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The protective effects of lixisenatide against inflammatory response in human rheumatoid arthritis fibroblast-like synoviocytes

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ABSTRACT

Rheumatoid arthritis (RA) is a major debilitating systemic disease characterized by chronic inflammation of the synovium and joint destruction. Despite major advancements in our understanding of RA in recent decades, it remains a disease of unknown etiology. To our knowledge, this is the first study exploring the effects of agonism of the glucagon-like peptide-1 (GLP-1) receptor using lixisenatide, a licensed drug used for the treatment of type II diabetes, on the pathological characteristics of RA in human fibroblast-like synoviocytes. Our findings indicate that lixisenatide inhibited the inflammatory response through downregulation of proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8); inhibition of matrix metalloproteinases (MMPs); and blockade of cellular signaling pathways, including the c-Jun N-terminal kinase (JNK), activator protein 1 (AP-1), and nuclear factor κ B (NF-κB) pathways. Furthermore, lixisenatide improved oxidative stress, rescued mitochondrial membrane potential (ΔΨm), and prevented cell death in fibroblast-like synoviocytes. These findings suggest that agonism of the GLP-1 receptor using lixisenatide may serve as a novel therapeutic option for the treatment and prevention of RA.

1. Introduction

Rheumatoid arthritis (RA) is a common debilitating disease characterized by chronic painful inflammation, joint destruction, and subsequent loss of function. While recent research has focused on finding new targeted drug treatment options for RA, including inhibition of the release of cytokines such as tumor necrosis factor alpha (TNF-α) and interleukins (ILs), blockage of proinflammatory signaling pathways such as the c-Jun N-terminal kinase (JNK) and nuclear factor B (NF-κB) pathways, and blockade of B-cell depletion. Notably, the exact mechanisms driving the development and progression of RA remain poorly understood [1]. IL-1β is well-recognized as a major driver of inflammation in a wide range of chronic inflammatory diseases, including RA. Significantly elevated levels of IL-1β, as well as TNF-α and IL-6, have been found in the synovial fluid of RA patients [2]. Interestingly, injection of IL-1β alone into the knee joints of rabbits was found to produce nearly all of the pathologies associated with RA, thereby suggesting that stimulation with IL-1β is a suitable method for investigating the effects of RA [3]. In normal physiology, the synovial membrane encapsulates the joint cavity, wherein synovial fluid consisting of macrophage-like and fibroblast-like synoviocytes (FLSs) acts as lubricant between joint cartilages and provides joint tissues with nutrients as well as various collagens and proteins. Additionally, FLSs are equipped with the property of contact inhibition, which limits the concentration of FLSs in synovial fluid, thereby maintaining joint homeostasis [4,5]. However, in RA, FLSs undergo a change in phenotype and loss of contact inhibition, which leads to an overabundance of FLSs in synovial fluid and triggers an inflammatory response [4].

In addition to sustained excessive inflammation, pathological factors such as mitochondrial dysfunction, oxidative stress and degradation of collagen due to the release of matrix metalloproteinases (MMPs), including MMP-1, MMP-3 and MMP-13, have been observed in RA [6,7,8,9]. However, the exact mechanisms driving these processes remain elusive. In the present study, we investigated the involvement of mediation of the JNK, activator protein 1 (AP-1) and nuclear factor κ B (NF-κB) proinflammatory signaling pathways by the incretin hormone

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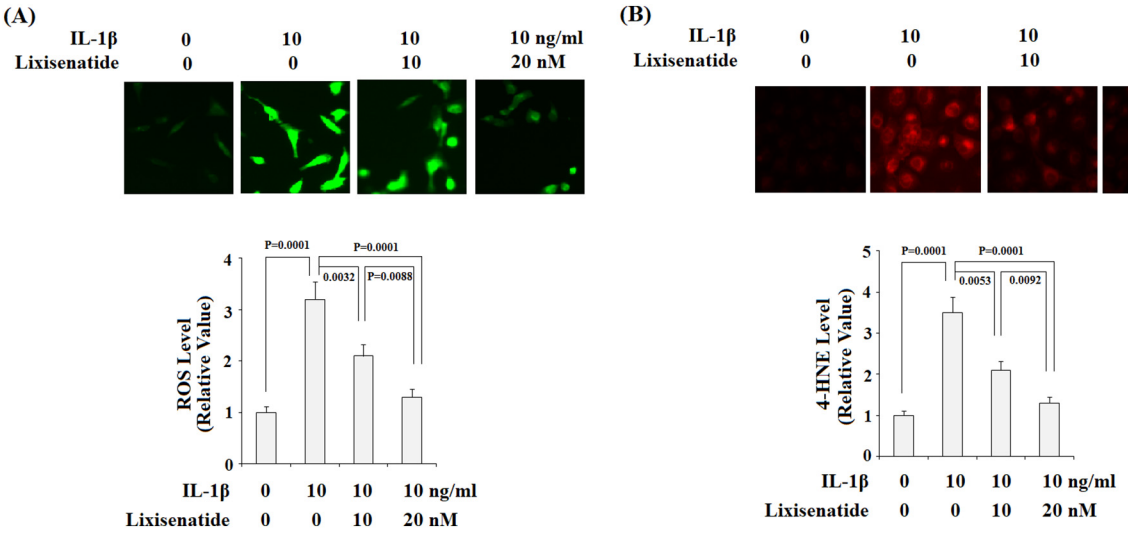

