Inflammation, Vol. 42, No. 2, April 2019 (© 2018)
DOI: 10.1007/s10753-018-0906-4

ORIGINAL ARTICLE

Protective Effects of Sweroside on IL-1β-Induced Inflammation in Rat Articular Chondrocytes Through Suppression of NF-κB and mTORC1 Signaling Pathway

Rui Zhang,[1] Chao-min Wang,[2] Hua-ji Jiang,[3] Xing-gui Tian,[4] WenJun Li,[3] Wei Liang,[3] Jianhua Yang,[3] Chunlan Zhong,[3] Yuhui Chen,[5,6] and Tao Li[5,6]

Abstract— Sweroside (SW), as a bioactive herbal ingredient, has anti-inflammatory effects. Protective effects of SW on IL-1β-stimulated articular chondrocytes, however, has not been fully understood. This study was to explore the anti-inflammatory effects and further to investigate the possible mechanism underlying SW effect on IL-1β-stimulated rat articular chondrocytes. Rat articular chondrocytes were cultured with or without SW for 1 h, and then stimulated with IL-1β for 24 h. ELISA analysis was used to measure the production of NO and PGE2. Western blot was to detect the expression of iNOS and COX-2. Furthermore, the mRNA expression of MMP-1, MMP3, MMP13, and ADAMTS-5 were measured by q-PCR. These results demonstrated that SW significantly inhibited IL-1β-induced NO and PGE2 production, as well as MMP-1, MMP3, MMP13, and ADAMTS-5 mRNA expression. Moreover, SW also suppressed IL-1β-induced NF-κB activation and iκ-B degradation, S6K1 and S6 phosphorylation. In conclusion, these results strongly demonstrated that the anti-inflammatory activity of SW is in part mediated by suppressing NF-κB and mTORC1 signaling, which was expected to be a promising drug target of osteoarthritis therapy.

KEY WORDS: Sweroside; Articular chondrocytes; IL-1β; NF-κB; mTORC1.

INTRODUCTION

Osteoarthritis (OA) is a progressive degeneration of joint tissues, loss of articular cartilage, and inflammation of synovium [1–3]. It is widely accepted that inflammatory mediators have been involved in the development and progression of OA [4, 5]. Interleukin-1β (IL-1β), one of major inflammatory mediators, exerts a vital role in the pathogenesis of OA [6, 7]. Previous studies showed that the chondrocytes stimulated by IL-1β can markedly increase the production of inflammatory mediators, among which are NO, PGE_2, COX-2, and iNOS [8, 9]. In addition, IL-1β could also stimulate chondrocytes to release several proteolytic enzymes, including the matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) [10, 11]. The MMPs and ADAMTS activities

Rui Zhang, Chao-min Wang and Hua-ji Jiang contributed equally to this work.

[1] Department of Cardiology (Cardiac Rehabilitation), The Third Affiliated Hospital of Southern Medical University, Tianhe District, Guangzhou, China
[2] Department of Trauma Treatment Center, The Third Affiliated Hospital of Southern Medical University, Tianhe District, Guangzhou, China
[3] Department of Pain, Yue Bei People's Hospital, No. 133 South Huimin Road,, Shaoguan, Guangdong, China
[4] Department of Orthopedics, The Affiliated Hospital of Southwestern Medical University, LuZhou, China
[5] Department of Orthopedics, The Third Affiliated Hospital of Southern Medical University, No. 183 West Zhongshan Road, Tianhe District, Guangzhou, 510630, China
[6] To whom correspondence should be addressed at Department of Orthopedics, The Third Affiliated Hospital of Southern Medical University, No. 183 West Zhongshan Road, Tianhe District, Guangzhou, 510630, China. E-mails: ah1990726@163.com; gukelitao@aliyun.com

are involved in degradation of the extracellular matrix in articular cartilage during the development of OA [5, 12]. Consequently, recent studies have explored to inhibition of IL-1β and IL-1β-induced inflammatory mediators for the prevention and treatment of OA [6, 10, 13, 14].

Sweroside (SW), an iridoid glycoside, has been exhibiting diverse biological activities, such as anti-fungal, anti-diabetic, anti-inflammatory, and anti-tumor effects [15–17]. Previous studies reported that SW ameliorated liver injury by decreasing oxidative damage and inhibiting the production of pro-inflammatory kinases [18, 19]. Recently, SW could also ameliorate α-naphthyl isothiocyanate-induced cholestatic liver injury in mice by regulating bile acids and suppressing pro-inflammatory responses [20]. In another study, SW has displayed bright prospects in prevention and therapy of osteoporosis [21]. However, the anti-inflammatory effects and the possible mechanism of SW on articular chondrocytes stimulated with IL-1β are not fully understood. Therefore, this study aimed to investigate the anti-inflammatory effects and further to explore the mechanism of SW on IL-1β-stimulated rat articular chondrocytes.

MATERIALS AND METHODS

Chemicals and Reagents

SW (purity > 98%) and cell counting kit-8 (CCK8) were obtained from Sigma Chemical Co. St. Louis, MO, USA. Primary antibodies specific against PCNA, Cyclin-D1, β-actin, iNOS, COX-2, p65, p-p65, IκB, p-IκB, S6K1, and S6 were purchased from Santa Cruz Biotechnology (Santa Cruz, NYCA,USA). Enzyme-linked immunosorbent assay (ELISA) kits of PGE_2, MMP-1, MMP-3, MMP-13, and ADAMTS5 were obtained from R&D systems (Minneapolis, MN, USA). Recombinant rat IL-1β was purchased from PeproTech (NJ, USA).

