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Loganin ameliorates cartilage degeneration and osteoarthritis development in an osteoarthritis mouse model through inhibition of NF-κB activity and pyroptosis in chondrocytes

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ABSTRACT

Ethnopharmacological relevance: *Corni Fructus* (CF), the red fruit of *Cornus officinalis Siebold & Zucc*, has been used both as food and medicinal herb in traditional Chinese medicine (TCM). Loganin is a major iridoid glycoside and one of the quality control indexes of CF. In TCM clinical practice, prescription containing CF is commonly used to treat osteoarthritis (OA), but the underlying mechanisms of loganin are not yet utterly understood.

Aim of the study: The aims of the present study are to confirm the therapeutic effects of loganin in an OA mouse model and to determine the mechanisms involved in the OA protective effects.

Materials and methods: The destabilization of the medial meniscus (DMM) procedure was performed on the right knee of 8-week-old C57BL/6 male mice. 30 or 100 μg/ml of loganin was then injected into articular space twice a week for 8 and 12-week. Safranin O/Fast green staining, H&E staining, micro-CT analysis were performed to analyze structural and morphological changes. The protein expression of collagen type II (Col2), metalloproteinase-3 (Mmp3), matrix metalloproteinase 13 (Mmp13) collagen type X (Col10), cryopyrin and caspase-1 were detected by immunochemistry staining. Immuno-fluorescence assay was performed to assess changes in expression of CD31, endomucin, p65 and p-I-κB.

Results: Results of histomorphometry showed that loganin delays the progression of OA in the DMM model. In cartilage, loganin decreased the OARSI score, increasing hyaline cartilage (HC) thickness and decreasing calcified cartilage (CC) thickness. Moreover, loganin inhibited osteophyte formation, reduced the bone volume fraction (BV/TV), lowered trabecular thickness (Tb.Th) and increased trabecular separation (Tb.Sp) in subchondral bone. Mechanistically, loganin increased the expressions of Col2, decreases the expression of Mmp3, Mmp13, Col10, cryopyrin and caspase-1 in cartilage. In parallel, loganin inhibited the expression of CD31 and endomucin in subchondral bone. Furthermore, loganin suppressed nuclear translocation of p65 protein, and decreased the amount of p-I-κB in chondrocytes.

Conclusions: In summary, these results uncovered that loganin inhibits NF-κB signaling and attenuates cartilage matrix catabolism and pyroptosis of chondrocytes in articular cartilage. Loganin may serve as a potential therapeutic agent for OA treatment.

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1. Introduction

Corni Fructus (CF) is one of the most popular plant products used in traditional Chinese medicine (TCM) and has been clinically used for long time in Osteoarthritis (OA) treatment (Chen et al., 2014). As one of the major iridoid glycoside and active components isolated from CF, loganin has a variety of biological effects such as hepatic and renal protection, antidiabetes activity, cardioprotection, antioxidation, neuroprotection, antitumor activity, anti-inflammation, analgesic effects, antiaging activity, antiamnesia, antiosteoporosis, and immunoregulation (Huang et al., 2018). Besides, loganin also exerts its anti-inflammatory effects through inhibition of NF-κB pathway (Kim et al., 2015a,b; Li et al., 2016). The fundamental roles of CF-composed formulas in OA treatment and anti-inflammatory effects of loganin lead us to speculate that loganin may have beneficial effect on OA treatment.

OA is one of the most prevalent joint diseases affecting the whole joint (Chen et al., 2017; Liao et al., 2017). Cartilage destructions during OA development can be seen in any joints, but mainly occur in knees, hips, hands and spine. The major pathological features of OA include progressive articular cartilage degeneration, increased osteophyte formation and subchondral bone sclerosis, disruption of tidemark accompanied by angiogenesis at the osteochondral junction (Suri and Walsh, 2012).

Articular cartilage contains the sole cell type, chondrocytes, surrounded by extensive extracellular matrix mainly composed by type II collagen (Col2) in the remainder of cartilage (Liao et al., 2017). Matrix metalloproteinase 3 (MMP3) and Matrix metalloproteinase 13 (MMP13) are the potent enzymes for degradation of the cartilage matrix (Chen et al., 2017). Human clinical and animal model studies have showed that MMP3 and MMP13 play key roles during articular cartilage degeneration, whose expression were strongly upregulated in late-stage OA specimens (Liao et al., 2017). During OA progression, chondrocyte hypertrophy and subsequent collagen X fragmentation also seem to be increased in a subset of patients with inflammatory OA (Gu et al., 2014). As a result, the protection of collagen from degradation of these enzymes is one of the most important strategies in OA treatment (Wang et al., 2013).

Pyroptosis is a new form of programmed inflammatory cell death, requiring the function of the enzyme caspase-1 (Vande and Lamkanfi, 2016). Another important component of inflammasome is cryopyrin, which is encoded by the *NLRP3* gene in humans and binds the adaptor, the CARD domain containing apoptosis-associated speck-like protein (ASC) to induce pro-caspase-1 recruitment. During this process, cryopyrin, ASC and pro-caspase-1 were recruited and cryopyrin inflammasomes are assembled. Then, activated caspase-1 is released, further inducing pyroptosis through Gasdermin D (Lamkanfi and Dixit, 2014). To date, literatures on the involvement of the NLRP3-inflammasome in OA pathogenesis and its potential use is limited. A recent study showed the NLRP3-inflammasome is involved in OA pathogenesis, leading to synovial inflammation through activation of toll-like receptors and transcription factor κB (NF-κB) signaling and deteriorate the development of OA (Zhang et al., 2019). However, the role of articular chondrocyte pyroptosis during the progression of OA remains obscure.

