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The pharmacological mechanism study of traditional prescription Meng-He Long-Huo-Tang on the treatment of rheumatoid arthritis

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Abstract: *A Chinese traditional prescription Meng-He Long-Huo-Tang (LHT) has been used for the treatment of rheumatoid arthritis (RA) for long time, but the pharmacological mechanism is unknown. A collagen-induced arthritis (CIA) rat model was established to study the effects of LHT on CIA rats by recording body weight, paw thickness, arthritis score and peripheral blood analysis. In vitro, rat fresh synovial fibroblasts (SFbs) were extracted, and the effects of LHT on SFbs proliferation, migration and apoptosis were investigated using cell viability and migration assays, gelatin zymography, western blot and immunocytochemistry. In vivo experiments show that LHT can reduce the joint swelling, decrease the levels of MMP-2/9 and inflammatory factors in the blood of CIA rats. In vitro experiments showed that LHT inhibited cell proliferation, migration and promoted apoptosis of activated SFbs. To sum up, this study revealed the underlying mechanism that LHT can effectively alleviate rheumatoid arthritis symptoms by reducing inflammatory factors and inhibiting SFbs excessive proliferation.*

Keywords: *Long Huo Tang; rheumatoid arthritis; synovial fibroblast; collagen induced arthritis rat; inflammatory factors*

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of unknown etiology, which is mainly characterized by inflammation of the synovium, damage to articular cartilage and bone, and ultimately leads to joint deformity and even disability[1, 2]. Synovial tissue is a kind of mesenchyme connective tissue around the joint, and synovial fibroblasts (SFbs) are the main cells secreting extracellular matrix proteins [3]. SFbs play an important role in all stages of RA, and their excessive activation and proliferation can lead to joint swelling and progressive destruction of bone tissue [4], which is the one of the key pathogenic factors of RA. When SFbs are activated, they proliferate excessively and produce a large number of soluble inflammatory mediators, such as TNF-α, interleukin-6 (IL-6), interleukin-1β (IL-1β) and matrix metalloproteinase (MMPs) to promote immune cells recruitment and activate inflammation and immune response [5, 6]. At the same time, these inflammatory factors can further promote the invasion and metastasis of SFbs to synovia cartage causing synovial degradation, and aggravating joint inflammation and damage [7, 8]. Traditional Chinese medicine classifies rheumatoid arthritis as "bi syndrome". It is believed that the occurrence of rheumatoid arthritis is due to the deficiency of vital qi in the body, and it is also due to the invasion of wind, cold and damp, which blocks the meridians, muscles, bones and joints. *Long Huo Tang (LHT)* is from the clinical record *"Yi Chun Sheng Yi"* (published in 1863) written by Fei Boxiong (1800~1879), a master of the Meng-He traditional academic genre. The prescription is composed by 14 traditional Chinese medicines: *Cistanche deserticola, Cinnamomum cassia Presl, Codonopsis pilosula, Poria cocos, Atractylodes macrocephala Koidz, Angelicasinensis, Radix Paeoniae Alba, Aucklandia lappa Decne, Dipsacus, Radix Angelicae Pubescentis, Cervusnippon Temminck, Faeces Bombycis, Ziziphus jujuba Mill* and *Zingiber officinale Roscoe*. It has the effects of removing dampness, dredging collaterals, removing cold, tonifying blood, activating blood circulation and relieving pain, and is widely used in the treatment of rheumatoid arthritis in Chinese medicine clinic. Although LHT has significant clinical efficacy, safety and little side effects, its pharmacological mechanism is still unclear. Therefore, this study established a rat model of collagen induced arthritis (CIA) and an in vitro cell model to explore its mechanism, strengthen the development and application of traditional ancient prescriptions, and provide experimental basis for the treatment of rheumatoid arthritis.

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2. Materials and instruments