Cell Culture

Primary rat chondrocytes were isolated from the knees and femoral heads of 4-week-old Sprague-Dawley (SD) rats under sterile conditions. Well-preserved rat cartilage was washed with PBS containing penicillin/streptomycin solution three times, and then cut into small pieces. Cartilage pieces were digested primarily with 0.25% Trypsin-EDTA solution for 30 min and subsequently with 0.2% collagenase type II in Dulbecco's modified Eagle's medium (DMEM) for 2 h at 37 °C. The released articular chondrocytes were resuspended in DMEM containing 10% FBS and seeded onto a 25-cm^2 culture flask at 37 °C in a humidified atmosphere of 5% CO_2–95% air. Up to approximately 90% confluency, chondrocytes were passaged at a ratio of 1:3. To avoid the phenotype loss, passages 1–3 chondrocytes were used in our experiments.

Cell Viability/Proliferation Assays

The CCK8 assay was used to assess the cytotoxicity of SW on rat chondrocytes. The cells were seeded onto 96-well culture plates at 1×10^5 cells/well and allowed to adhere overnight at 37 °C. Following incubation, cells were treated with various concentrations (0.1, 1, 10, 100 μg/ml) of SW and stimulated with IL-1β (10 ng/ml). For 24 h, the culture medium was removed and then 10 μg/100 μl CCK8 was added to each well for incubation at 37 °C for 4 h. The optical density was measured at 450 nm using a microplate reader.

ELISA Assay

The levels of PGE2 in the culture medium were detected using ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

NO Measurement

As described previously in many studies [22, 23] and our previous study [24], rat chondrocytes were treated with the indicated concentrations of SW 1 h prior to stimulation of IL-1β (10 ng/ml) for 24 h. The culture supernatants were collected for measuring nitrite production by the Griess reagent assay according to the instructions. Briefly, the supernatants were mixed with an equal volume of the Griess reagent and then incubated for 30 min. Optical density was measured at 540 nm.

Quantitative PCR (q-PCR)

Total RNA was extracted from rat chondrocytes with Trizol® reagent (Life Technologies, GrandIsland, NY). A total of RNA (1 μg) was used to synthesize complementary DNA (cDNA) with a complementary DNA synthesis kit (Takara Biotechnology Co Ltd., Dalian, China). Quantitative real-time PCR was performed in duplicate using the SYBR Premix ExTaq II kit (Takara Biotechnology Co Ltd) and a Rotor-Gene Qthermal cycler (Qiagen, Hilden, Germany). The primer sequences (Life Technologies) are shown in Table 1. The thermal cycling conditions were as follows: 1 cycle of 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 20 s, and 72 °C for 15 s. Expression levels were normalized to those of endogenous GAPDH, and the data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Table 1. The Primers Used for the Real-Time PCR

Gene	FP(5–3)	RP(5–3)	Fragments (bp)
MMP1	CCAGGTATTGGAGGGGATGC	CCAAGGGAATGGCCCAGTT	110
MMP3	AATCCCCTGATGTCCTCGTGGTA	GGTCCTGAGAGATTTTCGCCAA	161
MMP13	TATGACTATGCGTGGCTGGA	CCATTTGTGGTGTGGGAAGT	129
ADAMTS5	TGTGAAGAGACCTTTGGTTCC	TTCTGTGATGGTGGCTGAAG	172
GAPDH	TGGAGTCTACTGGCGTCTT	TGTCATATTTCTCGTGGTTCA	138

Western Blot

Total protein was extracted from rat chondrocytes using RIPA lysis buffer, and the protein concentration was determined by BCA protein assay kit according to the instructions (Thermo Scientific; IL, USA). A total of protein (30 μg) was separated by 12% SDS-PAGE and then transferred onto PVDF membranes. The membranes were probed with primary antibodies, after being incubated with 5% BSA for 2 h at room temperature. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 1 h, and signals were revealed using an enhanced chemiluminescence kit (Cell Signaling Technology).

Statistical Analysis

These data are shown as the mean ± SD. One-way ANOVA and Student's *t* test were used to analyze statistical differences among treatment groups. $P < 0.05$ was considered statistically significant.

RESULTS

SW Showed No Cytotoxicity Effects on Rat Chondrocytes

The cytotoxicity of SW on rat chondrocytes was assessed by CCK8 assay and western blots. At the three different concentrations (0.1, 1, 10 μg/ml), these results showed that SW had no cellular toxicity on chondrocytes (Fig. 1a). Moreover, the cell-proliferative protein PCNA and cell cycle protein cyclin-D1 had also no effect on the protein expression level at the three different concentrations (0.1, 1, 10 μg/ml) (Fig. 1b). However, SW is cytotoxic at the concentration 100 μg/ml, which has significantly difference compared that at the three different concentrations (0.1, 1, 10 μg/ml). Thus, SW at the three concentrations (0.1, 1, 10 μg/ml) were selected in subsequent experiments.

SW Attenuates IL-1β-Induced Production of NO and PGE2

In rat chondrocytes, the protective effects of SW on IL-1β-induced inflammatory mediators, NO, and PGE2 production were determined by ELISA. As shown in Fig. 2a, b, IL-1β treatment significantly induced of NO and PGE2 production in rat chondrocytes compared to control. However, the groups treated with different concentrations (0.1, 1, 10 μg/ml) SW attenuated NO (36.6%, 47.2%, 82.6%, respectively) and PGE2 (9.6%, 41.4%, 66.2%, respectively) production induced by IL-1β in a dose-dependent manner (Fig. 2a). Furthermore, IL-1β-induced the protein expressions of iNOS and COX-2 were evaluated by western blots. Similarly, SW also suppressed the expression level of iNOS (28.9%, 90.8%, 536.0%, respectively) and COX-2 (30.5%, 76.8%, 480.0%, respectively) protein in a dose-dependent manner, compared with the group treated with IL-1β in rat chondrocytes (Fig. 2b).

