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Chondroprotective Effect of Semaglutide in Osteoarthritis: Association with Macrophage Polarization

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Abstract

Objective: To investigate the chondroprotective effect of semaglutide in osteoarthritis (OA) and to observe whether this effect is associated with the regulation of synovial macrophage M1/M2 polarization. **Methods:** *In vivo*, a mouse OA model was established by destabilization of the medial meniscus (DMM) in C57BL/6J mice. Semaglutide (200 ng/mL) was administered via intraperitoneal (IP-Sema) or intra-articular (IA-Sema) injection. Synovial inflammation was evaluated by H&E staining (Krenn score), and cartilage degeneration was assessed by Safranin O-fast green staining (OARSI score). Immunohistochemistry was used to detect the expression of the M1 marker CD86 and the M2 marker CD206 in synovial tissues. *In vitro*, RAW264.7 macrophages were stimulated with LPS/IFN-γ to induce M1 polarization, followed by semaglutide treatment. Immunofluorescence staining was performed to assess the expression of CD86 and CD206. **Results:** Compared with the OA model group, semaglutide-treated groups exhibited significantly reduced synovial inflammation (decreased Krenn score) and delayed cartilage degeneration (decreased OARSI score), with the intra-articular injection showing superior efficacy. Immunohistochemistry revealed that semaglutide markedly decreased the number of CD86-positive cells (M1) and increased CD206-positive cells (M2) in synovial tissues. *In vitro* experiments confirmed that semaglutide directly inhibited LPS/IFN-γ-induced CD86 expression and promoted CD206 expression, indicating a shift from M1 to M2 macrophage phenotype. **Conclusion:** Semaglutide alleviates synovial inflammation and protects cartilage matrix in conjunction with an M1-to-M2 polarization shift. This study provides a new theoretical basis for the potential use of GLP-1 receptor agonists as a therapeutic option for OA.

Keywords

Osteoarthritis, Semaglutide, Macrophages, M1-M2 Polarization, Cartilage Repair

1. Introduction

Osteoarthritis (OA) is the most common arthritis [1], affecting more than 500 million people worldwide [2], and is projected to cause a "tsunami" of cases for healthcare systems by 2050. One-fifth of the general population and one-third of people over 50 years of age suffer from this disease [3] [4]. OA can be broadly divided into two categories: primary OA and secondary OA [5]. Typical OA presents with joint pain and loss of function; however, the disease is highly variable clinically, with considerable discordance between structural changes and symptoms [6]. OA can range from an asymptomatic incidental finding to a devastating, permanently disabling disease [5]. For many years, OA was described as a non-inflammatory "wear-and-tear" disease because it was considered a slow-burning condition that usually develops over a long period due to natural "wear and tear" of synovial joints. However, "wear and tear" is insufficient to describe all inflammation-driven disease mechanisms. OA, previously considered a disease of cartilage, is now recognized as a complex multi-tissue pathology. A modern understanding of OA also recognizes that it is not merely a disease of articular cartilage but a disease of the entire joint as an organ and with systemic manifestations, involving complex interactions of various biological processes, including chronic low-grade inflammation, abnormal joint mechanics, and alterations in the extracellular matrix, with low-grade chronic inflammation playing a central role [7]. Therefore, modern OA management focuses on multimodal approaches, including lifestyle modification, physical therapy, pharmacological interventions, and surgical procedures. Current treatment goals aim to control symptoms, improve joint function, and prevent further joint damage. Future therapies aim to modify the disease, promote reparative responses, and provide long-term joint protection, thereby inhibiting the initiation and progression of OA as much as possible and alleviating the burden on families and society.

Macrophages are among the most abundant immune cells in the inflamed synovium of OA patients and are considered important contributors to cytokine production in OA [8]. Macrophages in OA joints polarize into M1 or M2 phenotypes upon stimulation by inflammatory cytokines [9]. Pro-inflammatory cytokines, such as TNF-α, IL-1β, and IFN-γ, enhance M1 polarization and lead to the expression of inflammatory cytokines, subchondral bone remodeling, and osteoclastogenesis in OA [9]-[11]. Synovial macrophages and macrophage-derived mediators play a major role in the inflammatory and destructive responses in OA [7]. Depending on their origin, function, and characteristic molecules produced during regeneration, activated macrophages may polarize into M1 pro-inflammatory cells or M2 anti-inflammatory/resolutive cells. M1 macrophages are characterized by high production of pro-inflammatory cytokines and chemokines, such as TNF-*α*, IL-1*β*, and IL-6 [12]. As recently reported by Lee *et al.* [13], M1 polarization of synovial macrophages exacerbates experimental OA, suggesting that reprogramming macrophages away from the M1 phenotype is a potential strategy for OA treatment. In contrast, M2 macrophages are characterized by the secretion of vascular endothelial growth factor (VEGF), transforming growth factor-*β* (TGF-*β*), and arginase. Moreover, reprogramming of macrophages from M1 to M2 has been shown to be feasible in multiple tissue repair studies. Anti-inflammatory cytokines such as IL-4 and IL-13 promote M2 polarization and have tissue-repairing functions. These findings indicate that controlling M2 polarization should be a key target for anti-OA therapy.

