Original Article
Effects of nicotine on a rat model of early stage osteoarthritis

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Abstract: The objective of the study was to investigate the effects of nicotine on articular cartilage degeneration and inflammation in a rat model of early stage osteoarthritis (OA), using T2 mapping. In this study, a rat model of early stage OA was established by immobilizing the left knee joints of adult male rats for two weeks. Subsequently, rats were fed with nicotine for two and four weeks. Changes in the articular cartilage from the medial femoral condylar region of the knee were evaluated by gross observation and histological grading with the contents of cartilage matrix detected. T2 values of the articular cartilage were estimated through high-field magnetic resonance imaging (MRI) (7.0T). Levels of serum tumor necrosis factor-α (TNF-α) were assessed by ELISA. The expression of TNF-α and the cholinergic receptor, α7nAChR, in the synovial tissue was measured by RT-PCR. Nicotine treatment ameliorated cartilage destruction, promoted matrix production, reduced the serum level of TNF-α and the expression of TNF-α in the synovial tissue, and increased the expression of α7nAChR in the synovial tissue in the rat model of early stage OA. In conclusion, nicotine prevented cartilage damage and had an anti-inflammatory effect in a rat model of early stage OA. Thus nicotine may have potential as a therapeutic strategy for early stage OA.

Keywords: Nicotine, cartilage degeneration, early stage OA, α7nAChR, T2 mapping

Introduction

Osteoarthritis (OA) is the most common form of arthritis, affecting millions of people worldwide. It is the major cause of disability in the elderly, affecting about 60% of men and 70% of women above the age of 65 [1]. Currently, the efficacy of disease modifying anti-OA drugs is limited [2].

OA involves the progressive degeneration of articular cartilage including loss of chondrocytes and degradation of the extracellular matrix (ECM) [3]. Proinflammatory cytokines secreted by chondrocytes, including tumor necrosis factor-α (TNF-α), contribute to the progression of OA [4]. Therefore, targeted treatments inhibiting the production of proinflammatory cytokines may have potential as effective therapies in OA [5, 6].

Cholinergic mechanisms are important for regulation of immune function and suppressing inflammation [7]. T cells, B cells, and macrophages have the ability to produce acetylcholine (Ach). These cells also express various cholinergic receptors on their surface. In particular, the α7 (α7nAChR) subtype is important for immune regulation [8]. Specific stimulation of α7nAChR on monocytes leads to suppression of proinflammatory cytokine production, and this receptor is essential in the cytokine regulating neuro-immune mechanism known as the cholinergic anti-inflammatory pathway [9, 10].

Recent studies have investigated the anti-inflammatory and therapeutic effect of the cholinergic mechanism and nicotine on arthritis. Maanen et al. demonstrated that oral nicotine attenuated collagen-induced arthritis in mice and reduced TNF-α expression in the synovial tissue of knee joints [11]. Li et al. reported that treatment with nicotine improved arthritis and reduced serum TNF-α and IL-6 levels in a mouse model of collagen-induced arthritis [12]. Wald-
burger et al. revealed that the α7R subunit is expressed in the intimal lining of rheumatoid arthritis (RA) and OA synovium and in cultured fibroblast-like synoviocytes (FLS); stimulation of FLS by ACh suppressed the expression of several chemokines, including IL-8 and IL-6 [13]. However, research into the effects of nicotine on early stage OA is limited.

Magnetic resonance imaging (MRI) has become a common non-invasive method for diagnosis of OA. The recent development of high field strength imaging and functional MRI T2 mapping enables quantitative assessment of early degenerative changes in glycosaminoglycan (GAG) and collagen in articular cartilage [14-16]. Many spontaneous and artificially induced (surgery or drug) OA models [17, 18] have been used for testing potential anti-arthritic agents, and disease modifying effects have been reported [19, 20] for agents currently used to treat patients with OA. Joint immobilization is used as a model of OA [21]. The cartilage degeneration induced by this method is reported to match the characteristics of early stage OA and can be reversible [22, 23].

Therefore, in this study, T2 mapping was used to estimate early articular cartilage degeneration in a rat knee joint immobilization model. The objective of the study was to investigate the effects of nicotine on articular cartilage degeneration and inflammation in a rat model of early stage OA using T2 mapping.

Materials and methods

Experimental design and animals

This study included 105 adult male Sprague-Dawley rats aged 20 weeks (body weight 380-400 g) that were randomly assigned to four groups: 1) Sham-operated group (n = 30); 2) Immobilized nicotine-treated group (n = 30); 3) Immobilized saline-treated group (n = 30); and 4) Untreated controls (n = 15).