2.1. Main drugs and reagents

Cistanche deserticola 12g (Batch No. 20170501, origin: Inner Mongolia); Cinnamomum cassia Presl 3g (Batch No. 84120901, origin: Guangxi); Codonopsis pilosula 15g (Batch No.: 2105034, origin: Gansu); Poria cocos 10g (Batch No. 19120102, origin Yunnan); Atractylodes macrocephala Koidz 5g (Batch No. 2019004, origin Sichuan); Angelicasinensis 10g (Batch No. 190626001, origin: Gansu); Radix Paeoniae Alba 5g (Batch No. 200609, origin Anhui) ; Aucklandia lappa Decne 3g (Batch No.: 15091903, origin: Yunnan); Dipsacus 10g (Batch No.: 130701, origin: Sichuan); Radix Angelicae Pubescentis 5g (Batch No. 8050861, origin Hubei); Cervusnippon Temminck 15g (Batch No. 207001, origin: Jilin); Faeces Bombycis 12g (Batch No. 160722, origin Jiangsu); Ziziphus jujuba Mill 10g (Batch No. 20150601, origin: Xinjiang); Zingiber officinale Roscoe 5g (Batch number: 140405, origin: Shandong).The above medicines were purchased from Beijing Tongrentang Drugstore and identified by Professor Tang Decai of Nanjing University of Traditional Chinese Medicine according to the 2020 edition of the Chinese Pharmacopoeia.

Fetal bovine serum (Multicell, USA); DMEM (Gibco, USA); MTT (Solarbio, China); PBS (Solarbio, China); Cattle type II collagen (Chondrex, USA); Complete Freund's adjuvant (Sigma, MO, USA); Containing IL-6, IL-1β And TNF- α ELISA kit: IL-6, IL-1β And TNF-α (NOVUS Biotech, Hong Kong, China) was purchased from NOVUS Biotech in Hong Kong.

2.2. Experimental animal

Wistar rats (male, 5 weeks, 200 ± 10 g) were obtained from Sibeifu Biotechnology Co. Ltd. (license number SCXK (Jing) 2019-OD10, Beijing, China) and housed in a controlled environment under the Guidelines on Animals Ethics and Welfare. All procedures were undertaken with the approval of the Changzhou University Ethics Association (ethical approval number CCZU-BM2021R001). All animals in the experimental group were selected randomly and determined by double blind method.

2.3. Main instruments

Leica Optical Microscope (XSP-11C), Cai Kang Optics (Shanghai); Ultra low temperature refrigerator (BDF-60H458) Boke Science (Shandong); Gel imager (Bio RAD), USA;Fluorescence microscope (Carl Zeiss, Germany);ChemiDoc CRS + Molecular Imager (Bio-Rad Laboratories, USA); Full wavelength microplate reader (1510-00326C), Thermo Fisher Technology Co., Ltd; Electronic balance (FA3315B), Shanghai Jingke Balance Company; Circulating water multipurpose vacuum pump (SHZ-D (III)), rotary evaporator (LC-RE-52AA), cryogenic coolant circulating pump (LC-LTC-5/10), Shanghai Lichen Bangxi Instrument Technology Co., Ltd.

3. Methods

3.1. Collagen-induced arthritis (CIA) rat model

CIA rat model is established as described above[9]. In short, bovine type II collagen (2 mg/mL, 0.05 mol/L acetic acid solutions) was completely mixed with Freund's adjuvant in a 1:1 volume ratio. Wistar rats were injected subcutaneously at the root of their tails for 300 μ L collagen / IFA lotion for 3 times (the first injection is at the 1st day, 3rd day and 7th day of booster injection). Normal rats were injected with the same amount of normal saline. The severity of arthritis was assessed every 2 days according to the arthritis index and joint swelling count.

3.2. Preparing aqueous extracts and grouping administration of LHT

LHT is composed of 14 traditional Chinese medicines, including 12 g Cistanche deserticola, 3 g Cinnamomum cassia Presl, 15 g Codonopsis pilosula, 10 g Poria cocos, 5 g Atractylodes macrocephala Koidz, 10 g Angelicasinensis, 5 g Radix Paeoniae Alba, 3 g Aucklandia lappa Decne, 10 g Dipsacus, 5 g Radix Angelicae Pubescentis, 15 g Cervusnippon Temminck, 12 g Faeces Bombycis, 10 g Ziziphus jujuba Mill and 5 g Zingiber officinale Roscoe. Wash the medicinal materials and place them in the boiling container, add 10 times of the volume of distilled water to soak the medicinal materials, fully soak them for 1 hour, boil them over high fire, and cook them gently for 0.5 h. Since cinnamomum cassia presl

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needs to be cooked later, it needs to be cooked with other herbs 15 minutes before frying, and finally filtered with gauze. Add 5 times of the volume of distilled water to the dregs and decoct them again for 0.5 h, then filter them with gauze. Evenly mix the two filtrates, use the rotary evaporator to concentrate to the target crude drug amount of 1.25 g/mL, cool it and put it into the sterilization bottle, and place it in the 4°C refrigerator for standby. If the adult human body mass is calculated as 60 kg, the adult dosage of LHT is 2 g/kg. According to the formula of the US Food and Drug Administration, the dosage of rat LHT is 12.5 g/kg, and the in vivo experiment is conducted at this dosage.