SW Inhibits IL-1β-Induced MMP-1, MMP-3, MMP-13, and ADAMTS-5 Production

IL-1β is well known as the central inflammatory mediator of cartilage loss in OA, which was induced to upregulate the expression level of major extracellular proteolytic enzymes, such as MMPs, ADAMTS. To examine whether SW has effects on the gene expression of MMPs, ADAMTS induced by IL-1β. The mRNA expression level of MMP-1, MMP-3, MMP-13, and ADAMTS-5 were determined by q-PCR. As the data show (Fig. 3), the mRNA expression level of MMP-1, MMP-3, MMP-13, and ADAMTS-5 increased significantly in the group treated with IL-1β compared to the control group. As expected, the groups treated with SW in a concentration-dependent manner significantly inhibited the MMP-1 (22.6%, 53.2%, 84.2%, respectively), MMP-3 (28.2%, 46.8%, 82.0%, respectively), MMP-13 (30.2%, 54.8%, 83.0%, respectively), and ADAMTS-5 (39.0%, 57.6%, 77.1%, respectively) mRNA expression level stimulated by IL-1β in rat chondrocytes.

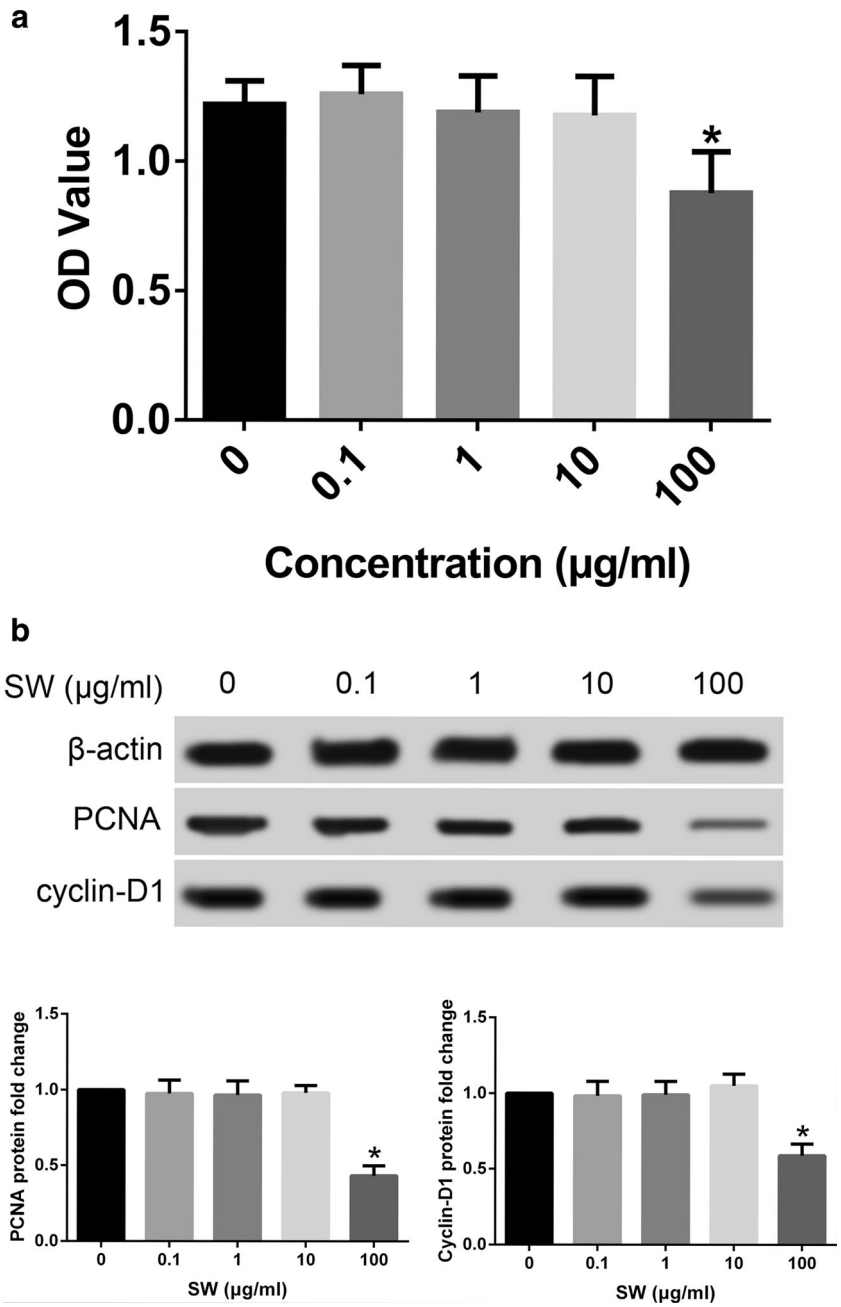

Fig. 1. Effects of SW on cell viability of rat chondrocytes. **a** The CCK8 assay demonstrated that SW had no cellular toxicity on rat chondrocytes at the three different concentrations (0.1, 1, 10 μg/ml). **b** SW had also no effect on the proliferative proteins(PCNA and cyclin-D1) expression at the three different concentrations (0.1, 1, 10 μg/ml). SW Sweroside; CCK8 cell counting kit-8.

