Original Research

Thymoquinone inhibits matrix metalloproteinase expression in rabbit chondrocytes and cartilage in experimental osteoarthritis

Wei-Ping Chen, Jing-Li Tang, Jia-Peng Bao and Li-Dong Wu

Department of Orthopedics Surgery, The Second Affiliated Hospital of Medical College, Zhejiang University, Jie Fang Road 88#, 310009 Hangzhou, People's Republic of China
Corresponding author: Li-Dong Wu. Email: ldwu@yahoo.com

Abstract

Thymoquinone (TQ) is the main constituent of *Nigella sativa* oil, which has been traditionally used against arthritis in the Middle East. In this study, we investigated the effect of TQ against matrix metalloproteinase (MMP) expression in both rabbit chondrocytes and animal mode of osteoarthritis (OA) induced by anterior cruciate ligament transection and tested whether or not nuclear factor kappa B (NF-κB) was involved in this process. TQ down-regulated MMP-1, MMP-3 and MMP-13 expression and up-regulated tissue inhibitors of metalloproteinase-1 expression as assessed by quantitative realtime polymerase chain reaction. In addition, NF-κB p65 protein level as well as its translocation induced by interleukin-1β were inhibited by TQ. Our findings suggest the potential of TQ in the treatment of OA.

Keywords: thymoquinone, osteoarthritis, matrix metalloproteinase, chondrocyte, nuclear factor kappa B, ACLT

Experimental Biology and Medicine 2010; **235**: 1425–1431. DOI: 10.1258/ebm.2010.010174

Introduction

Osteoarthritis (OA) is a degenerative joint disease of cartilage degradation as well as synovial inflammation and subchondral sclerosis. Previous studies have demonstrated that proteolytic enzymes such as matrix metalloproteinases (MMPs), aggrecanases and cathepsins are involved in the breakdown of cartilage.[1–3] Among them, MMPs have been considered as the key matrix-degradation enzymes due to the ability to cleave most components of extracellular matrix.[4,5] The activities of MMPs are antagonized by tissue inhibitors of metalloproteinases (TIMPs).[6] The imbalance between MMPs and TIMPs plays a pathophysiological role in OA. Recently, inflammation has been believed to play a pivotal role in the OA progression.[7–9] For instance, interleukin-1β (IL-1β), a proinflammatory cytokine, has been shown to contribute to OA progression via inducing MMP expression as well as other catabolic factors, e.g. prostaglandin E2 and nitric oxide.[10–14]

Nuclear factor kappa B (NF-κB) is a ubiquitous protein involved in inflammation. Previous studies have proved that activation of NF-κB was involved in the synthesis of cytokines such as IL-1β and tumor necrosis factor α (TNF-α).[15,16] Furthermore, the correlation between NF-κB activation and the induction of MMPs in arthritis is also well documented.[17,18] These findings suggest that NF-κB may be considered a potential target in the treatment of OA.

Recently, considerable attention has been drawn to herbal drugs for the treatment of OA. Thymoquinone (TQ) is the main constituent of *Nigella sativa* oil which has been traditionally used against arthritis in the Middle East.[19] TQ possesses antioxidative properties and exhibits anticancer activity against several tumor cells.[20,21] Additionally, TQ has been reported to exert anti-inflammatory properties.[22] Tekeoglu *et al.* found that TQ reduced the levels of TNF-α and IL-1 in blood in a rat model of adjuvant-induced arthritis.[23] These findings suggest the potential of TQ in the treatment of OA because inflammation plays an important role in the disease. In the present study, we investigated the effect of TQ in IL-1β-stimulated rabbit chondrocytes, and we also assessed the *in vivo* effect of TQ in experimental OA induced by anterior cruciate ligament transection (ACLT) in rabbits.

Material and methods

Animals

Twenty mature New Zealand rabbits weighting 2.0–2.5 kg were used for the *in vivo* study and 10 five-week-old female China white rabbits were used for chondrocyte culture (Animal center of Zhejiang University). This study was approved by the Institutional Animal Care and Use Committee of Zhejiang University (Hangzhou, China).

Reagents

TQ, recombinant human (rh) IL-1β, collagenase II and 3-(4,5-dimethylthiazolyl-2)-2,5-diphe-nyltetrazoliumbromide

(MTT) were obtained from Sigma (St Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, fetal bovine serum (FBS) and 0.25% trypsin were obtained from Gibco BRL (Grand Island, NY, USA). TQ was dissolved in dimethylsulfoxide (DMSO).

Culture of rabbit articular chondrocytes

Articular cartilage was isolated from knee and hip joints of five-week-old female China white rabbits under sterile conditions. Cartilage was digested with 0.1% collagenase II for four hours to obtain chondrocytes. Cell suspension was transferred to 75 cm^2 culture flasks at a density of 10^5 cells/cm^2 in 10 mL DMEM containing 10% FBS and antibiotics (100 units/mL penicillin, 100 μg/mL streptomycin). The chondrocytes were cultured in a 5% CO_2 atmosphere at 37°C. Confluent primary chondrocytes were passaged at a ratio of 1:3. Chondrocytes passages two to four were used.

