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Original Article

Effects of Maohuoside A on AMPK Activation in Rats with Osteoarthritis Induced by Anterior Cruciate Ligament Transection

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Abstract

Objective: To explore the therapeutic effects of Maohuoside A (MHA) on rats with osteoarthritis (OA) induced by anterior cruciate ligament transection, and to analyze its impact on the activation of adenosine monophosphate-activated protein kinase (AMPK). **Methods**: Sprague-Dawley male rats were divided into six groups: Sham, OA, L-MHA, M-MHA, H-MHA, and H-MHA + Compound C (n = 12 per group). The Sham group had sham surgery, while the OA, L-MHA, M-MHA, and H-MHA groups underwent OA via ACL severing. L-MHA, M-MHA, and H-MHA groups received low, moderate, and high doses of Maohuoside A, respectively, and the H-MHA + Compound C group received high-dose Maohuoside A plus Compound C. Hematoxylin-eosin (HE) staining and Safranin O/Fast Green staining were used to assess articular cartilage damage and degeneration, and the OARSI score was used to evaluate the extent of cartilage degeneration. **Results**: Compared with the OA group, the L-MHA, M-MHA, and H-MHA groups showed decreased OARSI scores, increased relative levels of Bcl-2 mRNA, decreased relative levels of Bax mRNA, reduced serum levels of IL-1β, IL-6, and TNF-α, increased relative levels of Collagen II mRNA, decreased relative levels of MMP-13 mRNA, and increased relative levels of BMP2, Runx2, and Osterix mRNA. Additionally, the relative level of cartilage AMPKα (Thr172) phosphorylation increased ($P<0.05$). **Conclusion**: This study suggests that Maohuoside A exerts anti-osteoarthritis (OA) effects by activating AMPK.

Keywords: Adenosine Monophosphate Activated Protein Kinase, Anterior Cruciate Ligament Transection, Cartilage Degeneration, Maohuoside A, Osteoarthritis

Introduction

Osteoarthritis (OA) is one of the most common types of arthritis and a chronic, degenerative, and disabling disease. It is characterized by articular cartilage destruction, subchondral bone loss, joint bony hypertrophy, and instability of tendons and ligaments. As of 2021, more than 22% of adults over 40 years old suffer from OA, with over 500 million people worldwide currently affected[1]. Due to the lack of long-term effective treatments, many advanced OA patients eventually require joint replacement surgery[2]. However, prosthetic joints have a limited lifespan and a risk of poor outcomes. Currently, there is a shortage of effective treatment protocols for OA in clinical practice.

Epimedium koreanum Nakai is a commonly used herb for OA treatment, with its main active ingredient, icariin, extensively studied for promoting osteogenesis and treating OA[3,4]. Another natural product from Epimedium, Maohuoside A (MHA), has shown potential in osteogenesis. It has been reported to promote osteogenesis in rat mesenchymal stem cells through BMP and MAPK signaling pathways[5] and acts in a BMP-dependent manner during osteogenesis[6]. These findings suggest that Maohuoside A may have significant potential for treating OA.

Adenosine monophosphate-activated protein kinase (AMPK) is a heterotrimeric complex consisting of one catalytic subunit (α) and two regulatory subunits (β and γ)[7]. AMPK plays a crucial role in regulating energy homeostasis by sensing low cellular ATP levels and responding to changes in the ATP-to-AMP ratio. It regulates metabolic enzymes to

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promote ATP production and inhibit ATP depletion. When cellular energy is low, phosphorylation of Thr172 in the α subunit activates the AMPK complex[7]. In patients with osteoarthritis (OA), Thr172 phosphorylation of AMPKα is reduced in articular cartilage compared to normal cartilage[7]. Similarly, AMPKα phosphorylation is significantly decreased in OA mouse and rat models[8]. Activation of AMPK represents an effective pathway for OA treatment[9,10]. Therefore, this study aimed to explore the therapeutic effects of Maohuoside A on OA rats and analyze its impact on AMPK activation, providing a potential candidate for clinical drug development in OA.

Materials and Methods

Experimental materials

- **Maohuoside A** (E-1893, purity ≥ 98%), Shanghai Tauto BIOTECH Co., Ltd.
- **AMPK Inhibitor Compound C** (8666430, purity ≥ 98%), Shanghai Yanhui Biotechnology Co., Ltd.
- **Interleukin-1β (IL-1β)** (AZ0647), **IL-6** (AZ0652), and **Tumor Necrosis Factor-α (TNF-α)** (AZ0673) ELISA Kits, Beijing APPLYGEN Gene Technology Co., Ltd.
- **Hematoxylin Eosin (HE) Staining Kit** (60524ES60), Yisheng Biotechnology (Shanghai) Co., Ltd.
- **Modified Safranin O/Fast Green Cartilage Staining Solution** (AG1371), Shanghai Acmec Biochemical Technology Co., Ltd.
- **Trizol** (15596018), Invitrogen Corporation, USA
- **PrimeScript RT Kit** (RR036A), Takara Corporation, Japan
- **SYBR Green PCR Master Mix** (4367659), ABI Corporation, USA
- **RIPA** (R0010), Beijing Solarbio Science & Technology Co., Ltd.
- p-**AMPKα (Thr172)** (ab314032), **AMPKα** (ab32047), and **β-actin** (ab213262) primary antibodies, **IgG H&L (HRP)** secondary antibody (ab205718), Abcam Corporation, USA

Laboratory animals

A total of 80 seven-week-old (250-300 g) Specific Pathogen-Free (SPF) grade male Sprague-Dawley (SD) rats were obtained for this study (Nanjing Anji Biotechnology Co., Ltd. [SYXK (SU) 2023-0051]). The rats were housed in an animal room maintained at 25°C with 55% humidity and were exposed to a 12-hour light/dark cycle.

