Empagliflozin ameliorated chondrocytes inflammation, catabolism and senescence and osteoarthritis via suppressing the nuclear factor kappa-B signal pathway.

Bin Ru[1], Zhonggai Chen[2], Langhai Xu[1], Shun Li[1], and Wenjun Cai[1]

[1]Zhejiang Provincial People's Hospital
[2]Yuying Children's Hospital of Wenzhou Medical College

January 30, 2024

Abstract

Background Extracellular matrix (ECM) degradation, chondrocyte inflammation, and cellular senescence contribute to the pathology of osteoarthritis (OA). Empagliflozin, a selective inhibitor of sodium-glucose cotransporter-2 (SGLT2), has been reported to show the anti-inflammatory properties in several conditions. However, whether empagliflozin can be used to improve OA is still unknown. Methods The protective effects and underlying mechanism of empagliflozin in OA were investigated in vitro and in vivo. Cell viability, catabolic markers, inflammatory mediators, cellular senescence level, cartilage degeneration were evaluated by CCK-8 assay, safranine O staining, ELISA, real-time PCR, western blot, β-galactosidase Staining, and histological analysis. Results We found that empagliflozin significantly downregulated the expression of catabolic enzymes (MMP9 and MMP13), and decreased the expression of inflammatory mediators (NO, PGE2, IL-6, COX2, and INOS), and reduced the cellular senescence level in IL-1β-treated chondrocytes by inhibiting the nuclear factor kappa-B (NF-ϰB) signaling pathway. What's more, empagliflozin prevented cartilage degeneration in DMM-induced OA mice model with significant lower OARSI grade. Conclusion Our findings reveal that empagliflozin inhibited chondrocytes ECM degradation, inflammation and cellular senescence in vitro and prevented cartilage degeneration in vivo by suppressing NF-ϰB signal pathway, indicating a therapeutic potential in OA treatment.

Empagliflozin ameliorated chondrocytes inflammation, catabolism and senescence and osteoarthritis via suppressing the nuclear factor kappa-B signal pathway.

Bin Ru[1#], Zhonggai Chen[2#], Langhai Xu[1#], Shun Li[1*], Wenjun Cai[1*]

[1]Department of Pain, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, China.

[2]The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China.

[#] Bin Ru, Zhonggai Chen, Langhai Xu contributed equally to this work

[*] Correspondence to:

Wenjun Cai (caiwj0302@163.com) and Shun Li (1148449287@qq.com), Department of Pain, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, China.

Contributions: Wenjun Cai & Shun Li: Conceptualization, Methodology. Bin Ru: Project administration, Investigation. Zhonggai Chen: Writing Original Draft, Data Curation. Langhai Xu: Acquisition, Analysis and interpretation of data.

ACKNOWLEDGMENTS: This study was supported by grants from the Department of Education of Zhejiang Province (NO. Y202044592), the Natural Science Foundation of Zhejiang Province (NO. LGF20H310004) and the National Natural Science Foundation of China (NO. 81901122).

CONFLICT OF INTERESTS: The authors declare that there is no conflict of interests.

DATA AVAILABILITY STATEMENT: The raw data of this study are available from the corresponding author upon reasonable request.

Abstract

Background

Extracellular matrix (ECM) degradation, chondrocyte inflammation, and cellular senescence contribute to the pathology of osteoarthritis (OA). Empagliflozin, a selective inhibitor of sodium-glucose cotransporter-2 (SGLT2), has been reported to show the anti-inflammatory properties in several conditions. However, whether empagliflozin can be used to improve OA is still unknown.

Methods

The protective effects and underlying mechanism of empagliflozin in OA were investigated in vitro and in vivo. Cell viability, catabolic markers, inflammatory mediators, cellular senescence level, cartilage degeneration were evaluated by CCK-8 assay, safranine O staining, ELISA, real-time PCR, western blot, β-galactosidase Staining, and histological analysis.

Results

We found that empagliflozin significantly downregulated the expression of catabolic enzymes (MMP9 and MMP13), and decreased the expression of inflammatory mediators (NO, PGE2, IL-6, COX2, and INOS), and reduced the cellular senescence level in IL-1β-treated chondrocytes by inhibiting the nuclear factor kappa-B (NF-ϰB) signaling pathway. What's more, empagliflozin prevented cartilage degeneration in DMM-induced OA mice model with significant lower OARSI grade.