Rats were anesthetized with ketamine (80 mg/kg) administered intraperitoneally. The left knee joint of each rat was fixed at 150° of flexion with a rigid Kirschner wire (diameter, 0.8 mm) (Fukang Company, Zhangjiagang, China). In immobilized rats, holes were drilled in the femur distal diaphysis and the middle segment of the tibia and the Kirschner wire was clipped to create a frame, similarly as previously described [21]. In sham-operated rats, holes were drilled in the femur and tibia and Kirschner wires inserted, but no frames were created. After 2 weeks, the Kirschner wires of immobilized and sham-operated rats were removed, and the rats were allowed to move freely in cages. At this time, nicotine (50 μg/ml; Sigma, St. Louis, MO) was added to the drinking water (normal saline) of the immobilized nicotine-treated rats to stimulate the peripheral part of the cholinergic pathway (nAChR). Immobilized saline-treated rats received normal saline. Drinking water was changed twice weekly. Fifteen rats from each group were killed at two weeks and four weeks after removing immobilization.

The protocols for these experiments were approved by the Animal Research Committee of Nanjing Medical University.

Tissue preparation

Rats were euthanized with an overdose of ketamine. For gross morphological observations, the capsule of the knee was cut with a surgical knife, and the joint was opened and fixed with 4% paraformaldehyde in 0.1 M phosphate-buffer, pH 7.4 by perfusion through the aorta. After totally resecting bilateral menisci, the articular cartilage of the femur and tibia was visible.

For histological observation and mechanical property testing, knee joints were resected and kept in the same fixative for 24 hours. The fixed specimens were decalcified in 10% EDTA in 0.01 M phosphate-buffer, pH 7.4 for 2 months at 4°C. After dehydration, the specimens were embedded in paraffin. Five-μm serial sections were obtained at the medial condylar region in the sagittal plane. Standardized serial sections were created in the medial condyle region of the knee.

Histology and histomorphometry

For histological analyses, sections (5 μm in thickness) were stained with hematoxylin-eosin (HE) and safranin 0-fast green (SO). HE staining was used to evaluate structures, cells, and tidemark. SO staining was used to evaluate loss of proteoglycan. Staining results were observed using a light microscope (Ci-L; Nikon, Tokyo, Japan). Semi-quantitative histopathological
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grading was performed according to a modified Mankin scoring system established for grading articular cartilage degeneration [24]. Three sections from each sample were scored by two different blinded observers, for a maximum possible score of 14.

7.0 MRI T2 and T2 mapping

Knees were kept frozen and intact until MRI was performed. Prior to MRI, 60 knee joints (10 from each group) were thawed and rehydrated in normal saline for 16 hours at 4°C. All MRI images of the knee joints were obtained with a specially designed 7.0-T micro-MR imager (Bruker PharmaScan; Bruker BioSpin, Karlsruhe, Germany) using a volume 60 mm coil.

Each joint was placed in a closed atmosphere in a sample tube. All samples were put in the same direction with regard to the magnetic field (anatomic axis parallel to B0) to limit the potential anisotropic effect. For morphological assessment in sagittal planes, T2 weighted images were acquired using a 2D rapid acquisition with refocused echoes RARE sequence (repetition time/echo time [TR/TE] = 2914/36 ms, matrix = 384 × 384, field of view [FOV] = 35 × 35 mm², section thickness [ST] = 0.5 mm, acquisition time [TA] = 11 min 39 sec 364 ms). T2 maps were reconstructed using an MSME acquisition. The T2 map images were obtained as follows: TR = 3852.4 ms; TE = -11.0 ms, 22.0 ms, 33 ms, 165 ms and 176 ms; matrix = 256 × 256; FOV = 35 × 35 mm; ST = 0.50 mm; TA = 12 min 19 sec 666 ms; four signals acquired; flip angle = 180°. The weight-bearing area of the femoral part of each knee joint was selected as the region of interest (ROI). The average T2 relaxation times were calculated using a Bruker ParaVision 5.0 system, and ROIs were analyzed by a senior musculoskeletal radiologist.

Quantitative measurement of GAG and collagen

After MRI, 70 femoral condyles (n = 10/each period) were used for biochemical analysis; 35 femoral condyles were used for assessment of proteoglycan content, and 35 femoral condyles were used for assessment of collagen content. Cartilage samples for biochemical assays were harvested from the weight-bearing portion of each femoral condyle.