Fig. 1. Lixisenatide ameliorates IL-1β-induced generation of reactive oxygen species (ROS) and 4-HNE expression in human FLSs. Human FLSs were treated with 10 ng/mL IL-1β with or without 10 and 20 nM lixisenatide for 48 h. (A). Intracellular ROS was measured by DCFH-DA assay; (B). 4-HNE was measured by immunostaining. Experimental data were normalized to the vehicle control (N = 5–6).

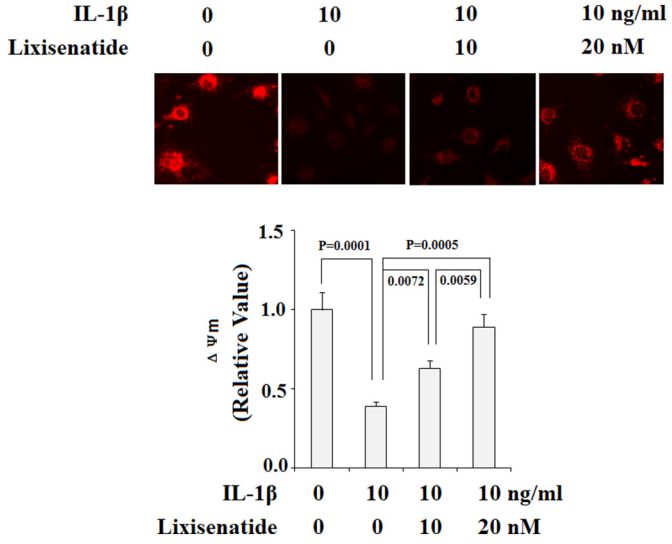

Fig. 2. Lixisenatide attenuates IL-1β-induced mitochondrial dysfunction in human FLSs. Human FLSs were treated with 10 ng/mL IL-1β with or without 10 and 20 nM lixisenatide for 48 h. Mitochondrial membrane potential (ΔΨm) was determined by TMRM staining. Experimental data were normalized to the vehicle control (N = 6).

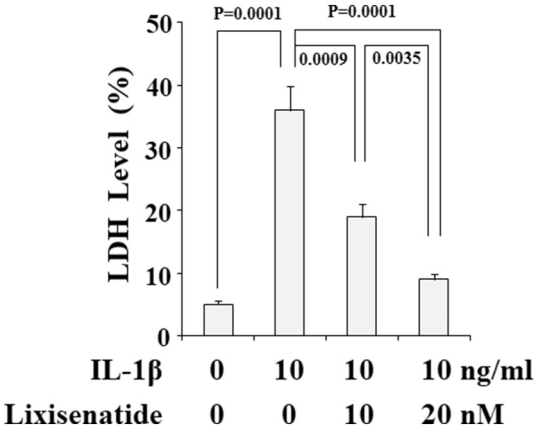

Fig. 3. Lixisenatide mitigates IL-1β-induced LDH release in human FLSs. Human FLSs were treated with 10 ng/mL IL-1β with or without 10 and 20 nM lixisenatide for 48 h. LDH release was determined (N = 6).

glucagon-like peptide 1 (GLP-1) in the pathogenesis of RA by stimulating RA-FLSs with lixisenatide, a selective GLP-1 agonist widely used in the treatment of type II diabetes mellitus. Insulin resistance has been shown to be positively correlated with RA, thereby suggesting that incretin hormones such as GLP-1 may play a role in the development of both type II diabetes and RA [10]. Furthermore, elevated expression of TNF-α and IL-6 has also been found to be directly correlated with the occurrence of ischemia/reperfusion (I/R) in RA [11]. Targeted blockade of the proinflammatory pathways involved in the pathogenesis of RA may have potential as a safe and effective novel treatment for RA. Thus, in the present study, we investigated the effects of treatment with lixisenatide, a selective GLP-1 agonist, on FLSs stimulated with IL-1β to determine the potential of this drug as a novel therapeutic option for RA.

