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Original Paper

Activation of $\alpha 7$ Nicotinic Acetylcholine Receptors Prevents Monosodium Iodoacetate-Induced Osteoarthritis in Rats

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Key Words

Osteoarthritis • Nicotinic acetylcholine receptor • Monosodium iodoacetate • Chondrocyte • Rat

Abstract

Background/Aims: Although some evidence suggests that the prevalence of osteoarthritis (OA) is lower in smokers compared to nonsmokers, the mechanisms of nicotine-induced protection remain unclear. Stimulation of the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ -nAChR) appears to be a critical mechanism underlying the anti-inflammatory potential of cholinergic agonists in immune cells. The inhibition of secreted inflammatory molecules and the subsequent inflammatory processes have been proposed as a novel strategy for the treatment of OA. The objective of the present study was to determine whether nicotine-induced protection in a monosodium iodoacetate (MIA) rat model of OA occurs via $\alpha 7$ -nAChR-mediated inhibition of chondrocytes. **Methods:** Both *in vivo* (MIA) and *in vitro* (MIA; Interleukin-1 β , IL-1 β) models of OA were used to investigate the roles and the possible mechanisms whereby $\alpha 7$ -nAChRs protect against knee joint degradation. Multiple experimental approaches, including macroscopic, histological analysis, chondrocyte cell cultures, confocal microscopy, and western blotting, were employed to elucidate the mechanisms of $\alpha 7$ -nAChR-mediated protection. **Results:** Systemic administration of nicotine alleviated MIA-induced joint degradation. The protective effects of nicotine were abolished by administration of the $\alpha 7$ -nAChR-selective antagonist methyllycaconitine (MLA). In primary cultured rat chondrocytes, pretreatment with nicotine suppressed both p38, extracellular regulated kinase (Erk) 1/2 and c-Jun-N-terminal

Y.Liu and D. Wu contributed equally to this work.

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kinase (JNK) mitogen-activated protein kinases (MAPK) phosphorylation and phosphorylated nuclear factor-kappa B (NF- κ B) p65 activation induced by MIA- or IL-1 β , and these effects were also reversed by MLA. **Conclusion:** Taken together, our results suggest that activation $\alpha 7$ -nAChRs is an important mechanism underlying the protective effects of nicotine.

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Introduction

Osteoarthritis (OA), the most common type of arthritis affecting the elderly [1], dramatically impacts health care because of its negative effect on mobility. The extent of articular inflammation is normally associated with disease progression, pointing to articular damage as a contributor to this disease [2]. Recent evidence from both *in vitro* and *in vivo* studies suggests that chondrocytes can release and respond to a number of different cytokines, and are key active players during the disease process [3-5]. Interleukin (IL)-1 β and tumor necrosis factor (TNF)- α are the foremost cytokines involved in the pathophysiology of OA [6]. Consequently, drugs that inhibit secreted inflammatory molecules, such as proinflammatory cytokines, and their ensuing inflammatory induction, may provide understanding into enduring pathogenic injuries, and identifying molecular targets for new therapeutic interventions in OA.

There is increasing evidence that nicotine employs anti-inflammatory and immunomodulatory effects on many cell types, such as T cells, B cells, dendritic cells, astrocytes and polymorphonuclear leukocytes, in the lung, spleen, liver, joints and the gastrointestinal tract [7]. The vagus nerve can modify immune responses and influence inflammation through an $\alpha 7$ -nicotinic acetylcholine receptor ($\alpha 7$ -nAChR) dependent anti-inflammatory pathway [8]. Yu et al. reported that nicotine-induced modulation of adjuvant-induced arthritis (AIA) involves specific alterations in the disease-related cellular and humoral immune responses in AIA [9]. $\alpha 7$ -nAChR knockout mice showed significant increases in both the incidence and severity of arthritis, and synovial inflammation as well as joint destruction were also demonstrated in murine collagen-induced arthritis (CIA) [10, 11]. Evidence of the cholinergic anti-inflammatory pathway in the murine CIA model of rheumatoid arthritis or other arthritis models has been established, however, little is known about the role of $\alpha 7$ -nAChRs in regulating pro-inflammatory cytokines in the pathophysiology of OA.

By injecting monosodium iodoacetate (MIA), a glyceraldehyde-3-phosphate dehydrogenase activity inhibitor, into a joint, one can rapidly induce OA-like lesions in both rodent and nonrodent models [12-15]. In rodents, this model yields cartilage lesions with loss of proteoglycan matrix and functional joint impairment much like human OA [16, 17].

