Am J Transl Res 2019;11(8):4800-4808
www.ajtr.org /ISSN:1943-8141/AJTR0092881

Original Article

Liraglutide suppresses TNF-α-induced degradation of extracellular matrix in human chondrocytes: a therapeutic implication in osteoarthritis

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Received February 21, 2019; Accepted April 29, 2019; Epub August 15, 2019; Published August 30, 2019

Abstract: Osteoarthritis (OA) is a major global health problem; however, the etiology of the disease remains unknown and a reliable treatment strategy has yet to be discovered. Modulation of the receptor for glucagon-like peptide 1 (GLP-1) has emerged as a potential treatment strategy for various diseases including OA. In the present study, we investigated the effects of the specific GLP-1 receptor agonist liraglutide on factors of the pathogenesis of OA induced by tumor necrosis factor-α (TNF-α), including oxidative stress, expression of proinflammatory cytokines, degradation of articular cartilage extracellular matrix, and activation of the nuclear factor-κB (NF-κB) pathway. Our findings demonstrate that liraglutide exerted a potent beneficial effect in human primary chondrocytes by downregulating generation of reactive oxygen species and NADPH oxidase 4, suppressing expression of interleukin-6 and monocyte chemoattractant protein 1, rescuing type II collagen and aggrecan from degradation my matrix metalloproteinases and a disintegrin and metalloproteinase with type I thrombospondin motif, and inhibiting activation of the proinflammatory NF-κB signaling pathway. These findings demonstrate a potential role of GLP-1 receptor in the pathogenesis of OA and lay a foundation for further research on the mechanisms behind the potential therapeutic application of liraglutide in the treatment and prevention of OA.

Keywords: Osteoarthritis, liraglutide, GLP-1 receptor, cartilage degradation, NF-κB, purinergic receptor

Introduction

Osteoarthritis (OA) is one of the most common debilitating diseases among the global population. Among the factors contributing to the pathogenesis of OA are age, mechanical loading, injury, and obesity [1, 2]. However, the exact mechanisms driving its development and progression remain incompletely understood. Currently, treatment strategies for OA are primarily aimed at slowing progression of the disease, with the first-line treatment for advanced OA being arthroplasty. While this procedure is known to have a high success rate, it has the disadvantages of potentially long recovery times, high costs, and an increased risk of complication in elderly patients [3, 4]. Thus, there is a significant demand for novel non-invasive therapies against the development and progression of OA.

Along with oxidative stress and expression of proinflammatory cytokines including interleukin 6 (IL-6) and monocyte chemoattractant protein 1 (MCP-1), the main hallmark of OA is irreversible degradation of the articular extracellular matrix (ECM), which is composed primarily of type II collagen and aggrecan. Aggrecan is the main large aggregating chondroitin sulfate proteoglycan in the articular ECM. Consisting of three globular domains, its gel-like structure provides cartilage with its load-bearing and shock-absorptive properties [5]. In normal physiology, aggrecan undergoes relatively rapid turnover via degradation by a class of proteases termed a disintegrin and metalloproteinase with type thrombospondin motif (ADAMTS), or aggrecanases [6]. The family of ADAMTS consists of a group of extracellular enzymes that play a role in collagen processing, degradation of matrix proteoglycans, angiogenesis, and

blood coagulation homeostasis, among other things [7]. In the pathogenesis of OA, ADAMTS-4 and ADAMTS-5 have been shown to have the greatest effect on aggrecan degradation through cleavage at the Glu^{373}, Glu^{1545}, Glu^{1714}, Glu^{1819}, and Glu^{1919} sites [8, 9]. Meanwhile, type II collagen undergoes very slow turnover, and thus, excessive degradation of type II collagen is largely considered irreversible. Matrix metalloproteinases (MMPs) are zinc-dependent catabolic enzymes that target type II collagen for degradation [10]. Of these, MMP-3 (stromelysin-1) and MMP-13 (collagenase-3) have been shown to play the most significant role in OA by unwinding the collagen triple helix at the P4-P11' site [11]. Thus, suppression of the actions of MMPs and ADAMTS is regarded as a promising therapeutic strategy against OA.

