Aminoguanidine inhibits IL-1β-induced protein expression of iNOS and COX-2 by blocking the NF-κB signaling pathway in rat articular chondrocytes

YUANQIANG MA, XIAOPENG SONG, TIANWEN MA, YUE LI, HUI BAI,
ZHIHENG ZHANG, HAILONG HU, RUI YUAN, YAJING WEN and LI GAO

Department of Clinical Surgery, College of Veterinary Medicine, Northeast
Agricultural University, Harbin, Heilongjiang 150000, P.R. China

Abstract. Osteoarthritis is a chronic joint disease which has a serious impact on the health and quality of life of affected humans and animals. As an inhibitor of inducible nitric oxide synthase (iNOS), aminoguanidine (AG) displays anti-inflammatory effects. The purpose of the present study was to investigate the effect of AG on the expression of iNOS and cyclooxygenase-2 (COX-2), and the activity of the NF-κB signaling pathway in rat chondrocytes stimulated by interleukin-1β (IL-1β). The viability of chondrocytes treated with AG (0.3, 1 or 3 mM) alone was determined using a Cell Counting Kit-8 assay. Subsequently, the chondrocytes were treated with either 10 ng/ml IL-1β alone, or co-treated with increasing concentrations of AG (0.3, 1 or 3 mM) and 10 ng/ml IL-1β. The protein levels of COX-2, iNOS, phosphorylated (p)-p65, p65, p-NF-κβ inhibitor α (IκBα), IκBα, p-inhibitor of NF-κβ-β (IKKβ) and IKKβ were evaluated by western blotting. NF-κB translocation was determined by immunofluorescence analysis. Western blotting and reverse transcription-quantitative PCR were used to detect expression levels of relevant proteins/genes. The results suggested that the inhibitory effect of AG on the protein and gene expression levels of iNOS and COX-2 in IL-1β-treated chondrocytes was dose-dependent. In addition, AG decreased the level of phosphorylation of IKKβ, IκBα and NF-κB p65, the degradation of IKKβ, IκBα and p65, and the translocation of NF-κB in IL-1β-stimulated chondrocytes. The most significant inhibitory effect of AG was observed at a concentration of 1 mM. Therefore, the present study suggested that AG may serve as a potential agent to reduce the inflammatory response of chondrocytes stimulated by IL-1β.

Correspondence to: Professor Li Gao, Department of Clinical Surgery, College of Veterinary Medicine, Northeast Agricultural University, 600 ChangJiang Road, Xiangfang, Harbin, Heilongjiang 150000, P.R. China
E-mail: gaoli43450@163.com

Introduction

Osteoarthritis (OA) is a common chronic and irreversible joint disease (1,2) that causes alterations to the morphology, structure and function of chondrocytes (3). Overexpression of inflammatory mediators, including cytokines [interleukin (IL)-1β], reactive oxygen species and matrix-degrading enzymes, leads to progressive deterioration of cartilage, synovium and subchondral bone (4). At present, no effective treatments to prevent or reverse progressive joint injury are available (5).

AG is an inducible nitric oxide synthase (iNOS) inhibitor, which can regulate the activity and expression levels of iNOS (6). iNOS inhibitors have been used to alleviate articular cartilage injury, pain and inflammation in a surgical model of OA (7). In addition, AG has various pharmacological effects, including anti-inflammatory effects that can modulate OA and articular inflammation (8). Additionally, it has been reported that AG protects against colonic inflammation by inhibiting the expression of NF-κB/p65 (9). In a previous study, AG treatment reduced the protein expression levels of iNOS and p65 in the liver of an animal model of diabetes (10). Furthermore, AG can reduce the level of osteocyte apoptosis during non-traumatic osteonecrosis (11). Although the pharmacological effects of AG have been studied in numerous cell types (12-17), it remains unclear whether AG can influence iNOS and cyclooxygenase-2 (COX-2) expression, and the NF-κB signaling pathway in rat chondrocytes.

The pathogenesis of OA is related to the activation of a number of different molecular pathways, such as the NF-κB and MAPK pathways (18). Activation of the NF-κB signaling pathway directly affects the pathogenesis and development of OA (19). Activated NF-κB molecules trigger increases in the levels of other proinflammatory cytokines, for example IL-1β, tumor necrosis factor α and IL-6, leading to increased extracellular degradation and reduced synthesis of collagen and proteoglycans, which further contribute to the onset of OA (20). The activation of NF-κB has been hypothesized

to be closely associated with IL-1β-stimulated OA (20). Furthermore, it has been hypothesized that the proinflammatory responses stimulated by IL-1β stimulate chondrocytes during OA, leading to increases in COX-2 and iNOS expression by activating NF-κB (21-23). In addition, NF-κB promotes articular cartilage breakdown by inducing the expression of matrix metalloproteinases and ADAMTs in OA chondrocytes (20,24). Elevated levels of NF-κB in OA chondrocytes lead to the overexpression of COX2 and iNOS, which further contribute to cartilage inflammation (25,26) and degeneration of articular cartilage (24).

Targeted therapies that interfere with NF-κB signaling may serve as a novel therapeutic strategy for the treatment of OA. In the present study, IL-1β-treated articular chondrocytes were used to investigate whether AG inhibited iNOS and COX-2 expression, and the NF-κB signaling pathway.

Materials and methods

Chondrocyte isolation and culture. Sprague-Dawley rats (age, 2-3 weeks; weight, 41±3.5 g; sex, male:female, 1:1) obtained from The Second Affiliated Hospital of Harbin Medical University (Harbin, China) were anaesthetized with isoflurane and sacrificed by cervical dislocation. Articular cartilage from the femoral heads and knees was isolated, and ground into small pieces under sterile conditions. All experiments were approved by the Departmental Animal Care and Use Committee at Northeast Agricultural University.

As previously described (27), cartilage debris was isolated with trypsin (Gibco; Thermo Fisher Scientific, Inc.) at 37°C for 30 min, and subsequently rinsed with PBS containing penicillin and streptomycin solution. The tissue was digested with collagenase Type II (Gibco; Thermo Fisher Scientific, Inc.) in PBS at 37°C for 4 h. Subsequently, the supernatant was collected and centrifuged at 500 x g, 27°C for 7 min. The harvested articular chondrocytes were placed in 25 m^2 culture flasks containing DMEM supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin, and were incubated at 37°C with 5% CO_2. When the monolayer on the bottom of the culture flask reached 80-90% confluence, the cells were passaged and the second-generation chondrocyte cells were isolated for subsequent experiments.

