ORIGINAL RESEARCH

Therapeutic Effects of Curcumin on Osteoarthritis and Its Protection of Chondrocytes Through the Wnt/B-Catenin Signaling Pathway

Tangbo Yuan, MD; Dawei Cai, MD; Bo Hu, MD; Yiran Zhu, MD; Jian Qin, PhD

ABSTRACT

Context • Osteoarthritis (OA) is a high-incidence, chronic condition, with an extremely high prevalence among older adults. OA seriously compromises the normal living of OA patients, and it's imperative to find a novel therapy as soon as possible to improve their prognosis and life quality.

Objective • The study intended to investigate the therapeutic effects of Curcumin (Cur) on OA and to explore its preliminary mechanism of action, with the aim of offering more accurate guidance for use of OA therapy.

Design • The research team designed a prospective non-randomized controlled trial.

Setting • The study took place in the Department of Orthopedics at Sir Run Run Hospital at Nanjing Medical University in Nanjing, China.

Participants • Participants were 107 OA patients treated at the hospital between March 2019 and January 2020.

Intervention • Participants were divided into two groups, 51 in the Cur group and 56 in the ibuprofen group.

Outcome Measures • The clinical efficacy and safety of the two groups were observed. In addition, the research team performed in-vitro studies. Chondrocytes HC-a and C28/I2 were purchased to evaluate the intracellular inflammatory response and apoptosis rate under the intervention of Cur and Wnt/β-catenin pathway inhibitors.

Results • No significant differences existed in the clinical-efficacy rate between the two groups ($P > .05$), but the Cur group show higher improvements in safety, joint mobility, and inhibition of inflammation ($P < .05$). In-vitro experiments showed that Cur inhibited the apoptosis rate of chondrocytes and the levels of inflammatory factors, while the Wnt/β-catenin inhibitor did the opposite ($P < .05$).

Conclusions • Cur can effectively decrease the pathological results of OA, with a remarkable safety profile; its mechanism may be the activation of the Wnt/β-catenin signaling pathway to inhibit the inflammatory reaction and apoptosis in chondrocytes. (*Altern Ther Health Med.* 2022;28(5):28-37)

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Osteoarthritis (OA) is a joint condition that mainly involves the functional degeneration of articular cartilage, which is usually characterized by multidirectional and multilevel bone-structure lesions. It's a high-incidence, chronic condition, with an extremely high prevalence among older adults.[1]

According to one investigation, 240-million people worldwide suffer from OA, 60%-80% of whom are patients over 60 years old.[2] Currently, the rate of OA in worldwide older adults is as high as 2%-5%, and the incidence even reaches approximately 10% in some coastal cities and countries with large temperature ranges.[3] Moreover, OA has been afflicting younger people in recent years.[4]

With recurrent pathological pain as the most typical clinical symptom, OA can induce obvious joint stiffness, swelling, dysfunction, and deformity as it develops, and it can hinder activity and cause substantial impacts on normal life.[5,6] OA can cause concurrent injuries, such as those in the ligaments, synovium, and joint capsule, which can greatly threaten patients' entire joints and may even give rise to joint-function loss or paralysis.[7]

In clinical practice, OA is usually treated conservatively, but drugs are able only to relieve the pain and joint dysfunction of patients and can't completely cure OA, which is one primary cause for the recurrent attacks of OA long-

term.[8] Currently, clinical therapy for OA is still highly limited, and a reliable and effective cure is still required.[9] It's imperative to find a novel therapy as soon as possible to improve patient's prognosis and life quality.

Curcumin (Cur)

In recent years, studies have found that natural extracts, such as Berberine, can effectively hinder the development of chronic diseases and that the Ligusticum chuanxiong extract can have anti-inflammatory and anti-oxidant effects.[10,11] These findings have gradually captured clinical attention for their use in therapy for various diseases.

Cur is a naturally bioactive polyphenol, with many functions according to some research, including hypoglycemic, antioxidant, lipid-lowering, and anti-inflammatory actions; urinary-protein reduction; kidney protection; and antitumor activity.[12,13] As a polyphenol compound, Cur mainly includes active components such as curcumin and volatile oil. The former has blood-lipid reduction, anticoagulation, antioxidation, choleretic, and anticancer functions, while the latter mainly possesses anti-inflammatory, antibacterial, and antitussive functions.[14]

Cur is known to be a natural compound with anti-inflammatory activity comparable to steroidal and nonsteroidal drugs, and its role in alleviating human inflammatory disease has been repeatedly verified.[15,16] It possesses various antipathological effects with the advantage of great safety.[17] Cur has demonstrated excellent effects for chronic diseases such as diabetes, with a strong ability to manage blood glucose and blood lipids and to lower the incidence of concurrent diseases.[18]

Cur has been shown to exert anticancer effects by inducing the differentiation of malignant tumor cells and apoptosis of tumor cells and by suppressing the growth of tumors in various stages while promoting the decomposition of amyloid in the brain to prevent nervous-system diseases.[19] Cur is able to downregulate forkhead box P3 (Foxp3) in regulatory T (Treg) cells, enhance the secretion of interferon gamma (IFN-γ), and give rise to the transformation of Treg cells into T helper type 1 (Th1) cells,[20] which indicates the remarkable regulatory impact of Cur on the immune function of the body.