Sulfated GAG was measured with a Blyscan™ sGAG assay kit (Biocolor, Newton Abbey, UK) according to the manufacturer’s instructions. Briefly, cartilage samples were cut into small pieces and digested with Papain Extraction Reagent (1 ml) in 1.5 ml labeled microcentrifuge tubes. The papain-digested solution was mixed with Blyscan dye reagent and centrifuged at 12,000 rpm for 10 min. Non-bound GAG dye was removed and dissociation reagent was added to the remaining sample. A microplate reader (Bio-Rad Laboratories, Richmond, CA, USA) was used to measure absorbance at 656 nm.

Collagen content in the cartilage was determined with the Sircol™ collagen assay (Biocolor, Newton Abbey, UK) according to the manufacturer’s instructions. Briefly, a pepsin-digested cartilage solution was mixed with Sircol dye reagent and centrifuged at 12,000 rpm for 10 min. After removing the supernatant, the remaining sample was washed with acid-salt wash reagent and dissolved in alkali reagent. A microplate reader (Bio-Rad Laboratories, Richmond, CA, USA) was used to measure the absorbance at 555 nm.

Cytokine quantification by enzyme-linked immunosorbent assay

Two and four weeks after removal from immobilization, five mice in each group were killed, and serum was harvested. Serum levels of TNF-α were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) according to manufacturer’s instructions (Keygen Biotech Company, Nanjing, China).

Quantitative real-time PCR

Synovial tissues around the left knees were obtained from five rats in each group. Samples were stored at -80°C until use. Samples were pulverized in liquid nitrogen, and lysed in Trizol (Invitrogen, California, USA) at room temperature for 10 mins. Total RNA was extracted according to the manufacturer’s instructions. Briefly, total RNA was reverse transcribed to cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). RT-PCR was performed for nACh-7α, TNF-α, and GAPDH.
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Histomorphologic analyses

Two weeks after removing immobilization, the morphological structure of the articular cartilage in the sham-operated and untreated control groups was normal (Figure 2A, 2D, 2G). In the saline-treated group, there was obvious chondrocyte hypertrophy, clon- ing of chondrocytes, and bone misalignment (Figure 2B); four weeks after immobilization, hypertrophic chondrocytes with an irregular morphological structure were present (Figure 2E). Apparent fibrillation and cleft formation were not observed. In the nicotine-treated group, mild chondrocyte hypertrophy and bone misalignment was noted 2 weeks after removing immobilization. However, there were more normal chondrocytes compared to the saline-treated group, and more normal chon- drocytes in the nicotine-treated group 4 weeks after removing immobilization compared to 2 weeks after removing immobilization (Figure 2C, 2F).

The severity of cartilage destruction was assessed using Mankin’s method (Table 2). Two weeks after removing immobilization, the severity of cartilage destruction in the saline-treated group (3.47 ± 1.19) was significantly greater compared to the untreated control (1.07 ± 0.96) (P < 0.001) and sham-operated (1.33 ± 0.97) (P < 0.001) groups. The nicotine-treated group (2.13 ± 0.92) showed signifi- cantly less severe cartilage damage compared to the saline-treated group (P = 0.001), but signifi- cantly more severe cartilage damage compared to the untreated control (P = 0.006) and sham- operated (P = 0.035) groups. Four weeks after removing immobilization, the nicotine-treated group (1.73 ± 0.96) still showed significantly less severe cartilage damage compared to the saline-treated group (3.63 ± 1.23) (P < 0.001). The severity of cartilage damage was not sig- nificantly different in the nicotine-treated group 4 weeks after removing immobilization com-

Table 1. Primer sequences used for RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Forward: 5-GGCCCTCCGTGTTCCTACC-3 Reverse: 5-CGCCGCTCACCACCTC-3</td>
<td>103 bp</td>
</tr>
<tr>
<td>nACh-7α</td>
<td>Forward: 5-GAGCCTGAGACCAAGACTCC-3 Reverse: 5-TTAGCAGAGTCGACGTC-3</td>
<td>128 bp</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Forward: 5-TCTTCTCATTCCTGTCGTCG-3 Reverse: 5-GGTCTGAGCCATAGCTA-3</td>
<td>128 bp</td>
</tr>
</tbody>
</table>

Results

Gross morphology

Two weeks after removing immobilization, there were no obvious differences in gross morphology between sham-operated, nicotine-treated, saline-treated, and untreated control rats. In all rats, the articular cartilage surfaces were smooth, and no obvious lesions were observed (Figure 1A-C, 1G). Similarly, four weeks after removing immobilization, no apparent degeneration of the articular cartilage was observed in the nicotine-treated and sham-operated groups (Figure 1D, 1F). In the saline-treated group, the surface of the femoral articular cartilage had lost some luster and was not as smooth as the nicotine-treated, sham-operated, and control groups (Figure 1E), but the difference was not significant.

with an ABI Prism 7500 sequence detection system (Applied Biosystems, CA, USA) according to the manufacturer’s instructions. GAPDH was used as the housekeeping gene for normalization. The system was programmed to run for 40 cycles. The primer sequences were designed using Primer 5 software and are listed in Table 1.

