Protective Effects Of Intra-Articular Formulated Liraglutide In Osteoarthritis

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ABSTRACT

Aim: Osteoarthritis (OA) is a degenerative joint disease affecting millions of individuals worldwide and is associated with cartilage degradation and inflammatory responses leading to pain, swelling and reduced function. Liraglutide, a Glucagon-Like-Peptide 1 Receptor (GLP-1R) agonist, is clinically used as a treatment for type 2 diabetes. Interestingly, immunomodulatory properties of the GLP-1 pathway have been recently described but its role in OA remains to be elucidated. The objective of this study was to evaluate the effects of intra-articular (IA) Liraglutide in *in vitro* and *in vivo* models of OA by evaluating surrogate markers of inflammation, cartilage degradation and pain.

Methods: IL-1β-stimulated chondrocytes were treated with different concentrations of Liraglutide. Production of matrix metalloproteinases (MMP) and prostaglandin E2 (PGE2) was measured by ELISA. IA injections of Liraglutide or vehicle were performed in two knee OA models. Paw withdrawal threshold and weight bearing distribution were performed for pain behavior assessment. Histopathological analyses (OARSI score) were conducted for evaluating cartilage degradation.

Results: Liraglutide significantly reduced the IL-1β-induced production of PGE2, MMP-3 and MMP-13 in chondrocytes. In both *in vivo* OA models, Liraglutide IA injections significantly attenuated pain symptoms with a similar response than IA injection of dexamethasone. Histological assessment revealed a significant decrease of the total joint score in the IA Liraglutide treated group compared to vehicle.

Conclusion: IA injection of Liraglutide has demonstrated anti-catabolic, anti-inflammatory and pain relieving effects in preclinical OA models, opening the way to considering now this molecule as a potential disease-modifying OA drug.

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INTRODUCTION

OA is a degenerative joint disease affecting 315 millions of individuals worldwide, making it the leading cause of functional disability. Its development has been reported to be associated with cartilage degradation and inflammatory responses leading to pain, swelling and reduced function. Osteoarthritis is a serious and frequent disease that can lead indirectly to death. Although OA is a disorder of the whole joint, the progressive destruction of cartilage extracellular matrix is considered as its hallmark. To date, approved OA treatments are only symptomatic. Therefore, there is an urgent need to explore disease-modifying OA drugs (DMOADs) that can mitigate, stop, or even reverse the development of OA. **In this context, the objective of this study was to assess the local effects of Liraglutide, a Glucagon-Like-Peptide 1 Receptor agonist approved for type 2 diabetes, in *in vitro* and *in vivo* models of OA by evaluating OA-related markers of inflammation and catabolism, cartilage degradation and pain.**

METHODS & MATERIALS

***In vitro* experiments:** IL-1β-(2ng/ml) stimulated mouse articular chondrocytes[(Ref 1)] were treated with three concentrations (3, 10 and 50nM) of Liraglutide for 24 hours. Production of matrix metalloproteinases MMP-3 and MMP-13 and prostaglandin E2 in supernatant was measured by ELISA.

***In vivo* experiments:** Intra-articular injections of formulated Liraglutide or vehicle were performed in two chemically-induced inflammatory knee OA models: the mouse monosodium iodoacetate (MIA) model[(Ref 2)] and the rat collagenase model[(Ref 3)] (IA injection into the right knee). Paw withdrawal threshold and weight bearing distribution were performed for pain behavior assessment. Histopathological analyses (OARSI score[(Ref 4)]) were conducted blindly by one observer in the rat collagenase OA model for evaluating cartilage degradation.

