Liraglutide As A Potential Intra-Articular Treatment For Cartilage Regeneration In Osteoarthritis: *In Vitro* And *In Vivo* Studies Supporting A Pro-Chondrogenic Effect

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INTRODUCTION & AIM

Osteoarthritis (OA) is an age-related joint disease affecting millions of individuals worldwide and associated with an extremely high burden largely attributable to disability. To date, there are only symptomatic treatments and no disease-modifying OA drugs (DMOADs) acting on both symptoms and structure are yet approved. Although OA is a disorder of the whole joint, progressive cartilage degeneration is considered as its hallmark. Indeed, differentiation and function of chondrocytes are impaired in OA, resulting in the breakdown of the cartilage matrix. Liraglutide is a Glucagon-Like-Peptide 1 Receptor (GLP-1R) agonist widely prescribed for the treatment of type 2 diabetes. We have previously shown that intra-articular injection (IA) of Liraglutide exerts anti-inflammatory and anti-degradative effects (Ref. 1). In this study, Liraglutide was assessed for its pro-chondrogenic properties.

MATERIAL AND METHODS

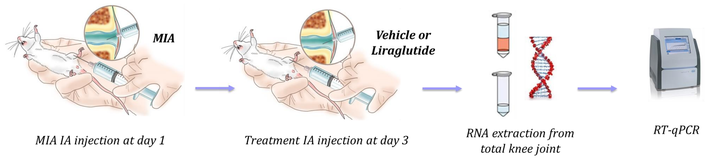


Fig 1: *Intra-articular (IA) injection of formulated Liraglutide or vehicle was performed in chemically-induced inflammatory knee mouse OA model: monosodium iodoacetate (MIA, IA injection into the right knee). RT-qPCR analyses of knee joint were performed 10 days following saline or MIA injection for evaluating pro-chondrogenic markers.*

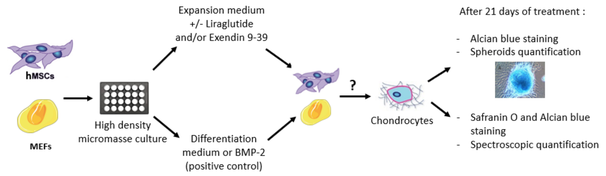


Fig. 2: *The capacity of Liraglutide (10, 100 and 500nM) to induce chondrogenesis was evaluated using human Mesenchymal Stem Cells (hMSCs) and Mouse Embryonic Fibroblasts (MEFs) high-density micromass in-well culture systems (Ref. 2, 3). Safranin O and/or Alcian blue staining was used to assess differentiation into chondrocytes. Exendin 9-39, a GLP-1R antagonist, was used to confirm target specificity. A commercial differentiation medium and Bone Morphogenetic Protein 2 (BMP-2) were used as positive controls for hMSC and MEFs models, respectively. For hMSC micromass model, spheroid formation count was conducted by 2 observers.*

RESULTS

(1) Liraglutide increases pro-chondrogenic gene expression in knee joint MIA mouse model

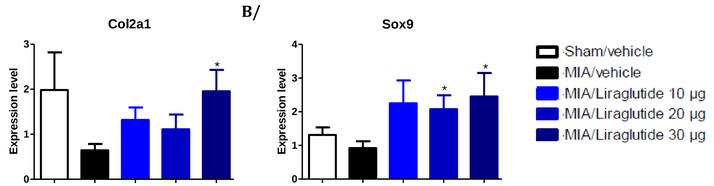
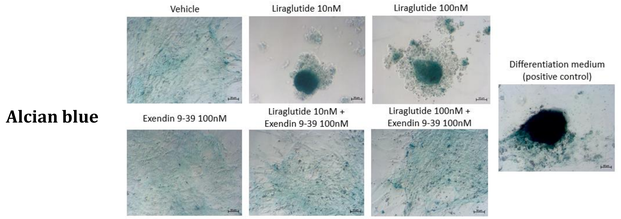


Fig. 3: **A/** *Col2a1 gene expression was significantly increased in total knee joints from MIA-induced mice treated with 30µg of Liraglutide compared to vehicle (At Day 11, Liraglutide= 1.96±1.34, vs vehicle= 0.64±0.46, p< 0.05, fold change related to saline treatment, n=10-11 animals per group).* **B/** *Moreover, there was a significant induction of Sox9 gene expression in MIA mice treated with 30µg and 20µg of Liraglutide (Fold 2.46±1.84; 2.08±1.36, respectively, p≤0.05) compared to vehicle (0.92±0.67, n=9-11 animals per group).*

