ISSN 1948-0210 (online)

World Journal of *Stem Cells*

World J Stem Cells 2024 November 26; 16(11): 900-989

Published by Baishideng Publishing Group Inc

World Journal of Stem Cells

Contents

Monthly Volume 16 Number 11 November 26, 2024

EDITORIAL

ORIGINAL ARTICLE

Basic Study

LETTER TO THE EDITOR

World Journal of Stem Cells

Monthly Volume 16 Number 11 November 26, 2024

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Stem Cells*, Anton Bonartsev, DSc, PhD, Associate Professor, Faculty of Biololy, M.V. Lomonosov Moscow State University, Moscow 119234, Russia. ant_bonar@mail.ru

AIMS AND SCOPE

The primary aim of *World Journal of Stem Cells* (*WJSC*, *World J Stem Cells*) is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, *etc*.

INDEXING/ABSTRACTING

The *WJSC* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Journal Citation Reports/Science Edition, PubMed, PubMed Central, Scopus, Biological Abstracts, BIOSIS Previews, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2024 Edition of Journal Citation Reports® cites the 2023 journal impact factor (JIF) for *WJSC* as 3.6; JIF without journal self cites: 3.5; 5-year JIF: 4.2; JIF Rank: 105/205 in cell biology; JIF Quartile: Q3; and 5-year JIF Quartile: Q2. The *WJSC*'s CiteScore for 2023 is 7.8 and Scopus CiteScore rank 2023: Histology is 11/62; Genetics is 78/347; Genetics (clinical) is 19/99; Molecular Biology is 131/410; Cell Biology is 104/285.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Stem Cells

ISSN
ISSN 1948-0210 (online)

LAUNCH DATE
December 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Shengwen Calvin Li, Carlo Ventura

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-0210/editorialboard.htm

PUBLICATION DATE
November 26, 2024

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2024 November 26; 16(11): 944-955

DOI: 10.4252/wjsc.v16.i11.944

ISSN 1948-0210 (online)

ORIGINAL ARTICLE

Basic Study

Efficacy of serum-free cultured human umbilical cord mesenchymal stem cells in the treatment of knee osteoarthritis in mice

Kai-Zhen Xiao, Gui Liao, Guang-Yu Huang, Yun-Long Huang, Rong-He Gu

Specialty type: Cell and tissue engineering

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade C

Novelty: Grade B, Grade B

Creativity or Innovation: Grade B, Grade B

Scientific Significance: Grade B, Grade C

P-Reviewer: Jiang YC; Redondo PC

Received: April 22, 2024

Revised: September 3, 2024

Accepted: October 18, 2024

Published online: November 26, 2024

Processing time: 217 Days and 16.1 Hours

Kai-Zhen Xiao, Gui Liao, Guang-Yu Huang, Yun-Long Huang, Rong-He Gu, Department of Orthopedic Surgery, The Fifth Affiliated Hospital of Guangxi Medical University, Nanning 530022, Guangxi Zhuang Autonomous Region, China

Kai-Zhen Xiao, Guang-Yu Huang, Yun-Long Huang, Graduate School of Guangxi Medical University, Guangxi Medical University, Nanning 530022, Guangxi Zhuang Autonomous Region, China

Rong-He Gu, Department of Guangxi Key Laboratory of Intelligent Medicine, The Fifth Affiliated Hospital of Guangxi Medical University, Nanning 530022, Guangxi Zhuang Autonomous Region, China

Co-first authors: Kai-Zhen Xiao and Gui Liao.

Corresponding author: Rong-He Gu, Doctor, Professor, Department of Orthopedic Surgery, The Fifth Affiliated Hospital of Guangxi Medical University, No. 89 Qixing Road, Qingxiu District, Nanning 530022, Guangxi Zhuang Autonomous Region, China. 201920518@sr.gxmu.edu.cn

Abstract

BACKGROUND

We investigated the efficacy of intra-articular injection of human umbilical cord mesenchymal stem cells (hUC-MSCs) for the treatment of osteoarthritis (OA) progression in the knee joint. Although many experimental studies of hUC-MSCs have been published, these studies have mainly used fetal bovine serum-containing cultures of hUC-MSCs; serum-free cultures generally avoid the shortcomings of serum-containing cultures and are not subject to ethical limitations, have a wide range of prospects for clinical application, and provide a basis or animal experimentation for clinical experiments.

AIM

To study the therapeutic effects of serum-free hUC-MSCs (N-hUCMSCs) in a mouse model of knee OA.

METHODS

Fifty-five male C57BL/6 mice were randomly divided into six groups: The blank control group, model control group, serum-containing hUC-MSCs (S-hUCMSC) group, N-hUCMSC group and hyaluronic acid (HA) group. After 9 weeks of modeling, the serum levels of interleukin (IL)-1β and IL-1 were determined.

Hematoxylin-eosin staining was used to observe the cartilage tissue, and the Mankin score was determined. Immunohistochemistry and western blotting were used to determine the expression of collagen type II, matrix metalloproteinase (MMP)-1 and MMP-13.

RESULTS

The Mankin score and serum IL-1 and IL-1β and cartilage tissue MMP-1 and MMP-13 expression were significantly greater in the experimental group than in the blank control group ($P < 0.05$). Collagen II expression in the experimental group was significantly lower than that in the blank control group ($P < 0.05$). The Mankin score and serum IL-1 and IL-1β and cartilage tissue MMP-1 and MMP-13 levels the experimental group were lower than those in the model control group ($P < 0.05$). Collagen II expression in the experimental group was significantly greater than that in the model control group ($P < 0.05$).

CONCLUSION

N-hUCMSC treatment significantly alleviate the pathological damage caused by OA. The treatment effects of the S-hUCMSC group and HA group were similar.

Key Words: Osteoarthritis; Serum-free culture; Mesenchymal stem cell; Human umbilical cord mesenchymal stem cells; Serum-free culture of human umbilical cord mesenchymal stem cells; Hyaluronic acid

Core Tip: Mesenchymal stem cells have been shown to alleviate osteoarthritic cartilage and could therefore be used to treat osteoarthritis to reduce or delay the need for knee replacement therapy. This study provides a favorable foundation for the future application of serum-free cultured human umbilical cord mesenchymal stem cells in clinical trials for treating knee osteoarthritis.

Citation: Xiao KZ, Liao G, Huang GY, Huang YL, Gu RH. Efficacy of serum-free cultured human umbilical cord mesenchymal stem cells in the treatment of knee osteoarthritis in mice. *World J Stem Cells* 2024; 16(11): 944-955
URL: https://www.wjgnet.com/1948-0210/full/v16/i11/944.htm
DOI: https://dx.doi.org/10.4252/wjsc.v16.i11.944

INTRODUCTION

Osteoarthritis (OA) is a degenerative disease of the joints that severely affects the lives of patients. More than 240 million patients worldwide have symptoms of limited mobility, and approximately 30% of people over 45 years of age have imaging evidence of knee OA, approximately half of whom have knee symptoms. This condition results in a major economic burden on patients, families and society, and its main symptoms are joint pain and dysfunction, which can even lead to disability. OA is treated with exercise therapy, physical therapy, medication and joint replacement surgery. The current conventional treatment for OA is aimed mainly at relieving pain, alleviating symptoms of stiffness, correcting deformities, and correcting and restoring joint function, thus improving the quality of life of patients[1]. However, these standard treatment options can only relieve pain and improve functioning. The only cells that maintain homeostasis of the extracellular matrix in the articular cartilage are chondrocytes, and the stability of the articular cartilage depends on the chondrocytes. Thus, chondrocyte function determines articular cartilage damage, and chondrocyte apoptosis is one of the etiological factors in degenerative joint changes[2]. These findings indicate that repairing articular cartilage in patients with knee OA is highly important for treating and controlling the progression of OA, and will likely constitute a new therapeutic direction; however, there are no effective drugs for repairing articular cartilage. Mesenchymal stem cells (MSCs) are multipotent stem cells, that show directed homing to sites of injury, can differentiate into different cell types, can secrete a various cytokines involved in the immune response, and have immunosuppressive properties[3,4]. These cells are widely used in clinical applications, such as for cartilage repair, bone defect disease[5,6], cancer[7], kidney disease[8,9], diabetes[10], and cirrhosis[11] of the liver. Human umbilical cord MSCs (hUCMSCs) are extracted from the umbilical cord, are waste products of fetal birth, do not have ethical or moral issue, are convenient, have abundant sources and are easy to collect. These cells show a low prevalence of transplant-associated viruses and pathogenic microorganisms, and no tumor cell contamination, have a high proliferative capacity and good biostability, and show low immunogenicity or weak immune rejection of allografts[12-15]. These are the only safe allogeneic steam cell among various multifunctional stem cells[16].

