Observational Study

Medicine®

OPEN

Cervus and cucumis peptides combined umbilical cord mesenchymal stem cells therapy for rheumatoid arthritis

Tao Qi, MD[a], Hongxiang Gao, MD[b], Yazheng Dang, MD[a], Shigao Huang, PhD[c,d,*], Minfei Peng, MD[e,*]

Abstract

Cervus and cucumis peptides (Lugua polypeptides, LG) are traditional Chinese medicine, which are active components of polypeptide extracted from Sika deer bone and melon seed, and they contain bone induced polypeptide biological factors. Umbilical cord mesenchymal stem cell, (UC-MSC) have tissue repair multiple effects, anti-inflammatory, and immune regulation function, which become a very promising start in rheumatoid arthritis (RA) treatment. Hence, LG combined UC-MSC can significantly enhance the UC-MSC treatment of rheumatoid arthritis (RA).

To explore the clinical curative effect and therapeutic mechanism of LG combined UC-MSC for treating RA.

119 patients were divided into control and treatment groups, and both groups were treated with methotrexate tablets, leflunomide, and UC-MSC. But, LG were added to the treatment group. In vitro, the effects of LG on UC-MSC cell secretion of anti-inflammatory factors were also performed.

The Health Assessment Questionnaire; the 28 joint disease activity score; C reactive protein; the erythrocyte sedimentation rate; rheumatoid factor; and anti-cyclic citrullinated peptide antibody were significantly reduced in treatment group 1 year after treatment ($P < .05$). In vitro, compared with the control group, the number of hepatocyte growth factor (HGF), the secretion of prostaglandin E2 (PGE2) and tumor necrosis factor-inducible gene 6 protein (TSG6) increased significantly ($P < .05$).

LG combined UC-MSCs can significantly improve the curative effect of RA patients, while LG may reduce inflammatory cytokines, regulate immunity, improve microcirculation, and are conducive to UC-MSCs migration and the repair of damaged tissue.

Abbreviations: Anti-CCP= anti-cyclic citrullinated peptide antibody, CRP = C reactive protein, DAS 28 = the 28 joint disease activity, ESR = the erythrocyte sedimentation rate, HAQ = the Health Assessment Questionnaire, LG = cervus and cucumis peptides, RA = rheumatoid arthritis, RF = rheumatoid factor, UC-MSC = umbilical cord mesenchymal stem cell, WBC = white blood cells.

Keywords: anti-inflammatory cytokines, deer and melon Polypeptides, mesenchymal stem cells, repair damage tissue, rheumatoid arthritis

1. Introduction

rheumatoid arthritis (RA), an autoimmune disease, is characterized by inflammatory synovitis, which can cause complete joint damage, as well as extra-articular and generalized body symptoms. Without timely diagnosis and treatment, these symptoms may eventually lead to disability and premature death.[1,2] So, exploring new and effective treatments for RA is pressing issues for medical personnel in the rheumatic field. Although many studies[3–5] have showed that RA is closely related to the expression of inflammation gene, the pathogenesis

Editor: Muhammad Shahzad Aslam.

TQ, HG, YD, and SH have contributed equally to this article.

Ethics approval and consent to participate: The study protocol was approved by the Ethics Committee of the Institute of 986 hospital of People's Liberation Army Air Force. All patients' written informed consent was obtained.

This study was supported by the National Natural Science Foundation of China (31440041) and Enze Medical Center Scientific Research Funds (NO 15EZA1).

The authors have no conflict of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] Department of Radiation Oncology, 986 Hospital of People's Liberation Army Air Force, [b] Department of Radiotherapy Oncology, Chang An Hospital, Xi'an, Shaan Xi, [c] Cancer Centre, [d] Institute of Translational Medicine, Faculty of Health Sciences, University of Macau, Taipa, Macao SAR, [e] Department of Clinical Laboratory, Taizhou Hospital of Zhejiang Province, Taizhou Enze Medical Center (Group), Linhai, Zhejiang Province, China.

