Am J Transl Res 2023;15(7):4727-4734
www.ajtr.org /ISSN:1943-8141/AJTR0149753

Original Article

Study on the protective effect of chondroitin sulfate from sturgeons on rat chondrocytes and its potential mechanisms

Xi Zhang[1], Qingsong Li[1], Lei Chen[2]

[1]*Department of Anesthesiology, The Second Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong, China;* [2]*Shandong Academy of Pharmaceutical Sciences, Jinan, Shandong, China*

Received February 19, 2023; Accepted April 24, 2023; Epub July 15, 2023; Published July 30, 2023

Abstract: Objective: To investigate the protective effect of Chondroitin Sulfate from Sturgeons on rat chondrocytes and its possible mechanism. Methods: The model of chondrocyte injury induced by hydrogen peroxide was established and chondrocytes were cultured and divided into the following groups: control group, sham group, model group, Sofast group, Low dose of Chondroitin Sulfate from Sturgeon B (CSSB-L) group, Moderate dose of Chondroitin Sulfate from Sturgeon B (CSSB-M) group and High dose of Chondroitin Sulfate from Sturgeon B (CSSB-H) group. The cell proliferation was analyzed by Cell Counting Kit-8 (CCK-8) assay. The cell apoptosis was detected by flow cytometer. The expression levels of Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Interferon gamma (IFN-γ) in cell supernatants were examined by Enzyme-linked immunosorbent assay (ELISA). Western blot analysis was used to detect the levels of proteins associated with Wnt signal pathway in chondrocytes. Results: Compared with the control group and sham group, the cell proliferation was decreased significantly, cell apoptosis was increased obviously, and the levels of IL-6, IL-8 and IFN-γ were remarkably increased in the model group. For Wnt signal pathway related proteins, the levels of Wnt3a, Frizzled5, Dsh, β-Catenin and C-myc proteins in the model group were significantly reduced, and p-GSK3β expression level was obviously increased (all $P<0.05$). Compared with the model group, CSSB could promote cell viability, and inhibit cell apoptosis and the levels of IL-6, IL-8 and IFN-γ (all $P<0.05$). The levels of Wnt signaling pathways related proteins in the CSSB-M group and CSSB-H group were obviously expressed. Conclusions: Chondroitin sulfate from sturgeons protected rat chondrocytes from injuries induced by hydrogen peroxide, which may be associated with the Wnt signaling pathway.

Keywords: Chondroitin sulfate from sturgeons, chondrocytes, hydrogen peroxide, Wnt signaling pathway

Introduction

The Wnt/β-catenin signaling pathway belongs to the canonical Wnt signaling pathways, which regulate the function, growth, development, and death of cells and plays an important role in these process [1, 2]. The Wnt/β-catenin signaling pathway is a hot topic at present. It is an important regulatory pathway in the differentiation, proliferation and apoptosis of bone cells from patients with osteoarthritis. The abnormities of the Wnt/β-catenin signaling pathway could lead to metabolic imbalance in the skeletal system and ultimately result in the development of osteoarthritis. Matrix metalloproteinases (MMPs) can destroy the extracellular matrix (ECM) of articular cartilage. It was reported that MMP-3 and MMP-13 were the most closely associated with the pathogenesis of knee osteoarthritis [3, 4]. The dynamic balance of the extracellular matrix is maintained by chondrocytes through anabolism and catabolism. The reduced anabolism and increased catabolism can further cause degradation of the extracellular matrix and in turn lead to knee osteoarthritis. Among the currently known matrix metalloproteinases, MMP-13 is the most effective type II collagenase, and overexpression of the MMP-13 gene could cause the destruction of articular cartilage. Some studies showed that the Wnt/β-catenin signal transduction pathway could regulate the expression levels of downstream genes MMPs and BMP family genes [5, 6]. It follows then that MMPs are

the key proteins of Wnt/β-catenin signal pathways in the development of osteoarthritis.