2. Materials and methods

2.1. Cell isolation, culture, and treatment

Experiments using human samples were performed in accordance with the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. These experiments were approved by the ethics committee of Soochow University. All participants have carefully read and signed written informed consent. 10 cases of synovial tissues of RA patients received joint replacement were enrolled in this study. These patients included 6 males and 4 females, and mean age was 50.1 ± 5.2 years. DAS28 score ranged from 4.01 to 5.56 (mean 4.68 ± 0.92). Fibroblast-like synoviocytes (FLSs) were isolated from synovial tissues of donors using procedures as previously described [12]. Passage 2–4 FLSs were cultured in 24-well culture plates at a density of 250,000 cells/well. 5 samples were used in each study. FLSs were treated with 10 ng/mL IL-1β in the presence or absence of 10 and 20 nM lixisenatide (Sanofi-Aventis Co., LTD, France) for 48 h.

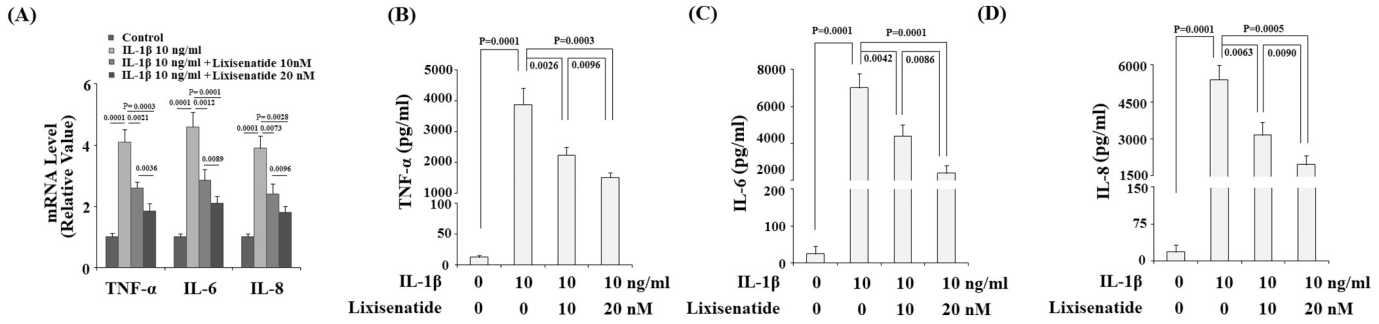

Fig. 4. Lixisenatide inhibits IL-1β-induced expression of TNF-α, IL-6, and IL-8 in FLSs. Human FLSs were treated with 10 ng/mL IL-1β with or without 10 and 20 nM lixisenatide for 48 h. (A). Expression of TNF-α, IL-6, and IL-8 at the gene level was determined by RT-PCR analysis. Experimental data were normalized to GAPDH and the vehicle control; (B-D). Secretion of TNF-α, IL-6, and IL-8 at the protein level was determined by the ELISA assay (N = 5–6).

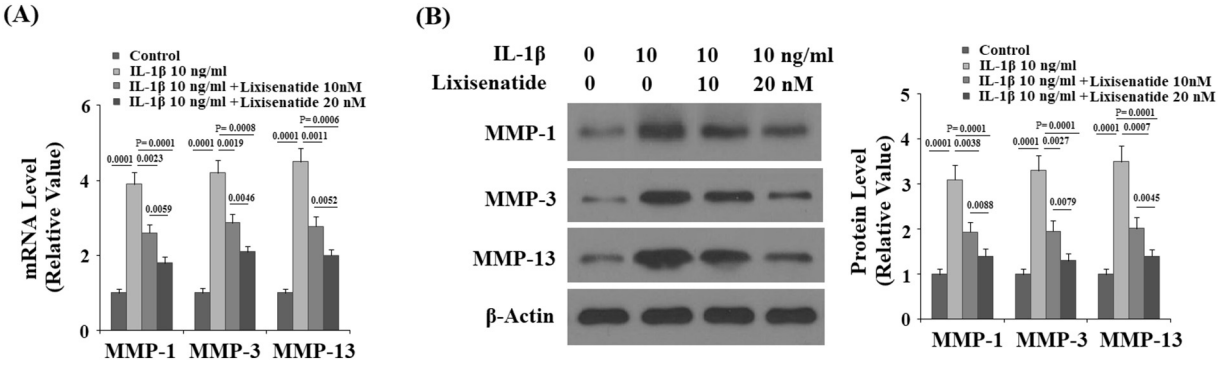

Fig. 5. Lixisenatide ameliorates IL-1β-induced expression of MMP-1, MMP-3 and MMP-13 in human FLSs. Human FLSs were treated with 10 ng/mL IL-1β with or without 10 and 20 nM lixisenatide for 48 h. (A). Expression of MMP-1, MMP-3, and MMP-13 at the gene level was determined by RT-PCR analysis. Experimental data were normalized to GAPDH and the vehicle control; (B). Expression of MMP-1, MMP-3, and MMP-13 at the protein level was determined by western blot analysis. Experimental data were normalized the vehicle control (N = 5–6).

