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Original Article

Matrine inhibits IL-1β-induced expression of matrix metalloproteinases by suppressing the activation of MAPK and NF-κB in human chondrocytes *in vitro*

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Abstract: Interleukin (IL)-1β plays an important role in promoting osteoarthritis (OA) lesions by inducing chondrocytes to secrete matrix metalloproteinases (MMPs), which degrade the extracellular matrix and facilitate chondrocyte apoptosis. Matrine was shown to exert anti-inflammatory effects. However, the role of matrine in OA is still unclear. Therefore, in this study, we investigated the effects of matrine on the expression of MMPs in IL-1β-treated human chondrocytes and the underlying mechanism. The cell viability of chondrocytes was detected by MTT assay. The cell apoptosis of chondrocytes was measured by flow cytometric analysis. The protein production of MMPs was determined by ELISA. The protein expression of phosphorylation of mitogen-activated protein kinases (MAPKs) and the inhibitor of kappaB alpha (IκBα) was determined by Western blot. Matrine significantly inhibited the IL-1β-induced apoptosis in chondrocytes. It also significantly inhibited the IL-1β-induced release of MMP-3 and MMP-13, and increased the production of TIMP-1. Furthermore, matrine inhibits the phosphorylation of p-38, extracellular regulated kinase (ERK), c-Jun-N-terminal kinase (JNK) and IκBα degradation induced by IL-1β in chondrocytes. Taken together, our results show that matrine inhibits IL-1β-induced expression of matrix metalloproteinases by suppressing the activation of MAPK and NF-κB in human chondrocytes in vitro. Therefore,-matrine may be beneficial in the treatment of OA.

Keywords: Matrine, interleukin (IL)-1β, chondrocyte, matrix metalloproteinases (MMPs)

Introduction

Osteoarthritis (OA) is a common arthritic disease which gradually leads to cellular changes, structural defects and dysfunction of all the joint compartments, i.e. cartilage, bone and synovium [1]. OA is characterized by degradation of extracellular matrix macromolecules and decreased expression of chondrocyte protein and resulted in severe joint pain, loss of movement, and progressive irreversible dysfunction [2]. It is well known that matrix metalloproteinases (MMPs) are considered critical to degrading ECM [3]. MMP-3 can aggravate inflammation via activating various pro-MMPs such as pro-MMP-1, pro-MMP-7, pro-MMP-8, pro-MMP-9, and pro-MMP-13 and cleaves extracellular components including collagen types III, IX, and X and telopeptides of collagen types I, II, and XI [4]. MMP-13 degrades the extracellular matrix, including the cartilagespecific component type II collagen during the progression of OA [5]. In addition, pro-inflammatory cytokines such as interleukin (IL)-1β, play an important role in the progression of OA. IL-1β caused cartilage damage through modulating the expression of MMPs, inducing proteoglycan degradation and causing cellular apoptosis [6]. Currently, although nonsteroidal anti-inflammatory drugs (NSAID) have been used clinically for the past few years to treat OA, these agents have negative effects on cartilage and potential adverse effects [7]. Therefore, there is a need to find new agents which can reverse cartilage degradation.

Recently, accumulating evidence suggests that active ingredients from natural products play an important role in the prevention and treatment of OA. For example, green tea polyphenol epigallocatechin-3-gallate (EGCG), a major green tea polyphenol, protects human chondcytes

from the catabolic degradation of cartilage matrix protein by inhibiting the tumor necrosis factor-α (TNF-α), MMPs production [8]. Curcumin inhibited the matrix degradation by decreasing the production of MMP-3, MMP-9 and MMP-13 in chondroctytes [9].

Matrine, one of the main alkaloid components extracted from Sophora [10], bitter beans, broad beans, and other leguminous Sophora root plants, belongs to tetracyclic quinolizidine alkaloids. It has been used to treat inflammatory diseases, such as enteritis and hepatitis [11, 12]. Several lines of evidence have shown that matrine exerts anti-inflammatory effects by inhibiting inflammatory signal activation and pro-inflammatory mediator production in fibroblasts [13], Kupffer cells [14], and rat intestinal microvascular endothelial cells [15]. In addition, it has been reported that matrine could down-regulate the increase in levels of TNF-α, and interleukin-6 (IL-6) induced by lipopolysaccharide [12]. However, the role of matrine in OA is still unclear. Therefore, in this study, we investigated the effect of matrine on the expression of MMPs in IL-1β-treated human chondrocytes *in vitro* and the underlying mechanism.