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by the gut upon meal ingestion that triggers insulin production by the pancreas. GLP-1 has a range of extrapancreatic functions related to its anti-inflammatory properties [14]-[16]. Currently, eight injectable GLP-1 receptor agonists (GLP-1RAs) are available, including semaglutide, exenatide, exenatide extended-release, liraglutide, benaglutide, lixisenatide, dulaglutide, and polyethylene glycol loxenatide. GLP-1RAs have emerged as promising therapeutic agents with potent anti-inflammatory properties and multiple clinical implications [17]; they improve lipid metabolism, promote fat redistribution, reduce insulin resistance, and decrease intrahepatic lipid accumulation, but their mechanisms of action are not yet fully understood and require further investigation. The GLP-1 receptor (GLP-1R) is expressed in pancreatic islets as well as in several extrapancreatic organs and cell lineages, indicating that GLP-1-based drugs may exert extrapancreatic functions. GLP-1 exhibits anti-inflammatory properties in pancreatic islets and adipose tissue, contributing to glycemic control in diabetic patients [18] [19]. GLP-1 may regulate macrophage polarization toward M2, thereby exerting a protective role in the progression of coronary atherosclerosis [20]. Beyond these tissues, emerging data suggest that GLP-1-based therapies exert anti-inflammatory effects on the liver, brain, kidney, lung, testis, skin, and vascular system (including aortic and venous endothelial cells) by reducing the production of inflammatory cytokines and the infiltration of immune cells in tissues [21] [22]. Chronic inflammation in adipose tissue is closely associated with activation of the I*κ*B kinase (IKK*β*)/nuclear factor-*κ*B (NF-*κ*B) pathway and the c-Jun N-terminal kinase (JNK) pathway. Activation of these pathways promotes the expression of pro-inflammatory cytokines, cell adhesion molecules, and inflammatory chemokines, as well as the recruitment and infiltration of monocytes/macrophages. For example, in spinal cord injury (SCI), during secondary injury, monocytes derived from blood and bone marrow differentiate into macrophages, are recruited to the injury site, and join the endogenous microglia in the spinal cord. In the cardiovascular system, GLP-1R may regulate macrophage polarization toward M2, thereby playing a protective role in the progression of coronary atherosclerosis. These phenomena are related to two macrophage phenotypes: "classically" activated M1 cells that participate in inflammation and tissue damage, and "alternatively" activated M2 cells that reduce inflammation and promote tissue repair. Studies have shown that liraglutide, a GLP-1 analog, can inhibit NF-κB activation and downregulate TNF-α-induced NF-κB activation [23], thereby alleviating inflammation.

Semaglutide is an approved drug, a GLP-1 receptor agonist indicated for patients with type 2 diabetes mellitus (T2DM). It has been shown to have potent anti-diabetic effects and effectively lowers blood glucose levels through multiple cellular pathways [24]. Recent evidence suggests that, like other GLP-1 receptor agonists, semaglutide [25]-[28] may provide additional benefits beyond glycemic control and can inhibit certain harmful pathways [29] [30]. Therefore, semaglutide may be beneficial for the treatment of OA due to its anti-inflammatory and anti-catabolic effects, as well as potential analgesic properties when injected intra-articularly. However, the anti-inflammatory mechanism of semaglutide is poorly understood.

Thus, the aim of this study was to investigate the analgesic and anti-inflammatory properties and effects of semaglutide on OA through *in vivo* and *in vitro* experiments. The results may provide potential therapeutic materials or targets for the treatment of OA.

2. Materials and Methods

2.1. *In Vivo* Experiments

2.1.1. Establishment of the Animal Model

Eight- to ten-week-old male C57BL/6J mice were acclimated for one week, and then an OA model was established by destabilization of the medial meniscus (DMM). Experimental groups: SHAM group, intraperitoneal PBS injection group (IP-OA), intraperitoneal semaglutide injection group (IP-Sema), intra-articular PBS injection group (IA-OA), and intra-articular semaglutide injection group (IA-Sema). Three mice per group were used for subsequent experiments. The intraperitoneal and intra-articular injection groups were designed to examine whether the route of administration significantly affected arthritis. Briefly, 8-week-old male C57BL/6J mice were anesthetized by intraperitoneal injection of 1.25% tribromoethanol (20 mg/kg). After shaving and disinfection of the right hind limb, a longitudinal incision was made along the medial patellar side to expose the joint capsule. The medial meniscotibial ligament was located and completely severed using microscissors, taking care to avoid damage to the adjacent articular cartilage. The joint capsule and skin were then sutured layer by layer. Sham-operated mice underwent the same surgical procedure without severing the medial meniscotibial ligament. All mice received intramuscular penicillin (40,000 U/kg) for three consecutive days postoperatively to prevent infection. Animals were randomly assigned to experimental and control groups. Mice were euthanized at week 6. The entire knee joint was excised and fixed in 4% paraformaldehyde (PFA) at 4˚C for 72 hours, followed by decalcification in 14% EDTA solution (pH