Many studies have shown that injecting hUCMSCs to treat OA can significantly improve pain and function, with good therapeutic effects[14,17,18]. Many models of spontaneous OA exist (*e.g.*, mice, guinea pigs, and dogs)[19-22]. However, surgical models can offer many advantages, such as reduced variability, reduced dependence on genotype, and faster onset of disease, which often shortens the study time of experiments and thus reduces feeding costs[23]. Serum-free cultures have advantages over traditional fetal bovine serum cultures in that serum-free cultures avoid the effects of the

presence of different species, reduce the risk of contamination with infectious agents and are not ethically restricted. Serum-free culture allows good amplification, multipotency and normal genotype maintenance of hUCMSCs, maintaining the pluripotency and surface antigen profile used to define human MSCs[24,25]. Therefore, the main purpose of this study was to verify the effectiveness of serum-free cultured hUCMSCs (N-hUCMSCs) in treating OA in mice, and to compare the ability of intra-articular injections of serum-cultured hUCMSCs (S-hUCMSCs), N-hUCMSCs, and hyaluronic acid (HA) to treat an artificially generated mouse model of OA. For these aim, repeated dose MSC therapy is superior single-dose MSC therapy and is safer[18,26]. Biochemical, histological, and microstructural analyses of articular cartilage and subchondral bone were performed after repeat injections, as were *in vivo* tracking of inflammatory factors. In addition, we evaluated whether N-hUCMSCs, S-hUCMSCs, and HA were equipotent for the treatment of knee OA in mice.

MATERIALS AND METHODS

Ethics statement

The animal ethics review followed the Guiding Opinions on the Treatment of Laboratory Animals issued by the Ministry of Science and Technology of the People's Republic of China and the Laboratory Animal Guidelines for Ethical Review of Animal Welfare issued by the National Standard GB/T35892-2018 of the People's Republic of China.

Animal handling

The research protocol was reviewed by the Animal Ethics Committee of Guangxi Medical University, and the content and process of this research followed the ethical requirements for biomedical research promulgated by international and national organizations. A total of 55 male C75BL/6 mice were used in this study. C75BL/6 mice were randomly grouped into the S-hUCMSC group (n = 10), N-hUCMSC group (n = 11), HA group (n = 7), model control group (n = 12) and blank control group (n = 15). The body weights of the mice [purchased from Spivey (Beijing) Biotechnology Co., Ltd. weight 20 ± 2 g for 6-8 weeks] were measured after purchase, and the mice were housed in ventilated groups (4 mice per cage) with a 12-hour light/dark cycle at the Guangxi Academy of Sciences Animal Breeding Room, with water and food provided ad libitum. The animals were allowed to adapt for one week, after which they were observed daily, and whether their feeding and drinking conditions and mental condition were normal was recorded. If mice with abnormal indicators were found, the animals were immediately eliminated and replaced.

Construction of a mouse model of knee OA

Except for those in the blank control group, the animals in all groups underwent anterior cruciate ligament (ACL) disarticulation surgery. The animals were placed in the induction anesthesia box. After anesthesia, both upper limbs of the animals were fixed, the nose of the animals were placed into respiratory anesthesia masks, a longitudinal incision of approximately 2.0 cm was made in the knee joint, the patellofemoral ligament was cut to approximately 1 cm to turn out the patella to expose the knee joint, the inferior patellar crease wall connected with the intercondylar fossa was clipped (the operation could be completed to visualize the ACL), and the ACL was cut with microscopic clippers. Then, wound debridement and suturing were performed[27]. After the operation, drugs were given to promote wound healing. The animals in the blank control group did not undergo ACL dissection, and the other treatments were the same as those used in the model group. After 9 weeks of modeling, the sera of all animals were monitored for interleukin (IL)-6 and IL-1β, and the cartilage tissues of the knee joints of 2 animals each in the blank control group and the model control group were collected bilaterally and placed in tissue fixative for hematoxylin-eosin (HE) staining. After verification of the success of the model, the animals in each group were dissected and sampled, and the whole femur and tibia of the left knee joint were placed in 4% formaldehyde for fixation and stored in a refrigerator at 4 °C; the cartilage tissues of the right knee joint and serum were separated and stored at -80 °C.

MSC harvest

The stem cells used in this study were reviewed by the Ethics Committee of Nanning First People's Hospital. P3 generation hUCMSCs were purchased from Guangxi Huaren & Sai Biotechnology Co., Ltd., divided into serum (10% fetal bovine serum) culture and, serum-free culture groups, and passaged to P5 (Figure 1). hUCMSCs from the 5th generation were used for all experiments.

Injection of hUC-MSCs into C75BL/6 knee joints

The experiment was conducted in the laboratory of Guangxi Academy of Sciences. C75BL/6 mice were randomly grouped and 1 × 10^7 S-hUCMSCs, 1 × 10^7 N-hUCMSCs, phosphate buffered saline (PBS) (blank control group and model control group), and HA were injected *via* a syringe into the posterior (knee) joints of the mice with knee OA. In addition, the animals in the blank control group were injected with PBS, and all the treatments were consistent with the model group. All injections were given initially 2 weeks after the mouse model was established and again 2 and 4 weeks thereafter. Each experiment was conducted in the morning. All the animals were housed in groups in miniature isolation cages with free movement. Three weeks after the last injection, model validation was carried out with 2 animals each in the blank control group and the model control group. Knee joint cartilage tissue sections were taken for HE staining, and sera from all the animals were tested for IL-6, and IL-1β. Uneven chondrocyte thickness, disorganized cartilage structure, and increased clefts were more pronounced in the model control group than in the blank control group, and the levels of

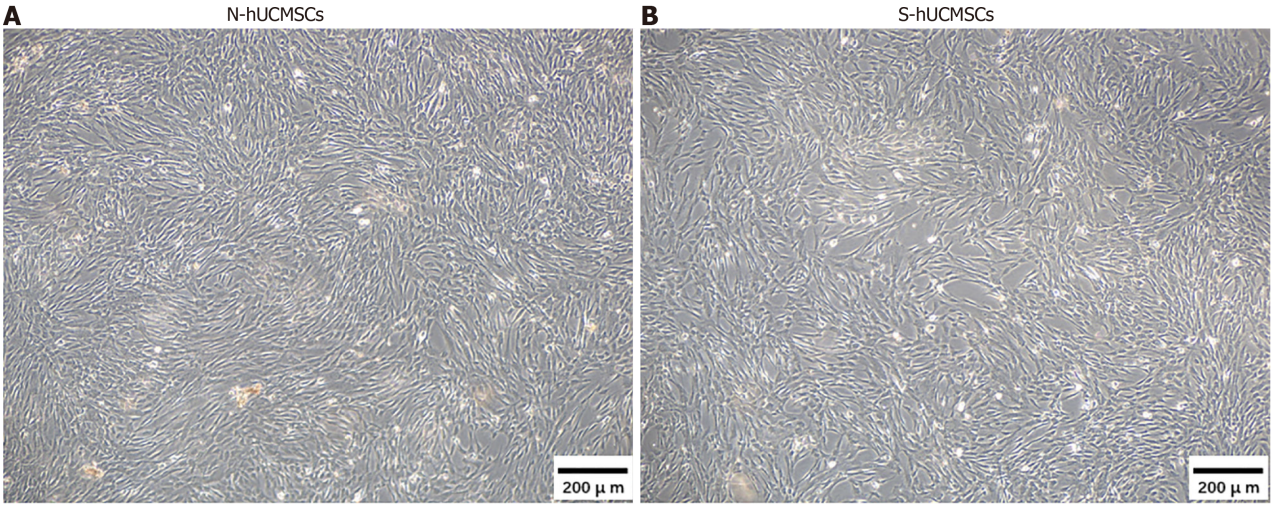

Figure 1 Collection of human umbilical cord mesenchymal stem. A: Serum-free human umbilical cord mesenchymal stem, scale bar = 200 μm; B: Serum-cultured human umbilical cord mesenchymal stem cells, scale bar = 200 μm. N-hUCMSCs: Serum-free human umbilical cord mesenchymal stems; S-hUCMSCs: Serum-cultured human umbilical cord mesenchymal stem cells.

the inflammatory factors IL-6 and IL-1β were significantly elevated by 2-3-fold in the model group, indicating that the modeling was successful. Mouse bilateral knee radiographs were obtained after model validation. Immediately after humane execution, the blood and knee joints of both lower limbs of the mice were harvested. The cartilage tissue of the right knee joint was labeled, quickly frozen at -80 °C in a freezer and prepared for multiple analyses (Table 1). Throughout the entire research process, only the designer was informed about the experimental groups; all the other authors were unaware.