Conclusion

Our findings reveal that empagliflozin inhibited chondrocytes ECM degradation, inflammation and cellular senescence in vitro and prevented cartilage degeneration in vivo by suppressing NF-ϰB signal pathway, indicating a therapeutic potential in OA treatment.

Keywords: empagliflozin; chondrocyte; inflammation; senescence; NF-ϰB.

1 Introduction

Osteoarthritis (OA) is a common joint disease with high rate of incidence and wide coverage in the elderly population, which causes a severe economic burden on families and society [1,2]. Current research has shown that OA is characterized by cartilage degradation, underlying bone remodeling, and synovial inflammation[3]. Several risk factors, such as age, gender, obesity and genetics, may affect the development of OA [2,4]. Nevertheless, the underlying molecular mechanism among OA remains obscure.

Increasing evidence suggests that low-grade inflammation is a key mediator of the pathogenesis of OA [5]. Chondrocytes, as the only cellular component of cartilage, play a central role in the synthesis of cartilage matrix. OA is defined as the accumulation of degenerative factors leading to the decease of chondrocytes and the degradation of extracellular matrix (ECM). During OA, pro-inflammatory factors, such as IL-1β, TNF-α, trigger a series of pathogenic reactions of chondrocytes, promoting inflammation, senescence, and apoptosis[6]. Current treatment is aimed at relieving the symptoms of OA but cannot slow down disease progression. Therefore, it is necessary to further facilitate identification of emerging pharmaceutical therapies.

Empagliflozin (Empa), a selective inhibitor of sodium-glucose cotransporter-2 (SGLT2), lowers blood sugar by reducing glucose reabsorption in the renal tubules. Various studies showed favorable effects of Empa

on glycaemic improvement, body weight, blood pressure, arterial stiffness, and endothelial function [7-9]. In recent years, Empa has been reported to regulate macrophage polarity and exert anti-inflammatory effects [10]. Also, Empa has hepatic protective effect against cholestatic liver injury via its anti-oxidant and anti-inflammatory properties [11]. However, the effect of Empa on inflammation and senescence in OA has not been reported yet

In our study, we hypothesized that Empa might inhibit inflammation in chondrocytes during the process of OA. We concentrated on the anti-inflammatory effect of Empa on chondrocytes and the underlying mechanism. We hope that our study will provide a new therapeutic approach for the treatment of OA in the future.

2 Materials and methods

Reagents

Recombinant mouse IL-1β was purchased from R&D Systems, UK. Empagliflozin was purchased from MedChemExpress, China. Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin, fetal bovine serum (FBS), and 0.5% trypsin were obtained from Gibco BRL, Grand Island, NY, USA. Collagenase type II was purchased from Sigma-Aldrich, St, Louis, MO, USA.

Chondrocyte isolation and culture

Primary chondrocytes were obtained from 5-day-old C57BL/6 mice as previously described [12]. Briefly, the articular cartilage from the knee joint of mice was cut into 1 mm^3 particles and then was digested with DMEM containing 1% penicillin/streptomycin, 0.5% trypsin, and 1% Collagenase type II for 2 h. After filtered through a 40 μm cell strainer, the digestive suspensions were placed in a centrifuge at 1500 rpm. The centrifuged cell deposits were resuspended and grown in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C with 5% CO_2. The cells were passaged to passage 3 and then used for the following experiments.

Cell viability assay

5 x 10^3 cells were seeded in 96-well plates and treated with different concentrations (0, 0.5, 1, and 5 μM) of empagliflozin with or without IL-1β (10 ng/ml) for 24 h. The equal volume of vehicle was used in the control group. To analyze cytotoxicity of empagliflozin on chondrocytes, CCK-8 assays (MCE, HY-K0301) were conducted according to the manufacturer's instruction. The optical density (OD) value was read at a wavelength of 450 nm.

Safranine O staining

Chondrocytes were incubated with different concentrations (0, 1, and 5 μM) of empagliflozin with or without IL-1β (10 ng/ml) for 24 h. Then the cells were fixed with 4% paraformaldehyde (PFA) solution for 20 min. After washing with PBS, 0.1% Safranine O dye solution were added to stain the cells for 5 min. After washing with PBS twice, cells were imaged by gross camera.

