Effect of Gubi prescription on caveolin-1 expression and phosphoinositide 3 kinase/protein kinase B and Fas signal pathways in rats with knee osteoarthritis


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Supported by Grants from the Training Project for Young and Middle-aged Academic Leaders of “Jiangsu 333 Project” and “High Level Health Personnel Six One Project” of the Jiangsu Health Planning Commission: Study on the Molecular Mechanism of Gubi Prescription in the Treatment of KOA Based on the Effect of Caveolin-1 on Chondrocyte Apoptosis Related Signal Transduction Pathway (LGY2016014) and Research Project for Clinical Talents of Chinese Medicine of Jiangsu Province

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Abstract

OBJECTIVE: To investigate the effects of Gubi prescription on the expression of caveolin-1, and the phosphoinositide 3 kinase/protein kinase B (PI3K/Akt) and Fas signal pathways in rats with knee osteoarthritis (KOA).

METHODS: Forty KOA model rats were established using a modification of Hulth’s method. Rats were divided into five groups by the random number method: model, positive drug (Vicolli group), and high-, medium-, and low-dose Gubi prescription groups (n = 8/group). In the sham surgery group (n = 8), only anterior and posterior cruciate ligaments of rats were exposed during surgery. A normal group (n = 8) consisted of rats with no treatment. Rats were intragastrically administered corresponding drugs once every day for eight consecutive weeks. Then, rat synovial membranes were extracted and histomorphological changes were recorded. mRNA expression was measured by q-PCR. Serum superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO), and interleukin 1β (IL-1β) levels were measured. Western blotting determined the effects of Gubi prescription on protein expressions of caveolin-1, Bax, Bcl-2, Fas, and caspase-3 in chondrocytes from KOA rats. The knee cartilage of rats was excised and cultured under aseptic conditions. After coincubation of chondrocytes with Gubi prescription-containing serum, IL-1β, and siRNA, Western blotting was used to determine the protein expressions of caveolin-1, Bax, Bcl-2, Fas, and caspase-3.

RESULTS: The morphological score of the articular synovium in the model group was significantly higher than in the normal group (P < 0.01). The morphological score in the high- and medium-dose Gubi prescription groups was lower than in the model group (P < 0.05). Chondrocytes from the decoction-containing serum group had a lower expression of Bax (P < 0.05), and higher expressions of Bcl-2 (P < 0.05) and caspase-3 (P < 0.05) compared with the model group. Chondrocytes in the decoction-containing serum group had higher expressions of Bax and Bcl-2 (P < 0.01) and lower ex-
pressions of caveolin-1 and Fas ($P < 0.05$) compared with the model group. Compared with the model group, Bax and caspase-3 expressions were reduced in the chondrocytes of all three Gubi prescription groups ($P < 0.05$) whereas Bcl-2 expression was increased ($P < 0.05$). Compared with the model group, the expressions of caveolin-1 and Fas ($P < 0.05$) were reduced in groups that received high- and medium-doses of Gubi prescription. Gubi prescription increased the serum level of SOD and significantly reduced those of MDA, NO and IL-1β ($P < 0.05$).

**CONCLUSION:** Gubi prescription suppressed the chondrocyte-related PI3K/Akt and Fas signal pathways and inhibited the overexpression of caveolin-1 in rat chondrocytes.

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**Keywords:** Osteoarthritis, knee; Caveolin 1; Phosphatidylinositol 3-kinase; Superoxide dismutase; Malondialdehyde; Nitric oxide; Interleukin-1beta; Gubi prescription