Chondrocyte treatment. To assess cell viability, chondrocytes were seeded in 96-well plates (5x10^4/ml, 100 μl/well), treated with 0, 0.3, 1, or 3 mM AG (Sigma-Aldrich; Merck KGaA) for 24, 48 and 72 h at 37°C and evaluated using a Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Inc.), as described below. For the evaluation of iNOS and COX-2 expression, chondrocyte cells were exposed to the following conditions for 24 h at 37°C: i) 10 ng/ml IL-1β (PeproTech, Inc.) alone or ii) co-treatment with different concentrations of AG (0.3, 1, 3 mM) and 10 ng/ml IL-1β for 24 h, after treatment with 10 ng/ml IL-1β for 24 h alone. The activity of the NF-κB signaling pathway was assessed in chondrocytes treated with either 10 ng/ml IL-1β alone for 0.5 h or co-treated with AG (0.3, 1, 3 mM) and 10 ng/ml IL-1β at 37°C for 2 h. Rat chondrocytes cultured without AG and IL-1β were used as controls.

Cell viability assay. The cytotoxicity of AG was determined using a CCK-8 assay. The assay was conducted on chondrocytes treated with varying concentrations of AG (0.3, 1 or 3 mM) for 24, 48, and 72 h, as aforementioned. Cells were incubated with 10 μl CCK-8 reagent at 37°C for 2 h. Subsequently, the absorbance at a wavelength of 450 nm was determined using a microplate reader.

Western blot analysis. AG-treated chondrocyte cells were washed with PBS and collected with cell scrapers. Total protein was extracted from AG-treated chondrocyte cells using RIPA lysis buffer (Beyotime Institute of Biotechnology) by centrifugation at 10,000 x g for 15 min at 4°C. Total protein was quantified using a bicinchoninic acid assay. Protein (15 μl/lane) was separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. Subsequently, the membranes were blocked with 5% skim-milk at room temperature for 1 h. The membranes were incubated at 4°C overnight with primary antibodies targeted against: p65 (cat. no. 8242; 1:1,000; Cell Signaling Technology, Inc.), NF-κβ inhibitor α (IκBα; cat. no. 9242; 1:1,000; Cell Signaling Technology, Inc.), inhibitor of NF-κβ-β (IKKβ; cat. no. ab124957; 1:1,000; Abcam), phosphorylated (p)-p65 (cat. no. 3033; 1:1,000; Cell Signaling Technology, Inc.), p-IκBα (cat. no. 9246; 1:1,000; Cell Signaling Technology, Inc.), p-IKKβ (cat. no. ab59195; 1:1,000; Abcam), iNOS (cat. no. WL0992a; 1:500; Wanleibio Co., Ltd.), COX-2 (cat. no. WL01750; 1:500; Wanleibio Co., Ltd.) and GAPDH (cat. no. TA-08; 1:3,000; OriGene Technologies, Inc.). Subsequently, the membranes were washed with TBST. Following primary incubation, the membranes were incubated for 2 h at 37°C with horseradish peroxidase-conjugated secondary antibody goat anti-mouse IgG and horseradish peroxidase-conjugated secondary antibody goat anti-rabbit IgG (cat. nos. ZB2305 and ZB-2301, respectively; 1:1,000; ZSGB-BIO). Protein bands were visualized using an ECL kit (Beyotime Institute of Biotechnology) and the signals were analyzed using a Tanon detection system (Tanon Science and Technology Co., Ltd.). GAPDH was used as the loading control. The densities of bands were analyzed using ImageJ (version 1.51; National Institutes of Health).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the chondrocyte cells using the BioSci™ Tissue/Cultured Cell Total RNA Extraction kit (Dakewe Bioengineering Co., Ltd.), according to the manufacturer's instructions. Total RNA (1 μg), dNTPs (cat. no. AD101-12; Beijing TransGen Biotech Co., Ltd.), primer (cat. no. AH101-02; Beijing TransGen Biotech Co., Ltd.) and TransScript®II reverse transcriptase (cat. no. AH101-02; Beijing TransGen Biotech Co., Ltd.) were used to synthesize cDNA. The temperature protocol was as follows: 50°C for 30 min and 85°C for 5 sec. Quantitative PCR (qPCR) was performed using SuperReal PreMix Plus (SYBR Green; cat. no. FP205; Tiangen Biotech Co., Ltd.) according to the manufacturer's protocols. The following thermocycling conditions used for qPCR: 15 min at 95°C for initial denaturation; followed by 40 cycles at 95°C for 15 sec, 60°C for 32 sec, and 72°C for 5 min. The following primer pairs were used for qPCR: GAPDH forward, 5'-GAT GCCCCCATGTTTGTGAT-3' and reverse, 5'-GGCATGGAC TGTGGTCATGAG-3'; iNOS forward, 5'-GAGACGCACAGG

CAGAGGTTG-3' and reverse, 5'-AGCAGGCACACGCAA
TGATGG-3'; and COX-2 forward, 5'-AGAAGCGAGGAC
CTGGGTTCA C-3' and reverse, 5'-ACACCTCTCCACCGA
TGACCTG-3'. Protein levels were quantified using the $2^{-\Delta\Delta Cq}$
method (28) and normalized to the internal reference gene
GAPDH.

Immunofluorescence assay. After treating with 10% FBS,
Second-generation chondrocytes cultured with DMEM
supplemented with 0.05% FBS were treated with 10 ng/ml
IL-1β, or co-incubated with 10 ng/ml IL-1β and 1 mM AG
for 2 h at 37°C. Subsequently, the cells were washed with
PBS and fixed with 4% paraformaldehyde for 1 h at 37°C.
The cells were washed with PBS, blocked with 10% goat
serum (cat. no. AR1009; 0.3% Triton, 1:10; Boster Biological
Technology) for 1 h at room temperature and rinsed with PBS.
Subsequently, the cells were incubated with primary antibodies
against p-p65 (cat. no. 3033; 1:100; Cell Signaling Technology,
Inc.) overnight at 4°C. Following primary incubation, the cells
were gently washed and then incubated with a horseradish
peroxidase-conjugated secondary antibody goat anti-rabbit
IgG (cat. no. ZB-2301; 1:250; OriGene Technologies, Inc.) for
2 h at 37°C. Subsequently, the cells were stained with DAPI
(Beyotime Institute of Biotechnology) at room temperature for
15 min. Cells were rinsed and observed under a fluorescence
microscope in six randomly-selected fields (magnification,
x400).