Western blot

After treatment, equal amounts of cells were lysed with RIPA for 60min. The lysates were quantified by BCA kit (Beyotime, China). The samples were then separated with SDS-polyacrylamide gels and transferred into nitrocellulose membranes. After being blocked and cut into proper sections, membranes were incubated with different primary antibodies against GAPDH (Abcam, #ab181602), MMP9 (Abcam, #ab76003), MMP13 (Abcam, #ab39012), COX2 (CST, #12282), INOS (Abcam, #ab3523), P53 (Abcam, #ab90363), P21 (Abcam, #ab215971), NF-κB P65 (CST, #8242), P-NF-κB P65 (Ser536) (CST, #3033), IκB (CST, #4812), and P-IκB (Ser32) (CST, #2859) at 4 °C overnight. The membranes were then incubated with luminesced secondary antibodies and detected with the BioRad System. GAPDH was used as control.

Real-time PCR (RT-PCR)

Chondrocytes were pretreated with empagliflozin (0, 1, and 5 μM) for 2 h, and followed by incubating with IL-1β (10 ng/ml) for 24 h. Afterward, RNAiso reagent (Takara, China) was used to extract total RNA from chondrocytes. The total RNA was reversely transcribed into cDNA with cDNA synthesis kit (Takara). SYBR Green PCR Master Mix (Takara) was used for RT-PCR with an ABI StepOnePlus System (Applied Biosystems, UK). The primers used for the RT-PCR were shown in Table 1.

Assessment of nitric oxide (NO)

The NO concentration in cell suspensions was measured with Griess reagent (Sigma, G4410). Chondrocytes were pretreated with empagliflozin at various concentrations (0, 1, and 5 μM) for 2 h and incubated with IL-1β (10 ng/ml) for 24 h, the NO levels in the cell culture medium were assessed with Griess reagent according to the manufacturer. The relative levels of NO were normalized to cell count.

ELISA

The levels of MMP9, MMP13, PGE2 and IL-6 were measured using ELISA kits (AdipoGen LIFE SCIENCES, Germany). Chondrocytes were incubated with IL-1β (10 ng/ml) for 24 h before pretreatment for 2 h with empagliflozin at various concentrations (0, 1, and 5 μM). The levels of MMP9, MMP13, PGE2 and IL-6 were detected by collecting the cell culture supernatants from each sample with ELISA kits according to the manufacturer. The relative levels were normalized to cell count.

Senescence analysis

β-galactosidase Staining assay was performed according to the manufacture's instruction. After treatment, chondrocytes were fixed for 20 min and then incubated with β-galactosidase staining buffer (Beyotime, China) at 37°C for 24h and subsequently observed under a microscope.

Animal experiments

DMM-induced OA mouse model was used in the animal experiments as previously described [13]. Thirty 12-week-old C57BL/6 mice were randomly divided into 3 groups. Mice in the OA group and the OA+Empa group were anesthetized and the medial meniscus in their knee joints was surgically removed while mice in the Sham group were anesthetized and performed a sham operation. A week after surgery, intra-articular injection of 5 μM empagliflozin was conducted once a week in the OA+Empa group, and equal volume of vehicle was conducted in the OA group and the Sham group. After 4 weeks of treatment, the mice were sacrificed and the knee samples were harvested for histological analysis. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, China.

Histological analysis

The mouse knee samples were fixed with 4% paraformaldehyde solution for 48 h and then decalcified with 0.5 M ethylenediaminetetraacetic acid (EDTA) until the tissues became soft. The samples were embedded in paraffin and cut into 5 mm sections by a microtome. The tissue sections were subsequently stained with safranin O and then observed by optical microscopy. The OARSI grading system (0–6) was used to evaluate the sections.

Statistical analysis

All data are presented as means ± SDs. One-way ANOVA with a subsequent post hoc Tukey's test was used for comparisons. The value of $p < 0.05$ was considered to indicate significant differences.

3 Results

3.1 Εφφεςτς οφ εμπαγλιφλοζιν ανδ ΙΛ-1β ον ςελλ ιαβιλιτψ ανδ ςηονδροςψτε πηενοτψπε μαιντενανςε

The effects of empagliflozin and IL-1β on cell viability and chondrocyte phenotype maintenance were evaluated by CCK-8 assay and the safranine O staining. The results demonstrated that empagliflozin had no cytotoxicity on mouse chondrocyte viability with concentration [?]5 μM at 24 h. Besides, chondrocytes

treated with 1 and 5 μM concentrations of empagliflozin could significantly improve the down-regulated cell viability caused by IL-1β (Figure 1A). Similarly, the results of safranine O staining revealed that chondrocytes were apparently less stained after 24 h IL-1β treatment, but the mouse chondrocytes treated with 1 and 5 μM of empagliflozin were observably reverse the IL-1β caused safranine O stain loss (Figure 1B). Thus, 1 and 5 μM concentrations of empagliflozin were used for the following in vitro tests, and a concentration of 5 μM was used for the following in vivo tests.

