Obeticholic acid suppresses expansion of hepatic immune cell populations and improves NAFLD Activity Score in the Gubra biopsy-confirmed DIO-NASH mouse model

ALBA MANRESA-ARRAUT[1], SANNE S. VEIDAL[1], MICHAEL FEIGH[1], NIELS VRANG[1], LISBETH N. FINK[1]
[1]Gubra ApS, Hørsholm, Denmark
Corresponding author: ama@gubra.dk

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 (Tølbøl *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 AMLN diet (40 % fat, 20 % fructose, 2 % cholesterol) for 30 weeks prior to liver pre-biopsy sampling. Only animals with histology-proven steatosis (score ≥2) and fibrosis (stage ≥F1) were included and stratified into treatment groups. DIO-NASH mice received vehicle (n=13) or OCA (30 mg/kg, PO, QD, n=13) 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 n=6, DIO-NASH n=9, DIO-NASH OCA n=9) 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 NK T-cells and analyzed by flow cytometry (Figure 2).

STUDY DESIGN

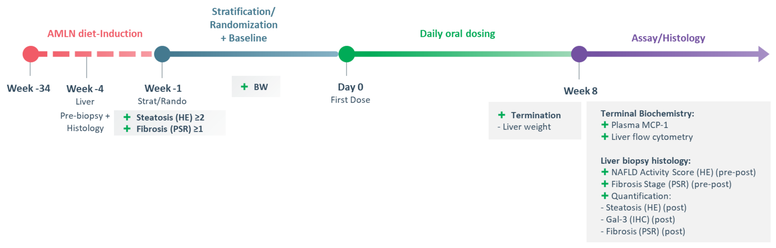


Figure 1 | Study outline. HE: Hematoxylin and eosin, PSR: Picosirius red, BW: Body weight, MCP-1: Monocyte chemoattractant protein-1 , IHC: Immunohistochemistry.

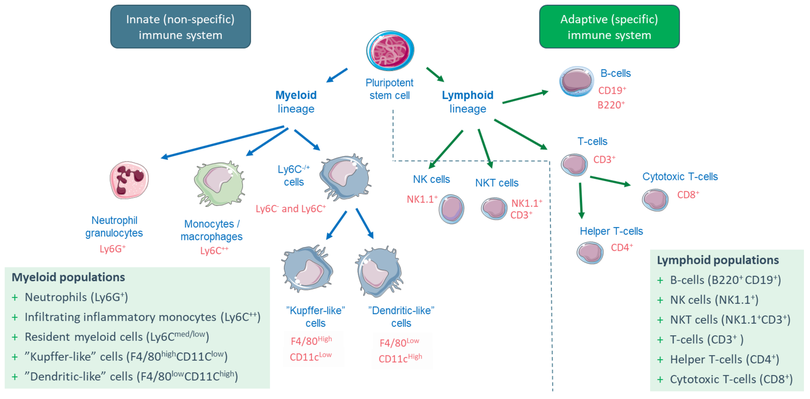


Figure 2 | Liver myeloid and lymphoid leukocyte sub-populations analyzed by flow cytometry

RESULTS

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

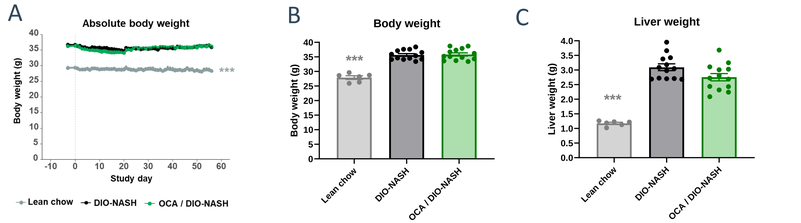


Figure 3 | Absolute body weight throughout the study period (**A**) and body weight (**B**) and liver weight (**C**) at termination. Data expressed as mean + S.E.M (n=6-13 per group). Dunnett's test, one-factor linear model. *** : P<0.001 compared to DIO-NASH.

OCA treatment reduced NAS via reduction of liver lobular inflammation in DIO-NASH mice

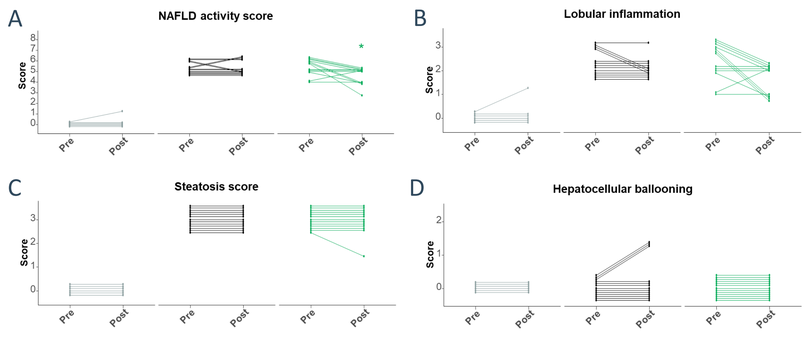


Figure 4 | Histopathological score of liver biopsies for all animals separated by groups. For each animal, the pre- to post-study biopsy score is indicated by a line (n=6-13 per group). **A** NAFLD activity score (NAS). **B** Lobular inflammation. **C** Steatosis score. **D** Hepatocellular ballooning. One-sided Fisher's exact test with Bonferroni correction. *: P < 0.05 compared to DIO-NASH.

