2016 1(2) 1-5

Submission: 2016/11/10, Peer review: 2016/11/20, Accepted: 2016/11/25, Published: 2016/12/30

International journal of sport

Publication state: Japan
ISSN: 2423-8287

Publisher: J-INSTITUTE
Website: http://www.j-institute.jp

Corresponding author
E-mail: yoont114@hanmail.net

Peer reviewer
E-mail: editor@j-institute.jp

http://dx.doi.org/10.22471/sport.2016.1.2.01

A Candidate SPORTS Nutrition Supplement, Lonicerae Flos, Attenuates Collagen-Induced Arthritis in Mice

Yoon Tae-gun[1], Hwang Byong-yong[2]*

Yongin University, Yongin, Republic of Korea

Ahn Sang-hyun[3]

Semyung University, Jecheon, Republic of Korea

Kim Ki-bong[4]

Pusan National University, Pusan, Republic of Korea

Abstract

Rheumatoid arthritis(RA) can occur in persons of all ages and of both sexes. Diverse studies recommend exercise to help diminish pain from RA symptoms. Although exercise for patients with RA is an effective therapy to improve functional impairment, patients experience reluctance to exercise because of pain. Several studies have focused on investigating the treatments for this disease. However, RA is currently an incurable disease because its pathogenic cause is still unclear. In view of the complex considerations, the treatment of RA with exercise requires more effective research for developing therapies with proven stability. For example, natural compounds are potential therapeutic agents and candidates as sports nutrition supplements. In the present study, we aimed to find a candidate sport nutrition supplement from natural compounds such as foods and tea. Lonicerae flos(LF) has been widely used in Korean traditional medicine and as a tea material. Therefore, we evaluated the effect of LF extract on an RA model. First, we found that the mRNA expression of macrophage migration inhibitory factor(MIF), cyclooxygenase-2(COX-2), and matrix metalloproteinase-9(MMP-9) in synoviocytes stimulated with phorbol-12-myristate-13-acetate was decreased by LF treatment(0.4–1.0 mg/mL). Furthermore, the distribution of MIF-, COX-2-, and MMP-9-positive cells in mice with collagen-induced arthritis treated with LF(45 mg/kg) was remarkably decreased. These data likely indicate that LF may act as an anti-inflammatory agent and may be a potential compound for the development of useful agents for RA treatment.

[Keywords] ***Sport Nutrition, Lonicerae Flos, Inflammation, Natural Compounds, Rheumatoid Arthritis***

1. Introduction

Rheumatoid arthritis(RA) is known to affect approximately 1% of the worldwide population. According to the National Health and Nutrition Survey in 2005, the incidence of RA in Korea was 21.1 per 1000[1]. RA is a chronic disease that can lead to synovitis, consequently inducing joint deformity and damage. Diverse studies have suggested that the etiology of RA is multifactorial, involving various genetic and environmental factors, autoimmune functions, and pathogenic mediators. Unfortunately, RA is still characterized as a chronic disease of unknown etiology, and the complete cure for this disease is yet unclear.

Exercise contributes to maintaining health and is associated with significant increases in the function of the immune system and in biological activities[2]. The review article by Metsios et al. suggested that therapy with prescription and exercise was a potential intervention for improving RA[3]. Because the medications for RA consist of disease-modifying anti-rheumatic drugs, biological response modifiers, glucocorticoids, and nonsteroidal anti-inflammatory medications, with the risk of adverse effects from long-term administrations, the treatment for RA requires more effective research for developing therapies with proven stability[4].

Especially, natural compounds may improve the risk of adverse effects. Lonicerae flos(LF) has been commonly used as a tea material. Some studies showed that the diverse pharmacological actions of LF extract include anti-inflammatory, anti-oxidant, and anti-cancer effects. However, the effects of LF on RA mediation have not yet been established. Hence, we designed this study to determine whether LF can mitigate RA through the inhibition of cyclooxygenase(COX)-2 and matrix metalloproteinase(MMP)-9 by means of regulation of macrophage migration inhibitory factor(MIF).

2. Methods

2.1. Materials

All cell culture materials and the MTT assay kit were purchased from Sigma, Gibco BRL(Gaithersburg, MD, USA). Antibodies such as anti-COX-2 and anti-MMP-9 were purchased from Santa Cruz Biotechnology(Santa Cruz, CA, USA). All other chemicals were purchased from Sigma(St. Louis, MO, USA).

2.2. Design of experiments

To determine the effect LF in a collagen-induced RA model, we performed reverse transcription polymerase chain reaction(RT-PCR), histological analysis, and immunohistochemistry.