Here, the main objective was the determination, using *in vivo* and *in vitro* models, of nicotine, acting on the $\alpha 7$ -nAChRs, as an inhibitor of chondrocyte-mediated inflammation triggered by MIA, and potentially a cartilage degeneration preventer. This possible anti-inflammatory mechanism, induced by nicotine, was further investigated using IL-1 β , the main proinflammatory cytokine involved in the pathophysiology of OA [6]. Moreover, we investigated whether $\alpha 7$ -nAChR function is associated with modulation of phosphorylation of the mitogen-activated protein kinases (MAPKs) and members of the nuclear factor-kappa B (NF- κ B) pathway.

Materials and Methods

Animals and treatments

All experiments were carried out in accordance with the guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals (publication number 85-23, revised 1985) and the Society for Neuroscience Guidelines for the Care and Use of Animals in Neuroscience Research, and were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University [18].

Male Sprague-Dawley rats (2 months old, 175 to 200 g in weight) were used. All animals were housed in groups of five per cage under standard laboratory conditions with free access to food and water, constant room temperature (22°C) and humidity (50% to 60%), and a natural day/night cycle.

Nicotine, methyllycaconitine citrate (MLA), and MIA were dissolved in sterile saline (0.9% NaCl). Rats were randomly divided into groups as described below. All drugs were acquired from Sigma Chemical Co. (St. Louis, MO, USA).

The chronic MIA induction protocol was similar to that described previously [19]: 1 mg of MIA in 50 μ L of sterile physiologic saline solution was injected once a week for 4 weeks into the right knees through the infrapatellar ligament. Rats were euthanized 1 week after final injection. Vehicle control rats were treated with saline. During nicotine treatment, rats were given intraperitoneal injections of nicotine 1 mg/kg once per day for 5 weeks (1 week before and throughout MIA administration). For all nicotine antagonism experiments MLA 1 mg/kg was injected 30 min before nicotine administration.

Macroscopic analysis

Macroscopic lesions were graded as follows: 0 = normal appearance; 1 = slight yellowish discoloration of the chondral surface; 2 = small cartilage erosions in load-bearing areas; 3 = large erosions extending down to the subchondral bone; and 4 = large erosions with large areas of subchondral bone exposure. Each of the chondral compartments of the knee (the femoral condyles, the tibial plateaus, the patella, and the femoral groove) was graded by two segregated observers who were blinded to the induction procedure. The four compartmental scores were combined (for a maximum possible score of 16) and the mean was determined for each group [20].

Histological analysis

Whole knee joints were fixed immediately in 4% paraformaldehyde, decalcified, and embedded in paraffin. Sagittal sections were stained with hematoxylin (HES) and toluidine blue. The severity of the OA lesions was graded according to Mankin's score [21] with minor modifications. This scale evaluates the severity of OA lesions based on proteoglycan integrity (scale 0–4), cellular changes (scale 0–3), collagen fiber integrity (scale 0–3), invasion of the tidemark by blood vessels (scale 0–1), bone modifications (scale 0–2) and structural changes (scale 0–5, where 0 = normal cartilage structure and 5 = erosion of the cartilage down to the subchondral bone) [22].

Chondrocyte cultures

Normal articular cartilage was obtained from Sprague-Dawley rats euthanized under anesthesia. After joint surgery, articular cartilage pieces were aseptically dissected from femoral head caps and chondrocytes were obtained by sequential digestion with pronase (2 mg/mL) and collagenase B (1.5 mg/mL) (Roche, Basel, Switzerland). The cells were washed twice in phosphate-buffered saline (PBS) and cultured to confluence in 75 mm² flasks at 37°C in a humidified atmosphere containing 5% CO₂. The medium used was DMEM/Ham's F-12 supplemented with L-glutamine (2 mM), gentamicin (50 μ g/mL), amphotericin B (0.5 g/mL), and heat-inactivated fetal calf serum (FCS, 10%). Chondrocytes were cultured under low FCS conditions (10%, v/v) and used at confluence [22].

Staining with α -bungarotoxin and confocal microscopy

Primary cultured chondrocytes (as described above) were passaged into six-well tissue-culture plates at 5×10^5 cells per well, and then cultured for 24 h. Thereafter, cultures were incubated with Alexa Fluor 488-conjugated α -bungarotoxin (2.5 μ g/mL; B13422, Invitrogen Corp., Carlsbad, CA) at 4°C for 15 min. Immediately after incubation, these cells were washed with PBS three times, and then fixed for 15 min in 4% paraformaldehyde in PBS at room temperature. After fixation, cells were washed once with PBS and then mounted for viewing under a laser scanning confocal microscope (Meta 710 Laser Scanning Microscope, Carl Zeiss Inc., Thornwood, NY) [18].