After 3 days of adaptive feeding, 18 rats were randomly divided into 3 groups (6 rats in each group): normal group (normal saline, 10 mL/kg/d), CIA group (normal saline, 10 mL/kg/d), and LHT + CIA group (LHT, 12.5 g/kg/d). All rats were gavaged from the 8th day for three consecutive weeks.

3.3. Detection of inflammatory factors by enzyme-linked immunosorbent assay

Detection of cytokines IL-6, IL-1β and TNF-α in serum samples by ELISA kit, and operations were according to the manufacturer's instructions.

3.4. Extraction and culture of primary rat SFbs in-vitro

Under aseptic conditions, cut the extracted synovial tissue to about 1 cm^3 in size and transfer it to a 15 mL centrifuge tube. Add 2 to 3 times the volume ratio of Type I collagenase digestion solution, keep away from light, and digest it for 2 to 4 hours in a shake at 37°C. After filtering through the cell filter screen, wash it with sterile PBS and centrifuge twice. Add completed DMEM containing 10% fetal bovine serum, and culture it in a 5% CO2, 37°C incubator. When the cells grew to about 90%, they were passaged by trypsin digestion and cultured for 3-6 generations for subsequent experiments.

3.5. Cell viability assay (MTT)

SFbs (5×10^3/well) were inoculated in 96 well plates and incubated overnight at 37 °C with 5% CO_2. Then, treat with LHT (7.5, 15, 30 mg/mL) for 24 h under the condition of serum free. The cells were washed with phosphate-buffered saline (PBS) twice and further incubated with MTT (5 mg/mL) for 4 h. After discarding the culture solution, add DMSO into each well 100μL. The optical density (OD) value is read at 590 nm by Bio Rad microplate reader. Microplate Manager®6 software was used for analysis. Calculate the inhibition rate of SFbs treated with LHT compared with untreated cells (n=3): inhibition rate compared with untreated cells (%) = [1 - (average value of treated cells)/(average value of untreated cells)] × 100%.

3.6. Gelatin Zymography analysis

The experiment was carried out according to the method reported in reference [14, 15]. The sample was diluted with PBS, and then mixed with SDS-PAGE non reducing buffer solution (0.5 mol/L Tris, glycol, 10% SDS, 0.1% bromophenol blue) of twice the concentration of the same volume. Use 8% separating gel and run gel vertically (100 V) until reaching the bottom. Then use 2.5% Triton X-100 eluent at room temperature, place it in a shaker (60 r/min), and elute it for 3 times, 20 min/time. The gel was incubated overnight in an active incubation solution containing 50 mmol/L Tris, 0.2 mol/L NaCl, 5 mmol/L CaCl2 and 0.02% Triton X-100 at 37 °C. The next day, dye with 0.5% Coomassie brilliant blue R-250 for 30 min, and then elute with V (methanol): V (acetic acid): V (water)=5 : 1 : 4 eluent until a clear white band appears on the background. Photos were taken with bio rad gel imager and analyzed semi quantitatively with imagelab software.

3.7. In-vitro cell-gap closure assessment (cell migration and proliferation)

SFbs were seeded into 24-well plate and cultured overnight. The cell-gaps were made with fine pipette-tips after cell-monolayer confluence, and then washed with PBS to remove the debris; and treated with LHT of different concentrations (15, 30 mg/mL) in serum free condition. Take pictures at 4, 8,16, 24 hours with the inverted microscope, calculate the width of the cell gap according to the pictures, and calculate the closure rate of the cell gap (%)=[1- (width of the cell gap at 8, 16, 24 hours)/(width of the cell gap at 4 hours)] × 100%.

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3.8. Western blot

The total protein in serum or cell lysates was determined using BCA assay kit (Sigma-Aldrich). Equal amount of protein in each sample was loaded and separated by 4–12% gradient SDS-PAGE; then transfer to PVDF membrane. The membranes were blocked with 2% BSA in PBS containing 0.1% Tween 20 (TPBS) at room temperature for 1 h, and incubated overnight at 4°C with corresponding primary antibodies primary antibodies against IL-6, IL-1β, TNF-α, TGF-β, Caspase-3, Bax, Bcl-2 and GAPDH; and then horseradish peroxidase-linked secondary antibody for 1h at room temperature, and enhanced chemiluminescent for signals. The images were captured using a ChemiDoc CRS + Molecular Imager (Bio-Rad Laboratories, USA).