NF-κB signaling plays a critical role in various inflammatory responses in many diseases including OA (Liu et al., 2017; Chang et al., 2019). NF-κB signaling pathway participates in OA pathogenesis by upregulating the expression of collagen degrading enzymes and promoting chondrocyte hypertrophy during OA progression. Due to its close connection with OA, NF-κB has been recognized as a promising target (Rigoglou and Papavassiliou, 2013). Moreover, NF-κB is also an essential upstream activator of NLRP3-inflammasome, which triggers the priming and assembling of inflammasome by inducing NLRP3 expression (Guo et al., 2015).

In this study, with the help of DMM OA model, we found that loganin has a chondroprotective effect by attenuating cartilage degradation and inhibiting chondrocyte pyroptosis through inhibition of NF-κB signaling.

2. Materials and methods

2.1. Chemicals and reagents

Loganin powder (≥98% of purity) was purchased from Manster TCM Co., Ltd (Sichuan, China) and was authenticated by the authors. A voucher specimen (No.: MUST-17080704) has been deposited at the First Affiliated Hospital of Zhejiang Chinese Medical University (Hangzhou, China). Primary antibodies against Col2, Mmp3, Mmp13, ColX, cryopyrin, caspase-1, endomucin and CD31 were purchased from Ruiying Biological (Jiangsu, China). Primary antibodies against p65 and p-I-κB were purchased from Cell Signaling Technology (Beverly, MA, USA). Unless otherwise mentioned, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Mice and DMM-induced OA model

Eight-week-old C57BL/6 male mice weighing 18–22 g were purchased from Zhejiang Chinese Medical University laboratory animal research center (Grade SPF, SCXK (Shanghai): 2017-0005), and housed under controlled pathogen-free conditions with a 12 h light/dark cycle. All mice were allowed free access to water and regular rodent chow. All protocols of mouse procedures were approved by the Ethics Committee of Zhejiang Chinese Medical University. The experimental mice were subjected to surgically induced OA by destabilization of the medial meniscus (DMM) as our previously described (Dong et al., 2018). Briefly, the mice were first anesthetized (1% pentobarbital sodium), joint capsule was incised, and then, the medial meniscotibial ligament was sectioned with microsurgical scissors. The lateral meniscotibial ligament was identified and protected during the surgery. For sham operation, an arthrotomy without the transaction of medial meniscotibial ligament, was also performed in the right knee joint of mice in sham control group. All mice were allowed to move freely in the cages after DMM surgery.

2.3. Experimental design and treatments

Eighty mice were randomly and equally divided into four groups (n = 20 in each group), including a sham control group (Sham group), an OA group (DMM group), an OA treated with low-dose loganin group (Low-dose group) or high-dose loganin group (High-dose group). Mice in Sham group and DMM group were injected with vehicle control, while mice in Low-dose group and High-dose group received 6 μL of loganin (30 and 100 μg/ml, respectively) per joint. The microinjection of loganin into articular cavity of mice began two weeks after DMM surgery, and were performed using 10 μl microinjector and 32 gauge needles (Hamilton Company, Reno, NV) twice a week for 8 and 12 weeks (Fig. 1). Animals were sacrificed by cervical dislocation at 8-week or 12-week post DMM surgery and the knee joint tissues were collected for further evaluation the severity of OA.

2.4. Histological staining, immunohistochemistry (IHC) and immunofluorescence (IF)

The knee joints of all mice were resected, fixed in 10% formalin for 24 h, decalcified with 10% EDTA for two weeks, dehydrated with graded ethanol and embedded in paraffin. Sections (4 μm) were cut and processed for Safranin O/Fast green and H&E staining. Histologic scoring for mouse cartilage degeneration was performed using a modified osteoarthritis research society international (OARSI) scoring system by double-blind observation as previously reported (Glasson et al., 2010). We measured the distance from the tidemark to articular cartilage surface as the thickness of hyaline cartilage (HC), and the distance from the tidemark to subchondral bone plate (SBP) as the

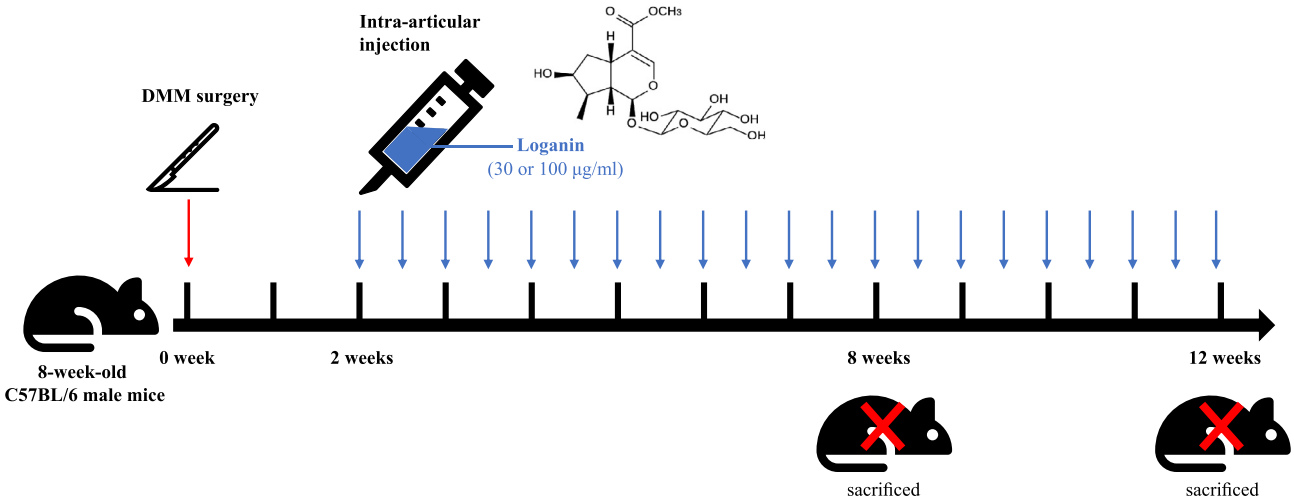

Fig. 1. Study design of the project.