Recently, the role of incretin hormone receptors in various diseases and cell types has been receiving increasing attention. Selective targeting of the receptor for glucagon-like peptide-1 (GLP-1R) is a common strategy for the treatment of type 2 diabetes [12]. Native GLP-1 is rapidly degraded to its N-terminally truncated metabolite GLP-1 (9-36) amide/(9-37) by the aminopeptidase dipeptidyl peptidase IV (DPP-4), and thus administration of GLP-1 is not a viable treatment option [13-15]. However, modulation of GLP-1R either through inhibition of DPP-4 or agonism of GLP-1R itself using GLP-1 analogues has become an attractive treatment option for type 2 diabetes and other diseases. Several recent studies have demonstrated the potential benefit of GLP-1 agonism in preventing bone loss [16, 17]. Liraglutide is a human GLP-1 analogue that has recently been shown to exert beneficial effects in terms of bone metabolism in non-diabetic rats and cardiovascular outcome in type II diabetes patients [18, 19]. In the present study, we explored the effects of GLP-1R agonism using liraglutide on TNF-α-induced oxidative stress, expression of proinflammatory cytokines, degradation of articular ECM, and activation of NF-κB in human primary chondrocytes (HPCs).

Materials and methods

Cell isolation and treatment

Human subject experiments were performed in accordance with the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. Human subject experiments were approved by the ethics committee of our institute. Samples were collected after written informed consent was received from all subjects. Primary chondrocytes were isolated from human articular cartilage subjects. Briefly, articular cartilage tissue was cut into small pieces and incubated with 0.2% type II collagenase (Life Technologies, USA) overnight at 37°C. Isolated chondrocytes were maintained in Dulbecco's Modified Eagle's medium (DMEM) high glucose medium (Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), 100 μg/ml streptomycin (Sigma-Aldrich, USA) and 50 μg/ml gentamicin (Sigma-Aldrich, USA) in a humid 37°C incubator with 5% CO_2. Cells were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (Novo Nordisk, Japan) (50 and 100 nM) for 24 h.

Determination of reactive oxygen species (ROS)

Intracellular levels of ROS in human primary chondrocytes was assessed using the 2',7'-Dichlorofluorescin diacetate (DCFH-DA) (Sigma-Aldrich, USA) staining method. Cells were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (50 and 100 nM) for 24 h. Cells were then washed 3 times with PBS and stained with 5 μM DCFH-DA for 30 min. After 3 washes, fluorescence signals were visualized with the IBE2000 inverted fluorescence microscope (Zeiss, Germany) (Excitation: 510 nm; Emission: 580 nm). The average fluorescence intensity was calculated and used to reflect intracellular ROS.

Real time polymerase chain reaction (PCR)

Total RNA in chondrocytes was isolated by the Qiazol reagent (Qiagen, USA). RNA quality and concentration were determined by NanoDrop microvolume spectrophotometers. Isolated RNA (2 μg) was reverse transcribed into cDNA using an iScript cDNA Synthesis kit (Bio-Rad, USA). Real time PCR experiments were performed to determine the expression of target genes using SYBR Green Master Mix (Thermo Fisher Scientific, USA) and primers. The internal gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for a reference gene. Expression of target genes was calculated by normalizing to GAPDH using the $2^{-\Delta\Delta CT}$ threshold

