International Immunopharmacology 18 (2014) 1–6

Contents lists available at ScienceDirect

International Immunopharmacology

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

Total glucosides of paeony inhibit the proliferation of fibroblast-like synoviocytes through the regulation of G proteins in rats with collagen-induced arthritis

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ARTICLE INFO

Article history:
Received 10 January 2013
Received in revised form 2 September 2013
Accepted 4 September 2013
Available online 22 October 2013

Keywords:
Total glucosides of paeony
Fibroblast-like synoviocytes
IL-1β
Proliferation
G protein

ABSTRACT

The aim of this study was to investigate the expression of G proteins in fibroblast-like synoviocytes (FLSs) from rats with collagen-induced arthritis (CIA) and to determine the effect of total glucosides of paeony (TGP). CIA rats were induced with chicken type II collagen (CCII) in Freund's complete adjuvant. The rats with experimental arthritis were randomly separated into five groups and then treated with TGP (25, 50, and 100 mg/kg) from days 14 to 35 after immunization. The secondary inflammatory reactions were evaluated through the polyarthritis index and histopathological changes. The level of cyclic adenosine monophosphate (cAMP) was measured by radioimmunoassay. The FLS proliferation response was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The toxin-catalyzed ADP-ribosylation of G proteins was performed through autoradiography. The results show that TGP (25, 50, and 100 mg/kg) significantly decreased the arthritis scores of CIA rats and improved the histopathological changes. TGP inhibited the proliferation of FLSs and increased the level of cAMP. Moreover, the FLS proliferation and the level of Gαi expression were significantly increased, but the level of Gαs expression was decreased after stimulation with IL-1β (10 ng/ml) *in vitro*. TGP (12.5 and 62.5 μg/ml) significantly inhibited the FLS proliferation and regulated the balance between Gαi and Gαs. These results demonstrate that TGP may exert its anti-inflammatory effects through the suppression of FLS proliferation, which may be associated with its ability to regulate the balance of G proteins. Thus, TGP may have potential as a therapeutic agent for the treatment of rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune and inflammatory disease that is characterized by an uncontrolled proliferation of synoviocytes, the infiltration of abundant inflammatory cells, and progressive joint erosion to result in the destruction of cartilage and bone [1]. Although the exact cause of the pathology of RA is not fully understood, an increasing body of evidence suggests that fibroblast-like synoviocytes (FLSs) play a crucial role in the propagation of inflammation and joint damage through their proliferation and the production of a variety of inflammatory mediators, such as prostaglandin E_2 (PGE_2), interleukin (IL)-1, and tumor necrosis factor (TNF)-α. IL-1β induces the proliferation of FLSs and is considered one of the most important cytokines in the pathogenic process of inflammation in RA [2,3]. The signaling pathways that regulate the production of IL-1β are implicated in RA pathogenesis. In this study, IL-1β was used to reproduce an inflammatory status in FLS cultures.

An increasing number of studies suggest that the G protein pathway is associated with the pathogenesis of RA [4,5]. Heterotrimeric GTP-binding proteins (G proteins) are composed of α, β, and γ subunits and convert receptor-bound signals into intracellular signals. The G protein α subunits can be divided into four families based on their primary sequence similarity: Gαs, Gαi, Gαq, and G_{12} [6]. The members of the Gαs family activate adenylyl cyclases (AC), whereas the Gαi family members can inhibit AC and thus control the intracellular concentrations of cyclic adenosine monophosphate (cAMP). In addition, cAMP and its downstream signaling pathway involve cell growth and proliferation, and cAMP may block the proliferation of many cell types through multiple targets [7]. Previous studies in our laboratory have shown that the mRNA expression of Gs is decreased and the mRNA expression of Gi is significantly increased in synoviocytes from collagen-induced arthritis (CIA) rats that are compared with normal rats [8]. However, the protein level and activity of the G proteins in FLS are unclear.