Western blotting

Cells were collected and homogenized in 200 μ L lysis buffer. After incubation for 20 min on ice, cell lysates were separated by centrifugation, and the protein concentration in the extracts was determined by the Bradford assay. Proteins in the cell extracts were denatured with SDS sample buffer and separated by 10% SDS-PAGE. Proteins were transferred to nitrocellulose membranes using a wet transfer unit (Miniprotean@-

III; Bio-Rad Laboratories, Inc., Hercules, CA). The membranes were then incubated with 5% BSA dissolved in Tris-buffered saline with Tween 20 (TBS-T, 10 mmol/L Tris-HCl, 150 mmol/L NaCl, and 0.1% Tween 20, pH 7.5) at room temperature for 1 h, washed three times, and incubated with different antibodies (p38, phospho-p38, extracellular regulated kinase1/2 [Erk1/2], phospho-Erk1/2, the c-Jun-N-terminal kinase [JNK], phospho-JNK and phospho-NF- κ B p65, 1:1000; Cell Signaling Technology Inc., Beverly, MA; $\alpha 7$ -nAChRs, 1:300, Santa Cruz Biotechnology Inc., Santa Cruz, CA) overnight at 4°C. The following day the membranes were washed three times with TBS-T buffer, and incubated with secondary antibody for 1 h, followed by four washes in TBS-T. Signal detection was performed with an enhanced chemiluminescence kit. The results were scanned using a gel imaging system (GelMax Imager; Ultra-Violet Products Ltd., Upland, CA) and measured using analyzing software (GelPro Analyzer software; Media Cybernetics, Inc., Bethesda, MD) [18].

Statistical analyses

All values are expressed as mean \pm standard error of the mean (SEM). Differences between means were analyzed using one-way or two-way analysis of variance (ANOVA) with time and treatment as the independent factors. When ANOVA showed significant differences, pairwise comparisons between means were further analyzed using the Newman-Keuls post hoc test. In all analyses, significance was set at $P = 0.05$ [18].

Results

Nicotine acting via $\alpha 7$ -nAChRs prevents MIA-induced cartilage degradation in rats.

To examine the effects of nicotine on cartilage degradation in rats, macroscopic and Mankin's scores of the knee joints acquired at the conclusion of the experiment were assessed. Intra-articular injection of 1 mg of MIA induced knee joint deterioration, as evidenced by the cartilage on the central load-bearing areas of each articular surface was thin and yellowish, with focal erosions of the femoral condyles and tibial plateaus, as can be observed from the macroscopic score (Fig. 1A) [11, 23]. HES and toluidine blue staining revealed major remodeling of subchondral bone, loss of the hypertrophic chondrocyte layer and a decrease in cellularity in the MIA group (Fig. 1B, C). Nicotine (1 mg/kg) suppressed increases in MIA-induced macroscopic and Mankin's scores ($P < 0.01$) compared with MIA treatment alone. Interestingly, the protective effect of nicotine against MIA-induced cartilage degradation was reversed by pretreatment with MLA (1 mg/kg) (Fig. 1D, E) ($P < 0.01$). Notably, there were significant differences between the control group and the experimental group treated with both MIA and nicotine ($P < 0.05$), implying that although MLA could entirely invert the protective effect of nicotine against MIA-induced cartilage degradation, nicotine cannot fully prevent the MIA-induced cartilage degradation effects. No significant cartilage degradation changes were seen in mice treated with nicotine and MLA alone ($P > 0.05$ compared with controls, data not shown).

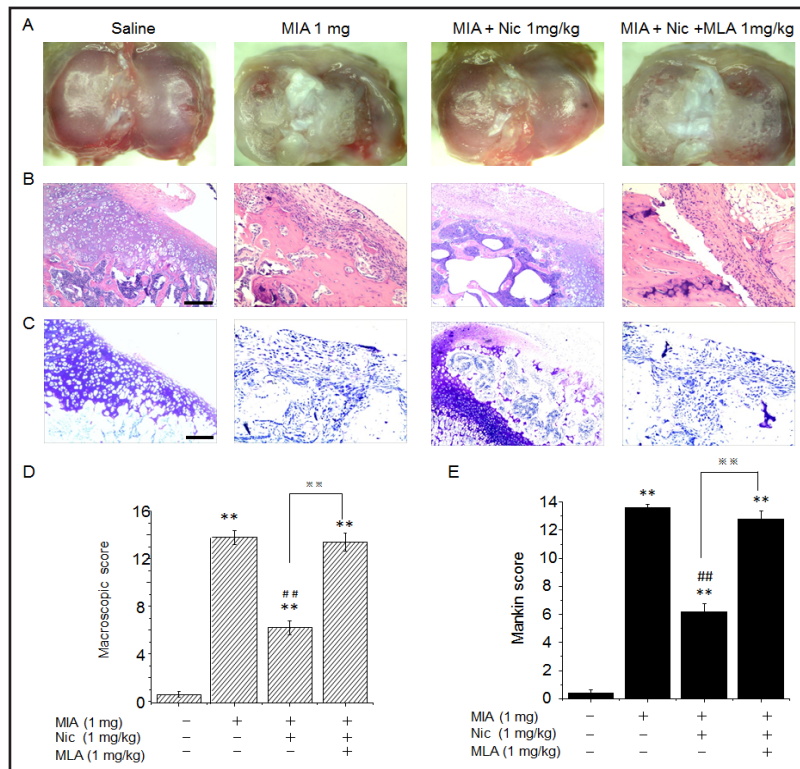
Expression of the $\alpha 7$ -nAChR subunit in chondrocytes