cycle method. The following primers were used in this study: MCP-1 (forward: 5'-ATGCAATCAATGCCCCAGTC-3'; reverse: 5'-TGCAGATTCTTGGGTTGTGG-3'); IL-6 (forward: 5'-GGTACATCCTCGACGGCATCT-3'; reverse: 5'-GTGCCTCTTTGCTGCTTTCAC-3'); NOX-4 (forward: 5'-CTTTTGGAAGTCCATTTGAG-3'; reverse: 5'-CGGGAGGGTGGGTATCTAA-3'); MMP-3 (forward: 5'-TTAAAATAAAACTGCTTTT-3'; reverse: 5'-AACTGGAGCATTTTTT-3'); MMP-13 (forward: 5'-AGG AGC ATG GCG ACT TCT AC-3'; reverse: 5'-TAA AAA CAG CTC CGC ATC AA-3'); ADAMTS-4 (forward: 5'-ACACTGAGGACTGCCCAAC-3'; reverse: 5'-GGTGAGTTTGCACTGGTCCT-3'); ADAMTS-5 (forward: 5'-GCAGAACATCGACCAACTCTACTC-3'; reverse: 5-CCAGCAATGCCCACCGAAC-3'); GAPDH (forward: 5'-ACT GGC GTC TTC ACC ACC AT-3'; reverse: 5'-AAG GCC ATG CCA GTG AGC TT-3').

Western blot analysis

Chondrocytes were seeded into 6-well plates. After the indicated treatment, protein was isolated using cell lysis buffer containing protease and phosphatase inhibitors (buffer: PMSF: PhosSTOP = 100:1:1). Then, 20 μg protein was separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. The non-specific sites on the membranes were blocked with 5% non-fat milk at room temperature (RT) and probed with primary antibodies overnight in a cold room. After 3 washes with phosphate buffered saline tween-20 (PBST), the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. Blots were developed with an enhanced chemiluminescence kit (Thermo Fisher Scientific, USA). The following antibodies were used in this study: Mouse monoclonal antibody (mAb) against type II collagen (1:1000, #MAB8887, Chemicon, USA); Mouse mAb against β-actin (1:10000, #3700, Cell Signaling Technology, USA); Mouse mAb against aggrecan (1:1000, #ab3778, Abcam, USA); Anti-rabbit IgG, HRP-linked secondary antibody (1:3000, #7074, Cell signaling technology, USA); Anti-mouse IgG, HRP-linked antibody (1:3000, #7076, Cell signaling technology, USA).

Enzyme-linked immunosorbent assay (ELISA)

Human chondrocytes were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (50 and 100 nM) for 24 h. Cell culture media was collected and cell lysates were prepared. Cell culture media were used to determine the secretion of IL-6 and MCP-1 and cell lysates were used to determine the expression of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5. The following ELISA kits from R&D systems were used in this study: Human IL-1β Quantikine ELISA Kit (#DLB50); human IL-6 Quantikine ELISA Kit (#D6050); human MCP-1 Quantikine ELISA Kit (#DCP00); Human MMP-3 Quantikine ELISA Kit (#DMP300); Human MMP-13 DuoSet ELISA (#DY511); Human ADAMTS4 DuoSet ELISA (#DY4307-05); Human ADAMTS5 DuoSet ELISA (#DY2198-05).

NF-κB luciferase activity

NF-κB luciferase promoter vector was purchased from Clontech, USA. Cells were cotransfected with a nuclear factor-κB (NF-κB) promoter and a firefly luciferase vector using Lipofectamine 2000 (Thermo Fisher Scientific, USA). Cells were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (50 and 100 nM) for 24 h. Cells were then lysed and luciferase activity was measured using a dual-luciferase reporter assay system protocol from Promega, USA.

Statistical analysis

Experimental data are shown as means ± standard error of measurement (S.E.M.). Experiments were repeated at least for 3 times. Statistical analysis was performed using the software SPSS (Version 21.0). One-way analysis of variance (ANOVA) was followed by the post hoc Bonferroni test. A value of $P<0$ 05 was considered statistically significant.

