Tripterygium glycosides inhibit inflammatory mediators in the rat synovial RSC-364 cell line stimulated with interleukin-1β

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Abstract. *Tripterygium* glycosides (TG) are extracted from a traditional Chinese medicinal herb. Using the compound, progress has been made in the treatment of rheumatoid arthritis (RA), but the underlying mechanism of its action is poorly understood. The purpose of the present study was to investigate the role of TG in preventing inflammatory arthritis. An inflammatory cell model was established in the rat synovial RSC-364 cell line via induction with interleukin (IL)-1β. The expression of IL-32 and matrix metalloproteinases (MMP-1 and MMP-9) was determined using an enzyme-linked immunosorbent assay. Compared with the control group (without IL-1β), IL-1β in the treatment group induced the expression of IL-32, MMP-1 and MMP-9 in RSC-364 cells. When a different dose of TG was added to RSC-364 cells stimulated with IL-1β, TG decreased the expression levels of IL-32, MMP-1 and MMP-9 in a dose-dependent manner. These results indicated that TG suppressed the inflammation response in RSC-364 cells. Taken together, these findings may contribute to a better understanding of the role of TG in the anti-inflammatory therapeutics for RA.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that affects multiple peripheral joints (1). RA is the most common form of inflammatory arthritis and is characterized by synovial hyperplasia, which results in the evolution of joint destruction (2,3). *Tripterygium* glycosides (TG) are a traditional Chinese medicinal herb with which progress has been made in the treatment of RA, but the underlying mechanism of its action is poorly understood.

Interleukin (IL)-32 is a relatively recently described pro-inflammatory cytokine reported to have a role in RA (4). IL-32 is produced mainly by T cells, natural killer cells, epithelial cells and monocytes. Notably, IL-32 is highly expressed in fibroblast-like synoviocytes (FLS) from RA patients and has been recently identified as a possible RA prognostic biomarker (5-7).

Matrix metalloproteinases (MMPs) are key enzymes in the degradation of extracellular matrices and MMP expression plays important roles in inflammatory diseases (8). Inflammatory cytokines, such as IL-1β and tumor necrosis factor-α, stimulate the production of MMPs, and enzymes, including MMP-1 and MMP-9, can degrade the components of the extracellular matrix in RA FLS (9).

Earlier studies reported that the key pro-inflammatory cytokines, such as IL-1β, are present in the synovial fluid of RA patients and play key roles in amplifying and perpetuating inflammation and joint destruction (10). In addition, IL-1β is also a potent activator of FLS, inducing them to produce cytokines, matrix-degrading metalloproteinases and other inflammatory mediators (11).

RA treatment has received increasing attention. TG is an extract derived from *Tripterygium wilfordii* Hook F and has been widely used in the treatment of RA, autoimmune disease and inflammatory disease in China (12). TG can evidently improve the symptoms and laboratory indicators of RA (13). Although the clinical efficacy of TG has been verified, its basic mechanism of action remains unclear. In the present study, to gain insight into the potential mechanisms underlying the therapeutic value of TG for RA, RSC-364 cells treated with IL-1β were employed to establish an inflammation study model *in vitro*. The observations indicated that TG reduced the expression levels of IL-32, MMP-1 and MMP-9.

Materials and methods

Cell culture. A rat synovial cell line, RSC-364 (donated by Beijing 301 Hospital, Beijing, China), was cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich,

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St. Louis, MO, USA), 100 U/ml penicillin and 100 U/ml streptomycin at 37˚C in a humidified atmosphere of 5% CO_2 and 95% air. The RSC-364 cells were grown to 70% confluence in 6-well plates (~5x10^5 cells) for testing. The enzyme-linked immunosorbent assay (ELISA) kits of IL-32, MMP-1 and MMP-9 were from R&D Systems (Minneapolis, MN, USA).

ELISA assay. RSC-364 cells were seeded in 6-well plates and cultured in DMEM with 10% FBS for 24 h before treatment. RSC-364 cells of the IL-1β groups were treated with IL-1β (10 ng/ml) for 3 h, and the control group cells had no treatment. Cell culture supernatants were then collected. To further study the effect of TG on IL-32, MMP-1 and MMP-9 expression in RSC-364 cells treated with IL-1β, the cells were pre-treated with IL-1β for 3 h, and subsequently, different doses (5, 10, 20 and 40 mg/dl) of TG (dissolved in anhydrous alcohol) were added to each well. After 24 h, the supernatants were collected for detection of the expression levels of IL-32, MMP-1 and MMP-9 using ELISA. ELISA was performed according to the manufacturer's instructions. All the experiments were performed in triplicate. Employing commercially available kits, the expression values were calculated on the basis of the standard curve constructed for each assay.

