Protective effects of Schisandrin C on chondrocyte damage by inhibiting MAPK and NF- κ B signal pathways

Ruipeng Li (✉ Liruipeng87@163.com)

Research

Keywords: Schisandrin C, osteoarthritis, inflammatory response, MAPK, NF- κ B

Posted Date: June 5th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-32659/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Osteoarthritis (OA) is a common joint disorder that affects the elderly population. The pathogenesis of OA is related to cartilage degradation and inflammatory response. Schisandrin C (Sch C), a dibenzocyclooctadiene derivative of *Schisandra chinensis*, has been demonstrated to exert anti-inflammatory effect in various inflammation diseases. However, the effect of Sch C on OA remains unclear. Thus, we aimed to investigate its action on chondrocytes and explore the mechanism associated with the inflammatory response.

Methods: In this study, we chose rabbits and SW1353 cells as in vivo/in vitro models.

Matrix metalloproteinase (MMP3), Nitric oxide (NO), IL-1 β and TNF- α were detected by ELISA kits. The expression of MAPK/NF- κ B related signaling molecules were determined by western blot.

Results: In vitro, Sch C suppressed the IL-1 β -induced production of NO and PGE2. Additionally, Sch C significantly decreased IL-1 β -induced p65 phosphorylation and mitogen-activated protein kinase (MAPK) activation, as evidenced by the reduced phosphorylation of p38, extracellular signal-regulated kinase (ERK), and c-Jun amino-terminal kinase (JNK). Moreover, Sch C prevented cartilage damage in rabbit OA model with lower Mankin's score than the model group. Sch C also inhibited the level of inflammatory cytokines in the articular cavity flushing fluid in rabbit OA model.

Conclusions: Our study suggested that Sch C inhibited the IL-1 β -induced inflammation and cartilage degradation through suppressing the MAPK and NF- κ B signal pathways, indicating a potential in OA treatment.

Background

Osteoarthritis (OA) is the most frequently occurring joint disorder that affects diarthrodial joints such as the hand and knee joints. It is also a degenerative disease characterized by degradation of cartilage and inflammatory responses^[1]. To date, the treatments of OA remain challenging, and its pathophysiology is still evolving. Present therapies such as Nonsteroidal anti-inflammatory drugs (NSAIDs) are at best moderately effective for the alleviation of the OA-related pain and swelling, which do not slow the progression of the disease^[2]. Moreover, long-term use of NSAIDs can cause serious side effects. Therefore, the development of more effective and safe drugs for the treatment of OA is imperative.

During the progression of OA, inflammatory responses are considered to be risk factors for the loss of chondrocytes^[3]. Previous Studies suggest that the pro-inflammatory cytokine IL-1 β played an important role in the development of OA^[4, 5]. Patients who suffered from OA exhibited elevated levels of IL-1 β in the synovial fluid, synovial membrane, and cartilage^[6]. It has been reported that IL-1 β can induce reactive oxygen species production and increase caspase-3 and caspase-9 activities in chondrocytes, eventually leading to chondrocyte apoptosis^[7]. Moreover, IL-1 β has been demonstrated to induce the overproduction of nitric oxide (NO) and promote the expression of matrix-degrading genes such as matrix

metalloproteinase (MMP), which are relevant to exacerbation of cartilage matrix degradation in OA [8]. It is well recognized that NF- κ B and mitogen-activated protein kinase (MAPK) signal pathway are important inflammatory signaling in cartilage degradation and could be therapeutic targets in OA treatment [9, 10]. Therefore, inhibition of inflammatory response may be a potential therapeutic target for future treatment of OA.

Schisandrin C (Sch C), which is extracted from *Schisandra chinensis*, was identified as the effective constituent, exhibiting anti-oxidative and anti-inflammatory effects [11, 12]. Sch C is also found to reduce inflammation and oxidation through the MAPK pathway in lipopolysaccharide-stimulated human dental pulp cells [13]. However, the effect of Sch C on chondrocyte inflammation and OA progression has not been reported. The present study aimed to investigate the anti-inflammatory effects and mechanism of Sch C on IL-1 β -induced chondrocyte in vitro. Furthermore, we investigated the effect of Sch C on rabbit OA model in vivo.