Materials and methods

Reagents

Matrine, IL-1β, pronase, collagenase, dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO, USA). Phosphate-buffered saline (PBS), TBST (Tris buffered saline and Tween 20) were obtained from Abcam (Cambridge, UK). Human MMP-3, MMP-13 and tissue inhibitor of metalloproteinase 1 (TIMP-1) ELISA kits were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). p38, p-p38, extracellular signal-regulated kinase (ERK) 1/2, p-ERK, c-Jun N-terminal kinase (JNK), p-JNK, inhibitor of κB < alpha > (IκBα) and β-actin were obtained from Invitrogen (Carlsbad, CA, USA).

Human articular cartilage chondrocytes culture

Human articular cartilage was obtained from the femoral chondyle and tibia plateau of 10 patients, six males and four females (mean ± SD age: 67.3 ± 6.4 years), who had undergone arthroplasty at the Chenzhou No. 1 People's Hospital affiliated the University of South China. Two orthopedists read sites from all regions of the knee joint under a microscope. Cartilage was collected according to the medical ethical regulations of the Chenzhou No. 1 People's Hospital affiliated the University of South China. Chondrocytes were isolated from cartilage as previously described [16]. Cartilages pieces were digested with 0.25% trypsin for 15 min and incubated with 0.2% (v/v) collagenase for 4 h at 37°C. The resulting cells were cultured in 24-well plates with 400 μl complete culture medium. The complete culture medium consisted of Dulbecco's modified Eagle's medium (DMEM), supplemented with 10 mM HEPES, penicillin (100 IU/ml), streptomycin (100 μg/ml), and 5% fetal bovine serum (FBS). After 24 h, cartilage medium was changed to basal culture medium (DMEM, supplemented with 10 mM HEPES, penicillin 100 IU/ml, streptomycin 100 μg/ml and 2% FBS).

Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide (MTT) assay was used to detect cell viability. In brief, human chondrocytes (1×10^4/well) in 96-well plates were pretreated with or without different concentrations (25, 50 and 100 μg/ml) of matrine for 2 h and then co-incubated in the absence or presence of IL-1β (10 ng/ml) for 24 h. Then MTT was added to the cells at a final concentration of 0.5 mg/ml before the end of the experiment and incubated for 4 h at 37°C. The supernatant was removed, and the crystals were dissolved in 100 μl DMSO. Absorbance at 490 nm was measured using a Bio-Rad microplate reader (Bio-Rad, Hercules, CA, USA).

Cell apoptosis assay

For flow cytometry experiment, the cells were divided into four groups: control, cells were cultured without any treatment; IL-1β, cells cultured for 24 h with 10 ng/ml IL-1β; matrine alone, cells cultured for 24 h with matrine (100 μg/ml) without IL-1β; IL-1β+matrine, cells cultured for 2 h with matrine (100 μg/ml) followed by 24 h with 10 ng/mL IL-1β. The cell apoptotic ratio was measured by annexin V-FITC and PI staining followed by analysis with flow cytometry (Beckman-Coulter, Brea, CA). In brief, 2×10^5 cells per well were plated in 24-well plates. Cells were trypsinized and harvested by centrifugation and then incubated with Annexin V and PI for 15 min at room temperature. Apop-