At present, preliminary studies of Cur for OA have occurred. For example, Chin KY et al and Bannuru RR et al have pointed out that Cur can effectively improve the clinical symptoms of OA with high safety.[21,22] Daily et al[23] have indicated the ability of Cur to substantially alleviate the symptoms of arthritis and to potentially provide another direction for arthritis therapy in the future. And Shep et al[24] suggested that the best treatment for OA is Cur not a nonsteroidal anti-inflammatory drug. Wu et al[26] have examined the use of Cur for OA and found the same conclusion as the above study.

OA's Pathogenesis

OA's specific pathogenesis is still under exploration, and various factors such as hyperactivity, heredity, and external injury are clinically deemed to be risk factors. Some research has indicated that the development of OA is strongly bound up with the degeneration of articular cartilage function and that the accelerated apoptosis of articular chondrocytes is a decisive factor for cartilage function.[25] The Wnt/β-catenin signaling pathway, one of the most classical signal pathways in clinical research, has a crucial part in cell function and has been implicated in changes in the cell growth and death cycle for diseases such as liver cancer and colorectal cancer.[26,27]

Cur and the Wnt/β-catenin Pathway

Cur has been shown to improve diseases such as cerebral ischemia injury and osteoporosis by affecting the Wnt/β-catenin pathway,[28,29] and the role of the Wnt/β-catenin pathway in OA, has been repeatedly documented.[30,31] Wnt/β-catenin has been confirmed to be in an abnormal state and be implicated in the development of OA.[32]

One study found that Curcumin (Cur) can inhibit the development of cervical cancer via the Wnt/β-catenin signaling pathway,[33] which suggests a certain potential association of Cur with Wnt/β-catenin.

The therapeutic mechanism of Cur on OA is studied relatively rarely. The specific action pathway and mechanism of Cur are still under exploration, which greatly limits the application of Cur. More research is warranted to further explore its relationship with OA and clarify the specific impact of Cur on OA, so as to realize its clinical popularization.

Current Study

In response to the increasingly high incidence of OA, understanding the mechanism of Cur is important for its future clinical application against OA. By analyzing the treatment of OA with Cur and the effects of Wnt/β-catenin pathway on chondrocytes, the current study can not only verify the therapeutic effects of Cur in OA but also further understand the mechanism of action of Cur on OA, which has important reference significance for clinical practice.

Accordingly, the current study intended to investigate the therapeutic effects of Curcumin (Cur) on osteoarthritis (OA) and to explore its preliminary mechanism of action, with the aim of offering more accurate guidance for use of OA therapy.

METHODS: CLINICAL TRIAL

Participants

The research team designed a prospective non-randomized controlled trial. The study took place in the Department of Orthopedics at Sir Run Run Hospital at Nanjing Medical University in Nanjing, China. Participants were OA patients treated in the hospital between March 2019 and January 2020. These patients were diagnosed as OA from March 2019 to January 2020 at Sir Run Run Hospital, Nanjing Medical University, and were collected by two joint surgeons, Yuan Tangbo and Qin Jian. A total of 143 patients with OA were collected during this period, among whom 36 were excluded based on the following exclusion criteria:

tumors, multiple cardio-cerebrovascular diseases, autoimmune diseases, organ dysfunction, infectious diseases, neurological disorders, drug allergies, inability to take care of themselves due to disabilities, and hospital referrals. Two doctors, Yuan Tangbo and Cai Dawei, were responsible for the relevant clinical examinations of these patients.

Potential participants were included in the study if they: (1) showed clinical manifestations of OA that had been confirmed after examination, (2) had detailed case data available, (3) had not received antibiotic therapy in the three months prior to admission, and (4) agreed to cooperate with the investigation of the medical staff in the hospital.

Potential participants were excluded from the study if they: (1) had comorbid tumors; (2) had multiple cardiovascular or cerebrovascular diseases, autoimmune diseases, organ dysfunction, infectious diseases, or neurological disorders; (3) were allergic to drugs, (4) were unable to take care of themselves due to disabilities, and (5) were referred patients.

This study was approved by the Human Research Ethical Committee of the Sir Run Run Hospital, Nanjing Medical University (NO.: 2020-SR-S012), and was conducted in strict accordance with the *Declaration of Helsinki*. Informed consent forms were signed by all enrolled individuals. was conducted.

Procedures

Patients were allowed to choose the drug therapy themselves after introducing two kinds of treatment drugs to them. Patients treated with ibuprofen were then selected as the ibuprofen group, and those treated with Cur were assigned to the Cur group.

Interventions

The Cur group orally took a 100 mg Cur capsule (Yangtze River Pharmaceutical Group, Jilin, China) twice a day. The ibuprofen group orally took a 0.3g, sustained-release tablet twice a day.

