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CO-016 - PHARMACOLOGICAL INHIBITION OF ATP-CITRATE LYASE PROMOTES A REDUCED AMPK SIGNALING AFFECTING TO MITOCHONDRIAL FUNCTION AND LIPID ACCUMULATION

A. Sola GarcÍa[a], D. GonzÁlez-MorÁn[a], M.Á. CÁliz-Molina[a], I. Espadas-Villanueva[a], N. El Kharoubi-Zamudio[a], R. LÓpez-FernÁndez-Sobrino[a] and A. MartÍn-Montalvo[a,b]

[a]Centro Andaluz de BiologÍa Molecular y Medicina Regenerativa (cabimer), Sevilla, Spain.[b]Centro de InvestigaciÓn BiomÉdica en Red de Diabetes y Enfermedades MetabÓlicas Asociadas (CIBERDEM), Madrid, Spain.

Resumen

Introduction and objectives: ATP-citrate lyase (Acly) is a central integrator of cellular metabolism in the interface of protein, carbohydrate, and lipid metabolism. This enzyme regulates several main cellular processes, such as de novo fatty acid synthesis or cholesterogenesis by producing acetyl coenzyme A (Ac-coA). However, the mechanism involved in long-term Acly inhibition over the regulation of energy metabolism remains unknown. Here we use the compound SB-204990 as a pharmacological intervention to elucidate the role of Acly inhibition.

Materials and methods: Cell culture studies were performed in primary hepatocytes from C57BL6 wild-type mice. Primary hepatocytes were treated with SB-204990 (10 μM) and were treated with glucose (25 mM), acetate (5 mM) or citrate (5 mM), bempedoic acid (30 μM) or Metformin (500 μM) for 16 hours, depending on the performed experiment. Hepatocytes lipid content was measured using Oil Red O staining and metabolic activity was identified by MTT experiments. Mitochondrial functional dynamics and glycolytic capacity were studied using a Seahorse equipment to determine the oxygen consumption rate and the rate of extracellular acidification of the media. Western Blots from hepatic lysates were used to evaluate protein expression levels.

Results: Using oil-red O staining, we found that primary hepatocytes treated with SB-204990 exhibited a significant increase in lipid content. Specifically, SB-204990 treatment under basal glucose conditions increased lipid content even when culture media was supplemented with citrate and acetate. MTT experiments showed reduced cellular metabolism in hepatocytes treated with SB-204990. In addition, Seahorse monitorization mitochondrial functional dynamics indicated a significant decrease in both basal and maximal oxygen consumption rate as well as in extracellular acidification rate in cells cultured in high-glucose conditions, suggesting glycolytic inhibition. Direct evaluation of glycolysis indicated that SB-204990 produces net decreases in ECAR in both, basal and high glucose conditions, suggesting the allosteric inhibition of glycolysis by intracellular accumulation of citrate. Experiments performed using the Acly inhibitor bempedoic acid exhibited similar effects. We found that SB-204990 reduces Ampk phosphorylation indicating a high energetic status. Primary hepatocytes co-treated with SB-204990 and Metformin, an Ampk activator, showed normalized metabolic activity and lipid levels, indicating that these effects require the blockade of

Ampk activity.

Conclusions: Taken together these results indicate that the effects of SB-204990 in hepatocytes require reduced Ampk signaling. Our study posits that pharmacological inhibitors of the Acly produce modulations on lipid and mitochondrial metabolism, which might have important consequences for metabolic health.