[*] Correspondence: Minfei Peng, Department of Clinical Laboratory, Taizhou Hospital of Zhejiang Province, Taizhou Enze Medical Center (Group), 150 Ximen Street of Linhai City, Linhai 317000, Zhejiang Province, China (e-mail: pengmf@enzemed.com); Shigao Huang, Faculty of Health Sciences, University of Macau, Taipa, Macao SAR, P.R. China (e-mail: huangshigao2010@aliyun.com).

Copyright © 2020 the Author(s). Published by Wolters Kluwer Health, Inc.
This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Qi T, Gao H, Dang Y, Huang S, Peng M. Cervus and cucumis peptides combined umbilical cord mesenchymal stem cells therapy for rheumatoid arthritis. Medicine 2020;99:28(e21222).

Received: 25 October 2019 / Received in final form: 1 May 2020 / Accepted: 10 June 2020

http://dx.doi.org/10.1097/MD.0000000000021222

of RA is still unclear.[6] In the past 2 decades, various new biological agents and the optimization of treatment principles and strategies, were executed in the treatment of RA, and the prognosis of patients were significantly improved.[7] However, due to the side effects of drugs and other reasons, various treatments have their limitations, and for some patients, the curative effect are not satisfactory. Therefore, it is urgent to explore new therapeutic methods through their mechanism of action.[8]

Umbilical cord mesenchymal stem cells (UC-MSCs) have tissue repair, multiple effects, anti-inflammatory and immune regulation function, which become a promising therapy method in RA therapeutic areas.[9] Many studies have shown that UC-MSCs therapy for RA can safely, effectively and persistently alleviate the RA symptoms.[10] Our preliminary study[11] also reported that UC-MSCs had very good safety and effectiveness in the treatment of RA, and the patient's tolerance was good.[12] In addition, cervus and cucumis peptides (LG) is a compound preparation,[13] which is the active component of polypeptides extracted from the bones of the sika deer and the seeds of melon. It contains bone-induced polypeptide biological factors, such as transforming growth factor -beta (TGF- beta),[14] which can promote the synthesis of type I collagen and osteoporosis.[14] They inhibit the inflammatory response, reduce the content of tumor necrosis factor-alpha (TNF-alpha) in serum,[15] and assist macrophage-derived cytokines to play a role in tissue repair.[16] According to the pathogenesis of RA in traditional Chinese and western medicine, UC-MSCs are pre-stimulated by the polypeptide of LG to promote the superposition of kidney-nourishing, bone strengthening, blood-activating, and collateral-opening, and also reduce the release of pro-inflammatory factors. While stabilizing the internal environment, the biological characteristics of UC-MSCs migration, homing, regeneration, repair, and immune regulation can be enhanced, which also conform to the principle of specimen co-treatment in traditional Chinese medicine.

Base on the above mechanism, we hypothesized that LG early intervention in UC-MSCs can significantly increase the effective treatment of RA. However, the specific mechanism was not clear. In this study, we observed the clinical effect of LG combined with UC-MSCs in the treatment of RA patients and studied the potential mechanism of LG to enhance the effectiveness of UC-MSCs therapy for RA, In addition, through a vitro-cell model, we focused the synergistic effect of LG on the secretion of anti-inflammatory factors to UC-MSCs.

2. Materials and methods

2.1. Patients selection

All 119 RA patients were selected from January 2011 to December 2018, and the inclusion criteria referred to the American College of Rheumatology (ACR) standard.[17] Patients were randomly divided into LG + UC-MSCs group (n = 59) and UC-MSCs group (n = 60). There were 6 males and 53 females in the treatment group with an average age of 43 years, and the average time of illness course was 10 years. There were 7 males and 53 females in the control group, with an average age of 44 years and the average time of illness course was 10 years (Table 1). The exclusion criteria were following: pregnant or lactating women; complicated with serious primary diseases of cardiovascular, liver, kidney and blood system; mentally ill; patients with skin diseases or skin lesions. And this study was registered in ClinicalTrials.gov (NCT01547091).

Table 1

Patients characteristics and demographics.