Outcome Measures

Efficacy assessment. The participants' symptoms were under good clinical control if: (1) the symptoms had disappeared, (2) the joint motion was normal, (3) the X-ray-examination results were normal, and (4) the symptom score had decreased by over 95%.

The treatment was determined to have been markedly effective if: (1) the symptoms had disappeared, (2) the joint motion was unlimited, (3) the X-ray-examination results had improved, and (4) the symptom score had decreased by over 70%.

The treatment was determined to have been effective if: (1) most symptoms had disappeared, (2) the joint motion was limited, (3) the X-ray-examination results had improved, and (3) the symptom score had decreased by over 30%.

The treatment was determined to have been ineffective if no improvement had occurred (1) in symptoms, (2) joint motion, or (3) X-ray results and (4) the symptom score had decreased by less than 30%.

The total effective rate was equal to the number of participants under good clinical control + those participants with markedly effective results + those participants with effective results, divided by the total number of participants × 100%.

Visual Analog Scale (VAS).[34] The VAS (full score: 10 points) was used to evaluate the pain of patients, with a score of 0 indicating no pain, 1-2 indicating mild pain, 4-6 indicating moderate pain, and 7-10 indicating severe pain. A higher VAS score indicates more serious pain.

Western Ontario and McMaster Universities (WOMAC) OA Index.[35] The WOMAC OA Index was used to evaluate the degree of OA of patients from three dimensions of pain, stiffness and difficulty, with a total of 24 items. A higher score on the WOMAC indicates more serious disease.

Range of motion (ROM).[36] The ROM test was used to assess patients' joint mobility, including the shoulder, elbow, forearm, wrist, hip, knee, and ankle joints. A higher score on the ROM indicates a wider range of joint motion.

Lysholm Knee Scoring Scale (LKSS).[37] The LKSS developed by Lysholm and Gillqui in 1982 was used to evaluate the knee function of patients from 8 items of pain, instability, locking, swelling, limp, stair-climbing, squatting and support. A higher score on the scale indicates better joint activity.

Serum inflammatory factors. Fasting venous blood (4mL) was extracted from patients and centrifuged to collect the serum and plasma. The mRNA levels of inflammatory factors IL-1β, IL-6 and IL-8 in the serum were detected by qRT-PCR. The total RNA extracted by Trizol from the sample was synthesized into cDNA using a reverse transcription kit. Thermal cycling conditions: initial denaturation at 95°C for 10min, followed by 35 cycles of 15s at 95°C and 40s at 55°C. Using GAPDH as an internal reference, the relative expression levels of genes were calculated by $2^{-\Delta\Delta CT}$.

METHODS: IN-VITRO STUDIES

Procedures

In vitro experiments were carried out after the completion of all the above-mentioned clinical research evaluations and tests.

Chondrocytes. Chondrocytes HC-a and C28/I2 cells were purchased from the American Type Culture Collection (ATCC; Manassas, Virginia, USA). The cells were treated by one-minute trypsinization using 0.25% trypsin after washing with phosphate-buffered saline (PBS) three times, followed by the addition of Dulbecco's Modified Eagle Medium (DMEM; Sigma, Shanghai, China) for reaction termination.

The suspended cells were repeatedly blown and then transferred to a centrifuge tube, followed by the removal of the supernatant and the addition of 4.5 mL of culture medium. Afterward, the cells were subjected to incubation at 37°C, using 5% CO_2, with the solution being replaced once every three days. Subsequent assays were carried out when the cells grew to cover 70%-80% of the bottle's bottom.

Curcumin interventions. Cur was added into the medium to culture HC-a and C28/I2 and the levels of

inflammatory factors were detected. With reference to research by Zhao et al,[15] the HC-a and C28/I2 cells were grouped into the Cur group, incubation with 10 μmol / L Cur during incubation, and the control group, incubation with the same amount of normal saline.

Intervention of Wnt/β-catenin pathway inhibitor. With reference to research by Lietman et al,[38] HC-a and C28/I2 cells were grouped into the ICG-001 group and the blank group. ICG-001 is a Wnt/β-catenin pathway inhibitor from Sigma (shanghai, China). The former group was subjected to 12 h of treatment using DMEM with 4 μmol/L, and the latter group was subjected to the same treatment with the same amount of normal saline.

Cell-apoptosis determination. Cells in the log-growth stage that had been seeded in a six-well plate, 2×10^5 cells/well, were resuspended with binding buffer after washing using PBS. The washing was followed by the addition of 5 μL of Annexin V-FITC (Abcam, Galveston, Texas, USA) and 5 μL of propidium iodide (PI) from manufacturer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), with 15 min of incubation in dark surroundings after even mixing. Subsequently, 400 μL of 1× binding buffer was added, and a flow cytometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was adopted for cell-apoptosis determination.

