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ScienceDirect
Phytomedicine 16 (2009) 538–546
Phytomedicine

Madecassoside attenuates inflammatory response on collagen-induced arthritis in DBA/1 mice

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Abstract

Madecassoside (MA), a triterpenoid product isolated from *Centella asiatica*, has been described to exhibit antioxidant and anti-inflammatory activities. The present study was undertaken to determine whether madecassoside (MA) is efficacious against collagen-induced arthritis (CIA) in mice and its possible mechanisms. DBA/1J mice were immunized with bovine type II collagen and treated with MA (3, 10 and 30 mg/kg d, i.g.) from days 21 to 42 after immunization. Arthritis was evaluated by hind paw swelling, polyarthritis index, and histological examination. *In vitro* proliferation of spleen cells was examined using 3-[4,5-dimethylthylthiazol-2-yl]-2, 5-diphenyltetrazoliumbromide (MTT) assay. Plasma levels of cytokines tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10) and the expression of prostaglandin E_2 (PGE_2), cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) in synovial tissues were also determined. The results showed that comparing with untreated CIA mice, treated with MA dose-dependently suppressed the clinical arthritis score and joints tissues pathological damage, reduced the proliferation of spleen cells, plasma levels of TNF-α and IL-6, synovial tissues PGE_2 production and COX-2 protein expression, however, the expression of COX-1 in symovial tissues did not change and the plasma levels of IL-10 were increased. These results suggest that MA can effectively alleviate inflammatory response on CIA, and anti-inflammatory effects of MA can be attributed, at least partially, to the inhibition of pro-inflammatory mediators, including COX-2 expression, PGE_2 production, TNF-α and IL-6 levels and the up-regulation anti-inflammatory molecule IL-10.

Keywords: Madecassoside; *Centella asiatica*; Rheumatoid arthritis; Collagen-induced arthritis; Pro-inflammatory cytokines; IL-10

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disorder of the joints and affects approximately 0.5–1% of the population worldwide, for which current treatment strategies remain suboptimal (Gabriel, 2001). As the disease advances, there is progressive destruction of bone. Clinically, RA manifests as a symmetric polyarthritis associated with swelling and pain in multiple joints, often initially occurring in the joints of the hands, wrists, and feet. If left untreated, RA can cause significant disability,

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doi:10.1016/j.phymed.2008.11.002

substantial economic costs, and higher mortality rates (Mitchell et al., 1986; Smolen et al., 1995; Sokka, 2003). Although the pathogenesis and the underlying mechanisms of RA remain rudimentary, it has been suggested that abnormalities of cytokines such as TNF-α, IL-6, IL-10, and PGE_2 play an important role in the pathogenesis (Dayer and Fenner, 1992; Karouzakis et al., 2006).

Several medicinal herbs have shown to promote and suppress immunity in different ways and can provide an alternative to conventional chemotherapy for a variety of diseases, especially when host defence mechanism has to be activated under the conditions of impaired immune responsiveness. *Centella asiatica*, a perennial creeper growing abundantly in moist areas and distributed widely in tropical and subtropical countries, has been used for centuries in Ayurvedic and traditional Chinese medicine to alleviate symptoms of venous insufficiency, striae gravidarum, wound, ulcer, arthritis, depression, and anxiety (Brinkhaus et al., 2000; Jayathirtha and Mishra, 2004; Sampson et al., 2001) In South China, *C. asiatica* is even widely used as a dietary supplement and an ingredient of special tea to promote positive health and keep immunomodulation by establishing body equilibrium. Madecassoside (MA) (Fig. 1), a major pentacyclic triterpenoid saponin component of *C. asiatica*, has been described to have wound healing, anti-apoptosis, antioxidant, and anti-inflammatory activities (Bian et al., 2008; Jia and Lu, 2008; Liu et al., 2004; Matsuda et al., 2001) However, there are few reports on the effects and mechanisms of MA in treatment of RA.

CIA in the arthritis susceptible DBA/1J mouse strain is one of the most commonly used immunization-based models in which many of the pathologic features of human RA are recapitulated. Therefore CIA is widely used in numerous studies to investigate the pathogenesis of RA and for identification of potential therapeutic targets (Kannan et al., 2005). Here, based on the immunological and anti-inflammatory feature of *C. asiatica* and MA, we examined the anti-inflammatory effects of MA in the mice CIA model. In particular, we investigated whether MA attenuated the progressive destruction of arthritic joints by regulating the production of TNF-α, IL-6, IL-10, and PGE_2 and modulating the expression of cyclooxygenase (COX) protein in CIA synovial tissues, which is a key enzyme in the biosynthetic metabolism of PGE_2, and plays an important role in the pathogenesis of CIA.

Materials and methods

Animals

Inbred male DBA/1J mice, aged 6 weeks, were purchased from Shanghai Experimental Animal Center of Chinese Academy of Sciences. The animals were housed in specific pathogen-free conditions (12 h light/12 h dark photoperiod, 23±1 °C, 55±5% relative humidity). All mice were allowed to acclimatize in our facility for 1 week before any experiments were started. All experiments were approved by Institute's Animal Ethical Committee and confirmed to national guidelines on the care and use of laboratory animals.

Reagents

MA ($C_{48}H_{78}O_{20}$, MW: 975.12, purity: ⩾98%) determined by HPLC as previously described (Günther and Wagner, 1996) was purchased from Guangxi Changzhou Natural Products Development Co. Ltd. (Nanning, China). Indomethacin was obtained from Sigma (St. Louis, MO, USA). RPMI 1640 medium was purchased from Gibco BRL Life Technologies (CA, USA). FBS (fetal bovine serum) was purchased from HyClone Laboratories (Logan, UT, USA). Bovine type II collagen (CII), complete Freund's adjuvant (CFA), dimethyl sulfoxide (DMSO), and MTT were obtained from Sigma Chemical Co. (St. Louis, MO, USA). BCA

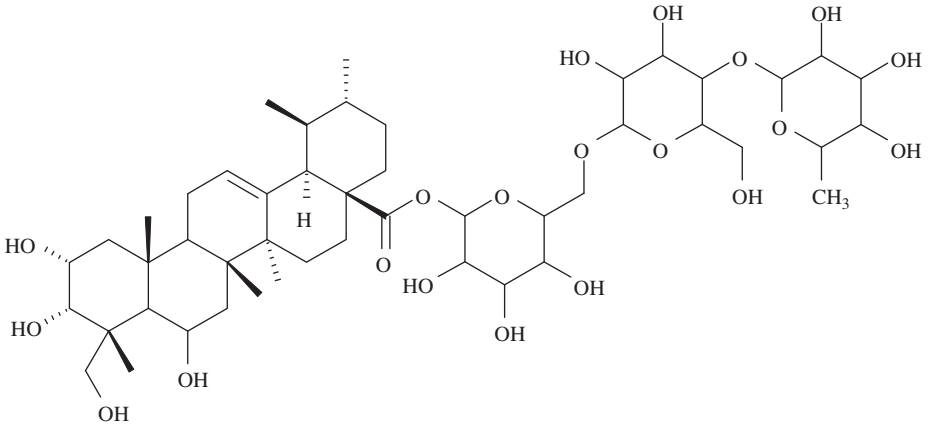

Fig. 1. Chemical structure of madecassoside.