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Periplocoside A ameliorated type II collagen-induced arthritis in mice via regulation of the balance of Th17/Treg cells

Yang Yang [a], Xudong Hu [a], Lei Cheng [b], Wei Tang [b], Weimin Zhao [c], Yifu Yang [a,*], Jianping Zuo [a,b,**]

[a] Laboratory of Immunology and Virology, Experiment Center For Science and Technology, Shanghai University of Traditional Chinese Medicine, China
[b] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China
[c] Department of Natural Products Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China

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ABSTRACT

Periplocoside A (PSA) has been extracted from the Chinese herbal medicine *Periploca sepium* Bge to treat rheumatoid arthritis (RA) via immune regulation. We previously found that PSA exhibits immunosuppressive activity both in vitro and in vivo. Balanced regulation of helper T 17 (Th17)/regulatory T (Treg) cells is the current therapeutic direction for the treatment of RA. The present study investigated the mechanism of PSA in treating collagen-induced arthritis (CIA). The therapeutic effects and potential pharmacological mechanisms of PSA were specifically clarified by examining its effects on CIA in DBA/1 mice. PSA administration significantly relieved the severity of the arthritis, and preventive administration of PSA reduced the incidence of arthritis in the mice with CIA and relieved joint damage in terms of morphology. PSA was also able to reduce the levels of anti-collagen II (CII) antibodies and pro-inflammatory cytokines in the serum. As a result, the proportion of Th17 cells decreased, and the proportion of Treg cells increased. A follow-up study of the ex vivo immunological reactions induced by a specific antigen found that PSA suppressed lymphocyte proliferation, inhibited the differentiation and reactivity of Th17 cells, and promoted the proportion of Treg cells among helper T cells. PSA also exhibited pharmacological effect in regulating the balance between Th17 and Treg cells in CIA through relevant signalling pathways. Thus, PSA played a specific role in CIA treatment. In particular, our results suggest that the therapeutic effects of PSA on RA are partially realized via the regulation of the balance of Th17/Treg cells.

1. Introduction

Periploca sepium Bge is a traditional Chinese herbal medicine that is used to treat rheumatoid arthritis (RA) in via both clinical and traditional applications. Periplocoside A (PSA) is extracted from *Periploca sepium* Bge [1] as a pregnane glycoside compound. It was previously found that PSA and a similar compound (periplocoside E, or PSE) exhibited immunoinhibitory activity both in vitro and in vivo [2,3]. PSA can specifically suppress T lymphocyte activation in vitro and prevent hepatic injury induced by concanavalin A (ConA) in mice via inhibition of natural killer T (NKT) cell-derived inflammatory cytokine production [4]. PSA also inhibits IL-17 production and Th17 cell differentiation to prevent experimental autoimmune encephalomyelitis (EAE) [5]. Therefore, PSA is an immunosuppressive compound that might exert therapeutic effects on IL-17-mediated autoimmune diseases.

Similar to EAE, RA is a typical autoimmune disorder that is primarily characterized by chronic inflammation within multiple joints' synovial tissue. RA also features synovial hyperplasia and inflammatory cell infiltration, which result in continuous destruction of the articular cartilage tissue and bone structures [6]. DBA/1 mice with collagen-induced arthritis (CIA) are a common experimental model used to shed light on CIA in human beings [7]. The high incidences of anti-type II collagen (CII) antibodies and CII-specific T cells indicate that CII is one of the major autoantigens in human RA [8]. The mechanism of the CII-induced CIA animal model is similar to the pathogenesis of human RA, especially with regard to the regulation of an imbalance of immune cells, including pro-inflammatory and anti-inflammatory helper T (Th) lymphocytes [9].

Th17 cells primarily produce cytokines (e.g., IL-17), and these cells are classified as the major pathological effector cells in a variety of autoimmune diseases, including RA [10]. IL-17 primarily activates various pathogenic cells in RA via activation of pro-inflammatory mediators, such as tumour necrosis factor α (TNF-α), IL-1β and IL-6. In contrast, regulatory T (Treg) cells play an important role in the maintenance of immunological homeostasis and tolerance. A deficiency of CD4 + CD25 + Treg cells causes experimental autoimmune diseases [11] and increases

* Corresponding author.
** Corresponding author at: State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China.
E-mail address: jpzuo@mail.shcnc.ac.cn (J. Zuo).

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the severity of CIA in mice [12]. The population of Treg cells is also reduced in patients with RA, in whom a loss of immunosuppressive function is often found [13]. Therefore, Treg cells play a key protective role in patients with autoimmune diseases or chronic inflammation.

An imbalance of differentiation and functionality of Th17/Treg cells could be a mechanism of autoimmune diseases. Regulation of the Th17/Treg cell balance is thus an important direction for RA treatment, and this balance might be a potential critical target for new drug development [14,15]. In this context, PSA exhibits potent immunosuppressive effects that might be beneficial for the treatment of CIA.