Characteristics	UC-MSCs N (%)	LG + UC-MSCs N (%)
Patients	60	59
Gender		
Male	7 (11.7%)	6 (10.2%)
Female	53 (88.3%)	53 (89.8%)
Age (yr)		
Median	48	47
Mean (Range)	44 (18–65)	43 (16–64)
Duration of the disease (years)		
Median	14	16
Mean (Range)	10 (0.5–33)	10 (0.5–35)
Joints pain number	4 (3–9)	4 (3–10)
Morning stiffness (h)	3.1 (2–5)	3.3 (2–5)
Along with the symptoms (n)		
JRA	3 (5.0%)	3 (5.1%)
AS	5 (8.3%)	4 (6.8%)

AS = ankylosing spondylitis, JRA = juvenile rheumatoid arthritis, UC-MSC = umbilical cord mesenchymal stem cell.

2.2. Treatment protocol

For LG + UC-MSCs group, all patients were firstly administrated intravenous drip with 100 mL 0.9% sodium chloride mixed with 24 mg LG injection (Harbin Yuheng pharmaceutical LTD, China); 1 time/d, totally 7 days and oral 5 mg methotrexate, 2 times/wk, oral 10 mg leflunomide, 1 day, then administrated intravenous drip with 40 ml UC-MSC cells (4×10^7/40 mL) (Shenzhen Bio LTD, China.). Patients in the UC-MSCs group were administrated oral 5 mg methotrexate, twice a week, oral 10 mg leflunomide, once a day, and 40 mL UC-MSC cells (4×10^7/40 mL).

2.3. Clinical effect evaluation

Based on our previous study,[18] the 28 joint disease activity score (DAS 28), the health assessment questionnaire (HAQ)score and RA clinical remission standard (ACR standard) were recorded 1 year before and after treatment.[19] The erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), and anti-cyclic citrullinated peptide antibody (Anti-CCP) were detected pretreatment and 1 year after treatment. The routine hematuria and liver and kidney functions were detected pretreatment and 1 year after treatment to evaluate adverse reactions and safety during treatment.

2.4. In vitro experiment

Experimental materials: LG injection (Harbin Yusheng pharmaceutical LTD). Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) and fetal calf serum (FBS) (Invitrogen, USA). Automatic enzyme marker (Thermo Fisher, USA). Hepatocyte growth factor (HGF), prostaglandin E2 (PGE2) and tumor necrosis factor-inducible gene 6 protein (TSG6) enzyme-linked immunosorbent assay (ELISA) Kit (Nanjing Jincheng Biological LTD, China). The UC-MSC cells (Tianjin Heze Bio LTD, China.) were cultured in DMEM/F12 medium containing 10 ng/mL Fibroblast growth factor-basic, (bFGF) (Gibco, USA), 10% FBS and 1% penicillin/streptomycin at 37.0°C, 5% CO2 and 95% humidity.

Experimental method: When the UC-MSC cells were growing in good condition, then they were inoculated in 4 wells in 6-well

Table 2

Comparison of clinical indicators change between the 2 groups before and after treatment.

	UC-MSCs			LG + UC-MSCs		
	Pretreatment	Posttreatment	*P* value	Pretreatment	Posttreatment	*P* value
DAS28	5.82 ± 1.4	3.96 ± 1.09	.011	6.05 ± 1.7	3.55 ± 1.40	.023
HAQ	2.51 ± 2.01	3.45 ± 1.21	.024	1.99 ± 1.17	1.11 ± 1.29	.019
CRP (m/L)	51.44 ± 37.09	27.153 ± 23.02	.687	33.16 ± 25.37	18.67 ± 19.01	.032
ESR (mm/h)	65.07 ± 32.87	45.97 ± 22.91	.071	52.22 ± 45.12	21.89 ± 14.53	.005
RF (RU/mL)	251.09 ± 65.98	198.08 ± 95.50	.099	323.01 ± 95.42	239.07 ± 85.58	.018
AntiCCP (RU/mL)	251.04 ± 89.98	204.82 ± 84.05	.159	356.03 ± 112.03	280.22 ± 110.01	.044

P value represents that the compare between pretreatment and posttreatment.