7.4). The decalcification solution was changed every three days. When the bone became soft upon needle puncture, the tissues were embedded in paraffin and sectioned at 5 μm thickness using a Polycut E microtome. Sections were mounted on slides, baked at 60˚C for 2 hours, deparaffinized, rehydrated, and used for subsequent analyses.

2.1.2. Immunofluorescence

Eight-week-old male C57BL/6J mice were randomly divided into the SHAM group, the intraperitoneal injection group (200 ng/mL), and the intra-articular injection group (200 ng/mL). Paraffin sections were placed in citrate buffer (pH 6.0) and heated in a 95˚C water bath for 20 minutes for antigen retrieval. For permeabilization and blocking, sections were incubated with PBS containing 0.2% - 0.5% Triton X-100 for 10 - 20 minutes at room temperature, washed with PBS, and then blocked with PBS containing 5% normal goat serum (or 5% BSA) and 0.1% Tween-20 for 1 hour at room temperature. Primary antibodies (CD86, CD206) diluted in blocking buffer were added and incubated overnight at 4˚C in a humidified chamber. For negative controls, a blocking buffer was used instead of the primary antibody. After washing with PBS, fluorescence-labeled secondary antibodies were added and incubated for 1 hour at room temperature in the dark. All subsequent steps were performed in the dark. After washing with PBS, sections were mounted with DAPI mounting medium, coverslipped, and sealed with nail polish. Images were acquired using a confocal laser scanning microscope. Acquisition settings (laser power, gain, pinhole) were kept consistent for all samples to allow quantitative comparison. Images were analyzed using ImageJ software.

2.1.3. Histological Analysis

After deparaffinization and rehydration, tissue morphology was assessed by Safranin O-fast green staining and hematoxylin and eosin (H&E) staining to evaluate cartilage degeneration and chronic synovitis according to the OARSI score and Krenn score. Scores were assigned blindly by two different examiners.

2.2. *In Vitro* Experiments

2.2.1. Cell Preparation

The mouse monocyte/macrophage cell line RAW 264.7 was cultured in DMEM high-glucose medium (4.5 g/L glucose) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were routinely passaged in a humidified incubator at 37˚C with 5% CO_2. After reaching optimal growth, M0 macrophages were induced to polarize to the M2 phenotype by adding 20 ng/mL IL-4 for 24 hours. M1 macrophages were induced by adding 20 ng/mL IFN-γ and 100 ng/mL LPS for 24 hours. This macrophage cell line was used for all experiments in this study.

2.2.2. Immunofluorescence

RAW 264.7 mouse macrophages were cultured in complete medium (DMEM high glucose + 10% fetal bovine serum + 1% penicillin/streptomycin). When cells in culture flasks reached 70% confluence, they were passaged into six-well plates. After drug treatment, cells were fixed with 4% paraformaldehyde for 30 minutes, then permeabilized with Tris buffer containing 0.05% Tween 20 for 10 minutes, washed three times with PBS (5 minutes each), and blocked with 2.5% normal fetal bovine serum for 30 minutes. Cells were then incubated with primary antibodies (iNOS, CD86, CD206) overnight at 4˚C, washed three times with PBS (5 minutes each), and then incubated with secondary antibodies for 1 hour at room temperature, followed by three PBS washes (5 minutes each). After mounting with DAPI-containing mounting medium, the cells were observed under a microscope. Images were captured using a confocal microscope and analyzed with ImageJ software.

2.2.3. Immunohistochemical Staining and Quantitative Analysis of Synovial CD86 and CD206

Synovial tissues were fixed in 4% paraformaldehyde for 24 hours and then routinely embedded in paraffin. Sections of 4 - 5 μm thickness were prepared. After deparaffinization and rehydration, antigen retrieval was performed using 10 mM sodium citrate buffer (pH 6.0) in a microwave oven: the buffer was heated to boiling and then maintained at medium-low power for 10 - 15 minutes, followed by natural cooling to room temperature. Endogenous peroxidase activity was blocked by incubation with 3% H_2O_2 for 10 minutes at room temperature, followed by blocking with 5% normal goat serum for 1 hour at room temperature. Primary antibodies used were rabbit anti-CD86 monoclonal antibody (clone E5W6H, CST, 1:500) and rabbit anti-CD206 polyclonal antibody (Abcam, 1:100), both incubated overnight at 4˚C. The next day, sections were incubated with HRP-labeled goat anti-rabbit secondary antibody for 30 minutes at room temperature, developed with DAB, counterstained with hematoxylin, dehydrated, and mounted. Positive controls (known positive tissues) and negative controls (using isotype IgG instead of the primary antibody) were included in each staining run.