Results

Liraglutide ameliorates TNF-α-induced oxidative stress

Oxidative stress plays a major role in cell health and survival. In OA, expression of proinflammatory cytokines such as TNF-α promotes generation of reactive oxygen species (ROS) by chondrocytes, which disrupts the oxidant/antioxidant balance and results in mitochondrial damage and apoptosis [20]. Additionally, NADPH oxidase 4 (NOX-4) has been shown to induce production of ROS and promote degradation of the articular ECM by triggering expression of MMPs, thereby playing a major role in the

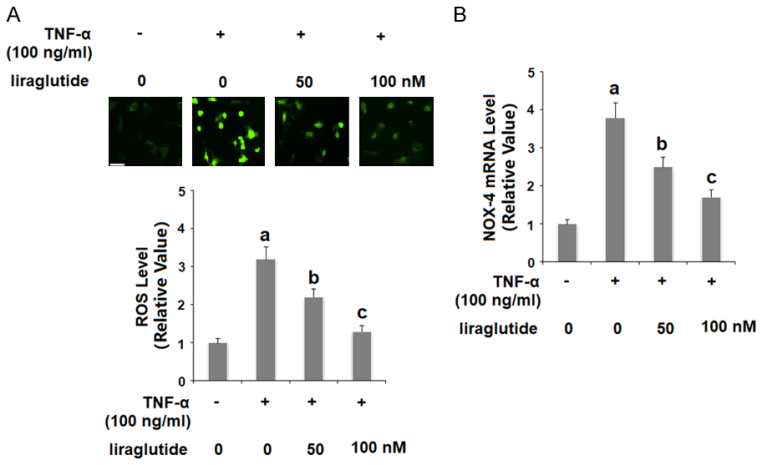

Figure 1. Liraglutide attenuates TNF-α-induced oxidative stress in human primary chondrocytes. Human primary chondrocytes were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (50 and 100 nM) for 24 h. A. The level of intracellular reactive oxygen species (ROS) was determined by DCFH-DA; Scale bars, 100 μm; B. Expression of NOX-4 at the mRNA level was determined by real time PCR analysis (a, b, c, P<0.01 vs. previous column group, n=5-6).

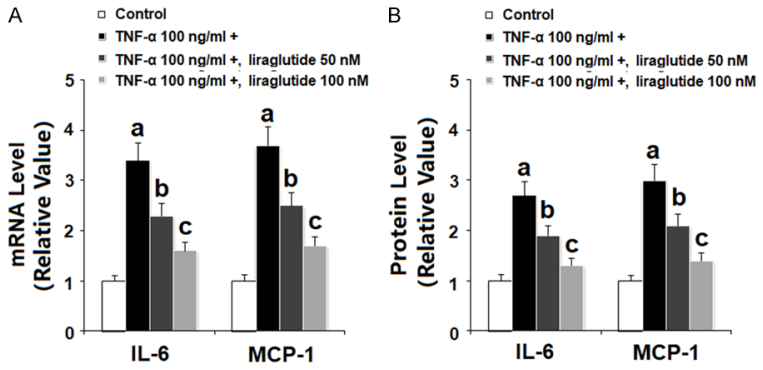

Figure 2. Liraglutide reduces TNF-α-induced expression and secretion of pro-inflammatory cytokines in human primary chondrocytes. Human primary chondrocytes were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (50 and 100 nM) for 24 h. A. Expression of IL-6 and MCP-1 at the mRNA level was determined by real time PCR analysis; B. Expression of IL-6 and MCP-1 at the protein level was determined by ELISA analysis (a, b, c, P < 0.01 vs. previous column group, n=5-6).

pathogenesis of OA [21]. To determine the effects of liraglutide on TNF-α-induced oxidative stress in OA, HPCs were treated with 100 ng/ml TNF-α in the presence or absence of 50 and 100 nM liraglutide. As shown in **Figure 1A**, the results of DCFH-DA staining revealed that TNF-α significantly increased ROS production, which was reduced to near basal levels by treatment with liraglutide in a dose-dependent manner. The results of real time PCR and western blot analyses in **Figure 1B** and **1C**, respectively, demonstrate that liraglutide also downregulated TNF-α-induced production of NOX-4 in a dose-dependent manner, returning NOX4 levels to near baseline. These findings suggest that agonism of GLP-1R by liraglutide may exert a powerful antioxidant effect in the context of OA.

