Glucagon-like peptide-1 receptor agonists inhibit inflammatory macrophage activation and improves the major components of NASH in an optimized CDAA mouse model

Xiaoyu Wang[1], Shih-Yen Weng[1], Tao chen[1,] Olena Molokanova[1], David Fraser [2,] Detlef Schuppan[1,3].

1 Institute of Translational Immunology and Research Center for Immune Therapy, University Medical Center, Mainz, Germany, 2 Jecurion Therapeutics, Oslo, Norway 3 Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA

BACKGROUND& AIMS

Glucagon-like peptide-1 (GLP-1) improves insulin sensitivity via enhanced glucose-dependent insulin secretion, inhibition of glucagon release, and delayed gastric emptying following its release into the circulation from the gut. We aimed to explore the utility of a long-acting GLP-1 receptor agonist (Bydureon, BY) on liver steatosis, inflammation and fibrosis in an optimized choline deficient, amino acid defined (CDAA), 0.2% cholesterol high fat diet model of NASH, focusing on immune cells and especially macrophages, considered a cell population with high relevance in the pathogenesis of NASH.

MATERIALS & METHODS

Male C57BL/6 mice were fed the CDAA (CSAA= choline sufficient, control) diet for 12 weeks, BY was administered twice weekly by subcutaneous injection of 0.4 (BY-L) or 2 (BY-H) mg/kg to C57BL/6 mice fed the CDAA diet for 6 weeks.

Hepatic fibrosis was assessed by morphometric analysis of Sirius red stained collagen and measurement of hydroxyproline content. Fibrosis, inflammation and metabolism related transcripts were measured by quantitative real-time polymerase chain reaction (qPCR). Select inflammatory and fibrosis markers were quantified by immunohistochemistry. Ex vivo analysis of hepatic inflammatory cells was performed by FACS.

RESULTS

Fig.1: Bydureon attenuated insulin resistance in injured livers of CDAA diet-fed mice

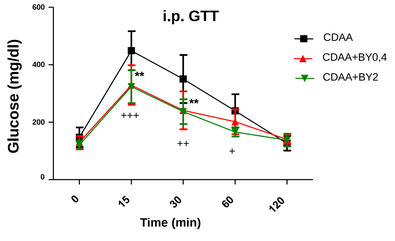


Figure 2: Food intake, Body, liver and epididymal weights, and serum biochemical parameters of CDAA diet–fed mice

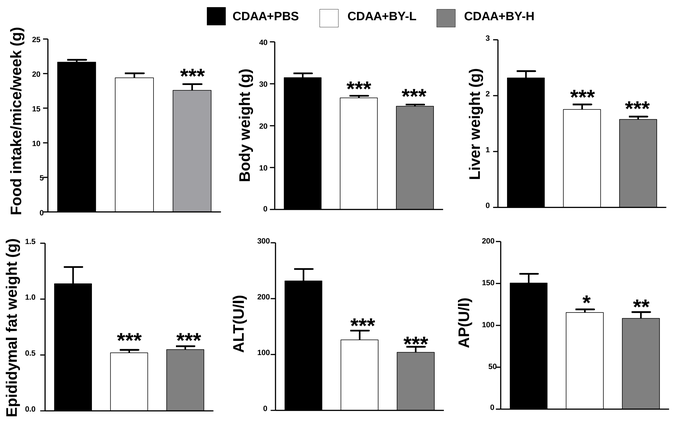


Fig. 3: Bydureon treatment reduces Hyp content in the CDAA diet mouse model

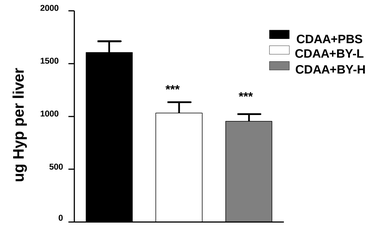


Fig.4: Bydureon treatment reduces fibrosis related transcript levels in the CDAA diet mouse model

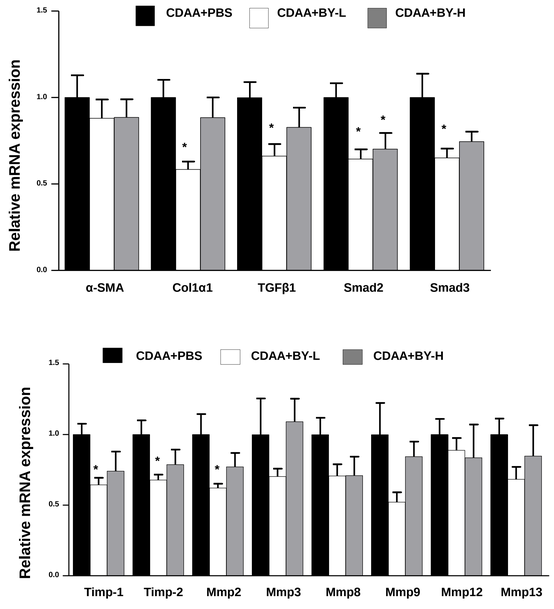


Fig. 5: Bydureon treatment suppress hepatic fibrosis in the CDAA diet mouse model