SW Suppresses IL-1β-Induced NF-κB Activation and iκB Degradation

Previous studies demonstrated that IL-1β can initiate several signal transduction pathways, such as NF-κB activation and iκ-B degradation. Therefore, we examined whether SW has anti-inflammatory protective effect on rat chondrocytes treated with IL-1β mediated by NF-κB activation and iκ-B degradation. As shown in Fig.4, the group treated with IL-1β significantly upregulated NF-κB p65 phosphorylation, not the total NF-κB p65 protein level

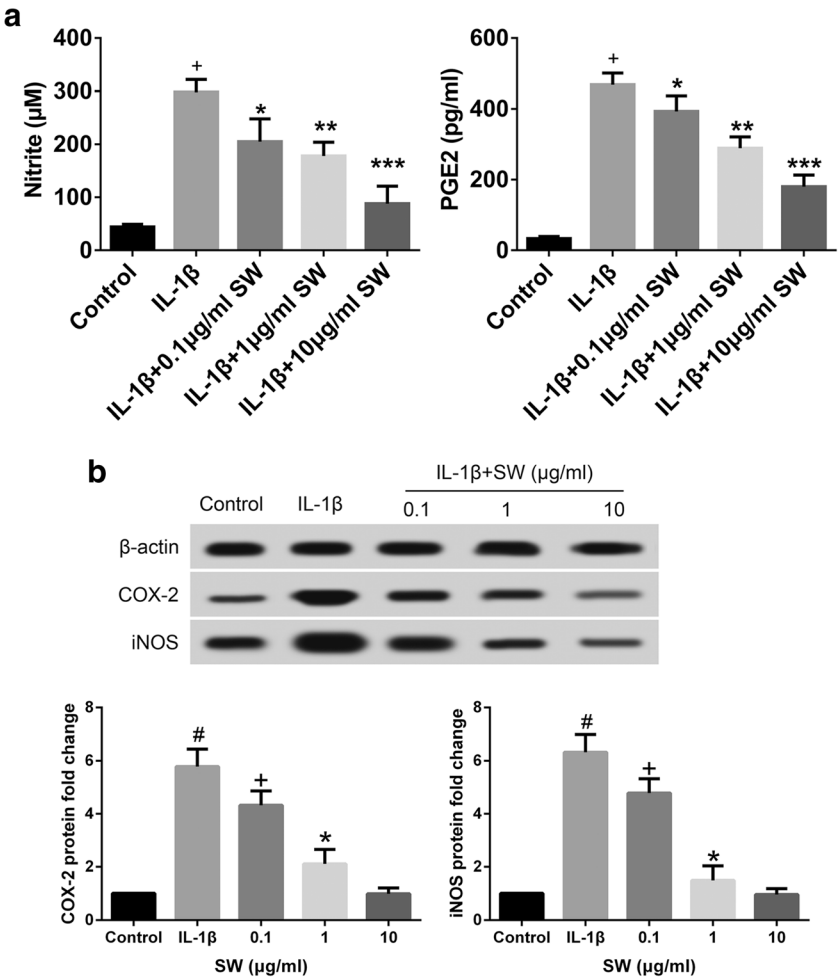

Fig. 2. SW attenuates IL-1β-induced NO and PGE2 production. **a**, **b** The Elisa data suggested that SW attenuated the production of NO (36.6%, 47.2%, 82.6%, respectively) and PGE2 (9.6%, 41.4%, 66.2%, respectively) induced by IL-1β in a dose-dependent manner (0.1, 1, 10 μg/ml). **c** Then, western blots also demonstrated that SW suppressed the protein expression of iNOS (28.9%, 90.8%, 536.0%, respectively) and COX-2 (30.5%, 76.8%, 480.0%, respectively) in IL-1β-stimulated chondrocytes in a dose-dependent manner (0.1, 1, 10 μg/ml). SW, Sweroside.

compared to the control group. While SW treatment in a dose-dependent manner attenuated NF-κBp65 phosphorylation (18.4%, 44.5%, 72.7%, respectively) induced by IL-1β in rat chondrocytes. As expected, iκB phosphorylation (17.5%, 33.8%, 60.8%, respectively) in the group treated with SW, as inhibitor of NF-κB, was also downregulated in a dose-dependent manner.

SW Has Effects on IL-1β-Induced S6K1 and S6 Phosphorylation

Moreover, to further clarify the effects of SW on possible signal transduction pathways, S6K1 andS6 phosphorylations induced by IL-1β were analyzed by western blots. As shown in Fig. 5, these results demonstrated that presence of SW in different concentrations (0.1, 1, 10 μg/ml) significantly inhibited IL-1β-induced S6K1 (17.7%, 34.1%, 45.2%, respectively) and S6 phosphorylation (4.3%, 22.5%, 80.8%, respectively) in a dose-dependent manner.

DISCUSSION

In the study, the anti-inflammatory effects and possible underlying mechanism of SW on rat articular chondrocytes stimulated with IL-1β were investigated. Our results demonstrated that SW attenuates inflammation induced by IL-1β, including inhibiting NO and PGE2 production, as well as iNOS and COX-2 expression at the

