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Suppressive effects of *Chelidonium majus* methanol extract in knee joint, regional lymph nodes, and spleen on collagen-induced arthritis in mice

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Abstract

Chelidonium majus L. has multiple applications in Korean traditional medicine because of its anti-tumoral, cytotoxic, anti-inflammatory and anti-microbial activities and has long been known to have anti-inflammatory effects. However, no study on the anti-arthritic activity of *Chelidonium majus* has been reported in vivo. Rheumatoid arthritis (RA) is a systemic autoimmune disease with chronic inflammation characterized by hyperplasia of synovial cells in affected joints, which ultimately leads to the destruction of cartilage and bone. Cytokine production and gene expression were assessed during CIA (collagen-induced arthritis) model mice in knee joint, lymph node (LN), and spleen, using ELISA and competitive RT-PCR. DBA/1J mice were immunized with bovine type II collagen. After a second collagen immunization, mice were treated with CME orally at 400, 40 mg/kg once a day for 4 weeks. The severity of arthritis within the knee joints was evaluated by histological assessment of cartilage destruction and pannus formation. Administration of CME significantly suppressed the progression of CIA and inhibited the production of TNF-α and IL-6 in spleen and lymph node. The erosion of cartilage was dramatically reduced in mouse knees after treatment with CME.

In conclusion, our results demonstrates that CME significantly suppressed the progression of CIA and that this action was characterized by the decreased production of TNF-α, IL-6, IFN-γ, B cells, γδ T cells (in spleen) and increased proportion of CD4+CD25+ regulatory T cells in vivo. In the serum of CME-treated mice, the levels of IgG and IgM RA factor were decreased.

Keywords: *Chelidonium majus*; Arthritis; TNF-α; IL-6; IFN-γ; CD4+CD25+ regulatory T cell

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease with chronic inflammation characterized by hyperplasia of synovial cells and angiogenesis in affected joints, which ultimately leads to the destruction of cartilage and bone (Koch, 1998). RA is characterized by the inflammation of synovial joints infiltrated by CD4+ T cells, macrophages, and plasma cells that play major roles in the pathogenesis of the disease (Feldmann et al., 1996; Wolfe and Hawley, 1998). T cells have a direct impact on TNF-α, IL-6, IFN-γ induction in RA joints. TNF-α is known to play a critical role in the pathogenic mechanisms of a number of chronic inflammatory diseases, including RA. B cells may play an important role in the pathogenesis of RA through cell–cell interaction with T cells, dendritic cells, synovial nurse-like cells and fibroblasts (Tran et al., 2005). CD4+CD25+ regulatory T cells are potent suppressors of T cell responses both in vitro and in vivo. CD4+CD25+ regulatory T cells from RA patients inefficiently suppress the IFN-γ and TNF-α production of effector T cells. CD4+CD25+ regulatory T cells were able to diminish the clinical severity of arthritis despite a lack of reduction in systemic CII-specific T and B cell responses (Morgan et al., 2005).

Several medicinal herbs have shown to promote and suppress immunity in different ways. They have shown to augment specific cellular and humoral immune response. *Chelidonium*

Abbreviations: RA, rheumatoid arthritis; RF, rheumatoid factor; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; LN, lymph node; CIA, collagen-induced arthritis; CME, *Chelidonium majus* methanol extract

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majus L. is a plant which grows in the wild in Southern and Central Europe, part of Asia, North America and in the Azores archipelago (Tin-Wa et al., 1972; Colombo and Bosisio, 1996). *Chelidonium majus* L. has multiple applications in folk medicine because of its antitumoral, cytotoxic, anti-inflammatory and antimicrobial activities (Kim et al., 1969; Saglam and Arar, 2003). The plant contains, as major secondary metabolites, isoquinoline alkaloids, such as sanguinarine, chelidonine, chelerythrine, berberine and coptisine. Isoquinoline alkaloids have anti-inflammatory activity (Küpeli et al., 2002). Sanguinarine, chelerythrine and quaternary benzophenanthridine fraction were screened for their anti-inflammatory activity in assays involving carrageenan-induced rat paw edema. Sanguinarine showed a higher anti-inflammatory activity than chelerythrine, which could be explained with the different oxygen electrodonating substituents (Lenfeld et al., 1981). Analgesic, diuretic, choleretic and spasmolytic properties have also been reported (Kery et al., 1987; Vahlensieck et al., 1995; Mitra et al., 1996). CME also contain berberine and this compound as previously reported to possess significant anti-inflammatory activity (Yesilada and Küpeli, 2002).

