



Anti-rheumatoid arthritis effects of traditional Chinese herb couple in adjuvant-induced arthritis in rats



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ABSTRACT

Ethnopharmacological relevance: *Clematis chinensis* Osbeck / *Notopterygium incisum* Ting ex H, T-Chang (CN) is a traditional Chinese herb couple with prominent efficacy. The herb couple has been commonly used for clinical treatment of arthralgia syndrome (“Bi Zheng” in Chinese) for centuries in China, including rheumatic arthritis, osteoarthritis and gout in modern medicine.

Aim of the study: To evaluate the anti-arthritis effect of CN herb couple in a rat model of rheumatoid arthritis (RA).

Materials and methods: Rats were divided randomly into six groups with eight each. Adjuvant-induced arthritis (AIA) model was established by intradermal injection of complete Freund's adjuvant (CFA). Rats were treated orally with different dosages of CN (0.7 g/kg, 2.1 g/kg, 6.3 g/kg) from day 16 till day 40. Ibuprofen (50.4 mg/kg) served as a positive control. Spontaneous activity, body weight, paw swelling, and arthritis index (AI) were monitored throughout drug treatment. Then serum levels of tumor necrosis factor α (TNF- α), interleukin-6 (IL-6), and vascular endothelial growth factor (VEGF) were determined by enzyme linked immunosorbent assay (ELISA) kits. In addition, histopathological examination and immunohistochemistry were used to assess the severity of arthritis.

Results: Three dosage of CN significantly ameliorated symptoms of RA via increasing body weight as well as reducing paw swelling (at dose of 6.3 g/kg, $p < 0.01$) in AIA rats. An extremely significant reduction of AI ($p < 0.001$) was also observed with treatment of CN (6.3 g/kg) compared with model group. In parallel, treatment of CN significantly down-regulated levels of TNF- α , IL-6, and VEGF both in serum ($p < 0.01$) and in joint synovial compared with model rats. And histopathology revealed noticeable reduction in synovial hyperplasia, cartilage damage, and inflammatory infiltration by CN treatment, especially at dose of 6.3 g/kg.

Conclusions: To conclude, all results suggest that CN possesses evident anti-arthritis effects in AIA rats.

1. Introduction

Rheumatoid arthritis (RA) is a kind of systemic autoimmune disease characterized by chronic progressive symmetrical synovitis and destruction of multiple joints (Scott et al., 2010). Generally, small diarthrodial joints are primarily affected in the progression of RA, while large joints and extra-articular tissues can also be involved if uncontrolled (Bax et al., 2011; McInnes and Schett, 2011). Typical pathological findings of RA are synovial hyperplasia, inflammatory cell infiltration, pannus formation, articular cartilage damage, bone erosion

as well as extra-articular manifestations (Firestein, 2003; Lee and Weinblatt, 2001).

RA affects 0.5–1% of global population, and varies considerably among age, gender, ethnic and geographic groups (Alamanos and Drosos, 2005). Further, genetic, non-genetic environmental factors and interaction were implicated in the initiation and progression of RA (Lerner and Matthias, 2015). The disease leads to a chronic syndrome of pain, stiffness and joints swelling, eventually causing deformity, dysfunction and disability. All of these lead to great decline in the quality of life. What is more, systemic complications of RA, especially

Abbreviations: RA, rheumatoid arthritis; CC, *Clematis chinensis* Osbeck; NI, *Notopterygium incisum* Ting ex H, T-Chang; CN, *Clematis chinensis* Osbeck / *Notopterygium incisum* Ting ex H, T-Chang; TCM, traditional Chinese medicine; AIA, Adjuvant-induced arthritis; CFA, complete Freund's adjuvant; AI, arthritis index; TNF- α , tumor necrosis factor α ; IL-6, interleukin-6; VEGF, vascular endothelial growth factor; ELISA, enzyme linked immunosorbent assay; NSAIDs, non-steroidal anti-inflammatory drugs; DMARDs, disease-modifying antirheumatic drugs; H & E, hematoxylin and eosin

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cardiovascular and neurologic disorders could also increase patients' mortality (Scott et al., 2010). RA has been ranked as a higher contributor to global disability (Cross et al., 2014) and brought heavy economic burden to society (Furneri et al., 2012).

Currently, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), glucocorticoids, and biological agents are commonly used for RA clinical therapy according to diagnosis and treatment guidelines (Scott et al., 2010). However, most of them greatly contributed to severe adverse effects and comorbidities, such as gastrointestinal injury, cardiovascular risk and renal irritations (Obiri et al., 2014). In traditional Chinese medicine (TCM) theory, RA is considered as part of arthralgia syndrome ("Bi Zheng" in Chinese), with arthralgia and dyskinesia of the joints and limbs. The occurrence of Bi Zheng may be due to attack of the meridians of the limbs by exogenous wind, dampness, and heat or cold pathogens (Liu et al., 2013). Traditional Chinese medicines (TCMs), with advantage of prominent efficacy but less side effects and costs, have been used for therapeutic and prophylactic managements of diseases for centuries, and worthy of attention for RA therapy.

