Extended Abstract Journal of Clinical and Bioanalytical Chemistry 2021
Vol.5 No.1

Antiarthritic Effects of Daphne giraldii Nitsche (Thymelaeaceae) Mainly through Suppression of the Secondary Inflammation

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The present study focuses on the anti-arthritic effect of Zushima cataplasms (ZC) in adjuvant arthritic (AA) rats. AA rats were treated with ZC from the 3rd day before immunization and continued until the 20th day after immunization. The severity of arthritis was evaluated by swelling, heat, nociceptive, histopathology and radiological changes. The levels of IL-1β, TNF-α in the serum were measured by ELISA. ZC showed no effect on alleviating swelling in primary inflammation, while ZC 0.75 g/kg dramatically reduced the swelling, and showed a significant increment of mechanical pain thresholds in secondary inflammation. The synovial hyperplasia and inflammatory cells infiltration were suppressed by ZC in secondary inflammation but not in primary inflammation. The radiographic studies further provided supportive evidence for histopathology analysis. Additionally, There was significant reduction in production of interleukin-1β (IL-1β), tumor necrosis factor (TNF-α) in serum of AA rats treated with ZC. Rheumatoid arthritis (RA), characterized by severe morbidity, functional impairment, permanent disability, and increased mortality, is a chronic, destructive inflammatory polyarticular joint and systemic autoimmune disease which leads to the destruction of synovial membranes, cartilage and bone . Although the etiology remains unknown, the recent advancements have demonstrated that many different cell components are involved in the RA development, including neutrophils, T and B lymphocytes, and monocytes/macrophages . Activation of these cells leads to the production of cytokines which are responsible for inflammation. However, the hierarchy of the immuneinflammatory and its relationship with synovial stroma activation is not fully understood. Overwhelming evidence indicates that the immunological response, both in its innate and adaptive immunity, can play a critical role in early and advanced stages of the disease . RA pathogenesis is a multistep process consisting early phase, involving the generation of autoantibodies, an “initiation phase”, where synovial inflammation emerges, and advanced stages, which is dominated by non-resolving synovial inflammation and joint destruction . Compelling evidence supporting this conclusion, a rat model of antigen-induced arthritis characterized by acute phase (primary inflammation) and a chronic phase (secondary inflammation), was triggered by systemic immunization followed by local challenge with Freund’s complete adjuvant (FCA) . In the acute phase, antigen challenge in peripheral tissue results in dendritic activation, with local activation of naïve or central T and B cells, and effector lymphocytes favor the removal of the antigen, leading to the primary inflammation . Besides, effector lymphocytes secrete inflammatory cytokines, such as tumor necrosis factor-a (TNFa), interleukin-1 (IL-1), and IL-6, which enter the systemic circulation and induce the secondary inflammation . The goals for antiarthritic are long-term relief. Drugs used for the treatment of RA exhibit antiarthritic effect by affecting the onset and symptoms of inflammation . Zushima (Chinese name), the dried stem bark and root bark of Daphne giraldii Nitsche (Thymelaeaceae), a traditional Chinese medicine used for over two thousand years in China for inflammation related symptoms, including joint pain, is mainly used to treat rheumatoid arthritis (RA) in clinics . Previous phytochemical studies reported that Daphne giraldii Nitsche mainly contained coumarin compounds, flavonoids and biscoumarin glycosides. And Zushima-Pian (ZP), a traditional Chinese medicine tablet, was officially recorded in the Ministry of Public Health of China for the treatment of arthritis, rheumatic arthritis . The above evidences indicate that Zushima has showed good activity for antiarthritic, but reports on anti-inflammation effect of Zushima were relatively limited in recent 5 years and underlying mechanisms of action were not clear. Thus, the present study was focused on the evaluation of the anti-arthritic effects of Zushima cataplasms (ZC) using FCA induced AA rats. The potential activities of ZC on controlling TNF-α and IL-1β production in serum were determined by ELISA. Moreover, the severity of arthritis in the knee joints was evaluated by histological assessment of cartilage destruction and X-ray photographs. All experiments were performed on male Sprague–Dawley (SD) rats, weighing 180-220 g, provided by the Animal Center of Shanghai Institute of Medical Sciences. Throughout the experiments, rats were maintained in plastic cages at 21 ± 2°C, on a 12 h light/dark cycle and with free access to water. Animal welfare and experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, and were approved by the animal ethics committee of Genetics and Cytology of Shanghai Institute of Pharmaceutical Industry. The registration number of animal ethic committee is 2013235 HM and the approved date of animal ethicis December 23, 2013. All possible efforts were made to minimize the animals’ suffering and to reduce the number of animals used. The method described by Talwar was followed for this investigation . SD rats were randomly divided into five groups, control, model and test groups (three different doses), ten rats in each group. Firstly, right hind paw of all rats were unhaired with 8% sodium sulfide (Na2 S) to promote transdermal absorption of ZC (Gansu Wuwei Pharmaceutical Co., Ltd.), and were fed for 5 days. Then, rats of test groups were pretreated topically with ZC (0.25, 0.5, 0.75 g/kg), while control and model rats were treated topically with inert paste as the control for 3 days, covered with gauze and a layer of plastic film, sealed, and fixed with desensitized adhesive plaster. The optimal doses of ZC were utilized based on the clinical doses. AA was induced by intradermal injection with 0.1 ml FCA into the right hind paw of the rats in model and test groups on the 4th day, and control group animals received an intradermal injection with an equal volume of PBS. After FCA immunization, the rats were treated

