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LIVRQNac (AXA1125) Increases Fatty Acid Oxidation in a Primary Human Hepatocyte Model of Nonalcoholic Steatohepatitis

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Introduction

- Complex diseases involve dysregulation of multiple biological pathways, limiting the effectiveness of single-targeted therapies[1]
- Endogenous metabolic modulators (EMMs) are naturally occurring compounds with signaling and regulatory properties that, when selectively combined, may elicit multifactorial effects in complex diseases
- In a 16-week clinical study, administration of AXA1125, a novel EMM composition of 5 specific amino acids (AAs; leucine [L], isoleucine [I], valine [V], arginine [R], glutamine [Q]) and an AA derivative, *N*-acetylcysteine (Nac), resulted in a greater reduction of hepatic fat than placebo as one of its multifactorial effects in patients with nonalcoholic fatty liver disease[2,3]
- An analogous decrease in triglyceride accumulation has been observed in primary human hepatocytes (PHHs) treated with LIVRQNac,[4] the nonclinical form of AXA1125 containing the same constituents; a mechanism explaining this effect might be promotion of fatty acid oxidation (FAO) in PHHs by LIVRQNac

Aim

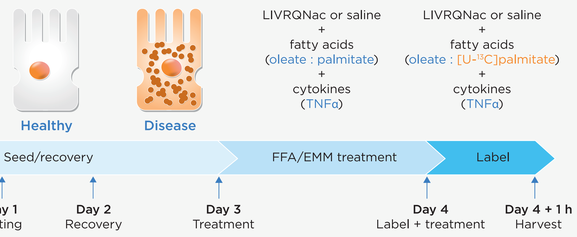
- To determine the *in vitro* effects of LIVRQNac on FAO in a PHH model of nonalcoholic steatohepatitis (NASH) using a stable isotope-labeled tracer with chromatography and mass spectrometry

Methods

- PHHs were seeded in collagen-coated 12-well plates on Day 1; 48 hours later, PHHs were switched to a custom medium containing physiological concentrations of AAs, 500 µM carnitine, 10 µg/mL insulin, 10 ng/mL epidermal growth factor, 1 µM dexamethasone, and LIVRQNac (10× or 30×) or control saline. Cells were then treated with free fatty acids (FFAs, 250 µM, 2:1 oleate:palmitate) and tumor necrosis factor alpha (1 ng/mL). Following a 24-hour exposure to disease stimulus and LIVRQNac, PHHs were retreated using a stable isotope-labeled [U-^{13}C]palmitate tracer for 1 hour (Figure 1)
- Following the 1-hour incubation with [U-^{13}C]palmitate, PHHs were lysed and analyzed for [U-^{13}C]palmitate and unlabeled palmitate metabolites using gas chromatography-mass spectrometry and liquid chromatography with tandem mass spectrometry (Figure 2)

FIGURE 1

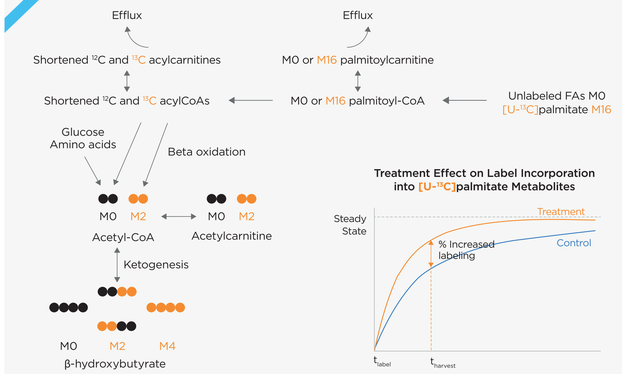
Culture, Treatment, and Processing of PHHs



EMM, endogenous metabolic modulator; FFA, free fatty acid; h, hour; LIVRQNac, leucine/isoleucine/valine/arginine/glutamine/*N*-acetylcysteine; PHH, primary human hepatocyte; TNF, tumor necrosis factor.

FIGURE 2

Use of [U-^{13}C]palmitate Tracer to Explore the Effect of LIVRQNac on FAO in PHHs



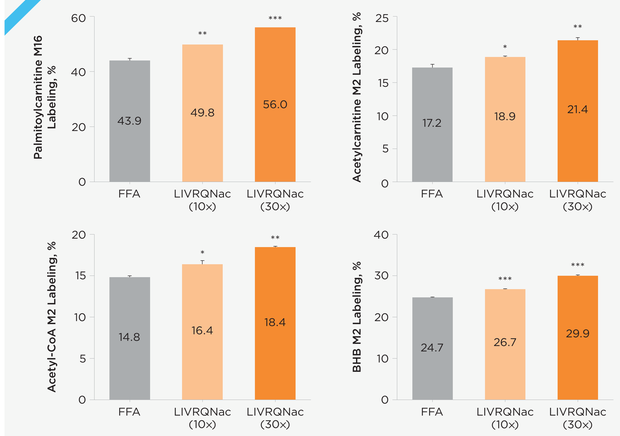
acylCoA, acyl-coenzyme A; CoA, coenzyme A; FA, fatty acid; FAO, fatty acid oxidation; LIVRQNac, leucine/isoleucine/valine/arginine/glutamine/*N*-acetylcysteine; M, mass isotopomer (the numbers 0 to 16 represent the number of heavy atoms in the molecules detected); PHH, primary human hepatocyte.

