DOCTORAL THESIS

The anti-arthritic effect and underlying mechanisms of QFGJS, a pharmaceutical preparation from a Chinese herbal formula

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The Anti-arthritic Effect and Underlying Mechanisms of QFGJS, a Pharmaceutical Preparation from a Chinese Herbal Formula

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ABSTRACT

QFGJS is a pharmaceutical preparation from an anti-arthritic Chinese herbal formula composed of five well-documented herbs, and intended to be approved by the State Food and Drug Administration (SFDA) of China as a novel botanical drug for the treatment of rheumatoid arthritis (RA). The objective of this research is to comprehensively and intensively investigate the anti-arthritic effect and underlying mechanisms of QFGJS on experimental models of arthritis in rats including adjuvant-induced arthritis (AIA) and collagen-induced arthritis (CIA).

Through combination of chemical, pharmacodynamic, and toxicological studies, the optimized and standardized pharmaceutical procedures and manufacturing processes for the pilot production of QFGJS were established. Quality analysis of the pilot product of QFGJS by high-performance liquid chromatography (HLPC) demonstrated that the chromatographic fingerprint profiles of three batches of QFGJS were almost identical and the contents of four characteristic and bioactive markers were relatively consistent. General toxicological studies showed a favorable safety profile of QFGJS. The maximum tolerated single dose of QFGJS was determined in both sexes of rats to be 33.63 g/kg body weight which is equivalent to 346 times of clinical dose. In the chronic oral toxicity study, significant loss of body weight of animals treated with doses of 3.89, 6.80, and 9.72 g/kg body weight (equivalent to 40, 70 and 100-fold clinical doses, respectively) was observed after 6 weeks of daily administration of QFGJS. However, the results of laboratory investigation showed that QFGJS caused no changes in all hematological parameters and blood biochemical parameters of rats. No mortality or specific toxic responses were observed in animals after 3 months of repeated dosing with QFGJS.

To establish a well-developed and well-characterized AIA model in the outbred Sprague-Dawley (SD) rats for the evaluation of anti-arthritic effect of QFGJS, the roles of different preparative techniques, inoculation routes and doses of *Mycobacterium tuberculosis* (MT) suspension as well as the sex preference in the induction of AIA in SD rats were comparatively studied using various examinations. The results demonstrated that the particle size and dose of MT in the suspension played a dominant role in the induction and severity of AIA. The same incidence and no significantly different severity of AIA were observed in the rats inoculated either intradermally or subcutaneously. Male rats manifested markedly more severe arthritic signs than female rats. After subcutaneous inoculation with the ground MT suspension containing 500 μg MT, male SD rats developed pronounced arthritis with 100% incidence and low variable clinical signs. Even only 62.5 μg MT was used for inoculation, AIA was efficiently induced in male rats, which showed up-regulated expression of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α. Moreover, we compared AIA in SD and Lewis rats in terms of clinical, histological, radiological, and immuno-inflammatory features. The results showed that, following inoculation with the ground MT suspension, both strains of rats experienced closely similar disease progression, with 100% incidence, similar severity and low variability in clinical arthritic signs. The development of arthritis was accompanied by significantly higher erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels than in control rats. Radiographic examination of the hind paws showed that both SD and Lewis AIA rats manifested conspicuous soft tissue swelling, bone matrix resorption, periosteal new bone formation, and bone erosion, while histopathological analysis of the synovial

joints revealed marked cellular infiltration, angiogenesis, synovial hyperplasia, pannus formation, narrowing of joint space, and focal erosions of cartilage and bone. Moreover, in relation to disease progression, serum TNF-α, IL-1β, and IL-6 levels were markedly overproduced in both SD and Lewis AIA versus control rats, and SD and Lewis AIA rats exhibited divergent profiles for the expression of TNF-α and IL-1β. Taken together, these results demonstrated that the SD rat AIA model shares several arthritic features with the comparable model in Lewis rats. Hence, given the more favorable characteristics of SD rats as compared with Lewis rats, i.e., lower cost, wider availability, and heterogenic background, this SD rat AIA model is much more cost effective and advantageous for studying the pathophysiology of arthritis in humans as well as for screening novel anti-arthritic agents. This is also one of the findings and contributions to the academic community in the current research.

To analyze the anti-arthritic effect of QFGJS on RA, the well-established AIA model in SD rats and CIA model in Wistar rats were used in the current study. Two treatment protocols, i.e., oral administration with different doses of QFGJS (0.97, 1.94, and 3.89 g/kg body weight), or indomethacin at the dose of 1 mg/kg body weight, or vehicle beginning on the day of the induction of arthritis (the prophylactic treatment protocol) or on the day after the onset of arthritis (the therapeutic treatment protocol), were initiated and continued until day 30 of the experiments. The results showed that prophylactic treatment with QFGJS significantly suppressed the onset of arthritis, while therapeutic treatment with QFGJS markedly reduced arthritic score, paw swelling, and ESR levels even in the established arthritis. Radiological and histopathological examinations showed markedly decreased tissue and bone destruction of arthritic joints in the QFGJS-treated rats. The current study demonstrates that oral treatment with QFGJS can effectively block the disease progression of arthritis, showing suppression of joint inflammation and of the radiological and histopathological progression of joint damage.

The possible molecular mechanisms underlying the anti-arthritic effect of QFGJS in which pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 are predominantly involved were further investigated. Our results showed that the inflammatory process in the arthritic rats led to substantial increases in systemic levels of TNF-α, IL-1β, and IL-6; while QFGJS decreased overexpressed TNF-α, IL-1β, and IL-6 levels in blood serum in a dose-dependent manner. Therefore, the anti-arthritic effect of QFGJS on both polyarticular inflammation and joint damage may basically correlate with its action of suppressing the abundant production of TNF-α, IL-1β, and IL-6 in blood serum.

Overall, QFGJS has been demonstrated to not only direct towards the control of pain and the inflammation associated with joint synovitis, but also prevent the structural damage of arthritic joints caused by tissue and bone breakdown. QFGJS is an example of combination therapy of Chinese medicinal herbs, which, increasingly, is being found to be the best approach to complex refractory and degenerative diseases. The results obtained in our studies provide substantial and well-documented evidence of the quality, safety, and effectiveness of QFGJS as an anti-arthritic agent. Hence, QFGJS is a strong candidate for development into an effective botanical drug for the treatment of RA.

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