3.2 Effects of empagliflozin on IL-1β-induced ECM degradation in mouse chondrocytes

Here the expression levels of catabolic markers of ECM were detected by ELISA, RT-PCR and western blot to evaluate the effects of empagliflozin on IL-1β-induced ECM degradation in mouse chondrocytes. As shown in Figure 2, administration of empagliflozin could counteract the increments of MMP9 and MMP13 secretion level in the cell medium caused by IL-1β. In addition, pretreatment with empagliflozin significantly decreased IL-1β-induced upregulation of matrix-degrading genes (MMP9, MMP13) in a dose-dependent manner at both mRNA level and protein level. Based on the results above, empagliflozin could protect mouse chondrocytes from ECM degradation by inhibiting the MMPs.

3.3 Effects of empagliflozin on IL-1β-induced inflammation in mouse chondrocytes

To discover the anti-inflammation effects of empagliflozin on mouse chondrocytes, the expression of several inflammatory mediators in mouse chondrocytes was assessed by ELISA, RT-PCR and western blot. As illustrated in Figure 3A, the production and secretion of proinflammatory factors (NO, PGE2, and IL-6) was increased under IL-1β treatment, and empagliflozin pretreatment could dose-dependently offset these increments. Moreover, the results also showed that empagliflozin significantly decreased IL-1β-induced upregulation of other inflammatory genes (COX2 and INOS) in both mRNA and protein expression levels (Figure 3B-C). These data demonstrated that empagliflozin could significantly inhibited the production of IL-1β-induced inflammatory mediators in mouse chondrocytes.

3.4 Effects of empagliflozin on IL-1β-induced senescence in mouse chondrocytes

Senescence analysis was conducted to evaluate the effects of empagliflozin on the IL-1β-induced senescence in mouse chondrocytes by western blot and β-galactosidase Staining Assay. It was found that the levels of senescence markers (P21 and P53) were upregulated by IL-1β treatment, but this effect was largely weakened by empagliflozin (Figure 4A). Besides, β-galactosidase Staining results showed that the proportion of SA-β-Gal-positive cells obviously increased after IL-1β treatment but decreased in the presence of empagliflozin (Figure 4B). These data collectively suggested that empagliflozin exerted the protective role in IL-1β-induced cellular senescence of mouse chondrocytes.

3.5 Effects of empagliflozin on IL-1β-induced NΦ-κB pathway activation in mouse chondrocytes

To explore the underlying mechanism, the effects of empagliflozin on NF-κB pathway activation were investigated. As illustrated in Figure 5, empagliflozin activated the NF-κB pathway in mouse chondrocytes represented as higher levels of the activated status of P65 (phosphor-P65, p-P65) and IκB (phosphor-IκB, p-IκB). The above IL-1β-induced NF-κB pathway activation was inhibited by empagliflozin pretreatment. The results indicated that empagliflozin could inhibit IL-1β-induced NF-κB pathway activation in mouse chondrocytes.

3.6 Effects of empagliflozin on cartilage degeneration in mouse OA model

To investigate the anti-osteoarthritic effects of empagliflozin in vivo, DMM-induced OA mouse model was used. The articular cartilage tissue samples from mice in the OA group exhibited typical OA features with reduced abundance of chondrocytes and loss of ECM. However, 4-week intra-articular injection of 5 μM empagliflozin could improve these changes (Figure 6A). The OARSI grade of the OA+Empa group was lower than the OA group with significant difference. The data demonstrated that empagliflozin showed anti-osteoarthritic effect in mouse model.

4 Discussion

OA is a common clinical degenerative joint disease in elderly population, which is one of the main causes of chronic pain and joint disability. OA can severely reduce the quality of life of affected individuals. Current therapeutic goal of OA in clinical practice is to relieve the symptoms but cannot slow down the disease progression. Non-steroidal anti-inflammatory drugs (NSAIDs) are generally considered to be the first-line pharmacological treatments for OA. However, NSAIDs cannot achieve the long-term pain relief for OA patients, and they are associated with significant side efforts such as gastric ulceration, renal impairment, and cardiovascular accidents [14]. At the end stage of OA, a replacement surgery remains to be the only treatment option. Thus, identification of emerging pharmaceutical therapies is urgently needed in the treatment of OA. Empagliflozin, a selective inhibitor of sodium-glucose cotransporter-2 (SGLT2), has been reported to show the anti-inflammatory properties in several conditions such as cardiovascular diseases, Alzheimer's disease, liver injury, and chronic kidney disease [15-19]. In this study, we discovered the protective effect of empagliflozin on cartilage degeneration via inhibition of NF-κB pathway.