Protein detection. After being lysed with radioimmunoprecipitation assay (RIPA) buffer, the cells were subjected to 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) from manufacturer (Abcam, Galveston, Texas, USA), followed by transfer to a polyvinylidene difluoride (PVDF) membrane from manufacturer (Abcam, Galveston, Texas, USA) and one hour of immersion in a sealing solution. Subsequently, the membrane was subjected to overnight incubation at 4°C, with Wnt3a, Wnt5a, β-catenin, and β-actin I antibody as the internal reference.

The next day, the membrane was subjected to one hour of incubation at 37°C with horseradish peroxidase (HRP)-coupled II antibody (Abcam, Galveston, Texas, USA), followed by development with enhanced chemiluminescence (ECL). (Abcam, Galveston, Texas, USA) Finally, Image-Pro Plus (Media Cybernetics, Silver Springs, Maryland, USA) was adopted for relative-expression calculation of protein bands.

Rescue assay. Some cells were incubation with both Cur and ICG-001 as Group A, and some cells by normal saline alone as Group B. Then their inflammatory reaction and apoptosis were quantified by the above methods.

Outcome Measures

Inflammatory Factors in Chondrocytes. qRT-PCR was used to detect the mRNA levels of L-1β, IL-6, and IL-8 in cells to evaluate the degree of inflammatory response. Higher detection results indicate more severe inflammatory responses in cells.

Chondrocyte Viability and Wnt/β-Catenin Pathway. The Wnt/β-Catenin pathway and cell activity were evaluated by detecting the expression of Wnt/β-Catenin pathway-related proteins and cell apoptosis rate. Increased Wnt/β-Catenin pathway protein expression indicates that the pathway is in an activated state, and vice versa. An increase in the apoptosis rate indicates a reduction in cell activity, and conversely, a decreased apoptosis rate suggests an increased cell activity.

Wnt/β-Catenin Pathway in Chondrocytes. To further confirm the role of the Wnt/β-catenin pathway in chondrocytes, the study first intervened chondrocytes with Wnt/β-catenin pathway inhibitors (ICG-001), and tested the expression of Wnt/β-catenin pathway proteins in HC-a and C28/I2 cells to verify the success of the intervention with the same method as mentioned in Protein detection. Under the action of ICG-001, the Wnt/β-catenin pathway protein expression in cells decreased, indicating that the inhibitor was successfully used.

Chondrocyte Viability. We observed changes in the apoptosis rate after ICG-001 administration to determine the effect of inhibiting Wnt/β-catenin pathway on cell activity.

Rescue Assay. To confirm that Cur affects the activity and inflammatory process of chondrocytes through the Wnt/β-catenin pathway, the study conducted a rescue experiment to verify and analyze the mechanism of action of Cur. If there was no difference in cell activity and levels of inflammatory factors between Cur and ICG-001 co-cultured cells and normal saline-treated cells, it indicated that there was a negative regulatory relationship between Cur and ICG-001, and that the combined use of the two could reverse the previously mentioned effects on cells.

Statistical Analyses

All assays of the study were performed with specimens three times, and the results were averaged. The study used SPSS, version 22.0 (IBM, Chicago, Illinois, USA) for statistical analyses. Intergroup comparisons of quantitative data (%) were conducted using the Chi-square test, and counting data (means ± SDs) were compared using the t test between groups and the variance test and LSD post hoc test among groups. A $P<.05$ indicated a significant difference.

RESULTS: CLINICAL TRIAL

Participants

Of the prospective OA participants, 107 were enrolled and prospectively analyzed, with 51 being assigned to the Cur group and 56 to the ibuprofen group. No significant differences were found between the two groups in the clinical data at baseline ($P>.05$).

Clinical Efficacy

The effective rate for the Cur group was 88.24%, as shown in Table 1, and it wasn't significantly different from that of the ibuprofen group, at 85.71% ($P>.05$). In both groups, the clinical efficacy was mainly markedly effective, 43.14% in the Cur group and 44.64% in the ibuprofen group, followed by participants whose OA was under good clinical control (data not shown).

Table 1. Comparison of Clinical Efficacy (N = 107)

	Under Good Clinical Control n (%)	Markedly Effective n (%)	Effective n (%)	Ineffective n (%)	Total Effective Rate (%)
Intervention group (n = 51)	15 (29.41)	22 (43.14)	8 (15.69)	6 (11.76)	88.24
Ibuprofen group (n = 56)	14 (25.00)	25 (44.64)	9 (16.07)	8 (14.29)	85.71
χ^2					0.149
P value					.699

Pain

The VAS scores of the two groups postintervention after two courses of therapy (Figure 1) weren't significantly different ($P > .05$), but after one course of therapy, the Cur group had significantly lower VAS scores than did the ibuprofen group ($P < .05$). The scores of both groups decreased gradually with treatment time ($P < .05$).

Joint Function

As treatment time increased (Figure 2), both group's WOMAC scores significantly decreased and ROM and Lysholm scores significantly increased ($P < .05$). Postintervention after two courses of therapy, the two groups' joint scores weren't significantly different ($P > .05$), but after one course of therapy, the Cur group had significantly lower WOMAC scores and significantly higher ROM and Lysholm scores than did the ibuprofen group ($P < .05$).