Statistical analysis

All statistical analyses were performed using SPSS for Windows, Version 13.0 (Chicago, IL: SPSS Inc). Data are expressed as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and the Fisher’s least significant difference (LSD) post-hoc test was used to compare differences in Mankin’s scores, GAG, collagen, serum levels of TNF-α, and gene expression. Differences between time points were compared with the Student’s t-test. P values < 0.05 were considered statistically significant.
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pared to 2 weeks after removing immobilization ($P = 0.253$).

Quantitative T2 mapping

Two weeks after removing immobilization, T2 mapping of the nicotine-treated group demonstrated a lower signal intensity compared to the saline-treated group (Figure 3B, 3C), but a higher signal intensity compared to the untreated control and sham-operated groups (Figure 3A, 3G). Four weeks after removing immobilization, the nicotine-treated group showed a lower signal intensity compared to the saline-treated group, but there was no difference compared to the sham-operated group (Figure 3D-F). Quantifying T2 values showed similar results (Figure 3H). Two weeks after removing immobilization, the mean T2 value of the nicotine-treated group ($29.52 \pm 4.96$ ms) was significantly lower compared to the saline-treated group ($38.73 \pm 1.58$ ms) ($P < 0.001$), but significantly higher compared to the sham-operated ($24.37 \pm 4.66$ ms) ($P = 0.009$) and untreated control ($23.56 \pm 4.65$ ms) ($P = 0.005$) groups. Four weeks after removing immobilization, the T2 value of the saline-treated group ($39.00 \pm 2.11$ ms) was significantly higher compared to the untreated control ($P < 0.001$), the sham-operated ($23.82 \pm 5.04$ ms) ($P < 0.001$), and nicotine-treated ($27.01 \pm 4.71$ ms) ($P < 0.001$) groups. The difference between the sham-operated group and the nicotine-treated group was not significant ($P = 0.105$).

Quantitative measurement of GAG and collagen

Two and four weeks after removing immobilization, there were no significant differences in GAG content of the articular cartilage in the untreated control (83.16 ± 5.21 µg/mg) and sham-operated groups (Figure 4A). Two weeks after removing immobilization, GAG content was significantly decreased in the saline-treated group (69.54 ± 3.09 µg/mg) and nicotine-treated group (74.40 ± 4.22 µg/mg) ($P = 0.001$) compared to the sham-operated group (82.49 ± 4.46 µg/mg). GAG content was higher in the nicotine-treated group compared to the saline-treated group, but the difference was not significant ($P = 0.131$). Four weeks after removing immobilization, GAG content of the articular cartilage was significantly decreased in the saline-treated group (68.55 ± 4.86 µg/mg) compared to the sham-operated group (82.06 ± 2.25 µg/mg) ($P < 0.001$). GAG content was significantly increased in the nicotine-treated group (79.31 ± 4.20 µg/mg) compared to the saline-treated group ($P = 0.002$). There were no significant differences between the sham-operated group and the nicotine-treated group ($P = 0.350$).
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Two weeks after removing immobilization, the saline-treated group (23.95 ± 5.62 pg/ml) had a significantly increased mean serum TNF-α level compared to the untreated control (6.22 ± 2.09 pg/ml) (P < 0.001) and sham-operated (6.03 ± 3.72 pg/ml) (P < 0.001) groups. The nicotine-treated group (12.83 ± 4.09 pg/ml) had a significantly decreased serum TNF-α level compared to the saline-treated group (P = 0.018). Similarly, four weeks after removing immobilization, the serum TNF-α level in the saline-treated group (24.57 ± 6.25 pg/ml) was significantly increased compared to the untreated control (P < 0.001), sham-operated (7.16 ± 1.96 pg/ml) (P < 0.001) groups. The nicotine-treated group (10.22 ± 4.20 pg/ml) (P < 0.001) groups. The nicotine-treated group had a lower serum TNF-α level four weeks after removing immobilization compared to two weeks after removing immobilization, but the difference was not significant (P = 0.347) (Figure 5).