2.3. Procedure

2.3.1. Cell isolation and culture

Infrapatellar fat pads(IFPs) were harvested from the knees of mice. Ten IFPs were minced and digested in 1 mg/mL collagenase(Wako, Osaka, Japan) for 15 min and passed through a 40-mm filter(Becton Dickinson, Franklin Lakes, NJ, USA). Dissociated cells and undigested IFPs were cultured together in a 100-mm culture dish. The mouse synovium cells(mSCs) were cultured in Dulbecco's modified eagle medium(DMEM) containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a 5% CO2 atmosphere. Primary cells were seeded at 5 × 105 cells/well in six-well plates containing DMEM, and incubated for 24 h. Cells were incubated with different concentrations of LF(0.4, 0.6, 0.8, and -1.0 mg/mL) in phorbol 12-myristate 13-acetate(PMA)-free medium or PMA-containing medium for 24 h.

2.3.2. RT-PCR

After cell culture, total RNA was isolated from cells by using TRI-reagent, and then cDNA was synthesized by using 1.0 μg total RNA in a Superscript II Reverse Transcription System. PCR amplification was performed with the following protocol: pre-denaturation at 95°C for 3 min and then either 30 cycles of denaturation at 94°C for 1 min, annealing at the melting temperature of each primer for 1 min, or extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. mRNA expression was quantified by using an ethidium bromide-stained 1.5% agarose gel. The following primers were used for the PCR reaction: MIF, sense primer 5'- CAC CAT GCC TAT GTT CAT CGT GAA CA-3', anti-sense primer 5'-GGG CTC AAG GCG AAG GTG GAA CCG TT-3'; COX-2, sense primer 5'-TCT CCA ACC TCT CCT ACT AC-3', anti-sense primer 5'-GCA CGT AGT CTT CGA TCA CT-3'; MMP-9, sense primer 5'-AAG CCT CTA CAG AGT CTT TG-3', anti-sense primer 5'-CAG TCC AAC AAG AAA GGA CG-3'; and β-actin, sense primer 5'-GGA GAA GAT CT GCA CCA CACC-3', anti-sense primer, 5'-CCT GCT TGC TGA TCC ACA TCTGCT GG -3'.

2.3.3. Animal care and analysis

The animals(DBA mice, 6 weeks; n = 20) were randomized into two groups: collagen-induced arthritis(CIA) group(CE group)[5] and RA treated with LF group(LT group). LF was dissolved in water and administered to the LT group at 45 mg/kg orally for 28 days. Animal care and all experiments were conducted in conformity with the institutional guidelines of Pusan National University(Institutional Animal Care and Use Committee no. PNU-2016-1290), South Korea, and conformed to the Guide for the Care and Use of Laboratory Animals from the US National Institutes of Health(publication no. 85-23, revised 2011). Segments from each half were embedded in paraffin, and 5-μm sections were prepared,

cleared with xylene, and hydrated with ethanol. The sections were stained with hematoxylin and eosin and observed with a BX51 light microscope. The slides with sections were treated with avidin–biotin block, exposed to diaminobenzidine with hematoxylin, and analyzed under a light microscope.

2.3.4. Statistical analysis

The results are expressed as the mean ± standard error of at least three independent experiments. The difference between the two groups was examined by using Student's t-test. A p-value of <0.05 was considered statistically significant. Statistical analysis was carried out with GraphPad Prism 4.0 software.

3. Results

3.1. LF attenuates the expression of MIF, COX-2, and MMP-9 in PMA-stimulated mSCs

As shown in <Figure 1>, PMA(1 ng/mL) significantly increased the expression levels of MIF, COX-2, and MMP-9 in mSCs. In contrast, this enhanced expression of MIF, COX-2, and MMP-9 was inhibited by treatment with LF(0.4–1.0 mg/mL) in a concentration-dependent manner. The expression levels of MIF, COX-2, and MMP-9 showed a maximal response at 1 mg/mL LF.