3.9. Immunocytochemistry (ICC)

SFbs were inoculated on the sterile glass coverslips (1.5×10^4 cells / piece) in 24 well plates and treated with or without LHT (15, 30 mg/mL). After 24 h, cells on coverslips were fixed with 100% chilled methanol for 30 min at 4°C; then incubated with 0.05% Triton X-100 aqueous solution for 15 min at 37°C and blocked with 3% BSA for 1 h at room temperature. All primary antibodies against focal adhesion kinase (FAK) were diluted in 3% BSA and incubated for 120 minutes at room temperature, and fluorescent conjugated secondary antibodies were applied, cell nuclei staining with propidium iodide (100μg/mL).Combine images using immunochemical microscope (X40/X100) (Carl Zeiss, ZEN-3-0-blue-Hotfix-4).

3.10. Statistics

Prism software version 8.0 was used for data analysis and graph preparations (Graph pad Prism software). All values are represented as the mean ± the standard error of the mean (SEM). The Mann-Whitney non-parametric t test was used to compare differences between groups. The Person r test was applied for the degree of association between variables. $^{*}P < 0.05$ was taken as significant; $^{**}P < 0.01$ very significant; $^{***}P < 0.001$ very very significant.

4. Result

4.1. LHT alleviated arthritis index and paw thickness in CIA rats

After immunization, the paw joint of CIA group and LHT group showed obvious swelling. Compared with the normal group, the arthritis index and paw thickness of CIA group increased significantly at day 12, 20 and 28 (P<0.01); Compared with CIA group, the arthritis index and paw thickness of LHT group decreased significantly at day 20, 28 (P<0.05, P<0.01) (Table 1 and Table 2).

Table 1: Arthritis index of CIA rats and LHT treated CIA rats ($\bar{x}\pm s$, n=6).

group	Day 4	Day 12	Day 20	Day 28
CIA	3.52±0.63	8.56±1.57**	10.51±2.68**	11.13±2.73**
LHT+CIA	3.84±0.34	7.87±3.35	4.95±2.84$^{\#\#}$	4.38±1.45$^{\#\#}$

Note: Compared with the normal group, $^{**}P$<0.01; Compared with CIA group, $^{\#\#}P$<0.01.

Table 2: Comparison of paw thickness of rats between groups ($\bar{x}\pm s$, n=6).

group	Day 4	Day 12	Day 20	Day 28
Normal	3.15±0.74	3.18±0.46	3.25±0.38	3.26±0.83
CIA	3.29±0.23	4.98±1.03**	5.67±1.35**	4.08±1.54**
LHT+CIA	3.16±0.42	4.09±1.22	4.08±1.86$^{\#}$	4.12±1.01$^{\#\#}$

Note: Compared with the normal group, **P<0.01; Compared with CIA group, $^{\#\#}$P<0.01 and $^{\#}$P<0.05.

4.2. LHT inhibited MMP-2/9 activity in serum of CIA rats

From the gelatin zymogram and quantitative analysis of band density (Figure 1), LHT can significantly inhibit the activity of MMP-2/9 in the serum of CIA rats (P<0.05).

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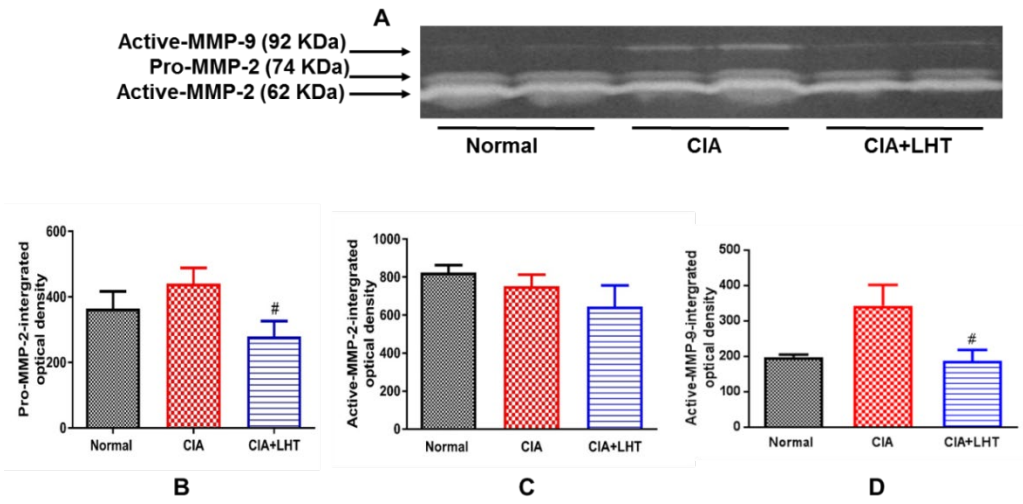