thickness of calcified cartilage (CC). IHC was performed on 4 μm tissue sections as described in the manufacturer's instructions of SP Link Detection Kits (ZSGB-BIO, Beijing, China). Briefly, the sections of paraffin-embedded knees were deparaffinized, rehydrated, and subjected to antigen retrieval in 10 mM sodium citrate. Then tissue sections were incubated with 3% H_2O_2 in PBS for 10 min to quench endogenous peroxidase followed by sequential incubation with normal serum for 30 min, control IgG or primary antibodies (Col2, 1:200; Mmp3, 1:800; Mmp13, 1:800; ColX, 1:1000; cryopyrin, 1:200; caspase-1, 1:200) overnight at 4 °C, and HRP-labeled secondary antibody for 20 min. Diaminobenzidine (DAB) was used to visualize the immunohistochemical reaction followed by being counterstained with hematoxylin. Finally, dark brown cells were considered to be positive. Photomicrographs were taken with microscope (Carl Zeiss, Göttingen, Germany). For IF analysis, the sections of paraffin-embedded knees were incubated overnight at 4 °C with primary antibody (endomucin, 1:200; CD31, 1:200; p65, 1:200 and p-I-κB, 1:200) were purchased from Cell Signaling Technology (Beverly, MA, USA). and then the fluorescent secondary antibody (Sungene Biotech, Tianjin, China) for 30 min in the dark. Followed with DAPI counterstaining, the sections were observed under the fluorescence microscope (Carl Zeiss, Göttingen, Germany).

2.5. Micro-CT analysis

The knee joint images of mice were scanned by micro-CT equipment (Skyscan 1176, Bruker micro-CT N.V., Kontich, Belgium) and reconstructed (NRecon v1.6). The data were analyzed using data analysis software (CTAn v1.9) and three-dimensional model visualization software (CTVol v2.0). In addition to the visual assessment of structural pictures, quantitative morphometry indexes were determined from microtomographic data based on the three-dimensional morphometry (Hildebrand et al., 1999). The region of interest was identified between the proximal tibia growth plate and tibial plateau. And the following indexes were evaluated subsequently: bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), and trabecular number (Tb.N, 1/mm).

2.6. Statistical analysis

Data are expressed as the mean ± SD and were analyzed using one-way ANOVA followed by Fisher's least significant difference (LSD) comparison. All analyses were performed using SPSS version 17.0 software. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Loganin attenuates cartilage degradation in DMM-induced OA model

To investigate the effect of loganin treatment on the cartilage degradation in DMM-induced OA, histological analysis with Safranin O/Fast green staining was performed 8 and 12 weeks after DMM surgery. Articular cartilage exhibited a regular morphological structure in Sham group. Comparing with the Sham group, cartilage superficial destruction, cartilage erosion, vast proteoglycan loss and apparent hypocellularity were observed in the DMM group. Comparing with the DMM group, intra-articular injection with different doses of loganin led to the dramatically increase in articular cartilage thickness and the amelioration of cartilage damage (Fig. 2A and B). The sections of Safranin O/Fast green staining were further assessed with the OARSI histological scoring system. OARSI scores showed that, a significantly elevated score in DMM group was observed when compared with the Sham group (Fig. 2C). Treatment with loganin displayed lower OARSI scores than DMM group (Fig. 2C) 8 weeks after DMM surgery. With time progression apparent reduction in OARSI scores was also observed in loganin treated mice 12 weeks after DMM surgery (Fig. 2C). We also found that DMM surgery lead to the decrease in hyaline cartilage (HC) thickness and the increase in calcified cartilage (CC), and treatment with loganin was able to reverse this change in the mice 8 and 12 weeks after DMM surgery (Fig. 2D and E). There was no significant difference between the low-dose and high-dose groups in OARSI scoring analysis. However, loganin does display a dose-dependent inhibition of CC thickness.

3.2. Loganin attenuates subchondral bone remodeling

To assess the structural changes of the bone in loganin-treated OA model, the three-dimensional image was carried out using micro-CT and quantitative morphometry indexes were analyzed. Loganin prominently reduced DMM-induced osteophyte formation (Fig. 3A and B). Low-dose of loganin significantly reduced the bone volume fraction (BV/TV), lowered trabecular thickness (Tb.Th) and increased trabecular separation (Tb.Sp) in the mice 8 weeks after DMM surgery. There was no statistically significant difference in trabecular number (Tb.N) among loganin treatment and control groups (Fig. 3C–F). No statistically significant difference among loganin treatment and control groups in the mice 12 weeks after DMM surgery, except treatment with loganin significantly increased Tb.Sp and high-dose of loganin treatment significantly reduced Tb.Th. The changes of aforementioned parameters indicate that loganin mainly suppresses the subchondral remodeling in