2. Materials and methods

2.1. Animals

Male DBA/1 mice, aged 7 or 8 weeks and weighing 20–22 g, were obtained from the Shanghai Laboratory Animal Centre of the Chinese Academy of Sciences. These mice were treated and fed under specific pathogen-free conditions. Humane care was provided following the National Institutes of Health Guide for the Care and Use of Laboratory Animals (SZY201412001). Additionally, the study protocol was approved by the Laboratory Animal Committee of Shanghai University of Traditional Chinese Medicine. Three groups of mice were randomly selected to evaluate the effects of PSA administration: untreated (non-immunized), vehicle-treated and PSA-treated (10, 20 or 40 mg/kg).

2.2. CIA induction and assessment

Bovine CII (250 μg, purchased from the Chemical Engineering Department of Sichuan University) was dissolved in 0.1 M acetic acid and emulsified in an equal volume of complete Freund's adjuvant (CFA) containing *Mycobacterium tuberculosis* strain H37Rv (Difco, U.S.A.). This solution was injected intradermally into the base of the tail to induce CIA, and a booster was delivered using the same method 21 days after the primary immunization, as described previously. An arthritis index was then used under double-blind conditions to evaluate the severity of CIA. Each limb had a ranking score from 0 to 4 based on defined indications (0: normal; 1: erythema and swelling of one or several digits; 2: erythema and moderate swelling extending from the ankle to the mid-foot (tarsals); 3: erythema and severe swelling extending from the ankle to the metatarsal joints; and 4: complete erythema and swelling encompassing the ankle, foot and digits, resulting in deformity and/or ankyloses) [16]. The scores of all four limbs were summed. The arthritis index was scored at the beginning of the booster immunization (day 0).

2.3. Compound and administration

PSA (MW = 1408 Da; purity over 98%) extracted from *Periploca sepium* Bge (stem barks) was provided by Prof Zhao WM from the Shanghai Institute of Materia Medica. Fig. 1 shows the chemical structure of PSA. The concentrated extract of PSA was dissolved in dimethyl sulfoxide (DMSO) and diluted with pure water to a dose containing a target concentration of DMSO of <1%. PSA (20 mg/kg, p.o.) was administered on a daily basis starting 1 day before booster immunization to allow assessment of its preventive effects. PSA administration was then initiated on day 8 after booster immunization and continued on a daily basis to further assess its therapeutic effects where indicated.

2.4. Histological evaluations

The mice were sacrificed on day 22 after booster immunization, and the left hind paws were fixed in 10% buffered formalin and decalcified in 5% EDTA. The paws were then embedded in paraffin, and a microscopic assessment of sagittal sections stained with haematoxylin and eosin (H&E) was performed. Histopathological changes were scored based on the following previously reported parameters: bone resorption, cartilage structure damage, cartilage cell damage, inflammatory cell infiltration, synovial inflammation and hyperplasia. Additionally, 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS, pH 7.4) was used to fix the right hind paws for 48 h, and micro-CT was used for analysis following storage in 70% ethanol. In particular, hind paws fixed in PFA as well as tibias were placed in separate microcentrifuge tubes containing 70% ethanol and were scanned using a Micro-CT80 scanner (SCANO Medical AG, Bassersdorf, Switzerland). All analyses were performed according to international guidelines [17].

2.5. Cell preparation and isolation

Joint cells were collected from DBA/1 mouse paws. The skin, muscles and bones were removed, and the joint tissues were minced and washed with PBS. Erythrocytes were lysed in Tris-buffered ammonium chloride, and the resultant cell suspensions were filtered using a cell strainer. The joint cells were then washed and resuspended in PBS containing 2% foetal bovine serum prior to staining and flow cytometry assays.

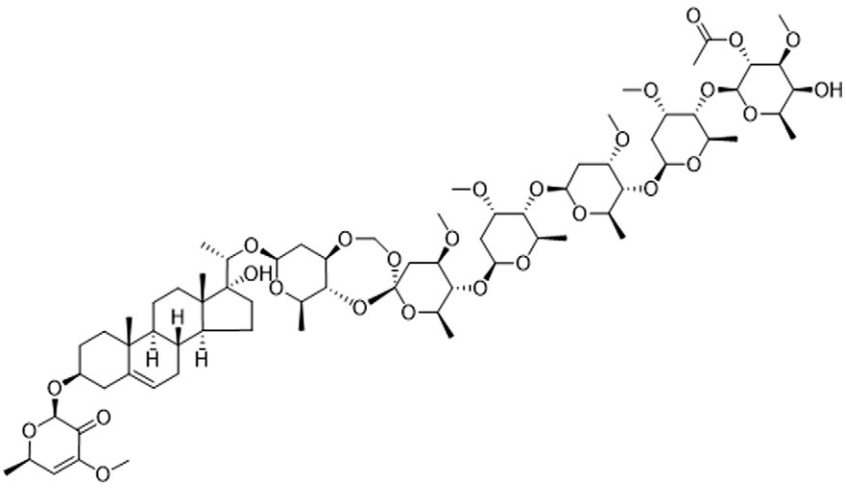

Fig. 1. The chemical structure of PSA. PSA is extracted from *Periploca sepium* Bge (purity ≥ 98% by HPLC analysis). Formula, $C_{72}H_{114}O_{27}$. Mol. wt., 1411.68 Da.