Materials And Methods

Materials

Sch C, dimethylsulfoxide (DMSO) were obtained from Sigma-Aldrich (MO, USA). Sch C was dissolved in DMSO and stored at -20°C. Human IL-1 β was purchased from R&D systems (MN, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were procured from Gibco (CA, USA). Antibodies against p65, p-p65, I κ B α , p-I κ B α , ERK1/2, p-ERK, p38, p-p38 were supplied by Wanlei Life Science (Shenyang, China). Antibodies against JNK, p-JNK were acquired from Wanlei Life Science (Shenyang, China).

Cell culture and treatment

The human chondrosarcoma cell line SW-1353 was purchased from the Shanghai Institute of Cell Biology (Shanghai, China), and it was cultured in 5% DMEM supplemented with 10% FBS, L-glutamine, and antibiotics (50 U/ml penicillin and 50 μ g/ml streptomycin). Cells grown to 70–80% confluency were washed twice with phosphate buffered saline prior to the treatment. Cells were incubated with various concentrations of Sch C in the absence or presence of IL-1 β (10 ng/ml, Sigma-Aldrich, MO, USA). Control group were added with vehicle DMSO in the experiment. Cell viability was detected with a cell counting kit-8 (CCK-8, Wanlei Life Science, China) after 24 h of incubation. Matrix metalloproteinase (MMP3) in cell supernatants was detected by ELISA kits. The absorbance at 450 nm was measured using a microplate reader (Bio-Rad, CA, USA).

NO and PGE2 Measurement

To detect the level of NO and PGE2, SW-1353 cell supernatants were harvested after 24 h treatment with IL-1 β (10 ng/ml) with or without different concentrations of Sch C. Griess reaction was conducted to

measure the NO level and PGE2 concentration was detected with an ELISA kit (Elabscience, China) following the manufacturer's protocol.

Western blot

The total protein was extracted from SW-1353 cells using ice-cold RIPA buffer containing protease inhibitor (Boster Biological Technology, China) and stored at -80°C . The protein concentrations were quantified using the BCA assay (Beyotime Institute of Biotechnology, Shanghai, China). Aliquots (40 μg) were separated on 10% SDS-PAGE and transferred to the equilibrated polyvinylidene difluoride (PVDF) membrane (Millipore, MA, USA), which were blocked in 5% fat-free milk or 5% BSA for 2 h at room temperature and incubated at 4°C overnight using the primary antibodies. Subsequently, blots were washed three times and incubated with horse-radish peroxidase (HRP)-labeled secondary antibody for 2 h at room temperature. Finally, the immunoreactive bands were detected with the chemiluminescence (ECL) kits (Millipore, MA, USA) and an imaging system (Bio-Rad, CA, USA). β -actin was used as the loading control and representative bands were shown.

Animals

Adult male New Zealand white rabbits (6 months old and weighing 3 ± 0.5 kg) were obtained from the Jinan Jinfeng Laboratory Animal Co. Ltd. The animals were housed singly and allowed freely with water and food. All animals were allowed to acclimate to their environment for 7 days before the experiment. All procedures were performed in agreement with the provision of Chinese Experimental Animals Administration Legislation and approved by the Animal Ethics Committee of Drug Nonclinical Evaluation and Research Center.

The animals were randomly divided into sham group and model group. Then rabbit OA models were established by open surgery including anterior cruciate ligament transection (ACL-T) and partial medial meniscectomy^[14]. After 4 weeks, the animals that had undergone surgery were randomly divided into model group and Sch C group. After grouping, intra-articular injection with Sch C (50 μM) or vehicle (equal volume) was performed every 7 days. After 4 weeks, the animals were sacrificed, and the knee samples were fixed with 4% paraformaldehyde solution.

Measurement of inflammatory cytokines in the articular cavity flushing fluid

1 ml normal saline was injected into the articular cavity to flush, and the flushing solution was extracted, centrifuged at $2000 \times g$ for 10 min, then the supernatant was absorbed and stored at -20°C . The level of IL-1 β and TNF- α in the articular cavity flushing fluid were measured according to the instructions of ELISA kits.