**INTRODUCTION**

Knee osteoarthritis (KOA) is the most common, chronic, progressive, and degenerative disease of joints in middle-aged and older adults. Patients may suffer from knee pain, stiffness, limited mobility, or even deformity as the disease progresses. Moreover, KOA severely endangers the health of middle-aged and elderly people, and it is ranked the fourth leading cause of disability worldwide. The pathogenesis of KOA is complex. First, the articular cartilage degenerates with local softening, then loses its normal elasticity until the surface becomes coarse. This process is accompanied by shedding cartilage and local small sunken on the cartilage surface. Furthermore, bone erosion occurs, and the subchondral bone plate is exposed after shedding of the cartilage. The third stage is mild synovitis. This complicated process is regulated by many cytokines and multiple signal pathways; chondrocyte apoptosis is closely related to the occurrence of KOA, and it plays a pivotal role in the degeneration of articular cartilage. The phosphoinositide 3 kinase/protein kinase B (PI3K/Akt) signal pathway is a classical anti-apoptotic pathway, which causes rapid signal transduction from the membrane to the nucleus. Its downstream pathways include Bad, Caspase and NF-κB, which are important anti-apoptosis pathways associated with cartilage degradation in patients with osteoarthritis. Previous studies have shown that the PI3K/Akt signal pathway is involved in the proliferation, apoptosis, differentiation, and regulation of many cells. It is one of the most important upstream pathways for the regulation of the proliferation, apoptosis, and matrix remodeling of chondrocytes. It can be activated by various factors in the early stages of osteoarthritis and it has a role in chondrocyte apoptosis.

The Fas pathway is an apoptotic signal pathway. Fas is a type I transmembrane glycoprotein located on cell membranes. It belongs to the tumor necrosis factor/nerve growth factor (TNF/NGF) receptor family. Fas ligand (FasL) is a type II membrane protein that belongs to the TNF family. The phosphorylation of tyrosine and threonine/serine is induced by the binding of Fas with FasL. This causes an increase in intracellular Ca²⁺ concentration, which further activates caspase-3 and causes the degradation of DNA. This eventually leads to the apoptosis of target cells. Previous studies have confirmed that Fas and FasL proteins play an important role in the chondrocyte apoptosis of KOA through a downstream signal pathway. Caveolin-1 is a primary functional protein located in small invaginations of cytoplasmic membrane (caveolae); caveolin-1 resembles a "space station" in which many signaling molecules perform transmembrane signal transduction. It interacts with several signaling proteins in caveolae to regulate several cell activities, such as cell proliferation, differentiation, and migration. Dai et al. reported that caveolin-1 positivity was associated with cartilage degeneration in humans and rats with osteoarthritis. They reported that when cartilage cells were treated with two differentiation inducers, IL-1β and hydrogen peroxide, the apoptosis of chondrocytes was promoted and the expression of caveolin-1 mRNA and protein was upregulated. They also reported that differentiation inducers and antisense oligonucleotides significantly inhibited chondrocyte apoptosis, which downregulated the expression of caveolin-1. This indicates that the expression of caveolin-1 is closely related to chondrocyte apoptosis in patients with osteoarthritis. Previous studies have confirmed that the expressions of PI3K and Akt were inhibited by the excessive activation of caveolin-1, leading to cell cycle arrest and apoptosis. Moreover, caveolin-1 may regulate the Fas signal pathway by mediating the apoptotic and mitochondrial pathways. Caspase-3 is a downstream target protein associated with the Fas pathway. It cleaves the carboxyl terminus of the target protein Bcl-2 downstream of the PI3K/Akt pathway, thereby antagonizing the anti-apoptotic effect of Bcl-2. In this study, we determined the effects of Gubi prescription on the expression of caveolin-1, and the PI3K/Akt, and Fas pathways. Furthermore, we also determined the morphology of articular chondrocytes and serological markers in KOA rats. The q-PCR and Western blotting were used to investigate ribonucleic acids and proteins. We also investigated the molecular mechanism of Gubi prescription treatment of KOA.
MATERIALS AND METHODS

Animals and experimental drugs

Overall, 56 (28 male rats and 28 female rats) Sprague-Dawley (SD) rats weighing between 200-250 g were obtained from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. [Laboratory animal production license of SXCC (Beijing) 2012-0001]. These rats were of specific pathogen free grade. The experimental animal waste was disposed of by strictly following the Experimental Animals’ Management and Protection Guidelines. The experimental animals were raised in clean-grade breeding rooms at the Animal Experimental Center, Nanjing University of Chinese Medicine, China. They had unlimited access to regular food pellets and water and were maintained at 24-25 °C with a relative humidity of ~40%.