2.2. Determination of reactive oxygen species (ROS)

To study oxidative stress in FLSs, the fluorescent dye 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) was used to assess the intracellular levels of ROS. Briefly, 5 μM DCFH-DA was added to the cell culture medium and incubated for 15 min in darkness in a cell culture incubator. FLSs were then washed 3 times, and fluorescent signals were visualized and captured in a fluorescent microscope.

2.3. 4-Hydroxynonenal (4-HNE) immunostaining

After the indicated treatment, expression of 4-HNE in FLSs was evaluated via immunostaining. Briefly, cells were incubated with 100% methanol at room temperature (RT) for 5 min. Permeabilization of cells was then carried out via incubation in 0.1% Triton X-100. Cells were then blocked with 1% BSA, 22.52 mg/mL glycine in PBST to block unspecific binding of the antibodies. FLSs were then incubated with primary antibody against 4-HNE. After a gentle wash with PBS, cells were incubated with TRITC-conjugated secondary antibody. After 3 washes, fluorescent signals were visualized and captured in a fluorescent microscope.

2.4. Measurement of mitochondrial membrane potential (ΔΨm)

To study mitochondrial function in FLSs, the fluorescent dye tetramethylrhodamine methyl ester (TMRM) was used to assess the intracellular levels of ΔΨm. Briefly, 5 μM TMRM was added to the cell culture medium and incubated for 60 min in darkness in a cell culture incubator. FLSs were then washed 3 times, and fluorescent signals were visualized and captured in a fluorescent microscope.

2.5. Lactate dehydrogenase (LDH) release assay

LDH is a stable cytosolic enzyme that is released from the cytoplasm to the cell culture medium upon cell death. Cells were plated in 96-well cell culture plates. After the indicated treatment, 50 μl aliquots were transferred from all test and control wells to a fresh 96-well flat clear bottom plate. Then, 50 μl LDH assay reagent (#88954, Thermo Fisher Scientific) was added and incubated for 30 min in darkness at RT. Stop buffer (50 μl) was then added to stop the reaction until a red formazan product was generated. Absorbance at 490 nm was measured to calculate LDH release.

2.6. Cell viability determination

FLSs were cultured in 96-well plates. Cells were treated with 10 ng/mL IL-1β with or without 10 and 20 nM lixisenatide for 48 h. MTT (5 mg/ml) in the culture medium was added into each well for 4 h at 37 °C. Then all the medium was removed and the product was dissolved with 100 μl DMSO. OD value was recorded at 490 nm to reflect cell viability.

2.7. Real-time polymerase chain reaction (RT-PCR)

RNA was extracted from FLSs using Qiazol (Qiagen, USA). Purified RNA (1 μg) from each of the test and control groups was used for reverse transcription PCR (RT-PCR) to synthesize cDNA using the SuperScript First-Strand Synthesis System (Invitrogen, USA). cDNA products were then used for RT-PCR analysis with SYBR Green Mix on an ABI 7500 real-time PCR system in accordance with the manufacturer's instructions. The internal gene GAPDH was used as a positive control. cDNA abundance was quantified by the $2^{-\Delta\Delta CT}$ threshold cycle