Enzyme-linked immunosorbent assay of serum

Enzyme-linked immunosorbent assay (ELISA) kits were purchased from ELISA Bio, (lot numbers YJ063159 and YJ301814). Blood was taken from the orbital vein and immediately centrifuged at 4 °C at 3000 rpm/minute for 10 minutes to obtain the serum. The ELISA was performed in an orderly manner according to the instructions of the kit.

C75BL/6 knee radiographs

After the animal, was anesthetized, it was fixed on the fixation plate and placed on the X-ray photography instrument table. The exposure parameters were adjusted, and the photo button was pressed to generate the X-ray image (the parameters were not debugged during the photo session and the position of the fixing point on the fixing plate was not moved to ensure that the animal's legs were in the same position).

HE staining

After fixation, decalcification, and dehydration, the knee cartilage tissue was transversely sectioned for routine paraffin embedding and sectioning. The sections were deparaffinized in xylene. The sections were rehydrated with ethanol, stained with hematoxylin stain, washed with distilled water to remove floating color, differentiated with differentiation solution, stained with eosin, sealed with neutral gum, and observed under a microscope.

Immunohistochemical assessment

Cartilage tissue sections were immunohistochemically stained for collagen II, matrix metalloproteinase (MMP)-1 and MMP-13, paraffin sections were deparaffinized in xylene, and rehydrated in ethanol, and an immunohistochemistry pen was used to draw a circle around the sample to prevent diffusion of the reagents. Citric acid restorative solution was added, and the sections were naturally cooled and then rinsed with PBS, and 3% hydrogen peroxide solution, repeatedly. After incubation with goat serum sealing solution, primary antibody and secondary antibody, DBA was used for color development, hematoxylin was used for restaining, alcohol was used for dehydration, neutral gum was used for sealing, and photographs were taken under the microscope.

Protein content of knee cartilage tissue

Protein extract preparation: According to the number of samples, the required volume of bone tissue protein extract, was prepared, and 2 μL of protease inhibitor and 2 μL of protein stabilizer were added to every 500 μL of cold bone tissue protein extract A, which was mixed well and prepared for use. After rinsing, grinding, and lysis, centrifugation and collection were performed to obtain the total protein of the bone tissue, the concentration of the protein was determined, denaturation treatment was performed, and the samples were stored at -80 °C. The protein content was determined with a BCA kit, purchased from Biyuntian Biotechnology, Lot No. P00125; the protein content of each sample was determined with an enzyme labeling instrument after strictly following the operating procedures of the kit.

Table 1 Weight of cartilage in the right knee joint of each mouse (the weighing time point is before extracting protein from knee cartilage)

Serial number	Animal number	Weights (g)	Serial number	Animal number	Weights (g)
1	2	0.019	18	D46	0.045
2	10	0.046	19	B92	0.078
3	98	0.027	20	D37	0.051
4	3	0.020	21	D28	0.032
5	8	0.048	22	D99	0.028
6	33	0.027	23	D33	0.039
7	34	0.067	24	B89	0.029
8	57	0.029	25	B97	0.036
9	17	0.048	26	B98	0.037
10	28	0.083	27	B96	0.054
11	22	0.027	28	B94	0.033
12	16	0.024	29	D36	0.033
13	6	0.030	30	D6	0.035
14	26	0.043	31	D31	0.043
15	36	0.037	32	D38	0.058
16	96	0.037	33	B95	0.034
17	D89	0.027	34	B93	0.045

Western blot analysis

A 10% Bis-Tris precast gel was placed in the electrophoresis tank, 20 μL of sample from each group was added, and electrophoresis was performed at 150 V constant voltage. The protein gel was removed, and the polyvinylidene fluoride (PVDF) membrane was activated with methanol. The transfer module was assembled according to the negative pole, sponge, filter paper, protein gel, PVDF membrane, filter paper, sponge and positive pole, and the membrane was transferred at 20 V constant voltage. The PVDF membrane was removed, and collagen II, MMP-13, MMP-1 and β-actin were separated separately. The membranes were incubated with blocking solution in a shaker, and the membranes were incubated with primary antibody, secondary antibody, and ECL developer solution and photographed with a gel imaging system.

Statistical analysis

The experimental data were statistically analyzed with SPSS 24.0 software and are presented as the mean ± SD. The experimental comparison of more than three groups was performed by one-way ANOVA for multiple comparisons, the analysis of the two groups was performed by an unpaired *t* test, and differences were defined as statistically significant at ($P < 0.05$). GraphPad Prism 9 software was used to draw bar graphs.

RESULTS

Clinical observations after modeling and drug injection

All the mice tolerated the knee arthritis model procedure, and no abnormal behavior was observed in any of the animals. Weight gain (Figure 2) was associated with no significant change in appetite during the feeding period. During the first phase of the experiment, 3 animals in the S-hUCMSC group died, 3 died in the N-hUCMSC group died, and 1 animal in the HA group died after knee injection. During the second phase of the experiment, no animals in the S-hUCMSC group died and 2 in the N-hUCMSC group died.

Throughout the experimental process, no animal death was observed in the blank control group and the model control group after injection into the knee joint cavity. In the cell injection group, the animal mortality rate was higher, and the deaths may be due to the injection of xenogeneic cells, which triggered immune stress. In addition, due to cellular injection into the knee joint cavity, hUCMSC and HA formation in response to the larger stimulus led to animal death.

Reduced IL-6 and IL-1β expression in the experimental group

The serum of the mice was tested for inflammatory factors (IL-6 and IL-1β) before modeling, and the baseline levels of the

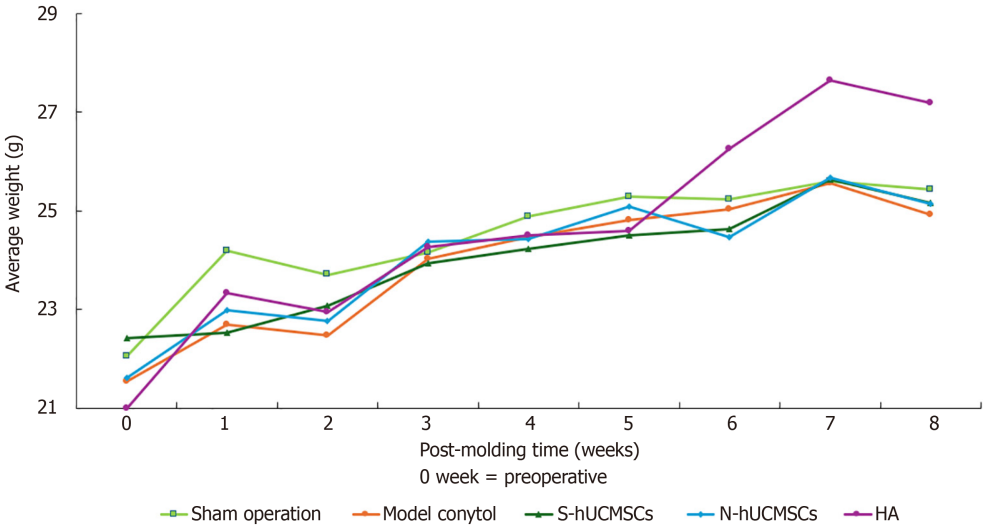

Figure 2 Average body weight of mice in each group. The weight of each group of mice showed an upward trend after surgical modeling. N-hUCMSCs: Serum-free human umbilical cord mesenchymal stems; S-hUCMSCs: Serum-cultured human umbilical cord mesenchymal stem cells; HA: Hyaluronic acid.

inflammatory factors, IL-6 and IL-1β, did not differ significantly between the groups, indicating that the levels of inflammatory factors were consistent among the animals in each group before modeling (Figure 3A and B). Serum samples were collected at week 6 after modeling, and inflammatory factors were assessed. The levels of the inflammatory cytokines IL-6 and IL-1β in the model group were significantly greater than those in the blank control group ($P < 0.05$) (Figure 3C and D). These findings indicate that the model was successfully prepared.