Serum Inflammatory Factors

Postintervention (Figure 3), both groups presented significantly lower mRNA levels of IL-6, IL-1β, and IL-8, with the Cur group's levels being significantly lower than those of the ibuprofen group postintervention ($P < .05$).

Adverse Reactions

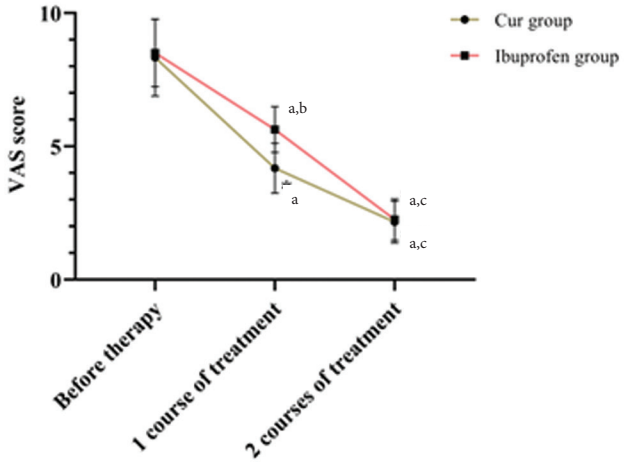
In the intervention group, adverse reactions were observed in one participant (1.96%), who developed a gastrointestinal reaction, and the incidence of adverse reactions was 1.96% (Table 2). In the ibuprofen group, two participants (3.57%) had a gastrointestinal reaction; one participant (1.79%) had upper abdominal pain; three participants (5.36%) had dizziness; and one participant (1.79%) had nausea and vomiting, with a total incidence of 12.50%. The incidence of adverse reactions in the intervention group was significantly lower than that in the ibuprofen group ($P < .05$), indicating that Cur is safer than ibuprofen.

RESULTS: IN-VITRO STUDIES

Impact on Inflammatory Factors in Chondrocytes

Figure 4 shows the levels of inflammatory factors in the chondrocytes. The mRNA levels of IL-6, IL-1β, and IL-8 in the Cur group were significantly lower than those in the blank group ($P < .05$), indicating that Cur can inhibit the inflammatory response of chondrocytes.

Figure 1. Comparison of VAS Scores Between the Groups



[*] $P < .05$, showing a significant difference between baseline and after one course of treatment for the Cur group as well as the ibuprofen group

[b] $P < .05$, showing a significant difference between the ibuprofen group and the Cur group after one course of treatment

[c] $P < .05$, showing a significant difference between the first course of treatment and the second course of treatment for the Cur group as well as the ibuprofen group

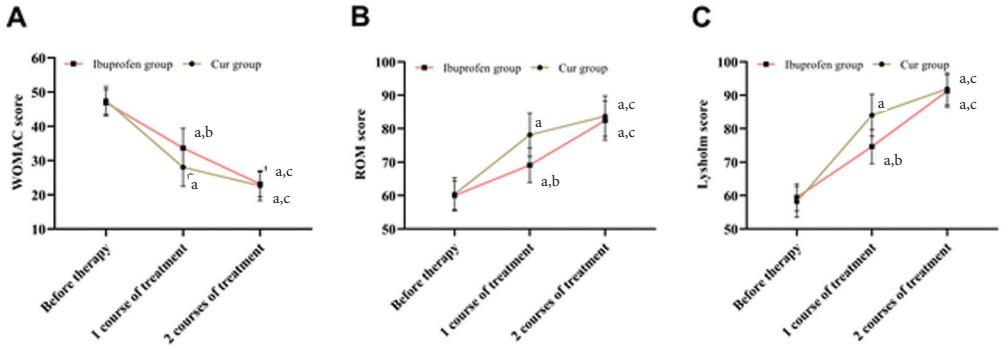
Impacts on Chondrocyte Viability and the Wnt/β-Catenin Pathway

Figure 5 shows the viability of chondrocytes after treatment with Cur. The apoptosis rate of the HC-a and C28/I2 in the Cur group was significantly lower than that in the blank group ($P < .05$), indicating that Cur can also inhibit chondrocyte apoptosis. Furthermore, the western-blot results showed that the protein levels of Wnt3a, Wnt5a, and β-catenin in the intervention group were significantly higher than those in the blank group ($P < .05$), suggesting that Cur can activate the Wnt/β-catenin pathway in chondrocytes.