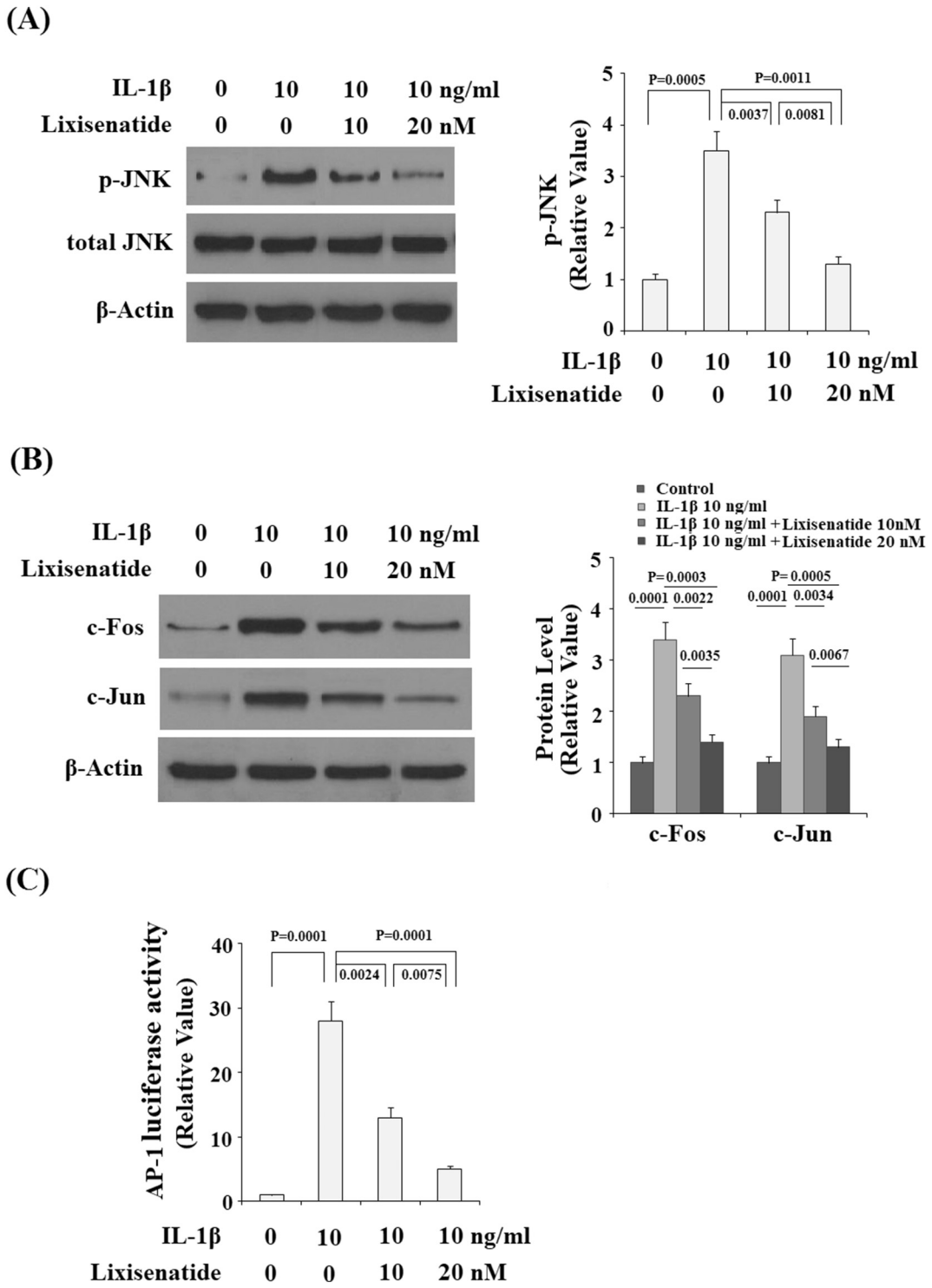

Fig. 6. Lixisenatide reduces IL-1β-induced activation of JNK and AP-1 in human FLSs. (A). Human FLSs were treated with 10 ng/mL IL-1β with or without 10 and 20 nM lixisenatide for 2 h. Phosphorylated and total levels of JNK were determined by western blot analysis. Experimental data were normalized to GAPDH and the vehicle control; (B–C) Human FLSs were treated with 10 ng/mL IL-1β with or without 10 and 20 nM lixisenatide for 48 h. Western blot analysis of the two AP-1 families (c-Fos and c-Jun); Luciferase reporter assay showed that lixisenatide treatment suppressed AP-1 activation in a dose-dependent manner. Experimental data were normalized to the vehicle control (N = 5–6).

method.

2.8. Enzyme-linked immunosorbent assay (ELISA)

The secretion of TNF-α, IL-6, IL-8 from human FLSs was determined by ELISA assay using commercial kits: TNF-α (#DTA00D, R&D systems); IL-6 (#D6050, R&D systems); IL-8 (#D8000C, R&D systems). After the indicated treatment, 50 μL of prepared cell culture medium was added to ELISA plates and incubated at RT for 2 h. The liquid was aspirated from the ELISA plates. After 4 washes, 100 μL of diluted detection antibody was added to the wells and incubated for 1 h. The liquid was then discarded, and the plates were washed 4 times. HRP