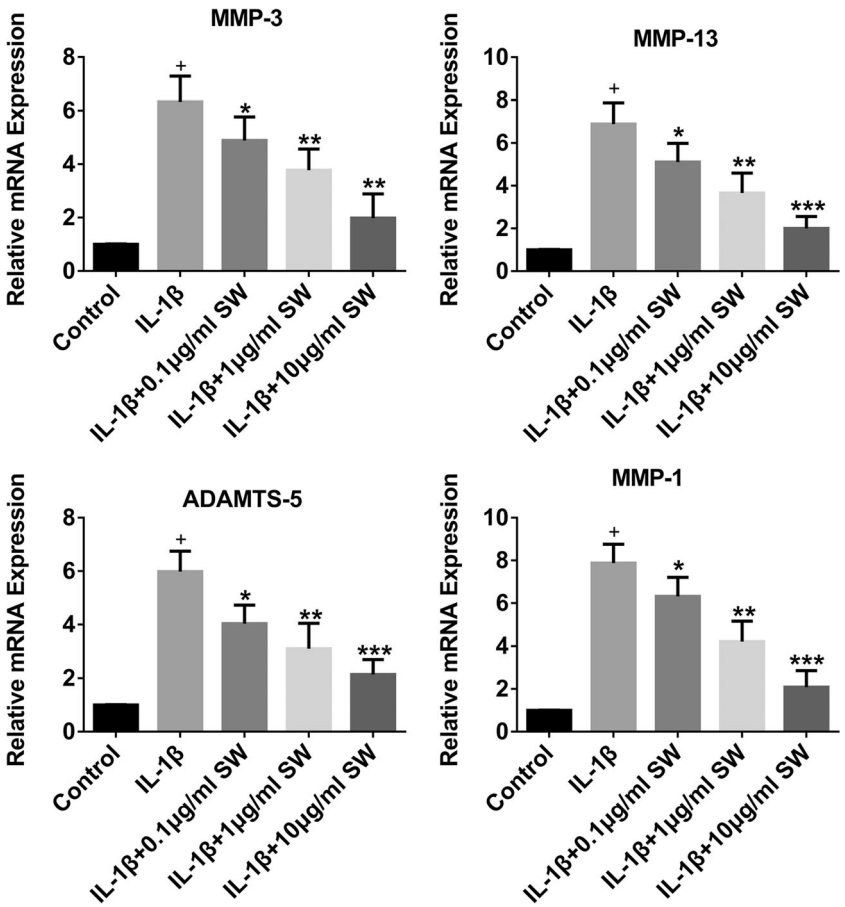

Fig. 3. SW inhibits MMP-1, MMP-3, MMP-13, and ADAMTS-5 production induced by IL-1β. Compared with the control group, SW had a concentration-dependent (0.1, 1, 10 μg/ml) inhibitory effect on **a** MMP-1 (22.6%, 53.2%, 84.2%, respectively), **b** MMP-3 (28.2%, 46.8%, 82.0%, respectively), **c** MMP-13 (30.2%, 54.8%, 83.0%, respectively), and **d** ADAMTS-5 (39.0%, 57.6%, 77.1%, respectively) mRNA expression level in rat chondrocytes treated with IL-1β. SW Sweroside, MMP matrix metalloproteinase, ADAMTS-5 a disintegrin and metalloproteinase with thrombospondin motifs 5.

protein level, *MMP-1, MMP-3, MMP-13*, and *ADAMTS-5* at the mRNA level in rat articular chondrocytes. The underlying mechanism of SW effects on rat articular chondrocytes was *via* suppressing IL-1β-inducing NF-κB and mTORC1 activation, which suggest that SW could be a promising drug target of OA therapy.

Accumulating studies showed that IL-1β exerted vital roles in the pathophysiology of OA [6, 10, 13]. Elevated IL-1β is detected in synovial fluid from patients with OA, which could cause cartilage degradation and pain [25]. It is well known that chondrocytes treated with IL-1β can induce increasing inflammatory mediators, including NO and PGE_2 [26, 27]. In the chondrocytes, IL-1β can markedly increase the production of NO, which simulates the production of MMPs and inflammatory cytokines including PGE_2. Moreover, PGE_2 has been involved in cartilage matrix degradation *via* enhancing MMPs activity [28]. Accumulating studies showed that it can attenuate the development and progression of OA through suppression of inflammatory mediators such as NO, PGE_2, iNOS, and COX-2 [6, 10, 11, 13]. In this study, our results demonstrated that SW significantly inhibited the production of NO and PGE2 induced by IL-1β in the rat chondrocytes. Furthermore, the other inflammatory mediators, iNOS and COX-2 proteins, were also suppressed by SW in a dose-dependent manner. Moreover, SW also markedly inhibited IL-1β-induced MMP-1, MMP-3, MMP-13, and ADAMTS-5 at the mRNA expression level. These results indicated that SW had anti-inflammatory effects against OA.

NF-κB, as a central transcription factor, regulates the expression of inflammatory mediators that has been associated with the pathogenesis of OA [29, 30]. Upon

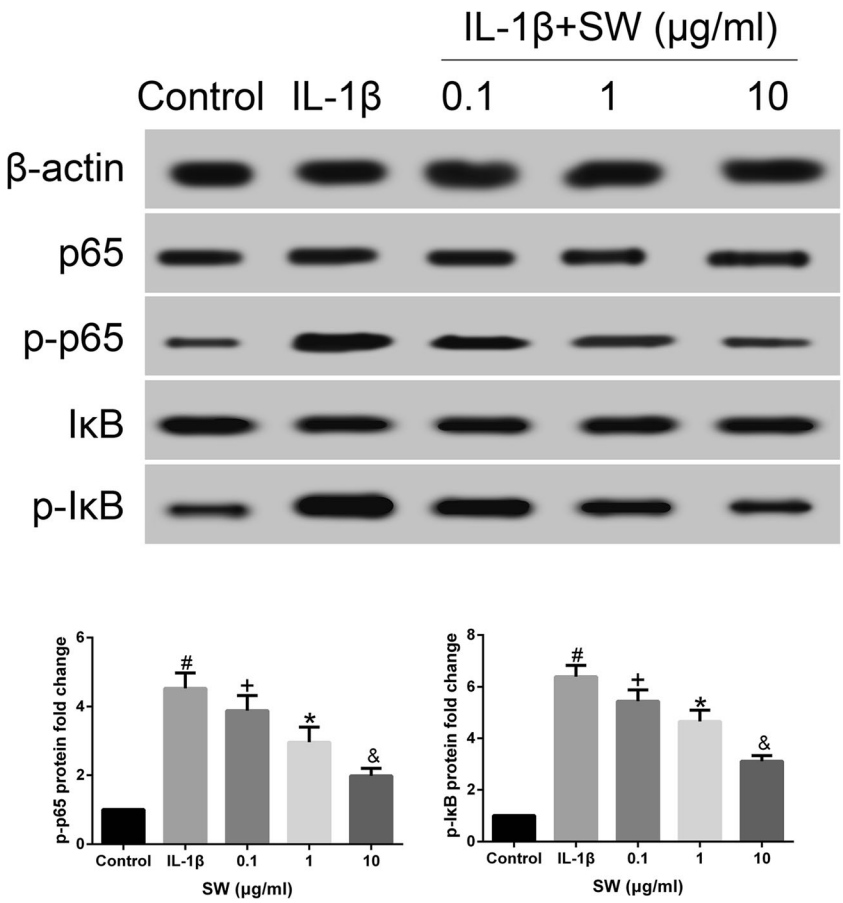

Fig. 4. SW suppresses IL-1β-induced NF-κB activation and iκB degradation. Compared to the control group, SW dose dependently attenuated IL-1β-induced NF-κB p65 phosphorylation (18.4%, 44.5%, 72.7%, respectively) in rat chondrocytes. Moreover, iκB phosphorylation (17.5%, 33.8%, 60.8%, respectively), as inhibitor of NF-κB, was also suppressed by SW in a dose-dependent manner (0.1, 1, 10 μg/ml). SW Sweroside.