Statistical analysis. All the data were entered into a computer database and analyzed using SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA). The results were expressed as the mean ± standard deviation and statistical comparisons were performed using a completely randomized design analysis of variance and least significant difference. In all the cases, $P<0.05$ was considered to indicate a statistically significant difference.

Results

IL-1β induces the expression of IL-32, MMP-1 and MMP-9 in RSC-364 cells. The group that did not receive IL-1β was used as a control group. In the treatment groups, IL-32, MMP-1 and MMP-9 levels were significantly increased ($P<0.01$) compared with the control group (Fig. 1). These findings showed that IL-1β induced the expression of IL-32, MMP-1 and MMP-9 in RSC-364 cells. These results also verified that the *in vitro* inflammatory cell model was successful.

TG inhibits the expression of IL-32, MMP-1 and MMP-9 in RSC-364 cells treated with IL-1β. As shown in Figs. 2-4, at 5 dose points, there was a significant difference in the expression of IL-32, MMP-1 and MMP-9 at the 10 ($P<0.05$), 20 ($P<0.01$) and 40 mg/dl dose ($P<0.01$) compared to that of the 0 mg/dl dose. The results showed that 5 mg/dl of TG decreased the expression of IL-32, MMP-1 and MMP-9 following stimulation with IL-1β in RSC-364 cells, but the difference was not clear when compared to that at the 0 mg/dl dose (without TG treatment). A decreased expression of IL-32, MMP-1 and MMP-9 was detected at the 10 mg/dl dose and reached its original levels at 40 mg/dl dose following TG treatment in RSC-364 induced with IL-1β. These findings indicated that TG downregulated the expression levels of IL-32, MMP-1 and MMP-9 in RSC-364 cells stimulated with IL-1β.

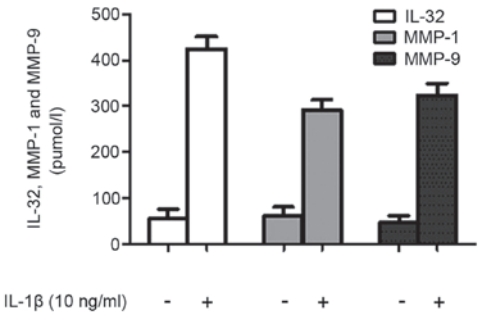

Figure 1. IL-1β induces the production of IL-32, MMP-1 and MMP-9 in RSC-364 cells. IL, interleukin; MMP, matrix metalloproteinase.

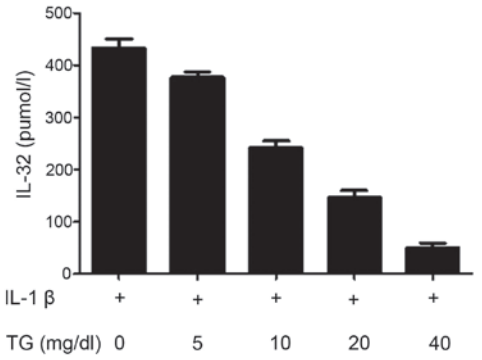

Figure 2. Effects of TG on the expression of IL-32 in RSC-364 cells stimulated with IL-1β. TG, *Tripterygium* glycosides; IL, interleukin; MMP, matrix metalloproteinase.

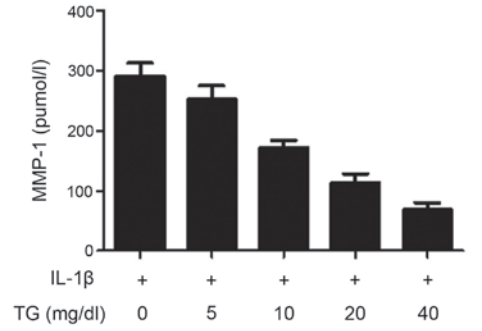

Figure 3. Effects of TG on the expression of MMP-1 in RSC-364 cells stimulated with IL-1β. TG, *Tripterygium* glycosides; IL, interleukin; MMP, matrix metalloproteinase.