The Gubi prescription was prepared by practitioners of Traditional Chinese Medicine. It is composed of the following eight herbs: Jixueteng (Caulis Spatholobi), Sangjiasheng (Herba Taxilli Chinensis), Gusuiibu (Rhizoma Drynariae), Niuxi (Radix Aconiti Asperijobi), Chuanxuuduan (Radix Dipsaci Asperoidis), Songjie (Lignum Pini Nodi), Qiannianjian (Rhizoma Homalomenae), and Tubiechong (Eupolyphaga). The specification of glucosamine sulfate capsule (Vicolli) is as follows: 0.25 g/capsule. This capsule was manufactured by Rottapharm Ltd., (Mulhuddart Dublin, Ireland) (National Medicine Permit No. H20009797).

Main reagents and instruments

In this study, we used the following reagents: cell culture serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), antibodies (Cell Signaling Technology, Danvers, MA, USA), type II collagenase (AngleGene Technologies, Shanghai, China), 0.9% saline, pentobarbital sodium, phosphate buffered saline (PBS), fluorescence quantitative reverse transcription PCR kits (PC04-50T, Nanjing Zhongding Biotechnology Co., Ltd., Nanjing, China), total RNA extraction reagent Trizol (RK-100, Nanjing Zhongding Biotechnology Co., Ltd., Nanjing, China), MMLV reverse transcriptase 200 U/μL (20 μL), reverse transcription reaction system (RT reaction buffer, Nanjing Zhongding Biotechnology Co., Ltd., Nanjing, China), Taq DNA polymerase 1 U/μL (40 μL), agarose (91622, Biowest, Shell Industrial Building, Catalonia, Spain), diethyl pyrocarbonate (DEPC, D5758, 25 mL, Orlando, FL, USA), Gelstain (GS101-01, Beijing TransGen Biotech Co., Ltd., Beijing, China), PCR primers (Shanghai Sangon Biotech Co., Ltd., Shanghai, China), and penicillin (North China Pharmaceuticals Co., Ltd., Shijiazhuang, China).

The Synergy Ultrapure Water Purification System was purchased from Millipore, (Burlington, MA, USA). The microplate reader BioTek-ELX800 was purchased from BioTek Instruments (Winooski, VT, USA). The nucleic acid and protein analyzer biophotometer, in-
Serum was prepared from Gubi prescription using a serum pharmacology method. As follows, we selected the last dose; the drug-containing serum was filtered with a 70-µm nylon mesh filter. Cells were cultured in a type I collagen coated plate with DMEM containing 10% fetal bovine serum, 2 mmol/L L-glutamate, 100 U/mL penicillin, and 100 µg/mL streptomycin, in a CO₂ incubator maintained at 37°C.

Cell processing and sample collection
Serum was prepared from Gubi prescription using a serum pharmacology method. As follows, we selected healthy SD rats, each weighing 260-300 g. For three consecutive days, rats were intragastrically administered twice a day with four times the normal dose of Gubi prescription. Blood was drawn one hour after administering the last dose; the drug-containing serum was obtained by centrifuging the blood sample at 14578.5 g/min for 10 min. This serum was then stored at 4°C for later use. Control serum was prepared with the same protocol; the Gubi prescription was replaced with saline solution. When cells grew to 80% confluency, they were treated with Gubi prescription-containing DMEM medium or control serum for 0, 4, 8, 12, 16 or 24 h. Cells were collected and added to cell lysates to extract total protein.