Under a microscope, five high-power fields (HPF, 400×) were randomly selected per section, avoiding tissue edges, folds, and necrotic areas, with focus on the synovial lining layer and the underlying inflammatory infiltrate. CD86- or CD206-positive cells showed brownish-yellow staining of the membrane or cytoplasm. Semi-quantitative analysis was performed by directly counting the number of positive cells and total cells (hematoxylin-stained nuclei) in each field to calculate the percentage of positive cells (%). The average of five fields per sample was used for subsequent statistical analysis, and the results were averaged.

2.3. Statistical Analysis

Statistical analysis was performed using SPSS 24.0 software. All quantitative data are expressed as mean ± standard deviation. Comparisons between two groups were made using independent samples t-tests. For comparisons among multiple groups, the Shapiro-Wilk test was first used to assess normality, followed by one-way ANOVA or Kruskal-Wallis rank sum test, depending on the homogeneity of variance. A P value < 0.05 was considered statistically significant.

3. Results

3.1. *In Vivo* Experiments

3.1.1. Semaglutide Treatment Inhibits Inflammatory Response and Delays Osteoarthritis Progression

H&E staining was used to evaluate synovial inflammation in the knee joints of experimental animals. As shown in Figure 1(A), the sham-operated group showed normal synovial tissue morphology with a thin lining layer (1 - 2 cell layers) and only scattered inflammatory cell infiltration in the sublining stroma. In contrast, the OA model group exhibited marked synovial hyperplasia, thickening of the lining layer to 5 - 7 cell layers, dense inflammatory cell infiltration, and increased vascularity, indicating severe synovitis.

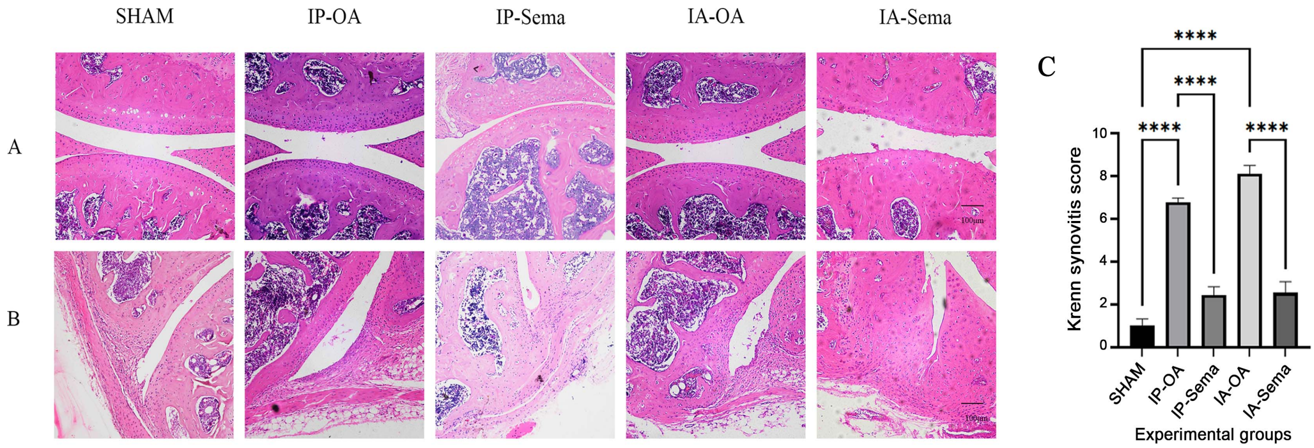

Figure 1. Semaglutide ameliorates synovial inflammation in osteoarthritic mice (H&E staining of knee joint sections from mice in each group (Sham, IP-OA, IP-Sema, IA-OA, IA-Sema); scale bars = 200 μm and 50 μm. The sham group showed a thin synovial lining layer (1 - 2 cell layers) and a smooth cartilage surface. The OA model group exhibited obvious synovial hyperplasia, inflammatory cell infiltration, and cartilage surface erosion. After semaglutide intervention, especially in the intra-articular injection group, synovial thickening and inflammatory infiltration were significantly reduced, and cartilage structure was well preserved. These results indicate that semaglutide has a protective effect on OA joint pathology).

Notably, semaglutide treatment significantly ameliorated these pathological changes. Compared with the OA model group, semaglutide-treated mice showed reduced lining layer thickness, decreased inflammatory cell infiltration, and lower vascular density (Figure 1(B)). Quantitative analysis using the Krenn synovitis scoring system showed that the total score of the OA model group was significantly higher than that of the sham group ($P < 0.01$; Figure 1(C)). Semaglutide effectively reduced the synovitis score, with statistically significant differences between the semaglutide groups and the OA model group ($P < 0.01$). Specifically, all three components of the synovitis score (lining layer hyperplasia, inflammatory cell infiltration, and vascular density) were reduced after semaglutide treatment. These results indicate that semaglutide effectively reduces synovial inflammation in OA joints.