OA animal modeling

An osteoarthritis (OA) rat model was established using anterior cruciate ligament transection[11]. Eight rats were excluded due to unsuccessful modeling, leaving a final count of 72 rats included in the study. Each rat was anesthetized with isoflurane. After shaving and disinfecting the area, the right knee joint was exposed via a medial parapatellar approach. The knee was flexed, and the anterior cruciate ligament was severed using miniature scissors. The incision was then sutured and sterilized. In sham-operated rats, only the skin was incised.

Table 1. Primer sequence.

Gene	Primer sequence
B-cell lymphoma/leukaemia 2 (Bcl-2)	F: 5′-CACCCCTGGCATCTTCTCCT-3′
	R: 5′-GTTGACGCTCCCCACACACA-3′
Bcl-2 associated X protein (Bax)	F: 5′-TGGCGATGAACTGGACAACAAC-3′
	R: 5′-CCCGAAGTAGGAAAGGAGGC-3′
Collagen II	F: 5′-TCCTAAGGGTGCCAATGGTGA-3′
	R: 5′-AGGACCAACTTTGCCTTGAGGAC-3′
Matrix metalloproteinase-13 (MMP-13)	F: 5′-TGATGATGAAACCTGGACAAGCA-3′
	R: 5′-GAACGTCATCTCTGGGAGCA-3′
Bone morphogenetic protein-2 (BMP2)	F: 5′-CCCCTATATGCTCGACCTGTACC-3′
	R: 5′-TGAAAGTTCCTCGATGGCTTCT-3′
Runt-related transcription factor 2 (Runx2)	F: 5′-TGATGAGAACTACTCCGCCGA-3′
	R: 5′-GACTGTTATGGTCAAGGTGAA-3′
Osterix	F: 5′-AGCGACCACTTGAGCAAACAT-3′
	R: 5′-GCGGCTGATTGGCTTCTTCT-3′
β-actin	F: 5′-AACCGTGAAAAGATGACCCAG-3′
	R: 5′-CTCCTGCTTGCTGATCCACAT-3′

Experimental Group Treatments

The final 72 Sprague-Dawley male rats were divided into the following six groups: Sham Group: Rats underwent a sham surgery, which involved all the procedural steps of the surgery without the actual OA-inducing manipulation. OA Group: Rats underwent anterior cruciate ligament (ACL) amputation to induce osteoarthritis (OA) but received no further treatment. L-MHA Group: OA rats received a low dose of Maohuoside A (10 mg/kg/d). M-MHA Group: OA rats received a moderate dose of Maohuoside A (20 mg/kg/d). H-MHA Group: OA rats received a high dose of Maohuoside A (40 mg/kg/d). H-MHA + Compound C Group: OA rats received a high dose of Maohuoside A (40 mg/kg/d) combined with an AMPK inhibitor, Compound C (20 mg/kg/d), administered intraperitoneally. Each group consisted of 12 rats. Rats that underwent a sham operation were included in the Sham Group, while the other groups consisted of OA model rats. Rats in the Sham and OA Groups were gavaged with 0.5% sodium carboxymethylcellulose (Na-CMC). Rats in the L-MHA, M-MHA, and H-MHA Groups were gavaged with 10, 20, and 40 mg/kg/day of MHA solution (MHA dissolved in 0.5% CMC-Na), respectively[6]. Rats in the H-MHA + Compound C Group received 40 mg/kg/day of MHA solution and 20 mg/kg/day of the AMPK inhibitor Compound C, which was injected intraperitoneally[12]. The treatment lasted for 4 weeks.