Histomorphological observation
Histological sections of articular synovial tissues were stained with HE and observed under an inverted biological microscope. The follow criterion was used to investigate and score vascular congestion, synovial swelling, inflammatory cell infiltration, and synovial necrosis. If the pathological degree was normal, mild, moderate and severe, the corresponding histopathological score is 0, 1, 2 and 3.

Protein expressions of caveolin-1, Bax, Bcl-2, Fas, and caspase-3 in chondrocytes from KOA rats were determined by Western blotting. Chondrocyte culture dishes were rinsed three times with D-Hanks solution. Then, 100 µL RIPA lysis buffer was added to each dish, which were kept on ice for five min. Cell lysates were collected with a cell scraper. Then, they were centrifuged at 12 000 r/min for 5 min at 4°C. The resultant supernatant was collected. A BCA protein assay kit was used to determine the protein content. For electrophoresis, the following procedure was used to prepare protein samples: 50 µg/well protein was mixed with loading buffer. Then, it was dehydrated at 99.9°C for 5 min in a PCR instrument. Finally, it was stored at 4°C. We prepared a 12% resolving gel and 5% stacking gel. Protein samples were loaded and subjected to electrophoresis. Then, these samples were transferred to membranes, and an electric current of 100 mA was passed through these membranes for 1 h. The membranes were blocked with 5% non-fat dry milk for 1 h. The membranes were rinsed with TBST and TBS. Finally, membranes were incubated overnight with primary antibodies at 4°C. The membranes were then rinsed with TBST and TBS. Finally, they were incubated with secondary antibodies at room temperature for 1 h. After exposure, compression, development, and fixation, films were analyzed by ImageJ software (Thermo Fisher Scientific, Waltham, MA, USA) to determine the absorbance values of the bands. The relative expression of proteins was defined as the ratio of the gray scale value of the target band to that of an internal reference band (β-actin).

mRNA expressions of caveolin-1, Bax, Bcl-2, Fas, and caspase-3 in chondrocytes from KOA rats were determined by q-PCR
Primers for caveolin-1, Bax, Bcl-2, Fas, and caspase-3 were designed and synthesized using GenBank data. Cultured chondrocytes from KOA rats were fully lysed with Trizol reagent (Nanjing Zhongding Biotechnology Co., Ltd., Nanjing, China) and then placed on ice for 5 min. After exposure, compression, development, and fixation, films were analyzed by ImageJ software (Thermo Fisher Scientific, Waltham, MA, USA) to determine the absorbance values of the bands. The relative expression of proteins was defined as the ratio of the gray scale value of the target band to that of an internal reference band (β-actin).
for 5 min to separate nucleic acids from proteins. After centrifuging the sample at 12 000 × g at 4 °C for 15 min, we obtained the following three phases: the lower organic phase was yellow in color, while the upper aqueous phase was colorless. An interphase was present between these two phases. The upper aqueous phase primarily consisted of RNA, of which 300-500 µL was carefully transferred to a new 1.5 mL Eppendorf tube. To precipitate the RNA, an equal amount of isopropyl alcohol was added and mixed gently in 1.5 mL Eppendorf tube. The resultant tube was kept on ice for 10 min. Then, the sample was centrifuged at 12 000 × g for 10 min at 4 °C. The supernatant was discarded, and the pellets RNA was extracted. Then, it was further rinsed with 75% ethanol. The RNA was reverse transcribed into cDNA by the Oligo (dT) method. Then, a PCR amplification reaction was conducted under the following conditions: 95 °C × 10 min and 40 cycles of 95°C × 15 s, 55 °C × 30 s, and 72 °C × 60 s.

**Expression of caveolin-1 in KOA rat chondrocytes and its correlation with the expressions of Bax and Bcl-2, downstream target proteins of the PI3K/Akt signal pathway**

Chondrocytes were co-incubated with siRNA which can down regulate caveolin-1 expression. Western blotting was used to determine the protein expressions of caveolin-1, Bax, and Bcl-2 in chondrocytes. q-PCR was used to determine the mRNA expressions of caveolin-1, Bax, and Bcl-2 in chondrocytes using the same methods before.