At the end of the experiment (9 weeks after modeling), serum was collected from the animals in each group to assess inflammatory factors. IL-6 and IL-1β levels were significantly greater in the model group than in the blank control group ($P < 0.05$). The levels of the inflammatory cytokines IL-6 and IL-1β levels were significantly lower in the experimental group than in the model control group ($P < 0.05$) (Figure 3E and F). There was no significant difference between the experimental groups.

The experimental groups showed promotion of cartilage tissue repair

At the 6th week after modeling, the cartilage tissues of two knees in the blank control group and two knees in the model control group were dissected and subjected to HE staining. Compared with those in the blank control group, the knee joints in the model control group were deformed, the articular cartilage was damaged, the synovial tissue of the articular cavity showed strong proliferation, the cartilage layer was thickened, and the articular cavity was narrowed. The model control group had a rougher and smoother cartilage surface, thickened cartilage layer, narrowed bone trabeculae, and increased number of osteoclasts in the bone marrow cavity, indicating that the model was successfully established (Figure 4A and B).

The HE staining results at the experimental endpoint (9 weeks after modeling) revealed that, in the blank control group, the cartilage surface was smooth, the chondrocytes were uniformly arranged, the tissue structure was clear, the matrix staining was uniform, and the tide line was complete and clear. The surface of the cartilage in the model group was severely damaged, the synovial tissue had proliferated, the chondrocytes were substantially reduced, the cartilage layer was thinned, the cartilage structure was disorganized, fissures were observed in many places, the matrix staining was heavily reduced, and the tidal line was incomplete. The HE staining results were similar in the experimental groups, showing a smoother cartilage surface, a small decrease in chondrocytes, a slight decrease in matrix staining, and a more complete tidemark line (Figure 4C). The Mankin scores were significantly higher in the model control group than in the blank control group ($P < 0.05$) (Figure 4D). The Mankin scores were significantly lower in the experimental group than in the model control group ($P < 0.05$). The above results indicated that N-hUCMSCs, S-hUCMSCs and HA had therapeutic effects on OA in mice.

The experimental group of mice exhibited reduced formation of osteoids in the knee joint

Before the animals, were dissected, they were photographed *via* X-ray to observe the status of the knee joints, and the blank control group had normal knee joint gaps, smooth joint surfaces, and no peripheral osteophytes. The knee joints of the mice in the model group were narrower than those in the blank group in terms of the interstitial space and had a high density of subchondral shadows, irregular and indistinct borders of the articular surfaces of the joints, and periarticular bony hyperplasia. Compared with the model control group, the experimental group showed varying degrees of improvement in various manifestations, including wider knee spaces and clearer articular cartilage boundaries (Figure 5). The above results indicated that the N-hUCMSC, the S-hUCMSC and the HA treatments had therapeutic effects on OA in mice.

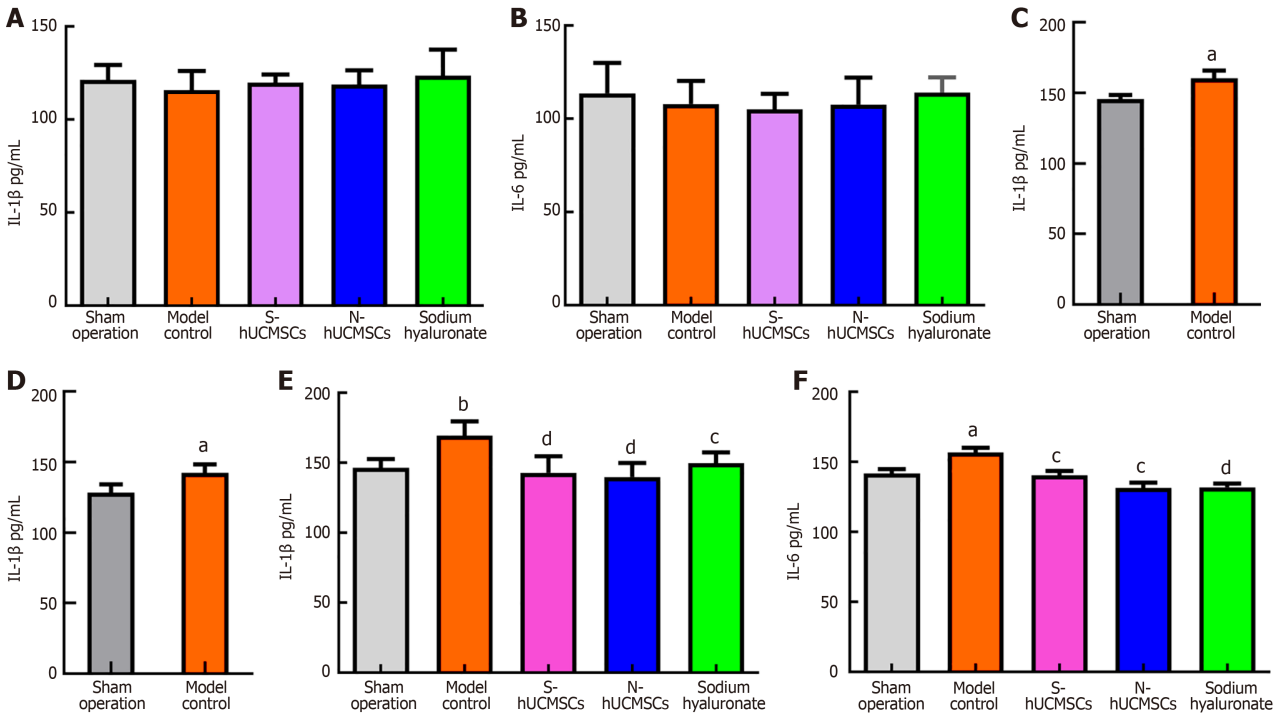

Figure 3 Reduced interleukin-6 and interleukin-1β expression in the experimental group. A: Baseline analysis: There was no statistically significant difference in the initial levels of interleukin (IL)-1β among the groups of animals; B: Baseline analysis: There was no statistically significant difference in the initial levels of IL-6 among the groups of animals; C: Model validation: The level of IL-1β in the model control group was significantly higher than that in the sham surgery group (blank control group); D: Model validation: The level of IL-6 in the model control group was significantly higher than that in the sham surgery group (blank control group); E: Experimental endpoints: The level of IL-1β in the experimental group was significantly higher than that in the model control group; F: Experimental endpoints: The level of IL-6 in the experimental group was significantly higher than that in the model control group. [a]$P < 0.05$, comparison with the sham operated group; [b]$P < 0.01$, comparison with the sham operated group; [c]$P < 0.05$, comparison with the model control group; [d]$P < 0.01$, comparison with the model control group. N-hUCMSCs: Serum-free human umbilical cord mesenchymal stems; S-hUCMSCs: Serum-cultured human umbilical cord mesenchymal stem cells; IL: Interleukin.

Immunohistochemical assessment

Immunohistochemical analysis of collagen II, MMP-1 and MMP-13 protein expression in each group revealed that collagen II staining was the strongest in the blank control group, and the weakest in the model control group. Collagen II expression was greater in the experimental group than in the model control group, and there was no difference between the experimental groups. MMP-1 and MMP-13 staining was the weakest in the blank control group and the strongest in the model control group, and there was no difference between the experimental groups (Figure 6). The above results indicated that N-hUCMSCs, S-hUCMSCs and HA had therapeutic effects on OA model mice.

Western blot

Collagen II expression was lowest in the model control group and highest in the blank control group, and the difference was statistically significant ($P < 0.05$) when the model control, blank control and experimental group subgroups were compared. There was no difference between the experimental groups. MMP-1 and MMP-13 expression was significantly greater in the model control group than in the blank control group ($P < 0.05$). The expression of MMP-1 and MMP-13 in the experimental group was significantly lower than that in the model group ($P < 0.05$). There was no difference between the experimental groups. In summary, N-hUCMSCs, S-hUCMSCs and HA have therapeutic effects on OA model mice (Figure 7).

DISCUSSION

OA is a degenerative disease of the joints that severely affects the lives of patients, and it is estimated that more than 240 million people worldwide suffer from symptomatic activity-limiting OA. With the increasing proportion of elderly and obese individuals, the incidence of OA is increasing annually[28,29]. OA is primarily a pathological change in the articular cartilage. Chondrocytes are the only cells in articular cartilage, that are needed to maintain the balance of the extracellular matrix, and the stability of the articular cartilage depends on chondrocytes. Owing to the absence of vascular structure in articular cartilage tissues, the weak regenerative ability of chondrocytes, and the lack of knowledge of the specific pathogenesis of OA, standard therapeutic options can alleviate pain and improve function, and there is currently no effective medication for the prevention and treatment of OA[28,30].