Anti-CCP = anti-cyclic citrullinated peptide antibody, CRP = C reactive protein, DAS 28 = the 28 joint disease activity, ESR = the erythrocyte sedimentation rate, HAQ = the Health Assessment Questionnaire, RF = rheumatoid factor, UC-MSC = Umbilical cord mesenchymal stem cell.

plates overnight. All medium were discarded and then we added medium containing 0 ug/mL,3 ug/mL, 6 ug/mL and 9 ug/mL LG injection, respectively, for co-culturing 48 hours. The supernatant were collected and centrifuged at 3000 rpm for 20 minutes, then stored for ELISA detection. Blank well, standard well and sample well were set for testing. The blank holes were used to adjust to zero, and the absorbance value of each well was measured at the wavelength of 450 nm.

2.5. Statistical analysis

SPSS statistics 21.0 (IBM) software was used for data statistical processing. The measurement data were presented as mean ± standard deviation (x ± s), and t-test was used for analyzing data, and $P < .05$ was considered statistically significant. The statistical analysis and figure plots were performed using GraphPad software (Prism 8 version, San Diego, CA).

3. Results

3.1. Curative effect evaluation

The comparison of DAS28 scores and HAQ scores of the 2 groups before and after treatment were shown in Table 2. The DAS28 scores and HAQ scores of 2 groups after treatment were both decreased significantly than those of pretreatment. What's more, the other immunology factors such as CRP, ESR, RF, and Anti-CCP had no significant difference in the UC-MSCs group, but they decreased significantly in the LG + UC-MSCs group.

3.2. Curative safety evaluation and adverse reactions

All the 59 patients in the LG + UC-MSCs group conformed the ACR20 index 3 months after treatment, and of which 14 cases conformed the ACR50 index. In the UC-MSCs group, 30 cases conformed the ACR20 index, of which seven cases conformed the ACR50 index. In the LG + UC-MSCs group after the infusion of LG combined UC- MSC, only 2 patients had gastrointestinal symptoms of different degrees. Six cases had mild leukocytopenia (white blood cells [WBC] $< 3.5 \times 10^9$). Among the 60 patients in the UC-MSCs group, 16 patients had adverse reactions, among which six had gastrointestinal symptoms of different degrees, 1 had moderate leukocytopenia (WBC $< 2.0 \times 10^9$), six had mild leukocytopenia (WBC $< 3.5 \times 10^9$), and 3 had mild transaminase elevation.

Routine laboratory indicators had no significant change during the pretreatment and 1-year posttreatment between UC-MSCs and LG + UC-MSCs group ($P > .05$). Routine hematuria and liver and kidney function tests were all normal 1 year after treatment (Table 3).

3.3. LG promoted the secretion of anti-inflammatory factors of UC-MSC

In the ELISA test, the LG treated UC-MSC cells group significantly increased the secretion of HGF, PGE2, and TSG6 than the UC-MSC cells group. Fig. 1 showed that LG could promote the secretion of anti-inflammatory factors of UC-MSC in a concentration-dependent manner ($P < .01$).

Table 3

Routine laboratory indicators change between the 2 groups before and after treatment.