**Expression of caveolin-1 in chondrocytes of KOA rats and its correlation with the expressions of Fas and its downstream target protein caspase-3**

Chondrocytes were co-incubated with siRNA which can down regulate caveolin-1 expression. Western blotting was used to determine the expressions of Fas and caspase-3 proteins in chondrocytes. q-PCR was used to determine mRNA expressions of Fas and caspase-3 in chondrocytes. The procedures were the same as those before.

**Effect of Gubi prescription on the expressions of caveolin-1, Bax, Bcl-2, Fas, and caspase-3 in chondrocytes of KOA rats**

Proteins were extracted from homogenized cartilage tissue by RIPA lysis. The concentration of the extracted protein sample was determined by bicinchoninic acid (BCA) assay. Protein samples were then denatured and prepared in loading buffer. Then, a 30 µL protein sample from each group was electrophoretically separated on a 10% SDS-polyacrylamide gel for approximately 1.5 h. Protein samples were electrotransferred onto PVDF membranes by the wet transfer method. Each membrane was blocked with 50 g/L non-fat dry milk for 1 h at room temperature. Then, each membrane was incubated overnight with its corresponding specific primary antibody in a refrigerator at 4 °C. The membranes were rinsed three times with TBST; each rinsing was carried out for 10 min. The membranes were then incubated in a shaker with horseradish peroxidase-conjugated secondary antibodies at 37 °C for 1 h. The results were developed and fixed in X-films with ECL solution. The films were scanned and saved. To determine the relative expression of proteins, we determined the ratio of the gray scale value of the target band to that of an internal reference band (β-actin).

**Serological testing**

Determination of superoxide dismutase (SOD) activity in serum: the SOD activity in serum was determined by the xanthine oxidase method, according to the kit instructions.

Determination of malondialdehyde (MDA) in serum: the thiobarbituric acid method was used to determine the MDA content in serum according to the kit instructions.

Determination of nitric oxide (NO) in serum: the NO content in serum was measured by the nitrate reduction method according to the kit instructions.

Determination of superoxide dismutase (SOD) activity in serum: the SOD activity in serum was determined by the xanthine oxidase method, according to the kit instructions.

**Statistical analysis**

The data was processed with SPSS 19.0 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY, USA). All measurement data are presented as mean ± standard deviation (±). A two-way analysis of variance, paired t-test, and Newman-Keuls test were conducted to test the differences between groups. P < 0.05 was statistically significant.

**RESULTS**

**Observation of articular synovium with HE staining**

Articular synovial cells in the normal group were well-distributed and aligned continuously. Cellular swelling, tissue necrosis, and inflammatory cell infiltration were not observed in articular synovial cells of the normal group. Synovial cells in the sham surgery group closely resembled those of the normal group. The cells were aligned continuously, well-ordered with slight vascular congestion; however, they did not show any cellu-
lar swelling, tissue necrosis, or inflammatory cell infiltration. Compared with the normal and sham groups, articular synovial cells in the model group exhibited various degrees of congestion and edema; moreover, these synovial cells were significantly swollen, with marked inflammatory infiltration and tissue necrosis. In the positive treatment group, mild edema, mild hyperplasia, and swelling were observed in the synovial membrane; however, there were no signs of tissue necrosis. This indicates that pathological changes in the positive treatment group were significantly greater than in the model group. In the group that received high doses of Gubi prescription, the articular synovium showed mild congestion and edema, mild proliferation of synovial cells, and low infiltration of inflammatory cells; however, there were no signs of tissue necrosis. In the group that received medium doses of Gubi prescription, the articular synovium of rats showed moderate congestion and edema with mild proliferation of synovial cells, and low infiltration of inflammatory cells; however, there were no signs of tissue necrosis. In the group that received low doses of Gubi prescription, the articular synovium of rats showed moderate congestion and edema with mild swelling and inflammatory infiltration; however, there were no signs of tissue necrosis. In the group that received medium doses of Gubi prescription, the articular synovium of rats showed moderate congestion and edema with mild swelling and inflammatory infiltration; however, there were no signs of tissue necrosis. In the group that received low doses of Gubi prescription, the articular synovium of rats showed moderate congestion and edema with mild swelling and inflammatory infiltration, but no sign of tissue necrosis (Figure 1).

