Obeticholic acid exerts anti-inflammatory action and suppress expansion of hepatic immune cell populations in the Gubra biopsy-confirmed DIO-NASH mouse model

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INTRODUCTION

Hepatic inflammatory mechanisms are key hallmarks in the development and progression of non-alcoholic steatohepatitis (NASH). Consistent with late-stage clinical NASH trials, we have previously reported that obeticholic acid (OCA), a semi-synthetic FXR agonist, improves metabolic, biochemical and liver histological endpoints in the Gubra biopsy-confirmed DIO-NASH mouse model (Tabal et al., World Journal of Gastroenterology, 2018). To further investigate the anti-inflammatory effect of OCA, we applied flow cytometry to profile liver leukocyte populations following chronic OCA treatment in Gubra biopsy-confirmed DIO-NASH mice.

METHODS

Male C57Bl/6j mice were fed the AIN93 diet (40 % fat, 20 % fructose, 2 % cholesterol) for 30 weeks prior to liver biopsy sampling. Only animals with histology-proven steatosis (score 1+2) and fibrosis (stage 1+2) were included and stratified into treatment groups. DIO-NASH mice received vehicle (n=12) or OCA (30 mg/kg, PO, QD; n=12) for 8 weeks. Vehicle-dosed Chow-fed mice (n=6) served as normal lean controls (Figure 1). Plasma MCP-1 was measured, and body weight, hepatic steatosis, inflammation and fibrosis were assessed. Single cell suspensions from liver lobular tissue samples (lean chow + OCA, DIO-NASH + OCA) were stained with two panels of fluorescently labelled antibodies to detect and quantify leukocyte subsets, including monocytes/macrophages, neutrophils, T-cells, B-cells, Natural Killer (NK) and NKT cells and analysed by flow cytometry (Figure 2).

STUDY DESIGN

RESULTS

OCA had no effect on liver weight in DIO-NASH mice

OCA treatment reduced hepatic myeloid and lymphoid cell infiltration in DIO-NASH mice

Figure 3 | Absolute body weight throughout the study period (A) and body weight (B) and liver weight (C) at termination. Data expressed as mean ± S.E.M (n=6-13 per group). Dunnett’s test, one-factor linear model. ***: *p<0.001 compared to DIO-NASH.

Figure 4 | Histopathological score of liver biopsies for all animals separated by groups. For each animal, the pre- to post-study biopsy score is indicated by a line (n=5-13 per group). A) NAFLD activity score (NAS). B) Lobular inflammation. One-sided Fisher’s exact test with Bonferroni correction. *: *p<0.05 compared to DIO-NASH.

Figure 5 | Quantification of the liver inflammation marker liver Galectin-3 (A), inflammatory foci (B) and plasma Monocyte Chemotactic Protein-1 (MCP-1) (C). Data are expressed as mean ± S.E.M (n=6-13 per group). Dunnett’s test one-factor linear model. *: *p<0.05, **: **: **: **p<0.001 compared to DIO-NASH. D) Representative images of liver Galectin-3 IHC staining at termination (magnification 20x, scale bar = 100 µm).

RESULTS SUMMARY:

Liver Myeloid cells

Liver Lymphoid cells

- DIO-NASH mice present marked hepatic expansion of resident and infiltrating monocyte/macrophage and specific T-cell populations
- The hepatic anti-inflammatory effect of OCA is associated with reduced monocyte/macrophage and cytokytic T-cell infiltrates
- Flow cytometry can provide detailed information of the anti-inflammatory mode of action of compounds tested in preclinical models of NASH

CONCLUSION

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