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Triptolide, a diterpenoid triepoxide, suppresses inflammation and cartilage destruction in collagen-induced arthritis mice

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Abbreviations:
CIA, collagen-induced arthritis
CII, type II collagen
COX, cyclooxygenase
DAB, diaminobenzidine
DMARDs, disease modifying antirheumatic drugs
IL, interleukin
MMPs, matrix metalloproteinases
NF, nuclear factor
PBS, phosphate buffered saline
PG, prostaglandin

ABSTRACT

Chinese herbal remedy *Tripterygium wilfordii* Hook. f. (TWHF) has been reported to be therapeutically efficacious in the treatment of rheumatoid arthritis (RA), but its *in vivo* actions have not been clarified. The purpose of this study was to investigate the effects of triptolide, a diterpenoid triepoxide extracted from TWHF, on inflammation and cartilage destruction in collagen-induced arthritis (CIA) model mice. Histological examination demonstrated that triptolide significantly reduced the inflammatory responses and cartilage damage in the joint tissues. Interestingly, triptolide interfered with CIA-augmented expression of matrix metalloproteinases-13 and -3, which are considered to be key enzymes in the pathological destruction of cartilage, and simultaneously augmented CIA-reduced tissue inhibitors of metalloproteinases-1 and -2 expression in the joints. Moreover, triptolide inhibited prostaglandin E_2 production via selective suppression of the production and gene expression of cyclooxygenase (COX)-2, but not COX-1. The levels of interleukin (IL)-1β, tumor necrosis factor α and IL-6 were also decreased by triptolide in the joint tissues and sera as well as the suppression of CIA-mediated expression of their mRNAs in the joints. In addition, triptolide treatment *in vivo* was able to reduce an abundance of nuclear factor-κB, the transcriptional factor closely related to the inflammatory process, in articular cartilage and synovium in CIA mice. These results suggest that triptolide exerts novel chondroprotective and anti-inflammatory effects on RA, and the therapeutic action of TWHF on RA is, in part, due to the triptolide activities.

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quantitative real-time RT-PCR, quantitative real-time reverse transcriptase-polymerase chain reaction
RA, rheumatoid arthritis
RIA, radio immunoassay
SABC, streptavidin–biotin complex
TIMPs, tissue inhibitors of metalloproteinases
TNF, tumor necrosis factor
TWHF, *Tripterygium wilfordii* Hook. f.

1. Introduction

Rheumatoid arthritis (RA) is characterized by chronic inflammation in joints and concomitant destruction of cartilage and bone. Inflammatory mediators, such as prostaglandins (PGs) and proinflammatory cytokines, are closely associated with this pathologic process and play important roles in RA [1,2]. On the other hand, matrix metalloproteinases (MMPs) are involved in the destruction of extracellular matrices in cartilage. In particular, MMP-13/interstitial collagenase-3, which specifically cleaves type II collagen (CII) of hyaline cartilage more efficiently than MMP-1/collagenase-1, and MMP-3/stromelysin-1, which digests proteoglycans and collagen types IX and X, are considered to be key enzymes in the pathological destruction of cartilage [3,4]. It is known that proinflammatory cytokines, interleukin (IL)-1, tumor necrosis factor (TNF) α, and IL-6 are pivotal factors, since they strictly enhance the biosynthesis and secretion of PGE_2 and MMPs from mesenchymal cells at inflammatory sites [5]. Under normal conditions, tissue inhibitors of metalloproteinases (TIMPs) bind to active MMPs in a ratio 1:1 to make an inactive complex. Therefore, an imbalance in the ratio of TIMPs to MMPs, which is generally caused by up-regulation of MMPs, results in continued matrix destruction in RA [6,7].

Currently, most treatments for RA are directly to normalize the immune system and to reduce inflammatory mediators. As therapeutic agents, disease modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs, and steroids are clinically common, and recently, TNFα-neutralizing therapy has been shown to provide sustained clinical benefits [8]. However, DMARD therapy has been impeded by the existence of a large number of nonresponders and by gravely adverse effects with a high frequency [6,9]. Therapy using soluble TNFα receptor or antibody against TNFα entails a high cost, hypersensitivity to medications, and infection due to TNFα blockage [10,11]. The validity of long-term treatment with these medicines has not yet been proven. To our knowledge no drugs have been developed for the purpose of cartilage protection.

Tripterygium wilfordii Hook. f. (TWHF) extracts have been found to be effective in traditional Chinese medicine for the treatment of immune inflammatory diseases including RA [12]. Triptolide, a diterpenoid triepoxide purified from TWHF, has been identified as the major component of TWHF and might account for its therapeutic effects [13]. Previous studies have shown that triptolide possesses both immunosuppressive and anti-inflammatory activities, including inhibition of cytokine gene expression in T cells [14]. These anti-inflammatory actions have been attributed to the inhibition of cyclooxygenase (COX)-2 and PGE_2 production in rheumatoid fibroblasts and other cell types [15]. Recently, we have reported the biological activity of triptolide *in vitro* and found novel evidence that triptolide suppressed the gene expression and production of proMMPs-1 and -3, and augmented those of TIMPs-1 and -2 in human synovial fibroblasts [16]. In addition, triptolide inhibited MMP-13 gene expression in human and bovine chondrocytes [17]. We also reported that triptolide interfered with the gene expression of proinflammatory cytokines in mouse macrophages [16]. These observations encouraged us to investigate the effects of triptolide, *in vivo*, on cartilage destruction and inflammation and its action mechanisms in RA model mice. Although an earlier study showed that prophylactic treatment of triptolide significantly reduced the incidence and severity of arthritis in the collagen-induced arthritis (CIA) rats [18], little detailed cellular or molecular evaluation has been performed to investigate the action mechanism of triptolide in this murine model. We have examined whether daily oral administration of triptolide could exert a therapeutic effect on CIA in mice. Specifically, the effects of triptolide treatment *in vivo* on inflammatory responses and cartilage destruction in this arthritis model were evaluated using immunohistochemistry, *in situ* hybridization, and quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR).

In the present study, we demonstrated that triptolide effectively interferes with cartilage destruction and the inflammatory responses accompanying the direct suppression of MMP gene expression and simultaneous up-regulation of TIMP production in the joints. In addition, triptolide down-regulated the expression of proinflammatory cytokines and COX-2 as well as a common transcription factor nuclear factor (NF)-κB [19,20] which closely participates in their expressions.

2. Materials and methods

2.1. Animals

Male DBA/1J mice (Charles River Laboratory Japan, Kanagawa, Japan), age 8–10 weeks, were used for the study of CIA. Filter-top cages were used, with five mice in each cage. During the