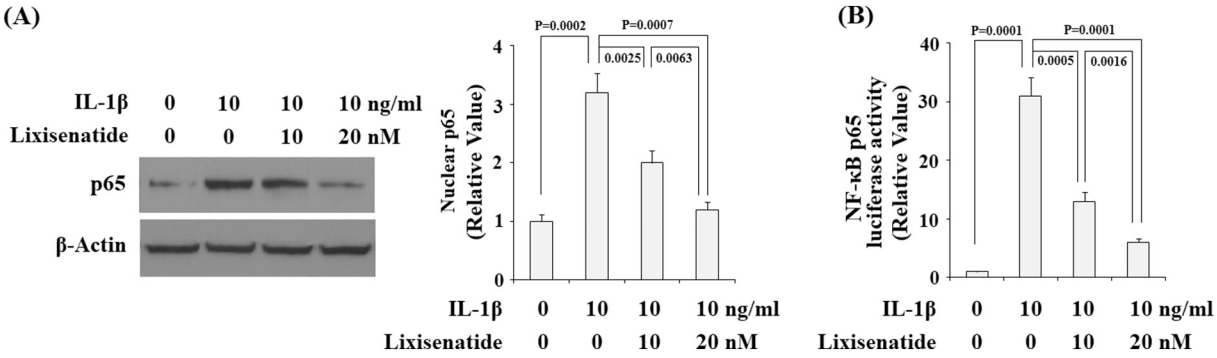

Fig. 7. Lixisenatide inhibits IL-1β-induced activation of NF-κB p65. Human FLSs were treated with 10 ng/mL IL-1β with or without 10 and 20 nM lixisenatide for 48 h. (A). Nuclear level of NF-κB p65; (B). NF-κB p65 luciferase activity. Experimental data were normalized to GAPDH and the vehicle control (N = 5–6).

conjugated antibody (100 μL) was then added to the ELISA plate and incubated for 30 min at RT. The liquid was then discarded, and the plates were washed 4 times. Chromogenic substrate (100 μL) was then added and developed for 30 min in darkness. Stop solution (100 μL) was then added to stop the reaction. Absorbance of each well at 450 nm was measured.

2.9. Western blot analysis

FLSs were seeded into 6-well plates. After the necessary treatment, cells were lysed with cell lysis buffer supplemented with 1% PMSF and 1% PhosSTOP. Protein extracts were collected, and the concentration was evaluated with a BCA protein assay kit (Thermo Scientific Pierce, USA). A total of 20 μg aliquots of the protein extracts was subjected to 10% SDS-PAGE and transferred to PVDF membranes, which were blocked with 5% non-fat milk at RT. After a gentle wash with TBST on a shaker, membranes were sequentially probed with primary antibodies overnight in a cold room and with horseradish peroxidase (HRP)-conjugated secondary antibodies. Blots in all of the groups were assessed by a chemiluminescence development kit. The following antibodies from Cell Signaling Technology were used in this study: p-JNK (#9255, 1:1000); JNK (#9252, 1:3000); c-fos (#2250, 1:2000); c-Jun (#9165, 1:3000); p65 (#8242, 1:2000); MMP-1 (#54376, 1:2000); MMP-3 (#14351, 1:3000); MMP-13 (#94808, 1:2000); β-actin (#3700, 1:10,000).

2.10. Luciferase activity assay

Transcriptional activities of the transcriptional factors NF-κB and AP-1 were evaluated via a luciferase activity assay. Cells were co-transfected with lipofectamine 2000 (Invitrogen, USA) for 3 h using either 0.5 μg of the NF-kB responsive firefly luciferase reporter gene (Clontech, USA) or the AP-1 firefly luciferase reporter gene (Clontech, USA), and 0.25 μg of a renilla luciferase reporter gene (Clontech, USA). At 24 h post transfection, FLSs were treated with 10 ng/mL IL-1β in the presence or absence of 10 and 20 nM lixisenatide for 48 h. Cells were then lysed and the dual activity of renilla and firefly luciferase were assayed with a dual-luciferase reporter assay (Promega, USA).

2.11. Statistical analysis

Results are expressed as means ± S.E.M. Statistical analyses of experimental data were performed using SPSS 21.0 software. Statistical comparisons were made using two-way ANOVA, and Tukey's test was used for post hoc comparisons. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Lixisenatide ameliorates IL-1β-induced oxidative stress, mitochondrial dysfunction, and apoptosis

As shown in Fig. 1A, the results of DCFH-DA staining reveal that treatment with 10 and 20 nM lixisenatide significantly reduced generation of ROS by human FLSs induced by stimulation with 10 ng/mL IL-1β for 48 h in a dose-dependent manner. Additionally, the immunostaining results in Fig. 1B demonstrate that lixisenatide (10 and 20 nM) also ameliorated IL-1β-induced expression of 4-hydroxynoneal (4-HNE), a byproduct of lipid peroxidation, in human FLSs stimulated with 10 ng/mL IL-1β for 48 h. These findings indicate that agonism of GLP-1 by lixisenatide provided a significant dose-dependent decrease in oxidative stress, one of the hallmarks of RA. As shown in Fig. 2, we next set out to determine the effects of lixisenatide on IL-1β-induced mitochondrial dysfunction. Preservation of mitochondrial membrane potential (ΔΨm) is critical for cell survival and energy production, however, exposure to 10 ng/mL IL-1β significantly decreased ΔΨm in FLSs as evidenced by TMRM staining. Remarkably, treatment with 10 and 20 nM lixisenatide significantly rescued ΔΨm, with the higher dose almost completely recovering ΔΨm in FLSs. Next, we set out to determine the involvement of the GLP-1 receptor in IL-1β-induced cell death. Lactose dehydrogenase (LDH) is released as a result of cell apoptosis. Human FLSs were exposed to 10 ng/mL IL-1β for 48 h in the presence or absence of 10 and 20 nM lixisenatide. As shown in Fig. 3, treatment with lixisenatide significantly decreased the level of LDH released into the culture medium, thereby indicating reduced cell death of FLSs exposed to IL-1β. Additionally, cell viability of FLS was measured by the MTT assay. Results in Supplementary Fig. 1 indicated that treatment with 10 ng/mL IL-1β significantly decreased cell viability of FLS, which was prevented by 10 and 20 nM lixisenatide dose dependently.