	LG + UC-MSCs			UC-MSCs		
	Pretreatment	Posttreatment	*P* value	Pretreatment	Posttreatment	*P* value
TP (g/L)	63.77 ± 4.05	62.99 ± 5.25	.058	60.22 ± 4.76	62.01 ± 4.72	.089
Albumin (g/L)	108.58 ± 12.52	110.50 ± 11.89	.122	120.51 ± 12.76	117.50 ± 10.54	.411
Platelet (10^9/L)	182.36 ± 70.46	182.20 ± 65.02	.368	190.02 ± 56.55	190.20 ± 55.76	.432
WBC (10^9/L)	6.31 ± 2.07	6.02 ± 2.55	.587	7.01 ± 3.02	6.95 ± 2.46	.285
RBC (10^{12}/L)	3.72 ± 0.44	3.83 ± 0.59	.232	4.07 ± 0.88	4.16 ± 0.74	.337
BUN (mmol/L)	4.80 ± 1.47	4.39 ± 1.55	.467	4.20 ± 1.66	4.89 ± 2.04	.535
Triglyceride (umol/L)	42.31 ± 11.26	43.52 ± 11.79	.593	43.11 ± 12.08	43.39 ± 10.89	.116
ALT (U/L)	20.42 ± 5.40	19.79 ± 10.36	.292	21.07 ± 5.77	19.88 ± 11.05	.557
AST (U/L)	29.42 ± 5.44	29.79 ± 12.26	.535	30.28 ± 4.97	32.06 ± 11.75	.820
Cholesterol (umol/L)	43.11 ± 11.36	43.12 ± 10.55	.191	40.55 ± 10.89	42.11 ± 9.57	.223

The data are shown as x ± s. *P* value represents that the compare between pretreatment and posttreatment.

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BUN = blood urea nitrogen, RBC = red blood cells, TP = total protein, *P* value represents that the compare between pretreatment and posttreatment, WBC = white blood cells.

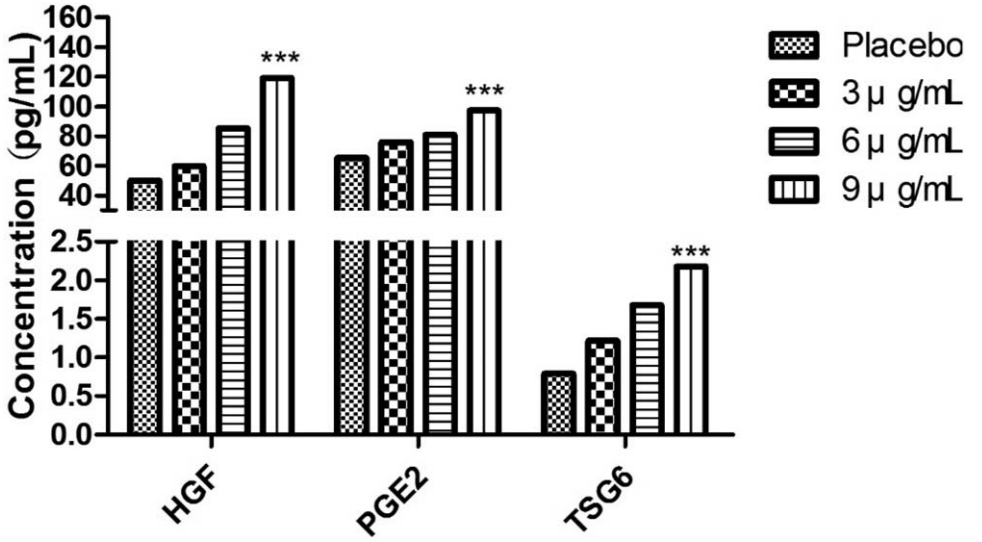

Figure 1. LG promoted the secretion of anti-inflammatory factors of UC-MSC. The data are shown as mean (SD) and *** represents $P < .01$. SD = standard deviation, UC-MSC = umbilical cord mesenchymal stem cell.

4. Discussion

To date, the treatment principle for RA is to control the joint inflammation and relieve the patient's pain, control disease progression and prevent joint destruction; promote joint repair and improve joint function.[20] The treatment strategies for RA are to early therapy, combination medication and long-term adherence.[21] Combination of using drugs with different mechanisms action is to avoid the same side effects arising. Clinical use nonsteroidal (Non-steroidal anti-inflammatory drugs, NSAIDs) type, anti-rheumatoid (diseases, modifying antirheumatic drugs, DMARDs) and hormone treatment were using for RA in past several years,[21] but the limits remain obvious that the recurrence rate is high, the adverse effects often occur, and they can't change the progress of the disease, also with no obvious repairability to diseased joints.