Previously thought to simply be damaged from "wear and tear", OA is now understood as a complex cell-mediated process. Current research has shown that OA is characterized by progressive cartilage degradation, but also underlying bone remodeling, osteophyte formation, and synovial inflammation [3,20]. A series of pathological factors such as ECM degradation, chondrocyte inflammation, oxidative stress, mitochondrial dysfunction, abnormal mechanical load, and senescent molecules contribute to the degeneration of chondrocytes, ultimately causing cartilage damage and OA development [21,22]. Chondrocytes, as the only cellular component of cartilage, play a central role in the balance of ECM metabolism through the synthesis of cartilage matrix. However, the inflammatory factors like IL-1β can disrupt the balance of ECM metabolism by increasing the expression of catabolic enzymes including MMPs, causing the gradual loss of ECM. MMPs inhibitors could be promising agents for the treatment of OA [23]. Our data revealed that IL-1β upregulated the expression of matrix-degrading enzyme (MMP9 and MMP13) and activated the inflammatory cascade reaction by the secretion of inflammatory cytokines (NO, PGE2, IL-6, COX2, and INOS) in mouse chondrocytes, whereas these effects could be offset by the administration of empagliflozin. These founds indicated that empagliflozin could protect mouse chondrocytes from IL-1β-induced ECM degradation and inflammatory reaction. What's more, chondrocyte senescence is also involved in the pathological process of OA. Jeon et al found that senescent cells accumulated in the articular cartilage and synovium after anterior cruciate ligament transection, and local clearance of these senescent cells attenuated the development of post-traumatic OA [24]. Therefore, the senescence analysis of chondrocytes was conducted in this study. Cellular senescence is generally regarded as a cell state characterized by an irreversible cell-cycle arrest [25]. P21 and P53 are the key factors for promoting senescence. We found that empagliflozin could counteract the elevated expression of senescence markers (P21 and P53) induced by IL-1β. Empagliflozin could also decrease the SA-β-Gal-positive cells. Thus, empagliflozin could alleviate IL-1β-induced senescence in mouse chondrocytes in vitro. All these results displayed the antidegradation role of empagliflozin on chondrocytes in vitro. The effect of empagliflozin on cartilage damage in vivo was also observed in DMM-induced OA mice model. We found that empagliflozin could also protect mouse knee cartilage from wear and matrix degeneration.

Multiple signaling pathways are involved in OA such as NF-κB, Wnt/β-catenin, HIFs, TGFβ/BMP and so on [26]. NF-κB signaling participates in many OA-associated events, including chondrocyte catabolism, survival, and inflammation [27]. NF-κB transcription factor has been considered as a disease-contributing factor of OA for a long time [27]. When NF-κB exists in the cytoplasm, it is in an inactive state and cannot enter the nucleus to play its function because it binds to the inhibitory protein IκB. Once cells are stimulated by certain inductive factors, IκB is phosphorylated and the molecular conformation of IκB changes, resulting in the activation and nuclear translocation of NF-κB[28,29]. Activation of NF-κB ,on the one hand, can directly bind to the promoters of MMPs genes to promote the expression of matrix-degrading enzyme [30]. On the other hand, activation of NF-κB regulates the transcription of many nuclear genes related to inflammation, including cyclooxygenase (COX2) and inducible nitric oxide synthase (iNOS) genes [31,32]. COX2 and iNOS can promote the production and secretion of induce prostaglandin (PGE2) and nitric oxide (NO). Increased PGE2 and NO levels can upregulate the expression of MMPs, ultimately causing cartilage degeneration.

NF-κB signaling pathway was focused in this study. Our results showed that empagliflozin inhibited the phosphorylation of IκB and P65, displaying the suppressive role on NF-κB signaling. Thus, the underlying mechanism of empagliflozin in mouse cartilage protective roles is related to the inhibition of NF-κB pathway.