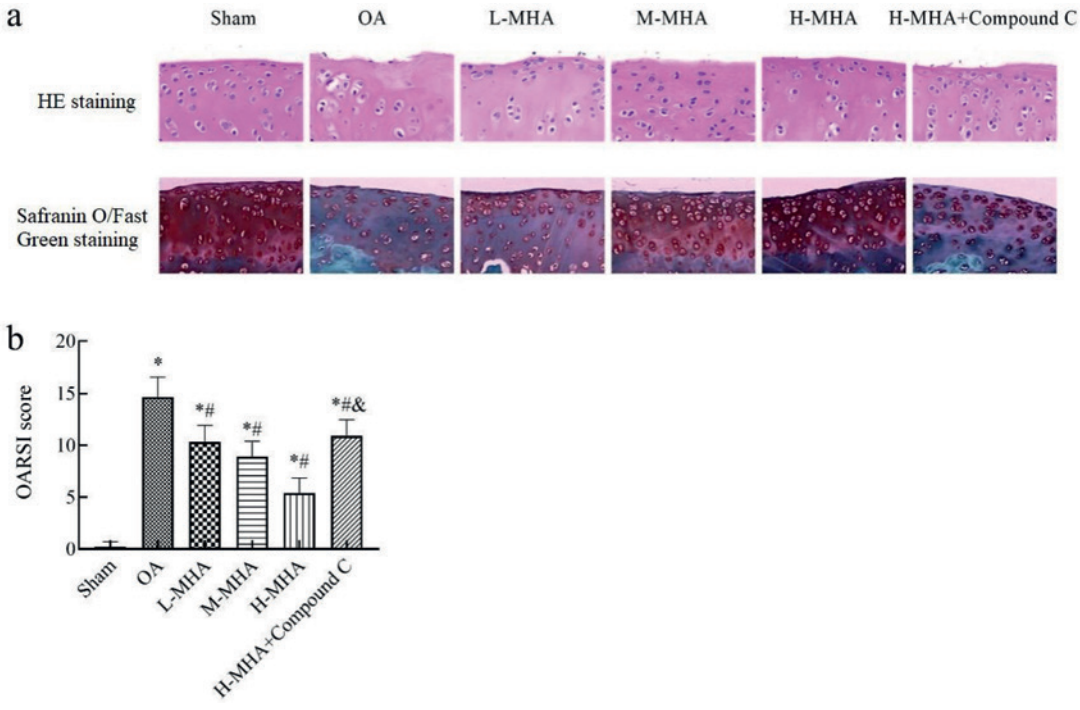

Figure 1. Effect of maohuoside A on cartilage morphology and cartilage degeneration in OA rats. Note: a: HE staining and saffron O/solid green staining images (×100); b: OARSI score; *P<0.05 vs Sham group; #P<0.05 vs OA group; &P<0.05 vs H-MHA group.

Histological staining

After 4 weeks of treatment, the rats were sacrificed, and articular cartilage samples were collected. The samples were fixed in paraformaldehyde for 24 hours and then decalcified in 10% ethylenediamine tetraacetic acid (EDTA; pH 7.4) for 21 days. Following decalcification, the cartilage tissue was embedded in paraffin and sectioned into 5 μm thick slices. The sections were dewaxed in xylene, rehydrated through a gradient of ethanol washes, and then stained with hematoxylin and eosin (HE) and Safranin O/Fast Green. The degree of cartilage degeneration was assessed using the International Association for Osteoarthritis Research (OARSI) score[13], based on the Safranin O/Fast Green staining results. The OARSI score ranges from 0 to 24 points, with higher scores indicating more severe cartilage degeneration.

Detection of serum inflammatory factors

Serum levels of IL-1β, IL-6, and TNF-α were measured using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's protocol.

RT-qPCR

Total cartilage RNA was extracted using Trizol. Reverse transcription was performed with a PrimeScript RT Kit (RR036A, Takara, Japan) according to the manufacturer's protocol to synthesize the first strand of cDNA. PCR was conducted with SYBR Green PCR Master Mix (4367659, ABI Corporation, USA) using a Bio-Rad CFX Connect system. The PCR conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 5 seconds, 60°C for 20 seconds, and 72°C for 15 seconds. Primer sequences are listed in Table 1. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method with β-actin as the internal reference gene.

Western blot

Cartilage tissue was shredded and homogenized, and total cartilage protein was extracted on ice using RIPA lysis buffer. The resulting supernatant was collected by centrifugation at 12,000 g for 30 minutes at 4°C. Protein concentrations were measured using the bicinchoninic acid (BCA) method. Equal amounts of protein samples were separated by electrophoresis on a 12% SDS-PAGE gel and then transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% skim milk for 1 hour at room temperature and then incubated overnight at 4°C with primary antibodies against p-AMPKα (Thr172), AMPKα, and β-actin. Following washing, the membranes were incubated with secondary antibodies for 1 hour at room temperature. Protein bands were visualized using a Bio-Rad ChemiDoc MP

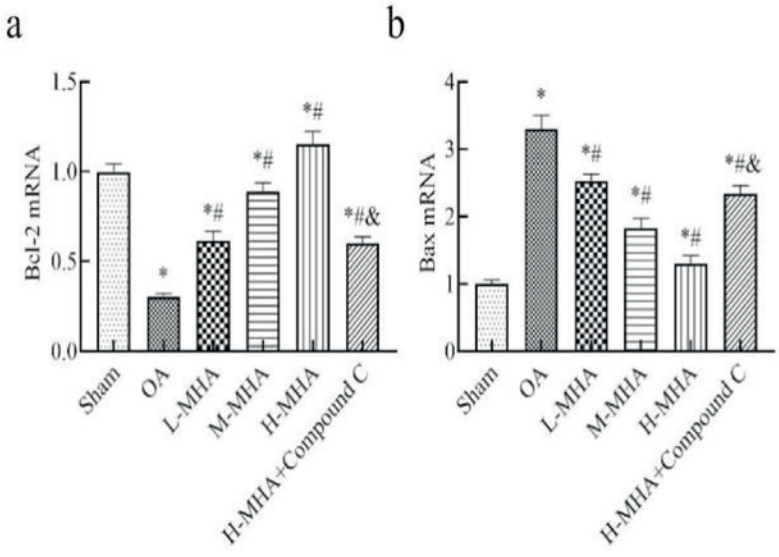

Figure 2. Effect of maohuoside A on the transcription of apoptosis-related genes in OA rats. Note: a and b: relative levels of Bcl-2 and Bax mRNA; *P<0.05 vs Sham group; #P<0.05 vs OA group; &P<0.05 vs H-MHA group.

chemiluminescence gel imaging system. Grayscale values of the protein bands were quantified using ImageJ, and the ratio of p-AMPKα to AMPKα was calculated.

Statistical Analysis

Data are presented as mean ± standard deviation. Differences between groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. All statistical analyses were conducted with IBM SPSS Statistics 22 software. A p-value of < 0.05 was considered statistically significant.

Results

Maohuoside A Improves Cartilage Morphology and Inhibits Cartilage Degeneration in OA Rats by Activating AMPKα

Histological analysis using HE staining and Safranin O/Fast Green staining revealed that articular cartilage in the Sham group appeared normal. In the OA group, the cartilage surface was rough, the thickness was reduced, the number of chondrocytes decreased, some cells were necrotic, and Safranin O staining was less intense. Compared to the OA group, cartilage lesions in the L-MHA, M-MHA, and H-MHA groups were significantly reduced, and Safranin O staining was more intense. In the H-MHA + Compound C group, cartilage lesions were exacerbated, and Safranin O staining was lighter compared to the H-MHA group.