Impacts of ICG-001 on Wnt/β-Catenin Pathway in Chondrocytes

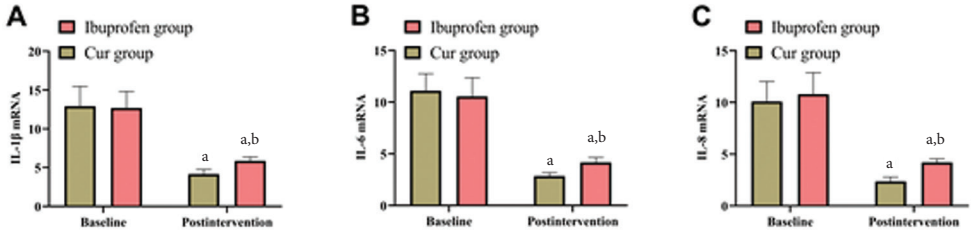
After ICG-001 intervention (Figure 6), the protein levels of Wnt3a, Wnt5a, and β-catenin in the HC-a and C28/I2 cells in the ICG-001 group were significantly lower than those in the control group ($P < .05$,), indicating the success of ICG-001 intervention.

Figure 2. Comparison of Joint Function. Figure 2A shows a comparison of the WOMAC scores; Figure 2B shows a comparison of ROMs; and Figure 2C shows a comparison of the Lysholm scores.



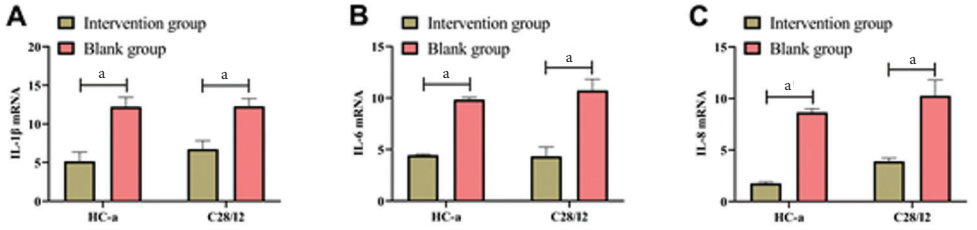
[a]$P < .05$, showing a significant difference between baseline and after one course of treatment for the Cur group as well as the ibuprofen group
[b]$P < .05$, showing a significant difference between the ibuprofen group and the Cur group after one course of treatment
[c]$P < .05$, showing a significant difference between the first course of treatment and the second course of treatment for the Cur group as well as the ibuprofen group

Figure 3. Comparison of Serum Inflammatory Factors Postintervention. Figure 3A shows a comparison of IL-1β mRNA; Figure 3B shows a comparison of IL-6 mRNA; and Figure 3C shows a comparison of IL-8 mRNA.



[a]$P < .05$, showing a significant difference between baseline and postintervention for the Cur group as well as the ibuprofen group
[b]$P < .05$, showing a significant difference between the ibuprofen group and the Cur group postintervention

Figure 4. Comparison of Inflammatory Factors. Figure 4A shows a comparison of IL-1β mRNA; Figure 4B shows a comparison of IL-6 mRNA; and Figure 4C shows a comparison of IL-8 mRNA.



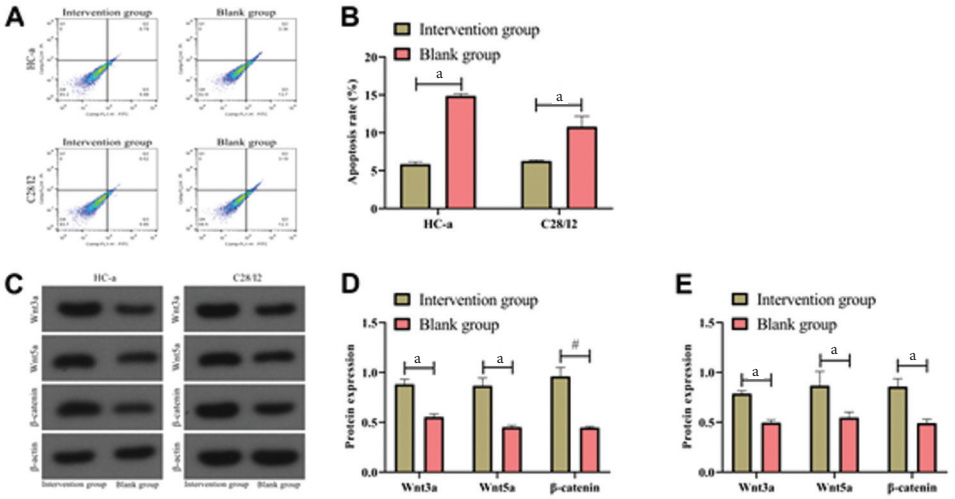
[a]$P < .05$, showing a significant difference between the ibuprofen group and the Cur group

Table 2. Comparison of the Incidence of Adverse Reactions (N = 107)

	Gastrointestinal Reaction n (%)	Upper Abdominal Pain n (%)	Dizziness n (%)	Nausea and Vomiting n (%)	Total Incidence (%)
Intervention group (n= 51)	1 (1.96)	0 (0.00)	0 (0.00)	0 (0.00)	1.96
Ibuprofen group (n= 56)	2 (3.57)	1 (1.79)	3 (5.36)	1 (1.79)	12.50
χ^2					4.286
P value					.038[a]