Mesenchymal stem cells (MSCs) have biological characteristics of regenerating and repairing parenchymal tissues and organs and immune regulation. Although the clinical treatment of RA has made breakthroughs, there are still some problems to be solved. One is that RA joint injuries still occur, which is not only associated with inflammation, and that anti-inflammatory therapy alone may not be enough to prevent RA progression. MSCs has shown exciting therapeutic prospects, especially for RA patients with poor response to current treatment.[22] MSC plays a therapeutic role in RA mainly incell differentiationand immune regulation. In the former, MSCs can be differentiated into damaged cells (such as osteocytes and chondrocytes) and thus they play a direct role in repair. Through the latter, MSC can regulate the immune response mediated by T cells, inhibit the production and function of various innate immune cells and play an anti-inflammatory role, regulate immunity and resist tissue injury.[23] Our previous study[18] has shown that UC- MSCs therapy for RA can reduce and alleviate the clinical symptoms of RA, and there is no significant change in human blood routine indicators, which showed good safety.

Blood stasis is an important feature of RA understanding in Chinese Traditional medicine (TCM), which leads to the differentiation and maturation of lymphocytes in intestinal related lymphoid tissues and obstructs their homing, and its effect on mucosal immunity is 1 of the pathogenesis of RA.[24] TCM syndrome of kidney deficiency and blood stasis can more accurately and comprehensively reflect the pathological essence of RA.[25] However, since RA is an autoimmune disease caused by multiple factors, it has been recognized by medical rheumatism personnel that the complementary advantages of the combination of traditional Chinese and western medicine together.[24]

Cervus and Cucumis polypeptide (LG) is a new drug of traditional Chinese medicine. Although it is not a therapeutic drug developed for RA, its function mechanism is applied to the pathological RA, so the LG treatment of RA maybe have certain efficacy. The LG are active components extracted from the bones of the sika deer and the seeds of muskmelon.[26] Melon seed extract can improve local capillary permeability, reduce exudation, promote blood circulation, inhibit the release of prostaglandins, and reduce pain. Therefore, LG injection has the effect of tonifying kidney, strengthening bone, removing blood stasis, dispersing the formation, dehumidifying and analgesic.[27] By down-regulating the expression of RA serum inflammatory factors TNF-alpha and IL-23, it can effectively regulate the internal environment of the body and maintain the homeostasis balance of the body.[28] The therapeutic effects of LG on reducing swelling and pain, regulating immunity, and inhibiting bone destruction. A study reported that an adjuvant RA treatment can significantly reduce the number of joint swelling and pain, improve the joint function and state, and increase grip strength, with fewer side effects and good patient compliance. Studies on the modernization of traditional Chinese medicine[29] showed that many traditional Chinese medicines can promote the proliferation and differentiation of MSCs.

We integrated the 2 medicine LG with UC-MSCs on basis of RA pathogenesis in Chinese and western medicine. LG polypeptides stimulated UC -MSCs to reduce the release of a pro-inflammatory factor instability of the internal environment, and increase UC-MSCs immune regulation and regenerative biology, which conformed to the principle of the main pathogenesis of TCM.[23,30] This study showed that the improvement of HAQ, DAS28, ESR, CRP, RF, and Anti-CCP

in the LG+UC -MSC group was significantly better than that in the UC -MSC group at 1 year after treatment ($P < 0.05$), and all patients conformed the ACR20 standard. This indicated that TCM intervention played an important role in anti-inflammation, regulating immunity and improving microcirculation in the treatment of UC-MSCs, which was beneficial to the migration of UC-MSCs and repair of damaged tissue. The combination of LG intervention UC-MSCs, DMADs and sequential therapy had a synergistic effect on the effectiveness and stability of RA treatment, especially for the remission of clinical symptoms in patients with active RA, and reduced the adverse reactions of DMARDs, which was safe and conformed to the treatment principles and strategies of RA.