However, there are still some limitations in our study. First at all, we confirmed the protective effect of empagliflozin on preventing cartilage degeneration via inhibiting the activation of NF-κB pathway. But whether NF-κB is the direct target of empagliflozin needs to be further identified. Secondly, we have only demonstrated the effects of empagliflozin on cellular inflammation and senescence in chondrocytes. The effects of empagliflozin on other aspects involving the onset and progression of OA deserve further investigation.

5 Conclusions

We demonstrated that empagliflozin inhibited chondrocytes ECM degradation, inflammation and cellular senescence in vitro and prevented cartilage degeneration in vivo by suppressing the NF-κB pathway activation. Our findings indicate a therapeutic potential of empagliflozin in OA treatment.

References

1 Roos EM,Arden NK. Strategies for the prevention of knee osteoarthritis. Nat Rev Rheumatol 2016; 12: 92-101.

2 O'Neill TW, McCabe PS,McBeth J. Update on the epidemiology, risk factors and disease outcomes of osteoarthritis. Best Pract Res Clin Rheumatol 2018; 32: 312-326.

3 Loeser RF, Goldring SR, Scanzello CR,Goldring MB. Osteoarthritis: A disease of the joint as an organ. Arthritis & Rheumatism 2012; 64: 1697-1707.

4 Palazzo C, Nguyen C, Lefevre-Colau MM, Rannou F,Poiraudeau S. Risk factors and burden of osteoarthritis. Ann Phys Rehabil Med 2016; 59: 134-138.

5 Robinson WH, Lepus CM, Wang Q, Raghu H, Mao R, Lindstrom TM,Sokolove J. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2016; 12: 580-592.

6 Liu-Bryan R,Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis. Nat Rev Rheumatol 2015; 11: 35-44.

7 Frampton JE. Empagliflozin: A Review in Type 2 Diabetes. Drugs 2018; 78: 1037-1048.

8 Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, Garcia-Ropero A, Ishikawa K, Watanabe S, Picatoste B, Vargas-Delgado AP, Flores-Umanzor EJ, Sanz J, Fuster V,Badimon JJ. Empagliflozin Ameliorates Diastolic Dysfunction and Left Ventricular Fibrosis/Stiffness in Nondiabetic Heart Failure: A Multimodality Study. JACC Cardiovasc Imaging 2021; 14: 393-407.

9 Kolijn D, Pabel S, Tian Y, Lódi M, Herwig M, Carrizzo A, Zhazykbayeva S, Kovács Á, Fülöp G, Falcão-Pires I, Reusch PH, Linthout SV, Papp Z, van Heerebeek L, Vecchione C, Maier LS, Ciccarelli M, Tschöpe C, Mügge A, Bagi Z, Sossalla S,Hamdani N. Empagliflozin improves endothelial and cardiomyocyte function in human heart failure with preserved ejection fraction via reduced pro-inflammatory-oxidative pathways and protein kinase Gα oxidation. Cardiovasc Res 2021; 117: 495-507.

10 Xu L, Nagata N, Nagashimada M, Zhuge F, Ni Y, Chen G, Mayoux E, Kaneko S,Ota T. SGLT2 Inhibition by Empagliflozin Promotes Fat Utilization and Browning and Attenuates Inflammation and Insulin Resistance by Polarizing M2 Macrophages in Diet-induced Obese Mice. EBioMedicine 2017; 20: 137-149.

11 Shakerinasab N, Azizi M, Mansourian M, Sadeghi H, Salaminia S, Abbasi R, Shahaboddin ME,Doustimotlagh AH. Empagliflozin Exhibits Hepatoprotective Effects Against Bile Duct Ligation-induced Liver Injury in Rats: A Combined Molecular Docking Approach to In Vivo Studies. Curr Pharm Des 2022; 28: 3313-3323.

12 Cho C, Kang LJ, Jang D, Jeon J, Lee H, Choi S, Han SJ, Oh E, Nam J, Kim CS, Park E, Jeong SY, Park CH, Shin YS, Eyun SI,Yang S. Cirsium japonicum var. maackii and apigenin block Hif-2α-induced osteoarthritic cartilage destruction. J Cell Mol Med 2019; 23: 5369-5379.

13 Park E, Lee CG, Han SJ, Yun SH, Hwang S, Jeon H, Kim J, Choi CW, Yang S,Jeong SY. Antiosteoarthritic Effect of Morroniside in Chondrocyte Inflammation and Destabilization of Medial Meniscus-Induced Mouse Model. Int J Mol Sci 2021; 22.

14 Gutthann SP, García Rodríguez LA,Raiford DS. Individual nonsteroidal antiinflammatory drugs and other risk factors for upper gastrointestinal bleeding and perforation. Epidemiology 1997; 8: 18-24.

