure 1: Liver weight

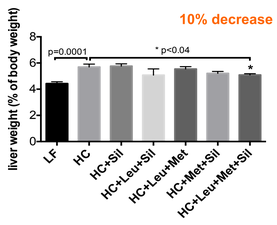


Figure 2: Liver triglycerides

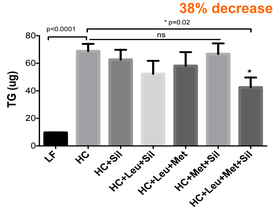


Figure 3: Alanine-Amino-Transferase (ALT) at 2 weeks

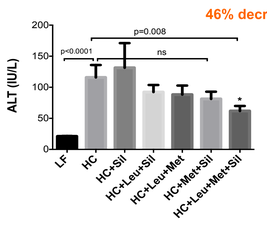


Figure 4: Alanine-Amino-Transferase (ALT) at 6 weeks

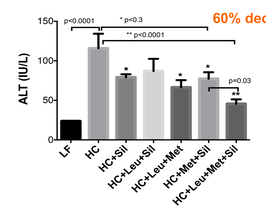
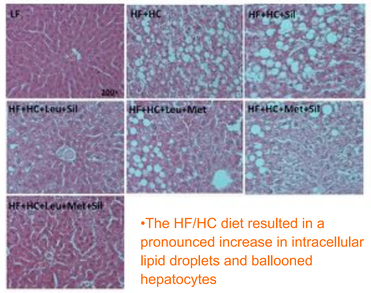


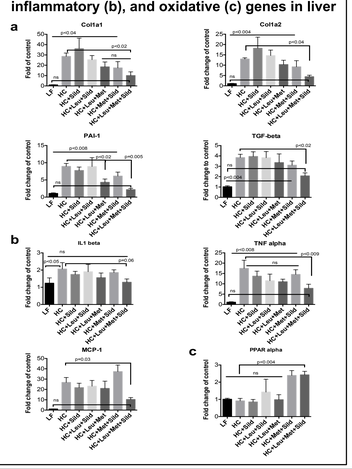
Figure 5: Liver Histology



- •The HF/HC diet resulted in a pronounced increase in intracellular lipid droplets and ballooned hepatocytes

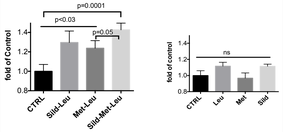
- leucine-metformin and leucine-sildenafil combinations attenuated, but did not reverse, these effects
- the triple combination of leucine, metformin and sildenafil resulted in a substantial reversal of this steatohepatitis

Figure 6: Gene expression of fibrogenetic (a), inflammatory (b), and oxidative (c) genes in liver

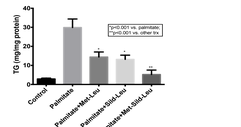


in vitro

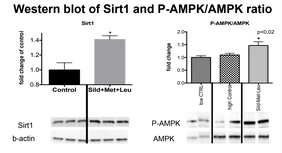
Oxygen consumption rate in HepG2



Hepatocyte Triglyceride

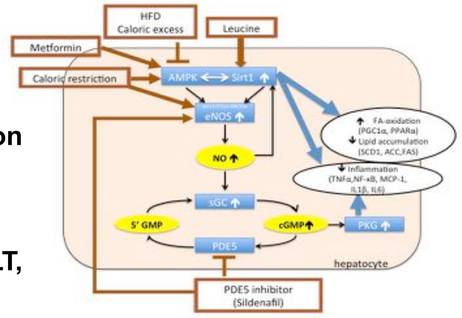


Western blot of Sirt1 and P-AMPK/AMPK ratio



Conclusions

- ✧ **The triple combination of leucine, low dose metformin and low dose sildenafil interacts on the AMPK-Sirt1-eNOS/NO pathway to increase fatty acid oxidation and to reduce lipid accumulation in hepatocytes**
- ✧ **Leu-Met-Sild feeding in mouse NASH-model resulted in significant reductions of liver weight, liver triglycerides and ALT, and reversed the diet-induced steatohepatitis**



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