Articular cartilage histopathology

Animals were euthanized and operated distal femur condyles were harvested. The specimens were fixed with 10% formalin solution for 24 h and then decalcified for 24 h. All specimens were cut into sections of

4 μm thickness and stained with hematoxylin-eosin (HE) for morphological analysis. Histological evaluation of OA was performed by eight individuals using modified Mankin's score ^[15].

Statistical analysis

Data analysis was performed using Graph Pad Prism 5.0. Data are presented as mean $\pm$ SD and statistically analyzed using one-way analysis of variance, followed by the Tukey's test. *P* values of > 0.05 were considered statistically significant.

Results

Effects of Sch C on cell viability and production of MMP3

CCK-8 assay was used to determine the effects of Sch C on cell viability. As shown in Fig. 1A, Sch C exhibited no cytotoxicity on SW1353 cells with concentrations $\leq 100 \mu\text{M}$ at 24 hours. The effect of Sch C on IL-1 β -induced MMP3 production was evaluated by ELISA kit. As shown in Fig. 1B, IL-1 β (10 ng/ml) significantly induced upregulation of MMP3 level in cell supernatants. Cells pretreated with Sch C in the concentrations ranging from 25 to 75 μM significantly decreased the production of MMP3 induced by IL-1 β in a concentration-dependent manner. Based on the results above, the concentration of 25 and 50 μM Sch C were used for in vitro studies and 50 μM was used for in vivo studies.

Effects of Sch C on IL-1 β -induced production of NO and PGE2

The Griess reagent was used to measure the NO level and a ELISA kit was used to detect PGE2 level. IL-1 β stimulation promoted production of NO and PGE2 in SW1353 cells, whereas Sch C at 25 and 50 μM decreased the production of NO and PGE2 (Fig. 2A, B).

Effects of Sch C on activation IL-1 β -induced MAPK signal activation in SW1353 cells

To explore the activation of MAPK in IL-1 β -induced chondrocytes and its regulation by Sch C, we detected the phosphorylation of p38, ERK and JNK through western blot. IL-1 β stimulation induced activation of MAPK pathway, as shown by increased expression of p-p38, p-ERK and p-JNK level, which were reduced by Sch C in a concentration-dependent manner (Fig. 3A). The results indicated that Sch C could suppress IL-1 β -induced MAPK activation in SW1353 cells.

Effects of Sch C on IL-1 β -induced NF- κ B signal activation in SW1353 cells

The NF- κ B signal activation was investigated by detecting the activation of p65 and degradation of I κ B α . Western blot was used to detect activation of p65 and degradation of I κ B α . The results demonstrated that IL-1 β stimulation significantly induced phosphorylation of I κ B α , and the upregulation of p-p65. However, pretreatment with 50 μ M Sch C could significantly reduce the phosphorylation of I κ B α and upregulation of p-p65 (Fig. 3B). The results indicated that Sch C could also suppress IL-1 β -induced NF- κ B signal activation in vitro.

Effects of Sch C on the level of IL-1 β and TNF- α in the articular cavity flushing fluid in rabbit OA model

As shown in Fig. 4, the level of IL-1 β and TNF- α in the flushing fluid was significantly increased in the model group ($p < 0.05$). Treatment with Sch C significantly decreased the level of IL-1 β and TNF- α in the flushing fluid ($p < 0.05$).

Effects of Sch C on articular cartilage in rabbit OA model

To evaluate the effect of Sch C on articular cartilage defects in vivo, rabbit animal model was constructed by removing ACL and partial medial meniscus. Compared with the model group, fewer fissuring was observed in cartilage from animals treated with 50 μ M Sch C (Fig. 6A). The average Mankin's score of Sch C group was lower than the model group with significant difference (Fig. 6B). These data suggested that Sch C could ameliorate OA in the rabbit model.

Discussion

It is well recognized that OA is a disease characterized by whole joint with cartilage degeneration and synovial inflammation, which often leads to chronic pain and joint disability^[16]. Evidence has shown the important role of inflammation in the pathophysiology of OA^[17]. Inhibition of chondrocyte inflammation was a potential therapeutic strategy in OA^[18]. In this study, we first reported that the effects of Sch C on the IL-1 β -induced inflammation and cartilage damage by inhibiting MAPK and NF- κ B signal pathway in SW1353 cells.