Statistical analysis. Statistical analyses were performed using
SPSS software (version 18.0; SPSS, Inc.). Data are presented
as the mean ± standard deviation. And all experiments were
performed at least three times. Differences were assessed
using one-way ANOVA followed by Tukey's post hoc test.
$P<0.05$ was considered to indicate a statistically significant
difference.

Results

Effect of AG on cell viability. To determine whether different
concentrations of AG were cytotoxic to rat chondrocytes, cells
were treated with AG at varying concentrations (0.3, 1 or
3 mM) for 24, 48, and 72 h. The results suggested that treat-
ment with AG did not significantly alter the viability of the
cells, as measured by a CCK-8 assay ($P>0.05$; Fig. 1).

*Effect of AG on IL-1β-induced expression levels of iNOS
and COX2 in chondrocytes.* Chondrocytes treated with IL-1β
displayed significantly increased COX-2 and iNOS expression
levels compared with the control cells (Fig. 2A-E). Compared
with cells stimulated with IL-1β alone, the protein expression
levels of iNOS and COX-2 were decreased in a dose-dependent
manner by AG ($P<0.05$; Fig. 2A-C). The gene expression levels
of iNOS and COX-2 were also decreased in a dose-dependent
manner by AG ($P<0.05$; Fig. 2D and E).

*Effect of AG on the activity of NF-κB in chondrocytes
induced by IL-1β.* Western blot analysis suggested that the
levels of p-IKKβ and p-IκBα were significantly increased in
IL-1β-stimulated chondrocytes compared with the control
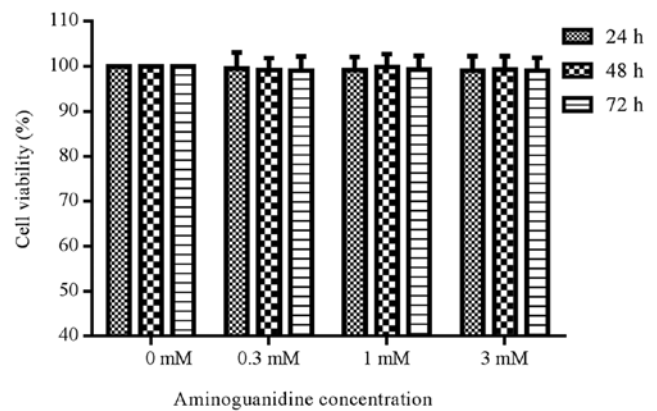cells ($P<0.05$; Fig. 3A, C and E). Moreover, the protein levels

Figure 1. Cytotoxicity of different concentrations of AG on chondrocytes.
AG did not alter the viability of rat chondrocytes. AG, aminoguanidine.

of p-IKKβ and p-IκBα were significantly reduced in chondro-
cytes co-treated with AG and IL-1β ($P<0.05$; Fig. 3C and E).
Interestingly, the lowest p-IKKβ and p-IκBα protein expression
levels were observed in chondrocytes treated with 1 mM AG
(Fig. 3C and E). Chondrocyte treatment with IL-1β significantly
reduced the protein expression levels of IκBα ($P<0.05$; Fig. 3D)
and IKKβ levels ($P<0.05$; Fig. 3B) compared with the control
cells. Chondrocytes co-cultured with AG and IL-1β displayed
decreased expression levels of IKKβ and IκBα compared
with the IL-1β group (Fig. 3B and D). Similar to the results
obtained for p-IKKβ/IKKβ and p-IκBα/IκBα, the highest
inhibitory effect of AG on IL-1β-induced p-IKKβ/IKKβ and
p-IκBα/IκBα alterations was observed in cells treated with
1 mM AG (Fig. 3C and D).

Treatment with IL-1β significantly reduced the protein
expression levels of NF-κB p65 in chondrocytes compared
with the control cells (Fig. 3F). By contrast, co-treatment
with AG and IL-1β resulted in significantly higher expres-
sion levels of p65 compared with cells stimulated with
IL-1β alone ($P<0.05$; Fig. 3F). Compared with cells stimu-
lated with IL-1β alone, co-treatment with 0.3, 1 or 3 mM
AG and IL-1β significantly reduced the p-p65/p65 ($P<0.05$;
Fig. 3G). Importantly, the greatest decrease in p-p65 levels
was observed in the group treated with 1 mM AG (Fig. 3G).
Taken together, the results suggested that AG inhibited the
activity of NF-κB in chondrocytes activated by IL-1β and
that AG displayed the strongest inhibitory effect at a concen-
tration of 1 mM.