stimulation by IL-1β, NF-κB dissociates from cytoplasm, and then translocates to nucleus to exert its functions that upregulates varieties of inflammatory mediators, such as NO, PGE2, iNOS, COX-2, MMPs, and ADAMTS [31, 32]. It has been involved in cartilage inflammation and contributes to the process of OA. Previous studies showed that targeted inhibition of NF-κB activation is a promising approach to treat for OA [31, 33]. Therefore, we further investigated whether the possible underlying mechanism of SW effecting on OA was *via* NF-κB signaling pathways. The effects of SW on NF-κB activation were determined at the protein expression level. As expected, our results showed that SW significantly inhibited NF-κB activation induced by IL-1β in the rat chondrocytes. It markedly suppressed phosphorylation of NF-κB P65, as well as iκ-B degradation in dose-independent manner. These results suggested that SW exerts its anti- inflammatory effects *via* suppression of NF-κB signaling pathway in OA.

The mammalian target of rapamycin complex 1 (mTORC1) plays critical roles in regulating cell growth, survival and metabolism [34]. Phosphorylation of ribosomal S6 protein kinase (S6) and 4E-BP1, as two key factors in translation initiation, are especially important for mTORC1 protein function stimulating protein synthesis to promote cell growth. Recent studies have reported that mTORC1 exerts vital effects on chondrocyte metabolism and has been involved in the pathophysiology of OA [35, 36]. In mouse models, it has been shown that cartilage-specific deletion of mTOR, as well as pharmacological mTORC1 inhibitor, were both benefiting from experimental OA mouse. Furthermore, mTORC1 suppression can activate autophagy, leading to a reduction in OA process [37]. Therefore, we proposed a hypothesis that inhibition of mTORC1 activation has been involved in delaying the process of OA. Interestingly, our results found that S6 and S6K1 proteins were downregulated by SW, suggesting that

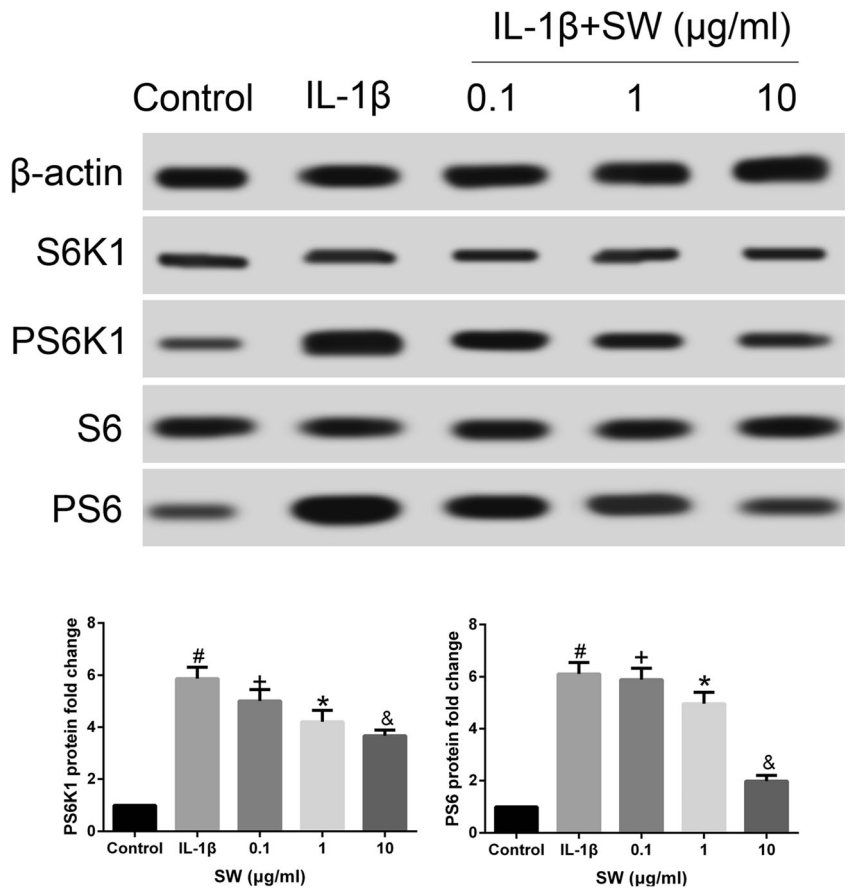

Fig. 5. Effects of SW on IL-1β-induced S6K1 and S6 phosphorylation. SW significantly also inhibited IL-1β-induced S6K1 (17.7%, 34.1%, 45.2%, respectively) and S6 phosphorylation (4.3%, 22.5%, 80.8%, respectively) in a dose-dependent manner (0.1, 1, 10 μg/ml). SW Sweroside, S6K1 p70 ribosomal S6 kinase 1, S6 p70 ribosomal S6.

SW suppressed the development of OA by inhibiting mTORC1 signaling. However, the exact mechanism underlying SW effect on rat chondrocytes is still unclear. Further studies are needed to investigate the exact mechanism of SW effect on OA in rat chondrocyte.