However, no study on the anti-arthritic and anti-inflammatory activity of *Chelidonium majus* has been reported. In order to verify its anti-arthritic effects, we have investigated the immunomodulatory and anti-inflammatory activities of the plant. The aim of this study was to evaluate the control activity of *Chelidonium majus* extract on TNF-α, IL-6, IFN-γ, IL-4 cytokines production, inhibition of αβ T cells, γδ T cells influx in lymph node and spleen and other factors. Therefore, we decided to investigate anti-inflammatory and anti-arthritic effects of *Chelidonium majus* extract in murine model of rheumatoid arthritis. The effects of *Chelidonium majus* extract on total cell number in joint, lymph node, spleen and thymus, T, B, regulatory T cell surface markers by flow cytometric analysis and cytokines production in spleen by ELISA were determined. Moreover, to determine whether CME (a methanol extract from *Chelidonium majus*) prevented articular destruction, we analyzed the knee joints of mice.

In this study, the various immunomodulatory effects of the CME were investigated in order to determine the potential bioactivity of CME on RA. In addition, the therapeutic effect of the CME on RA was examined using collagen-induced arthritis (CIA) mice.

2. Materials and methods

2.1. Plant material and preparation of extracts

The aerial part of *Chelidonium majus* were purchased from local market (Seoul, Korea) in September 2005. The plant was identified by Professor Young-Cheol Lee, College of Oriental Medicine, Sangji University in Wonju, Korea, and a voucher specimens (CME) are deposited in our laboratory (Department of Herbology, College of Oriental Medicine, Sangji University Wonju 220-702, Republic of Korea). Dried powdered herba samples of *Chelidonium majus* were separately extracted, using cold percolation method with methanol for 72 h each. Plant material (250 g) was extracted three times with 80% methanol. Then, the extract was filtered and evaporated on a rotatory evaporator (Rotary evaporator, BUCHI B-480, Switzerland) and finally dried by a freeze drier (Freeze dryer, EYELA FDU-540, Japan) to yield the extract CME (20 g). The yield (w/w) of the extract was about 8%.

2.2. Animals

Seven- to eight-week-old male DBA/1J mice were obtained at SLC (Hamamatsu, Japan). All animal procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee, Korea Research Institute of Bioscience and Biotechnology (Daejeon, Republic of Korea).

2.3. Preparations of murine CIA model

All of the animal procedures were approved by the Experimental Animal Commission of the Institute of Traditional Medicine and Bioscience at Daejeon University. Murine CIA model mice were induced in a modification of the method (Lee et al., 2005).

In brief, male DBA/1J mice (7–9 weeks old; SLC, Hamamatsu, Japan) received 200 μg of bovine type II collagen (Sigma) in Freund's complete adjuvant (Sigma) by intradermal injection at the base of the tail on day 0 and a booster injection on day 21 ($n = 6$ mice per group). Mice were monitored daily for signs of arthritis, and each paw was scored individually as follows: 0 = normal, 1 = slight erythema and edema, 2 = increased edema with loss of landmarks, 3 = marked edema, and 4 = marked edema with ankylosis on flexion. Each mouse was assigned an arthritis score (articular index) that equaled the sum of the scores for each paw, so that the possible maximum score per mouse was 20. In the prophylactic dosing model, mice were orally administered with CME (400 or 40 mg/kg dissolved in distilled water) daily from day 1 to day 28 after arthritis incidence, and monitored for disease incidence and the severity of arthritis up to day 28. CIA control mice received an intraperitoneal injection of PBS alone.

On the final day of above experiment, all of the mice were anesthetized with ethyl ether and then blood was collected from each by cardiac puncture; the mice were then killed by cervical dislocation. The mice spleen, thymus, and lymph node were taken out and used for ELISA analysis and total cell counts.

2.4. Antibodies and flow cytometric analysis

All antibodies (CD3, CD4, CD8, CD19, CD25, αβ TCR, γδ TCR) for flow cytometric analysis were purchased from Becton Dickinson (BD) PharMingen (San Diego, CA). Cells from lymph node and spleen were stained with the indicated antibodies in staining buffer (PBS containing 1% FBS and 0.01% NaN_3) for 10 min on ice, and analyzed by two color flow cytometry on a FACScan using CellQuest software (BD Biosciences, Mountain View, CA). Absolute cell numbers were counted manually in a hemocytometer chamber (Fisher). The 2×10^3 cells were spun onto glass slides (Cytospin centrifuge, Cellspin, Hanil, Korea)