Cell viability assays

Cytotoxicity of TQ was assessed by MTT assay. Chondrocytes were seeded on 96-well plates at a density of 1×10^4/well. TQ (1, 5 and 10 μmol/L) was added to cultured wells and incubated for 24 h. Twenty microliters of MTT solution (5 mg/L in phosphate-buffered saline [PBS]) was added to each well and incubated for four hours at 37°C. After aspiration of the supernatant, 150 μL of DMSO were added to each well. The plate was shaken for 10 min. Absorbance at 570 nm was measured with an ELISA reader (Bio-Rad, Hercules, CA, USA).

Quantitative realtime polymerase chain reaction

Cells (2.5×10^5/well) in six-well plates were serum-starved overnight. Then cells were pretreated with various concentrations of TQ for one hour followed by 10 ng/mL of IL-1β for 24 h in no serum media, then harvested for analysis of the mRNA level of MMPs. TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used for total RNA extraction according to the manufacturer's instructions. For the first strand cDNA synthesis, the Moloney murine leukemia virus (M-MLV) reverse transcriptase cDNA synthesis kit (Promega, Madison, WI, USA) was used, containing 2 μg of total RNA, 1 μL of Oligo $(dT)_{18}$ primer, 25 units of RNase inhibitor, 5 μL of M-MLV 5 × reaction Buffer and 2 μL of dNTPs (10 mmol/L). Quantitative realtime polymerase chain reaction (PCR) assays were carried out with the iCycler apparatus system (Bio-Rad) and SYBR Green I technology. PCR primers for MMP-1, MMP-3, MMP-13, TIMP-1 and 18S rRNA are shown in Table 1. 18s rRNA was used as an internal control to standardize mRNA levels. The realtime PCR data were quantified using the ΔCT method with the formula: $n = 100 \times 2^{-(\triangle \text{CT targeted gene} - \triangle \text{CT 18s rRNA})}$.

Preparation of nuclear extracts and Western blot analysis

Cells were pretreated with TQ (10 μmol/L) for 24 h and then stimulated with IL-1β (10 ng/mL) for one hour. Treated cells were harvested and washed with ice-cold PBS. A nuclear protein extract kit was used for nuclear protein extraction according to the manufacturer's instructions (Beyotime Institute of Biotechnology, Jiangshu, China). Protein concentration was determined using Bradford assay. Protein was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, proteins in the gel were electrotransferred to polyvinylidene fluoride membranes. Membranes were incubated with primary antibodies against NF-κB p65 and anti-Lamin B overnight at 4°C (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA) and then incubated for one hour at room temperature with horseradish peroxidase-linked secondary antibodies. Reactions were detected with Enhanced Chemiluminescence kit (Amersham Biosciences, Arlington Heights, IL, USA).

Immunofluorescence staining

Confluent cells in slides were serum-starved overnight and treated with TQ for 24 h, and stimulated with 10 ng/mL of IL-1β for one hour subsequently. Then, cells were washed with PBS, fixed with 4% paraformaldehyde and incubated with rabbit polyclonal anti-NF-κB p65 antibody overnight followed by PE-conjugated goat anti-rabbit antibody for 60 min (Santa Cruz Biotechnology). After washing, cells were counterstained with 4′-6-diamidino-2-phenylindole for 30 min, and viewed using confocal microscopy (Carl Zeiss, Oberkochen, Germany).

OA induced and rabbits treatment

Fourteen mature rabbits underwent bilateral ACLT in the knee as described previously.[24] Four weeks after surgery, rabbits received an intra-articularly injection of 0.3 mL of TQ (10 μmol/L) in the left knee and vehicle (DMSO) in the right knee. Weekly injections continued for five weeks. Rabbits were killed nine weeks after surgery. Six rabbits were used as the normal control.

Gross evaluations

The gross morphological changes of the femoral condyles were assessed in a blind manner using Indian ink staining, according to a previously described grading method as follows: Grade 1: intact surface; Grade 2: minimal fibrillation; Grade 3: overt fibrillation; and Grade 4: erosion.[25]

Histologic evaluations

Histologic evaluation was performed on sections from femoral condyles. Specimens were fixed in 10% neutral buffered formalin; after decalcification and dehydration, specimens were embedded in paraffin, cut into 5-μm thick sections, and stained with Safranin O-fast green, and then graded according to the Mankin score system.[26]

Table 1 Primers of targeted genes

Targeted genes	Sequence (5′–3′)*	Amplicon length (bp)	Accession number
MMP-1	S: AAGCCAGATGCTGAAACCCTG A: GACCCTTGGAGACTTTGGTGAAT	328	AH005676
MMP-3	S: ACACCGGATCTGCCAAGAGA A: CTGGAGAACGTGAGTGGAGTCA	89	NM001082280
MMP-13	S: TTGACCACTCCAAGGACCCAG A: GAGGATGCA GACGCCAGAAGA	252	NM001082037
TIMP-1	S: CAACTGCGGAACGGGCTCTTG A: CGGCAGCGTAGGTCTTGGTGAA	102	AY829730
18s rRNA	S: GACGGACCAGAGCGAAAGC A: CGCCAGTCGGCATCGTTTATG	119	EU236696

TIMP-1, tissue inhibitors of metalloproteinase-1; MMP, matrix metalloproteinase
*S = sense; A = antisense

Gene analysis of cartilage by quantitative realtime PCR

Cartilage was frozen in liquid nitrogen until use. Quantitative realtime PCR was performed as mentioned above to assess the expression of MMP-1, MMP-3, MMP-13 and TIMP-1.

