OP352 IMPROVING METABOLIC PARAMETERS IN NAFLD BY TARGETING NUCLEAR RECEPTORS
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Introduction: Non-alcoholic fatty liver disease (NAFLD) pathogenesis and treatment remain unsolved. microRNAs and bile acids were recently suggested to participate in disease pathogenesis and, as such, constitute potential therapeutic tools and targets. Moreover, nuclear receptors, namely peroxisome proliferator-activated receptors (PPAR) and the farnesoid X receptor (FXR) are currently under scrutiny as modulators of lipid and glucose metabolism in non-alcoholic steatohepatitis (NASH).

Aims & Methods: We aimed to elucidate the role of the miR-21/PPARα pathway in liver and muscle tissues of murine NASH models and ascertain the therapeutic potential of miR-21 abrogation alone or in combination with obeticholic acid (OCA). Wild-type (WT) and miR-21 KO mice were fed with chow (n = 10) or methionine and choline-deficient (MCD; n = 10) diets for 2 and 8 weeks. Alternatively, mice were fed either chow (n = 12) or fast food diet (FF; n = 12) for 25 weeks. Six animals from each group had their diet supplemented with OCA 10 mg/kg/day (Intercept Pharmaceuticals, Inc.). Human liver biopsies were obtained from morbid obese NAFLD patients (n = 28). Liver/muscle samples were used for histological analysis and assessment of miR-21, pro-inflammatory/pro-fibrogenic cytokines, PPARα and metabolic relevant genes, by qRT-PCR and immunoblotting. A Tagman® Array was performed to evaluate modulation of lipid regulated genes. ROS levels were analysed through the use of 2′,7′-dichlorodihydrofluorescin diacetate.

Results: WT mice fed with the MCD diet developed steatohepatitis and fibrosis, displaying increased levels of apoptosis, necroptosis and serum ALT and AST. In contrast, miR-21 KO mice displayed a significant decrease in steatosis severity, liver fibrosis, inflammation and did not develop fibrosis. WT FF-fed mice developed hepatomegaly, macrovesicular steatosis, inflammatory infiltrates and increased oxidative stress. miR-21 levels were increased in WT FF-fed mice, in both liver and muscle, concomitantly with decreased expression of PPARα, a key fat-oxidation gene. Similar findings were observed in NAFLD patients. Further, WT FF+OCA-fed mice exhibited decreased steatosis and miR-21 expression, compared with WT FF-fed mice. Importantly, KO FF+OCA-fed mice exhibited significantly reduced inflammation, oxidative stress and steatosis, in parallel with increased expression of PPARα and its metabolic targets, including PGC-1 and ACOX2. Finally, lipid regulated genes such as ACAT1, ALOX5 and FABP5 were found to be severely deregulated in WT FF-fed mice and reverted to control levels in KO FF+OCA-fed mice.

Conclusion: In conclusion, activation of PPARα as a result of miR-21 abrogation, together with FXR activation by OCA, significantly improves metabolic parameters in NASH, highlighting the therapeutic potential of multi-targeting therapies for NAFLD. (Supported by PTD/C/BM-MEC/0873/2012, SRFH BD/88212/2012, FCT, Portugal).

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OP354 TOLL LIKE RECEPTOR 2 MODULATES THE INHIBITORY MOTOR RESPONSE INDUCED BY HYDROGEN SULPHIDE IN MOUSE COLON
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Introduction: The recognition of intestinal microbiota is in part carried out by intestinal Toll-like receptors (TLR), which are responsible for initiating the innate immune response. Alterations in the intestinal microbiota and its recognition may contribute to the development of intestinal inflammatory pathologies. Otherwise, hydrogen sulphide (H2S) is an endogenous gaseous signalling molecule and it potentially plays a relevant role in the intestinal motility. In mammals, two pyridoxalphosphate-dependent enzymes are responsible for H2S synthesis: cystathionine-β-synthase (CBS) and cystathionine γ-lyase (CSE).

