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Xitong Wan attenuates inflammation development through inhibiting the activation of nuclear factor-κB in rats with adjuvant-induced arthritis

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ABSTRACT

Ethnopharmacological relevance: Xitong Wan (XTW), a traditional Chinese herbs formula, has been used to treat "Bi Zheng" in the clinical practice of traditional Chinese medicine (TCM) for hundreds of years. However, no scientific validation is available on the anti-rheumatic effect of XTW.

Aim of study: This study was carried out to investigate the effects of XTW on joints swelling, joints destruction, production of inflammatory mediators and nuclear factor-κB (NF-κB) activation in rats with adjuvant-induced arthritis (AIA).

Materials and methods: AIA was induced by intradermal injection of Complete Freund's adjuvant in the footpad of Wistar rats. Paw volume was measured every 7 days during XTW treatment. Histological score was calculated by hematoxylin and eosin staining. Osteoclast number in articular tissues was counted by tartrate-resistant acid phosphatase staining. Levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 in serum were detected by enzyme-linked immunosorbent assay. Levels of NF-κBp65 and inhibitor of NF-κB (IκB)α in synovium were analyzed by Western blot assay.

Results: Compared with AIA group rats, XTW significantly decreased the paw volume of AIA rats. Meanwhile, XTW significantly reduced the histological score and osteoclast number in articular tissues of AIA rats. In addition, XTW markedly abated the levels of TNF-α, IL-1β and IL-6 in serum, as well as enhanced the level of IκBα in synovium of AIA rats. However, XTW did not show significant effect on the level of p65 in *syn*ovium of AIA rats.

Conclusions: These results suggest that XTW attenuates the inflammation development through inhibiting the NF-κB-mediated proinflammatory cytokines production in AIA rats. Our study provides the scientific evidence of XTW on treatment of rheumatoid arthritis in the clinical practice of TCM.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic synovitis and damage of cartilage and bone in multiple joints. Ultimately, RA may result in the joint destruction and deformity, working disability, as well as an increased mortality (Choi et al., 2009).

Proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, play a key role in the pathological process of RA, as evidenced by the positive correlation of joint destruction with the levels of proinflammatory cytokines in serum or arthritic tissues of RA patients (Siebert et al., 2015). Joint destruction in RA involves both cartilage degradation and bone erosion. Cartilage degradation is mediated by pannus in synovial tissue and bone erosion is mediated by osteoclasts, a cell population derived from the monocyte/macrophage lineage (Amarasekara et al., 2015). TNF-α, IL-1β and IL-6 all can contribute to the pannus formation and osteoclasts differentiation, maturation and activation (Jung et al., 2014). Transgenic mice overexpressing TNF-α exhibit a severe inflammatory and rapidly destructive arthritis (Keffer et al., 1991). Conversely, in experimental animal models of human RA, IL-1-deicient mice (Koenders et al., 2005) or IL-6 deficient mice (Sasai et al., 1999) show a diminished tissue damage and synovial infiltrate. Meanwhile, biologic drugs against TNF-α (Infliximab and Adalimumab), IL-1 (Anakinra) or IL-6 (Tocilizumab) have shown to reduce both disease activity and radiographic progression of joint disease (Emery et al., 2013). These evidences strongly suggest that inhibition of proinflammatory cytokines may perform a protective action on joint destruction in RA.

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Nuclear factor-κB (NF-κB), mainly composed of p65 and p50 complex, plays a critical role in the transcriptional regulation of proinflammatory gene expression in RA (Brown et al., 2008). Activation of NF-κB requires the degradation of inhibitor of NF-κB (IκB)α, which facilitate the nuclear transport of NF-κBp65 (Spurlock et al., 2014). Expressions of cytokine genes were regulated by NF-κB activation, and conversely, cytokines may induce IκBα degradation and NF-κB activation through respective receptors, which finally enhance the inflammation development of RA.

Disease-modifying anti-rheumatic drugs, non-steroidal anti-inflammatory drugs, corticosteroids and biologic drugs are the main agents used to treat RA in current clinic. However, limited efficacy, serious side effects and expensive costs limit the application of these drugs (Gibofsky, 2014). It is reported that 60–90% RA patients tend to seek complementary and alternative medicine therapies including traditional Chinese medicine (TCM) (Lü et al., 2015).

Xitong Wan (XTW) is a traditional Chinese herbs formula which was first recorded in Ji-Shi-Yang-Sheng-Ji-Yi-Fang, a TCM book written by Mao Shihong in Qing dynasty of China. XTW is composed of *Siegesbeckia orientalis* L. and *Clerodendron trichotomum* Thunb., and has been used empirically to treat Bi Zheng for hundreds of years. In the current clinical practice of TCM, XTW is frequently used to treat various types of arthritis (Li and Tang, 2013). Although the anti-inflammation activities of *Siegesbeckia orientalis* or *Clerodendron trichotomum* Thunb were revealed in vitro (Park and Kim, 2007; Sun and Wang, 2006), the anti-rheumatic effect and potential mechanisms of XTW are not totally understood. In this study, we investigate the effects of XTW on foot swelling, joint destruction, production of inflammatory mediators and NF-κB activation in rats with adjuvant-induced arthritis (AIA).