Figure 1: Effects of LHT on matrix metalloproteinase-2/9 (MMP-2/9) in serum of rats in three groups. (A) The images and integrated optical density quantitation analysis of the bands within the gels for MMP-2/9; (B) The integrated optical density quantitation analysis of Pro-MMP-2; (C) The integrated optical density quantitation analysis of MMP-2; (D) The integrated optical density quantitation analysis of MMP-9. $^{\#}P < 0.05$ vs CIA group.

4.3. LHT reduced the inflammatory factor levels in CIA rats

Compared with the normal rats, the serum levels of IL-6, IL-1β, TNF-and TGF-β of CIA rats were increased significantly (P<0.01); Compared with untreated-CIA rats, LHT treatment significantly decreased the serum levels of IL-6, IL-1β, TNF-and TGF-β (P<0.01) (Figure 2, Table 3).

Table 3: Comparison of expression levels of inflammatory factors in each group ($\bar{x}\pm s$，n=3).

group	IL-6	IL-1β	TNF-α
Normal	91.05±17.39	446.31±62.34	163.55±30.55
CIA	228.92±43.92**	1 278.42±279.99**	493.53±43.76**
LHT+CIA	123.55±25.95$^{\#\#}$	645.84±103.44$^{\#\#}$	229.50±38.39$^{\#\#}$

Note: Compared with the normal group, **P<0.01; Compared with CIA group, $^{\#\#}$P<0.01 and $^{\#}$P<0.05.

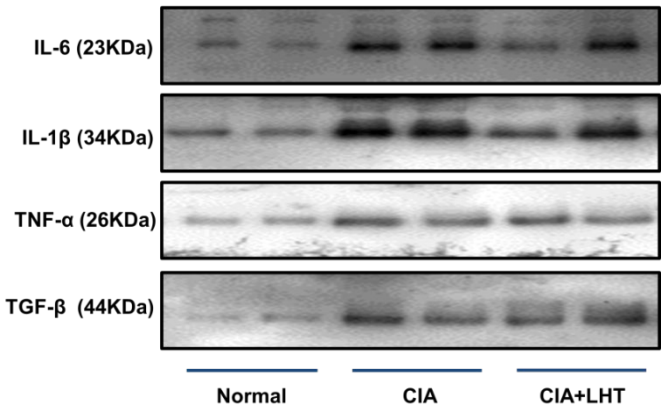

Figure 2: LHT reduced the serum level of IL-6, IL-1β, TNF-α and TGF-β in CIA rats in vivo. The serum samples collected at the final day of execution of rats in three groups, the levels of IL-6, IL-1β, TNF-α and TGF-β were detected by Western blotting and specific antibodies. .The images were taken by ChemiDoc CRS + Molecular Imager.

4.4. LHT decreased the activity of SFbs in vitro

Cell viability assay (MTT method) showed that LHT significantly reduced cell viability of SFbs after 24 hours treatment (Figure 3). Compared to the untreated cells, the inhibition rates of LHT (15, 30 mg/mL) treated cells were 36.95% and 72.81% respectively, which could effectively inhibit the

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proliferation of SFbs ($P<0.05$, $P<0.01$). There was no significant difference in the low dose LHT (7.5 mg/mL) treated cells, so the concentrations of LHT (15, 30 mg/mL) were selected as the intervention condition for the subsequent cell experiments.

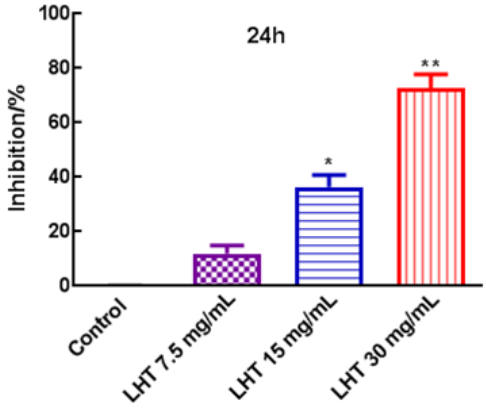

Figure 3: LHT inhibited the SFbs viability. SFbs were treated with different concentrations of LHT (7.5, 15, 30 mg/mL) for 24h.