OARSI scores were as follows: Sham group, 0.25 ± 0.45; OA group, 14.67 ± 1.92; L-MHA group, 10.33 ± 1.61; M-MHA group, 8.92 ± 1.51; H-MHA group, 5.42 ± 1.44; H-MHA + Compound C group, 10.92 ± 1.56 ($F = 135.232$, $P < 0.001$). The OARSI score in the OA group was significantly higher than that in the Sham group ($P < 0.05$). Compared to the OA group, the OARSI scores in the L-MHA, M-MHA, and H-MHA groups were significantly lower ($P < 0.05$). Additionally, the OARSI score in the H-MHA + Compound C group was higher compared to the H-MHA group ($P < 0.05$) (Figure 1).

Maohuoside A Inhibits Chondrocyte Apoptosis in OA Rats by Activating AMPKα

The relative levels of Bcl-2 mRNA were 1.00 ± 0.04, 0.30 ± 0.02, 0.62 ± 0.05, 0.89 ± 0.05, 1.15 ± 0.07, and 0.60 ± 0.04 for the Sham, OA, L-MHA, M-MHA, H-MHA, and H-MHA + Compound C groups, respectively ($F = 504.049$, $P < 0.001$). The relative levels of Bax mRNA were 1.00 ± 0.06, 3.30 ± 0.20, 2.53 ± 0.10, 1.83 ± 0.14, 1.30 ± 0.12, and 2.34 ± 0.12 for the same groups ($F = 509.162$, $P < 0.001$).

Compared to the Sham Group, the relative level of Bcl-2 mRNA in the OA Group decreased, while the relative level of Bax mRNA increased ($P < 0.05$). Compared to the OA Group, the relative levels of Bcl-2 mRNA in the L-MHA, M-MHA, and H-MHA groups were higher, and the relative levels of Bax mRNA were lower ($P < 0.05$). Compared to the H-MHA Group, the relative level of Bcl-2 mRNA in the H-MHA + Compound C Group decreased, and the relative level of Bax mRNA increased ($P < 0.05$) (Figure 2).

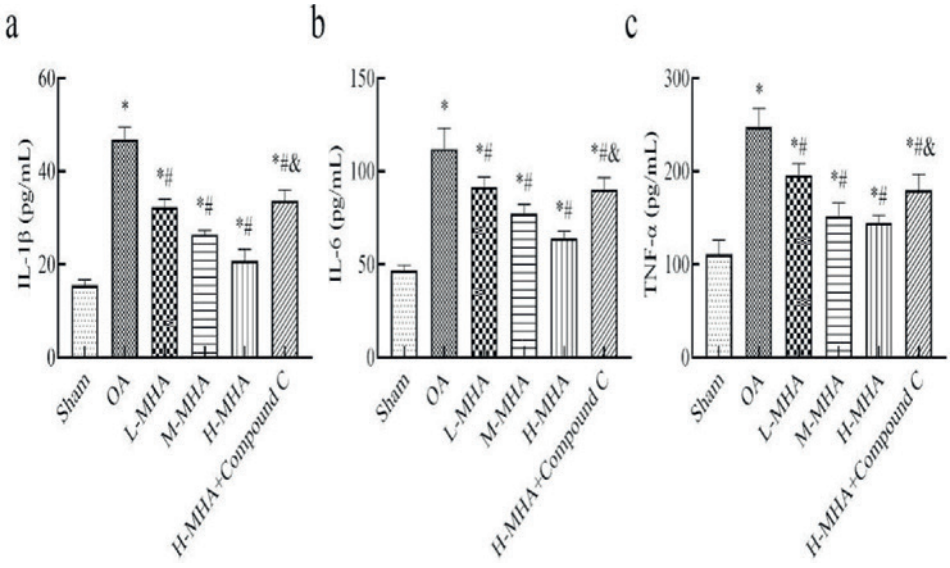

Figure 3. Effect of maohuoside A on serum inflammatory factors in OA rats. Note: a-c: IL-1β, IL-6 and TNF-α levels; *P<0.05 vs Sham group; #P<0.05 vs OA group; &P<0.05 vs H-MHA group.

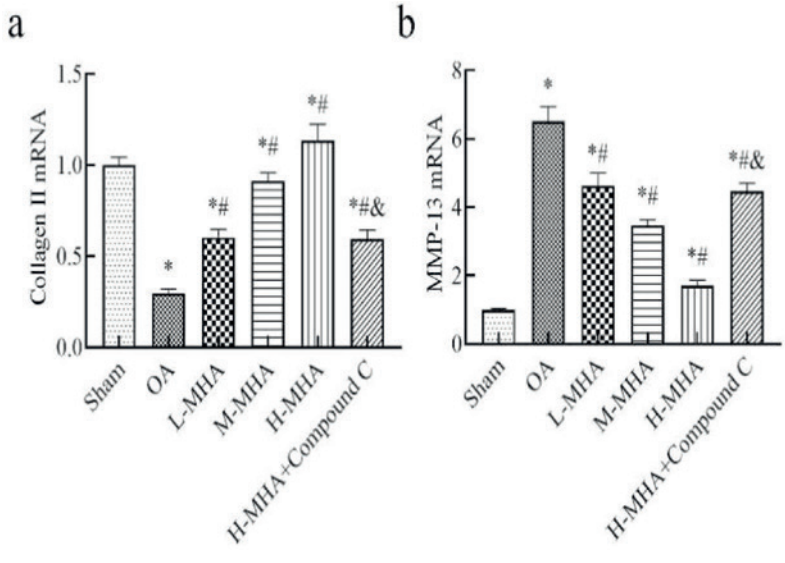

Figure 4. Effect of maohuoside A on cartilage matrix degradation in OA rats. Note: a and b: relative mRNA levels of Collagen II and MMP-13; *P<0.05 vs Sham group; #P<0.05 vs OA group; &P<0.05 vs H-MHA group.