3.2. Lixisenatide attenuates IL-1β-induced release of proinflammatory cytokines

Proinflammatory cytokines play a critical role in regulating the inflammatory response. In RA, however, excessive expression of cytokines induces an exacerbated inflammatory response resulting in joint damage, swelling and pain. To determine the effects of lixisenatide on the release of three major proinflammatory cytokines (TNF-α, IL-6, and IL-8) induced by IL-1β, we exposed human FLSs to 10 ng/mL IL-1β for 48 h in the presence or absence of 10 and 20 nM lixisenatide. As shown by the results of real time PCR and ELISA assay in Fig. 4, treatment with lixisenatide significantly reduced expression of TNF-α, IL-6 and IL-8 at both the mRNA and protein levels, respectively. Notably, protein expression of the three cytokines returned to near basal levels upon treatment with the higher dose of lixisenatide. These findings indicate

that GLP-1 may play an important role in regulating cytokine production in RA FLSs.

3.3. Lixisenatide reduces IL-1β-induced expression of MMPs

Overexpression of MMPs is well-recognized as a major event in the development and progression of joint damage associated with RA. Namely, MMP-1, MMP-3 and MMP-13 have been shown to drive excessive degradation of type II collagen, a major component of cartilage. To determine the involvement of the GLP-1 receptor in regulating the expression of MMPs induced by IL-1β, we exposed hum FLSs to 10 ng/mL IL-1β for 48 h in the presence or absence of 10 and 20 nM lixisenatide. As demonstrated by the results of real time PCR and western blot analysis in Fig. 5A and B, respectively, we found that lixisenatide significantly reduced expression of MMP-1, MMP-3, and MMP-13 at both the mRNA and protein levels in a dose-dependent manner. These findings indicate that expression of MMPs in RA may be regulated by the GLP-1 receptor.

3.4. Lixisenatide inhibits activation of proinflammatory pathways by IL-1β

Activation of pro-inflammatory pathways is a major event in a variety of chronic inflammatory diseases including RA. However, the exact mechanisms governing the activation of these pathways remain unclear. To determine the involvement of GLP-1 in activation of three major proinflammatory pathways, we investigated the effects of treatment with lixisenatide on the JNK, AP-1 and NF-κB signaling pathways. Another GLP-1 agonist, exendin-4, has been reported to inhibit phosphorylation of JNK [13]. To determine the effects of GLP-1 agonism on phosphorylation of JNK in RA, human FLSs were exposed to 10 ng/mL IL-1β for 2 h in the presence or absence of 10 and 20 nM lixisenatide. Using β-actin as a control, the results of western blot analysis in Fig. 6A indicate that treatment with lixisenatide significantly reduced the level of phosphorylated JNK in a dose-dependent manner, while the level of total JNK remained constant, thereby implying the involvement of GLP-1 in mediating activation of the JNK pathway. Next, we set out to determine the effects of GLP-1 agonism on activation of AP-1. Human FLSs were treated with 10 ng/mL IL-1β for 48 h in the presence or absence of 10 and 20 nM lixisenatide. As shown by the results of western blot analysis in Fig. 6B, protein expression of the two AP-1 subfamilies, c-fos and c-Jun, was significantly upregulated in response to treatment with IL-1β. However, treatment with lixisenatide strongly inhibited protein expression of c-fos and c-Jun in a dose-dependent manner, with 20 nM lixisenatide nearly recovering expression of these two factors to basal levels. Additionally, the results of luciferase reporter assay in Fig. 6C indicate that while activation of AP-1 at the basal level was barely detectable, exposure to 10 ng/mL IL-1β caused significant activation of AP-1, which was ameliorated by treatment with 10 and 20 nM lixisenatide in a dose-dependent manner. Lastly, we investigated the involvement of GLP-1 in nuclear translocation of p65 protein and subsequent activation of the NF-κB signaling pathway. Human FLSs were treated with 10 ng/mL IL-1β in the presence or absence of 10 and 20 nM lixisenatide for 48 h. As demonstrated by the results of western blot analysis and luciferase assay in Fig. 7A and B, respectively, IL-1β-induced nuclear translocation of p65 and activation of NF-κB, which were ameliorated by 10 and 20 nM lixisenatide in a dose-dependent manner. Notably, the dose of 20 nM lixisenatide almost completely rescued nuclear translocation of p65 to basal levels, thereby indicating the involvement of GLP-1 in activation of the NF-κB signaling pathway.