consecutively for 20 days. On the final day of treatment, all rats were anesthetized with ethyl ether, then the blood was collected from each rat by carotid artery cannulation. The rats were assessed for signs of arthritis by five independent observers who were blinded to the experimental design. The secondary arthritis, including left hind limb and whole body (hind limbs, forelimbs, ears and tail), were graded and scored for severity and swelling on a scale of 0-4 point at the 18th and 22th day separately: (0: normal, with no macroscopic signs of arthritis; 1: only redness; 2: redness and mild swelling; 3: redness and severe swelling; 4: joint deformation and arthrocleisis . Besides, one nodule on ears or tail scored as 1 point, and severity of secondary arthritis was evaluated by the cumulative scores. In our experiment, left hind paws (non-injected) of rats appeared swollen and red on day 15, and 80% rats in model group showed swelling or redness on day 18. Therefore, we evaluated the degree of swelling and joint score (left hind paw) on day 18 to assess the effect of ZC on secondary inflammation. The results indicated that the degree of swelling in model group was significantly higher than control ($P<0.01$). In the control group, the articular cavities were very clean. The articular surfaces were smooth, and there were loose connective tissues and fatty tissues under the synovial memb. In the model group, the histopathology of ankle joints showed multiple layers of synovium hyperplasia and inflammatory cell infiltration. Synovial cells became proliferated, enlarged in size and disorderly arranged. The loose connective tissues under the synovial membranes became hyperaemia and infiltrated with inflammatory cells. In the ZC-treated groups, there were obvious synovium hyperplasia and inflammatory cell infiltration in the primary inflammatory joint (right hind paw), showing no effects on alleviating primary inflamm. In contrast, in secondary inflammatory joint, ZC treatment significantly inhibited the synovium hyperplasia, and reduced the number of infiltrated inflammatory . The scores for synovium hyperplasia and cell infiltration were reduced significantly and dose-dependently by treatment with ZC compared with the model group ($P<0.01$). Based on this promising observation, we made a further study on the potential mechanism of antiarthritic effect. In terms of plasma level of IL-1β the IL-1β level of model group was significantly increased compared with that of control group . Currently, although available agents, including the neutralization of cytokines (by soluble receptors or monoclonal antibodies), receptor blockade, and the activation of anti-inflammatory pathways by bioengineered versions of immuno-regulatory cytokines, are effective in suppressing inflammation, but not capable of re-establishing immunological homeostasis, with disease relapse in the majority of patients on drug withdrawal . It is likely that further insights not only into the pathogenesis of inflammation and joint damage, but also into the upstream mechanisms sustaining these processes will be essential to switch off the disease . A large number of compounds of varied chemical structures isolated from medicinal plants have been shown to possess anti-inflammatory activity, and over 300 compounds isolated from plants with anti-inflammatory activity recorded in Chemical Abstracts from 1950-2000 . Zushima has been clinically used in China as an effective traditional Chinese medicine for the treatment of RA. In addition, Daphnetin, the chemical marker for quality evaluation of Zushima and its preparations, has been reported to make important contributions to antiarthritic activity of Zushima . Zushima cataplasms (ZC) are widely used to treat RA patients in clinic at present, and the efficacy is confirmed by amounts of clinical trials . However, there are scarcely reports about the anti-arthritic and antiinflammatory activity of ZC. Some scientific problems remain unclear. Firstly, the clinical efficacy is not supported by proper and scientific experiment data and the mechanism is not clear. Secondly, RA patients who see a doctor are generally in the secondary in health. 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