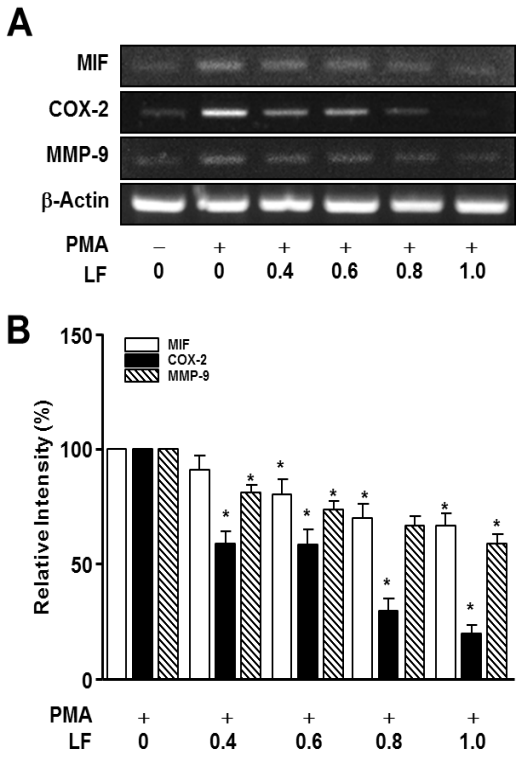
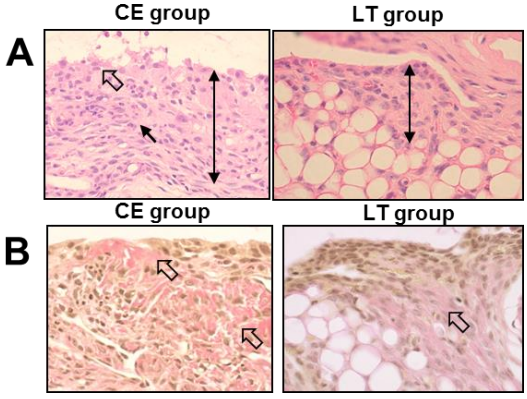


Figure 1. Effect of lonicerae flos(LF) extract on the expression of MIF, COX-2, and MMP-9 in phorbol 12-myristate 13-acetate(PMA)-stimulated mSCs. Primary cultured mSCs were incubated in the absence or presence of PMA(1 ng/mL) with LF(0.4, 0.6, 0.8, and 1.0 mg/mL) for 24 h, and then the total RNA was isolated. (A) Agarose gel image showing MIF, COX-2, and MMP-9 mRNA of expression in mSCs after LF treatment. (B) Relative band intensities of MIF, COX-2, and MMP-9. Data are means ± standard error(*p < 0.05 compared with the untreated group).

3.2. LF reduces damage in CIA-induced RA mice

As shown in <Figure 2>, LF(45 mg/kg) administration significantly reduced the synovial cell hyperplasia in the CIA-induced RA model <Figure 2A>. Moreover, LF administration reduced the fibrosis in the CIA-induced RA model <Figure 2B>.

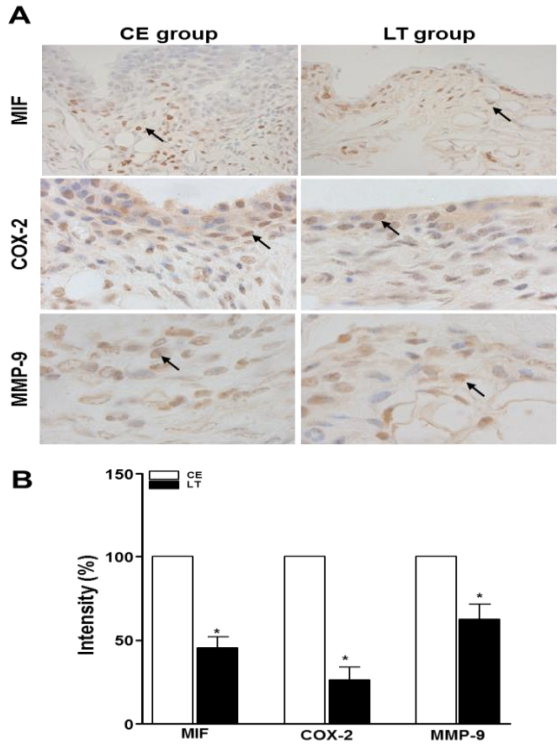
Figure 2. Effect of lonicerae flos(LF) extract on the CIA(collagen-induced arthritis)-induced RA model. (A) Inhibition of damaged synovial membrane as filopodia (wide open arrow), hyperplasia of synoviocytes (↕) ;(hematoxylin and eosin, ×400) (B) Inhibition of fibrosis (wide open arrow) ;(van Gieson's, ×400).



3.3. LF modulates the expression of MIF, COX-2, and MMP-9 in CIA-induced RA mice

We used immunohistochemistry assay for COX-2 and MMP-9 to identify the inflammatory response in the synovial tissue of the RA model. As shown in <Figure 3>, the intensity of COX-2 and MMP-9(arrow indicates positive cells) was lower in the LF-administration group than in the vehicle-treated group.

Figure 3. Effect of Lonicerae flos(LF) extract on the expression of MIF, COX-2, and MMP-9 in CIA (collagen-induced arthritis)-induced RA model. (A) Expression levels of MIF, COX-2, and MMP-9 in the CE and LT groups. (B) Relative expression intensities of MIF, COX-2, and MMP-9. Data are means ± standard error(*p < 0.05 compared with the CE group).