Previous studies showed that chondroitin sulfate from sturgeons could play an important role in improving osteoarthritis pain in rats through regulating the protein expression of MMP-1 and MMP-13 [7, 8]. This study intended to detect the related expression of proteins involved in the Wnt signaling pathways to investigate the molecular mechanism of chondroitin sulfate from sturgeons in protecting chondrocytes, treating and relieving osteoarthritis and related pain. Canonical Wnt signaling pathway includes Wnt secretion proteins, Wnt receptor proteins, glycogen synthesis kinase 3 (GSK-3), adenomatous polyposis coli protein (APC), β-catenin and other transcriptional regulatory factors and so on [9, 10]. Wnt3a is the main protein from the canonical Wnt signaling pathway, which is associated with bone loss, bone mineral density loss and osteoblast function [11]. The receptor protein Frizzled-5 can induce the β-catenin pathway by specific interaction with Wnt-5A. Dsh (Dishevelled) is the key component of Wnt receptor complex associated with cell membranes and can inhibit GSK-3, APC and other proteins after activation. C-myc protein is the expressed product of C-myc proto-oncogene and can induce cell apoptosis. In this study, we focused on the Wnt/β-catenin signaling pathway and explored the relationship between the protective effect of chondroitin sulfate from sturgeons on rat chondrocytes and the Wnt signal pathway by detecting the related proteins expression levels from the Wnt signaling pathway in chondrocytes under different conditions through Western blot. The results of this study provide experimental basis and research direction to further clarify the molecular mechanism of chondroitin sulfate from sturgeons in the treatment of osteoarthritis and related pain, and provide a research foundation for the application of a patented medicine of chondroitin sulfate from sturgeons.

Material and methods

The model of chondrocyte injury induced by hydrogen peroxide

This research was authorized by the Animal Ethics and Welfare Committee (AEWC) of the Second Hospital, Cheeloo College of Medicine, Shandong University (Ethics approval number: No. 2019-135). Chondrocytes were separated from the knees of 4-week-old male Sprague Dawley rats. Cartilage was isolated from the knee joints, and then cut and chopped into small pieces, and washed with phosphate-buffered saline (PBS) three times. The cartilage was digested using 0.25% trypsin in a 37°C constant temperature bath for 30 minutes. The digested cartilage was filtered through 150 mesh strainer and centrifuged at 1200 rpm for 5 minutes, and then collagenase II was added and incubated for 2 hours at 37°C. Finally, chondrocytes were collected after centrifugation at 1200 rpm for 5 minutes.

Chondrocytes were cultured in DMEM medium containing 10% fetal bovine serum (FBS) and penicillin/streptomycin in a 37°C constant temperature incubator with 5% CO_2. The confluent chondrocytes were separated in 1:2 ratios up to passage 2-3 and these chondrocytes were used for the subsequent experiments.

Chondrocytes were cultured in 6-well plates and 200 μmol/L H_2O_2 was added to incubate for 24 h at 37°C when chondrocytes reached 80% confluence [12]. These chondrocytes were divided into groups as follows: (1) Control group: normally cultured chondrocytes. (2) Sham group: The cultured chondrocytes were treated with PBS. (3) Model group: The cultured chondrocytes were treated with H_2O_2. (4) Sofast group: The H_2O_2-processed chondrocytes were further treated with 400 μg/mL chondroitin sulfate Sofast® (Shandong Freda Pharmaceutical Group Co., Ltd.). (5) Low dose of Chondroitin Sulfate from Sturgeon B (CSSB-L) group: The H_2O_2-processed chondrocytes were further treated with 100 μg/mL Chondroitin sulfate from Sturgeon bone (CSSB). (6) Moderate dose of Chondroitin Sulfate from Sturgeon B (CSSB-M) group: The H_2O_2-processed chondrocytes were further treated with 200 μg/mL Chondroitin sulfate from Sturgeon bone. (7) High dose of Chondroitin Sulfate from Sturgeon B (CSSB-H) group: The H_2O_2-processed chondrocytes were further treated with 400 μg/mL Chondroitin sulfate from Sturgeon bone. All the cells from different groups were treated for 24 h.

CCK-8 analysis

Cells viability was determined by CCK-8 assay according to the procedure reported by previ-

ous studies [13]. Briefly, 5 × 10^5 cells/ml were cultured in 96-well plates. Then, chondrocytes were treated with H_2O_2 and various doses of CSSB as before for 24 h. Next, 10 μL CCK-8 solution was added in each well and cultured for 2 h under the conditions of 37°C and 5% CO_2. OD values were detected at 450 nm by the microplate reader.

Cell apoptosis

Cell apoptosis was determined with Flow cytometry according to the procedure reported by previous studies [14]. Briefly, 5 × 10^5 cells per well were cultured in 6-well plates. Chondrocytes were treated with H_2O_2 and various doses of CSSB as before. The Chondrocytes in different groups were collected after washing with cool PBS. Then the cells were resuspended in 100 μL binding buffer. Next, 5 μL propidium iodide (PI) and 5 μL FITC Annexin V were added in the above buffer for 15 minutes at room temperature in the dark. Finally, 400 μL binding buffer was added prior to detecting the apoptosis rate by flow cytometer.