Liraglutide inhibits expression of IL-6 and MCP-1

Proinflammatory cytokines are major mediators of inflammation and cartilage degradation. IL-6 has been shown to mediate inflammation in OA as well as contribute to cartilage and subchondral bone destruction [22]. The C-C chemokine MCP-1 is located on chromosome 17 and composed of 76 amino acids with a molecular weight of 13 kDa [23]. MCP-1 has been shown to contribute to the onset and progression of OA by inducing cartilage degradation [24]. In the present study, we set out to determine the effects of GLP-1R agonism on the expression of IL-6 and MCP-1 induced by TNF-α in HPCs using liraglutide. Briefly, cells were exposed to 100 ng/ml TNF-α in the presence or absence of 50 and 100 μM liraglutide for 24 h. As shown in **Figure 2A**, the results of real time PCR reveal that TNF-α upregulated expression of IL-6 and MCP-1 to roughly 3.5-fold baseline at the mRNA level, which was reduced in a dose-dependent manner to approximately 1.5-fold by treatment with liraglutide. Additionally, the results of ELISA in **Figure 2B** show that liraglutide exerted a similar effect at the protein level, restoring TNF-α-induced protein expression of both IL-6 and MCP-1 to near basal levels.

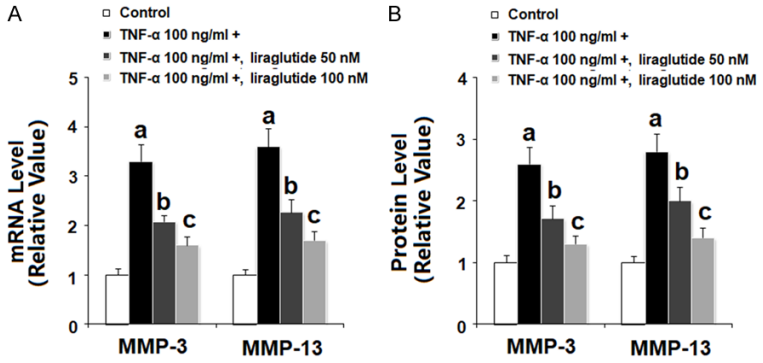

Figure 3. Liraglutide mitigates TNF-α-induced expression of MMP-3 and MMP-13 in human primary chondrocytes. Human primary chondrocytes were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (50 and 100 nM) for 24 h. A. Expression of MMP-3 and MMP-13 at the mRNA level was determined by real time PCR analysis; B. Expression of MMP-3 and MMP-13 at the protein level was determined by ELISA analysis (a, b, c, $P < 0.01$ vs. previous column group, n=5-6).

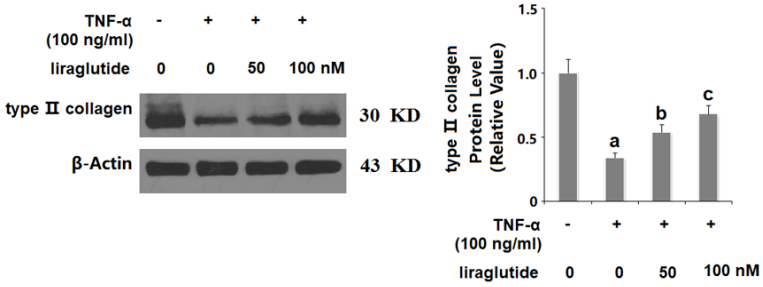

Figure 4. Liraglutide mitigates TNF-α-induced degradation of type II collagen in human primary chondrocytes. Human primary chondrocytes were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (50 and 100 nM) for 24 h. Expression of type II collagen was determined by western blot analysis (a, b, c, $P < 0.01$ vs. previous column group, n=5-6).