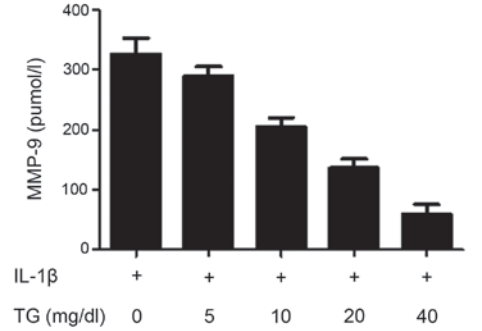

Figure 4. Effects of TG on the expression of MMP-9 in RSC-364 cells stimulated with IL-1β. TG, *Tripterygium* glycosides; IL, interleukin; MMP, matrix metalloproteinase.

Discussion

RA is a chronic inflammatory disease characterized by inflammation of the synovial lining and destruction of the adjacent bone and cartilage (14). It is well-known that TG is an effective RA treatment (15,16). However, extremely little is known regarding the detailed mechanism underlying the effects of TG in RA. IL-1β is known to induce the production of a variety of cytokines in fibroblasts. It has been reported that IL-32, MMP-1 and MMP-9 are considered to play critical roles in the pathogenesis of RA (17-19).

In the present study, whether IL-1β induced the production of IL-32, MMP-1 and MMP-9 in RSC-364 cells was explored. The results showed that IL-1β clearly increased the expression levels of L-32, MMP-1 and MMP-9 compared with that of the control group (without IL-1β treatment) in RSC-364 cells. This is consistent with a study by Kim *et al* (20), which noted that IL-32 has the properties of pro-inflammatory mediators via stimulation of IL-1β and is found in large quantities in the synovial fibroblasts of RA patients. Of note, it also suggested that the inflammatory cell model *in vitro* was extremely successful.

TG has anti-inflammatory and immunosuppressive activities in human clinical trials for inflammatory and autoimmune disease and has been historically used in traditional Chinese medicine to treat RA (21). Recently, IL-32 has been identified with a high expression in RA patients and mice models of experimental inflammatory arthritis (22). Furthermore, IL-32 is a pro-inflammatory cytokine expressed by activated natural killer cells, T cells and fibroblasts (23). *In vitro* studies confirm that TG strongly inhibits proliferation of T and B cells, and has demonstrated immunosuppressive activity (24). The present findings showed that TG could hamper the expression levels of IL-32 in a dose-dependent manner in RSC-364 cells stimulated with IL-1β. Therefore, we assumed that TG decreased the expression levels of IL-32 in RSC-364 cells stimulated with IL-1β, which may be associated with the suppression of the fibroblasts cells activity.

MMPs are involved in the articular tissue destruction processes in the pathogenesis of RA and have been shown to dissolve the extracellular matrix, initiating and promoting new vessel formation in RA. A recent study of the effects of TG extracts on inflammatory enzymes, such as metalloproteinases, showed an inhibition of metalloproteinase production by blocking mRNA transcription (25). In addition, several studies show that TG also decreases the production of MMPs by suppressing the proliferation of T and B cells and synovial fibroblasts in RA (26,27). In the present study, the results identified that TG clearly decreased the production of MMP-1 and MMP-9 in a dose-dependent manner in RSC-364 cells stimulated with IL-1β. Earlier clinical studies suggest that the anti-inflammatory effects of TG in the treatment of RA are due to the suppression of the MMPs (28). Consequently, we thought that TG could suppress the expression of MMP-1 and MMP-9 by inhibiting the proliferation of cells and blocking *MMP-1* and *MMP-9* mRNA transcription in RSC-364 cells stimulated with IL-1β.

In conclusion, the present study suggested that TG suppressed the expression of IL-32, MMP-1 and MMP-9 in a dose-dependent manner. These results contribute to further explanation of the mechanism of TG in the treatment of RA. TG may be an attractive agent for the development of potential RA therapeutic agents. However, the present study was an *in vitro* experiment and thus, reliable *in vivo* conclusions cannot be drawn from these results. Therefore, further investigations are required to confirm the results of the study.

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