(2) Liraglutide promotes chondrogenic 3D spheroids formation of Mesenchymal Stem Cells (hMSCs) via GLP-1R



B/

Percentage of wells with alcian blue positive chondrocyte spheroids	Day	Basal medium with						Commercial differentiation medium (positive control)
		Vehicle	Liraglutide 10nM	Liraglutide 100nM	Exendin 9-39 100nM	Liraglutide 10nM + Exendin 9-39 100nM	Liraglutide 100nM + Exendin 9-39 100nM	
	21	0/6	5/6	4/6	0/6	0/6	0/6	5/6

Fig. 4: *Using hMSC, after 21 days of treatment,* **A/** *alcian blue staining was used to assess differentiation into chondrocytes. Liraglutide, but not vehicle, induced their differentiation into chondrogenic 3D spheroids.* **B/** *Liraglutide 10nM= 5 alcian-blue positive spheroids out of 6 counted wells, p< 0.05; Liraglutide 100nM= 4/6, p=0.06, vs vehicle= 0/6. 5/6 alcian-blue positive spheroids were also observed for the positive control. The use of Exendin 9-39 confirmed that the effect of Liraglutide on chondrogenesis was GLP-1R dependent in hMSCs in vitro model (0/6 alcian-blue positive spheroids alone and in combination with Liraglutide).*

(3) Liraglutide promotes chondrocyte differentiation of Mouse Embryonic Fibroblasts (MEFs) via GLP-1R

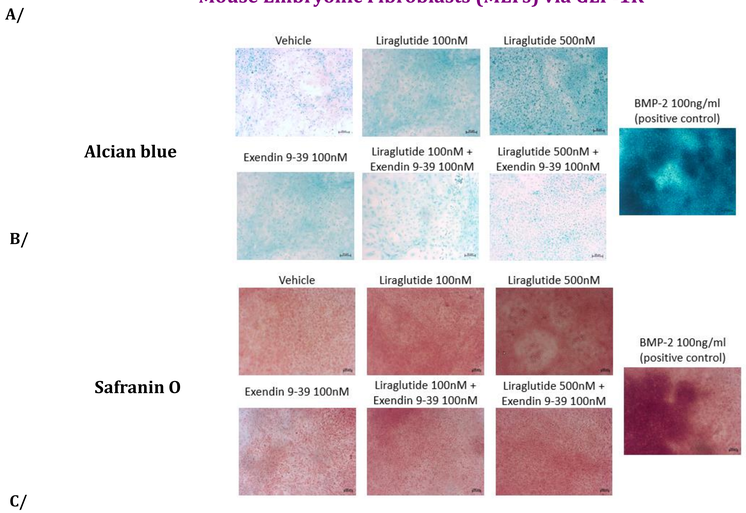
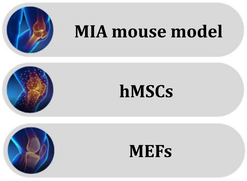


Fig. 5: *Using MEFs, after 21 days of treatment, Alcian blue (***A/***) and Safranin O (***B/***) staining was used to assess differentiation into chondrocytes. Liraglutide or BMP-2 induced MEFs to differentiate into chondrocytes. The use of Exendin 9-39 confirmed that the effect of Liraglutide on chondrogenesis was GLP-1R dependent in MEFs in vitro model (low color intensity).* ***C/*** *Spectroscopic quantification (in arbitrary unit, AU) of the Safranin O stain in MEFs after 21 days of chondrogenic differentiation indicated a significant increase of absorbance for Liraglutide 500nM (2.90±0.03 AU, p< 0.001) and BMP-2 (3.17±0.06, p< 0.001) vs vehicle (1.99±0.34).*

CONCLUSION



1. Liraglutide treatment in *in vivo* OA model has shown significant increase of pro-chondrogenic gene expression in knee joint.
2. Liraglutide promotes chondrocyte differentiation of human Mesenchymal Stem Cells (hMSCs).
3. Liraglutide promotes chondrocyte differentiation of Mouse Embryonic Fibroblasts (MEFs).

Liraglutide promotes chondrocyte differentiation, which could facilitate cartilage regeneration in OA. Previously, we have shown that intra-articular injection of Liraglutide exerts anti-inflammatory, anti-degradative and analgesic effects (Ref. 1).
Thus, Liraglutide represents a potential DMOAD treatment for knee OA.

REFERENCES

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