Obeticholic acid suppresses expansion of hepatic immune cell populations and improves NAFLD Activity Score 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
Make C57BL/6J mice fed the AIN-93G diet (40 % fat, 20 % fructose, 2 % cholesterol) for 30 weeks prior to liver biopsy sampling. Only animals with histology-proven steatosis (score ≥2) and fibrosis (stage ≥1F1) were included and stratified into treatment groups. DIO-NASH mice received vehicle (n=15) or OCA (20 mg/kg, PO, QD, n=15) for 8 weeks. Vehicle-dosed chow-fed mice (n=6) served as normal control (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 n=6, DIO-NASH n=5, DIO-NASH OCA n=5) 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

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=6-13 per group). A NAFLD activity score (NAS). B Lobular inflammation. C Steatosis score. D Hepatocellular ballooning. One-sided Fisher’s exact test with Bonferroni correction. *: P < 0.05 compared to DIO-NASH.

Figure 5 | Quantification of the liver and plasma inflammation markers liver Galexin-3 (A), inflammatory foci (B) and plasma Monocyte Chemoattractant 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. \( *: P < 0.05, **: P < 0.01, ***: P < 0.001 \) compared to DIO-NASH. B Representative images of liver Galexin-3 IHC staining at termination (magnification 20×, scale bar = 100 µm).

Figure 6 | Flow cytometric analysis of hepatic leukocyte subsets. Quantification of leukocytes in liver (A), and distribution of hepatic myeloid (B) and lymphoid (C) cell subsets. Quantification of hepatic myeloid (B-42) and lymphoid (B-41) cell subsets. Data are expressed as mean ± S.E.M. (n=6-8 per group). Dunnett’s test, one-factor linear model. \( *: P < 0.05, **: P < 0.01, ***: P < 0.001 \) compared to DIO-NASH.

CONCLUSION
- 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 monocytes/macrophage and cytotoxic T-cell infiltration.
- Flow cytometry can provide detailed information of the anti-inflammatory mode of action of compounds tested in preclinical models of NASH.