Poster

Evaluating the cellular consequences of ACLY inhibition in primary hepatocytes and pancreatic islets

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ABSTRACT

Motivation: Metabolic diseases and their pandemic proportions cause a significant loss in quality and expectancy of life in around 25% of the population. Therefore, the development of more efficient therapies should be a clinical priority. This project aims to evaluate the cellular consequences of a chronic Ac-CoA cytosolic pool reduction induced by the inhibition of ATP-citrate lyase (ACLY), core enzyme in metabolism and major source of Ac-CoA within the cytoplasm. Ac-Coa is a central molecule in many metabolic pathways involved in de novo lipogenesis, cholesterol production and protein acetylation.

Methods: Most of the assays were performed on mouse primary cultured hepatocytes and islets of Langerhans. Hepatocytes were obtained by perfusing the mouse liver with collagenase and isolated by filtering and differential centrifugation. Islets of Langerhans were obtained by perfusing the mouse pancreas with collagenase and were isolated by handpicking. Cells were treated with 0, 10, 30, 50 and 100 μM of SB-204990 (SB), an ACLY inhibitor with a Ki of 1 μM. Metabolic cell activity, cell death and urea secretion were measured. Radioactive metabolite tracing was performed to assess ACLY-dependent metabolic functions. Proteins of treated hepatocytes were extracted to perform western blots.

Results: In primary hepatocytes, SB neither affected metabolic activity nor urea production when concentration was <50 μM SB. However, exposure to this drug resulted in a significant increase in cell toxicity at ≥30 μM SB. We detected a dose-dependent decrease in liponeogenesis upon treatment. β-oxidation of fatty acids was affected by SB following a hormetic effect, with a rise at 10 μM and a reduction at 50 and 100 μM. The GAPDH protein expression was higher at 10 μM SB, suggesting a modulation of glycolysis/gluconeogenesis. LC3B protein expression patterns indicated autophagy was reduced at 10 μM SB and incremented at 100 μM SB. In primary islets, SB did not impair metabolic activity, while cell death, determined by ELISA, showed a reduction at 30 μM and no effects at other concentrations.

Conclusions: The effects of ACLY inhibition showed that this enzyme plays a key role in the cellular homeostasis of primary hepatocytes. Fatty acids catabolism and glucose utilization relies on the presence of functional ACLY. In islets, SB revealed to be non toxic. Our results suggest that ACLY may represent a promising target for the treatment of metabolc disorders.