OA development is accompanied with local inflammatory responses. IL-1 β , an important pro-inflammatory cytokine, induced inflammatory response in the chondrocytes, causing the damage to chondrocytes. Interference with IL-1 β could ameliorate inflammation-induced damage to chondrocytes^[19, 20]. Previous study confirmed that IL-1 β (10 ng/ml) could significantly increase the expression of inflammatory cytokines including NO and PGE2, causing damage to chondrocytes^[21]. Our results revealed that IL-1 β significantly upregulate the expression of NO and PGE2 and cause damage to SW1353 cells. However, Sch C could restore the changes induced by IL-1 β stimulation. In addition, we construct rabbit OA model to observe the effects of Sch C on cartilage damage in vivo. The histological analysis further confirmed the protective effects of Sch C by ameliorating cartilage damage.

Due to the crucial role in chondrocyte inflammation and cartilage degradation in OA development [22, 23], MAPK and NF- κ B were investigated in this study. In inactive state, NF- κ B p65 presents with its inhibitor I κ B α in cytoplasm. When treated by IL-1 β , p65 is separated from I κ B α with increased phosphorylation and translocate to nucleus, in which p65 promotes transcription of inflammation genes such as iNOS, COX-2 and cartilage degrading enzymes such as MMP3 [24, 25]. In this study, Sch C decreased phosphorylation of p65 and its inhibitor I κ B α in IL-1 β -induced SW1353 cells. MAPK includes ERK1/2, p38 and JNK, which can be activated by phosphorylation. In accordance with the previous study [26], our results revealed that Sch C decreased the phosphorylation of ERK1/2, p38 and JNK induced by IL-1 β stimulation. These results indicated that the protective effect of Sch C on cartilage degradation is related to the suppression of MAPK and NF- κ B pathways.

Conclusions

Our study is the first paper illustrating that Sch C inhibited IL-1 β -induced chondrocyte inflammation in vitro and ameliorated cartilage damage in vivo, which is related to the inhibition of NF- κ B and MAPK signaling pathways. Our study indicated that Sch C may be a potential drug candidate in OA treatment.

Abbreviations

OA: osteoarthritis; Sch C: Schisandrin C; MMP: matrix metalloproteinase; NO: Nitric oxide; NSAIDs: Nonsteroidal anti-inflammatory drugs; IL-1 β : interleukin-1 β ; TNF- α : tumor necrosis factor- α ; PGE: Prostaglandin E; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; JNK: c-Jun amino-terminal kinase; DMSO: dimethylsulfoxide; DMEM: Dulbecco's modified Eagle's medium; CCK-8: cell counting kit-8; PVDF: polyvinylidene difluoride; HRP: horse-radish peroxidase; ECL: chemiluminescence; ACL-T: anterior cruciate ligament transection; HE: hematoxylin-eosin; NF- κ B: nuclear factor- κ B; I κ B: inhibitory protein of κ B; iNOS: inducible nitric oxide synthase; COX-2: cyclooxygenase-2.

Declarations

Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Experiments were approved by Drug Nonclinical Evaluation and Research Center of Guangzhou General Pharmaceutical Research Institute Co. Ltd. Consent to participate is not relevant to this manuscript.

Consent for publication

Not relevant to this manuscript.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Author's contributions

LRP conceived and designed the work, analyzed the data and wrote the original draft. All authors read and approved the final manuscript.

Acknowledgements

Not relevant to this manuscript.