3.1.2. Semaglutide Promotes Cartilage Repair in Osteoarthritic Joints

Safranin O-fast green staining was performed for histological analysis of knee joint sections from each group. As shown in **Figure 2(A)** and **Figure 2(B)**, the sham group showed a smooth cartilage surface and strong positive Safranin O staining (red), indicating abundant proteoglycan content. In contrast, the OA model group showed severe cartilage erosion, surface fibrillation, and marked loss of Safranin O staining, especially in the superficial and middle zones. Notably, semaglutide treatment alleviated these pathological changes. The intra-articular semaglutide group (200 ng/mL) exhibited better-preserved cartilage structure and significantly higher Safranin O staining intensity than the OA model group.

Quantitative analysis using the OARSI scoring system showed that the OA model group had a significantly higher score than the sham group ($P < 0.01$; **Figure 2(C)**). Semaglutide effectively reduced the OARSI score, with both the intra-articular and intraperitoneal injection groups showing statistically significant differences compared with the OA model group ($P < 0.01$). Thus, semaglutide effectively promotes cartilage repair in OA joints by protecting proteoglycan content.

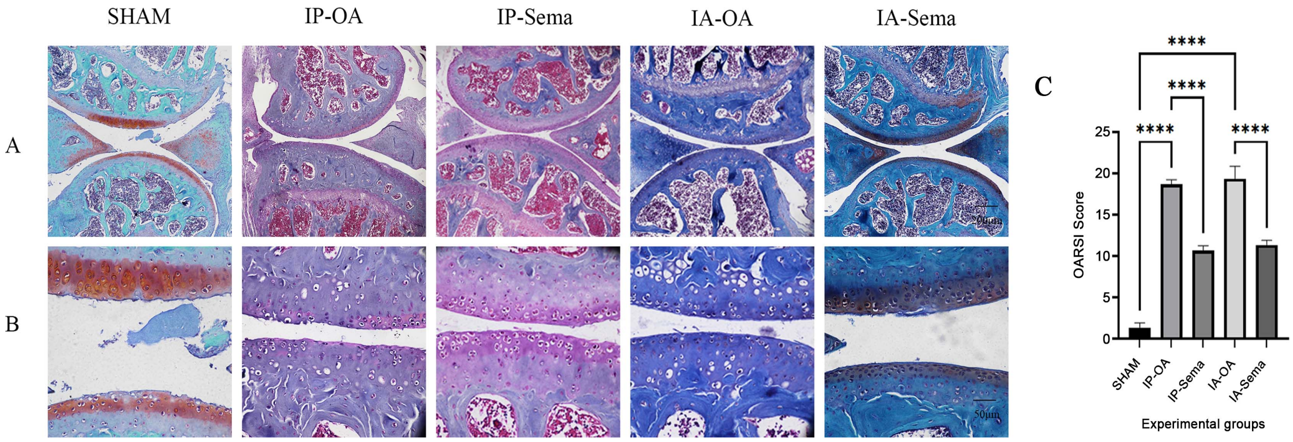

Figure 2. Semaglutide alleviates cartilage matrix loss in osteoarthritic mice (**Figure 2(A)** and **Figure 2(B)** show Safranin O-fast green staining of articular cartilage from mice in each group (Sham, IP-OA, IP-Sema, IA-OA, IA-Sema) at 100× and 400× magnification, respectively. Scale bars = 200 μm (upper row) and 50 μm (lower row). Representative images show that the sham group had well-preserved cartilage proteoglycan (red staining); the OA model group showed marked proteoglycan loss, surface erosion, and structural disruption. Notably, semaglutide intervention, especially intra-articular administration, significantly restored Safranin O staining intensity and maintained cartilage structural integrity. Quantitative analysis using the OARSI score further confirmed that cartilage degeneration scores were significantly reduced in semaglutide-treated groups compared with the model group).

3.2. *In Vitro* Experiments

3.2.1. Semaglutide Promotes M2 Polarization and Inhibits M1 Polarization

To further investigate the direct effect of semaglutide on macrophage polarization, we performed immunofluorescence staining for the M1 marker CD86 and the M2 marker CD206 in LPS/IFN-γ-stimulated RAW264.7 macrophages. As shown in **Figure 3(A)**, unstimulated control cells showed weak staining for both M1 and M2 markers. LPS/IFN-γ stimulation significantly increased the fluorescence intensity of the M1 marker CD86 (green), indicating successful induction of M1 polarization.