Maohuoside A Inhibits Inflammation in OA Rats by Activating AMPKα

Serum levels of IL-1β, IL-6, and TNF-α were measured as follows:

- **IL-1β:** 15.55 ± 1.19, 46.88 ± 2.66, 32.29 ± 1.71, 26.37 ± 0.88, 20.82 ± 2.38, 33.73 ± 2.23 pg/mL (F = 383.650, P < 0.001)
- **IL-6:** 46.63 ± 2.77, 111.89 ± 11.25, 91.58 ± 5.34, 77.27 ± 4.84, 63.88 ± 3.92, 90.24 ± 6.21 pg/mL (F = 158.060, P < 0.001)

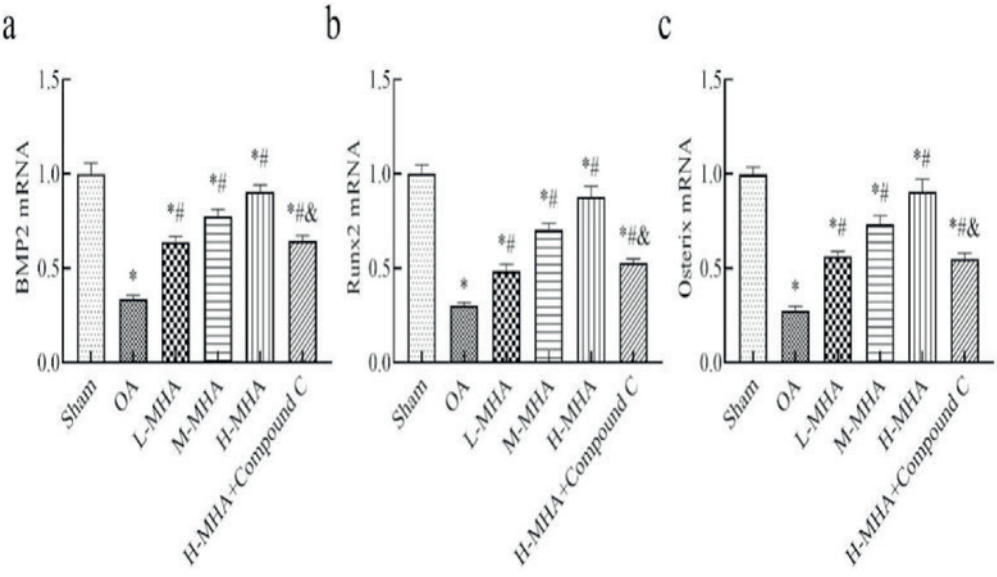

Figure 5. Effect of maohuoside A on cartilage formation in OA rats. Note: a-c: relative levels of BMP2, Runx2 and Osterix mRNA; *P<0.05 vs Sham group; #P<0.05 vs OA group; &P<0.05 vs H-MHA group.

- **TNF-α:** 110.95 ± 15.10, 247.32 ± 20.06, 195.59 ± 12.57, 151.75 ± 14.43, 144.67 ± 7.85, 179.77 ± 16.84 pg/mL (F = 119.879, P < 0.001)

In the OA group, serum levels of IL-1β, IL-6, and TNF-α were significantly increased compared to the Sham group (P < 0.05). Compared to the OA group, serum levels of IL-1β, IL-6, and TNF-α were significantly decreased in the L-MHA, M-MHA, and H-MHA groups (P < 0.05). However, in the H-MHA + Compound C group, serum levels of IL-1β, IL-6, and TNF-α were elevated compared to the H-MHA group (P < 0.05) (Figure 3).

Maohuoside A Inhibits Cartilage Matrix Degradation in OA Rats by Activating AMPKa

The relative levels of Collagen II mRNA and MMP-13 mRNA were as follows:

- **Collagen II mRNA:** 1.00 ± 0.04, 0.30 ± 0.02, 0.60 ± 0.04, 0.91 ± 0.05, 1.13 ± 0.09, 0.60 ± 0.05 (F = 410.290, P < 0.001)
- **MMP-13 mRNA:** 1.00 ± 0.04, 6.52 ± 0.42, 4.63 ± 0.38, 3.46 ± 0.18, 1.71 ± 0.16, 4.46 ± 0.25 (F = 684.876, P < 0.001)

In the OA group, the relative level of Collagen II mRNA decreased, and the relative level of MMP-13 mRNA increased compared to the Sham group (P < 0.05). Compared to the OA group, the relative levels of Collagen II mRNA were significantly higher and the relative levels of MMP-13 mRNA were significantly lower in the L-MHA, M-MHA, and H-MHA groups (P < 0.05). In the H-MHA + Compound C group, the relative level of Collagen II mRNA decreased, and the relative level of MMP-13 mRNA increased compared to the H-MHA group (P < 0.05) (Figure 4).