RESULTS

(1) Liraglutide decreases inflammatory and catabolic mediators production in IL1-β-stimulated mouse chondrocytes

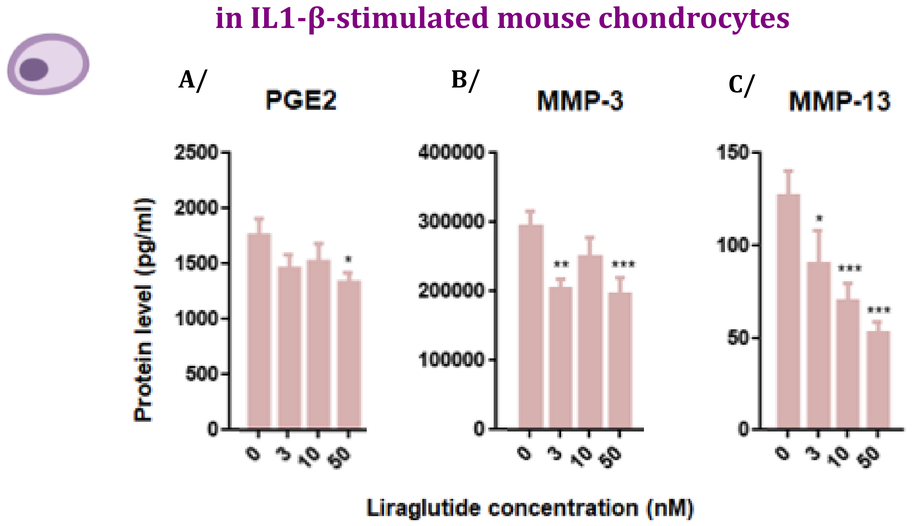

Fig. 1: **A/** *Liraglutide significantly reduced the IL-1β-induced production of PGE2 (1341pg/ml ± 86pg/ml vs 1766pg/ml ± 145pg/ml for vehicle, p≤0.05, 50nM dose) and* **B/** *cartilage matrix catabolic enzymes MMP-3 (294 000pg/ml ± 23 000pg/ml for vehicle vs 204 000pg/ml ± 15 000pg/ml, p≤0.01, 3nM dose; vs 197 000 ± 23 000 pg/ml, p≤0.001, 50nM dose) and* **C/** *MMP-13 with a dose response (127pg/ml ± 14pg/ml for vehicle vs 90pg/ml ± 18pg/ml, p≤0.01, 3nM dose; vs 70pg/ml ± 10ng/ml, p≤0.001, 10nM dose; vs 52pg/ml ± 6ng/ml, p≤0.001, 50nM dose) in murine chondrocytes.*