Effect of AG on the nuclear translocation of NF-κB p65. No
p65 staining was observed in the nuclei of untreated control
cells, indicated by no green fluorescence inside the nucleus
(Fig. 4). However, IL-1β-stimulated chondrocytes displayed
p65 staining in the nucleus, but not in the cytoplasm (Fig. 4).
However, IL-1β-stimulated chondrocytes displayed p-p65
staining in the nucleus. Therefore, strong green fluorescence
was observed in the nucleus (Fig. 4). In cells co-treated with
1 mM AG and 10 ng/ml IL-1β, the intensity of green fluo-
rescence in the nucleus decreased. The levels of cytoplasmic
staining were also low in this group (Fig. 4). Therefore, these
results suggested that the entry of p-p65 into the nucleus was
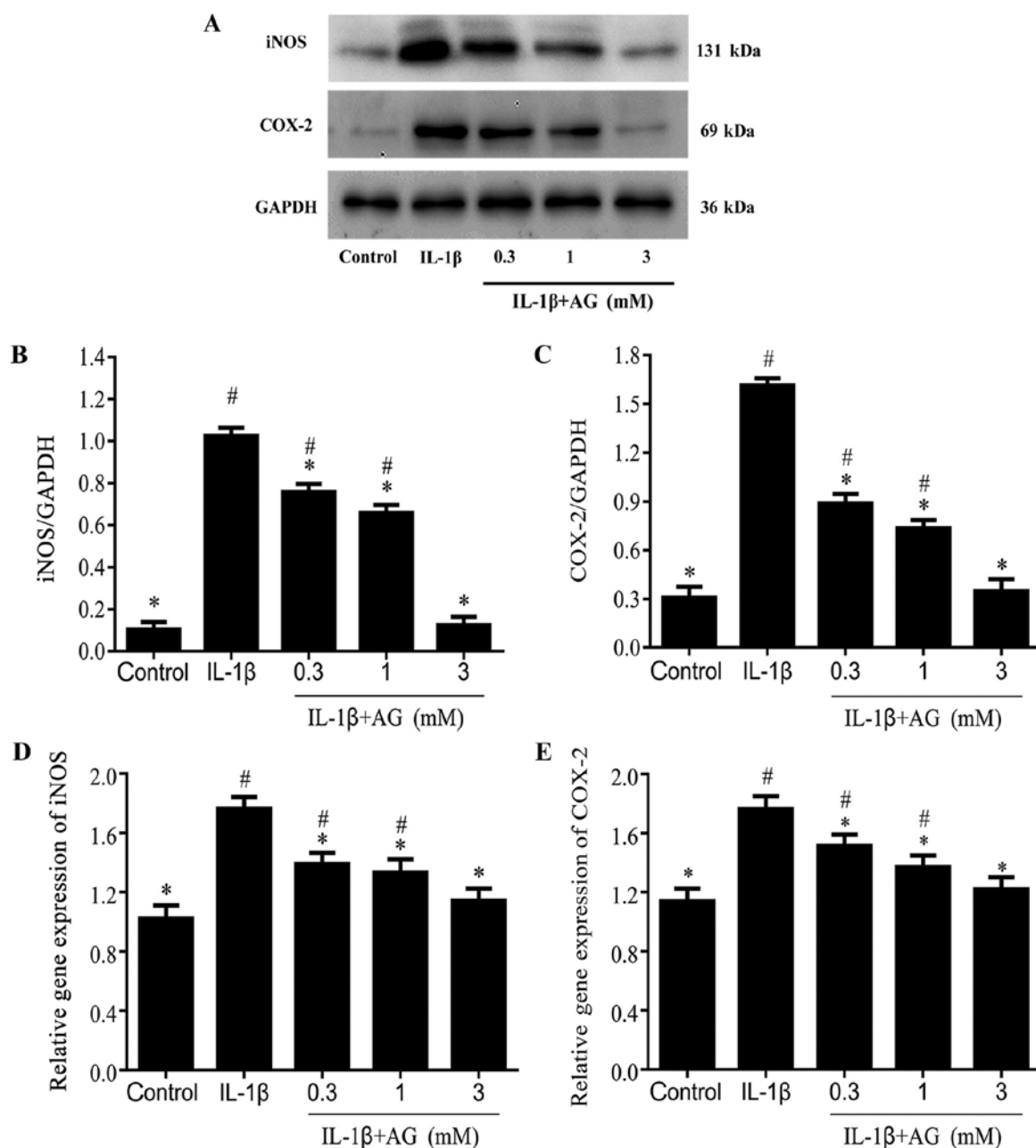limited in cells co-treated with AG and IL-1β.

Figure 2. Protein and mRNA expression levels of iNOS and COX-2 in the different treatment groups. (A) Western blotting was used to determine the protein expression levels of iNOS and COX-2. Quantification of western blotting for (B) iNOS and (C) COX-2. mRNA expression levels of (D) iNOS and (E) COX-2. #P<0.05 vs. the control group. *P<0.05 vs. the IL-1β group. iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; IL, interleukin; AG, aminoguanidine.

Discussion

OA is a chronic joint disease (1) and at present, no effective preventive or therapeutic drugs are available for the treatment of the disease (2). Therefore, the development of effective compounds for the treatment of OA is important. In the present study, the potential of AG, a compound that inhibits iNOS, to prevent or delay the progression of OA was investigated.