To further examine the hypothesis that nicotine-induced cartilage fortification in an MIA rat model is mediated through its actions on $\alpha 7$ -nAChRs on chondrocytes, the expression of $\alpha 7$ -nAChRs was evaluated using primary cultured chondrocytes. The $\alpha 7$ -nAChR subunit protein was identified by western blotting in primary cultured cells (Fig. 2A). Furthermore, to evaluate whether α -bungarotoxin, an $\alpha 7$ -nAChR subunit-selective blocker, could bind to this receptor, primary chondrocytes were stained with Alexa Fluor 488 conjugated α -bungarotoxin. Strong binding of α -bungarotoxin was detected on the chondrocyte surface (Fig. 2B).

Nicotine acting via $\alpha 7$ -nAChRs suppresses p38, Erk1/2 and JNK MAPK activation induced by MIA in chondrocytes

MAPKs collectively constitute crucial signaling molecules in the development of inflammation. Phosphorylation of MAPKs leads to the commencement of signal cascades

Fig. 1. Evaluation of nicotine-mediated protection against MIA-induced knee joint degradation by macroscopic and histological analysis. (A) Representative macroscopic photographs of tibial plateaus of the rat knee joints. (B) Photomicrographs showing representative hematoxylin staining of the rat knee joints. (C) Photomicrographs showing representative toluidine blue staining of the rat knee joints. (D) Semiquantitative macroscopic scores for joint destruction. (E) Semiquantitative Mankin's score for joint destruction. Data are presented as mean \pm SEM (n = 6). * P < 0.05 and ** P < 0.01 vs. the control (saline



alone) group; ## P < 0.01 vs. the MIA treatment group. *** P < 0.01 between the MIA + nicotine treatment group and the MIA + nicotine + MLA treatment group. Scale bar: 200 μ m.

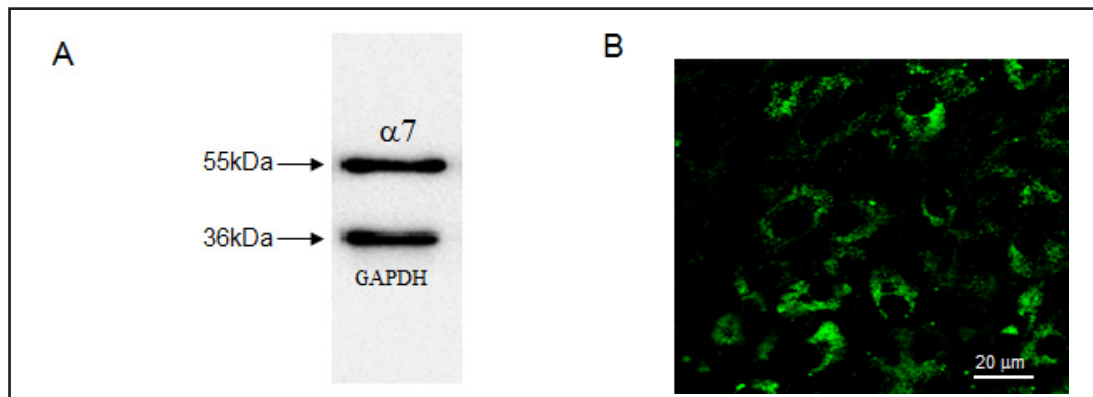
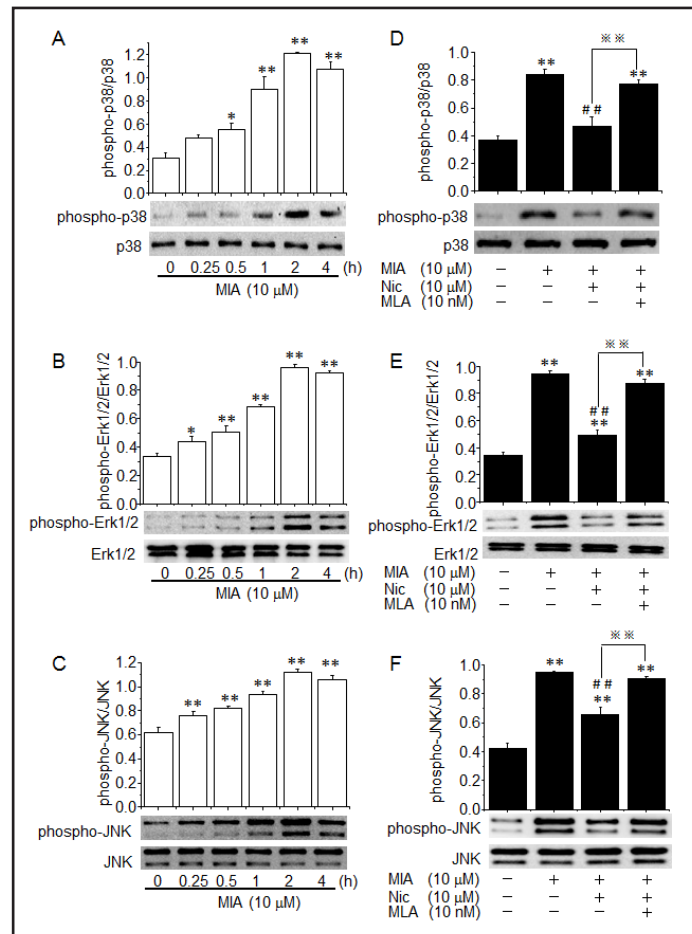