Herb couple, the simplest composition of Chinese herbal formulations, is characterized by both basic therapeutic features and special clinical efficacy. *Clematis chinensis* Osbeck (CC), the roots and rhizomes of the plant of genus *Clematis* (Ranunculaceae) named Weilingxian in Chinese, displays antioxidant, anti-inflammatory, analgesic (Chawla et al., 2012; Ding et al., 2009), anti-rheumatism (Wu et al., 2013), antibacterial, anticancer and diuretic activities (Wu et al., 2009). *Notopterygium incisum* Ting ex H, T-Chang (NI), the roots and rhizomes of the plant of the genus *Notopterygium* (Apiaceae) named Qianghuo in Chinese, is commonly used for colds, inflammation, headache (Atanasov et al., 2013), arthritis, analgesic (Liu et al., 2014), diaphoretics, and antifebrile agents (Wang and Huang, 2015). It was reported that the herbal composition comprising Radix Clematidis, Radix Angelicae Pubescentis, Rhizoma et Radix Notopterygii, Radix Saphoshnikoviae and Radix Gentianae Macrophyllae had preventive and therapeutic effects on relieving symptoms associated with inflammatory and arthritic diseases, such as acute or chronic rheumatoid arthritis, osteoarthritis, and infectious arthritis in humans and livestock (Li, 2004). Indeed, CC and NI have been extensively used for clinical treatment of Bi Zheng in TCM. They have potent effects of expelling wind, eliminating dampness, dissipating external cold pathogen, dredging the meridian passage and relieving pain according to the Chinese Pharmacopoeia (Committee, 2015). Our previous study was performed to explore the chemical compounds in the herb couple and total 69 compounds were tentatively identified, including coumarins, triterpenoid saponins, lignans, flavonoid, polyacetylenes, and alkaloids. Coumarins and triterpenoid saponins were quantitatively dominant among all of the components. Moreover, eight ingredients namely nodakenin, bergaptol, bergapten, notopteron, isoimperatorin, bergamottin, hederagenin and oleanolic acid were quantified by HPLC-QTOF-MS (Cheng et al., 2016). To the best of our knowledge, the reports concerning the efficacy of CN for RA are limited. The present study was designed to evaluate the anti-arthritic effects of CN in vivo.

2. Materials and methods

2.1. Materials, reagents and instruments

The roots and rhizomes of *Clematis chinensis* Osbeck (Ranunculaceae) and *Notopterygium incisum* Ting ex H, T-Chang (Apiaceae) were purchased from Simcere Drugstore (Nanjing, China) and identified by Professor Ping Li (Department of Traditional Chinese Medicine, China Pharmaceutical University, Nanjing, China). The voucher specimen (No. 2014001 and 20141027, respectively) has been deposited in state key laboratory of natural medicines in China Pharmaceutical University. Ibuprofen sustained release capsules were

obtained from Simcere Drugstore (Nanjing, China). Complete Freund's adjuvant (CFA) was brought from Chondrex, Inc. (Redmond, WA, USA). Disodium ethylenediaminetetraacetate dihydrate was purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China); sodium carboxymethyl cellulose was purchased from Tianjin kemiu Chemical Reagent Co., Ltd. (Tianjing, China). ELISA kits of IL-6 and VEGF were purchased from SenBeiJia Biotechnology Co., Ltd. (Nanjing, China); ELISA kits of TNF- α was purchased from Guangzhou RayBiotech, Inc. (Guangzhou, China). Anti-goat IL-6 antibody, anti-goat TNF- α antibody and anti-rat VEGF antibody were acquired from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

2.2. Preparation of wine-processed CC and CN extracts

Crude CC was cut into sections with 15–20 mm length. Then the sections were mixed well with Chinese rice yellow wine (25 mL per 100 g) which was from Hualian supermarket (Nanjing, China), soaked for 45 min until rice wine were completely absorbed, and stir-fried with mild fire (120 °C).

Wine-processed CC and crude NI (650 g, respectively) were soaked overnight with 1300 mL 50% ethanol and extracted by reflux 3 times, 1 h for each time. The ethanol extract was filtrated and the combined filtrates were concentrated and dried. Then the CN extraction was dissolved with 0.5% CMC-Na to obtain suspensions of low, middle and high concentration (0.065 g/mL, 0.194 g/mL, and 0.580 g/mL), respectively. The suspensions were stored at 4 °C till use.

2.3. Animals

Grade SPF male Sprague-Dawley rats, with weight of 140–180 g, were purchased from Shanghai Sippr-B & K laboratory animal Ltd. (Shanghai, China). All animals were housed in temperature-controlled room (22 ± 2 °C) and free access to standard pelleted forage and tap water. All rats were adaptively fed for 7 days before experiments. All animal experiments were performed in accordance with the Animal Ethics Committee of China Pharmaceutical University.

2.4. Adjuvant-induced arthritis (AIA) and drug administration

After adaptive feeding, 48 rats were divided randomly into 6 groups with 8 each: normal control (Con), AIA model (Mod), ibuprofen (Ibu), CN low-dose (CN-L), CN middle-dose (CN-M), CN high-dose (CN-H). AIA rat model was induced by an intradermal injection of a single dose of 0.1 mL complete Freund's adjuvant (CFA) into the right hind footpad of rats except the control group (day 1). All drugs were administered orally at the same time every day from day 16–40 when AIA model was established. Rats in CN groups were treated with CN of different doses respectively (0.7 g/kg, 2.1 g/kg, 6.3 g/kg). The suspension of ibuprofen in 0.5% CMC-Na (50.4 mg/kg) was given as positive control. Meanwhile, rats in Con group and Mod group were treated with 0.5% CMC-Na.

2.5. Determination of weight and paw edema

During drug treatment, body weight and living state of rats were monitored every 4 days. And paw swelling was also evaluated in terms of the volume of right hind paw with PV-200 plethysmometer (Taimeng Technology Co., Ltd., Chengdu, China). Paw edema (%) = $(V_t - V_n) / V_n \times 100\%$, where V_n and V_t were the volume of right hind paw before and after induction, respectively. Original weight and paw volume were determined before modeling.

2.6. Evaluation of arthritis index (AI)

The pathologic changes of arthritis were observed every 4 days from day 12. The severity of arthritis was evaluated by a five-grade scoring

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