References

1. Martel-Pelletier J, Barr AJ, Cicuttini FM, et al. Osteoarthritis [J]. *Nat Rev Dis Primers*. 2016;2:16072. DOI:10.1038/nrdp.2016.72.
2. 10.5435/JAAOS-21-09-571
Jevsevar DS. Treatment of osteoarthritis of the knee: evidence-based guideline, 2nd edition [J]. *J Am Acad Orthop Surg*, 2013, 21(9):571–576. DOI: 10.5435/JAAOS-21-09-571.
3. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis [J]. *Nat Rev Rheumatol*. 2015;11(1):35–44. DOI:10.1038/nrrheum.2014.162.
4. Kapoor M, Martel-Pelletier J, Lajeunesse D, et al. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis [J]. *Nat Rev Rheumatol*. 2011;7(1):33–42. DOI:10.1038/nrrheum.2010.196.
5. Wang D, Ren H, Li C, et al. Orexin A prevents degradation of the articular matrixes in human primary chondrocyte culture [J]. *Mol Immunol*. 2018;101:102–7. DOI:10.1016/j.molimm.2018.05.012.
6. Calich AL, Domiciano DS, Fuller R. Osteoarthritis: can anti-cytokine therapy play a role in treatment? [J]. *Clin Rheumatol*. 2010;29(5):451–5. DOI:10.1007/s10067-009-1352-3.
7. Kim J, Xu M, Xo R, et al. Mitochondrial DNA damage is involved in apoptosis caused by pro-inflammatory cytokines in human OA chondrocytes [J]. *Osteoarthritis Cartilage*. 2010;18(3):424–32. DOI:10.1016/j.joca.2009.09.008.

8. Heinegard D, Saxne T. The role of the cartilage matrix in osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1):50–6. DOI:10.1038/nrrheum.2010.198.
9. Saklatvala J. Inflammatory signaling in cartilage: MAPK and NF-kappaB pathways in chondrocytes and the use of inhibitors for research into pathogenesis and therapy of osteoarthritis [J]. *Curr Drug Targets*. 2007;8(2):305–13. DOI:10.2174/138945007779940115.
10. Rigoglou S, Papavassiliou AG. The NF-κB signalling pathway in osteoarthritis [J]. *Int J Biochem Cell Biol*. 2013;45(11):2580–4. DOI:10.1016/j.biocel.2013.08.018.
11. Kim JS, Yi HK. Schisandrin C enhances mitochondrial biogenesis and autophagy in C2C12 skeletal muscle cells: potential involvement of anti-oxidative mechanisms [J]. *Naunyn Schmiedebergs Arch Pharmacol*. 2018;391(2):197–206. DOI:10.1007/s00210-017-1449-1.
12. Guo M, An F, Wei X, et al. Comparative Effects of Schisandrin A, B, and C on Acne-Related Inflammation [J]. *Inflammation*. 2017;40(6):2163–72. DOI:10.1007/s10753-017-0656-8.
13. Oh SY, Kim YH, Bae DS, et al. Anti-inflammatory effects of gomisin N, gomisin J, and schisandrin C isolated from the fruit of *Schisandra chinensis* [J]. *Biosci Biotechnol Biochem*. 2010;74(2):285–91. DOI:10.1271/bbb.90597.
14. Appleton CT, McErlain DD, Pitelka V, et al. Forced mobilization accelerates pathogenesis: characterization of a preclinical surgical model of osteoarthritis [J]. *Arthritis Res Ther*. 2007;9(1):R13. DOI:10.1186/ar2120.
15. van der Sluijs JA, Geesink RG, van der Linden AJ, et al. The reliability of the Mankin score for osteoarthritis [J]. *J Orthop Res*. 1992;10(1):58–61. DOI:10.1002/jor.1100100107.
16. Loeser RF, Collins JA, Diekman BO. Ageing and the pathogenesis of osteoarthritis [J]. *Nat Rev Rheumatol*. 2016;12(7):412–20. DOI:10.1038/nrrheum.2016.65.
17. Scanzello CR. Role of low-grade inflammation in osteoarthritis [J]. *Curr Opin Rheumatol*. 2017;29(1):79–85. DOI:10.1097/BOR.0000000000000353.
18. Shen J, Abu-Amer Y, O'Keefe RJ, et al. Inflammation and epigenetic regulation in osteoarthritis [J]. *Connect Tissue Res*. 2017;58(1):49–63. DOI:10.1080/03008207.2016.1208655.
19. Jenei-Lanzl Z, Meurer A, Zaucke F. Interleukin-1β signaling in osteoarthritis- chondrocytes in focus [J]. *Cell Signal*. 2019;53:212–23. DOI:10.1016/j.cellsig.2018.10.005.
20. Daheshia M, Yao JQ. The interleukin 1beta pathway in the pathogenesis of osteoarthritis [J]. *J Rheumatol*. 2008;35(12):2306–12. DOI:10.3899/jrheum.080346.
21. Huang Y, Wan G, Tao J. C1q/TNF-related protein-3 exerts the chondroprotective effects in IL-1β-treated SW1353 cells by regulating the FGFR1 signaling [J]. *Biomed Pharmacother*. 2017;85:41–6. DOI:10.1016/j.biopha.2016.11.128.
22. Sondergaard BC, Schultz N, Madsen SH, et al. MAPKs are essential upstream signaling pathways in proteolytic cartilage degradation—divergence in pathways leading to aggrecanase and MMP-mediated articular cartilage degradation [J]. *Osteoarthritis Cartilage*. 2010;18(3):279–88. DOI:10.1016/j.joca.2009.11.005.