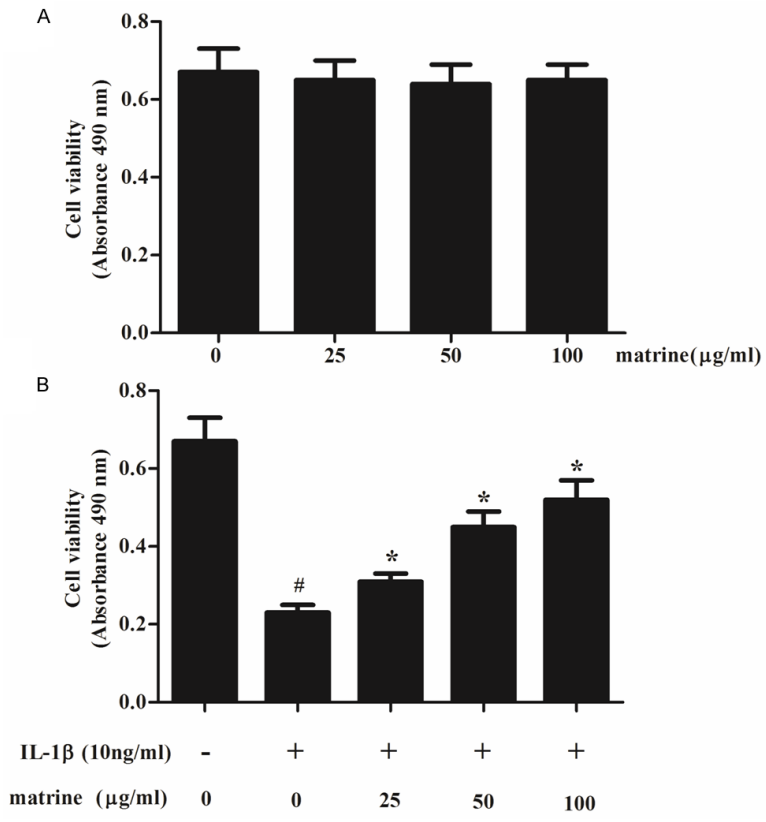

Figure 1. Effects of matrine on chondrocyte viability. A. Human chondrocytes (1×10^4/well) in 96-well plates were pretreated with various concentrations of matrine (25, 50 and 100 μg/ml) for 24 h, and the MTT assay was performed to detect cell viability. B. the chondrocytes were divided into five groups: control, cells were cultured without any treatment; IL-1β, cells cultured with IL-1β (10 ng/ml); cells were incubated with matrine (25, 50 and 100 μg/ml) for 2 h, followed by co-incubation with IL-1β (10 ng/ml). After 24 h, cell viability was determined using the MTT reagent. All data are mean ± SD obtained from five separate experiments performed in triplicate. $^{\#}P < 0.05$ compared with the control group; $^{*}P < 0.05$ compared with the IL-1β group.

tosis was examined by flow cytometry using Annexin V-FITC/PI kit (Abcam, Cambridge, UK). After 30 min, the cells were ready for the analysis by the flow cytometry and the cell apoptotic ratio was determined.

ELISA

Culture medium was collected for ELISA assay using human MMP-3, MMP-13 and TIMP-1 ELISA kits according to the manufacturer's instructions (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Western blot

Chondrocytes were pretreated with matrine (100 μg/ml) for 24 h, followed by stimulation with IL-1β (10 ng/ml) for 30 min, then chondrocytes were placed in 7 volumes of cold homogenization buffer (100 mM Tris, 150 mM NaCl, 1% triton X-100) to which a cocktail of protease inhibitors had been freshly added. The total protein was extracted by using RIPA lysis buffer (Beyotime, Nantong, China) according to the operating protocols. The protein concentration in the lysates was determined by BCA protein assay kit (Beyotime, Nantong, China). Protein aliquots (30 μg) were separated on 12% SDS-PAGE gels and transferred to a PVDF membrane (Millipore, Boston, MA, USA). Membranes were hybridized with antibodies against p38, p-p38, ERK 1/2, p-ERK, JNK, p-JNK, IκBα and β-actin (Invitrogen, Carlsbad, CA, USA). After washing with TBST, blots were then incubated with horseradish peroxidase-linked secondary antibodies (Invitrogen, Carlsbad, CA, USA) at room temperature for 1 h. The specific protein bands were developed using a chemiluminescent substrate and imaged using a gel scanner. Protein levels were normalized to β-actin as a reference.

Statistical analysis

Data were analyzed using SPSS 13.0 statistical software (SPSS Inc, Chicago, IL, USA). Results are shown as mean ± SD unless stated otherwise. One-way ANOVA analysis or Student's t test was used for the statistical comparison of multiple groups. $P < 0.05$ was considered statistically significant.