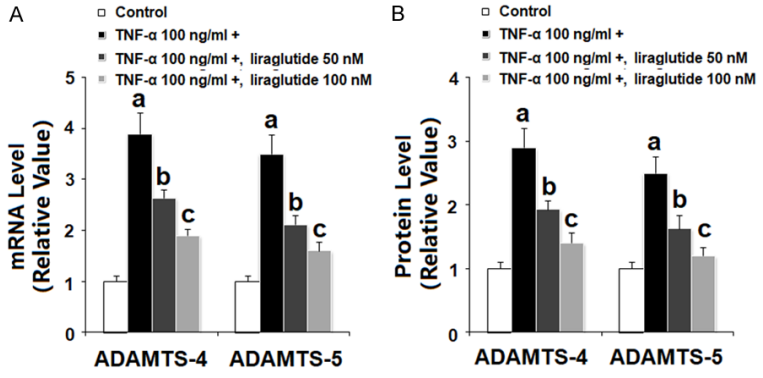

Figure 5. Liraglutide mitigates TNF-α-induced expression of and ADAMTS-4 and ADAMTS-5 in human primary chondrocytes. Human primary chondrocytes were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (50 and 100 nM) for 24 h. A. Expression of ADAMTS-4 and ADAMTS-5 at the mRNA level was determined by real time PCR analysis; B. Expression of ADAMTS-4 and ADAMTS-5 at the protein level was determined by ELISA analysis (a, b, c, $P < 0.01$ vs. previous column group, n=5-6).

Liraglutide downregulates expression of MMPs and degradation of type II collagen

Degradation of type II collagen is a major characteristic of the irreversible joint destruction observed in OA. To determine the effects of GLP-1 agonism using liraglutide on TNF-α-induced degradation of type II collagen, we first investigated expression of MMP-3 and MMP-13 by HPCs stimulated with 100 ng/ml TNF-α in the presence or absence of 50 and 100 μM liraglutide for 24 h. As shown in **Figure 3A**, TNF-α increased expression of MMP-3 and MMP-13 to roughly 3.4- and 3.6-fold basal levels, respectively. However, treatment with liraglutide significantly reduced expression of these enzymes to only roughly 1.5-fold and 1.6-fold baseline, thereby demonstrating a potent ability of GLP-1R to regulate expression of degradative enzymes including MMP-3 and MMP-13 (**Figure 3B**). Next, we confirmed whether this inhibition of MMP-3 and MMP-13 indeed reduced degradation of type II collagen induced by TNF-α. As shown in **Figure 4**, TNF-α degraded type II collagen by more than 50%, which was rescued by treatment with liraglutide in a dose-dependent manner with the higher dose reducing degradation of type II collagen to only approximately 20%. Therefore, agonism of GLP-1R may prevent degradation of type II collagen induced by TNF-α by downregulating expression of MMP-3 and MMP-13 by chondrocytes.

Liraglutide downregulates expression of ADAMTS and degradation of aggrecan

Expression of ADAMTS-4 and ADAMTS-5 has been shown to

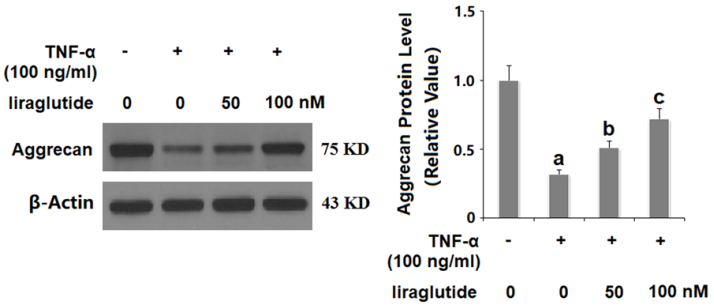

Figure 6. Liraglutide abrogates TNF-α-induced degradation of aggrecan in human primary chondrocytes. Human primary chondrocytes were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (50 and 100 nM) for 24 h. Expression of aggrecan was determined by western blot analysis (a, b, c, $P < 0.01$ vs. previous column group, n=5).