Aims & Methods: The aim of these studies was to investigate the influence of H2S in the motor response induced by H2S on the motor response induced by H2S and the enzymes responsible for H2S synthesis (CBS and CSE) in mouse colon. Colon strips from male C57/BL10 wild-type (WT) and TLR2−/− mice of 8–12 weeks old were suspended in an organ bath in the direction of circular smooth muscle. We studied the effect of NaHS (10 μM–1 mM), DL-propargylglycine (PAG, 10 μM–10 mM), an inhibitor of CSE, and amino-oxyacetic acid (AOAA, 10 μM–10 mM), an inhibitor of CBS, on WT and TLR2−/− mice colonic motility. Gene expression (mRNA) of CSE and CBS were determined by real time-PCR and protein expression of CSE and CBS were quantified by Western blotting in colon from WT and TLR2−/− mice.

Results: The NaHS, as a source of exogenous H2S, reduced the frequency but not the amplitude of the spontaneous contractions in colon from WT mice. The inhibition of CSE or CBS with PAG or AOAA, respectively, increased the frequency but not the amplitude of the spontaneous contractions in colon from WT mice. The NaHS induced a higher reduction of the frequency of the spontaneous contractions in TLR2−/− respect to WT mice. The PAG and AOAA did not modify the spontaneous contractions in colon from TLR2−/− mice. The mRNA and protein expression of CBS resulted decreased in colon of TLR2−/− compared with WT mice. The mRNA but not the protein expression of CSE resulted decreased in TLR2−/− compared with WT mice.

Conclusion: These results suggest that endogenous and exogenous H2S may regulate the colonic spontaneous contractions in WT mouse, reinforcing the hypothesis that H2S is a gaseous inhibitory mediator of intestinal motility. TLR2
OP355 DIRECT INHIBITION OF HMGB1 BY NEUTRALIZING ANTI- HMGB1 ABMLERATES EXPERIMENTAL COLITIS IN MICE VIA MODULATION OF MACROPHAGES’ PLASTICITY

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Introduction: Macrophages play a major role in inflammatory bowel disease (IBD) pathogenesis through an inappropriate response to migration, and an impaired transition from a pro-inflammatory (classical activated macrophages (CAMs)) to an anti-inflammatory (alternative activated macrophages (AAMs)) phenotype which is gaining awareness as a relationship between Chromogranin (Cg)-A and a susceptibility to inflammatory conditions, the specific interaction between CgA-derived peptides and macrophage plasticity in IBD is unknown. Recently, we have shown a linear correlation between CgA and colitic markers in mice with active ulcerative colitis, and colitic CgA-deficient mice demonstrated a significant decrease of colitis associated to a modulation of macrophage activation. As Cg-A is a prohormone, herein, we assessed the functional role of a specific CgA-derived peptides (Chromofungin (CHR); Kg-A7-46) in the regulation of acute colitis and the functional plasticity of murine macrophages.

Aims & Methods: Colitis was induced in C57BL/6 mice (7–8 weeks old) by administrating dextran sodium sulfate (DSS 3%) in drinking water for 5 days. Colitis was monitored by CHR (2.5 μg/kg, i.p. once per day). Control experiments started 1 day before induction of colitis and lasted for a total of 6 days. Disease activity index (DAI) was evaluated daily and mice were sacrificed on day 5 post-DSS induction to assess the extent of colitis. At sacrifice macroscopic scores were evaluated, and the functional role of CgA-derived peptides (CHR) was quantified using ELISA, and colonic interleukin (IL)-1β, IL-6, TNF-α and ARG-1 were assessed by using ELISA and RT-qPCR. Naïve peritoneal macrophages were isolated from non-colitic C57BL/6 mice and treated by CHR (200 ng/ml) then exposed for 6 h to LPS (100 ng/ml) to promote CAMs, or to IL-4/IL-13 (20 ng/ml) to promote AAMs. CAMs markers (IL-6, IL-1β, TNF-α, MIP-1α, MIP-1β) and AAMs markers (ARG-1) were quantified by using ELISA and RT-qPCR.

Results: Preventive treatment with CHR significantly reduced the DAI onset and severity of colitis associated to rectal bleeding, stool consistency and weight loss. Macrophage-derived scores, serum-CRP, colonic IL-1β, IL-6, TNF-α, MIP-1α, MIP-1β were significantly decreased, while ARG-1 was significantly increased. In vitro, CHR-conditioned CAMs expressed significantly less IL-1β, IL-6, TNF-α, MIP-1α, MIP-1β, but, surprisingly, more ARG-1 when compared to LPS control condition. Moreover, CHR-conditioned AMS expressed significantly more ARG-1 when compared to IL-4/IL-13 control condition.