Fig. 2. nAChR $\alpha 7$ subunit expression in primary chondrocyte cell cultures. (A) Western blot of $\alpha 7$ nAChR protein in primary cultured chondrocytes. (B) Primary rat chondrocytes were stained with Alexa Fluor 488 conjugated α -bungarotoxin and viewed by fluorescent confocal microscopy. Scale bar: 20 μ m.

that controls the creation of a variety of pro-inflammatory factors such as TNF- α in chondrocytes [24-26]. In the current study, we considered whether nicotine abolished the MIA-induced phosphorylation of p38, Erk1/2 and JNK MAPKs in chondrocytes. MIA treatment (10 μ mol/L) induced rapid and transient phosphorylation of p38, Erk1/2 and JNK, reflecting their activation, and peak levels of phosphorylated p38, Erk1/2 and JNK occurred after 2 h (Fig. 3A, B, C). These outcomes imply that p38, Erk1/2 and JNK MAPKs are activated in response to MIA stimulation in chondrocytes. Then, we investigated the effects of nicotine on MIA-induced p38, Erk1/2 and JNK MAPK phosphorylation. Pretreatment with 10 μ mol/L nicotine attenuated MIA-induced increases of phosphorylated p38, Erk1/2

Fig. 3. The effects of nicotine on MIA-induced p38, Erk1/2 and JNK MAPK activation in chondrocytes. MIA increased p38, Erk1/2 and JNK MAPK phosphorylation, indicative of their activation. (A,B,C) Chondrocytes were treated with 10 μ M MIA for various lengths of time as indicated. (D,E,F) Chondrocytes were pretreated with nicotine (10 μ M) in the absence or presence of MLA (10 nM) for 30 min and then challenged with MIA (10 μ M) for 30 min. After treatment, chondrocytes were harvested and levels of phosphorylated p38 (D), Erk1/2 (E) and JNK (F) MAPKs were analyzed. Lower: representative blots are shown. Upper: densitometric analysis of the phosphorylated forms of p38, Erk1/2 and JNK MAPK. Data are presented as the mean \pm SEM of four independent experiments. * P < 0.05 and ** P < 0.01 vs. the control (saline alone) group; ## P < 0.01 vs. the MIA treatment group. *** P < 0.01 between the MIA + nicotine treatment group and the MIA + nicotine + MLA treatment group.