Results

Effects of matrine on chondrocyte viability

We first examined whether matrine could promote the viability of human chondrocytes incubated with IL-1β. As shown in **Figure 1A**, compared with untreated chondrocytes, treatment

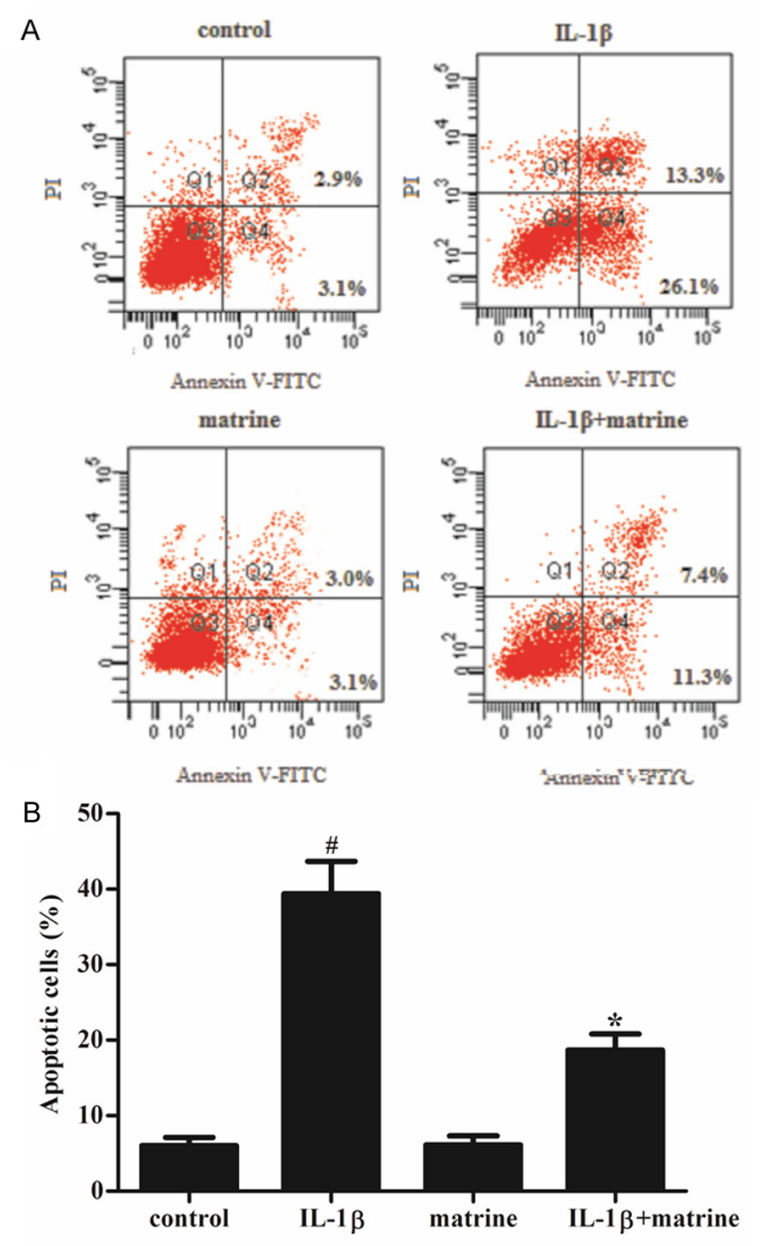

Figure 2. Effects of matrine on IL-1β-induced apoptosis in chondrocytes. Chondrocytes were treated with 100 μg/ml of matrine for 2 h prior to 24 h treatment with 10 ng/ml IL-1β. A. Annexin V/PI staining and flow cytometry assays were employed to assess chondrocyte apoptosis. B. apoptotic chondrocytes were quantified as % of total cells. All data are mean ± SD obtained from five separate experiments performed in triplicate. $^{\#}P < 0.05$ compared with the control group; $^{*}P < 0.05$ compared with the IL-1β group.

with matrine alone did not obviously affect cell viability. Furthermore, IL-1β significantly reduced the cell viability, however, pretreatment with matrine reversed this effect, exhibiting a dosedependent manner (**Figure 1B**). Highest inhibition was observed with 100 μg/ml matrine treatment.

Effects of matrine on IL-1β-induced apoptosis of chondrocytes

To study whether IL-1β induced cytotoxicity was mediated by apoptotic process, we used flow cytometry assay to assess chondrocyte apoptosis. Chondrocytes were pretreated with matrine (100 μg/ml) for 2 h before adding IL-1β (10 ng/ml) to treat the cells for 24 h. As shown in **Figure 2**, IL-1β significantly increased the apoptosis of chondrocytes, however, pretreatment with matrine significantly reduced the percentage of IL-1β induced apoptotic chondrocytes.