Results

- LIVRQNac treatment significantly and dose-dependently increased ^{13}C labeling of palmitate metabolites (palmitoylcarnitine, acetylcarnitine, acetyl-coenzyme A [acetyl-CoA], and β-hydroxybutyrate [BHB]), compared with the saline control (Figure 3)
  - The increased palmitoylcarnitine labeling likely represents LIVRQNac facilitation of FAO initiation allowing acylcarnitine transition into mitochondria
  - The increased acetylcarnitine and acetyl-CoA labeling indicates an increase in β-oxidation, during which successive 2-carbon units are cleaved from acyl-coenzyme A chains as FAO product acetyl-CoA, in equilibrium with acetylcarnitine
  - BHB labeling and concentration are good indexes of FAO as both tend to increase with increased FAO

FIGURE 3

Effect of LIVRQNac on ^{13}C Labeling of [U-^{13}C]palmitate-Derived Metabolites in PHHs

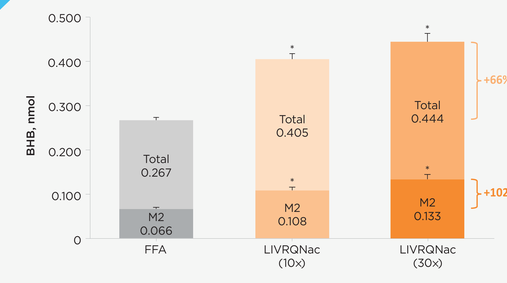


*P<0.05; **P<0.001; ***P<0.0001 (analysis of variance).
BHB, β-hydroxybutyrate; FFA, free fatty acid; LIVRQNac, leucine/isoleucine/valine/arginine/glutamine/*N*-acetylcysteine; M, mass isotopomer (the numbers 2 and 16 represent the number of heavy atoms in the molecules analyzed); PHH, primary human hepatocyte.

- The total amount of BHB increased dose dependently by 52% and 66% following treatment with LIVRQNac 10× and LIVRQNac 30×, respectively, compared with the saline control (Figure 4)
  - These increases demonstrate upregulation of the FAO pathway with LIVRQNac in PHHs, since BHB is a terminal end product of FAO
- Notably, the amount of mass isotopomer 2 (M2)–labeled BHB increased dose-dependently by 64% and 102% in PHHs treated with LIVRQNac 10× and LIVRQNac 30×, respectively, compared with the saline control (Figure 4)
  - M2-labeled BHB is derived from the [U-^{13}C]palmitate tracer and thus confirms it is derived from LIVRQNac-dependent FAO, as opposed to other sources, like ketogenic AA

FIGURE 4

Effect of LIVRQNac on M2-Labeled BHB (Terminal FAO Product) in PHHs



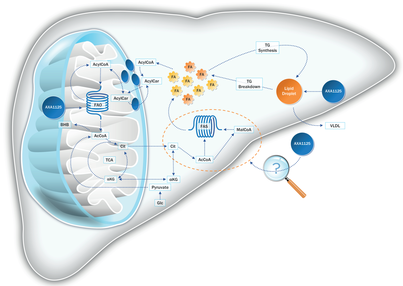
*P<0.0001 (analysis of variance).
BHB, β-hydroxybutyrate; FAO, fatty acid oxidation; FFA, free fatty acid; LIVRQNac, leucine/isoleucine/valine/arginine/glutamine/*N*-acetylcysteine; M, mass isotopomer (2 refers to the number of heavy atoms in the molecule analyzed); PHH, primary human hepatocyte.

Conclusions

- Statistically significant increases in labeled palmitoylcarnitine, acetylcarnitine, acetyl-CoA, and BHB in comparison with control reflected an increase in FAO in PHHs treated with LIVRQNac
- Consistently, there was a statistically significant increase in total intracellular BHB and percentage of label incorporation in LIVRQNac-treated cells, indicating an increase in ketogenesis, with ketone body terminal end products derived from oxidized fatty acids
- Taken together, these data support a mechanism for the clinical effect of AXA1125 of decreasing liver fat by increasing FAO and increasing ketogenesis from fatty acids
- We are also investigating the role of LIVRQNac in modulating additional lipid biology (Figure 5)
  - Mitochondrial fatty acid metabolism contributes to cellular energy production with implications in NASH and beyond
- The multifactorial effects of LIVRQNac, previously reported in PHHs and other NASH-relevant cell types,[4] include additional anti-inflammatory and antifibrotic benefits that complement its effects on lipid metabolism, improving biological mechanisms driving NASH disease progression
- AXA1125 is currently being studied in a phase 2b study in individuals with NASH (NCT04880187)

FIGURE 5

Potential Mechanisms of LIVRQNac (AXA1125) on FAO



αKG, α-ketoglutarate; AcCoA, acetyl-coenzyme A; AcylCar, acylcarnitine; AcylCoA, acyl-coenzyme A; BHB, β-hydroxybutyrate; Cit, citrate; FA, fatty acid; FAO, fatty acid oxidation; FAS, fatty acid synthase; Glc, glucose; LIVRQNac, leucine/isoleucine/valine/arginine/glutamine/*N*-acetylcysteine; MalCoA, malonyl coenzyme A; TCA, tricarboxylic acid cycle; TG, triglyceride; VLDL, very low-density lipoprotein.

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