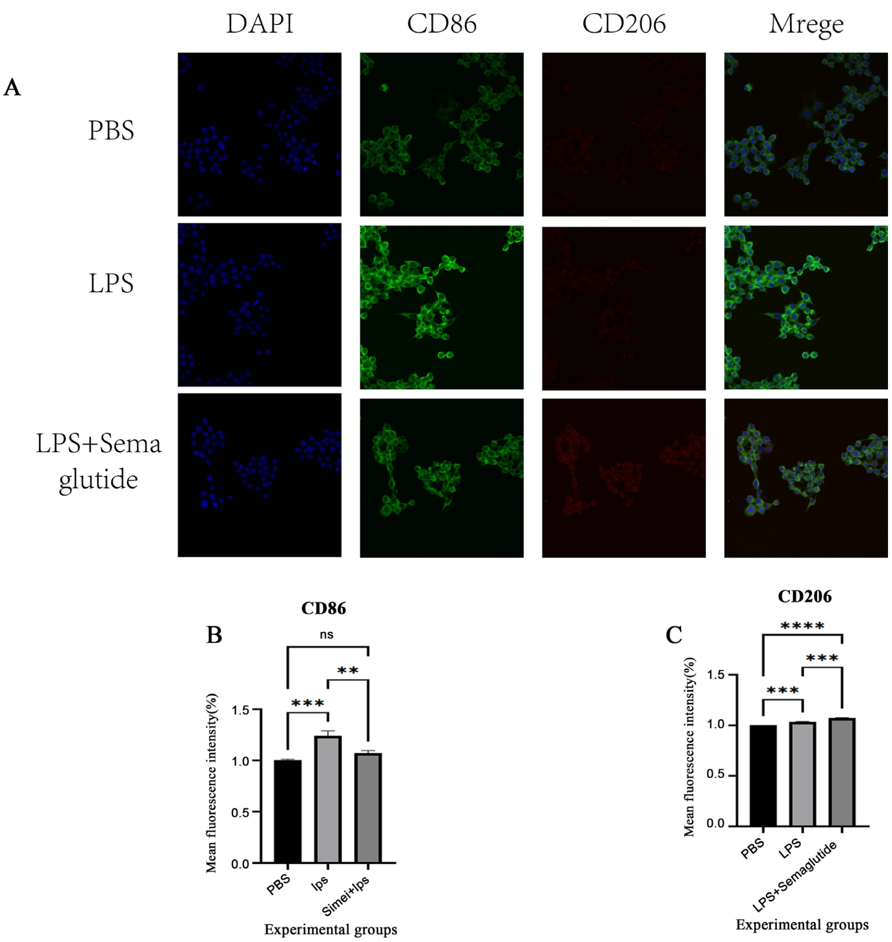

Figure 3. Semaglutide modulates M1/M2 polarization in RAW264.7 macrophages (RAW264.7 macrophages were treated with PBS (control), LPS/IFN-γ (to induce M1 polarization), LPS/IFN-γ + semaglutide (200 ng/mL), IL-4 (to induce M2 polarization), or IL-4 + semaglutide for 24 hours. (A) Representative immunofluorescence images of CD86 (green, M1 marker) and CD206 (red, M2 marker). Nuclei were counterstained with DAPI (blue). Scale bar = 50 μm. (B) (C) Quantitative analysis of mean fluorescence intensity for CD86 and CD206, respectively. Data are presented as mean ± SD (n = 3). ***$P < 0.001$ vs. PBS group; **$P < 0.001$ vs. LPS/IFN-γ group; ****$P < 0.0001$ vs. IL-4 group).

However, semaglutide treatment significantly reduced the fluorescence intensity of CD86 compared with the LPS/IFN-γ group. Quantitative analysis of mean fluorescence intensity confirmed that semaglutide reduced CD86 expression by approximately 25% compared with the LPS/IFN-γ group ($P < 0.001$; Figure 3(B)). Conversely, immunofluorescence staining for the M2 marker CD206 showed that semaglutide treatment enhanced CD206 expression in LPS/IFN-γ-stimulated RAW264.7 cells (Figure 3(A)). The mean fluorescence intensity of CD206 was increased by approximately 5% in the semaglutide-treated group compared with the LPS/IFN-γ alone group ($P < 0.001$; Figure 3(C)).

Merged images showed that semaglutide treatment shifted the macrophage population from an M1-dominant (iNOS$^+$/CD86$^+$) to an M2-dominant (CD206$^+$) phenotype. These results indicate that semaglutide directly promotes M2 polarization and inhibits M1 polarization in RAW264.7 macrophages (without verification of GLP-1R dependence), providing cell-level evidence for its anti-inflammatory effect in OA.

3.2.2. Semaglutide Promotes M2 Polarization and Inhibits M1 Polarization in RAW264.7 Macrophages

To explore the regulatory effect of semaglutide on synovial macrophage polarization, we performed immunohistochemical staining for the M1 marker CD86 and the M2 marker CD206 in synovial tissues of mice from each group.