Effects of matrine on the protein production of MMP-3, MMP-13 and TIMP-1

It is well known that MMPs and TIMPs play a critical role in bone remodeling and arthritis, therefore, we investigated the effects of ma-trine on the protein production of MMP-3, MMP-13 and TIMP-1 in IL-1β-induced chondrocytes by ELISA. As shown in **Figure 3**, chondrocytes stimulated with IL-1β (10 ng/ml) showed enhanced release of MMP-3 and MMP-13 compared to untreated controls ($P < 0.05$). Furthermore, the protein production of TIMP-1 was significantly decreased by IL-1β. However, treatment of chondrocytes in the presence of matrine significantly inhibited the IL-1β-induced release of MMP-3 and MMP-13, and increased the production of TIMP-1.

Effects of matrine on MAPK signaling pathway

Activation of MAPK signaling pathway has been reported to participate in chondrocyte apoptosis induced by various stimuli. Therefore, to understand the molecular mechanism by which matrine inhibits IL-1β-induced apoptosis, we

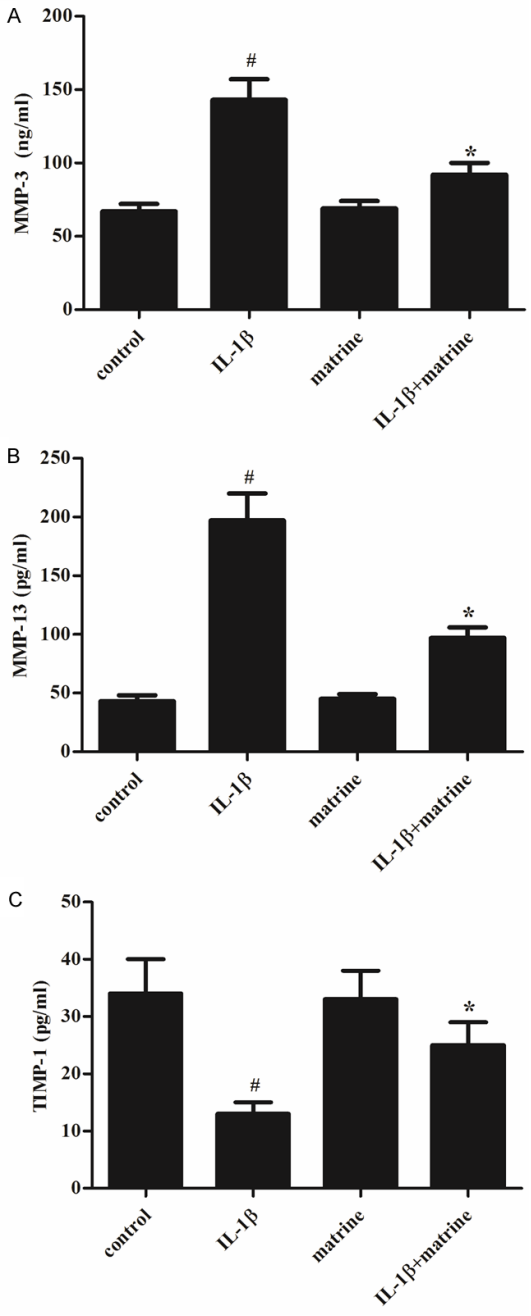

Figure 3. Effects of matrine on the protein production of MMP-3, MMP-13 and TIMP-1 in chondrocytes. Chondrocytes were pretreated with matrine (100 μg/ml) for 2 h, followed by stimulation with IL-1β (10 ng/ml) for 24 h. ELISA was performed to determine the protein production of MMP-3, MMP-13 and TIMP-1 in chondrocytes. All data are mean ± SD obtained from five separate experiments performed in triplicate. $^{\#}P < 0.05$ compared with the control group; $^{*}P < 0.05$ compared with the IL-1β group.

investigated any involvement of MAPK pathway by Western blot. As shown in **Figure 4**, IL-1β significantly increased the protein expression levels of p-p38, p-ERK and p-JNK, however, matrine could decreased the protein expression levels of p-p38, p-ERK and p-JNK induced by IL-1β.