CONCLUSION

Taken together, our data demonstrated that SW has anti-inflammatory effects on rat chondrocytes treated with IL-1β. It significantly inhibits IL-1β-induced NO and PGE_2 production, suppressing iNOS, COX-2 protein at the expression level, and MMP-1, MMP-3, MMP-13, and ADAMTS-5 expressions at the mRNA level in rat chondrocytes. Furthermore, it is proposed that the anti-inflammatory molecular mechanism of SW is through inhibiting NF-κB and mTORC1 activation, which suggested that SW may be served as a promising drug target of OA therapy.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no competing interests.

REFERENCES

1. Ehrlich, G.E. 1975. Osteoarthritis beginning with inflammation. Definitions and correlations. *JAMA* 232: 157–159.
2. Pelletier, J.P., J. Martel-Pelletier, and S.B. Abramson. 2001. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. *Arthritis and Rheumatism* 44: 1237–1247.
3. Benito, M.J., D.J. Veale, O. FitzGerald, W.B. van den Berg, and B. Bresnihan. 2005. Synovial tissue inflammation in early and late osteoarthritis. *Annals of the Rheumatic Diseases* 64: 1263–1267.

4. Kobayashi, M., G.R. Squires, A. Mousa, M. Tanzer, D.J. Zukor, J. Antoniou, U. Feige, and A.R. Poole. 2005. Role of interleukin-1 and tumor necrosis factor alpha in matrix degradation of human osteoarthritic cartilage. *Arthritis and Rheumatism* 52: 128–135.
5. Kapoor, M., J. Martel-Pelletier, D. Lajeunesse, J.P. Pelletier, and H. Fahmi. 2011. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nature Reviews Rheumatology* 7: 33–42.
6. Feng, Z., W. Zheng, X. Li, J. Lin, C. Xie, H. Li, L. Cheng, A. Wu, and W. Ni. 2017. Cryptotanshinone protects against IL-1beta-induced inflammation in human osteoarthritis chondrocytes and ameliorates the progression of osteoarthritis in mice. *International Immunopharmacology* 50: 161–167.
7. Guerne, P.A., B.L. Zuraw, J.H. Vaughan, D.A. Carson, and M. Lotz. 1989. Synovium as a source of interleukin 6 in vitro. Contribution to local and systemic manifestations of arthritis. *The Journal of Clinical Investigation* 83: 585–592.
8. Yang, G., H.J. Im, and J.H. Wang. 2005. Repetitive mechanical stretching modulates IL-1beta induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. *Gene* 363: 166–172.
9. Amin, A.R., M. Dave, M. Attur, and S.B. Abramson. 2000. COX-2, NO, and cartilage damage and repair. *Current Rheumatology Reports* 2: 447–453.
10. Zheng, W., Z. Feng, S. You, H. Zhang, Z. Tao, Q. Wang, H. Chen, and Y. Wu. 2017. Fisetin inhibits IL-1beta-induced inflammatory response in human osteoarthritis chondrocytes through activating SIRT1 and attenuates the progression of osteoarthritis in mice. *International Immunopharmacology* 45: 135–147.
11. Park, J.W., Y.P. Yun, K. Park, J.Y. Lee, H.J. Kim, S.E. Kim, and H.R. Song. 2016. Ibuprofen-loaded porous microspheres suppressed the progression of monosodium iodoacetate-induced osteoarthritis in a rat model. *Colloids and Surfaces. B, Biointerfaces* 147: 265–273.
12. Koenders, M.I., R.J. Marijnissen, I. Devesa, E. Lubberts, L.A. Joosten, J. Roth, P.L. van Lent, F.A. van de Loo, and W.B. van den Berg. 2011. Tumor necrosis factor-interleukin-17 interplay induces S100A8, interleukin-1beta, and matrix metalloproteinases, and drives irreversible cartilage destruction in murine arthritis: rationale for combination treatment during arthritis. *Arthritis and Rheumatism* 63: 2329–2339.
13. Zheng, G., Y. Zhan, Q. Tang, T. Chen, F. Zheng, H. Wang, J. Wang, D. Wu, X. Li, Y. Zhou, X. Wang, Y. Wu, Y. Zhou, H. Xu, N. Tian, and X. Zhang. 2018. Monascin inhibits IL-1beta induced catabolism in mouse chondrocytes and ameliorates murine osteoarthritis. *Food & Function* 9: 1454–1464.
14. Zheng, W., C. Chen, C. Zhang, L. Cai, and H. Chen. 2018. The protective effect of phloretin in osteoarthritis: an in vitro and in vivo study. *Food & Function* 9: 263–278.
15. Tan, R.X., J.L. Wolfender, W.G. Ma, L.X. Zhang, and K. Hostettmann. 1996. Secoiridoids and antifungal aromatic acids from Gentiana algida. *Phytochemistry* 41: 111–116.
16. Hase, K., J. Li, P. Basnet, Q. Xiong, S. Takamura, T. Namba, and S. Kadota. 1997. Hepatoprotective principles of Swertia japonica Makino on D-galactosamine/lipopolysaccharide-induced liver injury in mice. *Chemical and Pharmaceutical Bulletin (Tokyo)* 45: 1823–1827.
17. Mahendran, G., G. Thamotharan, S. Sengottuvelu, and B.V. Narmatha. 2014. RETRACTED: Anti-diabetic activity of Swertia corymbosa (Griseb.) Wight ex C.B. Clarke aerial parts extract in streptozotocin induced diabetic rats. *Journal of Ethnopharmacology* 151: 1175–1183.