Maohuoside A Promotes Chondrogenesis in OA Rats by Activating AMPKa

The relative levels of BMP2, Runx2, and Osterix mRNA were as follows:

- **BMP2 mRNA:** 1.00 ± 0.06, 0.34 ± 0.02, 0.64 ± 0.03, 0.78 ± 0.03, 0.91 ± 0.04, 0.64 ± 0.03 (F = 494.162, P < 0.001)
- **Runx2 mRNA:** 1.00 ± 0.05, 0.30 ± 0.01, 0.49 ± 0.04, 0.70 ± 0.03, 0.88 ± 0.06, 0.53 ± 0.02 (F = 588.673, P < 0.001)
- **Osterix mRNA:** 1.00 ± 0.04, 0.28 ± 0.02, 0.57 ± 0.02, 0.73 ± 0.05, 0.91 ± 0.06, 0.55 ± 0.03 (F = 516.798, P < 0.001)

In the OA group, the relative levels of BMP2, Runx2, and Osterix mRNA were significantly lower compared to the Sham group (P < 0.05). In the L-MHA, M-MHA, and H-MHA groups, the relative levels of BMP2, Runx2, and Osterix mRNA were significantly higher compared to the OA group (P < 0.05). In the H-MHA + Compound C group, the relative levels of BMP2, Runx2, and Osterix mRNA decreased compared to the H-MHA group (P < 0.05) (Figure 5).

Effect of Maohuoside A on AMPK Phosphorylation in Cartilage of OA Rats

The relative levels of cartilage AMPKα (Thr172) phosphorylation were:

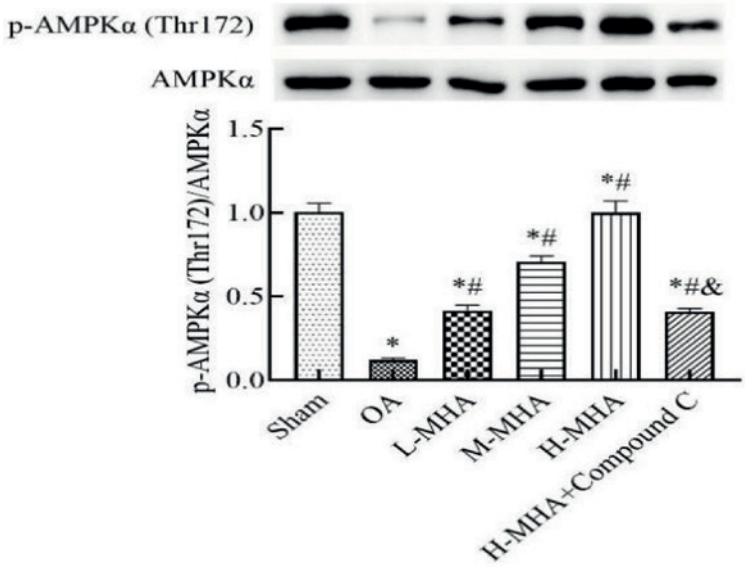

Figure 6. Effect of maohuoside A on AMPK phosphorylation in OA rat cartilage. Note: Relative phosphorylation level of AMPKα (Thr172); *P<0.05 vs Sham group; #P<0.05 vs OA group; &P<0.05 vs H-MHA group.

- **Sham Group**: 1.00 ± 0.05
- **OA Group**: 0.12 ± 0.01
- **L-MHA Group**: 0.42 ± 0.03
- **M-MHA Group**: 0.71 ± 0.03
- **H-MHA Group**: 1.00 ± 0.07
- **H-MHA + Compound C Group**: 0.41 ± 0.02 (F = 886.554, P < 0.001)

In the OA group, the relative level of cartilage AMPKα (Thr172) phosphorylation was significantly lower compared to the Sham group (P < 0.05). In the L-MHA, M-MHA, and H-MHA groups, the relative levels of AMPKα (Thr172) phosphorylation in cartilage were significantly higher compared to the OA group (P < 0.05). However, in the H-MHA + Compound C group, the relative level of cartilage AMPKα (Thr172) phosphorylation was lower compared to the H-MHA group (P < 0.05) (Figure 6).

Discussion

Epimedium is a commonly used herb for treating osteoarthritis (OA)[14,15], with most research focusing on its primary component, icariin[16]. However, Maohuoside A, a flavonoid glycoside isolated from *Epimedium koreanum*, has also garnered attention. Yang et al.[5] reported that Maohuoside A is more effective than icariin in promoting osteogenesis in rat bone marrow-derived mesenchymal stem cells (rMSCs), enhancing osteogenesis through BMP and MAPK signaling pathways. Cai et al.[6] found that oral administration of Maohuoside A (4.36 mg/kg) increased bone mineral density and osteopontin (OPN) transcription levels in the lumbar spine of mice. These findings suggest that Maohuoside A has potential as a therapeutic agent for OA.

In this study, HE staining and Safranin O/Fast Green staining demonstrated that Maohuoside A mitigates cartilage damage and degeneration in OA rats. Apoptosis, a critical physiological process impacting development and tissue homeostasis, is prevalent in OA cartilage. Literature indicates a clear correlation between the extent of cartilage damage and chondrocyte apoptosis[17]. This study demonstrated that Maohuoside A inhibits chondrocyte apoptosis in OA rats. Inflammatory mediators such as IL-1β, IL-6, and TNF-α are strongly associated with chondrocyte apoptosis in OA[18]. These inflammatory cytokines disrupt intra-articular homeostasis, decrease lubricin levels, and accelerate the degradation of the chondrocytic extracellular matrix (ECM)[19,20]. Our results showed that Maohuoside A reduced serum levels of these inflammatory cytokines in OA rats, suggesting its therapeutic potential in OA through the inhibition of chondrocyte apoptosis and inflammation.