23. 10.3390/ijms20246275
 Jimi E, Fei H, Nakatomi C. NF-κB Signaling Regulates Physiological and Pathological Chondrogenesis [J]. *Int J Mol Sci*, 2019, 20(24). pii: E6275. DOI: 10.3390/ijms20246275.

24. Roman-Blas JA, Jimenez SA. NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis [J]. *Osteoarthritis Cartilage*. 2006;14(9):839–48.
 DOI:10.1016/j.joca.2006.04.008.

25. Roman-Blas JA, Jimenez SA. Targeting NF-kappaB: a promising molecular therapy in inflammatory arthritis [J]. *Int Rev Immunol*. 2008;27(5):351-3LP74. DOI:10.1080/08830180802295740.

26. Takanche JS, Lee YH1, Kim JS, et al. Anti-inflammatory and antioxidant properties of Schisandrin C promote mitochondrial biogenesis in human dental pulp cells [J]. *Int Endod J*. 2018;51(4):438–47.
 DOI:10.1111/iej.12861.

Figures

Figure 1

Effects of Sch C on cell viability and MMP3 production in cell supernatants. (A) SW-1353 cells were exposed to Sch C alone in the concentrations ranging from 12.5 to 100 μM for 24h (n = 5). (B) MMP3 production was detected by ELISA kit (n=3). Data was shown as means ± SD. #p < 0.05 when compared with the control group; *p < 0.05 when compared with the IL-1β treatment group.

Figure 2

Effects of Sch C on IL-1 β -induced production of NO and PGE2 in SW-1353 cells. Cells were exposed with Sch C (25, 50 μ M) in the presence of IL-1 β (10ng/ml) for 24h. Cell culture supernatants were harvested. (A) Griess reaction was used to detect NO concentration. (B) PGE2 level was measured by ELISA kit. Data was shown as means $\pm$ SD (n = 6). #p < 0.05 when compared with the control group; *p < 0.05 when compared with the IL-1 β treatment group.

Figure 3

Effects of Sch C on MAPK pathway. Cells were exposed with Sch C (25, 50 μ M) with IL-1 β . (A) phosphorylations of ERK, p38, JNK were detected by western blot. (B) Relative protein expression was qualified by Image J software. ERK, p38 and JNK were used as the internal control, respectively (n=3). #p < 0.05 when compared with the control group; *p < 0.05 when compared with the IL-1 β treatment group.

Figure 4

Effects of Sch C on NF-κB signaling pathway. Cells were exposed with Sch C (25, 50μM) with IL-1β. (A) Protein levels of p-IκB, IκB, p-p65, p65 were detected by western blot. (B) Relative protein expression was qualified by Image J software. IκB, p65 and β-actin were used as the loading control, respectively (n=3). #p < 0.05 when compared with the control group; *p < 0.05 when compared with the IL-1β treatment group.

Figure 5

Effects of Sch C on the level of IL-1 β and TNF- α in the articular cavity flushing fluid in rabbit OA model. The concentrations of (A) IL-1 β and (B) TNF- α were detected with ELISA kits ($n = 8$). # $p < 0.05$ when compared with the control group; * $p < 0.05$ when compared with the OA model group.

A

Sham

Model

Sch C

B

Figure 6

Effects of Sch C on cartilage damage in rabbit OA model. (A) Microscopic images of rabbit knee joint sections. Scale bar=100μm. (B) Mankin's score of OA group and Sch C group (n = 8). *p < 0.05 when compared with the OA model group.