18. He, Y.M., S. Zhu, Y.W. Ge, K. Kazuma, K. Zou, S.Q. Cai, and K. Komatsu. 2015. The anti-inflammatory secoiridoid glycosides from gentianae scabrae radix: the root and rhizome of Gentiana scabra. *Journal of Natural Medicines* 69: 303–312.
19. Jeong, Y.T., S.C. Jeong, J.S. Hwang, and J.H. Kim. 2015. Modulation effects of sweroside isolated from the Lonicera japonica on melanin synthesis. *Chemico-Biological Interactions* 238: 33–39.
20. Yang, Q.L., F. Yang, J.T. Gong, X.W. Tang, G.Y. Wang, Z.T. Wang, and L. Yang. 2016. Sweroside ameliorates alpha-naphthylisothiocyanate-induced cholestatic liver injury in mice by regulating bile acids and suppressing pro-inflammatory responses. *Acta Pharmacologica Sinica* 37: 1218–1228.
21. Han, X.L., J.D. Li, W.L. Wang, C. Yang, and Z.Y. Li. 2017. Sweroside eradicated leukemia cells and attenuated pathogenic processes in mice by inducing apoptosis. *Biomedicine & Pharmacotherapy* 95: 477–486.
22. Wang, C., L. Zeng, T. Zhang, J. Liu, and W. Wang. 2016. Tenuigenin Prevents IL-1beta-induced Inflammation in Human Osteoarthritis Chondrocytes by Suppressing PI3K/AKT/NF-kappaB Signaling Pathway. *Inflammation* 39: 807–812.
23. Chen, W.P., Y. Xiong, P.F. Hu, J.P. Bao, and L.D. Wu. 2015. Baicalein Inhibits MMPs Expression via a MAPK-Dependent Mechanism in Chondrocytes. *Cellular Physiology and Biochemistry* 36: 325–333.
24. Ouyang, J., H. Jiang, H. Fang, W. Cui, and D. Cai. 2017. Isoimperatorin ameliorates osteoarthritis by downregulating the mammalian target of rapamycin C1 signaling pathway. *Molecular Medicine Reports* 16: 9636–9644.
25. Daheshia, M., and J.Q. Yao. 2008. The interleukin 1beta pathway in the pathogenesis of osteoarthritis. *The Journal of Rheumatology* 35: 2306–2312.
26. Studer, R., D. Jaffurs, M. Stefanovic-Racic, P.D. Robbins, and C.H. Evans. 1999. Nitric oxide in osteoarthritis. *Osteoarthritis and Cartilage* 7: 377–379.
27. Futani, H., A. Okayama, K. Matsui, S. Kashiwamura, T. Sasaki, T. Hada, K. Nakanishi, H. Tateishi, S. Maruo, and H. Okamura. 2002. Relation between interleukin-18 and PGE2 in synovial fluid of osteoarthritis: A potential therapeutic target of cartilage degradation. *Journal of Immunotherapy* 25: S61–S64.
28. Koskinen, A., K. Vuolteenaho, R. Nieminen, T. Moilanen, and E. Moilanen. 2011. Leptin enhances MMP-l, MMP-3 and MMP-13 production in human osteoarthritic cartilage and correlates with MMP-1 and MMP-3 in synovial fluid from OA patients. *Clinical and Experimental Rheumatology-Incl Supplements* 29: 57.
29. Baker, R.G., M.S. Hayden, and S. Ghosh. 2011. NF-kappaB, inflammation, and metabolic disease. *Cell Metabolism* 13: 11–22.
30. Lawrence, T., and C. Fong. 2010. The resolution of inflammation: anti-inflammatory roles for NF-kappaB. *The International Journal of Biochemistry & Cell Biology* 42: 519–523.
31. Roman-Blas, J.A., and S.A. Jimenez. 2006. NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. *Osteoarthritis and Cartilage* 14: 839–848.
32. Jacques C, Gosset M, Berenbaum F, Gabay C (2006) The role of IL-1 and IL-1Ra in joint inflammation and cartilage degradation. Vitam Horm 74:371-403
33. Jeong, J.W., H.H. Lee, E.O. Choi, K.W. Lee, K.Y. Kim, S.G. Kim, S.H. Hong, G.Y. Kim, C. Park, H.K. Kim, Y.W. Choi, and Y.H. Choi. 2015. Schisandrae Fructus Inhibits IL-1beta-Induced Matrix Metalloproteinases and Inflammatory Mediators Production in SW1353 Human Chondrocytes by Suppressing NF-kappaB and MAPK Activation. *Drug Development Research* 76: 474–483.
34. De Luna-Preitschopf, A, Zwickl, H, Nehrer, S, Hengstschlager, M, Mikula, M. 2017. Rapamycin Maintains the Chondrocytic Phenotype and Interferes with Inflammatory Cytokine Induced Processes. *International Journal of Molecular Sciences* 18.
35. Huang, M.J., L. Wang, D.D. Jin, Z.M. Zhang, T.Y. Chen, C.H. Jia, Y. Wang, X.C. Zhen, B. Huang, B. Yan, Y.H. Chen, S.F. Li, J.C. Yang,

Y.F. Dai, and X.C. Bai. 2014. Enhancement of the synthesis of n-3 PUFAs in fat-1 transgenic mice inhibits mTORC1 signalling and delays surgically induced osteoarthritis in comparison with wild-type mice. *Annals of the Rheumatic Diseases* 73: 1719–1727.

36. Zhang, H., H. Wang, C. Zeng, B. Yan, J. Ouyang, X. Liu, Q. Sun, C. Zhao, H. Fang, J. Pan, D. Xie, J. Yang, T. Zhang, X. Bai, and D. Cai. 2017. mTORC1 activation downregulates FGFR3 and PTH/PTHrP receptor in articular chondrocytes to initiate osteoarthritis. *Osteoarthritis and Cartilage* 25: 952–963.
37. Lopez, D.F.P., M.K. Lotz, F.J. Blanco, and B. Carames. 2015. Autophagy activation and protection from mitochondrial dysfunction in human chondrocytes. *Arthritis & Rhematology* 67: 966–976.