Gene expression analysis of the synovial tissues

Two and four weeks after removing immobilization, the saline-treated group had a significantly increased mean TNF-α RNA level compared to the untreated control (P = 0.001) and sham-operated (7.16 ± 1.96 pg/ml) (P < 0.001) groups. The nicotine-treated group had a lower serum TNF-α level four weeks after removing immobilization compared to two weeks after removing immobilization, but the difference was not significant (P = 0.347) (Figure 5).

Table 2. Histological score of articular cartilage

<table>
<thead>
<tr>
<th>Femoral condyle</th>
<th>Sham group</th>
<th>Saline group</th>
<th>Nicotine group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>1.33 ± 0.97</td>
<td>3.47 ± 1.19^ab</td>
<td>2.13 ± 0.92^abc</td>
<td>1.07 ± 0.96</td>
</tr>
<tr>
<td>4 weeks</td>
<td>1.27 ± 1.08</td>
<td>3.63 ± 1.23^ab</td>
<td>1.73 ± 0.96</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean ± SD. 2 weeks = 2 weeks after removing immobilization, 4 weeks = 4 weeks after removing immobilization. ^P < 0.05 vs. control group; ^P < 0.05 vs. sham-operated group; ^P < 0.05 vs. saline-treated group.

There were no significant differences in collagen content of the articular cartilage at any time point between any groups (Figure 4B).

Levels of serum TNF-α

Two and four weeks after removing immobilization, the saline-treated group had a significantly increased mean serum TNF-α level compared to the untreated control (P < 0.001) and sham-operated (P < 0.001) groups. The nicotine-treated group had a lower serum TNF-α level four weeks after removing immobilization compared to two weeks after removing immobilization, but the difference was not significant (P = 0.347) (Figure 5).

Figure 2. Hematoxylin-eosin (A-G) and Safranin-O staining (H-N). (A and H) Sham-operated group 2 weeks after removal of immobilization; (B and I) Saline-treated group 2 weeks after removal of immobilization; (C and J) Nicotine-treated group 2 weeks after removal of immobilization; (D and K) Sham-operated group 4 weeks after removal of immobilization; (E and L) Saline-treated group 4 weeks after removal of immobilization; (F and M) Nicotine-treated group 4 weeks after removal of immobilization; (G and N) Untreated control group. Magnification × 100.
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operated ($P < 0.001$) groups. In the nicotine-treated group, TNF-α expression level was significantly decreased compared to the saline-treated group. Two and four weeks after removing immobilization, mean $\alpha$7nAChR RNA levels were significantly increased in the saline-treated and nicotine-treated groups compared to the untreated control and sham-operated groups ($P < 0.001$). Four weeks after removing immobilization, $\alpha$7nAChR RNA levels were significantly increased in the nicotine-treated and saline-treated groups ($P < 0.001$) (Figure 6).

Discussion

In this study, pathological assessments and T2 mapping showed that nicotine ameliorated cartilage destruction and promoted matrix produc-
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In addition, nicotine reduced the serum level of TNF-α and the expression of TNF-α in the synovial tissue, and increased the expression of α7nAChR, indicating that nicotine-induced effects in OA are likely due to the peripheral stimulation of α7nAChR.

To our knowledge, there is one of the first studies to investigate the effect of nicotine on early stage OA. Van Maanen et al. established a mouse model of clinical arthritis using type II collagen. They observed bone degradation, extensive proteoglycan loss, and inflammatory cell infiltration, which were attenuated by treatment with nicotine [11]. Li et al. showed similar results [12]. Wu et al. reported that activating the cholinergic anti-inflammatory pathway with nicotine attenuated collagen-induced arthritis via suppression of the Th17 response [25]. The current study focused on early stage OA. Compared to saline, nicotine increased the number of normal chondrocytes, and the proteoglycan and GAG content in the articular cartilage. The effect seemed to be mediated by decreased serum and expression levels of TNF-α [11, 12].

The current study showed a strong expression of α7nAChR in salinetreated animals, which was unexpected. Westman et al. used immunohistostaining and found a higher expression of α7nAChR in RA patients’ synovial tissue compared to that of healthy subjects [10], but the difference did not reach statistical significant. Waldburger et al. used quantitative PCR and showed that α7R mRNA levels were similar in both RA and OA patients’ synovium [13]. Schubert and Beckmann measured nicotinic and muscarinic ACh receptor expression in synovial tissue from patients with RA and OA using RT-PCR and immunofluorescence labeling and also found no obvious difference between the samples [26].