Statistical analysis

All data are expressed as mean $\pm$ standard deviation (SD). Cell viability data were performed by the unpaired *t*-test. Gross morphology data were analyzed by the non-parametric Mann-Whitney *U* test, while histologic and gene expression data were analyzed by the paired *t*-test. Differences were considered significant when P was less than 0.05.

Results

Effects of TQ on MMP-1, MMP-3, MMP-13 and TIMP-1 expression in IL-1β-induced rabbit chondrocytes

As expected, stimulation with IL-1β resulted in the increase of MMP-1, MMP-3 and MMP-13 expression and the decrease of TIMP-1 expression. Although 1 μmol/L of TQ showed no effects, a higher concentration of TQ inhibited the expression of MMP-1, MMP-3 and MMP-13 and increased the expression of TIMP-1 ($P < 0.05$) (Figure 1). MTT assays showed that TQ at concentrations of 1, 5 or 10 μmol/L exhibited no cytotoxicity (Figure 2). Therefore, 10 μmol/L of TQ were used in the further study.

TQ inhibited IL-1β-stimulated NF-κB p65 expression and translocation in chondrocytes

In the present study, we investigated NF-κB p65 protein levels in the nuclei of IL-1β-stimulated chondrocytes by Western blots and immunofluorescence. Figure 3 shows that IL-1β increased the protein level of NF-κB p65 in the nuclei compared with normal chondrocytes and the elevated protein level was inhibited in TQ treated cells. Accordingly, TQ inhibited IL-1β-induced NF-κB p65 translocation as assessed by immunofluorescence staining (Figure 4).

Gross evaluations

Rabbits which underwent ACLT showed varying cartilage lesions. Condyles in knees treated with TQ showed a trend of less cartilage damage than that in the vehicle-treated group, but the difference was not significant ($P > 0.05$) (data not shown).

Histologic evaluations

Intra-articular injection of TQ reduced histological lesions. The Safranin-O staining reduction was less in TQ-treated cartilage while a severe reduction of Safranin-O staining was noted in the vehicle-treated cartilage (Figure 5). When evaluated according to the Mankin score system, the vehicle-treated cartilage had average values of 10.93 ± 3.12 while the TQ-treated cartilage showed significantly lower values of 8.71 ± 3.24 ($P < 0.05$) (Table 2).

Gene expression in cartilage

As seen in Figure 6, gene expression of MMP-1, MMP-3 and MMP-13 was increased, while TIMP-1 expression was decreased, in cartilage from vehicle-treated knees as compared with the normal cartilage. TQ significantly decreased the expression of MMP-1, MMP-3 and MMP-13, and increased the expression of TIMP-1 compared with the vehicle-treated cartilage ($P < 0.05$).

Discussion

The current study demonstrated that TQ inhibited the expression of MMP-1, MMP-3 and MMP-13 and increased the expression of TIMP-1 in both chondrocytes and cartilage. Our findings also suggest the involvement of NF-κB in the inhibition of MMPs by TQ.

In general, cartilage degradation in OA was mainly mediated by MMPs, and the pivotal roles of the MMP family in cartilage degradation are well accepted.[27] Among MMPs, MMP-1 and MMP-13 are the two main MMPs contributing to cartilage degradation. Therefore, in the current study, we focused on the inhibition of MMPs in rabbit chondrocytes to investigate whether TQ has chondroprotective properties. TQ significantly inhibited MMP-1, MMP-3 and MMP-13 expression in response to IL-1β in rabbit chondrocytes. Since the activities of MMPs were affected by TIMPs, in this study we also investigated the effect of TQ on TIMP-1 expression and found the increase of TIMP-1 expression by TQ in IL-1β-stimulated chondrocytes as shown in Figure 1. Thus, we speculated that TQ