However, the limits of this study are the following: First, the effect of LG on the immune regulation of UC-MSCs needs to be further verified by the follow-up in vivo studies. Second, the potential mechanism of LG enhances the effectiveness of UC-MSCs in treating RA needs further research. In addition, the underlying molecular mechanism of the LG joint UC-MSCs therapy for RA is needed to clarify.

5. Conclusions

Cervus and Cucumis peptide (LG) combined with UC-MSC can significantly increase the UC-MSC effectiveness for the treatment of RA, which can improve clinical curative effect and reduce side effects. Also, an in-vitro cell study indicated that LG could enhance the immune regulatory function of UC-MSC by promoting the secretion of HGF, PGE2 and TSG6 anti-inflammatory factors in a concentration-dependent manner. Thereby, UC-MSC combined LG synergistic treatment maybe provide novelty, safety and effective method for RA patients.

Author contributions

Conceptualization: Tao Qi, Hongxiang Gao, Yazheng Dang, Minfei Peng.
Funding acquisition: Hongxiang Gao.
Investigation: Minfei Peng.
Methodology: Tao Qi, Yazheng Dang.
Project administration: Tao Qi, Hongxiang Gao.
Resources: Yazheng Dang.
Supervision: Minfei Peng, Shigao Huang.
Visualization: Minfei Peng.
Writing – review & editing: Tao Qi, Hongxiang Gao.