The roles of AG as an inhibitor of oxidation (29), apoptosis (30,31) and inflammation (32-34) have been widely recognized and accepted. In addition to these well-recognized roles, AG can inhibit the generation of isoproterenol-induced reactive oxygen species, and restore levels of antioxidant superoxide dismutase, glutathione and catalase in the heart (35). AG can also suppress the production of nitric oxide and the expression of iNOS in osteocytes, leading to the release of cytochrome C and the induction of osteocyte apoptosis (11). Moreover, AG can decrease the accumulation of glycosylation products, which can lead to endoplasmic reticulum stress-induced cell apoptosis (36). Furthermore, AG mediates articular inflammatory processes by downregulating IL-1β production in human osteoarthritic synovial tissue and cartilage (8). Therefore, in the present study, the possible associations between the inhibitory effect of AG on inflammation and the NF-κB signaling pathway were investigated.
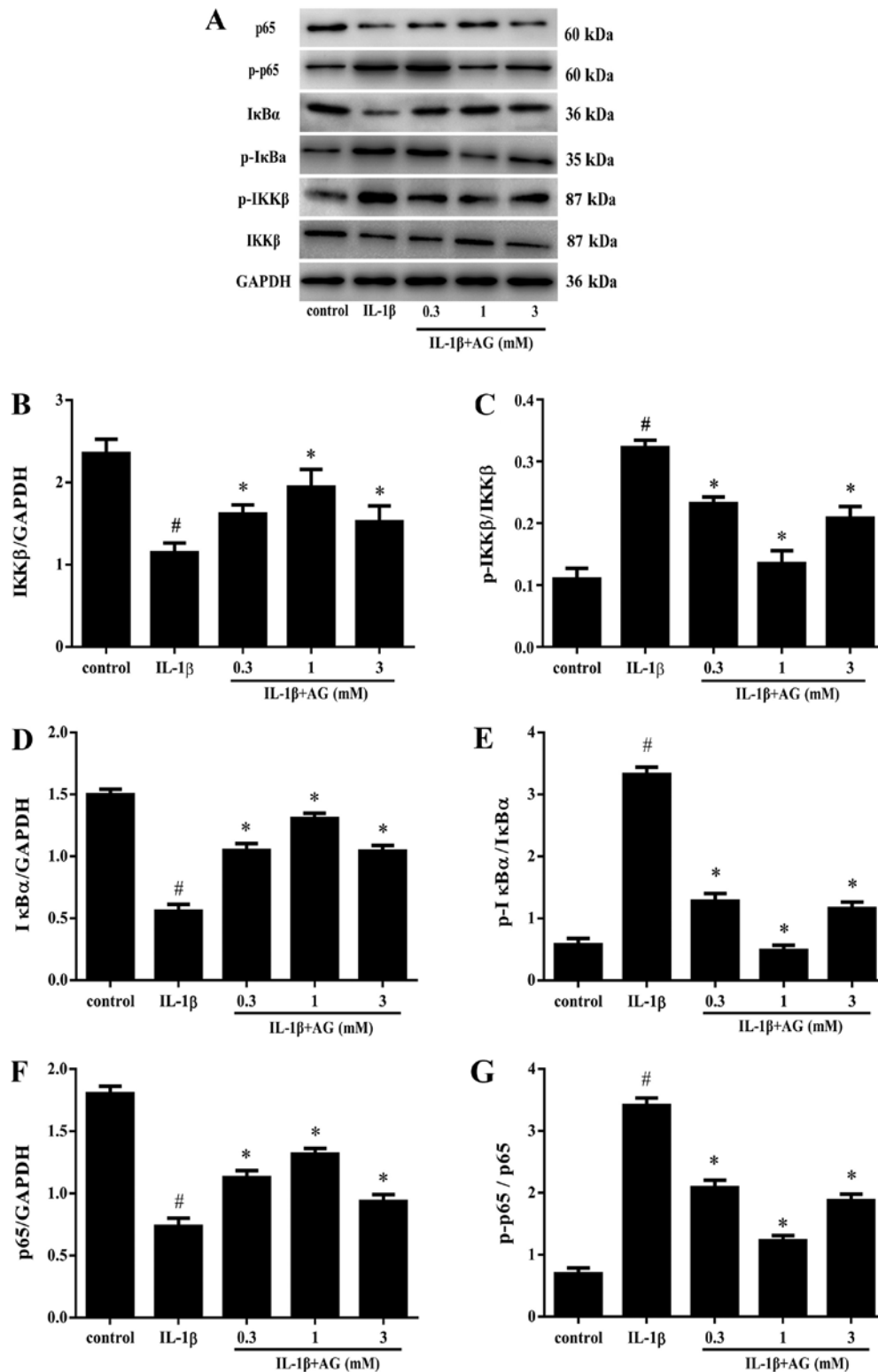
Figure 3. Protein levels of IKKβ, p-IKKβ, IκBα, p-IκBα, p65 and p-p65 in the different treatment groups. (A) Western blotting was performed to assess the protein expression levels of p-IKKβ, p-IκBα, IKKβ, IκBα, p65, and p-p65. Densitometric analysis of western blotting for (B) IKKβ, (C) p-IKKβ/IKKβ, (D) IκBα, (E) p-IκBα/IκBα, (F) p65 and (G) p-p65/p65. #P<0.05 vs. the control group. *P<0.05 vs. the IL-1β group. IKKβ, inhibitor of NF-κB-β; p, phosphory-lated; IκBα, NF-κB inhibitor α; IL, interleukin; AG, aminoguanidine.

IL-1β is involved in the pathogenesis of OA and it may result in marked alterations to the cartilage, including matrix degradation, inflammation, chondrocyte phenotypic changes and chondrocyte apoptosis (26,37-39). Previous studies have reported that 10 ng/ml IL-1β induces an

inflammatory response in chondrocytes (27,40); therefore, 10 ng/ml was used as the working concentration of IL-1β in the present study. IL-1β induces iNOS and COX-2 expression, promotes the synthesis of inflammatory mediators, including prostaglandin E2 and nitric oxide, and stimulates
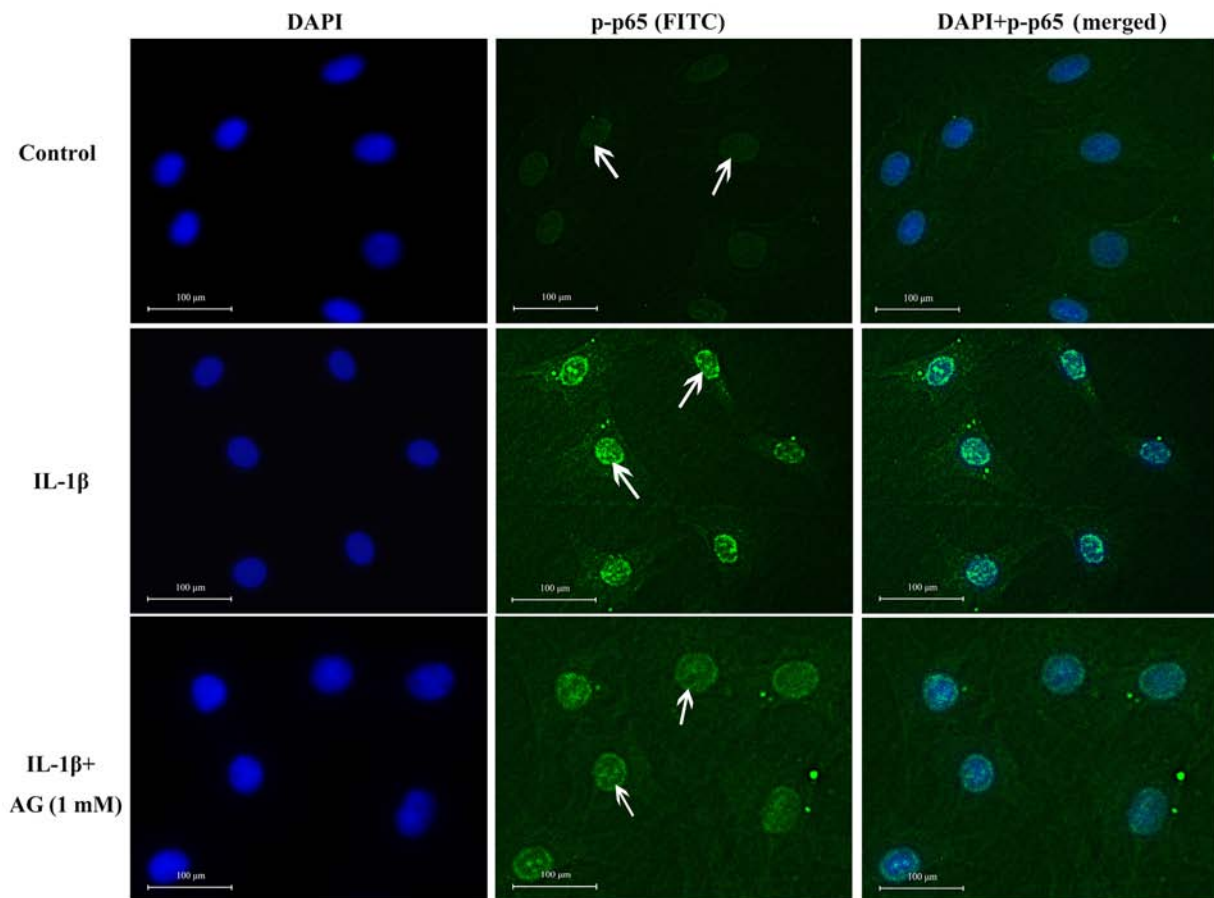
Figure 4. Immunofluorescence assay for the nuclear translocation of NF-κB p65. The control group displayed no intense green fluorescence in the nucleus. Chondrocytes treated with IL-1β displayed intense green fluorescence in the nucleus. The green fluorescence inside the nucleus was reduced in chondrocytes co-treated with IL-1β and AG. The white arrows indicate intense green fluorescence. IL, interleukin; AG, aminoguanidine; p, phosphorylated.