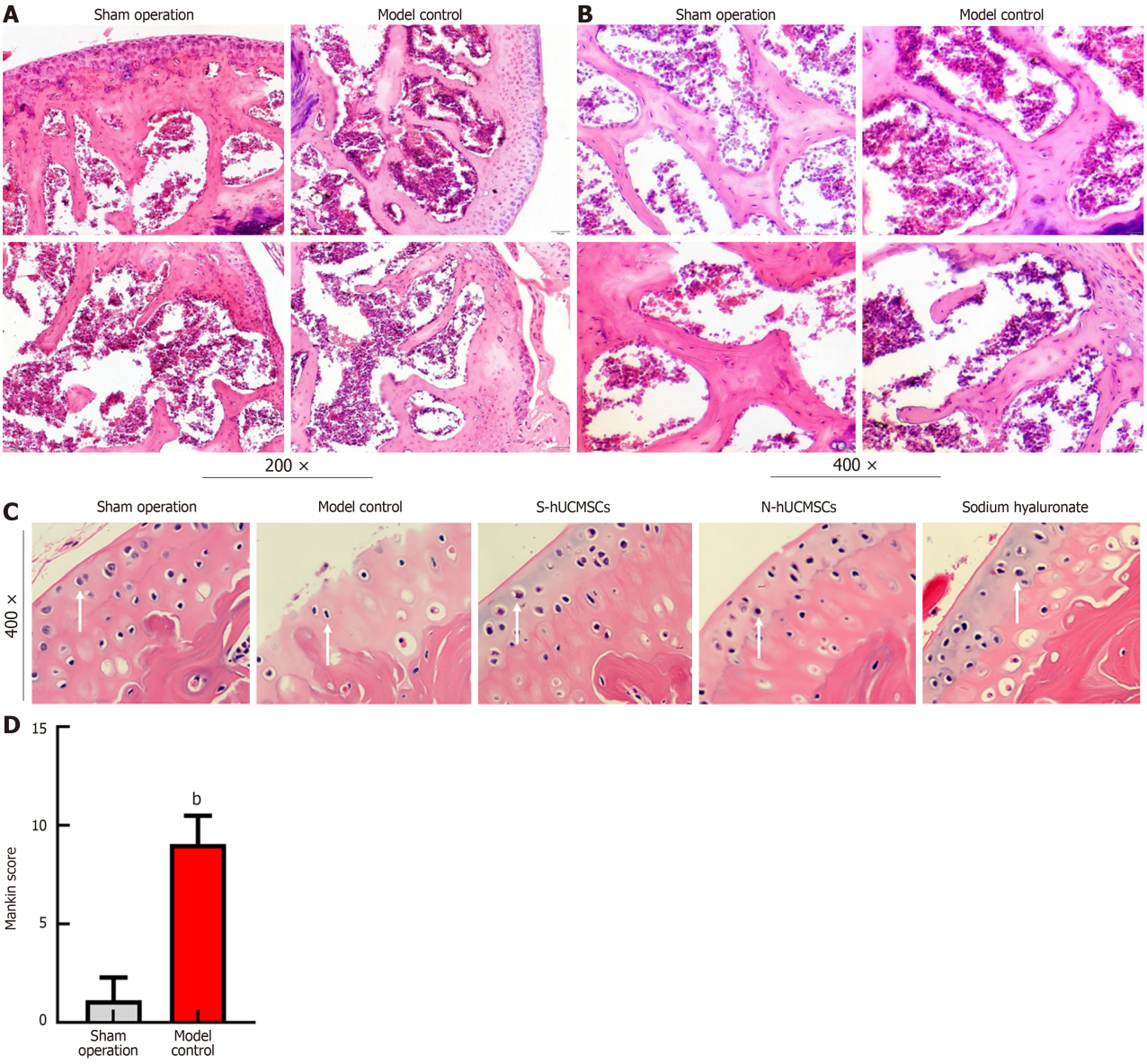

Figure 4 The experimental group favored the repair of cartilage tissue. A and B: Model validation: In the control group, the integrity of the articular cartilage was disrupted, the cartilage surface became rough and uneven, the synovial tissue in the joint cavity proliferated significantly, and the number of osteoclasts in the bone marrow cavity increased; C: Experimental endpoints: The number of chondrocytes in the treatment group was significantly increased compared to the model control group; D: Experimental endpoints: The Mankin score of the model control group was significantly higher than that of the sham surgery group (blank control group). $^{b}P < 0.01$, comparison with the sham-operated group. N-hUCMSCs: Serum-free human umbilical cord mesenchymal stems; S-hUCMSCs: Serum-cultured human umbilical cord mesenchymal stem cells.

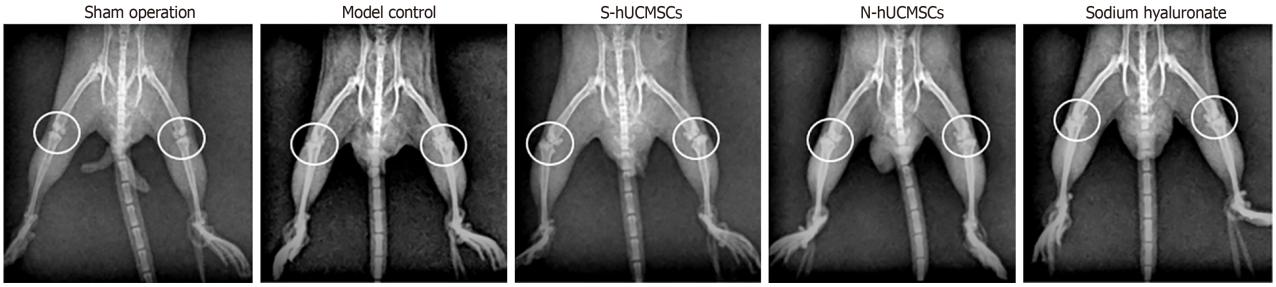

Figure 5 X-ray. Radiograph of the knee joint before the anatomical experiment. The experimental group can mice with reduced formation of osteoid in the knee joint. N-hUCMSCs: Serum-free human umbilical cord mesenchymal stems; S-hUCMSCs: Serum-cultured human umbilical cord mesenchymal stem cells.

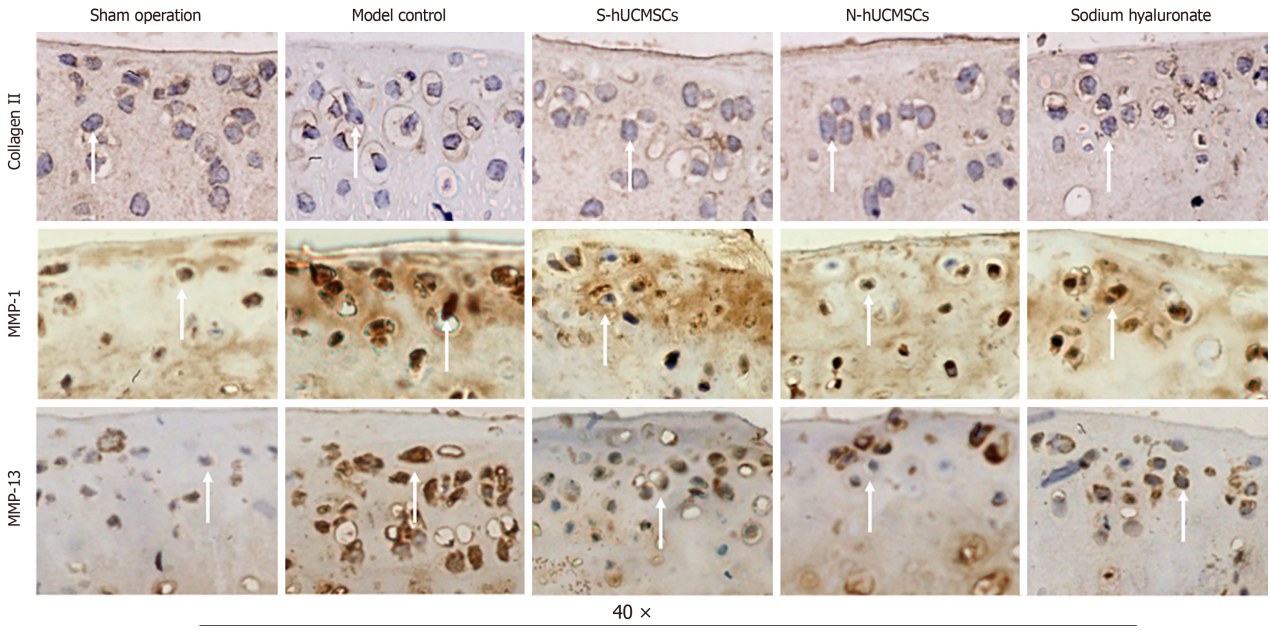

Figure 6 Histological images of cartilage tissue of knee joints of each group. The collagen II staining was strongest in the sham surgery group, weakest in the model control group, weakest in matrix metalloproteinase-1 and matrix metalloproteinase-13 staining in the sham surgery group (blank control group), and strongest in the model control group. MMP: Matrix metalloproteinase; N-hUCMSCs: Serum-free human umbilical cord mesenchymal stems; S-hUCMSCs: Serum-cultured human umbilical cord mesenchymal stem cells.