**Histological morphology score of articular synovial tissue**

The histological morphological score of articular synovia in the model group was significantly greater than that in the normal group ($P < 0.01$). In the groups that received middle and high doses of Gubi prescription, the morphological scores were significantly lower than those in the model group ($P < 0.05$) (Table 1).

**Protein expressions of caveolin-1, Bax, Bcl-2, Fas, and caspase-3 in chondrocytes from KOA rats**

Compared with the normal group, the expressions of caveolin-1, Bax and FAS proteins were significantly higher (normal group, $P < 0.01$) as well as caspase-3 (normal group, $P < 0.05$) in the model group (Figure 2) whereas Bcl-2 protein was reduced (normal group, $P < 0.05$). Compared with the model group, Bax and caspase-3 expressions were decreased in the decoction-containing serum group (Gubi prescription-containing serum group, $P < 0.05$) whereas Bcl-2 was increased (Gubi prescription-containing serum group, $P < 0.05$). Figure 2 shows protein expressions of caveo-

![Figure 1 HE staining of articular synovial membranes in each group (× 200)](image)

A: normal group (0.9% saline solution, equal volume, 8 weeks); B: model group (0.9% saline solution, equal volume, 8 weeks); C: sham surgery group (0.9% saline solution, equal volume, 8 weeks); D: positive drug group (Vicolli, 0.017 g/kg, 8 weeks); E: low-dose Gubi prescription group (Gubi prescription, 6.05 g/kg, 8 weeks); F: medium-dose Gubi prescription group (Gubi prescription, 12.1 g/kg, 8 weeks); G: high-dose Gubi prescription group (Gubi prescription, 24.2 g/kg, 8 weeks). HE: hematoxylin and eosin.

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<tr>
<td>Sham surgery</td>
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<td>0</td>
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<td>24.2</td>
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</table>

Table 1 Histological morphology score of rat articular synovial tissue ( ± s)

Notes: normal group (0.9% saline solution, equal volume, 8 weeks); model group (0.9% saline solution, equal volume, 8 weeks); sham surgery group (0.9% saline solution, equal volume, 8 weeks); positive drug group (Vicolli, 0.017 g/kg, 8 weeks); Low-dose Gubi prescription group (Gubi prescription, 6.05 g/kg, 8 weeks); medium-dose Gubi prescription group (Gubi prescription, 12.1 g/kg, 8 weeks); high-dose Gubi prescription group (Gubi prescription, 24.2 g/kg, 8 weeks). The model group compared with the normal group, *$P < 0.01$; the positive drug group compared with the model group, **$P < 0.01$; the medium and high-dose Gubi prescription groups compared with the model group, **$P < 0.05$. **
tein levels were significantly decreased (\(P < 0.01\)).

The expression of caspase-3 protein was decreased in the high- and medium-dose Gubi prescription groups (\(P < 0.05\)). The expression of Bcl-2 protein was higher in groups that received high and medium doses of Gubi prescription (\(P < 0.05\)). The expression of caspase-3 protein was decreased in the high-, medium-, and low-dose Gubi prescription groups (\(P < 0.05\)). The expressions of caveolin-1 and Fas were reduced in the high- and medium-dose Gubi prescription groups (\(P < 0.05\)). These results indicate that Gubi prescription inhibited the expression of caveolin-1 in chondrocytes from KOA rats. Consequently, the expression of Fas was upregulated, and the expression of its downstream target protein caspase-3 was inhibited. Thus, Gubi prescription reduces the expression of Bax protein and increases the expression of Bcl-2 protein to inhibit the apoptosis of chondrocytes (Figure 4).