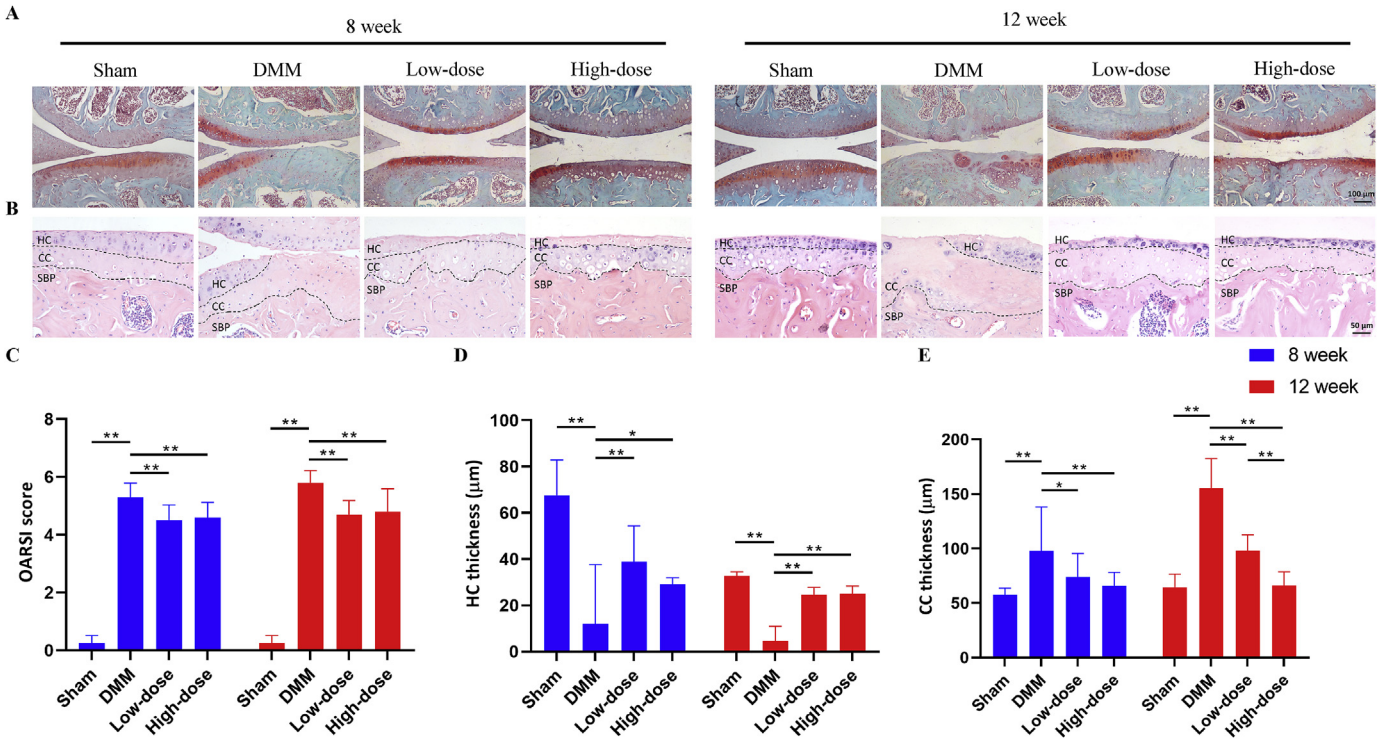

Fig. 2. Effects of loganin on cartilage degradation of DMM model. A. Safranin O/Fast green staining of cartilage. B. H&E staining of cartilage. Hyaline cartilage (HC) and calcified cartilage (CC) thickness are marked by dotted line. C. OARSI scores for cartilage structure damage on mice. D-E. HC and CC thickness of cartilage. Data are expressed as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, n = 10. Scale bar = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

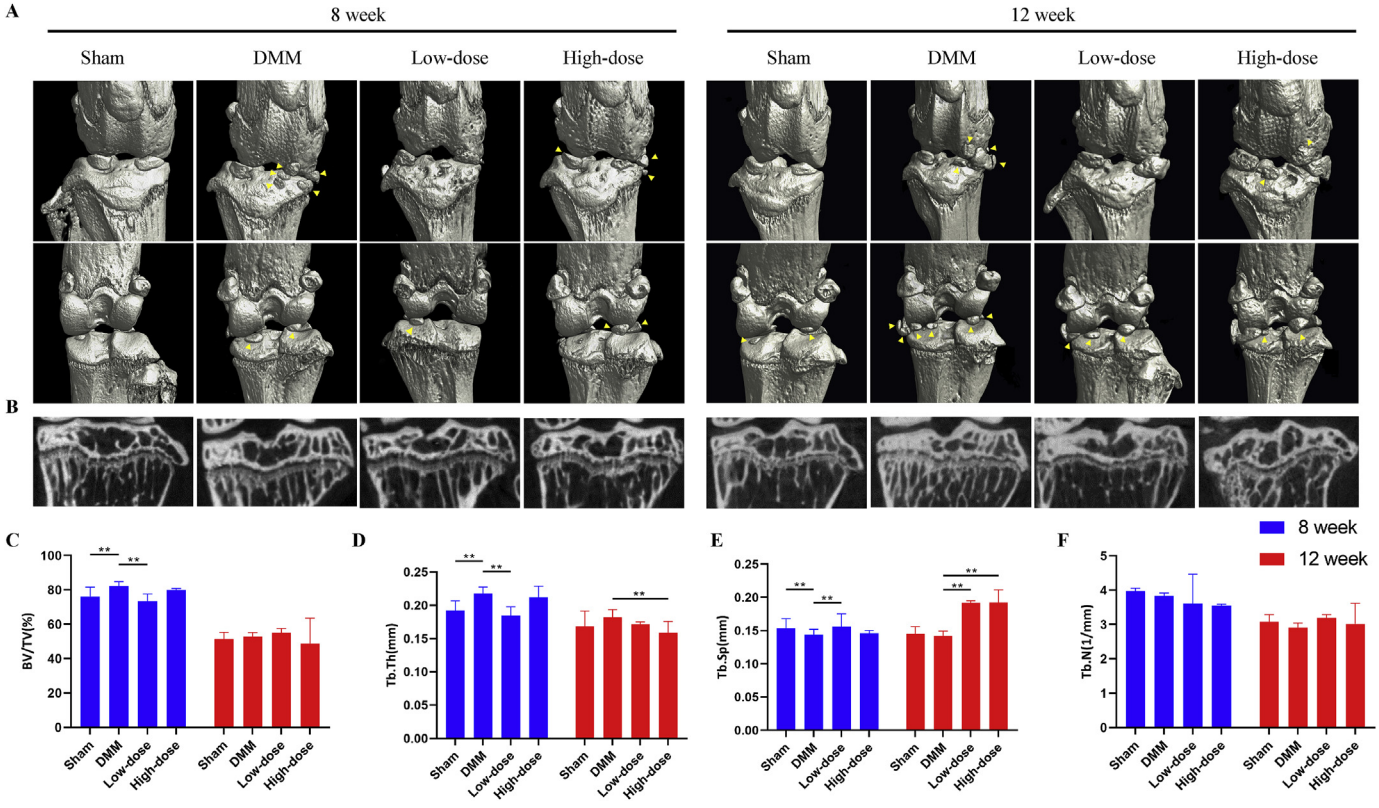

Fig. 3. Micro-CT analysis of bone structure in DMM model and loganin treatment. A. Representative micro-CT images. Osteophyte are marked by yellow triangle. B. Micro-CT two-dimensional reconstructions of tibial subchondral bone. C-F. Quantification of the bone morphological parameters (BV/TV, Tb.Th, Tb.Sp and Tb.N). Data are expressed as the mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$, n = 10 per group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