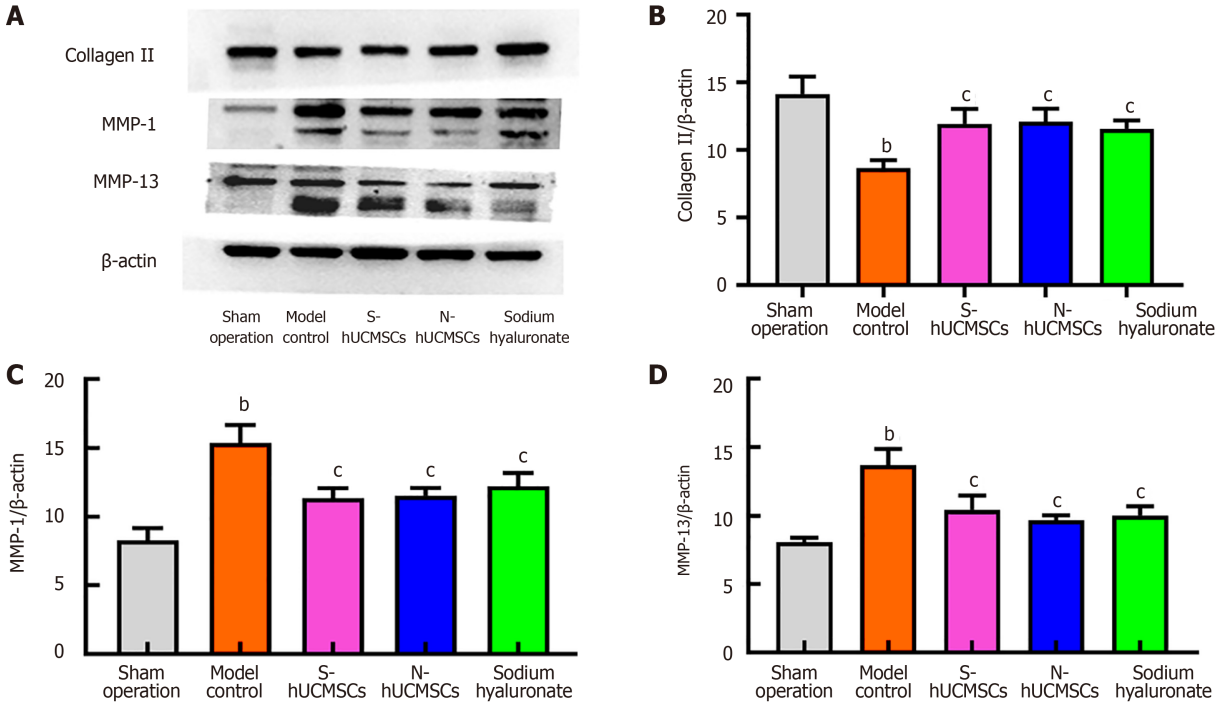

Figure 7 Western bolt. A: Western bolt original picture; B: The model control group had the lowest expression level of collagen II, while the blank control group had the highest expression level; C: The model control group had the highest expression level of matrix metalloproteinase-1, while the blank control group had the lowest expression level; D: The model control group had the highest expression level of matrix metalloproteinase-13, while the blank control group had the lowest expression level. $^{b}P < 0.01$, comparison with the sham-operated group; $^{c}P < 0.05$, comparison with the modeled control. MMP: Matrix metalloproteinase; N-hUCMSCs: Serum-free human umbilical cord mesenchymal stems; S-hUCMSCs: Serum-cultured human umbilical cord mesenchymal stem cells.

Inflammatory factors are key components of most inflammatory processes. Therefore, multiple inflammatory factors are associated with the pathogenesis of OA. In patients with OA, cartilage matrix homeostasis is disrupted by proinflammatory cytokines and chemokines[31,32]. Studies on the cytokines and chemokines involved in the progression of OA have shown that IL-1, IL-6, and IL-8 are upregulated[33-35]. IL-6 is produced mainly by osteoblasts in the subchondral bone, as well as adipocytes in osteoarthritic osteophytes and infrapatellar fat pads under mechanical loading[36-38]. Stimulated chondrocytes produce IL-1. IL-1 and IL-6 can stimulate synovial cell proliferation and osteoclast activation,

thereby inducing the production of MMPs. MMPs are a zinc-dependent protein hydrolase family that can degrade extracellular matrix proteins. MMPs can be divided into MMP-1, MMP-8 and MMP-13. Their function is to degrade collagen and facilitate the decomposition of joint cartilage. These MMPs, especially MMP-13, are closely related to damage to the extracellular matrix of osteoarthritic joint cartilage. One of the substrates of MMP-13 is type II collagen, which is one of the most abundant structural components in joint cartilage[39]. Moreover, MMP-13 can activate various matrix MMPs, including MMP-9 and MMP-1, which are involved in the complete pathological process of OA[40]. Compared with that in wild-type mice, the degree of articular cartilage degradation in *MMP-13* gene knockout mice is significantly lower[39]. Consistent with these findings, *MMP-13* gene knockout in mice prolonged the time to end-stage OA in the early and middle stages (8 weeks, 12 weeks, and 16 weeks after meniscus injury, after surgery)[41]. In this study, knee OA was induced in mice through artificial modeling, and the therapeutic effect of knee joint injection of N-hUCMSCs on OA was verified by determining the expression of IL-1β, IL-6, MMP-1, and MMP-13. hUCMSCs have been proven to treat and alleviate OA, but stem cell therapy has safety and ethical issues, and most of the studies utilized fetal bovine serum cultures, which can result in adverse immune rejection caused by xenogeneic cells. Therefore, this study aimed to identify specific N-hUCMSCs to assess their potential for treating OA.

The only difference in the primary control in this experiment was the different compositions of the drugs injected into the knee joint cavities of the mice with of OA. The therapeutic effects of intra-articular administration of S-hUCMSCs, N-hUCMSCs and HA were demonstrated in this study. S-hUCMSCs, N-hUCMSCs and HA reduced the levels of the inflammatory from IL-6, and IL-1β ($P < 0.05$). Western blot and immunohistochemical analyses revealed increased collagen II expression and decreased MMP-1 and MMP-13 protein expression ($P < 0.05$). X-ray imaging and HE staining verified the alleviation of the progression of knee OA in mice ($P < 0.05$). HA is used as a clinical first-line agent in the conventional nonsurgical treatment of OA, and no differences were detected among the S-hUCMSC, N-hUCMSC and HA groups in this study. Therefore, the present study verified that N-hUCMSCs could effectively treat OA and were equipotent with conventional treatments.

Another finding of this study was that there were no animal deaths in either the blank control group or the model control group after knee joint cavity injection throughout the experiment, that animal mortality was greater in the experimental group, and that animals died after all three injections. In this experiment, the first phase of knee injections was performed without anesthesia, the second phase of knee injections was performed under anesthesia, and the mortality rate of the animals in the experimental group was substantially reduced; however, there were no animal deaths in the control group, either in the first phase or the second phase. Accordingly, the knee joints of the C75BL/6 mice injected with S-hUCMSCs, N-hUCMSCs or HA were overstressed, leading to the death of the animals; this process could be carried out under anesthesia to reduce stress, and the concentration of the cells in the knee joint cavity of the C75BL/6 mice and the dose have yet to be investigated. Ao *et al*[18] and Wei *et al*[42] reported that low concentrations and multiple injections increased safety and reduced adverse events while ensuring efficacy. As with any study, this report has some limitations. First, the number of mice studied in this study was small, and increasing the sample size could increase the reliability of the study. Second, multiple animal deaths occurred in this study, and more adequate pretesting should have been performed before the experiment began. Finally, to mitigate any uncertainties potentially caused by N-hUCMSCs, future preclinical studies should have an expanded study sample size and utilize primate animal experiments.