Enzyme-linked immunosorbent assay (ELISA) analysis

The cell supernatants from different groups were collected through centrifugation at 3500 rpm for 10 minutes. The levels of IL-6 (No. PI330, Beyotime Biotech. Inc., China), IL-8 (No. PI641, Beyotime Biotech. Inc., China) and IFN-γ (No. PI325, Beyotime Biotech. Inc., China) in cell supernatants were detected by ELISA according to the protocols of the manufacturer. The optical density was examined at 450 nm by the microplate reader.

Western blot analysis

The expression levels of proteins associated with the Wnt signal pathway in chondrocytes from different groups were detected by Western blot analysis. The Cells were lysed in Radio Immunoprecipitation Assay (RIPA) buffer solution containing protease inhibitors and centrifuged at 12,000 rpm for 10 min at 4°C, and cell supernatant was harvested. Next, proteins were separated with SDS/polyacrylamide gel electrophoresis and transferred to Polyvinylidene Fluoride (PVDF) membranes. Then, PVDF membranes were blocked in a TBS-T buffer containing 5% skim milk for 1 hour. After washing with Tris Buffered Saline-T (TBS-T) buffer, these membranes were incubated with the primary antibodies including Wnt-3a (Dilution: 1:500, No. AF8352, Beyotime Biotech. Inc., China), Frizzled-5 (Dilution: 1:1000, No. ab-75234, Abcam Company, USA), Dsh (Dilution: 1:1000, No. ab126745, Abcam Company, USA), p-GSK-3β (Dilution: 1:800, No. sc-373800, Santacruze Company, USA), β-Catenin (Dilution: 1:1000, No. ab32572, Abcam Company, USA), C-myc (Dilution: 1:1000, No. ab32072, Abcam Company, USA), β-actin (Dilution: 1:2000, No. ab8226, Abcam Company, USA) overnight under the condition of 4°C. Next, after rinsing, HRP-labeled Goat Anti-Rabbit IgG (Dilution: 1:1500, No. A0208, Beyotime Biotech. Inc., China) was added and incubated for 2 hours at room temperature. Finally, the PVDF membrane was detected using an enhanced chemiluminescence reagent, and the images were captured through the Bio-rad Gel Imaging System (Bio-Rad Laboratories, Inc., USA). The expressional levels of proteins associated with the Wnt signal pathway were normalized to the intensity level of β-actin.

Statistical analysis

All data included in this study were analyzed with SPSS 20.0. The measurement data were expressed by mean ± standard deviation ($\bar{x}$ ± SD), and the independent sample t-test was used for the comparison between two groups. One-way ANOVA followed by post hoc Bonferroni analysis was performed among groups. $P<0.05$ was considered as statistically significant difference.

Results

CSSB could promote the cell viability in chondrocytes caused by H_2O_2

As shown in **Figure 1**, compared with control group or sham group, the cell viability in the model group was obviously reduced ($P<0.01$). The cell viability was presented in a dose-dependent manner when chondrocytes were treated with different doses of CSSB and H_2O_2. Compared with CSSB-L group and CSSB-M group, the cell viability in CSSB-H group was significantly increased, but there was no difference in cell viability between the Sofast group and CSSB-H group. This indicated that 200 μg/

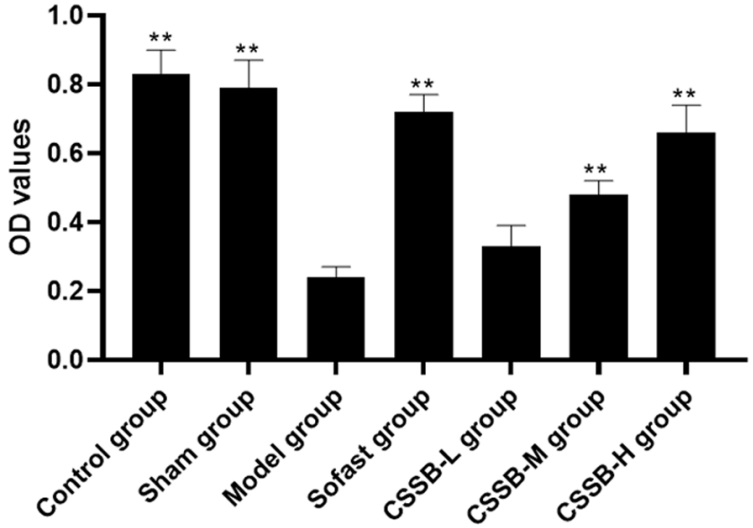

Figure 1. The viability of chondrocytes treated with H_2O_2 and different doses of CSSB was detected by CCK-8 analysis. Compared with the Model group, **P<0.01.