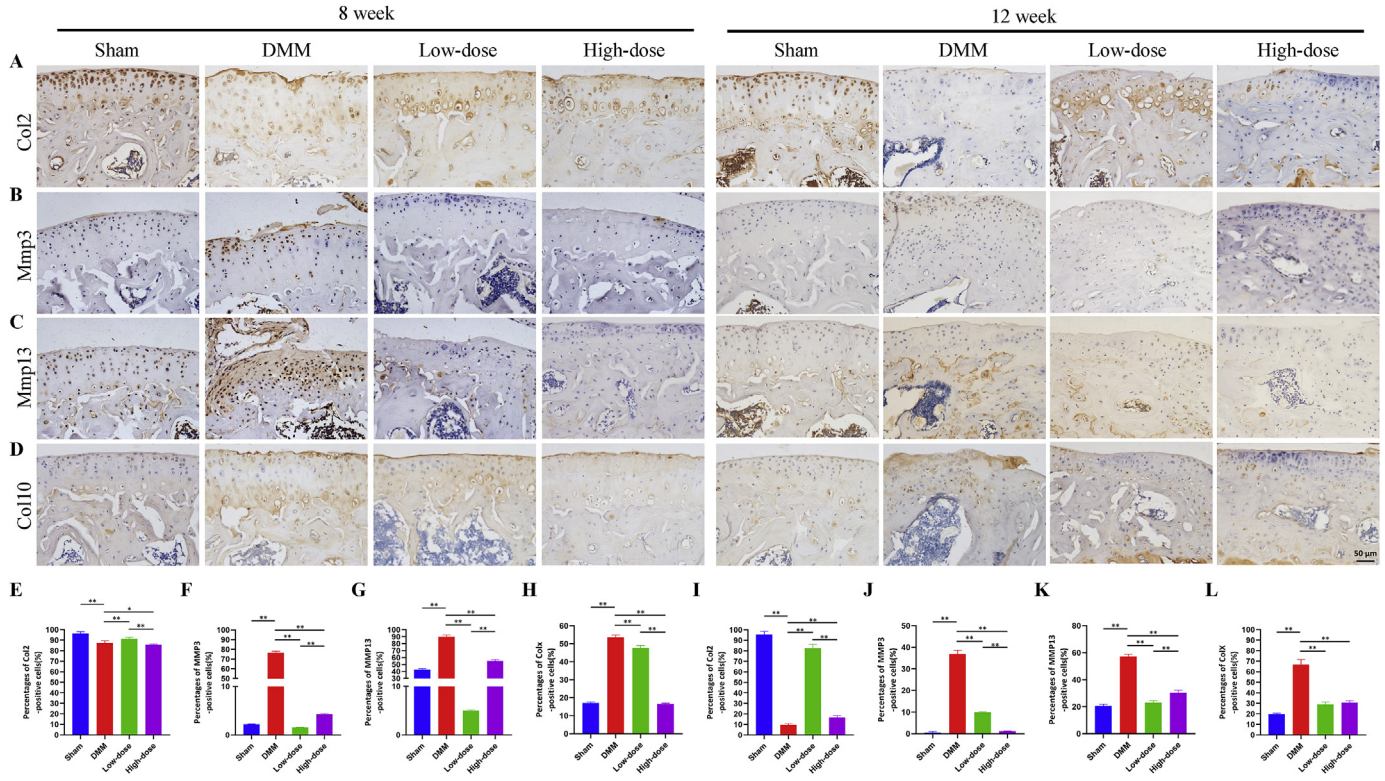

Fig. 4. Effects of loganin on expressions of Col2, Mmp3, Mmp13 and Col10 of cartilage in DMM model. A-D. Immunohistochemistry staining of Col2, Mmp3, Mmp13 and Col10. E-H. The ratios of immunoreactive positive cells at 8-week. I-L. The ratios of immunoreactive positive cells at 12-week. Data are expressed as the mean ± SD, $*P < 0.05$, $**P < 0.01$, n = 10 per group. Scale bar = 50 μm.

early OA progression.

3.3. Loganin inhibits cartilage matrix catabolism and hypertrophy of articular cartilage

To explore the mechanism underlying the delayed progression of cartilage damage in loganin-treated OA model. IHC staining for Col2, Mmp13, Mmp3 and Col10 were performed. The IHC analysis showed that the Col2-positive cells in DMM group was greatly diminished to 90.6% and 10.3% compared with Sham group 8 and 12 weeks after DMM surgery, respectively. Treatment with loganin reversed the DMM-induced Col2 reduction. There was statistically significant difference between low-dose and high-dose group, and the low-dose loganin showed better protective effect on Col2 (Fig. 4A, E and I). Compared with the Sham group, the expression of Mmp3 in DMM group was much higher at both time points, 8 and 12 weeks after DMM surgery, and expression of Mmp3 at 8-week time point was much higher than those at 12-week time point. Treatment with low-does loganin showed better effect of Mmp3 than the high-does loganin at 8-week, but a dose-dependent inhibitory effect was observed at 12-week (Fig. 4B, F and J). Similarly, loganin inhibited the Mmp13 expression, and the low-dose loganin has statistically better inhibitory effect on Mmp13 than high-dose loganin in both 8- and 12-week groups (Fig. 4C, G and K). As for Col10, loganin suppress Col10 protein expression in 8- and 12-week time points. A dose-dependent and statistically significant difference was detected in 8-week but not at 12-week group (Fig. 4D, H and L). To sum up, loganin treatment on DMM-induced OA model protected Col2, but greatly inhibited the expression of Mmp3, Mmp13 and Col10.