CONCLUSION

N-hUCMSCs had therapeutic effects on mice with OA, and the therapeutic efficacy of N-hUCMSCs was not significantly different from that of S-hUCMSCs or HA.

ACKNOWLEDGEMENTS

We would like to thank all the professionals who contributed to the discussion and elaboration of this minireview.

FOOTNOTES

Author contributions: Xiao KZ and Gu RH designed the study; Xiao KZ, Liao G, Huang GY, and Huang YL performed the experiments, acquired and analyzed data; Xiao KZ and Liao G interpreted the data; Xiao KZ, Liao G, and Gu RH wrote the manuscript; Xiao KZ and Liao G contributed equally to this work; all authors approved the final version of the article.

Supported by the Cultivated by Outstanding Young Scientific and Technological Innovation and Entrepreneurship Talents in Nanning City, China, No. RC20210107; and Self-Funded Research Project by Guangxi Health Commission, China, No. Z20191090.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Animal Ethics Committee of Guangxi Medical University (IACUC protocol number: Protocol No. 201907878).

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country of origin: China

ORCID number: Kai-Zhen Xiao 0009-0003-1368-6427; Rong-He Gu 0009-0008-6359-5706.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Yu HG

REFERENCES

1 **McAlindon TE**, Bannuru RR, Sullivan MC, Arden NK, Berenbaum F, Bierma-Zeinstra SM, Hawker GA, Henrotin Y, Hunter DJ, Kawaguchi H, Kwoh K, Lohmander S, Rannou F, Roos EM, Underwood M. OARSI guidelines for the non-surgical management of knee osteoarthritis. *Osteoarthritis Cartilage* 2014; **22**: 363-388 [PMID: 24462672 DOI: 10.1016/j.joca.2014.01.003]

2 **Chen D**, Shen J, Zhao W, Wang T, Han L, Hamilton JL, Im HJ. Osteoarthritis: toward a comprehensive understanding of pathological mechanism. *Bone Res* 2017; **5**: 16044 [PMID: 28149655 DOI: 10.1038/boneres.2016.44]

3 **Salinas Tejedor L**, Skripuletz T, Stangel M, Gudi V. Mesenchymal stem cells require the peripheral immune system for immunomodulating effects in animal models of multiple sclerosis. *Neural Regen Res* 2016; **11**: 90-91 [PMID: 26981093 DOI: 10.4103/1673-5374.175050]

4 **Kim HY**, Kumar H, Jo MJ, Kim J, Yoon JK, Lee JR, Kang M, Choo YW, Song SY, Kwon SP, Hyeon T, Han IB, Kim BS. Therapeutic Efficacy-Potentiated and Diseased Organ-Targeting Nanovesicles Derived from Mesenchymal Stem Cells for Spinal Cord Injury Treatment. *Nano Lett* 2018; **18**: 4965-4975 [PMID: 29995418 DOI: 10.1021/acs.nanolett.8b01816]

5 **He L**, He T, Xing J, Zhou Q, Fan L, Liu C, Chen Y, Wu D, Tian Z, Liu B, Rong L. Bone marrow mesenchymal stem cell-derived exosomes protect cartilage damage and relieve knee osteoarthritis pain in a rat model of osteoarthritis. *Stem Cell Res Ther* 2020; **11**: 276 [PMID: 32650828 DOI: 10.1186/s13287-020-01781-w]

6 **Kim YG**, Choi J, Kim K. Mesenchymal Stem Cell-Derived Exosomes for Effective Cartilage Tissue Repair and Treatment of Osteoarthritis. *Biotechnol J* 2020; **15**: e2000082 [PMID: 32559340 DOI: 10.1002/biot.202000082]

7 **Weng Z**, Zhang B, Wu C, Yu F, Han B, Li B, Li L. Therapeutic roles of mesenchymal stem cell-derived extracellular vesicles in cancer. *J Hematol Oncol* 2021; **14**: 136 [PMID: 34479611 DOI: 10.1186/s13045-021-01141-y]

8 **Lee PW**, Wu BS, Yang CY, Lee OK. Molecular Mechanisms of Mesenchymal Stem Cell-Based Therapy in Acute Kidney Injury. *Int J Mol Sci* 2021; **22** [PMID: 34768837 DOI: 10.3390/ijms222111406]

9 **Lu Y**, Wang L, Zhang M, Chen Z. Mesenchymal Stem Cell-Derived Small Extracellular Vesicles: A Novel Approach for Kidney Disease Treatment. *Int J Nanomedicine* 2022; **17**: 3603-3618 [PMID: 35990308 DOI: 10.2147/IJN.S372254]

10 **Pixley JS**. Mesenchymal stem cells to treat type 1 diabetes. *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**: 165315 [PMID: 30508575 DOI: 10.1016/j.bbadis.2018.10.033]

11 **Suk KT**, Yoon JH, Kim MY, Kim CW, Kim JK, Park H, Hwang SG, Kim DJ, Lee BS, Lee SH, Kim HS, Jang JY, Lee CH, Kim BS, Jang YO, Cho MY, Jung ES, Kim YM, Bae SH, Baik SK. Transplantation with autologous bone marrow-derived mesenchymal stem cells for alcoholic cirrhosis: Phase 2 trial. *Hepatology* 2016; **64**: 2185-2197 [PMID: 27339398 DOI: 10.1002/hep.28693]

12 **Joshi NS**, Bansal PN, Stewart RC, Snyder BD, Grinstaff MW. Effect of contrast agent charge on visualization of articular cartilage using computed tomography: exploiting electrostatic interactions for improved sensitivity. *J Am Chem Soc* 2009; **131**: 13234-13235 [PMID: 19754183 DOI: 10.1021/ja9053306]

13 **Bansal PN**, Joshi NS, Entezari V, Grinstaff MW, Snyder BD. Contrast enhanced computed tomography can predict the glycosaminoglycan content and biomechanical properties of articular cartilage. *Osteoarthritis Cartilage* 2010; **18**: 184-191 [PMID: 19815108 DOI: 10.1016/j.joca.2009.09.003]

14 **Dhillon J**, Kraeutler MJ, Belk JW, Scillia AJ. Umbilical Cord-Derived Stem Cells for the Treatment of Knee Osteoarthritis: A Systematic Review. *Orthop J Sports Med* 2022; **10**: 23259671221104409 [PMID: 35859650 DOI: 10.1177/23259671221104409]

15 **Li K**, Yan G, Huang H, Zheng M, Ma K, Cui X, Lu D, Zheng L, Zhu B, Cheng J, Zhao J. Anti-inflammatory and immunomodulatory effects of the extracellular vesicles derived from human umbilical cord mesenchymal stem cells on osteoarthritis *via* M2 macrophages. *J Nanobiotechnology* 2022; **20**: 38 [PMID: 35057811 DOI: 10.1186/s12951-021-01236-1]

16 **Fong CY**, Chak LL, Biswas A, Tan JH, Gauthaman K, Chan WK, Bongso A. Human Wharton's jelly stem cells have unique transcriptome profiles compared to human embryonic stem cells and other mesenchymal stem cells. *Stem Cell Rev Rep* 2011; **7**: 1-16 [PMID: 20602182 DOI: 10.1007/s12015-010-9166-x]

17 **Tong W**, Zhang X, Zhang Q, Fang J, Liu Y, Shao Z, Yang S, Wu D, Sheng X, Zhang Y, Tian H. Multiple umbilical cord-derived MSCs administrations attenuate rat osteoarthritis progression *via* preserving articular cartilage superficial layer cells and inhibiting synovitis. *J Orthop Translat* 2020; **23**: 21-28 [PMID: 32455113 DOI: 10.1016/j.jot.2020.03.007]

18 **Ao Y**, Duan J, Xiong N, Qian N, Zhang R, Yang L, Yu S, Wang F. Repeated intra-articular injections of umbilical cord-derived mesenchymal stem cells for knee osteoarthritis: a phase I, single-arm study. *BMC Musculoskelet Disord* 2023; **24**: 488 [PMID: 37312112 DOI: 10.1186/s12891-023-06555-y]