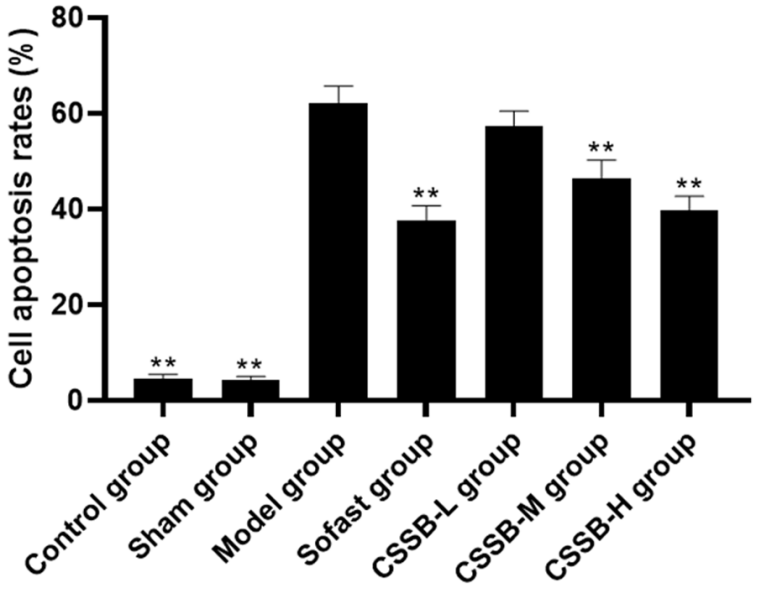

Figure 2. The cell apoptosis analysis of chondrocytes treated with H_2O_2 and different doses of CSSB. Compared with the Model group, **P<0.01.

mL and 400 μg/mL CSSB could reverse the decrease of cell viability induced by H_2O_2.

CSSB could inhibit the cell apoptosis in chondrocytes caused by H_2O_2

As shown in **Figure 2**, the rate of cell apoptosis in the model group was obviously increased in contrast to the control group or sham group (P<0.01). There was no significant difference in cell apoptosis between the model group and CSSB-L group. The cell apoptosis rates in the CSSB-M group and CSSB-H group were significantly lower than that in the model group (P<0.01). Moreover, no statistical differences were found between the CSSB-H group and Sofast group. This indicated that CSSB could inhibit the cell apoptosis caused by H_2O_2 in chondrocytes.

CSSB could inhibit the expression levels of inflammatory cytokines

As shown in **Table 1**, compared with the control group and sham group, the levels of IL-6, IL-8 and IFN-γ were significantly increased in the model group; compared with the model group, no statistical differences were observed in the CSSB-L group. The expression levels of IL-6, IL-8 and IFN-γ in the CSSB-M or CSSB-H group were significantly lower than those in the model group. Moreover, there were no obvious differences between the CSSB-H group and Sofast group. This further showed that CSSB could inhibit the levels of inflammatory cytokines in chondrocytes treated by H_2O_2.

Effects of CSSB on Wnt signaling pathway related proteins in Chondrocytes

As shown in **Figure 3**, there was no difference in Wnt signaling pathway related proteins levels between the control group and sham group; compared with the control group and sham group, the levels of Wnt3a, Frizzled5, Dsh, β-Catenin and C-myc proteins in the model group were significantly decreased, and p-GSK3β expression level was obviously increased (all P<0.001). Compared with the model group, the levels of Wnt3a, Frizzled5, Dsh, β-Catenin and C-myc

Table 1. Effect of CSSB on IL-6, IL-8 and IFN-γ in Chondrocytes induced by H_2O_2

Groups	IL-6 (pg/ml)	IL-8 (pg/ml)	IFN-γ (pg/ml)
Control group	1.58±0.26	2.74±0.61	1.24±0.20
Sham group	1.51±0.22	2.70±0.58	1.26±0.24
Model group	3.57±0.41***	7.45±0.87***	16.74±2.55***
Sofast group	2.08±0.34***	4.17±0.48***	11.62±1.21***
CSSB-L group	3.17±0.52***	6.97±0.72***	16.08±1.97***
CSSB-M group	2.64±0.37###,***	5.09±0.66###,***	13.12±1.65###,***
CSSB-H group	2.13±0.48###,***	4.32±0.57###,***	12.14±1.35###,***
F value	116.235	57.419	78.642
P value	<0.001	<0.001	<0.001

Note: Compared with Control group or Sham group, ***P<0.001; Compared with Model group, ###P<0.001.

proteins in the CSSB-M group and CSSB-H group were significantly elevated, while and p-GSK3β expression level was obviously decreased (all P<0.01 or 0.001). Moreover, the expression levels of Dsh and C-myc in CSSB-H group were higher than in the Sofast group.