Previous reports identify a role for the α7nAChR receptor in the anti-inflammatory cholinergic pathway [7]. Wang et al. demonstrated that in wild-type mice binding of ACh to α7-nAChR reduced the production of proinflammatory cytokines, including TNF-α, IL-1 [7]. van Maanen et al. found that stimulation of α7nAChR by selective agonists such as AR-R17779 reduced the level of serum TNF-α in a mouse model of collagen-induced arthritis [11] and described an increase in proinflammatory cytokines in α7nAChR knockout mice with experimentally-induced arthritis [27]. Other studies show that TNF-α and IL-1 play an important role in the degradation of the ECM and the destruction of cartilage in OA [28]. Taken together, these data suggest that nicotine

Figure 4. Biochemical analysis of cartilage. (A) GAG content analysis (B) collagen content analysis. 2 weeks = 2 weeks after removing immobilization, 4 weeks = 4 weeks after removing immobilization. *P < 0.05, **P < 0.005 vs. sham-operated group; #P < 0.05, ##P < 0.005 vs. saline-treated group.

Figure 5. Serum TNF-α levels. 2 weeks = 2 weeks after removing immobilization, 4 weeks = 4 weeks after removing immobilization. *P < 0.05, **P < 0.005 vs. sham-operated group; #P < 0.05, ##P < 0.005 vs. saline-treated group.
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could reduce the level of TNF-α through α7nAChR in OA.

In addition, our study indicated that GAG synthesis was upregulated by nicotine, which promoted the repair of the articular cartilage. Akmal et al. showed that nicotine, at physiological levels found in smokers, increased DNA, GAG, and collagen synthesis of nucleus pulposus cells [29]. However, Gullahorn et al. found that nicotine increased collagen synthesis, but had no effect on GAG synthesis in vitro [30]. Similarly, Ying et al. showed nicotine promotes proliferation and collagen synthesis of chondrocytes isolated from normal human and OA patients without increasing GAG synthesis [31].

MRI T2 mapping is a useful noninvasive method for the detection of early degenerative changes of cartilage in OA [14, 32]. In particular, GAG content causes variation in T2 values [33]. Watrin-Pinzano et al. induced rat joint cartilage degradation by hyaluronidase. They observed the signal intensities and T2 values of degraded cartilage increased with loss of GAG content, with no obvious change in collagen content [16]. McAlindon reported that delayed gadolinium enhanced MRI of cartilage (dGEMRIC) detected changes in proteoglycan content in knee cartilage among individuals taking collagen hydrolysate and that T2 mapping showed little variability [34]. In the present study, there were higher signal intensities and T2 values in the cartilage of the saline-treated group compared to that of the untreated control and sham-operated groups, as well as a reduced GAG content. In contrast, signal intensities and T2 values were down-regulated in the nicotine group compared to the saline group, which was accompanied by an increased GAG content.

The mechanism of nicotine’s anti-inflammatory effect on arthritis through α7-nAChR has been studied extensively, but remains controversial. JAK-2/STAT-3 and the NF-κB pathways have been implicated in α7R signaling in mouse macrophages [35, 36]. However, Waldburger et al. suggested that cholinergic signaling does not influence NF-κB-driven promoter activity in FLS from RA patients. Conversely, Zheng showed that nicotine reduced IL-1β-induced cartilage apoptosis through activation of PI3K/Akt signaling by regulating Bcl-2/Bcl-xl expression. They also observed that nicotine triggered the phosphorylation of S6 through p70S6K or Akt, regulating protein synthesis including the increase of TIMP-1 and the inhibition of MMP-13, thereby mediating ECM degradation and synthesis [6].

This study is associated with several limitations. First, we did not attempt to distinguish between different types of collagen. Total collagen content was unchanged, but nicotine is reported to up-regulate synthesis of collagen II; therefore, further investigations are warranted [31]. Second, our data suggested a tendency for a higher expression of α7nAChR in the synovial tissue of the nicotine-treated group compared to the saline-treated group, but no published literature supports these findings.

In conclusion, we demonstrated that nicotine treatment attenuated cellular damage, increa-
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sed the synthesis of the ECM, and reduced the serum TNF-α level in a rat model of early stage OA. These data suggest that nicotine prevented cartilage damage and had an anti-inflammatory effect. In the future, nicotine may have potential as a therapeutic strategy for early OA.

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Disclosure of conflict of interest

None.

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