3.4. Loganin attenuates chondrocyte pyroptosis in DMM model

To detect whether cartilage degradation is related to chondrocyte pyroptosis, IHC staining for cryopyrin and caspase-1 were performed using samples collected from 8-week groups. The expression of cryopyrin was much higher after DMM surgery, and both low- and high-dose loganin treatment markedly decreased the cryopyrin expression. No statistical significant difference was found between the low- and high-dose groups (Fig. 5A and E). At the same time, the increased level of caspase-1 expression in DMM group was inhibited by the loganin treatment. The low-dose loganin displays better inhibitory effect on caspase-1 and there was statistically significant difference between Low- and High-dose groups (Fig. 5B and F).

3.5. Loganin suppresses NF-κB signaling pathway in chondrocytes

As mentioned before, NF-κB signaling regulates the expression of MMPs and Col10. NF-κB signaling is a mediator of NLRP3-inflammasome activation. To confirm whether the protective effects of loganin on OA are related to its regulation of NF-κB signaling in articular cartilage in the DMM model, we analyzed the location of p65 and protein levels of p-I-κB by IF. The results showed that a significant increase in nuclear translocation of p65 protein was observed in DMM group and loganin suppressed nuclear translocation of p65 protein (Fig. 5C). Furthermore, treatment with low and high doses of loganin significantly decreased the number of p-I-κB-positive cells to 54.2% and 6.4% compared with the DMM group without loganin treatment (Fig. 5D and G). Taken together, these results suggest that loganin is a potent suppressor of NF-κB activity in chondrocytes during OA development.

3.6. Loganin attenuates aberrant angiogenesis in subchondral bone in DMM model

Aberrant angiogenesis in subchondral bone is another characteristic of OA development. To examine the potential effects of loganin on subchondral bone angiogenesis, immunofluorescence staining for CD31

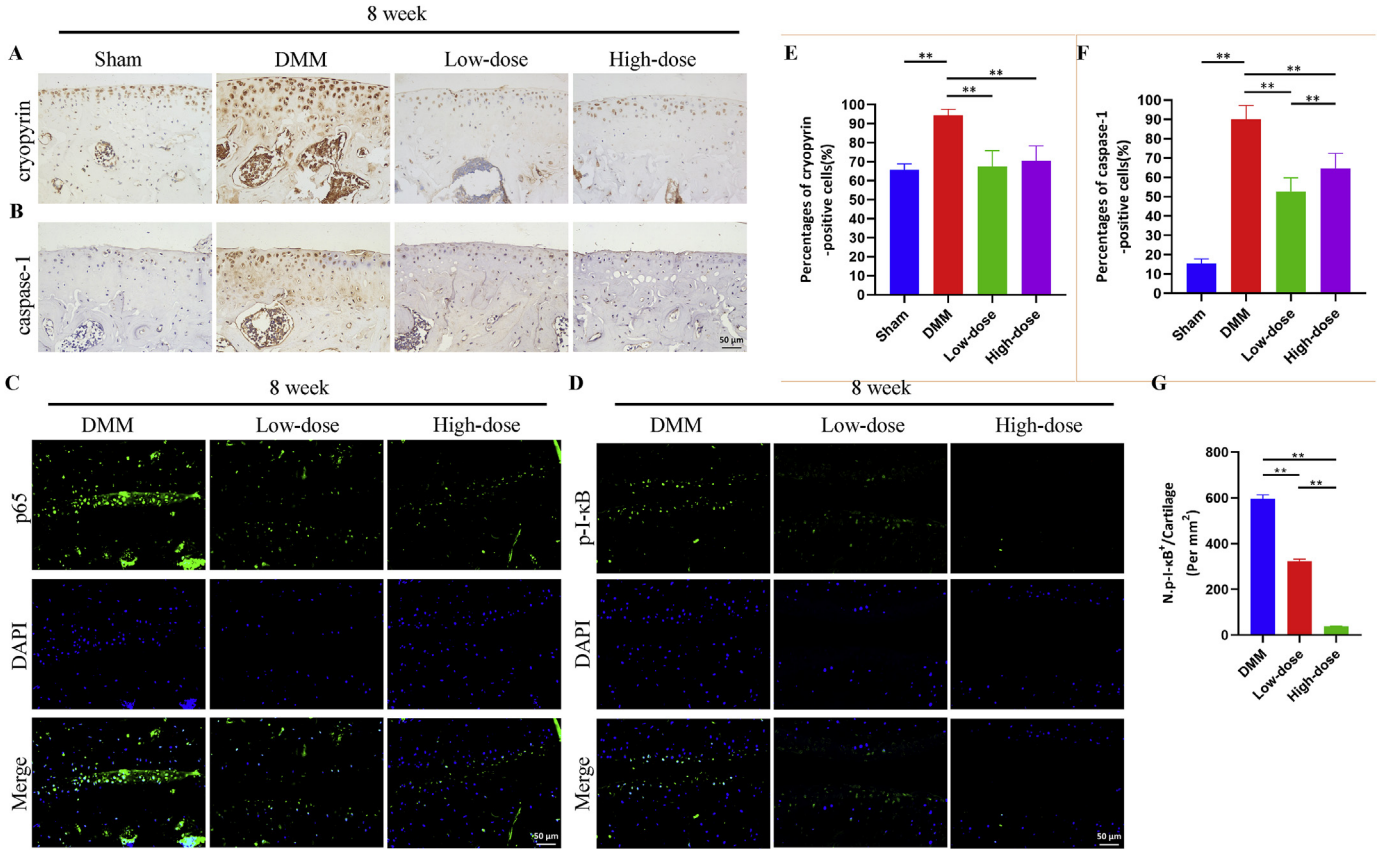

Fig. 5. Effects of loganin on pyroptosis and NFκB signaling in chondrocytes of DMM-induced OA model at 8-week. A-B. Immunohistochemistry staining of cryopyrin and caspase-1. C-D. Immuno-fluorescence staining of p65 and p-IκB in DMM model at 8-week. E-F. The ratios of immunoreactive positive cells in DMM model at 8-week. G. Quantification of p-I-κB positive cells in cartilage. Data are expressed as the mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$, n = 10 per group. Scale bar = 50 μm.

and endomucin was performed. Levels of endomucin statistically increased in DMM group and this elevation was reversed in the loganin treated groups (Fig. 6A and C). Likewise, increase of CD31 in DMM group was significantly reducted after loganin treatment (Fig. 6B and D).