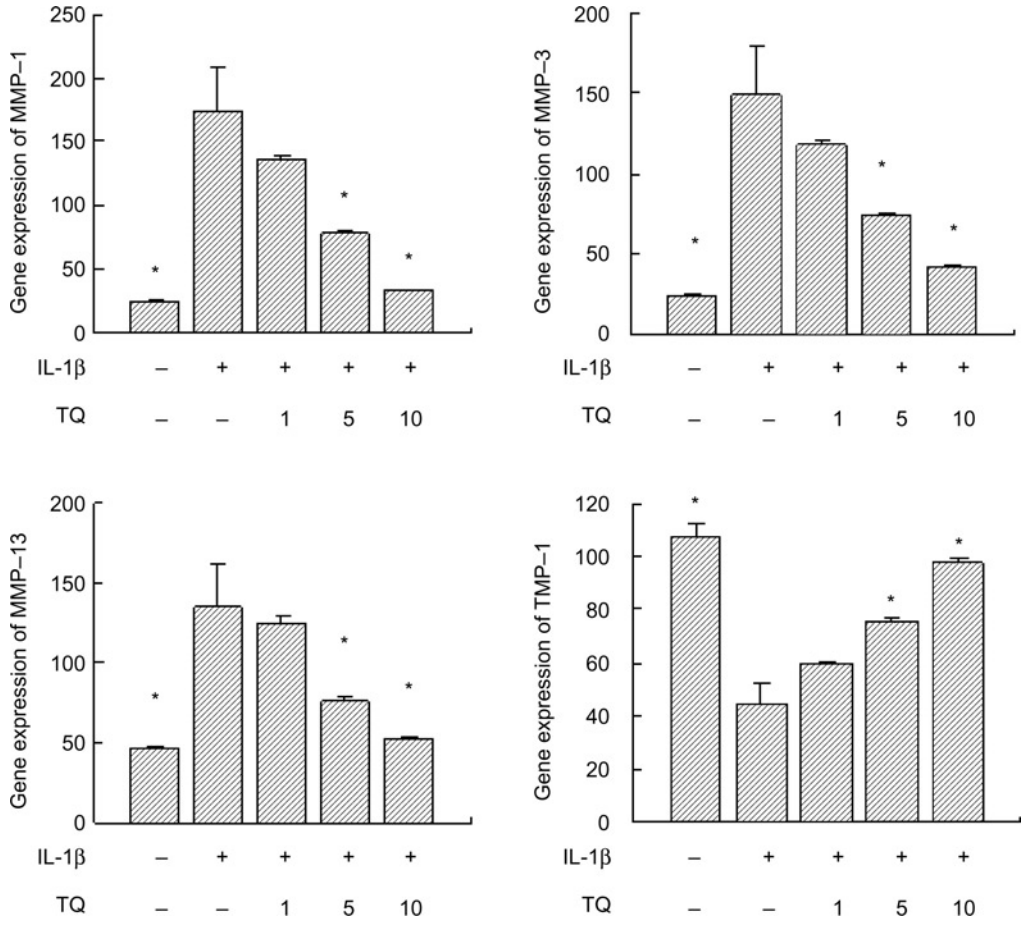

Figure 1 Effects of TQ on the expression of MMP-1, MMP-3, MMP-13 and TIMP-1 in IL-1β-induced rabbit chondrocytes. Chondrocytes were treated with TQ (1, 5, 10 μmol/L) for one hour and then followed by 10 ng/mL of IL-1β for 24 h. Data are expressed as mean $\pm$ SD. $^{*}P < 0.05$ compared with cells stimulated with IL-1β. MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; TQ, thymoquinone; IL-1β, interleukin-1β

may affect the ratio of MMPs to TIMPs to exert its biological effect in chondrocytes. Our data are partly supported by previous studies showing that agents inhibited MMPs' exerted beneficial effects in the treatment of OA *in vitro* and *in vivo*.[28,29]

IL-1β has been known as a critical proinflammatory cytokine during the progression of OA. Induction of MMPs as well as other catabolic factors such as iNOS and COX-2 by IL-1β in chondrocytes has been well documented.[30,31] Our previous study showed elevated IL-1β expression in cartilage in a rabbit model of OA induced by ACLT.[32] Similar findings were reported by Pelletier *et al*;[33] they found that production of IL-1 increased in both synovium and cartilage in ACLT-induced OA in dogs. Furthermore, recombinant IL-1 receptor antagonist has been proved to reduce cartilage damage in both cartilage explant culture and animal model.[14,34] In light of the importance of IL-1 in OA,

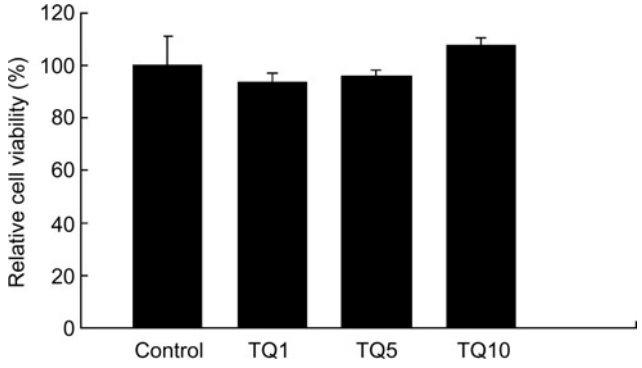

Figure 2 Effects of TQ on cell viability. Cells were treated with different concentrations of TQ for 24 h. The effects of TQ on rabbit chondrocytes viability were evaluated using an MTT assay. TQ, thymoquinone; MTT assay, 3-(4,5-dimethylthiazolyl-2)-2,5-diphe-nyltetrazoliumbromide assay