**Effect of Gubi prescription on the serum levels of SOD, MDA, and NO in KOA rats**

Compared with the model group, the SOD level in the high- and medium-dose Gubi prescription groups was increased significantly (\(P < 0.01\)), whereas the MDA and NO levels were significantly decreased in the medium-dose group (\(P < 0.01\)). There were no significant...
of synovial cells, and no signs of tissue necrosis were noted. This indicates that Gubi prescription had a therapeutic effect on KOA; however, the underlying mechanism of Gubi prescription remains elusive.

**Correlations of caveolin-1 protein expression with protein expressions of Bax, Bcl, Fas and caspase-3 in chondrocytes from KOA rats**

Chondrocyte apoptosis is a complicated pathological process, which involves several cell signal transduction pathways. The Fas and PI3K/Akt signal pathways are considered classic, essential pathways of anti-chondrocyte apoptosis. 15-17 By regulating the expression of caveolin-1, we investigated its effects on PI3K/Akt and Fas signal pathways. We confirmed that Gubi prescription inhibited the apoptosis of articular chondrocytes through several targets.

In 1953, American biologist Paladel used an electronic microscope to identify some flask-shaped invaginations on the surface of vascular endothelial cells, termed caveolae, which are now known as specific invaginated structures of the cytoplasmic membrane. 16,17 Many receptors, kinases, and proteins that are associated with signal transduction in these invaginations have an important role in signal transduction pathways. Caveolin-1 is a member of the caveolin family, which is a transmembrane scaffold. Moreover, it is an essential structural component of caveolae. Caveolin-1 directly affects and regulates the activities of signaling molecules through the caveolin scaffolding domain, by increasing or inhibiting the activities of caveolin-related proteins. Caveolin-1 performs various functions, such as increasing or inhibiting the activities of caveolin-related proteins.
as signal transduction, cell growth regulation, and apoptosis. PI3K/Akt is a serine/threonine-specific protein kinase that has an important role in multiple cellular processes, such as cell differentiation, proliferation, apoptosis, glucose transport, cell transcription, and migration. Moreover, PI3K is an intracellular phosphoinositide kinase activated by several cytokines and signaling controlling...
The Akt family consists of Akt, Akt

20

P2 and PIP3, which are intracellular secondary messengers. 25–26 Thus, PI3K and phosphoinositide-dependent protein kinase (PDK) activate and translocate Akt into the plasma membrane where it becomes activated. Then, activated Akt is transported into the cytoplasm, where it phosphorylates and inhibits activation of the downstream target protein Bax — a death-promoting factor in the Bcl-2 family, which activates Bcl-2 and enables cell survival.

Fas and its ligand Fasl are apoptosis-inducing factors. Rapid apoptosis of cells is promoted by the binding of Fas with Fasl; therefore, these are also known as death molecules. The phosphorylation of tyrosine and threonine/serine is induced by the binding of Fas with Fasl, which increases the concentration of intracellular Ca2+ ions. This activates caspase-3 and destroys cells by dismantling the cytoskeleton, blocking the replication and repair of DNA, interfering with mRNA scission, and damaging DNA and nuclear structures. Therefore, it degrades cells into apoptotic bodies that express phagocytosed signals that eventually mediate cell apoptosis. 27–35

The expression of caveolin-1 was upregulated by stimulating chondrocytes with IL-1β. In the model group, the expression of Bax was significantly higher than that in the normal group. The expression of Bcl-2 was lower than that in the normal group. The expression of caveolin-1 was downregulated when chondrocytes were co-incubated with siRNA. In the model group, the expression of Bax was also higher than in the normal group whereas the expression of Bcl-2 was slightly lower. These results indicate that the activities of PI3K/Akt can be inhibited by the overexpression of caveolin-1. This increases the expression of the pro-apoptosis regulator Bax, and reduces the expression of anti-apoptosis regulator Bcl-2. This leads to chondrocyte apoptosis. Compared with the normal group, the expressions of Fas and its downstream factor caspase-3 were increased in the model group. The expression of caveolin-1 was stimulated by IL-1β in chondrocytes. In the model group, the expressions of Fas and caspase-3 were in-