4. Discussion

Articular cartilage and subchondral bone act together as a functional unit in the knee joint. Combining biochemical approaches with DMM-induced OA mouse model, we have uncovered that loganin shows protective effects on articular cartilage and subchondral-bone in experimental OA model, which is consistent with our previous findings that CF-comprised Chinese traditional formulas could effectively attenuate OA progression in OA animal model and clincical studies (Dong et al., 2018; Wang et al., 2018; Zhang et al., 2017). Loganin exerts its cartilage-protective effects not only by reversing the HC thickness and decreasing the CC thickness, OARSI score and osteophyte formation, but also attenuated the catabolism of cartilage matrix and occurrence of pyroptosis in articular cartilage. The results also indicate that loganin may be a strong eradication of the activity of NF-κB signaling including decrease the phosphorylation status of I-κB and subsequently the nuclear translocation of p65, which acts as upstream activator of chondrocyte dysfunction and pyroptosis (Fig. 7). Our data provided detailed molecular evidence that loganin ameliorates osteoarthritis development.

Pyroptosis is increasingly associated with differential pathophysiological outcomes in chronic inflammatory diseases (Strowig et al., 2012). As far as we known, whether pyroptosis occurs in chondrocytes during OA progression remains unclear. Early study states that OA cartilage can be degraded independently of inflammasome activity (Bougault et al., 2012). However, other researchers hold the opposite view, arguing that low dose of indomethacin and Hedgehog signaling inhibitor administration synergistically attenuates cartilage damage in osteoarthritis by controlling chondrocytes pyroptosis (Liu et al., 2019). In our study, the expression of cryopyrin and caspase-1, two key proteins in pyroptosis, increased after DMM surgery but remarkably reversed by loganin treatment. Moreover, loganin suppressed the activated NF-κB signaling pathway in chondrocytes in DMM cartilage. Our results indicate that inhibition of chondrocyte pyroptosis may be another important mechanism in OA cartilage degeneration. Besides, human clinical and animal model studies have showed that pyroptosis of fibroblast-like synoviocytes also contribute to synovial fibrosis and aggravate the OA progression (Zhang et al., 2019; Zhao et al., 2018). Different approaches are currently being attempted to target various components of the inflammasome directly, or upstream regulators or downstream targets of the inflammasome (Di, 2013; McAllister et al., 2018). Further investigation on OA therapy may need to pay more attention on regulation of pyroptosis, and the NLRP3 inflammasome is an attractive target for OA therapy.

Several animal studies have confirmed that changes in the subchondral bone microarchitecture takes place in the early stages of OA, and is related to articular cartilage degeneration. (Ji et al., 2018; Lin et al., 2019). Subchondral bone undergoes modelling and remodeling in response to an abnormal mechanical loading environment, resulting in abnormal bone remodeling at the early stage of OA and osteophyte formation (Hu et al., 2018). Our micro-CT analysis reveals the subchondral bone changes in OA was reversed by loganin treatment, indicating that loganin attenuates OA subchondral bone remodeling with less osteophyte formation. Additionally, abnormal subchondral bone remodeling is associated with aberrant angiogenesis in subchondral bone (Baek et al., 2018), and increased CD31 and endomucin positive