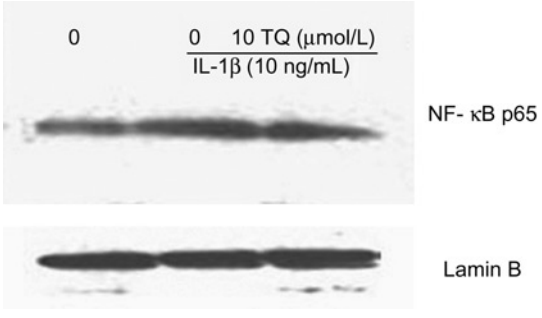

Figure 3 NF-κB expression in IL-1β-induced rabbit chondrocytes pretreated by TQ. Chondrocytes were precultured with 10 μmol/L of TQ for 24 h then stimulated with IL-1β (10 ng/mL) for one hour. NF-κB, nuclear factor kappa B; TQ, thymoquinone; IL-1β, interleukin-1β

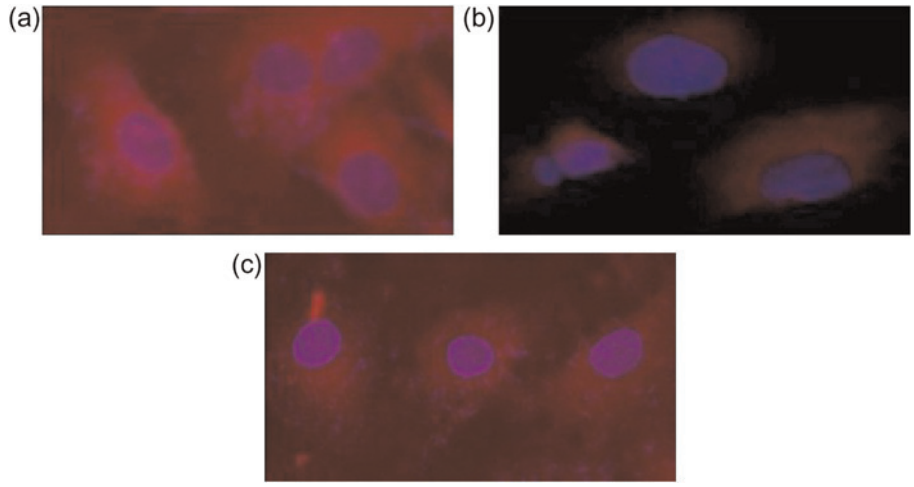

Figure 4 Immunofluorescence staining of NF-κB p65. Cells were pretreated with TQ for 24 h and stimulated with 10 ng/mL of IL-1β for one hour. Immunofluorescence staining was performed to assess the translocation of NF-κB p65. (a) Normal cells; (b) IL-1β treatment; (c) TQ plus IL-1β treatment. TQ, thymoquinone; IL-1β, interleukin-1β; NF-κB, nuclear factor kappa B (A color version of this figure is available in the online journal)

inhibition of IL-1 may be a strategy in the treatment of OA. TQ has been reported to reduce serum sample IL-1β concentration in animal studies.[23,35] In the present study, TQ inhibited the induction of MMPs by IL-1β in chondrocytes; this suppressive effect indicates the therapeutic potential of TQ in the treatment of OA.

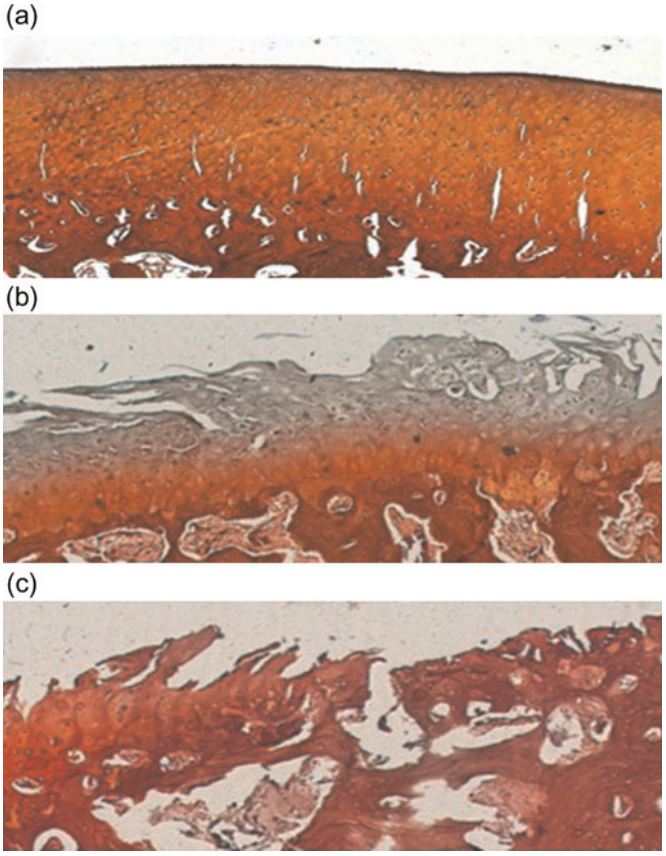

Figure 5 Photographs of articular cartilage stained by Safranin-O. Intra-articular injections of TQ or vehicle were performed once a week for five weeks starting at four weeks after ACLT. Knee joints were obtained at nine weeks after ACLT; cartilage was stained with Safranin-O. (a) Normal cartilage showed no reduction of Safranin staining. (b) TQ-treated cartilage showed reduction in loss of Safranin O staining as compared with the (c) vehicle-treated cartilage (original magnification $\times$50). TQ, thymoquinone; ACLT, anterior cruciate ligament transection (A color version of this figure is available in the online journall)