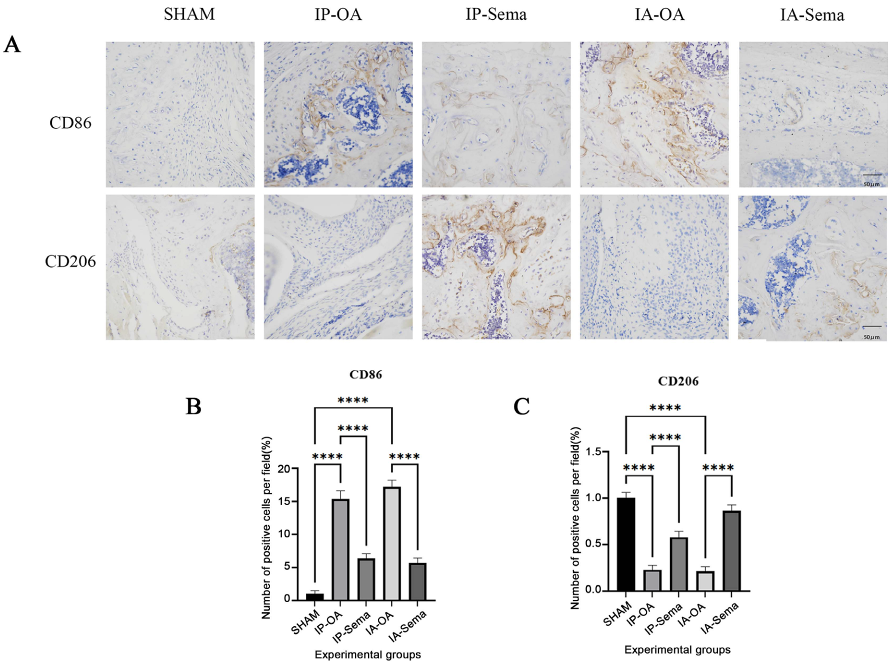

Figure 4. Semaglutide regulates M1/M2 polarization of synovial macrophages in OA mice (Immunohistochemical staining for the M1 marker CD86 and the M2 marker CD206 in synovial tissues (Sham, IP-OA, IP-Sema, IA-OA, IA-Sema groups). (A) Representative images show that the OA group had increased CD86-positive cells and decreased CD206-positive cells, and semaglutide intervention reversed these changes, with the IA-Sema group showing the most pronounced effect. (B) (C) Quantitative analysis of the percentage of positive cells per field. Data are presented as mean ± SD (n = 3 per group). $^*P < 0.05$, $^{**}P < 0.01$ vs. corresponding OA model group. Scale bar = 50 μm).

Immunohistochemical staining results (Figure 4) showed that in the sham group, the proportion of CD86-positive cells (M1 macrophage marker) in synovial tissues was approximately 20%, and the proportion of CD206-positive cells (M2 macrophage marker) was approximately 1.0%. Compared with the sham group, the intraperitoneal OA model group (IP-OA) and intra-articular OA model group (IA-OA) showed reductions in CD86-positive cell proportion to approximately 10% and 15%, respectively, while CD206-positive cell proportions increased to approximately 10% and 12%, respectively, indicating that OA induction resulted in a predominance of M1 polarization and suppression of M2 polarization in synovial macrophages.

After semaglutide treatment, this trend was reversed. In the intraperitoneal semaglutide group (IP-Sema), the proportion of CD86-positive cells decreased to approximately 10% compared with the IP-OA group, and the proportion of CD206-positive cells increased to approximately 0.5%. In the intra-articular semaglutide group (IA-Sema), the proportion of CD86-positive cells further decreased to approximately 4%, and the proportion of CD206-positive cells returned to approximately 1.0%, close to the level of the sham group.

Thus, semaglutide effectively inhibits the infiltration of M1 macrophages and promotes the polarization of M2 macrophages in synovial tissues, thereby correcting the OA-induced imbalance of the synovial immune microenvironment. Among the routes of administration, intra-articular injection showed superior efficacy in restoring the number of M2 macrophages.

4. Discussion

Osteoarthritis (OA), as a degenerative disease involving the whole joint, is characterized not only by progressive cartilage loss but also by chronic low-grade synovial inflammation. For a long time, clinical interventions for OA have focused mainly on symptom control, and there is a lack of treatments that can effectively slow disease progression. In recent years, glucagon-like peptide-1 receptor agonists (GLP-1RAs) have attracted widespread attention for their anti-inflammatory effects beyond glycemic regulation. In this study, we applied semaglutide, a representative GLP-1RA, to an OA model for the first time and systematically investigated its effects on cartilage protection and immunomodulation. The results showed that semaglutide significantly reduced synovial inflammation and delayed cartilage degradation, and these effects were associated with a shift of macrophages from the M1 to the M2 phenotype.