Effects of matrine on NF-κB signaling pathway

NF-κB is considered another important factor in cartilage degradation in OA. And, it has been reported that in human chondrocytes, MMPs are direct target genes of NF-κB signaling, therefore, we further explored whether the effect of matrine on MMPs expression was due to a cross-talk with NF-κB. As shown in **Figure 5**, IL-1β stimulation resulted in IκB-α degradation, however, in chondrocytes treated with matrine, the IL-1β-induced degradation of IκBα was prevented.

Discussion

OA is regarded as a non-inflammatory arthropathy with symptoms of local inflammation characterized primarily by cartilage degradation [17]. Currently, there are no effective pharmacological treatments to treat OA although some drugs reduce symptoms and slow the progression of OA. Several lines of evidence have shown that matrine possesses multiple biological functions, including anti-inflammatory effects. In this study, our results show that matrine inhibited IL-1β-induced apoptosis of chondroctytes, and it also inhibited the protein production of MMP-3 and MMP-13 and enhanced TIMP-1 production. Furthermore, matrine suppressed IL-1β-induced activation of MAPK and NF-κB signaling pathways.

Chondrocytes apoptosis is a critical step in pathogenesis of OA [18]. It can be induced by various stimuli, such as mechanical stress, cytokines, and inflammatory mediators. IL-1β, a cytokine released by synovial cells and macrophages, plays an important role in amplifying inflammation in OA. In this study, we found that IL-1β induced the apoptosis of chondrocytes. The results of this study agree with previous reports showing that IL-1β suppresses viability and induces apoptotic signaling in human chondrocytes harvested from articular cartilage and OA cartilage [19, 20]. Furthermore, matrine significantly reduced the percentage of IL-1β induced apoptotic chondrocytes, suggesting that matrine prevents the degradation of artic-