15 Fu J, Xu H, Wu F, Tu Q, Dong X, Xie H,Cao Z. Empagliflozin inhibits macrophage inflammation through AMPK signaling pathway and plays an anti-atherosclerosis role. Int J Cardiol 2022; 367: 56-62.

16 Gohari S, Reshadmanesh T, Khodabandehloo H, Karbalaee-Hasani A, Ahangar H, Arsang-Jang S, Ismail-Beigi F, Dadashi M, Ghanbari S, Taheri H, Fathi M, Muhammadi MJ, Mahmoodian R, Asgari A, Tayaranian M, Moharrami M, Mahjani M, Ghobadian B, Chiti H,Gohari S. The effect of EMPAgliflozin on markers of inflammation in patients with concomitant type 2 diabetes mellitus and Coronary ARtery Disease: the EMPA-CARD randomized controlled trial. Diabetol Metab Syndr 2022; 14: 170.

17 La Grotta R, de Candia P, Olivieri F, Matacchione G, Giuliani A, Rippo MR, Tagliabue E, Mancino M, Rispoli F, Ferroni S, Berra CC, Ceriello A,Prattichizzo F. Anti-inflammatory effect of SGLT-2 inhibitors via uric acid and insulin. Cell Mol Life Sci 2022; 79: 273.

18 Li N,Zhou H. SGLT2 Inhibitors: A Novel Player in the Treatment and Prevention of Diabetic Cardiomyopathy. Drug Des Devel Ther 2020; 14: 4775-4788.

19 Maayah ZH, Ferdaoussi M, Takahara S, Soni S,Dyck JRB. Empagliflozin suppresses inflammation and protects against acute septic renal injury. Inflammopharmacology 2021; 29: 269-279.

20 Charlier E, Deroyer C, Ciregia F, Malaise O, Neuville S, Plener Z, Malaise M,de Seny D. Chondrocyte dedifferentiation and osteoarthritis (OA). Biochem Pharmacol 2019; 165: 49-65.

21 Motta F, Barone E, Sica A,Selmi C. Inflammaging and Osteoarthritis. Clin Rev Allergy Immunol 2023; 64: 222-238.

22 Abramoff B,Caldera FE. Osteoarthritis: Pathology, Diagnosis, and Treatment Options. Med Clin North Am 2020; 104: 293-311.

23 Hu Q,Ecker M. Overview of MMP-13 as a Promising Target for the Treatment of Osteoarthritis. Int J Mol Sci 2021; 22.

24 Jeon OH, Kim C, Laberge RM, Demaria M, Rathod S, Vasserot AP, Chung JW, Kim DH, Poon Y, David N, Baker DJ, van Deursen JM, Campisi J,Elisseeff JH. Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. Nat Med 2017; 23: 775-781.

25 Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, Campisi J, Collado M, Evangelou K, Ferbeyre G, Gil J, Hara E, Krizhanovsky V, Jurk D, Maier AB, Narita M, Niedernhofer L, Passos JF, Robbins PD, Schmitt CA, Sedivy J, Vougas K, von Zglinicki T, Zhou D, Serrano M,Demaria M. Cellular Senescence: Defining a Path Forward. Cell 2019; 179: 813-827.

26 Yao Q, Wu X, Tao C, Gong W, Chen M, Qu M, Zhong Y, He T, Chen S,Xiao G. Osteoarthritis: pathogenic signaling pathways and therapeutic targets. Signal Transduct Target Ther 2023; 8: 56.

27 Choi MC, Jo J, Park J, Kang HK,Park Y. NF-κB Signaling Pathways in Osteoarthritic Cartilage Destruction. Cells 2019; 8.

28 Wang XF, Zhang AP, Sun ZY, Liu C, Kuang LH,Tian JW. [Expression of NF-κB in a degenerative human intervertebral disc model]. Zhonghua Yi Xue Za Zhi 2017; 97: 1324-1329.

29 Mortezaee K, Najafi M, Farhood B, Ahmadi A, Shabeeb D,Musa AE. NF-κB targeting for overcoming tumor resistance and normal tissues toxicity. J Cell Physiol 2019; 234: 17187-17204.

30 Zheng Y, Lin Y, Chen Z, Jiao Y, Yuan Y, Li C, Xu X,Cao P. Propionibacterium acnes induces intervertebral discs degeneration by increasing MMP-1 and inhibiting TIMP-1 expression via the NF-κB pathway. Int J Clin Exp Pathol 2018; 11: 3445-3453.