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<th>MDA (nmol/mL)</th>
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<td>54.1±12.4**</td>
<td>4.4±1.6**</td>
<td>29.4±13.3**</td>
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Notes: normal group (0.9% saline solution, equal volume, 8 weeks); model group (0.9% saline solution, equal volume, 8 weeks); sham surgery group (0.9% saline solution, equal volume, 8 weeks); positive drug group (Vicoll, 0.017g/kg, 8 weeks); low-dose Gubi prescription group (Gubi prescription, 6.05 g/kg, 8 weeks); medium-dose Gubi prescription group (Gubi prescription, 12.1 g/kg, 8 weeks); high-dose Gubi prescription group (Gubi prescription, 24.2 g/kg, 8 weeks). Compared with the normal group, *P < 0.01 (the model group); compared with the model group, P < 0.01 (the medium-dose Gubi prescription group, P < 0.05 (the low-dose Gubi prescription group), the high-dose Gubi prescription group, the positive group); compared with the positive control group, *P > 0.05 (the low-dose Gubi prescription group, the medium-dose Gubi prescription group, the high-dose Gubi prescription group).
increased when the expression of caveolin-1 was downregulated. This indicates that the Fas pathway was regulated by the overexpression of caveolin-1; moreover, chondrocyte apoptosis occurred when the expression of caspase-3 was increased.

**Effect of Gubi prescription on caveolin-1, and the PI3K/Akt and Fas signal pathways in chondrocytes from KOA rats**

On the basis of these findings, we speculate that Gubi prescription regulates the Fas signal pathway by inhibiting the overexpression of caveolin-1 in KOA chondrocytes. Thus, the activity of caspase-3 is inhibited, blocking cleavage of the carboxyl terminus of Bcl-2 and the downstream target proteins in the PI3K/Akt signal pathway. This promotes the activation of Bcl-2, reducing the expression of the pro-apoptotic regulator Bax. Thus, chondrocyte apoptosis is inhibited. The mechanism through which Gubi prescription ameliorates KOA is explained as follows: Gubi prescription affects chondrocyte related PI3K/Akt and Fas signal pathways by inhibiting the overexpression of caveolin-1 in chondrocytes. Thus, anti-chondrocyte apoptosis effects are achieved, improving the histopathology of knee osteoarthritis, and preventing the degeneration of knee cartilage.

When KOA occurs, synovial fluid, synovium and chondrocytes in the joint capsule release a large amount of inflammatory mediators and activators. The equilibrium between these inflammatory mediators (such as NO) and activators (such as IL-1β and TGF-β) determines the degree of chondrocyte damage. At the same time, the activity of oxygen free radicals is increased, promoting membrane lipid peroxidation, leading to an increase in lipid peroxidation metabolites such as MDA that inhibits the normal growth and development of chondrocytes and the synthesis of matrix, which together cause damage to the articular cartilage. Correspondingly, enzyme systems in the body scavenge destructive oxygen radicals such as superoxide dismutase and catalase to help remove them and prevent inflammation, and thus protect chondrocytes.

In conclusion, we demonstrated that Gubi prescription reduced the scores related to rat knee cartilage synovial injury, effectively increased the activity of SOD, decreased the contents of MDA, NO and IL-1β in the serum of KOA rats, and reduced damage to the articular cartilage by inflammatory cytokines and pro-lytic cytokines. Therefore, Gubi prescription may prevent destruction of joints, surrounding tissues and articular cartilage by reducing synovial inflammation, and thus it might have an important role in KOA prevention and treatment in rats.

**REFERENCES**