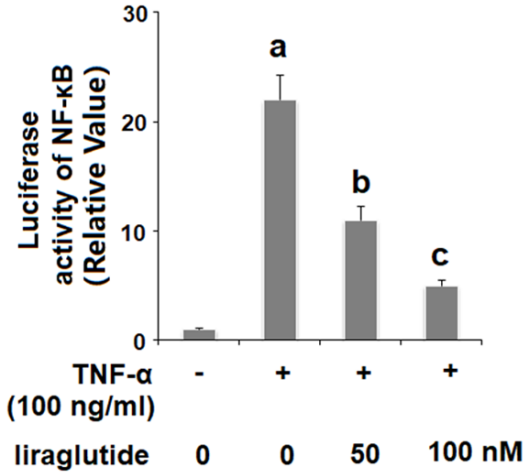

Figure 7. Liraglutide mitigates TNF-α-induced activation of NF-κB. Human primary chondrocytes were incubated with 100 ng/ml TNF-α in the presence or absence of liraglutide (50 and 100 nM) for 24 h. Luciferase activity of NF-κB was determined (a, b, c, $P < 0.01$ vs. previous column group, n=5-6).

play a major role in driving degradation of aggrecan in OA [25]. Here, we investigated the potential effects of GLP-1R agonism on degradation of aggrecan by exposing HPCs to 100 ng/ml TNF-α in the presence or absence of 50 and 100 μM liraglutide for 24 h. As shown in **Figure 5**, we first investigated the effects of liraglutide on expression of ADAMTS-4 and ADAMTS-5 induced by TNF-α. TNF-α significantly upregulated expression of both ADAMTS-4 and ADAMTS-5 to roughly 3.5- and 4-fold baseline at the mRNA level and roughly 2.5- and 3-fold baseline at the protein level. However, treatment with liraglutide reduced expression of ADAMTS-4 and ADAMTS-5 to less than 2-fold baseline at the mRNA levels and near baseline at the protein level (**Figure 5A** and **5B**, respectively). Next, we confirmed whether this regulatory effect of liraglutide on the expression of aggrecanases translated to downregulation of aggrecan degradation. As shown in **Figure 6**, using β-actin as a control, the results of western blot analysis revealed that TNF-α induced roughly 75% degradation of aggrecan, which was reduced to approximately 25% by treatment with liraglutide in a dose-dependent manner. These findings demonstrate a potential role of GLP-1R in regulating cartilage degradation in OA.

Liraglutide suppresses activation of the NF-κB pathway

Activation of the NF-κB pathway is a recognized as one of the main forces governing the pathogenesis of OA. Expression of cytokines including TNF-α can induce nuclear translocation of p65 protein and subsequent activation of the NF-κB proinflammatory cellular signaling pathway. Additionally, modulation of NF-κB is considered as a valuable treatment target for OA [26]. In the present study, we investigated the effects of liraglutide on activation of NF-κB induced by TNF-α. HPCs were treated with 100 ng/ml TNF-α in the presence or absence of 50 and 100 μM for 24 h. As shown in **Figure 7**, liraglutide reduced TNF-α-induced luciferase activity of NF-κB from more than 20-fold basal levels to only roughly 5-fold in a dose-dependent manner. Importantly, these findings show that agonism of GLP-1R using liraglutide exerted a significant inhibitory effect on TNF-α-induced activation of NF-κB.

Discussion

OA is a painful, debilitating disease of unknown etiology that affects millions of people worldwide. In the present study, we investigated the effects of agonism of GLP-1R on various factors related to the development and progression of OA, including oxidative stress, expression of cytokines such as IL-6 and MCP-1, degradation of type II collagen by MMPs-3 and -13, degradation of aggrecan by ADAMTS-4 and -5, as well as activation of the NF-κB proinflammatory signaling pathway. Our findings demonstrate that agonism of GLP-1R may indeed exert beneficial effects in OA by downregulating oxidative stress, expression of proinflammatory

cytokines, degradation of articular ECM and activation of NF-κB. However, the exact mechanisms behind these promising findings are complicated and remain unclear.