4.5. LHT inhibited SFbs migration

Cell-gap closure assessment (cell migration and proliferation) used to observe the effect of LHT (15, 30 mg/mL) at different times (4, 8, 16, 24 h) on the migration of SFbs (Figure 4A). The results showed that the width of cell-gap in LHT 30 mg/mL group decreased significantly with the increase of time, and the lowest value appeared at 24h ($P<0.01$); After treated with LHT (15 mg/mL), the width of cell-gap decreased gradually, but there was no significant difference compared with the control group(Figure 4B). It can be observed that with the increase of time, the intervention of LHT (15, 30 mg/mL) inhibits cell migration; Compared with no intervention, LHT reduced the rate of gap closure (Figure 4C).

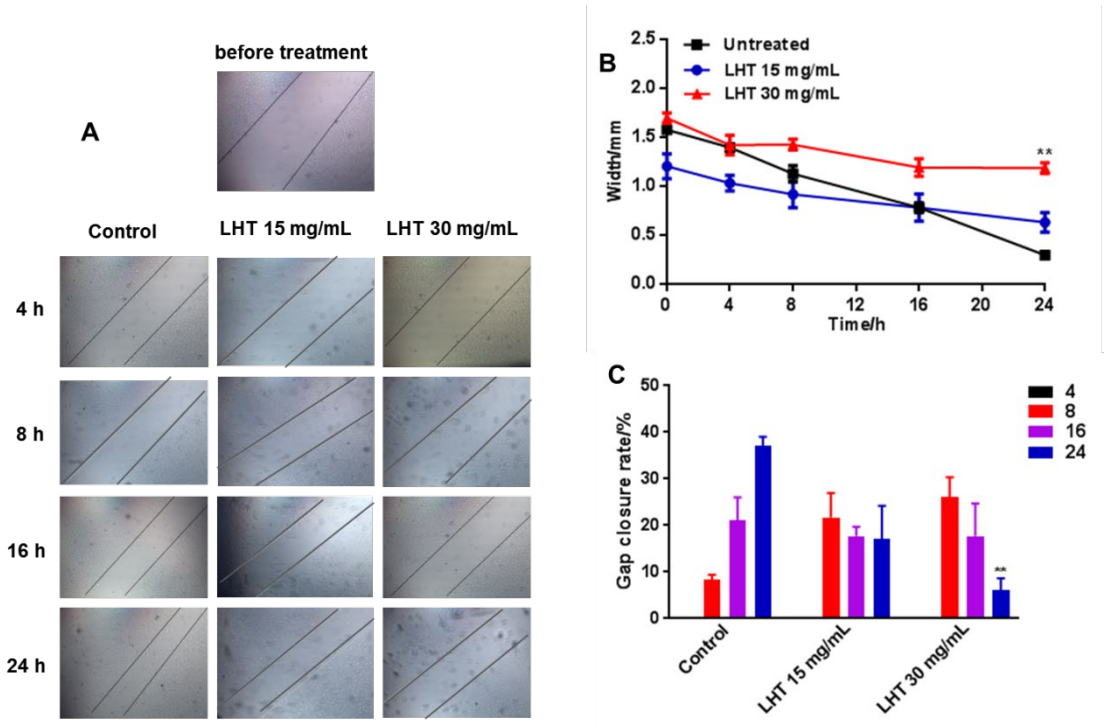

Figure 4: LHT inhibited SFbs migration and proliferation. (A) Healing of scratches photographed at different times; (B) Changes of cell gap width in each group; (C) The change of cell gap closure rate in each group. $^{**}P < 0.01$ vs untreated control cells.

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4.6. LHT decreases MMP-2 activity in SFbs

The result of gelatin zymography analysis showed that the MMP-9activity of SFbs had no significant change after 24 hours treated with LHT (15, 30 mg/mL); but the MMP-2 activity was decreased significantly ($P<0.05$, $P<0.01$) (Figure 5).

