Liver spheroids: a robust human in vitro system for testing the therapeutic options of cyclooxygenase 2 in NAFLD/NASH

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Background: NAFLD is the most prevalent chronic liver disease in the world and is considered the hepatic manifestation of metabolic syndrome. It begins with an accumulation of fat in the liver, which can lead to cellular damage and inflammation (NASH) and, if unaddressed, progresses to liver fibrosis, where parenchymal tissue is replaced by extracellular matrix. Cyclooxygenase (COX) is a key regulatory step in the biosynthesis of prostanoids. The COX-2 isoform is expressed and induced by different stimuli in various tissues and cell types; however, in liver, COX-2 expression is restricted to those situations in which proliferation or dedifferentiation occur (1). Our previous results revealed that COX-2 expression in hepatocytes protects against hyperglycemia-induced liver damage and against peripheral insulin resistance and adiposity in mice subjected to a high-fat diet (2). Furthermore, COX-2 expression in hepatocytes protects against experimental nonalcoholic steatohepatitis and fibrosis in mice (3).

Aims: Since our previous results are performed in mice, we wanted to bridge from animal models to a more translational research, close to human. Therefore, we wanted to establish an in vitro model based on 3D human cell culture to evaluate the role of COX-2, specifically the role of prostaglandins, in a human model of NAFLD/NASH.

Methods: To analyze the role of COX-2 in steatosis and fibrosis, we established a 3D spheroid culture composed of HepG2 and LX-2 cells. These spheroids were treated with palmitic acid and TGFβ to induce NAFLD/NASH and fibrosis, respectively. They were then treated with PGE_2 to evaluate their effect in this model.

Results: In summary, our results demonstrate that PGE_2 treatment reduces lipid levels after fatty acid exposure. Moreover, it reduces fatty acid-induced cell damage and insulin resistance. Furthermore, PGE_2 slows fibrotic progression by decreasing stellate cells activation after TGFβ stimulation.

Conclusions: These preliminary results indicate that a therapeutic strategy for the treatment of NAFLD/NASH may be COX-2-derived prostaglandin therapy.

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