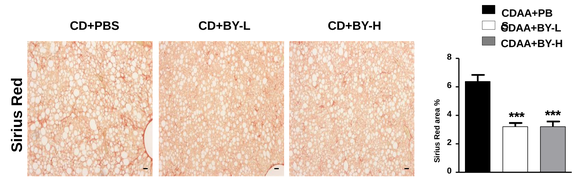


Fig. 6: Bydureon treatment reduces inflammation related transcript levels in the CDAA diet mouse model

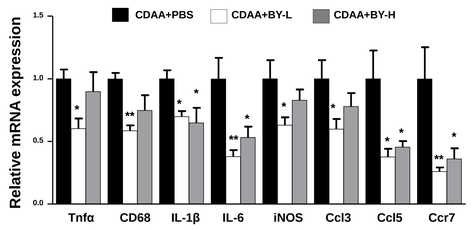


Fig.7: Fluorescence-activated cell sorting analysis of liver leukocytes in PBS and BY-treated mice.

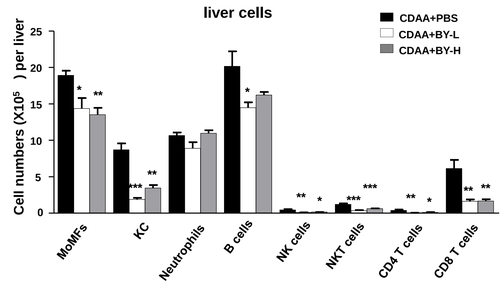
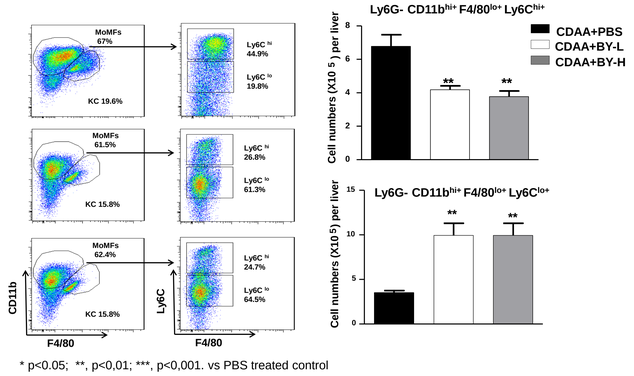


Fig. 8: Representative flow cytometry analysis of monocyte-derived macrophages (MoMF).



* p<0.05; **, p<0,01; ***, p<0,001. vs PBS treated control

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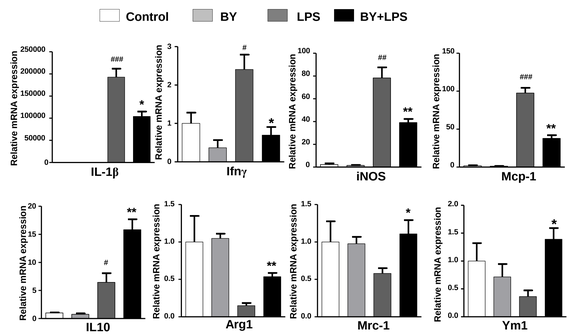
Fig. 9: *In vitro* treatment, Bydureon suppress LPS-stimulated M1 macrophage markers, increased M2 macrophage related markers.



* p<0.05; **, p<0,01; ***, p<0,001.LPS+BY vs LPS, # p<0.05; ##, p<0,01; ###, p<0,001.LPS vs control

RESULTS

All mice gained weight. At the lowest dose, BY significantly maximally decreased body and liver weight, ALT, AST, fasting glucose and insulin resistance, liver collagen accumulation, fibrosis related (Col1α1, α-SMA, TIMP-1, TGFβ1), several MMP) and inflammation related transcripts levels (Tnfα, CD68, IL-1β, IL-6, CCL3).

Flow cytometry analysis of liver mononuclear cells showed a reduction of Kupffer cells and monocyte derived macrophages, natural killer T cells, helper and cytotoxic T cells in BY treated CDAA diet fed animals. In vitro BY polarized LPS-stimulated macrophages towards M2, increasing their IL-10, Mrc1, Arg1 and Ym1 expression, and decreasing pro-inflammatory cytokines and chemokines.

CONCLUSIONS

The optimized CDAA model reflects all features of NASH. Direct GLP-1 receptor agonists improve all these pathologies, including fibrosis. Apart from their antidiabetic and weight lowering activities, a key mechanism is their suppression of pro-inflammatory M1, and promotion of anti-inflammatory (M2) macrophage populations that may also inhibit fibrogenesis. Therefore, further clinical evaluation of the utility of GLP-1R agonists for the treatment of patients with NASH and liver fibrosis is warranted.