3.5. Lixisenatide suppresses TNF-α-induced insults in FLSs

To further confirm the protective effects of lixisenatide against pro-inflammatory cytokines-induced insults in FLSs, another pro-inflammatory cytokine TNF-α was used. Exposure to TNF-α significantly increased the levels of IL-6 and IL-8, which were inhibited by lixisenatide (10 and 20 nM) (Supplementary Fig. 2A). Importantly, the presence of lixisenatide significantly decreased TNF-α-induced the expression of MMP-1, MMP-3, and MMP-13 (Supplementary Fig. 2B).

4. Discussion

While RA is a relatively common autoimmune disease, the exact mechanisms behind its pathogenesis remain poorly understood. In the present study, we investigated the involvement of the GLP-1 receptor on FLSs in the pathogenesis of RA. Namely, we sought evidence that GLP-1 plays a role in regulating some of the mechanisms shown to be involved in the development and progression of RA, such as oxidative stress, mitochondrial dysfunction, cell apoptosis, degradation of articular extracellular matrix, release of proinflammatory cytokines, and activation of proinflammatory signaling pathways. Our results indicate that treatment with lixisenatide had a significant effect on all of these factors, among which the most notable effects were restoration of the levels of ROS and ΔΨm, protein levels of TNF-α, IL-6, IL-8, MMP-1, MMP-3, and MMP-13, phosphorylated JNK, expression of c-Jun and nuclear translocation of p65, all of which returned to near basal levels upon treatment with 20 nM lixisenatide.

Recently, numerous studies have explored the effects of the family of GLP-1 agonists in diseases beyond diabetes, including chronic heart failure, osteoarthritis and RA, among others [14,15,16]. Additionally, research on the role of GLP-1 in bone loss and bone formation has been receiving attention, although solid evidence of the role of GLP-1 in this context has yet to be presented [17,18]. Consistent with our findings, a contemporary study showed that agonism of GLP-1 can ameliorate oxidative stress in type II diabetes patients [19]. Another GLP-1 agonist, exenatide, has been demonstrated to improve mitochondrial dysfunction in type II diabetes, cardiac disease and neurodegenerative disorders [20,21,22]. Lixisenatide, a selective, potent, once-daily GLP-1 receptor agonist for the treatment of type 2 diabetes, shares the same fundamental mechanism of action on the GLP-1 receptor with other GLP-1 receptor agonists. Lixisenatide is more resistant to proteolysis than native GLP-1 and activates the GLP-1 receptor for around 6 h after each injection. It primarily exerts its main effect during the prandial period and substantially reduces postprandial glucose (PPG) for the meal immediately following injection. It has been shown that lixisenatide affects numerous factors involved in glucose regulation [1,23]. RA-FLS have been identified as the main effector cells causing synovial inflammatory response and destruction of joint bone and cartilage in RA patients. The proliferation of synovial membranes is mainly caused by the abnormal proliferation of FLS. Several inflammatory cytokines are released in synovial tissues, such as TNF-α, IL-1β, and IL-6, which in turn promoting abnormal apoptosis of RA-FLS. However, there are no approved drugs that are known to target FLS in RA, and the underlying mechanisms driving FLS activation remain unresolved. Therefore, RA-FLS have been widely used for modelling RA in previous studies [24,25,26]. Notably, to our knowledge, our study is the first to demonstrate that lixisenatide can exert these effects in human FLSs. Additionally, we show that lixisenatide can ameliorate degradation of the articular extracellular matrix by downregulating the expression of MMP-1, MMP-3 and MMP-13, the three major enzymes responsible for degradation of type II collagen. In normal physiology, MMPs play important roles in a variety of processes including regular cell turnover in cartilage, but in RA, large amounts of MMPs are released by FLSs in response to oxidative stress and proinflammatory cytokines, thereby resulting in extensive and irreversible cartilage degradation [22]. To explore the effects of lixisenatide on activation of proinflammatory signaling pathways, we tested for activation of the JNK, AP-1 and NF-κB molecular pathways, all of which have been shown to be involved in the pathogenesis of RA [27,28,29]. Here, our findings indicate that agonism of GLP-1 by lixisenatide downregulated activation of all three of these important pathways, thereby suggesting that GLP-1 may serve as a valuable upstream target for regulation of the

inflammatory response in RA.

In conclusion, our findings show that GLP-1 may serve as a novel therapeutic target in RA and provide ample evidence to support further investigation into the potential benefit of lixisenatide in the treatment and prevention of chronic inflammatory disease including RA.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105732.

Declaration of Competing Interest

None of the authors of this work have any conflicts of interest that require disclosure.

Acknowledgments

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