articular chondrocytes to produce high levels of NF-κB (26). Therefore, in the present study, chondrocytes were stimulated with IL-1β to mimic the pathophysiology of OA. The results of the present study were consistent with previous studies, suggesting that IL-1β induced increased iNOS and COX-2 expression (37,41,42).

iNOS is not only an inflammatory mediator, but is also essential for the initiation and promotion of the inflammatory response (43). iNOS can significantly increase the production of nitric oxide, which leads to destruction of articular cartilage and chondrocyte apoptosis (44). In addition, iNOS can regulate other inflammatory processes, for example, cortisol can interact with or induce iNOS to increase the extent of DNA damage, and the formation of reactive oxygen/nitrogen species (45). Increased gene and protein expression levels of iNOS and COX-2 contribute to pain and joint inflammation in patients with OA (46). Moreover, current OA treatment strategies focus on the use of anti-inflammatory drugs that reduce COX-2 levels, which can alleviate joint pain and swelling (47). Therefore, reducing the expression of iNOS and COX-2 in chondrocytes could potentially ameliorate joint inflammation and the degeneration of articular cartilage in patients with OA. In a recent study, treatment with kaempferol reduced the expression levels of iNOS and COX-2 in a dose-dependent manner in IL-1β-stimulated rat chondrocytes (23). Consistently, the results of the present study indicated that AG decreased

the gene and protein expression levels of iNOS and COX-2 in IL-1β-stimulated rat articular chondrocytes. Therefore, the present study suggested that AG might reduce inflammatory responses.

Furthermore, the present study suggested that the expression of COX-2 and iNOS may be closely associated with the activation of the NF-κB signaling pathway. In a study conducted by Lianxu *et al* (39), an small interfering RNA targeted against NF-κB p65 reduced iNOS and COX-2 expression levels in rat chondrocytes stimulated by IL-1β. Additionally, IL-1β-mediated iNOS expression was reduced following suppression of NF-κB activity in rat chondrocytes (48). The aforementioned studies suggested that increased iNOS and COX-2 protein expression levels in IL-1β-stimulated rat chondrocytes are dependent upon the activation of NF-κB. Furthermore, it has been reported that the protein expression level of IκBα and the activity of NF-κB are decreased by IKKβ phosphorylation (49). Activated NF-κB translocates into the nucleus to induce the expression of iNOS and COX-2 (50) and other proinflammatory cytokines, such as NO and PGE2 (26), which further aggravate OA symptoms. The results reported in the aforementioned studies were consistent with the results of the present study, which indicated that IL-1β successfully induced an inflammatory response in chondrocytes. Therefore, suppressing NF-κB activity might serve as a novel therapeutic option for OA (11,25,36,46,47). In the present

study, AG inhibited IKKβ phosphorylation, IκBα degradation and NF-κB/p65 activation. Similar effects have been reported with pomegranate fruit extract (51). Pomegranate fruit extract inhibited IL-6 production, the expression of IKKβ mRNA, the degradation of IκBα and the nuclear translocation of NF-κB/p65 in OA chondrocytes. Furthermore, AG inhibited expression of COX-2 and iNOS, and similar effects have been observed with chrysin (52), suggesting that there is a common mechanism of action among these drugs. The present study suggested that AG inhibited NF-κB activation and suppressed the inflammatory response in IL-1β-stimulated chondrocytes. Therefore, it was hypothesized that AG inhibited nuclear translocation of NF-κB/p65 by inhibiting the phosphorylation of IκBα and IKKβ, thereby reducing the expression of iNOS and COX-2, and suppressing the inflammatory response. Collectively, these results indicated that AG has the potential to protect articular chondrocytes. Further investigation into the clinical application of AG is required.

In conclusion, AG downregulated iNOS and COX-2 expression by blocking the NF-κB signaling pathway; therefore, AG may protect chondrocytes. Additionally, 1 mM AG was the most effective concentration for the inhibition of inflammation. Furthermore, the present study indicated that AG may serve as a potential therapeutic for OA, therefore, providing the theoretical basis for future clinical studies.