In the present study, we also investigated the *in vivo* effect of TQ on cartilage degradation in experimental OA induced by ACLT in rabbits. We demonstrated that intra-articular injection of TQ ameliorated cartilage degradation during OA development. Histological analyses show that severity of cartilage degradation as well as structure changes in cartilage was less severe in the TQ-treated knees than the vehicle-treated knees. In addition, MMP-1, MMP-3 and MMP-13 expression decreased while TIMP-1 expression increased in TQ-treated cartilage compared with the vehicle-treated cartilage. The results are consistent with the findings obtained from *in vitro* study. Together, these observations confirm the therapeutic potential of TQ in the treatment of OA.

The mechanisms by which TQ affected MMPs and TIMPs in chondrocytes are still unclear. It is well established that NF-κB-dependent signaling pathways participate in the process of OA via inducing cytokine and MMP expression.[36,37] NF-κB is present in the cytoplasm in an inactive form; when activated by stimuli, NF-κB p65 shuttled from the cytoplasm to the nuclei. TQ has been shown to suppress the NF-κB activation pathway in other system.[38] Therefore, in the present study, we investigated whether NF-κB was involved in the inhibition of MMPs by TQ. We demonstrated that TQ inhibited the IL-1β-induced NF-κB p65 protein expression in rabbit chondrocytes. In addition,

Table 2 Histological quantification of articular cartilage

	TQ (N = 14)	OA (N = 14)
Structural changes	3.43 $\pm$ 1.50*	4.29 $\pm$ 1.49
Cellular changes	2.14 $\pm$ 0.53*	2.71 $\pm$ 0.61
Safranin staining	2.79 $\pm$ 0.89*	3.14 $\pm$ 0.86
Tidemark	0.36 $\pm$ 0.50*	0.79 $\pm$ 0.43
Total score	8.71 $\pm$ 3.24*	10.93 $\pm$ 3.12

Values are the means $\pm$ SD. *$P < 0.05$ compared with OA group analyzed by paired-samples t-test

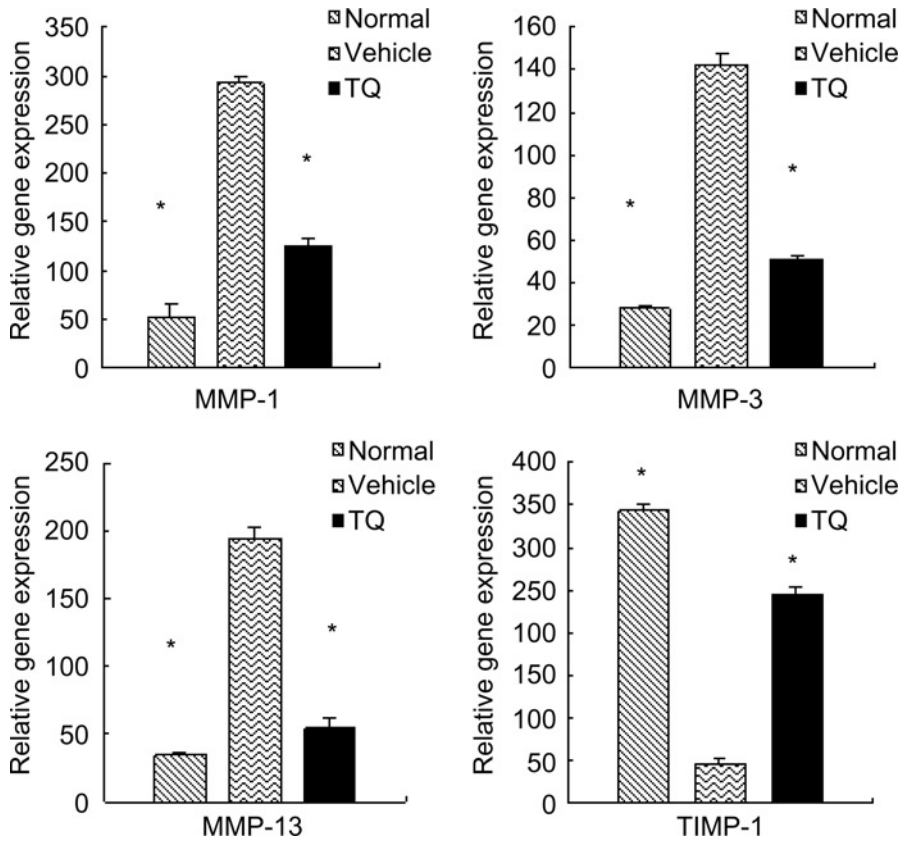

Figure 6 Gene expression in cartilage. Relative gene expression data were normalized with 18S rRNA. TQ inhibited MMP-1, MMP-3 and MMP-13 expression and increased expression of TIMP-1. Data are presented as mean ± SD. $^{*}P < 0.05$ when compared with vehicle-treated cartilage. MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; TQ, thymoquinone

we found that TQ inhibited the NF-κB p65 translocation as assessed by immunofluorescence staining. Thus, we speculated that NF-κB may be involved in the inhibition of MMPs by TQ in chodrocytes. Since other signaling pathways such as mitogen-activated protein kinase also play an important role in the regulation of MMPs,[39] further studies are needed to reveal the precise mechanisms that underlie the regulation of MMPs by TQ.