References

[1] Kucharz EJ, Stajszczyk M, Kotulska-Kucharz A, et al. Tofacitinib in the treatment of patients with rheumatoid arthritis: position statement of experts of the Polish Society for Rheumatology. Reumatologia 2018; 56:203–11.
[2] Kilic L, Erden A, Sener YZ, et al. Rituximab therapy in renal amyloidosis secondary to rheumatoid arthritis. Biomolecules 2018;8:136.
[3] Skalska U, Kozakiewicz A, Masliński W, et al. HLA-B27 detection - comparison of genetic sequence-based method and flow cytometry assay. Reumatologia 2015;53:74–8.
[4] Roelandse-Koop EA, Buisman B, van Hannen EJ, et al. Rapid HLA-B27 screening with real-time TaqMan PCR: a clinical validation in the Dutch population. Clin Chem Lab Med 2011;49:1979–85.
[5] Blasczyk R, Weber M, van Lessen A, et al. Discrimination of HLA-B27 alleles by group-specific amplification followed by solid-phase sequencing. Hum Immunol 1996;45:117–23.
[6] Van der Heijde DM, van't Hof M, van Riel PL, et al. Development of a disease activity score based on judgment in clinical practice by rheumatologists. J Rheumatol 1993;20:579–81.
[7] Smolen JS, van der Heijde D, Machold KP, et al. Proposal for a new nomenclature of disease-modifying antirheumatic drugs. Ann Rheum Dis 2014;73:3–5.
[8] Zhao C, Zhang L, Kong W, et al. Umbilical cord-derived mesenchymal stem cells inhibit cadherin-11 expression by fibroblast-like synoviocytes in rheumatoid arthritis. J Immunol Res 2015;2015:137695.
[9] Chen J, Li Y, Hao H, et al. Mesenchymal stem cell conditioned medium promotes proliferation and migration of alveolar epithelial cells under septic conditions in vitro via the jnk-p38 signaling pathway. Cell Physiol Biochem 2015;37:1830–46.
[10] Zou C, Luo Q, Qin J, et al. Osteopontin promotes mesenchymal stem cell migration and lessens cell stiffness via integrin beta1, FAK, and ERK pathways. Cell Biochem Biophys 2013;65:455–62.
[11] Wang L, Zhang Y, Li H, et al. Clinical observation of employment of umbilical cord derived mesenchymal stem cell for juvenile idiopathic arthritis therapy. Stem Cells Int 2016;2016:9165267.
[12] Liu Y, Mu R, Wang S, et al. Therapeutic potential of human umbilical cord mesenchymal stem cells in the treatment of rheumatoid arthritis. Arthritis Res Ther 2010;12:R210.
[13] Yang X, Niu K, Zhang X, et al. The effectiveness and safety of cervus and cucumis polypeptide injection in promoting fracture healing after bone fracture surgeries: protocol for a systematic review and meta-analysis. Medicine 2019;98:e14571–14571.
[14] Xiao W, Yang X, Wang Y, et al. Splenectomy delays fracture healing by affecting the level of tumor necrosis factor alpha, interleukin 6 and bone morphogenetic protein. Adv Clin Exp Med 2018;27:165–71.
[15] Bian J, Cao D, Shen J, et al. N-methyl pyrrolidone promotes ankle fracture healing by inhibiting inflammation via suppression of the mitogen-activated protein kinase signaling pathway. Exp Ther Med 2018;15:3617–22.
[16] Lin Z-M, Liu Y-T, Xu Y-S, et al. Cervus and cucumis peptides ameliorates bone erosion in experimental arthritis by inhibiting osteoclastogenesis. Lupus Sci Med 2019;6:e000331–1331.
[17] Da niel Aletaha, Tuhina Neogi, Alan J, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010;69:1580–8.
[18] Wang LM, Huang SG, Li SM, et al. Efficacy and safety of umbilical cord mesenchymal stem cell therapy for rheumatoid arthritis patients: a prospective phase i/ii study. Drug Des Dev Ther 2019;13:4331–40.
[19] Pincus T, Swearingen C, Wolfe F. Toward a multidimensional health assessment questionnaire (MDHAQ): assessment of advanced activities of daily living and psychological status in the patient-friendly health assessment questionnaire format. Arthritis Rheum 1999;42: 2220–30.
[20] Mian A, Ibrahim F, Scott DL. A systematic review of guidelines for managing rheumatoid arthritis. BMC Rheumatol 2019;3:42.
[21] Nam JL, Takase-Minegishi K, Ramiro S, et al. Efficacy of biological disease-modifying antirheumatic drugs: a systematic literature review informing the 2016 update of the EULAR recommendations for the management of rheumatoid arthritis. Annals of the rheumatic diseases 2017;76:1113–36.
[22] Weiss ML, Anderson C, Medicetty S, et al. Immune properties of human umbilical cord Wharton's jelly-derived cells. Stem Cells (Dayton, Ohio) 2008;26:2865–74.
[23] Vieira Paladino F, de Moraes Rodrigues J, da Silva A, et al. The immunomodulatory potential of Wharton's Jelly mesenchymal stem/stromal cells. Stem Cells Int 2019;2019:3548917.
[24] Pan H-D, Xiao Y, Wang W-Y, et al. Traditional chinese medicine as a treatment for rheumatoid arthritis: from empirical practice to evidence-based therapy. Engineering 2019;5:895–906.
[25] Jiang M, Lu C, Zhang C, et al. Syndrome differentiation in modern research of traditional Chinese medicine. J Ethnopharmacol 2012;140: 634–42.
[26] Li Q, Liu J, Duan YJCJOO. Influence of cervus and cucumis polypeptide injection on expression of BMP~ 2 in cancellous bone, and its bone mineral density and biomechanical properties in ovariectomized rats. Chin J Osteopor 2006;12:602–6.
[27] Zhang K, Niu L, Yuan F, et al. traumatology, research on promotory effect of traditional Chinese medicine on fracture healing in cell and molecular level. Chin J Pract 2017;30:777–82.

[28] Shin TH, Kim HS, Kang TW, et al. Human umbilical cord blood-stem cells direct macrophage polarization and block inflammasome activation to alleviate rheumatoid arthritis. Cell Death Dis 2016;7:e2524.
[29] Li Z, Zhang S, Cao L, et al. Tanshinone IIA and Astragaloside IV promote the angiogenesis of mesenchymal stem cell-derived endothelial cell-like cells via upregulation of Cx37, Cx40 and Cx43. Exp Ther Med 2018;15:1847–54.
[30] Miranda JP, Camões SP, Gaspar MM, et al. The secretome derived from 3D-cultured umbilical cord tissue MSCs counteracts manifestations typifying rheumatoid arthritis. Front Immunol 2019;10:18.