Liraglutide Has Potent Anti-inflammatory And Anti-catabolic Activity In Two Cell-types Implicated In Osteoarthritis

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INTRODUCTION & AIM

Osteoarthritis (OA) is an age-related joint disease affecting 300 million of individuals worldwide which provokes chronic pain and limits mobility leading to increasing cardiovascular-related mortality. The disease progression is associated with inflammatory responses and cartilage degradation. Both chondrocytes, the only cell type present in the cartilage, and macrophages from the synovium, play a major role in OA pathophysiology.

Liraglutide is a Glucagon-Like-Peptide 1 Receptor (GLP-1R) agonist widely prescribed for the treatment of type 2 diabetes. Interestingly, immunomodulatory and anti-inflammatory properties of the GLP-1 pathway have been recently reported in various diseases.

In this study, we evaluated the anti-inflammatory and anti-catabolic effects of 4P004-Liraglutide in two *in vitro* models relevant to OA by evaluating surrogate markers of inflammation, cartilage matrix proteolysis and cartilage matrix anabolism.

MATERIAL AND METHODS

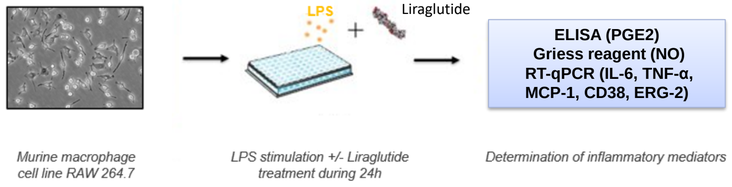

Fig. 1: *The capacity of Liraglutide to reduce inflammation was evaluated using mouse macrophages cell line (Raw 264.7) model. LPS (100ng/ml)-stimulated mouse macrophages were treated with different concentrations of Liraglutide (6.6nM to 3.4µM) for 24h, to determine the half maximal inhibitory concentration (IC_{50}). Production of Nitrite and prostaglandin E2 (PGE2) in supernatant was measured by Griess reagent or ELISA, respectively. RT-qPCR analyses were also performed with 3 selected doses of Liraglutide (13.3nM, 53.1nM and 1.7µM).*

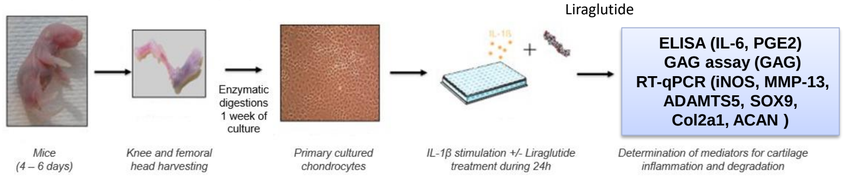

Fig. 2: *The capacity of Liraglutide to reduce inflammation and degradation was evaluated using murine primary chondrocytes model. IL-1β (2ng/ml)-stimulated mouse articular chondrocytes were treated with different concentrations of Liraglutide (6.6nM to 3.4µM) for 24h, to determine IC_{50}. Production of interleukin-6 (IL-6), matrix metalloproteinase 13 (MMP-13) and glycosaminoglycans (GAGs) in medium was measured by ELISA or GAG assay, respectively. RT-qPCR analyses were also performed with 3 selected doses of Liraglutide (13.3nM, 53.1nM and 1.7µM).*

RESULTS

(1) Liraglutide decreases inflammatory mediators production in LPS-stimulated murine macrophages

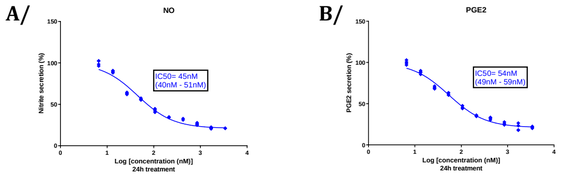

Fig. 3: *Liraglutide induced a dose-dependent inhibition of the LPS-induced production of* ***A/*** *NO (IC_{50}=45nM) and* ***B/*** *PGE_2 (IC_{50}=54nM) in Raw 264.7 murine macrophages.*