Recently, there has been mounting evidence suggesting that GLP-1 may play a role in the development and progression of bone diseases by mediating bone formation, remodeling, resorption, and osteoblast differentiation [27-29]. Endogenous GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase 4 (DPP-4), so GLP-1 agonists such as liraglutide and exenatide as well as DPP-4 inhibitors have emerged as attractive treatment options for type II diabetes and other diseases [30, 31]. Liraglutide was approved for the treatment of type II diabetes in 2010, obesity in 2014 and has been shown to display a good safety profile [32-34]. Since obesity is a major risk factor for OA due to the increased mechanical burden on joints [34], it is worth noting that the ability of liraglutide to reduce weight in obese patients may also be favorable for OA patients in this population. To further elucidate the potential role of GLP-1R in mediating the pathogenesis of OA, we exposed HPCs to TNF-α to simulate OA conditions. Oxidative stress induced by TNF-α has been shown to play a major role in OA by regulating intracellular signaling processes and mitigating synthesis and degradation of type II collagen and aggrecan, among other things [35]. Thus, we investigated the effects of liraglutide on two markers of oxidative stress in HPCs: generation of ROS and NOX-4. We found that liraglutide significantly attenuates overproduction of ROS and NOX-4 triggered by TNF-α (**Figure 1**). This suggests a powerful potential antioxidant capacity of liraglutide. Next, we explored the effects of liraglutide on expression of IL-6 and MCP-1. IL-6, TNF-α, and IL-1β have been cited as the three major cytokines involved in the pathogenesis of OA [36]. We found that treatment with liraglutide strongly inhibited expression of IL-6 induced by TNF-α (**Figure 2A**). We also investigated the effects of liraglutide on expression of MCP-1, as this chemokine has been shown to induce cartilage degradation in OA [24, 37]. Our results show that liraglutide had a similarly potent inhibitory effect on expression of MCP-1 (**Figure 2B**).

Next, we explored the effects of liraglutide on degradation of type II collagen and aggrecan by MMPs and ADAMTS, respectively. As a virtually irreversible pathology of OA, prevention of the degradation of type II collagen is regarded as an attractive target for the treatment and prevention of OA [38]. In the present study, we found that liraglutide exerted a strong inhibitory effect against TNF-α-induced expression of MMP-3 and MMP-13, and concordantly, had a remarkable preventative effect against degradation of type II collagen (**Figures 3** and **4**). As the main proteoglycan in articular cartilage, aggrecan cushions the joints, allowing them to withstand compression and absorb shock. Excessive degradation of aggrecan mediated by ADAMTS-4 and ADAMTS-5 leads to loss of joint function and eventual destruction of the joint [25, 39]. Here, we demonstrated that liraglutide had a similar noteworthy inhibitory effect against TNF-α-induced expression of ADAMTS-4 and ADAMTS-5 and subsequent degradation of aggrecan, thereby demonstrating a novel potential role of liraglutide in preserving cartilage by preventing degradation of both type II collagen and aggrecan induced by TNF-α (**Figures 4** and **6**). Regarded as playing an essential role in cartilage degradation in OA, the NF-κB signaling pathway has been shown to mitigate a wide range of pro-inflammatory responses as well as expression of MMPs and ADAMTS in chondrocytes [40, 41]. In the present study, we found that agonism of GLP-1R by liraglutide strongly inhibited activation of NF-κB (**Figure 7**). This finding demonstrates a potential role of liraglutide as a valuable inhibitor of NF-κB in the context of OA.

Taken together, our findings demonstrate that agonism of GLP-1R by liraglutide may exert a valuable protective effect against oxidative stress, inflammation, cartilage degradation, and activation of NF-κB induced by TNF-α in human primary chondrocytes. To our knowledge, this is the first study to explore the potential of liraglutide for the treatment and prevention of OA. Further study is required to gain a deeper understanding of the mechanisms behind these promising findings.

Acknowledgements

This work was supported by the Wuhan City Health Bureau of Medical Research project (No. WX15B06).

Disclosure of conflict of interest

None.

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