Discussion

Osteoarthritis has been considered as a degenerative disease, with a high incidence, which severely affects the life quality of patients and becomes the primary reason for disability in elderly people. However, in clinical practices, currently available treatments are not satisfying. Therefore, it is imperative to find drug targets and new treatment regimens for osteoarthritis. Pain and cartilage extracellular matrix destruction are the primary symptom and features of OA. Some studies demonstrated that inflammasome-signaling molecules were important regulators of pyroptosis, which were also involved in osteoarthritis progression [15]. Other studies revealed that repeated injuries on the articulation could induce the increased release of inflammation cytokines including IL-1β, tumor necrosis factor-α (TNF-α) etc., which were confirmed to exert an important role in the development of osteoarthritis [16]. In chondrocytes, extracellular signal-regulated kinase 1/2 (Erk1/2) and p38 mitogen-activated protein kinase (p38MAPK) could be activated by IL-1β, and then the nuclear translocation of the nuclear factor-κB (NF-κB) and the activator protein-1 (AP-1) were induced. These transcription factors bind to consensus sequences of many pro-inflammatory genes and initiate and maintain the inflammatory reaction in chondrocytes [17]. These inflammatory reactions could also induce the expression of matrix metalloproteases, TNF-α and so on. Chondroitin Sulfate from Sturgeons is a natural glycosaminoglycan expressed in the extracellular matrix surrounding cells, especially in the cartilage and it could form an essential component of proteoglycans. This study used H_2O_2 as the source of the free radicals to induce the damages of chondrocytes in an early-stage OA model. It was also reported that H_2O_2 can induce chondrocyte apoptosis, enhance the secretion of inflammatory factor and promote inflammation [18]. The results of this study showed that CSSB could inhibit the levels of pro-inflammation factors such as IL-6, IL-8 and IFN-γ in H_2O_2 induced chondrocyte pyroptosis. Moreover, Jomphe et al. reported that CSSB could diminish IL-1β induced NF-κB nuclear translocation and sodium nitroprusside-induced chondrocyte apoptosis [19], which was similar with this study.

In previous studies [20], chondroitin sulfate from sturgeons could relieve the osteoarthritis pain caused by sodium iodoacetate in rats by down-regulating expression of matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-13 (MMP-13), and up-regulating the expression of matrix metalloproteinases tissue inhibitor-1 (TIMP-1) protein. ECM components from cartilage are synthesized by chondrocytes to maintain their proper functioning. Cartilage ECM mainly includes glycosaminoglycans, collagen II fibers and proteoglycans. The composition and organization of ECM not only provides a specialized environment for the chondrocytes, but also determines the biomechanical properties of cartilage. During the cartilage degeneration and osteoarthritis progression, the ECM is gradually degraded, and components may serve as biomarkers of cartilage degeneration. In addition, Matrix metalloproteinases are also the key proteins in the Wnt/β-catenin signaling pathway involved in osteoarthritis development. Therefore, we speculated that the expression of Wnt/β-catenin signaling pathway related proteins might play an impor-