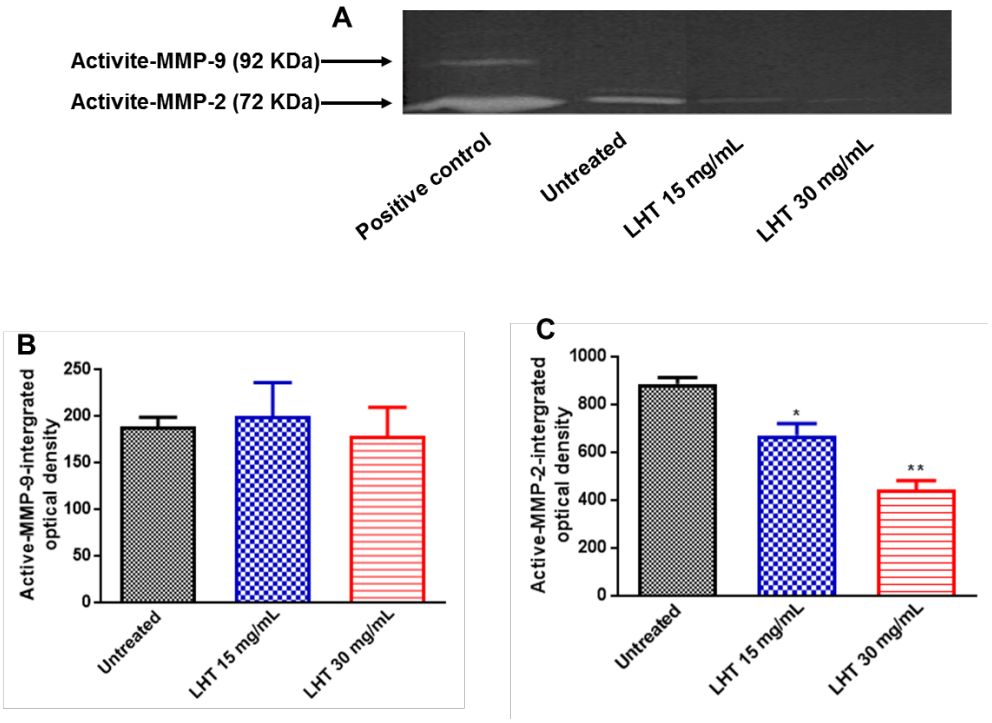

Figure 5: Effects of LHT on MMP-2/9 activities of SFbs. (A) The images of the bands within the gel for MMP-2/9; (B) The integrated optical density quantitation analysis of MMP-9; (C) The integrated optical density quantitation analysis of MMP-2. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs untreated cells.

4.7. LHT decreased FAK expression in SFbs

The data from immunocytochemistry illustrated that compared with untreated cells, FAK expressions were decreased by LHT treatment (Figure 6).

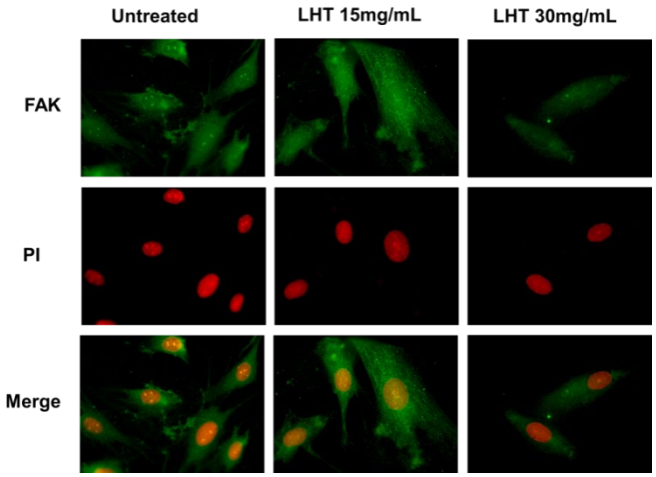

Figure 6: LHT suppressed FAK expression of SFbs. SFbs treated with or without LHT (15, 30 mg/mL) for 24h. Images were taken and combined using immunochemical microscope (Carl Zeiss, ZEN-3-0-blue-Hotfix-4, X100). The green fluorescence indicates FAK expression and the red fluorescence is propidium iodide (PI) for cell nucleus stain.

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4.8. LHT promoted apoptosis of SFbs

The data from western blot showed that LHT increased the expression of Caspase-3 and Bax in SFbs, while LHT significantly reduces the expression of Bcl-2 (Figure 7).