19 **Jimenez PA**, Glasson SS, Trubetskoy OV, Haimes HB. Spontaneous osteoarthritis in Dunkin Hartley guinea pigs: histologic, radiologic, and biochemical changes. *Lab Anim Sci* 1997; **47**: 598-601 [PMID: 9433695]

20 **Liu W**, Burton-Wurster N, Glant TT, Tashman S, Sumner DR, Kamath RV, Lust G, Kimura JH, Cs-Szabo G. Spontaneous and experimental osteoarthritis in dog: similarities and differences in proteoglycan levels. *J Orthop Res* 2003; **21**: 730-737 [PMID: 12798075 DOI: 10.1016/S0736-0266(03)00002-0]

21 **Kyostio-Moore S**, Nambiar B, Hutto E, Ewing PJ, Piraino S, Berthelette P, Sookdeo C, Matthews G, Armentano D. STR/ort mice, a model for spontaneous osteoarthritis, exhibit elevated levels of both local and systemic inflammatory markers. *Comp Med* 2011; **61**: 346-355 [PMID: 22330250]

22 **Kwapisz A**, Bowman M, Walters J, Cosh H, Burnikel B, Tokish J, Ye T, Mercuri J. Human Adipose- and Amnion-Derived Mesenchymal Stromal Cells Similarly Mitigate Osteoarthritis Progression in the Dunkin Hartley Guinea Pig. *Am J Sports Med* 2022; **50**: 3963-3973 [PMID: 36300544 DOI: 10.1177/03635465221126683]

23 **Perry J**, McCarthy HS, Bou-Gharios G, van 't Hof R, Milner PI, Mennan C, Roberts S. Injected human umbilical cord-derived mesenchymal stromal cells do not appear to elicit an inflammatory response in a murine model of osteoarthritis. *Osteoarthr Cartil Open* 2020; **2**: 100044 [PMID: 32596691 DOI: 10.1016/j.ocarto.2020.100044]

24 **Wang Y**, Wu H, Yang Z, Chi Y, Meng L, Mao A, Yan S, Hu S, Zhang J, Zhang Y, Yu W, Ma Y, Li T, Cheng Y, Wang Y, Wang S, Liu J, Han J, Li C, Liu L, Xu J, Han ZB, Han ZC. Human mesenchymal stem cells possess different biological characteristics but do not change their therapeutic potential when cultured in serum free medium. *Stem Cell Res Ther* 2014; **5**: 132 [PMID: 25476802 DOI: 10.1186/scrt522]

25 **Hwang SH**, Lee W, Park SH, Lee HJ, Park SH, Lee DC, Lim MH, Back SA, Yun BG, Jeun JH, Lim JY, Kang JM, Kim SW. Evaluation of characteristic of human turbinate derived mesenchymal stem cells cultured in the serum free media. *PLoS One* 2017; **12**: e0186249 [PMID: 29049314 DOI: 10.1371/journal.pone.0186249]

26 **Matas J**, Orrego M, Amenabar D, Infante C, Tapia-Limonchi R, Cadiz MI, Alcayaga-Miranda F, González PL, Muse E, Khoury M, Figueroa FE, Espinoza F. Umbilical Cord-Derived Mesenchymal Stromal Cells (MSCs) for Knee Osteoarthritis: Repeated MSC Dosing Is Superior to a Single MSC Dose and to Hyaluronic Acid in a Controlled Randomized Phase I/II Trial. *Stem Cells Transl Med* 2019; **8**: 215-224 [PMID: 30592390 DOI: 10.1002/sctm.18-0053]

27 **Naveen SV**, Ahmad RE, Hui WJ, Suhaeb AM, Murali MR, Shanmugam R, Kamarul T. Histology, glycosaminoglycan level and cartilage stiffness in monoiodoacetate-induced osteoarthritis: comparative analysis with anterior cruciate ligament transection in rat model and human osteoarthritis. *Int J Med Sci* 2014; **11**: 97-105 [PMID: 24396291 DOI: 10.7150/ijms.6964]

28 **Katz JN**, Arant KR, Loeser RF. Diagnosis and Treatment of Hip and Knee Osteoarthritis: A Review. *JAMA* 2021; **325**: 568-578 [PMID: 33560326 DOI: 10.1001/jama.2020.22171]

29 **Hawker GA**, Lohmander LS. What an earlier recognition of osteoarthritis can do for OA prevention. *Osteoarthritis Cartilage* 2021; **29**: 1632-1634 [PMID: 34461227 DOI: 10.1016/j.joca.2021.08.007]

30 **Hunter DJ**, Bierma-Zeinstra S. Osteoarthritis. *Lancet* 2019; **393**: 1745-1759 [PMID: 31034380 DOI: 10.1016/S0140-6736(19)30417-9]

31 **Akkiraju H**, Nohe A. Role of Chondrocytes in Cartilage Formation, Progression of Osteoarthritis and Cartilage Regeneration. *J Dev Biol* 2015; **3**: 177-192 [PMID: 27347486 DOI: 10.3390/jdb3040177]

32 **Chow YY**, Chin KY. The Role of Inflammation in the Pathogenesis of Osteoarthritis. *Mediators Inflamm* 2020; **2020**: 8293921 [PMID: 32189997 DOI: 10.1155/2020/8293921]

33 **Abramson SB**, Attur M. Developments in the scientific understanding of osteoarthritis. *Arthritis Res Ther* 2009; **11**: 227 [PMID: 19519925 DOI: 10.1186/ar2655]

34 **Hoff P**, Buttgereit F, Burmester GR, Jakstadt M, Gaber T, Andreas K, Matziolis G, Perka C, Röhner E. Osteoarthritis synovial fluid activates pro-inflammatory cytokines in primary human chondrocytes. *Int Orthop* 2013; **37**: 145-151 [PMID: 23212731 DOI: 10.1007/s00264-012-1724-1]

35 **Kapoor M**, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol* 2011; **7**: 33-42 [PMID: 21119608 DOI: 10.1038/nrrheum.2010.196]

36 **Sanchez C**, Gabay O, Salvat C, Henrotin YE, Berenbaum F. Mechanical loading highly increases IL-6 production and decreases OPG expression by osteoblasts. *Osteoarthritis Cartilage* 2009; **17**: 473-481 [PMID: 18974013 DOI: 10.1016/j.joca.2008.09.007]

37 **Sakao K**, Takahashi KA, Arai Y, Saito M, Honjo K, Hiraoka N, Asada H, Shin-Ya M, Imanishi J, Mazda O, Kubo T. Osteoblasts derived from osteophytes produce interleukin-6, interleukin-8, and matrix metalloproteinase-13 in osteoarthritis. *J Bone Miner Metab* 2009; **27**: 412-423 [PMID: 19333684 DOI: 10.1007/s00774-009-0058-6]

38 **Distel E**, Cadoudal T, Durant S, Poignard A, Chevalier X, Benelli C. The infrapatellar fat pad in knee osteoarthritis: an important source of interleukin-6 and its soluble receptor. *Arthritis Rheum* 2009; **60**: 3374-3377 [PMID: 19877065 DOI: 10.1002/art.24881]

39 **Little CB**, Barai A, Burkhardt D, Smith SM, Fosang AJ, Werb Z, Shah M, Thompson EW. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. *Arthritis Rheum* 2009; **60**: 3723-3733 [PMID: 19950295 DOI: 10.1002/art.25002]

40 **Haeusler G**, Walter I, Helmreich M, Egerbacher M. Localization of matrix metalloproteinases, (MMPs) their tissue inhibitors, and vascular endothelial growth factor (VEGF) in growth plates of children and adolescents indicates a role for MMPs in human postnatal growth and skeletal maturation. *Calcif Tissue Int* 2005; **76**: 326-335 [PMID: 15868281 DOI: 10.1007/s00223-004-0161-6]

41 **Wang M**, Sampson ER, Jin H, Li J, Ke QH, Im HJ, Chen D. MMP13 is a critical target gene during the progression of osteoarthritis. *Arthritis Res Ther* 2013; **15**: R5 [PMID: 23298463 DOI: 10.1186/ar4133]

42 **Wei P**, Bao R. Intra-Articular Mesenchymal Stem Cell Injection for Knee Osteoarthritis: Mechanisms and Clinical Evidence. *Int J Mol Sci* 2022; **24** [PMID: 36613502 DOI: 10.3390/ijms24010059]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: office@baishideng.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com