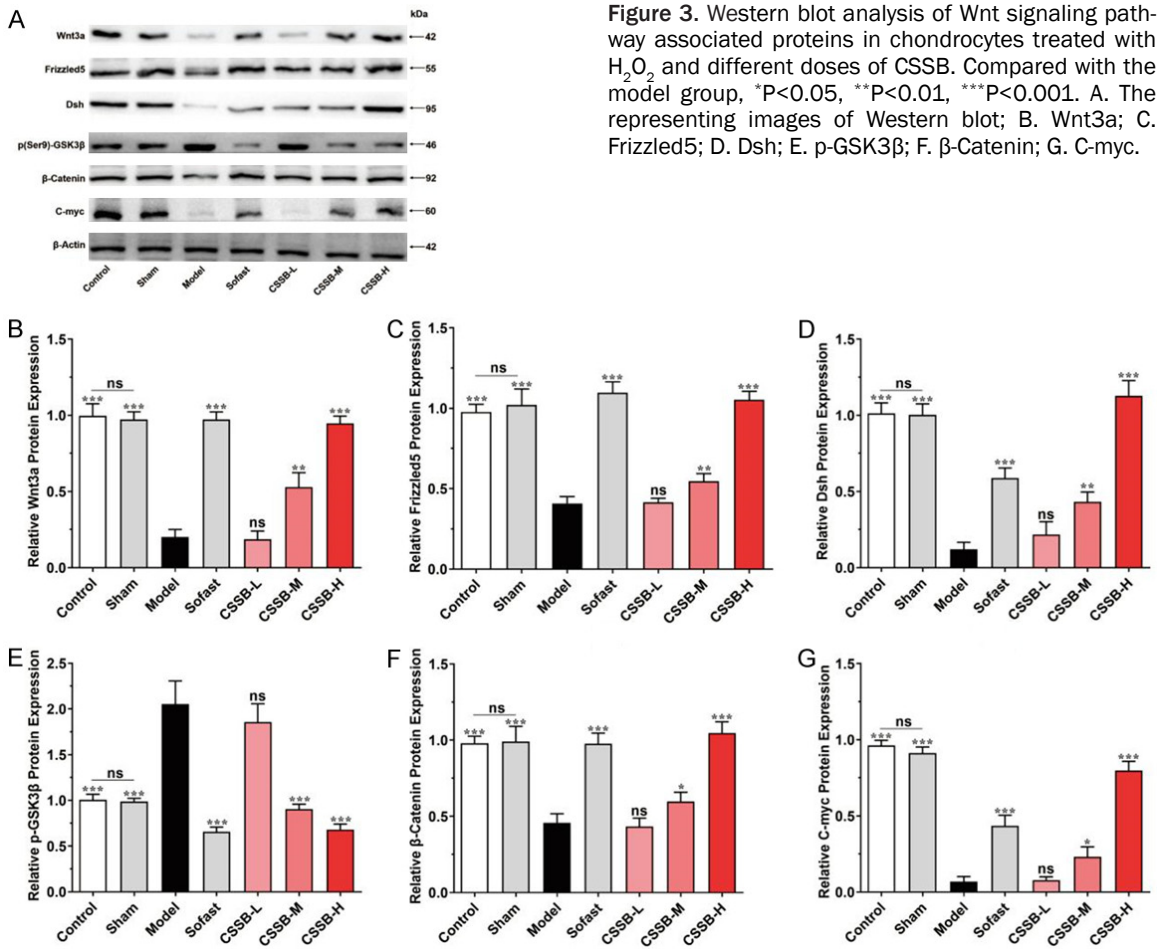

Figure 3. Western blot analysis of Wnt signaling pathway associated proteins in chondrocytes treated with H_2O_2 and different doses of CSSB. Compared with the model group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. A. The representing images of Western blot; B. Wnt3a; C. Frizzled5; D. Dsh; E. p-GSK3β; F. β-Catenin; G. C-myc.

tant role for chondroitin sulfate from sturgeons in the treatment and alleviation of osteoarthritis pain. In this study, the protective effect of chondroitin sulfate from sturgeons on rat chondrocyte was investigated and Western Blot method was conducted to analyze the expression levels of associated proteins before and after the treatment in hydrogen peroxide-induced chondrocyte injury model *in vitro*. The results of this study confirmed that chondroitin sulfate from sturgeons showed a significant effect in recovering expression levels of Wnt signaling pathway related proteins in injured chondrocytes in a dose-dependent manner.

Among many signal pathways, the Wnt signaling pathway plays an important role in regulating the homeostasis of the internal environment in joints [21, 22]. It was generally believed that the activation of cellular Wnt signaling pathway could lead to the overexpression of signaling proteins associated with catabolism in chondrocytes, which in turn results in the imbalance of the homeostasis in cell physiological processes, and ultimately causes tissue damage and functional inactivation [23, 24]. It was reported that the classic Wnt signaling pathway was summarized as follows: Wnt→Frizzled→Dsh→depolymerization of β-catenin degradation complexes (GSK-3β played an important role)→accumulation of β-catenin in the endonuclear→TCF/LEF1→transcription of downstream targeted genes such as MMPs, BMP, c-myc, cyclinD1, and so on [25].