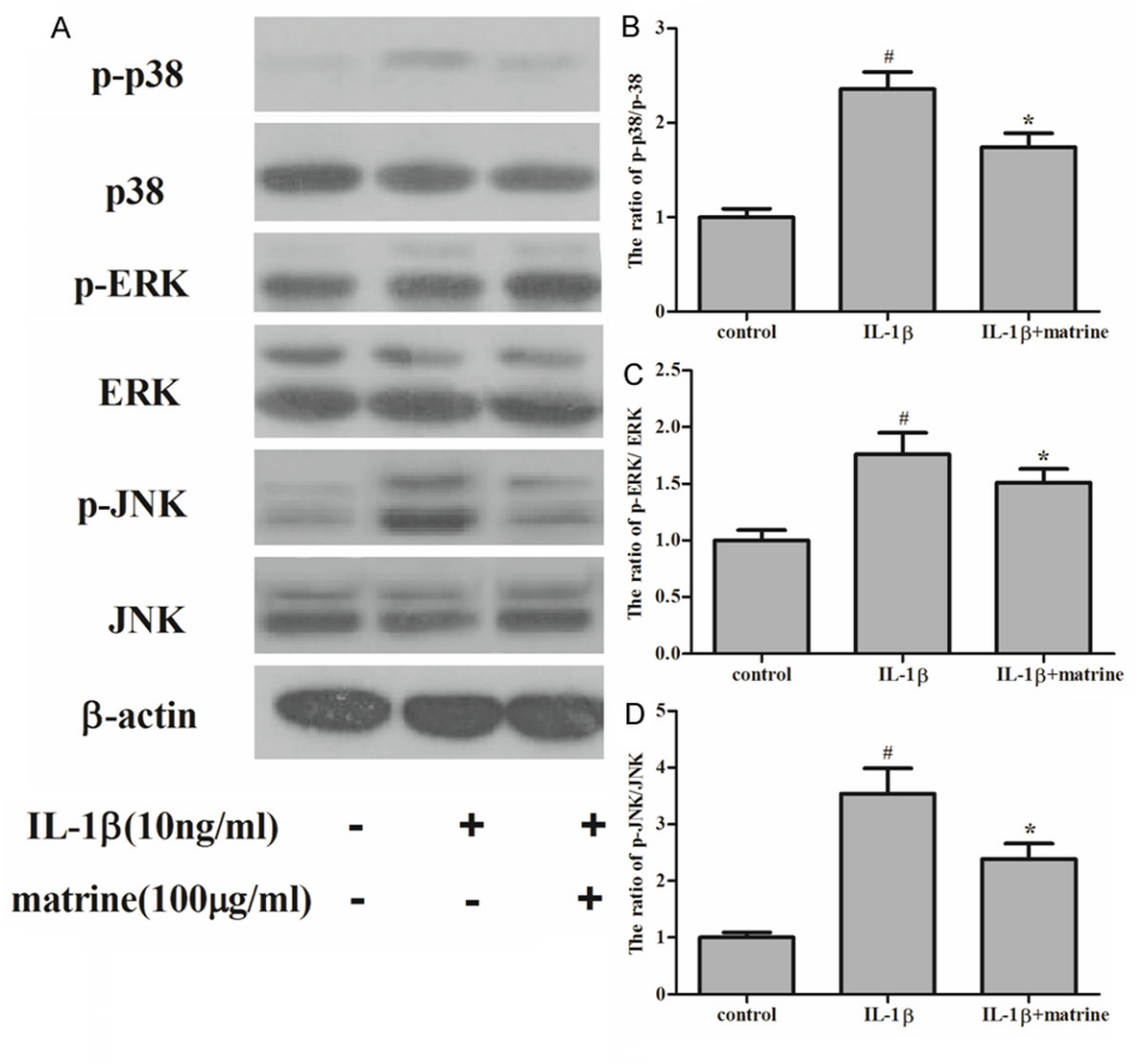

Figure 4. Effects of matrine on the phosphorylation of MAPK. Chondrocytes were divided into three groups: control, cells were cultured without any treatment; IL-1β, cells cultured with IL-1β (10 ng/ml); cells were pretreated with matrine for 24 h, followed by stimulation with IL-1β (10 ng/ml) for 30 min. Equal amounts (30 μg protein per lane) of total proteins were separated by SDS-PAGE and analyzed by immunoblotting with anti-p-p38, anti-p38, anti-p-ERK, anti-ERK, anti-p-JNK and anti-JNK antibodies. A. Matrine significantly inhibited the IL-1β-induced phosphorylation of p38, ERK and JNK. B-D. Relative protein expression was quantified using Image-Pro Plus 6.0 software and normalized to β-actin. The results are representative of three separate experiments. #P < 0.05 compared with the control group; *P < 0.05 compared with the IL-1β group.

ular cartilage by inhibiting chondrocyte apoptosis.

Increasing evidences have demonstrated that the levels of MMPs were increased in OA [21-23], therefore, it is reasonable to reduce cartilage degradation via inhibiting the MMPs activities. In this study, we found that matrine obviously decreased the protein production of MMP-3 and MMP-13, and increased the protein production of TIMP-1 in IL-1β-treated human chondrocytes. Similar to our results, an earlier study found that matrine significantly reduced the levels of MMP-2 and MMP-9 in breast cancer cells [24]. These results suggest that matrine controlled cartilage loss by down-regulating MMP-3 and MMP-13, and up-regulating TIMP-1 in IL-1β-treated chondrocytes.

MAPK consist of three subfamilies: ERK1/2, p38, and JNK. These kinases play a critical role in regulating a variety of cellular activities, such as cell growth, differentiation, and apoptosis [25, 26]. It has been reported that IL-1β stimu-