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents were purchased from Sigma-Aldrich (USA) unless otherwise specified. Enzyme-linked immunosorbent assay (ELISA) kits for TNF-α, IL-1β and IL-6 were purchased from Pierce (USA). *Mycobacterium tuberculosis* was purchased from Shanghai biochemical factory (China). Methotrexate (MTX) was purchased from Shanghai Xinyi pharmaceutical factory (China). Antibodies against NF-κBp65, IκBα and β-actin were purchased from Santa Cruz (USA). EDTA-free protease inhibitor cocktail was purchased from Roche (Switzerland). Polyvinylidene fluoride (PVDF) membrane and enhanced chemiluminescence (ECL) reagents were purchased from Milipore (USA). Enhanced bicinchoninic acid (BCA) protein assay kit was purchased from Beyotime Biotech (China).

2.2. Preparation of XTW

Siegesbeckia orientalis L. and *Clerodendron trichotomum* Thunb. were purchased from Chongqing Tongjunge Pharmacy (Chongqing, China) and were identified by Dr. Jifen Zhang, college of pharmaceutical sciences, Southwest University (Chongqing, China). Morphological, microscopic authentications and thin layer chromatography were performed in accordance to Chinese Pharmacopoeia (2010). Herbarium voucher specimens of the tested herbs were deposited at the Department of Combination of Chinese and Western Medicine, the First Affiliated Hospital of Chongqing Medical University, with voucher specimen numbers as follows: TCM (2015)-101 (*Siegesbeckia orientalis* L.) and TCM (2015)-102 (*Clerodendron trichotomum* Thunb.). Raw herbal materials (1:1, w/w) were extracted thrice with boiling water (1:10, w/v) for 1 h respectively. The solution was filtered, concentrated and then made into freeze-dried powder. The extraction yield of XTW was 20.8% (w/w) and XTW was kept at −70 °C until use. In this study, XTW was found to contain 1.36 mg kirenol and 0.22 mg hyperoside per g freeze-dried powders by high performance liquid chromatography method (Fig. 1).

2.3. Rats and AIA induction

Male Wistar rats weighing 180–200 g were purchased from Chongqing Medical University (China). All rats were housed in a temperature-controlled room (22 ± 2 °C) under a light/dark cycle with lights on from 7:00 am to 7:00 pm. They were allowed food and water ad libitum. All animal procedures were approved by the institutional animal care and use committee of Chongqing Medical University (Ethics No. CMU2014-121). Complete Freund's adjuvant (CFA) was prepared by suspending heat-killed *Mycobacterium tuberculosis* in sterile mineral oil (10 mg/ml). Arthritis was induced by a single injection of 100 μl of CFA intradermally in the left hind paw pad of the rat.

2.4. Drugs treatment

According to the clinical practice of TCM, the dosage of XTW for adults (60 kg/person) is 20 g/60 kg/d (*Siegesbeckia orientalis* L. 10 g

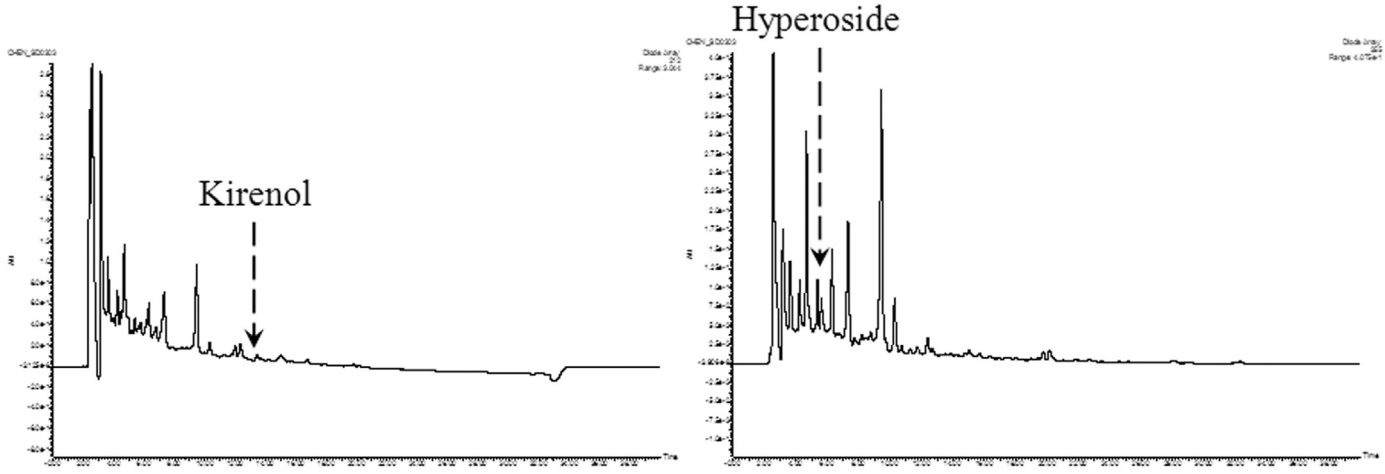

Fig. 1. Representative high performance liquid chromatography chromatograms of kirenol and hyperoside in Xitong Wan.