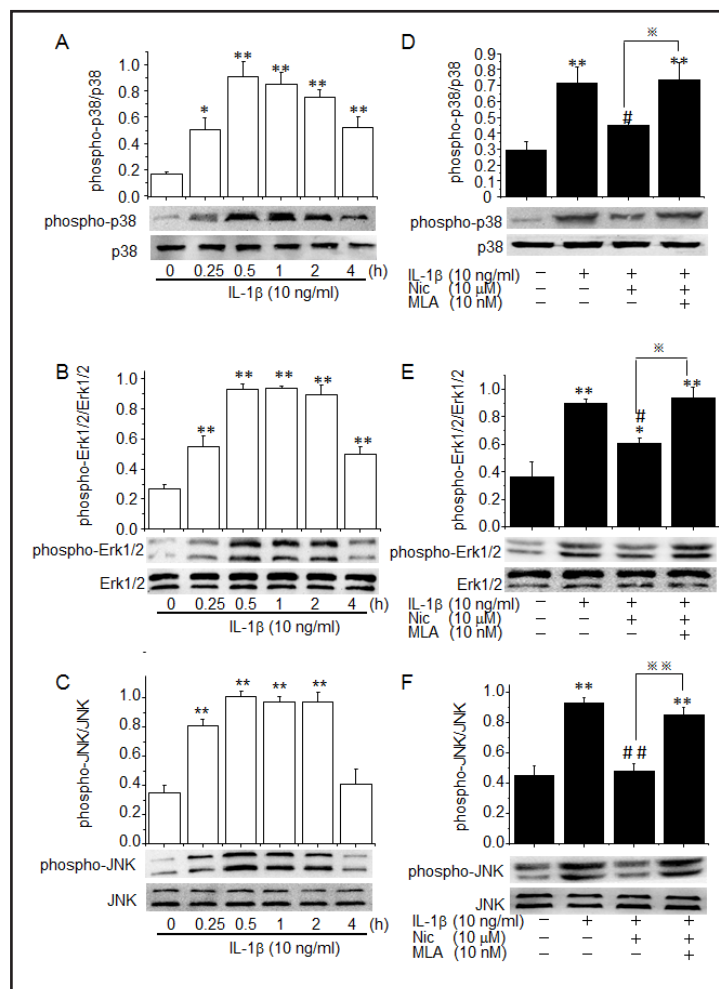


and JNK (Fig. 3D, E, F). Furthermore, the attenuating effects of nicotine on p38, Erk1/2 and JNK phosphorylation were completely blocked by 30 min of pretreatment with MLA at 10 nmol/L. No significant changes of phosphorylated p38, Erk1/2 and JNK were seen in chondrocytes treated with nicotine and MLA alone ($P > 0.05$ compared with controls, data not shown). Collectively, These data infer that nicotine, acting on the $\alpha 7$ -nAChRs, suppresses MAPK signal transduction, thereby influencing inflammation systems.

Nicotine acting via $\alpha 7$ -nAChRs suppresses p38, Erk1/2 and JNK MAPK activation induced by IL-1 β in chondrocytes

Amongst the proinflammatory cytokines implicated in OA, IL1 β is believed to be the foremost in cartilage degradation [6]. We explored the possibility that nicotine also regulated IL1 β -induced MAPK signaling transduction. Chondrocytes were treated with IL1 β at 10 ng/mL for different lengths of time to determine the extent of MAPK activation after IL1 β stimulation. Treatment with IL1 β also resulted in rapid and transient phosphorylation of p38, Erk1/2 and JNK, with peak levels also occurring at 30 min (Fig. 4A, B, C). These results suggest that p38, Erk1/2 and JNK are triggered in reaction to IL1 β stimulation in chondrocytes. We then investigated whether nicotine could regulate IL1 β -induced p38, Erk1/2 and JNK phosphorylation. Pretreatment with nicotine at 10 μ mol/L suppressed IL-1 β -induced increases in phosphorylated p38, Erk1/2 and JNK (Fig. 4D, E, F). Additionally, the suppressive effects of nicotine on p38, Erk1/2 and JNK phosphorylation were entirely reversed by MLA (10 nmol/L). These data suggest that nicotine, through its actions on $\alpha 7$ -nAChRs, also inhibits IL1 β -induced chondrocyte activation via inhibition of MAPK signal transduction.

Fig. 4. The effects of nicotine on IL1 β -induced p38, Erk1/2 and JNK MAPK activation in chondrocytes. IL1 β also increased p38, Erk1/2 and JNK MAPK phosphorylation, reflecting their activation. (A,B,C) Chondrocytes were treated with 10 ng/mL IL1 β for various lengths of time as indicated. (D,E,F) Chondrocytes were pretreated with nicotine (10 μ M) in the absence or presence of MLA (10 nM) for 30 min and then challenged with IL1 β (10 ng/mL) for 30 min. After treatment, chondrocytes were harvested and levels of phosphorylated p38 (D), Erk1/2 (E) and JNK (F) MAPKs were analyzed. Lower: representative blots are shown. Upper: densitometric analysis of the phosphorylated forms of p38, Erk1/2 and JNK MAPK. Data are presented as the mean \pm SEM of four independent experiments. * P < 0.05 and ** P < 0.01 vs. the control (saline alone) group; # P < 0.05 and ## P < 0.01 vs. the IL1 β treatment group. * P < 0.05 and ** P < 0.01 between the IL1 β + nicotine treatment group and the IL1 β + nicotine + MLA treatment group.



Nicotine or MLA administration alone did not significantly affect p38, Erk1/2 and JNK phosphorylation in chondrocytes ($P > 0.05$ compared with controls, data not shown).