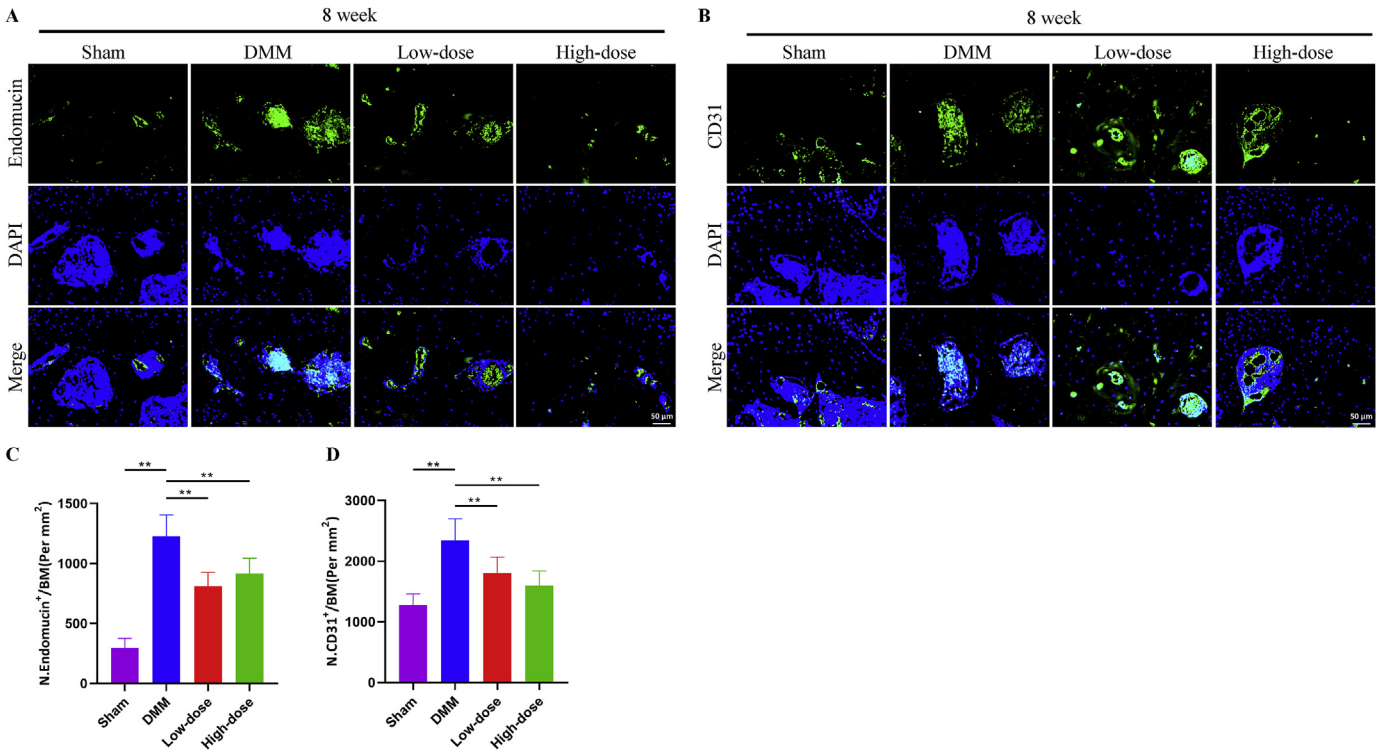

Fig. 6. Effects of loganin on angiogenesis in subchondral bone in DMM-induced OA model at 8-week. A. Immuno-fluorescence staining of endomucin in DMM model at 8-week. B. Immuno-fluorescence staining of CD31 in DMM model at 8-week. C. Quantification of endomucin positive cells in subchondral bone marrow (BM). D. Quantification of CD31 positive cells in subchondral bone marrow (BM). Data are expressed as the mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$, n = 10 per group. Scale bar = 50 μm.

vessels in subchondral bone has been observed during OA development (Cui et al., 2016). Capillaries with high expression of CD31 and endomucin, also called H-type vessels, is a specific vessel subtype. The area with H-type vessels represents environment with privileged access to oxygen and nutrients (Kusumbe et al., 2014), and is found in subchondral bone marrow (Cui et al., 2016; Li et al., 2019). In our study, CD31 and endomucin expression was significantly higher in subchondral bone of DMM group. Loganin significantly attenuates the pathological angiogenesis in subchondral bone.

As one of the major iridoid glycoside and active components

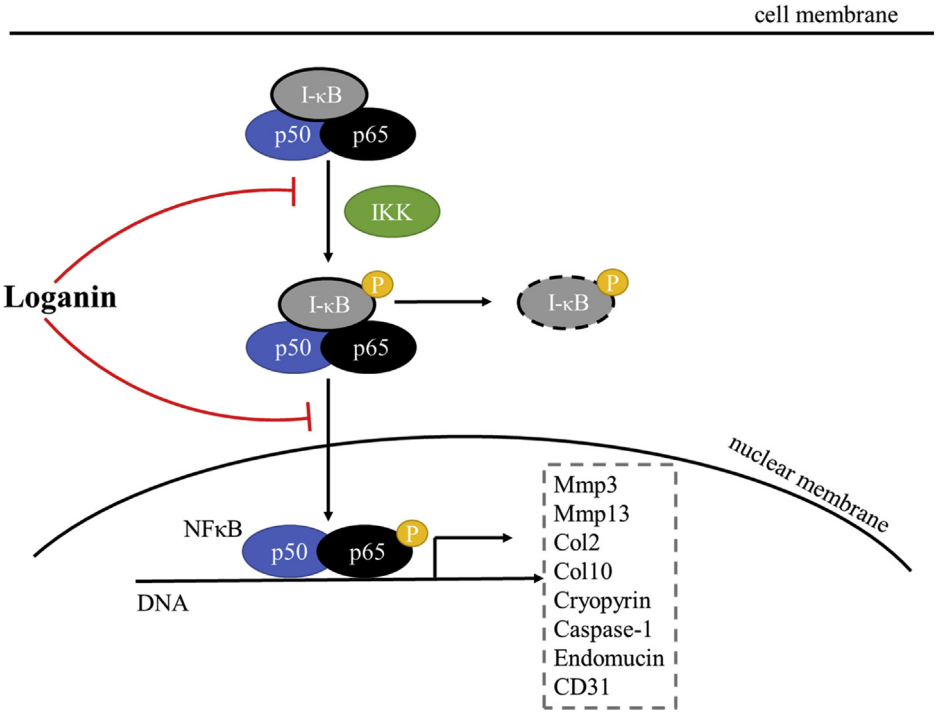

Fig. 7. Working model for the effects of loganin on OA progression via inactivation of NF-κB activation.

isolated from CF, loganin has been listed as one of the quality control indexes of CF by Chinese pharmacopoeia since 2005, the content of which must not be less than 0.6%. Our findings have increased our understanding of the roles of loganin in OA treatment. In clinical practice of TCM, the relationship between dose and effect of TCM is complex and diverse. In our study, we found different dose of loganin has different protective effects on OA, which further clarify a concise content level of loganin (as CF components) is required when treating with OA. The most important limitation lies in the fact that we did not determine the other gradients in quality control indexes of CF, and further research need to be undertaken to determine their roles in OA treatment.

5. Conclusions

In conclusion, we found that loganin, a natural product of CF, ameliorated OA progression in DMM-induced OA mouse model. Loganin attenuated cartilage degradation, preserved bone structure, inhibited chondrocyte pyroptosis and reduced aberrant angiogenesis via inhibition of NF-κB activation. Thus, these results uncover NF-κB inactivation and subsequent attenuation of cartilage matrix catabolism and pyroptosis of chondrocytes in articular cartilage as a hitherto uncharacterized mechanism controlling loganin-mediated preventive effect on OA progression.

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Appendix A. Supplementary data

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