In this study, it was shown that the down-regulation of Wnt3a, Frizzled5, Dsh, β-Catenin and C-myc proteins expression, and up-regulation of p-GSK3β protein expression were significant in hydrogen peroxide-induced chondrocyte injury model (Model group) *in vitro*, which indicated that the development of osteoarthritis was associated with Wnt signaling pathway [26]. Chondroitin Sulfate from sturgeons could increase the expression levels of Wnt signaling pathway related proteins such as Wnt3a, Fri-

zzled5, Dsh, β-Catenin, C-myc, etc. in rat chondrocytes stimulated by hydrogen peroxide-mediated oxidative stress, and decrease the expression level of catabolite gene protein p-GSK3β in a dose-dependent manner. Compared with the commercial chondroitin sulfate Sofast® (Sofast group) derived from porcine cartilage, 400 μg/mL chondroitin Sulfate from sturgeons showed a more significant effect in recovering the expression levels of Dsh and C-myc proteins in injured chondrocytes. The results of this study confirmed that the roles of chondroitin sulfate from sturgeons in protecting rat chondrocytes from injury induced by hydrogen peroxide and relieving and treating osteoarthritis pain were associated with the Wnt signaling pathway. Chondroitin sulfate from sturgeons played an important role in a dose-dependent manner.

In conclusion, it was found that the protective effect of chondroitin sulfate from sturgeons in rat chondrocytes induced by hydrogen peroxide may be correlated with the Wnt signaling pathway, and it showed a dose-dependent manner. However, there are still some limitations about this research: it was a single-center study, with a small sample size and cell experiments. This study was not involved in animal or human experiments, which may be more superior than cell experiments. The Wnt signaling pathway was not intervened with to explain whether the protective effects of CSSB were due to the Wnt signaling. In the future, a multicenter and deeper study with animal models or human experiments is required for further confirmation.

Disclosure of conflict of interest

None.

Address correspondence to: Xi Zhang, Department of Anesthesiology, The Second Hospital, Cheeloo College of Medicine, Shandong University, No. 247 Beiyuan Road, Jinan 250033, Shandong, China. Tel: +86-0531-88197777; Fax: +86-0531-88197777; E-mail: cookiebye2018@163.com

References

[1] Clevers H and Nusse R. Wnt/beta-catenin signaling and disease. Cell 2012; 149: 1192-1205.

[2] Huang P, Yan R, Zhang X, Wang L, Ke X and Qu Y. Activating Wnt/beta-catenin signaling pathway for disease therapy: challenges and opportunities. Pharmacol Ther 2019; 196: 79-90.

[3] Milaras C, Lepetsos P, Dafou D, Potoupnis M and Tsiridis E. Association of matrix metalloproteinase (MMP) gene polymorphisms with knee osteoarthritis: a review of the literature. Cureus 2021; 13: e18607.

[4] Lee YJ, Lee EB, Kwon YE, Lee JJ, Cho WS, Kim HA and Song YW. Effect of estrogen on the expression of matrix metalloproteinase (MMP)-1, MMP-3, and MMP-13 and tissue inhibitor of metalloproternase-1 in osteoarthritis chondrocytes. Rheumatol Int 2003; 23: 282-288.

[5] Zhang HX, Sun C, Yu HC, Song B and Pan ZX. Targeted inhibition of beta-catenin by miR-320 and decreased MMP-13 expression in suppressing chondrocyte collagen degradation. Eur Rev Med Pharmacol Sci 2018; 22: 5828-5835.

[6] Tang CY, Wu M, Zhao D, Edwards D, McVicar A, Luo Y, Zhu G, Wang Y, Zhou HD, Chen W and Li YP. Runx1 is a central regulator of osteogenesis for bone homeostasis by orchestrating BMP and WNT signaling pathways. PLoS Genet 2021; 17: e1009233.

[7] Sun Y, Zhang G, Liu Q, Liu X, Wang L, Wang J and Liang L. Chondroitin sulfate from sturgeon bone ameliorates pain of osteoarthritis induced by monosodium iodoacetate in rats. Int J Biol Macromol 2018; 117: 95-101.

[8] Ma N, Wang T, Bie L, Zhao Y, Zhao L, Zhang S, Gao L and Xiao J. Comparison of the effects of exercise with chondroitin sulfate on knee osteoarthritis in rabbits. J Orthop Surg Res 2018; 13: 16.

[9] Li VS, Ng SS, Boersema PJ, Low TY, Karthaus WR, Gerlach JP, Mohammed S, Heck AJ, Maurice MM, Mahmoudi T and Clevers H. Wnt signaling through inhibition of beta-catenin degradation in an intact Axin1 complex. Cell 2012; 149: 1245-1256.