Collagen II is a crucial component of the cartilage matrix, and numerous animal and clinical studies have shown that oral administration of undenatured Collagen II can significantly reduce OA incidence or alleviate symptoms of articular cartilage damage[21]. OA cartilage degeneration is often linked to elevated expression of matrix metalloproteinases (MMPs), which degrade proteoglycans and collagen in the cartilage[22]. MMP-13, a key MMP involved in cartilage degradation by cleaving Collagen II, is highly expressed in OA patients and

represents a significant therapeutic target[23,24]. This study found that Maohuoside A increased Collagen II mRNA levels and decreased MMP-13 mRNA levels in the cartilage of OA rats, thereby inhibiting cartilage degradation.

Activation of the BMP2/Runx2/Osterix signaling pathway is crucial for osteoblast formation and differentiation[25]. Bone morphogenetic protein (BMP) is a powerful initiator of osteogenesis[26]. BMP2 binds to its receptors and activates Smads (Smad1/5/8) during phenotypic transformation. Smads also function as transcription factors that regulate essential osteogenic genes involved in osteoblast proliferation, such as Runx2 and Osterix[27-29]. Research highlights the significance of Runx2 as a key transcriptional regulator in osteoblast differentiation[30]. Osterix, a zinc finger-containing transcription factor, is specifically expressed in developing bone; its mutation in mice results in the loss of bone formation[31]. As an osteoblast-specific transcription factor and downstream molecule of Runx2, Osterix is upregulated alongside Runx2[32]. Runx2 directs cells to form cartilage, while Osterix further guides cartilage towards osteogenesis[33]. This study shows that Maohuoside A increases BMP2, Runx2, and Osterix mRNA levels in the cartilage of OA rats, thereby promoting chondrogenesis.

Mitochondrial dysfunction and impaired mitochondrial biosynthesis in osteoarthritis (OA) chondrocytes are linked to abnormal AMPK activity[34]. Additionally, nuclear factor-κB (NF-κB), a critical inflammatory pathway, is directly or indirectly regulated by AMPK, which inactivates NF-κB by promoting the proteasomal degradation of p65[35]. AMPK activation mitigates synovial inflammation in rats primarily by inhibiting the NF-κB pathway[36]. Elevated levels of inflammatory cytokines can decrease AMPK activity in OA chondrocytes[8]. Restoring AMPK activity not only reduces inflammation but also prevents OA progression. For instance, the selective AMPK agonist AICAR has been shown to inhibit chondrocyte catabolic responses to inflammatory cytokines[8]. It has been reported that metformin alleviates osteoarthritis (OA) by upregulating the expression of both total and phosphorylated AMPK in mouse and rat articular cartilage tissues[9,10]. This study demonstrated that Maohuoside A increased AMPKα phosphorylation levels in the cartilage of OA rats. Additionally, when OA rats were treated with high doses of Maohuoside A alongside the AMPK inhibitor Compound C, Compound C diminished the anti-OA effects of Maohuoside A. These findings indicate that Maohuoside A exerts its anti-OA effects through the activation of AMPK.

In summary, this study suggests that Maohuoside A exerts anti-OA effects by activating AMPK. These findings indicate that Maohuoside A holds promise as a natural therapeutic agent for osteoarthritis and has significant potential for development.

References

1. Quicke JG, Conaghan PG, Corp N, Peat G. Osteoarthritis year in review 2021: epidemiology & therapy. Osteoarthritis Cartilage 2022;30(2):196-206.
2. Little CB, Hunter DJ. Post-traumatic osteoarthritis: from mouse models to clinical trials. Nat Rev Rheumatol 2013;9(8):485-497.
3. Hsieh TP, Sheu SY, Sun JS, Chen MH. Icariin inhibits osteoclast differentiation and bone resorption by suppression of MAPKs/NF-κB regulated HIF-1α and PGE(2) synthesis. Phytomedicine 2011;18(2-3):176-185.
4. Zu Y, Mu Y, Li Q, Zhang ST, Yan HJ. Icariin alleviates osteoarthritis by inhibiting NLRP3-mediated pyroptosis. J Orthop Surg Res 2019;14(1):307.
5. Yang L, Wang NL, Cai GP. Maohuoside A promotes osteogenesis of rat mesenchymal stem cells via BMP and MAPK signaling pathways. Mol Cell Biochem 2011; 358(1-2):37-44.
6. Cai M, Li G, Tao K, Yang Y, Lou L, Cai Z, et al. Maohuoside A acts in a BMP-dependent manner during osteogenesis. Phytother Res 2013;27(8):1179-1184.
7. Yan Y, Zhou XE, Xu HE, Melcher K. Structure and Physiological Regulation of AMPK. Int J Mol Sci 2018; 19(11):3534.
8. Terkeltaub R, Yang B, Lotz M, Liu-Bryan R. Chondrocyte AMP-activated protein kinase activity suppresses matrix degradation responses to proinflammatory cytokines interleukin-1β and tumor necrosis factor α. Arthritis Rheum 2011;63(7):1928-1937.
9. Li J, Zhang B, Liu WX, Lu K, Pan H, Wang T, et al. Metformin limits osteoarthritis development and progression through activation of AMPK signalling. Ann Rheum Dis 2020;79(5):635-645.
10. Wang C, Yao Z, Zhang Y, Yang Y, Liu J, Shi Y, et al. Metformin Mitigates Cartilage Degradation by Activating AMPK/SIRT1-Mediated Autophagy in a Mouse Osteoarthritis Model. Front Pharmacol 2020; 11:1114.
11. Gao H, Gui J, Wang L, Xu Y, Jiang Y, Xiong M, et al. Aquaporin 1 contributes to chondrocyte apoptosis in a rat model of osteoarthritis. Int J Mol Med 2016;38(6): 1752-1758.
12. Cheng XY, Li YY, Huang C, Li J, Yao HW. AMP-activated protein kinase reduces inflammatory responses and cellular senescence in pulmonary emphysema. Oncotarget 2017;8(14):22513-22523.
13. Pritzker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, et al. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14(1):13-29.
14. Tong X, Wang Y, Dong B, Li Y, Lang S, Ma J, et al. Effects of genus Epimedium in the treatment of osteoarthritis and relevant signaling pathways. Chin Med 2023;18(1):92.
15. Zhang W, Li R, Wang S, Mu F, Jia P. Effect of Chinese traditional herb Epimedium grandiflorum C. Morren and its extract Icariin on osteoarthritis via suppressing NF-kappaB pathway. Indian J Exp Biol 2013;51(4):313-321.
16. Zhang J, Fan F, Liu A, Zhang C, Li Q, Zhang C, et al. Icariin: A Potential Molecule for Treatment of Knee