31 Lin Y, Tang G, Jiao Y, Yuan Y, Zheng Y, Chen Y, Xiao J, Li C, Chen Z,Cao P. Propionibacterium acnes Induces Intervertebral Disc Degeneration by Promoting iNOS/NO and COX-2/PGE(2) Activation via the ROS-Dependent NF-κB Pathway. Oxid Med Cell Longev 2018; 2018: 3692752.

32 Wang J, Hu J, Chen X, Huang C, Lin J, Shao Z, Gu M, Wu Y, Tian N, Gao W, Zhou Y, Wang X,Zhang X. BRD4 inhibition regulates MAPK, NF-κB signals, and autophagy to suppress MMP-13 expression in diabetic intervertebral disc degeneration. Faseb j 2019; 33: 11555-11566.

Tables

Table 1 Primers used for RT-PCR in this study

Gene	Forward	Reverse
MMP9	CTTCACCGGCTAAACCACCT	TGTCCCTAACGCCCAGTAGA
MMP13	TGATGGACCTTCTGGTCTGGC	CATCCACATGGTTGGGAAGTTCTG
COX2	GGTCTGGTGCCTGGTCTGATGAT	GTCCTTTCAAGGAGAATGGTGC
INOS	GCAAACCCAAGGTCTACGTTCA	GAGCACGCTGAGTACCTCATTG
GAPDH	TCACTGCCACCCAGAC	TGTAGGCCATGAGGTCCAC

Figure legends

Figure 1. Effects of empagliflozin and IL-1β on cell viability and chondrocyte phenotype maintenance of mouse chondrocytes. (A) CCK-8 analysis, *p<0.05 versus the IL-1β group. (C) Gross view of safranine O stained mouse chondrocytes that treated with empagliflozin at various concentrations (0, 1, and 5 μM) with or without IL-1β (10 ng/ml) for 24 h.

Figure 2. Effects of empagliflozin on IL-1β-induced ECM degradation in mouse chondrocytes. Chondrocytes were pretreated with empagliflozin at various concentrations (0, 1, and 5 μM) for 2 h and then incubated with IL-1β (10 ng/ml) for 24 h. (A) ELISA analysis of MMP9 and MMP13 in treated cells. (B) The mRNA expression levels of MMP9 and MMP13 in treated cells. (C) The protein expression levels of MMP9 and MMP13 in treated cells. *p<0.05 versus control group, #p<0.05 versus model group.

Figure 3. Effects of empagliflozin on IL-1β-induced inflammation in mouse chondrocytes. Chondrocytes were pretreated with empagliflozin at various concentrations (0, 1, and 5 μM) for 2 h and then incubated with IL-1β (10 ng/ml) for 24 h. (A) Nitrite production, PGE2 secretion, and IL-6 secretion in treated cells. (B) The mRNA expression levels of COX2 and INOS in treated cells. (C) The protein expression levels of COX2 and INOS in treated cells. GAPDH as an internal control. *p<0.05 versus control group, #p<0.05 versus model group.

Figure 4. Effects of empagliflozin on IL-1β-induced senescence in mouse chondrocytes. Chondrocytes were pretreated with empagliflozin at various concentrations (0, 1, and 5 μM) for 2 h and then incubated with IL-1β (10 ng/ml) for 24 h. (A) The protein expression levels of P21 and P53 in treated cells. GAPDH as an internal control. (B) β-galactosidase staining results of treated cells. *p<0.05 versus control group, #p<0.05 versus model group. Bar = 50 μm

Figure 5. Effects of empagliflozin on the IL-1β-induced NF-ϰB pathway activation in mouse chondrocytes. Chondrocytes were pretreated with empagliflozin at various concentrations (0, 1, and 5 μM) for 2 h and then incubated with IL-1β (10 ng/ml) for 15 min. The protein expression levels of P-P65, P65, p-IϰB, and IϰB in treated cells. The ratio of p-P65 to P65 as well as the radio of p-IϰB to IϰB was calculated. GAPDH as an internal control. *p<0.05 versus control group, #p<0.05 versus model group.

Figure 6. Effects of empagliflozin on cartilage degeneration in mouse OA model. (A) Microscopic images of safranin O stained mouse knee joint sections. Bar = 500 μm. (B) The OARSI grades. *p<0.05 versus control group, #p<0.05 versus model group.