Acknowledgements

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YM designed the study and prepared the manuscript. LG designed the study and produced the final draft of manuscript before submitting. YM, TM and XS analyzed the data. YM, ZZ, HB, YL, HH, RY and YW performed the experiments and processed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Departmental Animal Care and Use Committee at Northeast Agricultural University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bijlsma JW, Berenbaum F and Lafeber FP: Osteoarthritis: An update with relevance for clinical practice. Lancet 377: 2115-2126, 2011.
2. Madry H and Cucchiarini M: Advances and challenges in gene-based approaches for osteoarthritis. J Gene Med 15: 343-355, 2013.
3. Scanzello CR and Goldring SR: The role of synovitis in osteoarthritis pathogenesis. Bone 51: 249-257, 2012.
4. Litwic AE, Parsons C, Edwards MH, Jagannath D, Cooper C and Dennison EM: Comment on: Inflammatory mediators in osteoarthritis: A critical review of the state-of-the art, prospects, and future challenges. Bone 106: 28-29, 2018.
5. McCulloch K, Litherland GJ and Rai TS: Cellular senescence in osteoarthritis pathology. Aging Cell 16: 210-218, 2017.
6. Hafez HM, Ibrahim MA, Ibrahim SA, Amin EF, Goma W and Abdelrahman AM: Potential protective effect of etanercept and aminoguanidine in methotrexate-induced hepatotoxicity and nephrotoxicity in rats. Eur J Pharmacol 768: 1-12, 2015.
7. Balaganur V, Pathak NN, Lingaraju MC, More AS, Latief N, Kumari RR, Kumar D and Tandan SK: Effect of S-methylisothiourea, an inducible nitric oxide synthase inhibitor, in joint pain and pathology in surgically induced model of osteoarthritis. Connect Tissue Res 55: 367-377, 2014.
8. Shirazi I, Yaron I, Wollman Y, Blum M, Chernihovsky T, Judovich R, Iaina A and Yaron M: Down regulation of interleukin 1beta production in human osteoarthritic synovial tissue and cartilage cultures by aminoguanidine. Ann Rheum Dis 60: 391-394, 2001.
9. Farghaly HS and Thabit RH: L-arginine and aminoguanidine reduce colonic damage of acetic acid-induced colitis in rats: Potential modulation of nuclear factor-κB/p65. ClinExp Pharmacol Physiol 41: 769-779, 2014.
10. Di Naso FC, Rodrigues G, Dias AS, Porawski M, Fillmann H and Marroni NP: Hepatic nitrosative stress in experimental diabetes. J Diabetes Complications 26: 378-381, 2012.
11. Wang J, Kalhor A, Lu S, Crawford R, Ni JD and Xiao Y: iNOS expression and osteocyte apoptosis in idiopathic, non-traumatic osteonecrosis. Acta Orthop 86: 131-141, 2015.
12. Kim J, Kim CS, Sohn E, Lee YM, Jo K, Shin SD and Kim JS: Aminoguanidine protects against apoptosis of retinal ganglion cells in Zucker diabetic fatty rats. Eur Rev Med Pharmacol Sci 18: 1573-1578, 2014.
13. Li W, Hu Q, Ren X, He P, Xu H, Dai H and Chen Z: Aminoguanidine suppresses methylglyoxal-mediated oxygen-glucose deprivation injury in human brain microvascular endothelial cells. Zhejiang Da Xue Xue Bao Yi Xue Ban 42: 261-266, 2013 (In Chinese).
14. Tian M, Qing C, Niu Y, Dong J, Cao X, Song F, Ji X and Lu S: Effect of aminoguanidine intervention on neutrophils in diabetes inflammatory cells wound healing. Exp Clin Endocrinol Diabetes 121: 635-642, 2013.
15. Sliman SM, Eubank TD, Kotha SR, Kuppusamy ML, Sherwani SI, Butler ES Kuppusamy P, Roy S, Marsh CB, Stern DM and Parinandi NL: Hyperglycemic oxoaldehyde, glyoxal, causes barrier dysfunction, cytoskeletal alterations, and inhibition of angiogenesis in vascular endothelial cells: Aminoguanidine protection. Mol Cell Biochem 333: 9-26, 2010.
16. Saiko P, Graser G, Giessrigl B, Lackner A, Grusch M, Krupitza G, Basu A, Sinha BN, Jayaprakash V, Jaeger W, et al: A novel N-hydroxy-N'-aminoguanidine derivative inhibits ribonucleotide reductase activity: Effects in human HL-60 promyelocytic leukemia cells and synergism with arabinofuranosylcytosine (Ara-C). Biochem Pharmacol 81: 50-59, 2011.
17. Zheng B, Zheng T, Wang L, Chen X, Shi C and Zhao S: Aminoguanidine inhibition of iNOS activity ameliorates cerebral vasospasm after subarachnoid hemorrhage in rabbits via restoration of dysfunctional endothelial cells. J Neurol Sci 295: 97-103, 2010.
18. Berenbaum F: Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis Cartilage 21: 16-21, 2013.
19. Rigoglou S and Papavassiliou AG: The NF-κB signalling pathway in osteoarthritis. Int J Biochem Cell Biol 45: 2580-2584, 2013.

20. Wojdasiewicz P, Poniatowski ŁA and Szukiewicz D: The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm 2014: 561459, 2014.

21. Liao S, Zhou K, Li D, Xie X, Jun F and Wang J: Schisantherin A suppresses interleukin-1β-induced inflammation in human chondrocytes via inhibition of NF-κB and MAPKs activation. Eur J Pharmacol 780: 65-70, 2016.

22. Rasheed N, Alghasham A and Rasheed Z: Lactoferrin from camelus dromedarius inhibits nuclear transcription factor-κB Activation, Cyclooxygenase-2 expression and prostaglandin E2 production in stimulated human chondrocytes. Pharmacognosy Res 8: 135-141, 2016.

23. Zhuang Z, Ye G and Huang B: Kaempferol alleviates the interleukin-1β-induced inflammation in rat osteoarthritis chondrocytes via suppression of NF-κB. Med Sci Monit 23: 3925-3931, 2017.

24. Chen YJ, Tsai KS, Chan DC, Lan KC, Chen CF, Yang RS and Liu SH: Honokiol, a low molecular weight natural product, prevents inflammatory response and cartilage matrix degradation in human osteoarthritis chondrocytes. J Orthop Res 32: 573-580, 2014.

25. Zhou HF, Yan H, Pan H, Hou KK, Akk A, Springer LE, Hu Y, Allen JS, Wickline SA and Pham C: Peptide-siRNA nanocomplexes targeting NF-κB subunit p65 suppress nascent experimental arthritis. J Clin Invest 124: 4363-4374, 2014.

26. Wang D, Qiao J, Zhao X, Chen T and Guan D: Thymoquinone inhibits IL-1β-induced inflammation in human osteoarthritis chondrocytes by suppressing NF-κB and MAPKs signaling pathway. Inflammation 38: 2235-2241, 2015.

27. Li Y, Wang J, Song X, Bai H, Ma T, Zhang Z, Li X, Jiang R, Wang G, Fan X, *et al*: Effects of baicalein on IL-1β-induced inflammation and apoptosis in rat articular chondrocytes. Oncotarget 8: 90781-90795, 2017.

28. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.

29. Ahmad R, Ahmad N, Naqvi AA, Exarchou V, Upadhyay A, Tuenter E, Foubert K, Apers S, Hermans N and Pieters L: Antioxidant and Antiglycating Constituents from Leaves of Ziziphus oxyphylla and Cedrela serrata. Antioxidants(Basel) 5: 9, 2016.