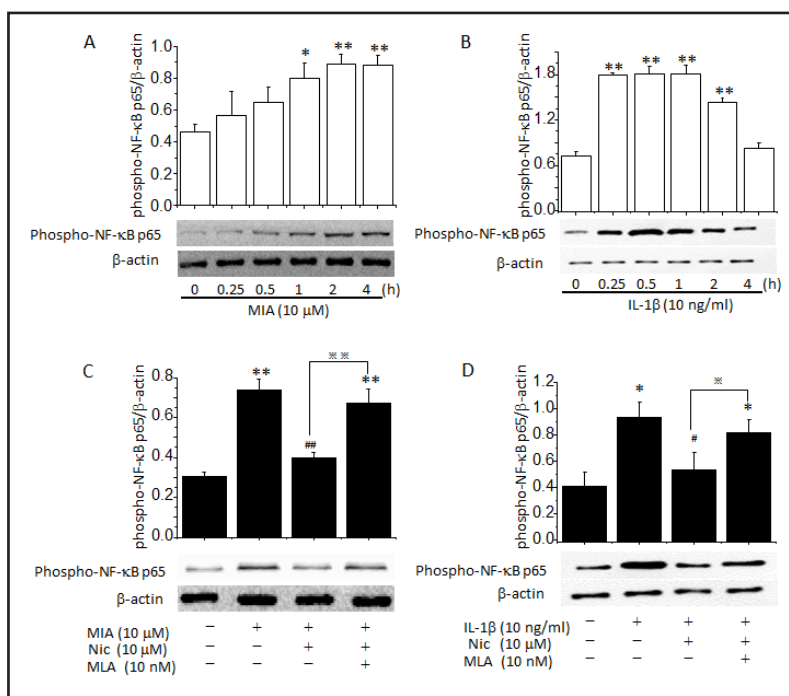
Nicotine acting via $\alpha 7$ -nAChRs suppresses MIA or IL-1 β -induced phospho-NF- κ B p65 activation in chondrocytes

To further examine the mechanisms of nicotine-mediated suppression of inflammatory responses in MIA or IL1 β -activated chondrocytes, we focused on the activation of NF- κ B, a family of transcription factors related to inflammatory responses and cartilage degradation. The results suggest that treatment with MIA at 10 μ mol/L or IL-1 β at 10 ng/mL induced phosphorylation of NF- κ B p65 (Fig. 5A, B). These results suggest that NF- κ B is activated in response to MIA or IL-1 β stimulation in chondrocytes. Pretreatment with nicotine at 10 μ mol/L suppressed IL-1 β - or MIA-induced increases in phosphorylated NF- κ B p65, and these effects could be prevented by 10 nmol/L MLA (Fig. 5C, D). Nicotine or MLA administration alone did not significantly affect NF- κ B p65 phosphorylation in chondrocytes ($P > 0.05$ compared with controls, data not shown). This data point to nicotine acting, via $\alpha 7$ -nAChRs, dampens phosphorylation of NF- κ B p65.

Discussion

Both the initiation and advancement of OA are now thought to include inflammation even in the preliminary stages of the disease [27]. Several studies show a distinct connection

Fig. 5. The effects of nicotine on MIA- or IL1 β -induced NF- κ B p65 activation in chondrocytes. (A,B) Chondrocytes were treated with 10 μ M MIA or 10 ng/mL IL1 β for various lengths of time as indicated. (C,D) Chondrocytes were pretreated with nicotine (10 μ M) in the absence or presence of MLA (10 nM) for 30 min and then challenged with MIA (10 μ M) or IL1 β (10 ng/mL) for 30 min. After treatment, chondrocytes were harvested and levels of phosphorylated NF- κ B p65 were analyzed. Lower: representative blots are shown. Upper: densitometric analysis of the phosphorylated forms of NF- κ B p65. Data are presented as the mean \pm SEM of four independent experiments. * P < 0.05 and ** P < 0.01 vs. the control (saline alone) group; # P < 0.05 and ## P < 0.01 vs. the IL1 β treatment group. * P < 0.05 and ** P < 0.01 between the IL1 β + nicotine treatment group and the IL1 β + nicotine + MLA treatment group.



between the evolution of tibiofemoral cartilage damage and the occurrence of activated synovial macrophages [28, 29]. Discharged inflammatory factors such as proinflammatory cytokines are essential mediators of the disturbed metabolism and enhanced catabolism of joint tissue involved in OA [6]. Consequently, drugs that can inhibit the performance of secreted inflammatory molecules, such as proinflammatory cytokines, and the ensuing inflammatory progression may contribute to articular fortification.

During this investigation, we found that systemic administration of nicotine significantly improved MIA-induced cartilage degradation assessed by macroscopic and Mankin's score of knee joints. Additionally, we discovered that nicotine inhibited MIA- or IL1 β -stimulated elevation of phosphorylation of p38, Erk1/2 and JNK MAPKs and of NF- κ B p65 from reactive chondrocytes. The p38, Erk1/2 and JNK MAPKs signaling pathways are essential in cartilage depletion [30]. Sondergaard et al. found that inhibition of the MAPK p38, p44/42 and Src family abolished proteolytic cartilage degradation by blocking MMP synthesis and activity [31]. Moreover, growing evidence demonstrates that NF- κ B signaling as not only playing a key role in the pro-inflammatory stress-related responses of chondrocytes to extra- and intra-cellular insults, but also in the management of their differentiation program [32]. Martel-Pelletier et al. found that MAPKs and NF- κ B together regulate interleukin-17-induced nitric oxide production in human osteoarthritic chondrocytes [33]. Taken together, our data indicate that the p38, Erk1/2 and JNK MAPKs and NF- κ B signaling-transduction pathways are involved in the inhibitory effects of nicotine on MIA- or IL1 β -induced chondrocyte activation.