4. Discussion and Conclusion

Although the complete therapy for RA is still unknown, a common pathological phenomenon in patients with RA is chronic inflammation. Abnormal immune response is relevant to inducing serious joint damage by pro-inflammatory cytokines, MIF, and type II collagen. In the viewpoint of enzyme signals, elevated levels occur mostly in inflamed tissue, whereas unexpressed levels are found in the normal condition. Elevated MMP-9 is also involved in the inflammatory indication[5].

The inflammatory process of the synovium in RA indicates synovial cell hyperplasia and fibrosis. In addition, excessive expression levels of COX-2 and MMP-9 are also identified in RA. In the present study, we investigated whether LF can regulate RA through the regulation of COX-2 and MMP-9 in vitro and in vivo. Our results showed that LF treatment significantly reduced inflammation in the RA model. In conclusion, we recommend the combination therapy with exercise and biological supplementation for RA. Rehabilitative exercise may be effective for patients with RA, and the prescription against RA demand excluding these risk factors in the long term.

5. References

5.1. Journal articles

[1] Hur NW & Choi CB & Uhm WS & Bae SC. The Prevalence and Trend of Arthritis in Korea: Results from Korea National Health and Nutrition Examination Surveys. *Journal of Rheumatic Disease*, 15(1), 11-26 (2008).

[2] Kim JS & Lim KW & Hwang YY. Research Trend of Sport Nutrition Science in the Republic of Korea. *International Journal of Sport*, 1(1), 1-7 (2016).

[3] Metsios GS & Stavropoulos KA & Kitas GD. The Role of Exercise in the Management of Rheumatoid Arthritis. *Expert Review of Clinical Immunology*, 11(10), 1121-1130 (2015).

[4] Smolen JS & Aletaha D & McInnes IB. Rheumatoid Arthritis. *Lancet*, 38(10), 2023-2038 (2016).

[5] Trentham DE & Townes AS & Kang AH. Autoimmunity to Type II Collagen: An Experimental Model of Arthritis. *Journal of Experimental Methods*, 146, 857-868 (1997).

Lead Author
Yoon Tae-gun / Yongin University Researcher
B.S. Dongju University

Research field
- A Candidate Spores Nutrition Supplement, Lonicerae Flos, Attenuates Collagen-induced Arthritis in Mice, International Journal of Sport, 1(2) (2016).

Major career
- 2016~present. Yongin University Department of Physical Therapy, Researcher
- 2016~present. International Society for Sport Science, Member

Corresponding Author
Hwang Byong-yong / Yongin University Professor
B.S. Korea National Open University
M.S. Yonsei University
Ph.D. Keimyung University

Research field
- Effects of Proprioceptive Control on the Balance in Patients with Chronic Hemiplegia, Physical Therapy Korea, 11(1) (2004).
- The Effects of the Proprioceptive Control and Visual Feedback for the Limits of Stability in Patients with Chronic Hemiplegia, The Journal of Korean Society of Physical Therapy, 19(6) (2007).

Major career
- 2003~2013. Korean Bobath Association, President
- 2006~pesent. Kliniken Schmieder, Education Consultant

Co-Author
Ahn Sang-hyun / Semyung University Professor
B.S. Dongguk University
M.S. Dongguk University
Ph.D. Dongguk University

Research field
- Protective Effect of Areca Catechu Leaf Ethanol Extract against Ethanol-induced Gastric Ulcers in ICR Mice, Journal of Medicinal Food, 19(2) (2016).
- Hataedock Treatment Has Preventive Therapeutic Effects in Atopic Dermatitis (AD)-induced NC/Nga Mice under High-fat Diet Conditions, Evidence-based Complementary and Alternative Medicine (2016).

Major career
- 1992~present. Korean Anatomy Association, Member
- 1993~present. Korean Immunology Association, Member

Co-Author
Kim Ki-bong / Pusan National University Professor
B.S. Dongguk University
M.S. Dongguk University
Ph.D. Dongguk University

Research field
- Douchi(Fermented Glycine Max Merr) Alleviates Atopic Dermatitis-like Skin Lesions in NC/Nga Mice by Regulation of PKC and IL-4, BMC Complementary and Alternative Medicine, 16(1) (2016).
- Hataedock Treatment Has Preventive Therapeutic Effects in Atopic Dermatitis (AD)-induced NC/Nga Mice under High-fat Diet Conditions, Evidence-based Complementary and Alternative Medicine (2016).

Major career
- 1999~present. Korean Pediatrics Association, Member
- 1999~present. Korean Medicine Association, Member