Journal of Ethnopharmacology 187 (2016) 42–48

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Anti-inflammatory and anti-arthritic effects of taraxasterol on adjuvant-induced arthritis in rats

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ARTICLE INFO

Article history:
Received 30 January 2016
Received in revised form
10 April 2016
Accepted 20 April 2016
Available online 22 April 2016

Keywords:
Taraxasterol
Adjuvant arthritis
Inflammation
Rats
Anti-arthritis

ABSTRACT

Ethnopharmacological relevance: Taraxasterol was isolated from the traditional Chinese medicinal herb *Taraxacum* which has been frequently used as a remedy for inflammatory diseases. In the present study, we determined the in vivo anti-arthritic effect of taraxasterol on arthritis induced by Freund's complete adjuvant (FCA) in rats.

Materials and methods: Rats were immunized with FCA by intradermal injection into the right hind metatarsal footpad, and were orally treated daily with taraxasterol at 2, 4 and 8 mg/kg from day 2–28 after immunization. Paw swelling, arthritis index, body weight, spleen index and thymus index were evaluated. The levels of TNF-α, IL-1β, PGE_2, OPG and RANKL in sera were measured using ELISA. Histopathological changes in joint tissues were examined using hematoxylin and eosin (H&E).

Results: Taraxasterol significantly suppressed paw swelling and arthritis index, attenuated body weight loss, decreased the spleen index and thymus index induced by FCA. Furthermore, taraxasterol significantly inhibited the overproduction of serum TNF-α, IL-1β, PGE_2 and RANKL, and increased serum OPG production in FCA-induced rats. Histopathological examination indicated that taraxasterol attenuated synovial hyperplasia, bone and cartilage damage, and inflammatory cell infiltration.

Conclusions: These results suggest that taraxasterol has the potential protective effect against FCA-induced arthritis in rats.

1. Introduction

Rheumatoid arthritis (RA) is a chronic and systemic autoimmune joint disease that is characterized by nonspecific inflammation of peripheral joints and destruction of cartilage and bone with resultant disability (Klareskog et al., 2009). In particular, it is reported that the inflammatory mediators play key roles in the inflammation and joint damages during the development of RA (William, 1996). The risk of morbidity and mortality still remains high in the last decade (Praveen and Suchita, 2013). Although strategies for treatment of RA have changed from traditional nonsteroidal anti-inflammatory drugs (NSAIDS) supplemented with steroids or disease-modifying antirheumatic drugs (DMARDs) to novel biologics, such as tumor necrosis factor-α (TNF-α) antibody and the decoy TNF-α receptor (Smolen et al., 2007), and these treatments are accompanied by concerns about the side effects and the high costs. The best ways to prevent long-term joint damage and functional decline is still unknown (Goekoop-Ruiterman et al., 2005). Therefore, the development of efficient, minimum side effect and low-cost therapeutic drugs for RA is urgently needed.

Taraxacum (dandelion) is the whole herb of *Taraxacum mongolicum* Hand.-Mazz, *Taraxacum sinicum* Kitag, or same genus plants (Composite), locally called 'pugongying'. It has long been used in traditional oriental medicine for its lactating, choleretic, diuretic, anti-angiogenic, antirheumatic and anti-inflammatory properties (Ahmad et al., 2000; Schütz et al., 2006; Jeon et al., 2008). It is widely used for treating various inflammatory or infectious diseases such as hepatitis, upper respiratory tract infections, bronchitis, pneumonia, and as a compress for its anti-mastopathy activity (Leu et al., 2005; Sweeney et al., 2005). In vivo, the aqueous extract of *Taraxacum* was assessed to contain acute anti-inflammatory activity by showing its protective effects against cholecystokinin-induced acute pancreatitis in rats (Seo et al., 2005), and we also reported its protective effects against lipopolysaccharide (LPS)-induced acute lung injury in mice (Liu et al., 2010). In vitro, *Taraxacum* extracts have been shown to inhibit LPS-induced inflammatory responses by reducing nitric oxide (NO), prostaglandin E_2 (PGE_2) and pro-inflammatory cytokines production via inactivation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signal pathway in RAW 264.7

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http://dx.doi.org/10.1016/j.jep.2016.04.031
0378-8741/

cells (Koh et al., 2010; Park et al., 2011). Taraxasterol, a pentacyclic-triterpene, was one of the main active constituents in *Taraxacum*, and its structural identity was determined by one- and two-dimensional nuclear magnetic resonance (NMR) spectroscopic analysis (Schütz et al., 2006). Recently, we have reported that taraxasterol has the in vitro anti-inflammatory activity by suppressing the production of various cytokines and inflammatory mediators in LPS-induced murine RAW 264.7 macrophages (Zhang et al., 2012; Xiong et al., 2014). Our studies have also shown that taraxasterol has the in vivo protective effects on ovalbumin-induced allergic asthma (Liu et al., 2013) and LPS-induced endotoxic shock in mice (Zhang et al., 2014), and protective effect of taraxasterol on acute lung injury in mice also has been studied (San et al., 2014). Recently, it has also been reported that taraxasterol inhibits IL-1β-induced inflammatory response in human osteoarthritic chondrocytes (Piao et al., 2015). However, no study thus far has addressed whether taraxasterol has in vivo anti-arthritic effect in the treatment of RA and what the underlying mechanisms. Therefore, as a part of our on-going screening program to evaluate the anti-inflammatory potentials of natural compounds, the present study was designed to confirm the in vivo anti-arthritic effect and clarify the potential mechanism of taraxasterol on adjuvant-induced arthritis in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–240 g) were purchased from Changchun Yisi Experiment Animals Co. Ltd., China (certificate no. SCXK (Ji) 2011-0004). The rats were kept in microisolator cages (room temperature 24 ± 1 °C, relative humidity 50–60%) and received food and water ad libitum. Before experimentation, the rats were allowed to adapt to the experimental environment for a minimum of one week. All animal experimental procedures were performed in accordance with the guidelines of the Ethical Committee for the Experimental Use of Animals at Yanbian University (Yanji, Jilin, China).