In conclusion, we demonstrated that TQ down-regulated MMP-1, MMP-3 and MMP-13 expression and up-regulated TIMP-1 expression in both rabbit chondrocytes and cartilage. These effects may partly associate with the inhibition of NF-κB. *In vivo*, TQ reduced cartilage degradation in the progression of experimental OA. The findings suggest that TQ can be considered as a therapeutic agent for OA.

Author contributions: All authors participated in the design, interpretation and review of the manuscript, and conducted the experiments; W-PC and L-DW wrote the paper and analyzed the data. J-LT and J-PB equally contributed the chondrocytes culture and the animal study.

ACKNOWLEDGEMENTS

This study was supported by grants from the Health Bureau of Zhejiang Province (2006A055).

REFERENCES

1 Little CB, Meeker CT, Golub SB, Lawlor KE, Farmer PJ, Smith SM, Fosang AJ. Blocking aggrecanase cleavage in the aggrecan interglobular domain abrogates cartilage erosion and promotes cartilage repair. *J Clin Invest* 2007;**117**:1627–36
2 Vasiljeva O, Reinheckel T, Peters C, Turk D, Turk V, Turk B. Emerging roles of cysteine cathepsins in disease and their potential as drug targets. *Curr Pharm Des* 2007;**13**:387–403
3 Murphy G, Knauper V, Atkinson S, Butler G, English W, Hutton M, Stracke J, Clark I. Matrix metalloproteinases in arthritic disease. *Arthritis Res* 2002;**4**(Suppl. 3):S39–49
4 Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 1993;**4**:197–250
5 Mengshol JA, Mix KS, Brinckerhoff CE. Matrix metalloproteinases as therapeutic targets in arthritic diseases: bull's-eye or missing the mark? *Arthritis Rheum* 2002;**46**:13–20
6 Martel-Pelletier J, Welsch DJ, Pelletier JP. Metalloproteases and inhibitors in arthritic diseases. *Best Pract Res Clin Rheumatol* 2001;**15**:805–29
7 Malemud CJ. Cytokines as therapeutic targets for osteoarthritis. *BioDrugs* 2004;**18**:23–35
8 Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. *Arthritis Rheum* 2001;**44**:1237–47
9 Sharif M, Shepstone L, Elson CJ, Dieppe PA, Kirwan JR. Increased serum C reactive protein may reflect events that precede radiographic progression in osteoarthritis of the knee. *Ann Rheum Dis* 2000;**59**:71–4
10 Mengshol JA, Vincenti MP, Brinckerhoff CE. IL-1 induces collagenase-3 (MMP-13) promoter activity in stably transfected chondrocytic cells: requirement for Runx-2 and activation by p38 MAPK and JNK pathways. *Nucleic Acids Res* 2001;**29**:4361–72

