DOCTORAL THESIS

Anti-arthritic properties of a herbal formula comprising Rosae Multiflorae Fructus and Lonicerae Japonicae Flos

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ABSTRACT

Rheumatoid arthritis (RA), the most common autoimmune disease, affects billions of people worldwide. Conventional therapeutics do not provide satisfactory efficacy and even cause severe adverse reactions. Researchers are seeking new approaches for RA management. Toll-like receptor 4 (TLR4) signalling plays a pivotal role in the pathogenesis of RA, and has been proposed as a potential therapeutic target for RA. Chinese medicines are believed to be alternative options for conventional RA therapeutics. A herbal formula RL, consisting of Rosae Multiflorae Fructus and Lonicerae Japonicae Flos, has traditionally been used in treating various inflammatory disorders including RA. In this study we assessed the anti-arthritic efficacy of RL in animals, and investigated the involvement of TLR4 signalling in RL's effects *in vivo* and *in vitro*.

In vivo anti-arthritic efficacy of RL was evaluated using CIA (collagen-induced arthritis) rats, a model that is well established for studying human RA. Articular disease manifestations were investigated grossly, radiographically, and histologically. Isolated splenocytes were used to determine the effects of RL on immune responses. Molecular events in the TLR4 pathways upon RL treatment were examined in sera and joint tissues of CIA rats as well as in cultured lipopolysaccharide (LPS)-stimulated murine RAW264.7 and human THP-1 cells.

In CIA rats, RL significantly increased food intake and weight gain of CIA rats without any observable adverse effect; ameliorated joint erythema and swelling; and inhibited immune cell infiltration, bone erosion and osteophyte formation in joints. RL also reduced the upregulated protein expression levels of TLR4, phospho-transforming growth factor β-activated kinase 1 (p-TAK1), phospho-nuclear factor-κB (p-NF-κB), phospho-c-Jun, and phospho-interferon regulatory factor 3 (p-IRF3) in

joint tissues; modulated the levels of inflammatory factors [lowered tumour necrosis factor α (TNFα), interleukin (IL)-6, IL-1β, IL-17A and monocyte chemoattractant protein-1 (MCP-1) in sera, and TNFα, IL-6, IL-1β and IL-17A in joints; and elevated IL-10 in sera and joints]; reinvigorated the declined activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in liver tissues and sera; reduced T helper 17 (Th17) cell proportions in splenocytes; inhibited splenocyte proliferation and activation; and lowered serum immunoglobulin G (IgG) levels.

In LPS-stimulated RAW264.7 and THP-1 cells, RL inhibited the production of pro-inflammatory mediators (e.g. TNFα, IL-6, IL-1β, and MCP-1), the phosphorylation and nuclear localization of transcription factors downstream of TLR4 [NF-κB, activator protein 1 (AP-1) and IRF3], and the activation/phosphorylation of inhibitor NF-κB α (IκBα), IκB kinase α/β (IKKα/β), TAK1, TANK-binding kinase 1 (TBK1) and interleukin-1 receptor-associated kinase 1 (IRAK1). RL's inhibitory effects on IRF3 phosphorylation reduced gradually along with the increase in LPS concentrations.

In conclusion, RL possesses anti-arthritic effects in CIA rats and had no observable adverse effect. The therapeutic effect of RL is, at least in part, attributed to its inhibition on the IRAK1/TAK1/NF-κB, IRAK1/TAK1/AP-1, and TBK1/IRF3 pathways. Findings of this study provide a pharmacological justification for the use of RL in the control of RA, and suggest that RL is a safe and effective alternative anti-RA agent.

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