2.2. Drugs and reagents

Taraxasterol (Fig. 1) was obtained from Chengdu Fenruisi Biotechnology Co. (Chengdu, Sichuan, China), and its purity was 99.5% based on HPLC analysis. Methotrexate (MTX, No.036140404) was purchased from Shanghai Xinyi Pharmaceutical Co. (Shanghai, China). Freund's complete adjuvant (FCA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rat TNF-α, IL-1β, PGE_2, osteoprotegerin (OPG) and receptor activator of nuclear factor-kB ligand (RANKL) ELISA kits were purchased from R&D Systems (Minneapolis, MN). All the other chemicals used in this study were of analytical grade.

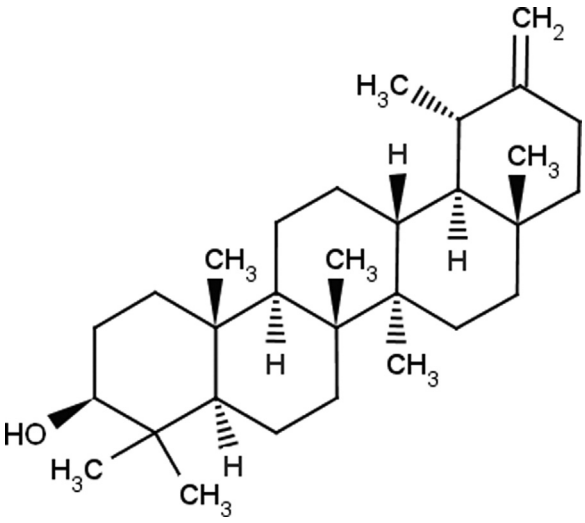

Fig. 1. Chemical structure of taraxasterol.

2.3. Establishment of rat adjuvant arthritis and treatment regimen

FCA was prepared by suspending heat-killed bacillus calmette guerin (BCG) in liquid paraffin at 10 mg/ml. The rats were immunized with 0.1 ml of FCA by intradermal injection into the right hind metatarsal footpad, as previously described (Lin et al., 2014). The rats were randomly divided into six groups (n=7): control group, CFA group, taraxasterol (at doses of 2, 4 and 8 mg/kg, respectively)+FCA groups, and MTX+FCA group. On second day after FCA immunizing, taraxasterol (2, 4 and 8 mg/kg) in 0.5% Sodium Tvlose was administered orally once per day consecutively. MTX at 3 mg/kg was administered orally twice a week as a positive control. The rats in control group (unsensitized) and FCA group were administered the equal volume of 0.5% Sodium Tvlose at the same time.

2.4. Evaluation of arthritis swelling

The volume of the right hind paw swelling of each rat was measured by using plethysmometer (Chengdu Taimeng Technology Co. Ltd., China) before FCA immunization and then at 4-day intervals up to day 28 as primary swelling. The left hind paw volume of rats was measured as secondary swelling. The change of paw swelling was calculated using the following formula: The paw swelling degree=(ΔV treated − ΔV untreated)/ΔV untreated). Where ΔV treated is mean changes in paw volume of treated rat on respective day. ΔV untreated is mean changes in initial paw volume (volume day 0).

The visual arthritis index was used to evaluate the severity of arthritis as described previously (Fan et al., 2012). In this scoring system, rats were examined visually for the appearance of arthritis in the peripheral joints, tail, ears, nebula of eyes and were scored for arthritis severity. The paw, ears, nose, eyes and tail were graded separately and cumulative scoring depended on redness and swelling. Observations were recorded by observer who was blind to the study. The arthritis score ranged from 0 to 3, where 0 indicated no change, 1 indicated mild inflammation, 2 indicated moderate inflammation and 3 indicated significant inflammation.

2.5. Measurement of body weight

The body weight of rats was measured every 4 days.

2.6. Serum TNF-α, IL-1β, PGE_2, OPG and RANKL assays

On day 28 after FCA immunization, the rats were anesthetized with diethylether 1 h after the last administration. Blood was drawn from the retro-orbital vein puncture, sera were collected by centrifugation at 3000 rpm for 10 min. The concentrations of TNF-α, IL-1β, PGE_2, OPG and RANKL in sera were measured by sandwich ELISA using commercially available reagents according to the manufacturer's instructions. Briefly, microwell plates were coated overnight at 4 °C with rat TNF-α, IL-1β, PGE_2, OPG and RANKL capture antibody and blocked at room temperature for 1 h with 1% BSA in phosphate-buffered saline with shaking. Samples from the sera and internal standard were incubated at room temperature for 2 h with shaking, followed by rat TNF-α, IL-1β, PGE_2, OPG and RANKL biotinylated detection antibody for 1 h and an avidin