Osteoarthritis. Front Pharmacol 2022;13:811808.
17. Hwang HS, Kim HA. Chondrocyte Apoptosis in the Pathogenesis of Osteoarthritis. Int J Mol Sci 2015; 16(11):26035-26054.
18. Xiao SQ, Cheng M, Wang L, Cao J, Fang L, Zhou XP, et al. The role of apoptosis in the pathogenesis of osteoarthritis. Int Orthop 2023;47(8):1895-1919.
19. Elsaid KA, Fleming BC, Oksendahl HL, Machan JT, Fadale PD, Hulstyn MJ, et al. Decreased lubricin concentrations and markers of joint inflammation in the synovial fluid of patients with anterior cruciate ligament injury. Arthritis Rheum 2008;58(6):1707-1715.
20. Nguyen TH, Duong CM, Nguyen XH, Than UTT. Mesenchymal Stem Cell-Derived Extracellular Vesicles for Osteoarthritis Treatment: Extracellular Matrix Protection, Chondrocyte and Osteocyte Physiology, Pain and Inflammation Management. Cells 2021; 10(11):2887.
21. Xu R, Wu J, Zheng L, Zhao M. Undenatured type II collagen and its role in improving osteoarthritis. Ageing Res Rev 2023;91:102080.
22. Carbone A, Rodeo S. Review of current understanding of post-traumatic osteoarthritis resulting from sports injuries. J Orthop Res 2017;35(3):397-405.
23. Hu Q, Ecker M. Overview of MMP-13 as a Promising Target for the Treatment of Osteoarthritis. Int J Mol Sci 2021;22(4):1742.
24. Wan Y, Li W, Liao Z, Yan M, Chen X, Tang Z. Selective MMP-13 Inhibitors: Promising Agents for the Therapy of Osteoarthritis. Curr Med Chem 2020;27(22):3753-3769.
25. Liou SF, Hsu JH, Chu HC, Lin HH, Chen IJ, Yeh JL. KMUP-1 Promotes Osteoblast Differentiation Through cAMP and cGMP Pathways and Signaling of BMP-2/Smad1/5/8 and Wnt/β-Catenin. J Cell Physiol 2015; 230(9):2038-2048.
26. Urist MR, Strates BS. Bone morphogenetic protein. J Dent Res 1971;50(6):1392-1406.
27. Bae JS, Gutierrez S, Narla R, Pratap J, Devados R, van Wijnen AJ, et al. Reconstitution of Runx2/Cbfa1-null cells identifies a requirement for BMP2 signaling through a Runx2 functional domain during osteoblast differentiation. J Cell Biochem 2007;100(2):434-449.
28. Ito Y, Fukushima H, Katagiri T, Seo Y, Hirata S, Zhang M, et al. Lactacystin, a proteasome inhibitor, enhances BMP-induced osteoblastic differentiation by increasing active Smads. Biochem Biophys Res Commun 2011; 407(1):225-229.
29. Li X, Yang HY, Giachelli CM. BMP-2 promotes phosphate uptake, phenotypic modulation, and calcification of human vascular smooth muscle cells. Atherosclerosis 2008;199(2):271-277.
30. Komori T. Whole Aspect of Runx2 Functions in Skeletal Development. Int J Mol Sci 2022;23(10):5776.
31. Liu Q, Li M, Wang S, Xiao Z, Xiong Y, Wang G. Recent Advances of Osterix Transcription Factor in Osteoblast Differentiation and Bone Formation. Front Cell Dev Biol 2020;8:601224.
32. Matsubara T, Kida K, Yamaguchi A, Hata K, Ichida F, Meguro H, et al. BMP2 regulates Osterix through Msx2 and Runx2 during osteoblast differentiation. J Biol Chem 2008;283(43):29119-29125.
33. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 2002;108(1): 17-29.
34. Wang Y, Zhao X, Lotz M, Terkeltaub R, Liu-Bryan R. Mitochondrial biogenesis is impaired in osteoarthritis chondrocytes but reversible via peroxisome proliferator-activated receptor γ coactivator 1α. Arthritis Rheumatol 2015;67(8):2141-2153.
35. Zhu H, Yan H, Ma J, Zhang H, Zhang J, Hu Z, et al. CCAL1 enhances osteoarthritis through the NF-κB/AMPK signaling pathway. FEBS Open Bio 2020;10(12):2553-2563.
36. Wang Y, Xian H, Qi J, Wei F, Cheng X, Li S, et al. Inhibition of glycolysis ameliorate arthritis in adjuvant arthritis rats by inhibiting synoviocyte activation through AMPK/NF-κB pathway. Inflamm Res 2020;69(6):569-578.