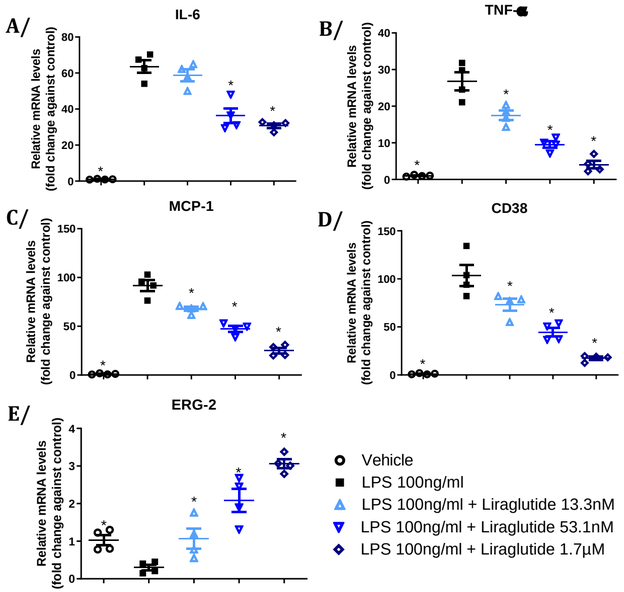

Fig. 4: ***A/****IL-6 and* ***B/*** *TNF gene expressions were significantly and dose-dependently decreased in LPS-stimulated Raw 264.7 cells treated with Liraglutide compared to LPS alone.* ***C/*** *Liraglutide induced also a significant and dose-dependent reduction of MCP-1 and* ***D/*** *CD38 (M1 macrophage markers) gene expression compared to LPS alone.* ***E/*** *On the contrary, a dose-dependent increase of ERG-2 (M2 macrophage marker) gene expression is induced by Liraglutide in LPS-stimulated Raw 264.7 cells. These results suggest that Liraglutide can polarize M1 macrophages towards the M2 phenotype.*

(2) Liraglutide decreases inflammatory and catabolic mediators and induces anabolic mediators in IL1-β-stimulated mouse chondrocytes

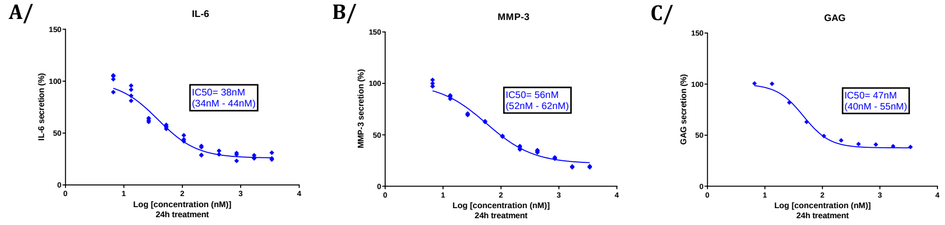

Fig. 5: *Liraglutide significantly reduced, in a dose-dependent manner, the IL-1β-induced release of* ***A/*** *IL-6 (IC_{50}=38nM),* ***B/*** *cartilage matrix catabolic enzyme MMP-3 (IC_{50}=56nM) and* ***C/*** GAGs *(IC_{50}=47nM) in murine articular chondrocytes.*

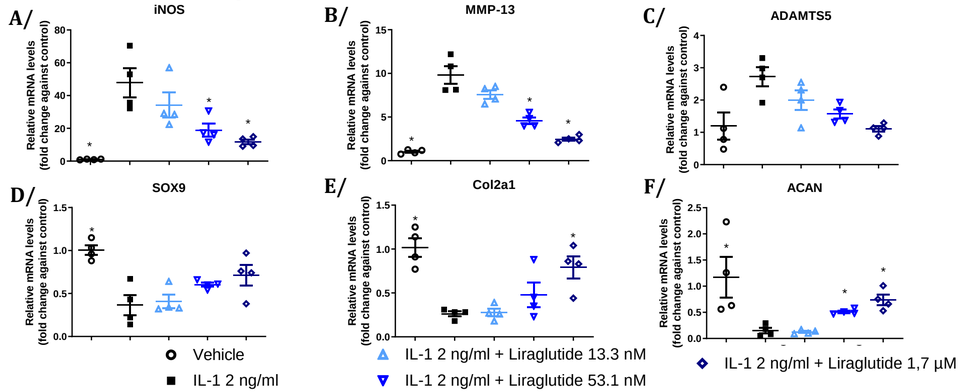

Fig. 6: *Liraglutide treatment dose-dependently decreased the IL-1β-induced gene expression of* ***A/*** *iNOS,* ***B/*** *MMP-13 and* ***C/*** *ADAMTS-5. Finally, IL-1β decreased gene expression of* ***D/*** *Sox9,* ***E/*** *Col2a1 and* ***F/*** *Acan anabolic markers, which could be rescued in a dose-dependent manner by Liraglutide.*

CONCLUSION

1. 4P004-Liraglutide treatment decreases the release and gene expression of OA inflammatory markers in an *in vitro* macrophage model (Raw 264.7 cell line) .
2. Moreover, in the same *in vitro* model, 4P004-Liraglutide treatment can polarize LPS-stimulated macrophages towards the M2 anti-inflammatory phenotype.
3. 4P004-Liraglutide treatment decreases the release and gene expression of OA inflammatory and catabolic markers and stimulates anabolic markers gene expression in an *in vitro* chondrocytes model .

A shift in M1/M2 macrophage phenotype and the inhibition of chondrocyte expression of several mediators involved in inflammation and cartilage degradation explain, at least in part, our previous results from rodent OA models that showed an analgesic, anti-inflammatory and anti-degradative effect of 4P004-Liraglutide.