Conclusion: These findings suggest that CHR can modulate the severity of experimental colitis. CHR treatment can attenuate the severity of experimental colitis and the inflammatory process via the modulation of the functional plasticity of murine macrophages and their functions. Targeting CgA-derived peptides may lead to novel therapeutic strategies in ulcerative colitis.

Disclosure of Interest: All authors have declared no conflicts of interest.

OP357 CHROMOFUNGIN (CHR) AMELIORATES EXPERIMENTAL COLITIS IN MICE VIA MODULATION OF MACROPHAGES’ PLASTICITY

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Introduction: Macrophages play a major role in inflammatory bowel disease (IBD) pathogenesis through an inappropriate response to migration, and an impaired transition from a pro-inflammatory (classical activated macrophages (CAMs)) to an anti-inflammatory (alternative activated macrophages (AAMs)) phenotype. As CgA-deficient mice demonstrated a significant decrease of colitis associated to a modulation of macrophage activation. As Cg-A is a prohormone, herein, we assessed the functional role of a specific CgA-derived peptides (Chromofungin (CHR); Kg-A7-46) in the regulation of acute colitis and the functional plasticity of murine macrophages.

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Conclusion: These findings suggest that CHR can modulate the severity of experimental colitis. CHR treatment can attenuate the severity of experimental colitis and the inflammatory process via the modulation of the functional plasticity of murine macrophages and their functions. Targeting CgA-derived peptides may lead to novel therapeutic strategies in ulcerative colitis.

Disclosure of Interest: All authors have declared no conflicts of interest.

OP358 DEFICIENCY OF PH-SENSING RECEPTOR TDAG8 AMELIORATES T-CELL TRANSFER COLITIS

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Introduction: Phagocytes are pivotal in the control of bacterial infections and the induction of endogenous protective mechanisms, such as TGFβ and glucagon-like-peptide-2 (GLP-2) through inhibition of DPP IV-dependent pathways. Experimental data indicate that PETIR, the inhibitory effect of EMDB-1 on DPP IV was characterized in vitro using the HPLC system measuring the degradation rate of endorphin-2 (EM2, natural DPP IV substrate) in the presence of the test compound. PETIR activity of EMDB-1 was investigated in the model of acute and semi-chronic colitis induced by trinitrobenzenesulfonic acid (TNBS). Body weight, macroscopic score, ulcer score, colon length and thickness, as well as myeloperoxidase (MPO) activity were recorded. Emdb-1 was used as control.

Results: EMDB-1 is a potent and specific DPP IV inhibitor as shown by significantly decreased degradation rate of EM2 by DPP IV (t0.5 = 1.73 vs. 3.60 min in the absence and the presence of EMDB-1, respectively). The intracolon (i.c.) administration of EMDB-1 (0.1, 1 and 3mg/kg, twice daily) attenuated both acute and semi-chronic TNBS-induced colitis in mice in a dose-dependent manner, as indicated by significantly reduced macroscopic parameters and MPO activity. Anti-inflammatory effect of EMDB-1 was not blocked by nalox-

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OP359 NEW, PEPTIDE INHIBITOR OF Dipeptidyl Peptidase IV, EMD-1 ATTENUATES COLITIS IN MICE VIA MODULATION OF MACROPHAGES’ PLASTICITY

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Introduction: PETIR (Peptide-Targeted IinmunoRegulation) is a novel therapeutic strategy which takes for the purpose restoration of the immune balance by limiting the activation of immune cells and induction of endogenous protective mechanisms, such as TGFβ and glucagon-like-peptide-2 (GLP-2) through inhibition of DPP IV-dependent pathways. Experimental data indicate that PETIR results in suppression of cell proliferation and reduced synthesis of pro-inflammatory cytokines without affecting cellular vitality.

Aims & Methods: The objective of this study was to test the anti-inflammatory activity of a novel DPP IV inhibitor EMDB-1 in the mouse models of colitis. The inhibitory effect of EMDB-1 on DPP IV was characterized in vitro using the