(2) Liraglutide has analgesic effect in MIA-induced model in mice

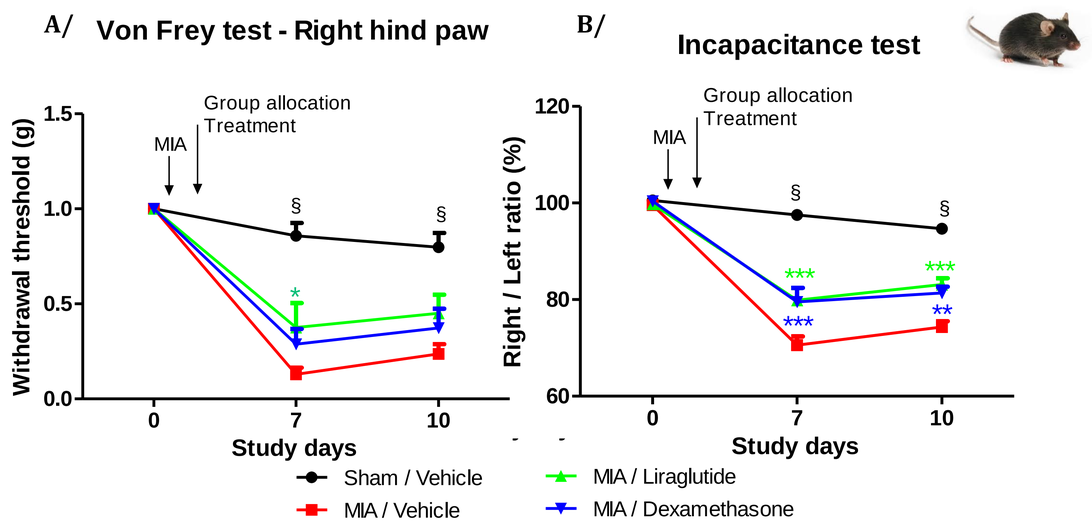

Fig. 2: *In mouse MIA model (0.75mg/mouse, n= 8-10, IA injection on Day 1),* **A/** *a single IA injection of formulated Liraglutide at day 3 increased significantly paw withdrawal threshold (0.37g ± 0.39g vs 0.13g ± 0.11g for vehicle, p≤0.05, day 7) and* **B/** *improved weight distribution to the affected limb (80% ± 7% at day 7 and 83% ± 4% at day 10, p≤0.001) compared to vehicle (71% ± 6% at day 7 and 74% ± 4% at day 10). The response was found similar to the one after an IA injection of Dexamethasone (79% ± 8% at day 7 and 81% ± 4% at day 10).*

(3) Liraglutide has analgesic properties and cartilage protection effects in collagenase-induced model in rats

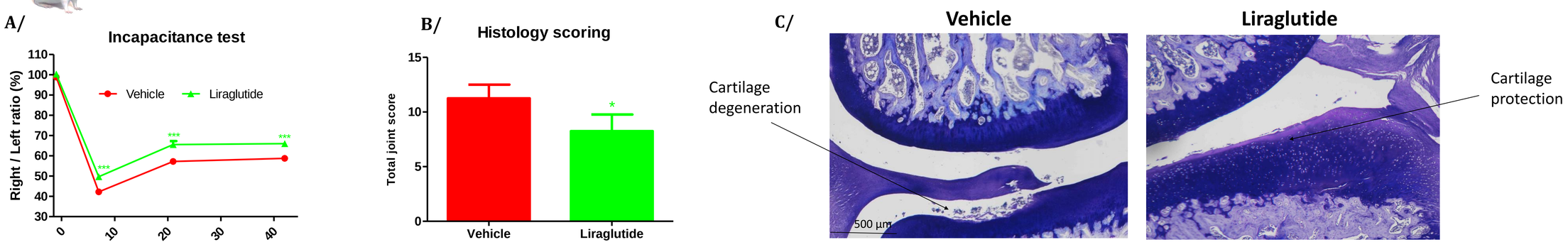

Fig. 3: *In the rat collagenase OA model (500U in 25µl/ rat, n=8-10, IA injections at day 1 and 4), repeated IA injections of formulated Liraglutide or vehicle were performed once a week for 5 weeks.* **A/** *Liraglutide improved weight bearing deficit at multiple time-points (50% ± 4% at week 1, 66% ± 5% at week 3 and 66% ± 4% at week 6, p≤0.001) compared to vehicle (42% ± 4% at week 1, 57% ± 4% at week 3 and 59% ± 3% at week 6).* **B/** *Histological assessment of rat collagenase-injected right knee joint revealed a significant (p≤0.05) decrease of the total joint score in the IA Liraglutide treated group (8±4) compared to vehicle (11±4).* **C/** *Representative pictures of right knee sections stained with toluidine blue showing that Liraglutide (right image) is able to mitigate focal extensive fibrillation and cartilaginous degeneration as compared to vehicle (left image), indicating cartilage protection.*

CONCLUSIONS

1. Liraglutide treatment in *in vitro* OA model has shown significant decrease in the release of OA inflammatory and catabolic markers.
2. Intra-articular administration of formulated Liraglutide decreases pain in two animal models of OA.
3. Intra-articular administration of formulated Liraglutide protects the joint from OA-related cartilage degradation.

Intra-articular administration of formulated Liraglutide reduces pain, inflammation and cartilage degradation in *in vitro* and animal models of OA, opening the way to considering this drug as a DMOAD.

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