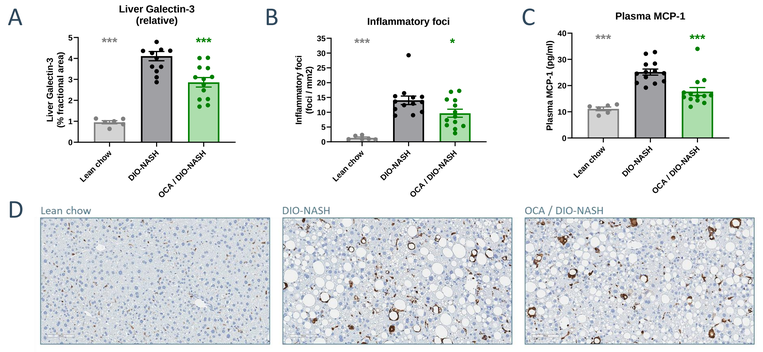


Figure 5 | Quantification of the liver and plasma inflammation markers liver Galectin-3 (**A**), inflammatory foci (**B**) and plasma Monocyte Chemoattractant Protein-1 (MCP-1) (**C**). Data are expressed as mean ± S.E.M. (n=6-13 per group). Dunnett's test one-factor linear model. *: P < 0.05, ***p<0.001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

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

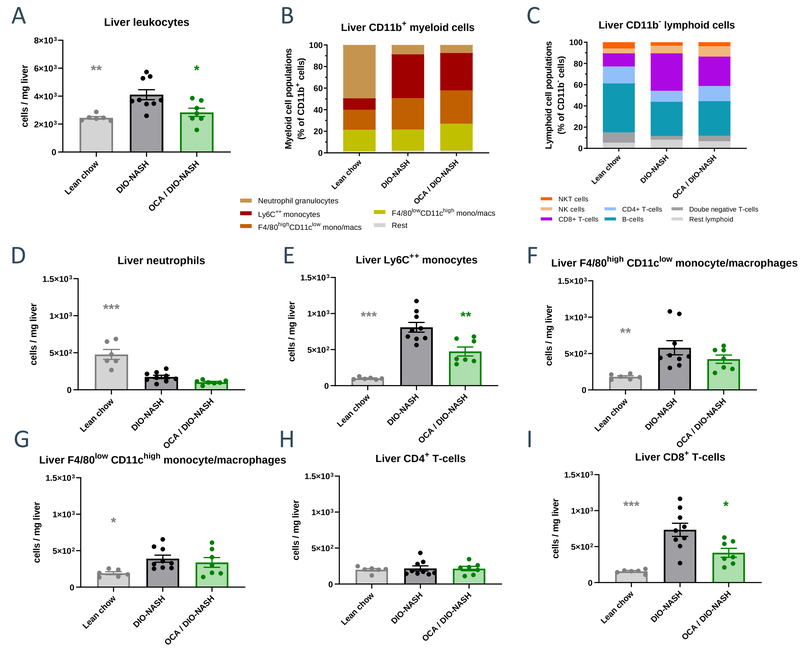
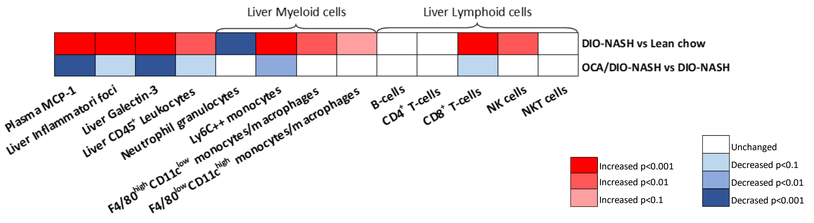


Figure 6 | Flow cytometric analysis of hepatic leukocyte subsets. Quantification of leukocytes in liver (**A**), and distribution of hepatic myeloid (**B**) and lymphoid (**C**) cell subsets. Quantification of hepatic myeloid (**D-G**) and lymphoid (**H-I**) cell subsets. Data are expressed as mean ± S.E.M. (n=6-9 per group). Dunnett's test, one-factor linear model. *p<0.05, **p<0.01, ***p<0.001 compared to DIO-NASH.

RESULTS SUMMARY:



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 monocyte/macrophage and cytotoxic T-cell infiltrates**
- **Flow cytometry can provide detailed information of the anti-inflammatory mode of action of compounds tested in preclinical models of NASH**