The Framingham study was one of the first to generate the hypothesis that smoking may protect against OA. The negative association was identified in both cross-sectional [34] and cohort analyses [35]. This supposition is also well advocated by several epidemiological reports [36-40], which have reported that the frequency of OA is lower in smokers than in

nonsmokers, leading to the hypothesis that the reduced occurrence of OA in smokers may be due to the presence of nicotine from tobacco. Considerable evidence supports that nicotine exerts its effects primarily by acting on nAChRs [41]. These subunits combine in the formation of a large family of nAChRs, the most abundant of which are heteromeric $\beta 2$ -containing nAChRs and homomeric $\alpha 7$ nAChRs. Recent studies suggest that the role of $\alpha 7$ -nAChRs in immune responses and inflammatory cascades has attracted much interest according to their apparent relevance to a variety of human diseases, such as diabetes, asthma, cystic fibrosis, Parkinson's disease, sepsis, ulcerative colitis and arthritis. Those diseases have been related to runaway cytokine-mediated inflammation [42]. Cytokine-based therapeutic agents play a major role in the treatment of these diseases. The 'nicotinic anti-inflammatory pathway' may have critical clinical implications, as treatment with nicotinic agonists can potentially moderate the production of pro-inflammatory cytokines from immune cells via $\alpha 7$ homomeric nAChRs ($\alpha 7$ -nAChRs) [43, 44].

In the current study, we found that the alleviation of MIA-induced cartilage degradation in the knee was reversed by the $\alpha 7$ -nAChR-selective antagonist MLA. In rat primary cultured chondrocytes, pretreatment with nicotine suppressed MIA- or IL1 β -induced chondrocyte activation, as shown by inhibition of phosphorylation of p38, Erk1/2 and JNK MAPKs and NF- κ B p65. These inhibitory effects of nicotine were also reversed by MLA. Those data demonstrated that nicotine-induced protection in a MIA rat model of OA occurs via $\alpha 7$ -nAChR-mediated inhibition of chondrocytes. It is of interest to explore the possible mechanisms by which $\alpha 7$ -nAChRs could trigger responses in different cells. At first, it was thought that the cell response was mediated by influx of calcium through activated nAChRs, causing an increase in the intracellular calcium concentration, and then activated variety signaling cascade. Moreover, $\alpha 7$ -nAChR agonists also can modulate several other signaling pathways such as Jak2/STAT3 signaling pathway [45]. Together with results from various other studies, these data suggest that additional and yet not fully clear mechanisms to link ligand gated ion channels to intracellular signaling pathways might exist [41].

However, nicotine might also play a much more direct part in this process. Data from a previous study showed that nicotine upregulates glycosaminoglycan and collagen synthesis by articular chondrocytes at physiological levels seen in those who smoke [46]. Ying et al. reported that nicotine promoted proliferation and collagen synthesis of chondrocytes isolated from normal human and OA patients [47]. The exact mechanism underlying the protection provided by nicotine must still be fully characterized.

Our results are the first to demonstrate that nicotine inhibits MIA-induced (*in vivo*) and MIA- or IL1 β -induced (*in vitro*) chondrocyte activation via $\alpha 7$ -nAChRs and decreases the resulting phosphorylation of p38, Erk1/2 and JNK MAPKs and NF- κ B p65. These data strongly support the hypothesis that inhibition of chondrocyte activation by stimulation of $\alpha 7$ -nAChRs may provide a new therapeutic strategy for treatment of inflammation-related disorders. Thus, nicotinic agonists that specifically target the $\alpha 7$ -nAChR might serve as potential therapeutic agents for OA and other inflammation-related diseases.

Abbreviations

OA (osteoarthritis); $\alpha 7$ -nAChR ($\alpha 7$ nicotinic acetylcholine receptor); MIA (monosodium iodoacetate); IL (interleukin); TNF (tumor necrosis factor); AIA (adjuvant-induced arthritis); CIA (collagen-induced arthritis); MAPKs (mitogen-activated protein kinases); NF- κ B (nuclear factor-kappa B); MLA (methyllycaconitine citrate); Erk1/2 (extracellular regulated kinase1/2); JNK (c-Jun-N-terminal kinase).

Disclosure Statement

The authors state that no conflicts of interest exist.

Acknowledgements

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