[10] Valvezan AJ, Zhang F, Diehl JA and Klein PS. Adenomatous polyposis coli (APC) regulates multiple signaling pathways by enhancing glycogen synthase kinase-3 (GSK-3) activity. J Biol Chem 2012; 287: 3823-3832.

[11] de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, Kujala P, Haegebarth A, Peters PJ, van de Wetering M, Stange DE, van Es JE, Guardavaccaro D, Schasfoort RB, Mohri Y, Nishimori K, Mohammed S, Heck AJ and Clevers H. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature 2011; 476: 293-297.

[12] Han I, Park HJ, Seong SC, Lee S, Kim IG and Lee MC. Role of transglutaminase 2 in apoptosis induced by hydrogen peroxide in human chondrocytes. J Orthop Res 2011; 29: 252-257.

[13] Zhang L, Cheng H, Yue Y, Li S, Zhang D and He R. H19 knockdown suppresses proliferation and induces apoptosis by regulating miR-

148b/WNT/beta-catenin in ox-LDL-stimulated vascular smooth muscle cells. J Biomed Sci 2018; 25: 11.
[14] Ye K, Wei Q, Gong Z, Huang Y, Liu H, Li Y and Peng X. Effect of norcantharidin on the proliferation, apoptosis, and cell cycle of human mesangial cells. Ren Fail 2017; 39: 458-464.
[15] Chang X, Kang Y, Yang Y, Chen Y, Shen Y, Jiang C and Shen Y. Pyroptosis: a novel intervention target in the progression of osteoarthritis. J Inflamm Res 2022; 15: 3859-3871.
[16] Liao CR, Wang SN, Zhu SY, Wang YQ, Li ZZ, Liu ZY, Jiang WS, Chen JT and Wu Q. Advanced oxidation protein products increase TNF-α and IL-1β expression in chondrocytes via NADPH oxidase 4 and accelerate cartilage degeneration in osteoarthritis progression. Redox Biol 2020; 28: 101306.
[17] Goldring MB and Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol 2011; 23: 471-478.
[18] Zhao C, Chen JY, Peng WM, Yuan B, Bi Q and Xu YJ. Exosomes from adipose-derived stem cells promote chondrogenesis and suppress inflammation by upregulating miR-145 and miR-221. Mol Med Rep 2020; 21: 1881-1889.
[19] Jomphe C, Gabriac M, Hale TM, Héroux L, Trudeau LE, Deblois D, Montell E, Vergés J and du Souich P. Chondroitin sulfate inhibits the nuclear translocation of nuclear factor-kappaB in interleukin-1beta-stimulated chondrocytes. Basic Clin Pharmacol Toxicol 2008; 102: 59-65.
[20] Tat SK, Pelletier JP, Mineau F, Duval N and Martel-Pelletier J. Variable effects of 3 different chondroitin sulfate compounds on human osteoarthritic cartilage/chondrocytes: relevance of purity and production process. J Rheumatol 2010; 37: 656-664.
[21] Zhou Y, Wang T, Hamilton JL and Chen D. Wnt/beta-catenin signaling in osteoarthritis and in other forms of arthritis. Curr Rheumatol Rep 2017; 19: 53.
[22] Wang Y, Fan X, Xing L and Tian F. Wnt signaling: a promising target for osteoarthritis therapy. Cell Commun Signal 2019; 17: 97.
[23] Sassi N, Laadhar L, Allouche M, Achek A, Kallel-Sellami M, Makni S and Sellami S. WNT signaling and chondrocytes: from cell fate determination to osteoarthritis physiopathology. J Recept Signal Transduct Res 2014; 34: 73-80.
[24] Xuan F, Yano F, Mori D, Chijimatsu R, Maenohara Y, Nakamoto H, Mori Y, Makii Y, Oichi T, Taketo MM, Hojo H, Ohba S, Chung UI, Tanaka S and Saito T. Wnt/beta-catenin signaling contributes to articular cartilage homeostasis through lubricin induction in the superficial zone. Arthritis Res Ther 2019; 21: 247.
[25] Chae WJ and Bothwell ALM. Canonical and non-canonical Wnt signaling in immune cells. Trends Immunol 2018; 39: 830-847.
[26] Blom AB, van Lent PL, van der Kraan PM and van den Berg WB. To seek shelter from the WNT in osteoarthritis? WNT-signaling as a target for osteoarthritis therapy. Curr Drug Targets 2010; 11: 620-629.