Introduction and objectives

Farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5) are interesting pharmacological targets for the treatment of liver and metabolic diseases. FXR-deficient mice on a high-fat diet (HFD) exhibit massive hepatic steatosis, non-alcoholic hepatic steatosis, and fibrogenesis. Moreover, pharmacological activation of TGR5 in mice promotes protective mechanisms in biliary epithelial cells, inhibits hepatic and systemic inflammation.

The aim of this study is to investigate the effect of FXR/TGR5 dual agonists on nonalcoholic steatohepatitis (NASH) in a rabbit model of high fat diet (HFD)-induced MetS.

Methods

We employed a recently established animal model of high fat diet (HFD)-induced MetS, characterized by insulin resistance, hypertension, atherosclerotic dyslipidemia, visceral adipose tissue accumulation and NASH (Filippi, et al., 2009; Maneschi et al., 2013). Subgroups of MetS rabbits were treated with increasing doses of the dual FXR/TGR5 agonist INT-767 (3, 10, 30mg/kg, orally, daily, 5 days a week for 12 weeks). We studied the effects of HFD and in vivo INT-767 treatments on liver function. Liver was studied by immunohistochemistry and RT-PCR.

Results

- Treatment with increasing doses of the dual FXR/TGR5 agonist INT-767 (3, 10, 30mg/kg/day, 5 days a week for 12 weeks) in a rabbit model of HFD-induced MetS, characterized also by NASH, dose-dependently reduced several MetS-associated alterations, including hepatomegaly, insulin resistance, increase of ALT, glucose and cholesterol levels, while significantly increasing HDL levels. ALT was positively associated with all MetS parameters; however introducing all MetS factors in a multivariate analysis, only total cholesterol levels resulted positively associated with ALT level (Adj.r: 0.493, p=0.014). High macrophage M1 pro-inflammatory/M2 anti-inflammatory ratio was observed in MetS-induced NASH, which was independently associated with serum ALT levels (Adj.r: 0.322, p=0.032). HFD-Induced increase in M1/M2 ratio was reduced by INT-767 treatment and M2 macrophage markers (IL10, TGF5) were increased.

- Genes related to neutrophil apoptosis/apoptotic-neutrophil clearance (lactoferrin, eNOS, RAGE) and to extracellular matrix degradation (MMP2, TIMP2) were also increased by INT-767 treatment. INT-767 also reduced liver expression of IL-6, which preferentially skewed the Th cell response towards a Th17-phenotype, while increasing Fox3 expression, a Treg cell marker (Fig. 1). Thus these data indicate that INT-767 can promote the neutrophil- and macrophage-driven resolution phase of inflammation and fibrosis regression. In addition, INT-767 increased genes related to hepatic fatty acid metabolism (PPARa, AR, CD36) and lipid droplet formation (SNAIP3, VAMP4, Syntaxin5, perilipin) therefore suggesting that INT-767 counteracts excess fatty acids mediated lipidopexy in the liver. Genes related to insulin signaling (IRS1, SREBP1, G6Pase, and PEPCK) were also increased by INT-767 (Fig. 1).

- Finally, immunohistochemical studies demonstrated that INT-767 treatment significantly reduced both HFD-induced liver inflammation (Fig. 2) and fibrosis (Fig. 3).

Conclusions

In conclusion, INT-767 treatment counteracts NASH in a rabbit model of HFD-induced MetS by promoting insulin sensitivity, resolution of inflammation and fibrosis regression.

References