30. Orman D, Vardi N, Ates B, Taslidere E and Elbe H: Aminoguanidine mitigates apoptosis, testicular seminiferous tubules damage, and oxidative stress in streptozotocin-induced diabetic rats. Tissue Cell 47: 284-290, 2015.

31. Wright C, Iyer AKV, Kulkarni Y and Azad N: S-Nitrosylation of Bcl-2 negatively affects autophagy in lung epithelial cells. J Cell Biochem 117: 521-532, 2015.

32. Madhu BP, Singh KP, Saminathan M, Singh R, Tiwari AK, Manjunatha V, Harish C and Manjunathareddy GB: Correlation of inducible nitric oxide synthase (iNOS) inhibition with TNF-α, caspase-1, FasL and TLR-3 in pathogenesis of rabies in mouse model. Virus Genes 52: 61-71, 2016.

33. Shafaroodi H, Hassanipour M, Mousavi Z, Rahimi N and Dehpour AR: The effects of sub-chronic treatment with pioglitazone on the septic mice mortality in the model of cecal ligation and puncture: Involvement of nitric oxide pathway. Acta Med Iran 53: 608-616, 2015.

34. Tian M, Qing C, Niu Y, Dong J, Cao X, Song F, Ji X and Lu S: Aminoguanidine cream ameliorates skin tissue microenvironment in diabetic rats. Arch Med Sci 12: 179-187, 2016.

35. Parthasarathy A, Gopi V, Devi KMS, Balaji N and Vellaichamy E: Aminoguanidine inhibits ventricular fibrosis and remodeling process in isoproterenol-induced hypertrophied rat hearts by suppressing ROS and MMPs. Life Sci 118: 15-26, 2014.

36. Lenin R, Mohan V and Balasubramanyam M: SEAP activity serves for demonstrating ER stress induction by glucolipotoxicity as well as testing ER stress inhibitory potential of therapeutic agents. Mol Cell Biochem 404: 271-279, 2015.

37. Chen WP, Wang YL, Tang JL, Hu PF, Bao JP and Wu LD: Morin inhibits interleukin-1β-induced nitric oxide and prostaglandin E2 production in human chondrocytes. Int Immunopharmacol 12: 447-452, 2012.

38. Li R, Cai L, Ding J, Hu CM, Wu TN and Hu XY: Inhibition of hedgehog signal pathway by cyclopamine attenuates inflammation and articular cartilage damage in rats with adjuvant-induced arthritis. J Pharm Pharmacol 67: 963-971, 2015.

39. Lianxu C, Hongti J and Changlong Y: NF-kappaBp65-specific siRNA inhibits expression of genes of COX-2, NOS-2 and MMP-9 in rat IL-1beta-induced and TNF-alpha-induced chondrocytes. Osteoarthritis Cartilage 14: 367-376, 2006.

40. Huang X, Pan Q, Mao Z, Zhang R, Ma X, Xi Y and You H: Sinapic acid inhibits the IL-1 β-induced inflammation via MAPK downregulation in rat chondrocytes. Inflammation 41: 562-568, 2018.

41. Ma Z, Wang Y, Piao T and Liu J: Echinocystic acid inhibits IL-1β-induced COX-2 and iNOS expression in human osteoarthritis chondrocytes. Inflammation 39: 543-549, 2016.

42. Ansari MY and Haqqi TM: Interleukin-1β induced stress granules sequester COX-2 mRNA and regulates its stability and translation in human OA chondrocytes. Sci Rep 6: 27611, 2016.

43. Suschek CV, Schnorr O and Kolb-Bachofen V: The role of NOS in chronic inflammatory processes in vivo: Is it damage-promoting, protective, or active at all? Curr Mol Med 4: 763-775, 2004.

44. Gómez R, Scotece M, Conde J, Lopez V, Pino J, Lago F, Gómez-Reino JJ and Guallilo O: Nitric oxide boosts TLR-4 mediated lipocalin 2 expression in chondrocytes. J Orthop Res 31: 1046-1052, 2013.

45. Flaherty RL, Owen M, Fagan-Murphy A, Intabli H, Healy D, Patel A, Allen MC, Patel BA and Flint MS: Glucocorticoids induce production of reactive oxygen species/reactive nitrogen species and DNA damage through an iNOS mediated pathway in breast cancer. Breast Cancer Res 19: 35, 2017.

46. Wang SN, Xie GP, Qin CH, Chen YR, Zhang KR, Li X, Wu Q, Dong WQ, Yang J and Yu B: Aucubin prevents interleukin-1 beta induced inflammation and cartilage matrix degradation via inhibition of NF-κB signaling pathway in rat articular chondrocytes. Int Immunopharmacol 24: 408-415, 2015.

47. Chin KY: The spice for joint inflammation: Anti-inflammatory role of curcumin in treating osteoarthritis. Dove Medical Press 10: 3029-3042, 2016.

48. Lei M, Wang JG, Xiao DM, Fan M, Wang DP, Xiong JY, Chen Y, Ding Y and Liu SL: Resveratrol inhibits interleukin 1β-mediated inducible nitric oxide synthase expression in articular chondrocytes by activating SIRT1 and thereby suppressing nuclear factor-κB activity. Eur J Pharmacol 674: 73-79, 2012.

49. Ying X, Chen X, Cheng S, Shen Y, Peng L and Xu HZ: Piperine inhibits IL-β induced expression of inflammatory mediators in human osteoarthritis chondrocyte. Int Immunopharmacol 17: 293-299, 2013.

50. Stuhlmeier KM: The anti-rheumatic gold salt aurothiomalate suppresses interleukin-1beta-induced hyaluronan accumulation by blocking HAS1 transcription and by acting as a COX-2 transcriptional repressor. J Biol Chem 282: 2250-2258, 2007.

51. Haseeb A, Khan NM, Ashruf OS and Haqqi TM: A polyphenol-rich pomegranate fruit extract suppresses NF-κB and IL-6 expression by blocking the activation of IKK and NIK in primary human chondrocytes. Phytother Res 31: 778-782, 2017.

52. Zheng W, Tao Z, Cai L, Chen C, Zhang C, Wang Q, Ying X, Hu W and Chen H: Chrysin attenuates IL-1β-induced expression of inflammatory mediators by suppressing NF-κB in human osteoarthritis chondrocytes. Inflammation 40: 1143-1154, 2017.