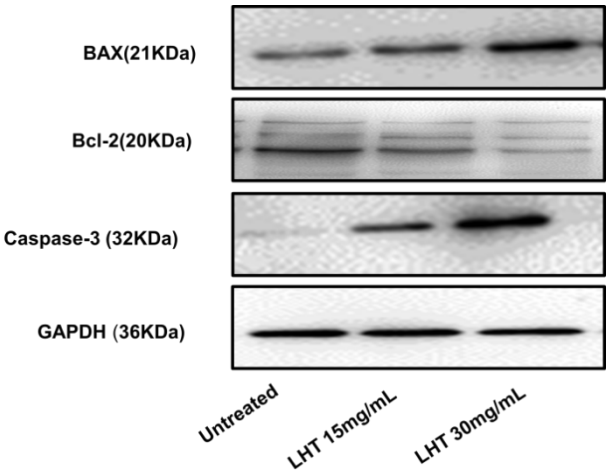

Figure 7: LHT regulated Bax, Bcl-2 and Caspase-3 protein levels in SFbs. SFbs treated with or without LHT (15, 30 mg/mL) for 24h. Western blotting and specific antibodies were applied to detect the protein expressions. The images were taken ChemiDoc CRS + Molecular Imager.

5. Discussion

Menghe traditional academic genre is one of the major Chinese medicine academics, originating from Menghe Town, Changzhou, China. In this study, a special self-prescription LHT of Menghe for treating "Bi syndrome" (RA), was selected from the book "*Yi Chun Sheng Yi (1863)*". According to the literature, Cistanche deserticola [10], Codonopsis pilosula [11], Poria cocos [12], Atractylodes macrocephala Koidz [13], Angelica sinensis [14] and other drugs in LHT have the effects of regulating immunity and anti-inflammation, Radix Paeoniae Alba has immunosuppressive function and been widely used in the treatment of RA and related research[15, 16]. The results of this study show that LHT can significantly delay the occurrence of joint inflammation, reduce the inflammation score and joint swelling, and prevent joint damage and deformation in rats.

Since the main pathological features of CIA rats and humans rheumatoid are the same, including synovial hyperplasia, infiltration of immune granulose cells, cartilage destruction, etc.[17], CIA animal models have been widely used to study the pathogenesis of RA [18]. Therefore, we established a CIA rat model to investigate the effects of LHT on RA in vivo. The occurrence and development of rheumatoid arthritis is closely related to the imbalance of inflammatory transmitters and cytokines. TNF-α, IL-1β, IL-6 are the main factors of inflammatory damage and immune response of the body, and secreted by monocytes and macrophages. TNF-α, IL-1β, IL-6 can stimulate and activate SFbs to produce MMPs, cause synovitis and cartilage damage, and accelerate osteolysis, as a key link in the pathological process of RA [19]. TGF-β can act as a proinflammatory factor in stimulating the secretion of cytokines such as TNF-α and IL-1β, acting as a potent chemoattractant for neutrophils, activating chemokines[20]. The results of this study show that LHT can reduce serum levels of IL-6, IL-1β, TNF-α and TGF-β in CIA rats, decrease the expression of MMP-2/9, associated with the reduced swelling of ankle joint and the arthritis index of CIA rats, indicating that LHT can relieving RA symptoms by suppressing inflammatory cytokines and the activity of enzymes.

SFbs are the key effector cells in the pathogenesis of RA, which can secrete a large number of inflammatory cytokines and aggravate the progress of the disease [21]. Stimulation of inflammatory signals can cause abnormal proliferation of SFbs, which is an important pathological mechanism of RA [22]. LHT could inhibit the activity, proliferation and migration of SFbs, and significantly reduce the activity of MMP-2; reflecting its anti-inflammatory effect. FAK links cytoplasmic cytoskeleton to ECM, MMP-2 and MMP-9 is involved in ECM remolding and associated with cell adhesion; the activity of FAK elicits intracellular signal transduction pathways that enhance the turn-over of cell contacts with the ECM, promoting cell migration [23, 24]. Interestingly, our data clearly shows that LHT can decrease the expression of FAK, implicating that it has an effect on the migration of SFbs. Bax, Bcl-2 and Caspase-3

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are key molecules in the process of cell apoptosis [25]. The activation of Bax/Bcl-2 and Caspase-3 families causes a cascade of cell apoptosis and leads to cell apoptosis [26]. LHT induced cell apoptosis of over-activated SFbs by decreasing Bcl-2 expression and up-regulating Bax, Caspase-3 expressions in vitro.

6. Conclusion

In conclusion, this study verified the pharmacological mechanism of LHT in relieving rheumatoid arthritis using in vivo rat model and in vitro cell model. LHT achieved this by reducing the levels of inflammatory factors and enzymes, and inhibiting the excessive activation, proliferation and migration of SFbs. This study has provided a theoretical basis and experimental evidences for the modem clinical application of Chinese traditional LHT.

Acknowledgements

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