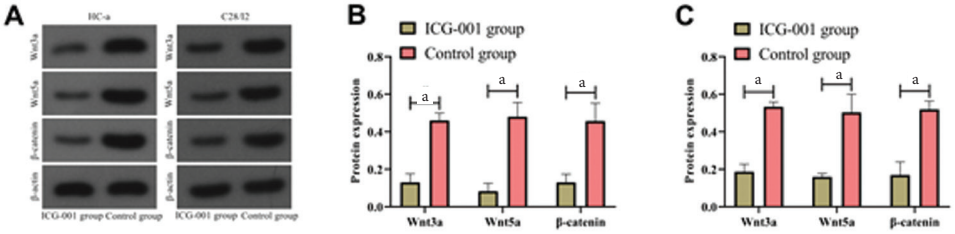
[a]$P < .05$, showing a significant difference between the intervention group and the ibuprofen group

Figure 5. Impacts of Cur on Chondrocyte Viability and Wnt/β-Catenin Pathway. Figure 5A shows flow cytometry; Figure 5B shows the apoptosis rate; Figure 5C shows a Western blot; Figure 5D shows the Wnt/β-catenin pathway protein expression in HC-a cells; and Figure 5E shows the Wnt/β-catenin pathway protein expression in C28/I2 cells.



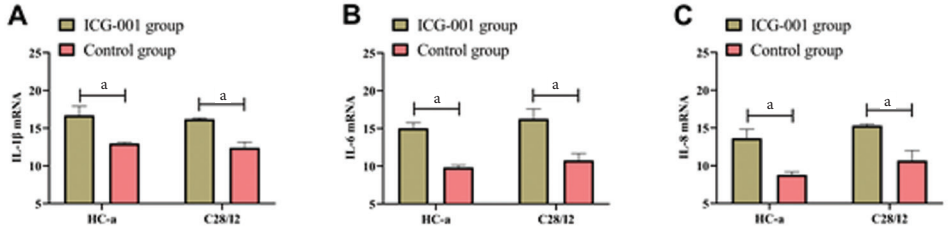
[a]$P < .05$, showing a significant difference between the ibuprofen group and the Cur group

Figure 6. Impacts of ICG-001 on Wnt/β-Catenin Pathway in Chondrocytes. Figure 6A shows a Western blot; Figure 6B shows the Wnt/β-catenin pathway protein expression in HC-a cells; and Figure 6C shows the Wnt/β-catenin pathway protein expression in C28/I2 cells.



[a]$P < .05$, showing a significant difference between the ibuprofen group and the Cur group

Figure 7. Second Comparison of Inflammatory Factors. Figure 7A shows a comparison of IL-1β mRNA; Figure 7B shows a comparison of IL-6 mRNA; and Figure 7C shows a comparison of IL-8 mRNA.

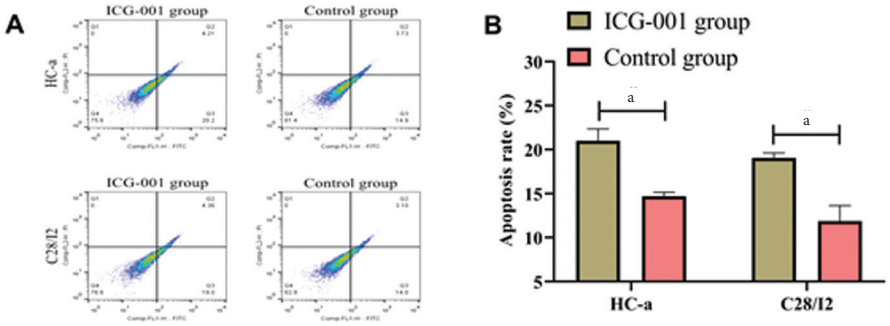


[a]$P < .05$, showing a significant difference between the ibuprofen group and the Cur group

Impacts of ICG-001 on Inflammatory Factors in Chondrocytes

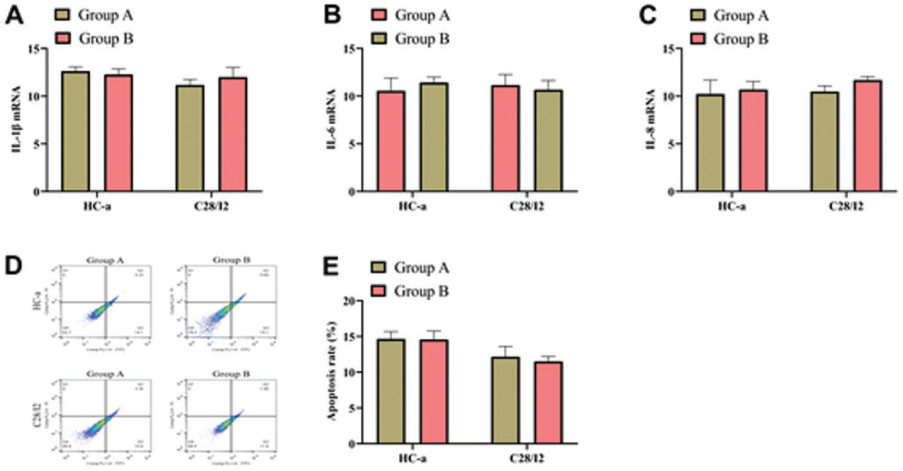
Subsequently, the levels of inflammatory factors in chondrocytes under ICG-001 intervention were measured again (Figure 7). The mRNA levels of IL-6, IL-1β, and IL-8 in HC-a and C28/I2 cells in the ICG-001 group were significantly higher than those in the control group ($P < .05$), suggesting that inhibiting the Wnt/β-catenin pathway can activate the inflammatory response in chondrocytes.