11 Vincenti MP, Coon CI, Lee O, Brinckerhoff CE. Regulation of collagenase gene expression by IL-1 beta requires transcriptional and post-transcriptional mechanisms. *Nucleic Acids Res* 1994;**22**:4818–27
12 Stichtenoth DO, Thoren S, Bian H, Peters-Golden M, Jakobsson PJ, Crofford LJ. Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. *J Immunol* 2001;**167**:469–74
13 Stadler J, Stefanovic-Racic M, Billiar TR, Curran RD, McIntyre LA, Georgescu HI, Simmons RL, Evans CH. Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. *J Immunol* 1991;**147**:3915–20
14 Kobayashi M, Squires GR, Mousa A, Tanzer M, Zukor DJ, Antoniou J, Feige U, Poole AR. Role of interleukin-1 and tumor necrosis factor alpha in matrix degradation of human osteoarthritic cartilage. *Arthritis Rheum* 2005;**52**:128–35
15 Tak PP, Firestein GS. NF-kappaB: a key role in inflammatory diseases. *J Clin Invest* 2001;**107**:7–11
16 Makarov SS. NF-kappa B in rheumatoid arthritis: a pivotal regulator of inflammation, hyperplasia, and tissue destruction. *Arthritis Res* 2001;**3**:200–6
17 Liacini A, Sylvester J, Li WQ, Zafarullah M. Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. *Matrix Biol* 2002;**21**:251–62
18 Vincenti MP, Brinckerhoff CE. Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Res* 2002;**4**:157–64
19 Abu-Irmaileh BE, Afifi FU. Herbal medicine in Jordan with special emphasis on commonly used herbs. *J Ethnopharmacol* 2003;**89**:193–7
20 El-Dakhakhny M, Madi NJ, Lembert N, Ammon HP. *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats. *J Ethnopharmacol* 2002;**81**:161–4
21 Yi T, Cho SG, Yi Z, Pang X, Rodriguez M, Wang Y, Sethi G, Aggarwal BB, Liu M. Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. *Mol Cancer Ther* 2008;**7**:1789–96
22 El Mezayen R, El Gazzar M, Nicolls MR, Marecki JC, Dreskin SC, Nomiyama H. Effect of thymoquinone on cyclooxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation. *Immunol Lett* 2006;**106**:72–81
23 Tekeoglu I, Dogan A, Ediz L, Budancamanak M, Demirel A. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. *Phytother Res* 2007;**21**:895–7
24 Bao JP, Chen WP, Feng J, Zhao J, Shi ZL, Huang K, Wu LD. Variation patterns of two degradation enzyme systems in articular cartilage in different stages of osteoarthritis: regulation by dehydroepiandrosterone. *Clin Chim Acta* 2009;**408**:1–7
25 Shikhman AR, Amiel D, D'Lima D, Hwang SB, Hu C, Xu A, Hashimoto S, Kobayashi K, Sasho T, Lotz MK. Chondroprotective activity of *N*-acetylglucosamine in rabbits with experimental osteoarthritis. *Ann Rheum Dis* 2005;**64**:89–94
26 Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *J Bone Jt Surg Am* 1971;**53**:523–37
27 Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. *Front Biosci* 2006;**11**:529–43
28 Sabatini M, Lesur C, Thomas M, Chomel A, Anract P, de Nanteuil G, Pastoureau P. Effect of inhibition of matrix metalloproteinases on cartilage loss *in vitro* and in a guinea pig model of osteoarthritis. *Arthritis Rheum* 2005;**52**:171–80
29 Janusz MJ, Bendele AM, Brown KK, Taiwo YO, Hsieh L, Heitmeyer SA. Induction of osteoarthritis in the rat by surgical tear of the meniscus: inhibition of joint damage by a matrix metalloproteinase inhibitor. *Osteoarthritis Cartilage* 2002;**10**:785–91
30 Ho LJ, Lin LC, Hung LF, Wang SJ, Lee CH, Chang DM, Lai JH, Tai TY. Retinoic acid blocks pro-inflammatory cytokine-induced matrix metalloproteinase production by down-regulating JNK-AP-1 signaling in human chondrocytes. *Biochem Pharmacol* 2005;**70**:200–8
31 Chabane N, Zayed N, Afif H, Mfuna-Endam L, Benderdour M, Boileau C, Martel-Pelletier J, Pelletier JP, Duval N, Fahmi H. Histone deacetylase inhibitors suppress interleukin-1beta-induced nitric oxide and prostaglandin E2 production in human chondrocytes. *Osteoarthritis Cartilage* 2008;**16**:1267–74
32 Chen WP, Bao JP, Hu PF, Feng J, Wu LD. Alleviation of osteoarthritis by trichostatin A, a histone deacetylase inhibitor, in experimental osteoarthritis. *Mol Biol Rep* 2010 [Epub ahead of print]
33 Pelletier JP, Jovanovic D, Fernandes JC, Manning P, Connor JR, Currie MG, Di Battista JA, Martel-Pelletier J. Reduced progression of experimental osteoarthritis *in vivo* by selective inhibition of inducible nitric oxide synthase. *Arthritis Rheum* 1998;**41**:1275–86
34 Caron JP, Fernandes JC, Martel-Pelletier J, Tardif G, Mineau F, Geng C, Pelletier JP. Chondroprotective effect of intraarticular injections of interleukin-1 receptor antagonist in experimental osteoarthritis. Suppression of collagenase-1 expression. *Arthritis Rheum* 1996;**39**:1535–44
35 El-Mahmoudy A, Shimizu Y, Shiina T, Matsuyama H, Nikami H, Takewaki T. Macrophage-derived cytokine and nitric oxide profiles in type I and type II diabetes mellitus: effect of thymoquinone. *Acta Diabetol* 2005;**42**:23–30
36 Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. *J Cell Physiol* 2007;**211**:19–26
37 Pulai JI, Chen H, Im HJ, Kumar S, Hanning C, Hegde PS, Loeser RF. NF-kappa B mediates the stimulation of cytokine and chemokine expression by human articular chondrocytes in response to fibronectin fragments. *J Immunol* 2005;**174**:5781–8
38 Sethi G, Ahn KS, Aggarwal BB. Targeting nuclear factor-kappa B activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. *Mol Cancer Res* 2008;**6**:1059–70
39 Gasparian AV, Fedorova MD, Kisselev FL. Regulation of matrix metalloproteinase-9 transcription in squamous cell carcinoma of uterine cervix: the role of human papillomavirus gene E2 expression and activation of transcription factor NF-kappaB. *Biochemistry (Mosc)* 2007;**72**:848–53

(Received May 30, 2010, Accepted September 7, 2010)