Synovial inflammation plays a key role in the initiation and progression of OA, especially in the early stages, where activated synovial macrophages release large amounts of inflammatory mediators that further exacerbate cartilage destruction. In this study, after semaglutide treatment, synovial lining layer thickening, inflammatory cell infiltration, and vascular proliferation in OA mice were significantly ameliorated, and the Krenn score was markedly decreased. This finding is consistent with the anti-inflammatory effects of GLP-1RAs observed in other tissues (e.g., adipose tissue, liver, blood vessels), suggesting that semaglutide may improve the intra-articular microenvironment through local or systemic anti-inflammatory mechanisms.

More importantly, this study revealed for the first time in the OA context the association of semaglutide with macrophage polarization. *In vivo* immunofluorescence staining showed that semaglutide treatment significantly reduced the expression of the M1 marker (CD86) and increased the level of the M2 marker

(CD206) in synovial tissues. *In vitro* experiments further validated these observations: in LPS/IFN-γ-stimulated RAW264.7 macrophages, semaglutide treatment significantly suppressed M1 polarization and enhanced CD206 expression. These results suggest that the effects of semaglutide are associated with a shift of macrophages from a pro-inflammatory to an anti-inflammatory phenotype, indicating a direct action on macrophages (receptor dependence remains to be verified).

Imbalance of macrophage polarization is considered an important mechanism for persistent inflammation and cartilage destruction in OA. Pro-inflammatory factors secreted by M1 macrophages, such as TNF-α and IL-1β, induce catabolic responses in chondrocytes and promote cartilage degradation, whereas M2 macrophages participate in tissue repair by releasing IL-10, TGF-β, and other factors. Therefore, inducing M2 polarization has become a hotspot in OA therapeutic research. Our results are consistent with the report [13] and further support the potential of "macrophage reprogramming" in OA treatment. Of note, it has been shown that GLP-1R is expressed on the surface of macrophages [19], and GLP-1 can alleviate inflammatory responses by inhibiting the NF-κB signaling pathway [23]. NF-κB is a key transcription factor for M1 polarization, whereas STAT6, PPAR-γ, and other pathways are involved in the regulation of M2 polarization. Therefore, it is reasonable to speculate that semaglutide may block M1 polarization by inhibiting the NF-κB pathway and simultaneously activating STAT6 or PPAR-γ pathways to promote M2 polarization; this hypothesis warrants further investigation.

In addition, we observed a direct protective effect of semaglutide on cartilage. In the OA model, semaglutide treatment significantly improved cartilage structural integrity, preserved proteoglycan content, and markedly reduced OARSI scores. This effect may be partly attributed to indirect protection resulting from reduced inflammation and may also be related to the expression of GLP-1R on chondrocytes themselves. Previous studies have shown that GLP-1RAs have anti-apoptotic and antioxidant effects in various cell types, such as cardiomyocytes and neurons [26] [30], suggesting that semaglutide may also directly act on chondrocytes to inhibit catabolism. Further studies using chondrocyte-specific GLP-1R knockout mice or *in vitro* chondrocyte culture models are needed to validate this hypothesis.

5. Limitations and Future Directions

Although this study provides relatively robust *in vivo* and *in vitro* evidence, several limitations should be acknowledged. First, the RAW264.7 cell line is widely used in macrophage polarization studies, but it may differ functionally from primary macrophages. Future studies should use mouse bone marrow-derived macrophages or human primary macrophages for validation. Second, this study only observed the short-term efficacy of semaglutide in the OA model; its long-term safety, optimal route of administration, and dosage need systematic evaluation. Third, the pathological process of OA involves multiple cell types, including fibroblast-like synoviocytes, chondrocytes, and osteoclasts; whether semaglutide also affects the function of these cells requires further investigation. Finally, although the GLP-1R signaling pathway is thought to play a key role in regulating macrophage polarization, this study did not use GLP-1R antagonists or knockout models for reverse validation, and this mechanism needs to be clarified in future studies.

In summary, semaglutide is associated with a shift of macrophages from the M1 to the M2 phenotype, alleviates synovial inflammation, and protects cartilage matrix, thereby delaying OA progression. This finding provides a new theoretical basis for semaglutide as a potential therapeutic agent for OA and opens a new direction for immunomodulatory intervention strategies in OA. Future clinical translational studies are needed to further evaluate the efficacy and safety of semaglutide in OA patients, which will provide stronger evidence for its clinical application.

6. Conclusions

1) Semaglutide inhibits the M1 pro-inflammatory phenotype and promotes the M2 anti-inflammatory phenotype by regulating the polarization balance of synovial macrophages M1/M2, thereby reducing synovial inflammation and protecting the cartilage matrix.

2) *In vitro* experiments confirmed that semaglutide directly acts on RAW264.7 macrophages to inhibit LPS/IFN-γ-induced M1 polarization and promote M2 polarization, providing direct evidence for its immunomodulatory role in OA treatment and suggesting that macrophage phenotype regulation is a potential therapeutic target.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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