Figure 8. Impacts of ICG-001 on Chondrocyte Viability. Figure 8A show flow cytometry, and Figure 8B shows the apoptosis rate.



[a]$P < .05$, showing a significant difference between the ibuprofen group and the Cur group

Figure 9. Rescue Assay. Figure 9A shows a comparison of IL-1β mRNA; Figure 9B shows a comparison of IL-6 mRNA; Figure 9C shows a comparison of IL-8 mRNA; Figure 9D shows flow cytometry; and Figure 9E shows the apoptosis rate.



Impacts of ICG-001 on Chondrocyte Viability

Similarly, the detection of the apoptosis of chondrocytes intervened by ICG-001 (Figure 8) revealed a significantly higher cell-apoptosis rate in the HC-a and C28/I2 cells in the intervention group than in the control group ($P < .05$), suggesting that inhibition of the Wnt/β-catenin pathway can also promote the apoptosis of chondrocytes.

Rescue Assay

Figure 9 shows a rescue experiment to verify and analyze the mechanism of action of Cur. No differences existed in the mRNA levels of the inflammatory factors IL-1β, IL-6, IL-8 or in the apoptosis rate between group A treated with Cur and ICG-001 and group B treated with saline ($P > .05$). This finding indicates that the inhibition of inflammatory factors and apoptosis rate of chondrocytes induced by Cur is completely reversed after the use of ICG-001, demonstrating that Cur affects chondrocytes through the Wnt/β-catenin pathway.

DISCUSSION

The current study found that Cur can effectively improve the condition of OA and inhibit the Wnt/β-catenin pathway in chondrocytes, and it demonstrated the mechanism of action of Cur on OA for the first time, which has important reference significance for clinical practice.

Similar to Wu et al's study,[39] the current study found no significant difference in clinical efficacy and in total effective rate between Cur and ibuprofen on OA. The current study also found no significant differences in VAS pain scores postintervention after two course of treatment. These results denote that both Cur and ibuprofen are stable and effective treatments for OA.

However, after one course of therapy, the current study found lower VAS scores in the Cur group than in the ibuprofen group. Moreover, the Cur group had lower WOMAC scores and higher ROM and Lysholm scores than the ibuprofen group after one course of therapy. The decreases in inflammatory factors in the Cur group after therapy fully suggest the ability of Cur to relieve the symptoms of OA patients more quickly and to greatly shorten the therapy cycle of patients.

The current study fully confirmed the remarkable effects of Cur on OA alleviation, which fully suggests a potential for Cur to be a novel direction in OA therapy in the future. Similar to the results of prior research,[40] the Cur group in the current study presented a lower incidence of adverse reactions than the ibuprofen group, which indicates the high safety of Cur as a clinical drug.

The mechanism for Cur on OA is still under exploration, and the current study suggests a possible association of Cur with Wnt/β-catenin in OA. It found that inflammatory factors in and apoptosis of chondrocytes dropped, while Wnt/β-catenin pathway-associated proteins in the chondrocytes elevated, which indicates the ability of Cur to suppress inflammatory reaction and apoptosis of chondrocytes. In addition, similar to previous studies' results,[41] cells showed more serious inflammation and a faster apoptosis after intervention by the Wnt/β-catenin pathway inhibitor, which confirmed the same ability of Wnt/β-catenin pathway to accelerate apoptosis of chondrocytes. Finally, the current study's rescue assay revealed the ability of Wnt/β-catenin pathway inhibitor to completely offset the influences of Cur on chondrocytes, which confirmed the research team's view that Cur alleviates OA via Wnt/β-catenin.

The current study had some limitations. The research team adopted only normal chondrocytes for analysis, so a more accurate modeling analysis is required to confirm the mechanism of Cur's influences on OA in chondrocytes. In addition, the lack of a follow-up hinders the research team in evaluating the influences of Cur on the prognosis of patients with OA. The team plans to carry out improved experiments as soon as possible and to perform a comprehensive analysis on the application of Cur in OA.

CONCLUSIONS

Cur can effectively decrease the pathological results of OA, with a remarkable safety profile; its mechanism may be the activation of the Wnt/β-catenin signaling pathway to inhibit the inflammatory reaction and apoptosis in chondrocytes.

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