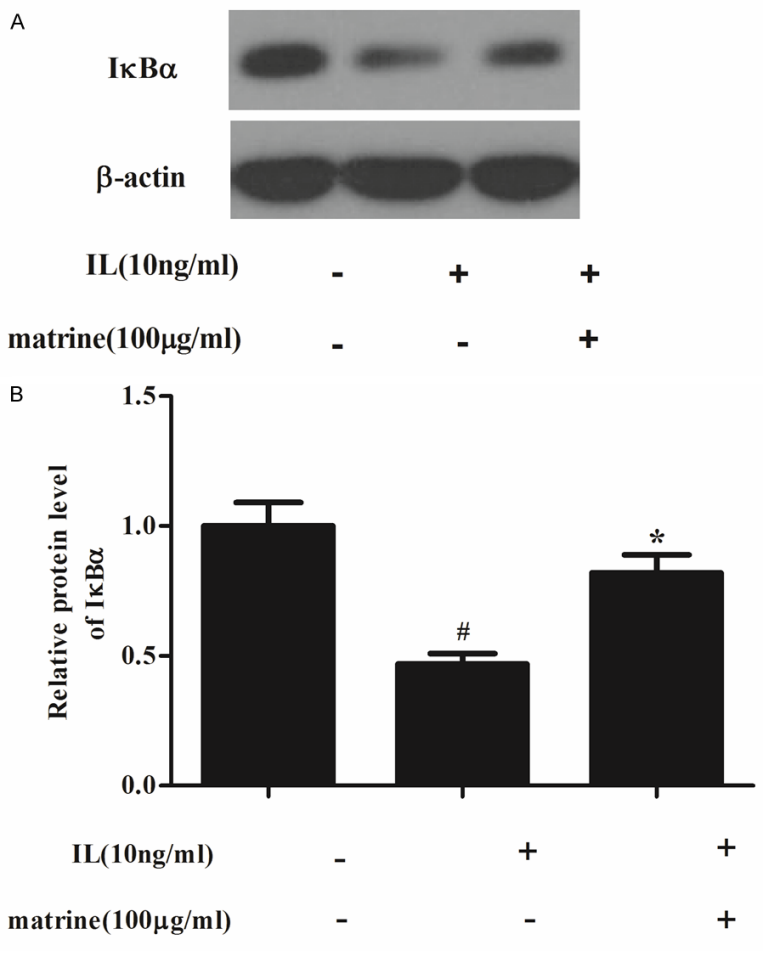

Figure 5. Effects of matrine on NF-κB signaling pathway. Chondrocytes were divided into three groups: control, cells were cultured without any treatment; IL-1β, cells cultured with IL-1β (10 ng/ml); cells were pretreated with matrine for 24 h, followed by stimulation with IL-1β (10 ng/ml) for 30 min. A. Equal amounts (30 μg protein per lane) of total proteins were separated by SDS-PAGE and analyzed by immunoblotting with anti-IκBα and anti-β-actin (control). IL-1β stimulation resulted in IκBα degradation while the degradation was inhibited by matrine. B. Relative protein expression of IκBα was quantified using Image-Pro Plus 6.0 software and normalized to β-actin. The results are representative of three separate experiments. #P < 0.05 compared with the control group; *P < 0.05 compared with the IL-1β group.

lation of human OA chondrocytes leads to increased phosphorylation and activation of MAPKs, such as p38, ERK1/2 and JNK kinase [27, 28]. In accordance with previous reports, we also found that IL-1β increased phosphorylation of p38, ERK and JNK kinase. It has been reported that matrine reduced the phosphorylated levels of ERK1/2 proteins in human osteosarcoma cells [29]. Another study reported that matrine obviously reduced the phosphorylation level of p38 in human colorectal cancer cells [30]. In this study, we found that matrine could decrease the protein expression levels of p-p38, p-ERK and p-JNK induced by IL-1β. These results suggest that matrine inhibits IL-1β-induced apoptosis through suppressing MAPK signaling pathway.

It is well known that NF-κB also plays a critical role in cartilage degradation in OA. Under unstimulated conditions, the NF-κB dimers are located in an inactive form in the cytoplasm bound to IκB molecules. The potentiation of NF-κB proteins occurs upon stimulation of cells by a variety of chemical and mechanical signals that leads to phosphorylation of IκBs by IκB kinases (IKKs) and their subsequent degradation through the ubiquitin-proteasome system [31]. In OA, the chondrocytes express a variety of NF-κB-mediated catabolic cytokines and chemokines, such as TNF-α, IL-1β, IL-6, receptor activator of NF-κB (RANK) ligand (RANKL) and IL-8, that increase the production of MMPs, decrease collagen and proteoglycan synthesis and act in a positive feedback loop to augment NF-κB activation [32]. Moreover, in human chondrocytes, the NF-κB inhibitor reduces IL-1β-induced MMP-3 and MMP-13 production [33]. Moreover, previous investigations have shown that matrine inhibited MMP-9 expression and the invasion of human HCC cells, and the inhibitory effects are partly associated with the downregulation of the NF-κB signaling pathway [34]. Matrine also protects neurons and astrocytes via inhibition of NF-κB signaling pathway [35]. In this study, we found that matrine suppressed IL-1β-induced IκBα degradation. These results suggest that matrine inhibits IL-1β-induced protein production of MMPs by suppressing the activation of NF-κB signaling pathway.

In conclusion, matrine inhibits IL-1β-induced expression of MMPs by suppressing the activation of MAPK and NF-κB signaling pathways in human chondrocytes. Therefore, matrine is a great candidate to develop further OA treatments.

Disclosure of conflict of interest

None.

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