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1567-5769/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.intimp.2013.09.007

The total glucosides of paeony (TGP) were extracted from the root of *Paeonia lactiflora* Pall, which is one of the most well-known herbs in China, Korea, and Japan. TGP, more than 90% of which is paeoniflorin (Pae), exerts both anti-inflammatory and immune-regulatory effects and has been used to treat RA in Chinese clinics. The anti-inflammatory and immunoregulatory activities of Pae and TGP have been extensively studied in our previous studies [9–13]. CIA is an arthritis that is induced through the sensitization of rats with type II collagen, and this model was developed because the antibody for type II collagen exists very frequently in the serum and synovial fluid of the joints in RA. The patterns of cell-mediated and humoral immunity in both RA and CIA exhibit similar characteristics. Thus, CIA is accepted as a disease model of RA and widely used to elucidate the pathogenesis of RA and to screen new drugs. To gain further insight into the mechanism of TGP, this study examined the effects of TGP on the function of FLSs derived from CIA *in vivo* and investigated whether the inhibitory effects of TGP on FLSs are achieved through the regulation of the expression of G proteins *in vitro*.

2. Materials and methods

2.1. Animals

Sprague–Dawley (SD) rats (male, 180 ± 20 g) were obtained from the Animal Department of Anhui Medical University, China. All of the rats were acclimatized under standard laboratory conditions. During the experimental period, the rats were given standard laboratory chow and tap water *ad libitum* and maintained on sawdust in plastic-bottomed cages at 20–25 °C. All of the experiments were approved by the Ethics Review Committee for Animal Experimentation of the Institute of Clinical Pharmacology at Anhui Medical University.

2.2. Reagents

Chicken type II collagen was purchased from Institute Jacques Boy (Reims, France). Bacillus Calmette–Guerin (BCG) was obtained from the Institute of Biological Products of Shanghai, China. 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Pertussis toxin (PTX) and cholera toxin (CTX) were obtained from List Biologic (Campbell, CA, USA). [^{32}P] NAD was purchased from Amersham Biosciences (Piscataway, NJ, USA). IL-1β was purchased from PeproTech. Anti-β-actin antibody was obtained from Santa Cruz Biotechnology, Inc. The ^{125}I-cAMP RIA kit was purchased from the Nuclear Medicine Laboratory of Shanghai College (Shanghai, China). The Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco Co. (CA, USA). All other chemicals used in this study were of analytical grade and were obtained from commercial sources.

2.3. Drugs

TGP was provided by the Chemistry Lab of the Institute of Clinical Pharmacology of Anhui Medical University (Hefei, Anhui Province, China). TGP was suspended in 0.5% sodium carboxymethylcellulose (CMC-Na) and distilled water at the required concentration before its use *in vivo* and *in vitro*.

2.4. Induction of collagen-induced arthritis (CIA)

Chicken type II collagen (CCII) was dissolved in 0.1 M acetic acid at a concentration of 2 mg/ml and emulsified with an equal volume of Freund's complete adjuvant that had been prepared using heat-killed BCG in Freund's incomplete adjuvant to a final concentration of 0.1 mg/ml. Rats were injected intradermally twice with a total of 1 ml of the emulsion. The first injection was made in the left hind paw with 0.1 ml and the tail and other 3–5 sites on the back with 0.9 ml; a provocation test was performed seven days later using a similar method.

2.5. Treatment of CIA

Before the onset of arthritis, rats were randomly separated into five groups: normal group, non-immunized rats; model CIA group, untreated CIA rats; and the TGP groups, CIA rats treated with 25, 50, and 100 mg/kg TGP. The rats in the TGP groups were administered TGP orally by gavage from days 14 to 35 after immunization. The rats in the normal and model CIA groups were given an equal volume of vehicle.

2.6. Evaluation of polyarthritis index

The rats were inspected daily for signs of arthritis by two independent observers who were not aware of the treatment. The arthritic severity in each paw was evaluated using a macroscopic scoring system ranging from 0 to 4: 0, paws with no swelling and focal redness; 1, paws with swelling of finger joints; 2, paws with mild swelling of the ankle or wrist joints; 3, paws with severe inflammation of the entire paw; and 4, paws with deformity or ankylosis. The cumulative score for all four paws of each rat was used as the polyarthritis index, which had a maximum value of 16 [14].

2.7. Histological examination

The rats were sacrificed by cervical dislocation after ether anesthesia on day 36 after immunization. The knees and hind paws of the rats were removed and fixed with 10% formaldehyde in PBS. The samples were then decalcified for 10 days with EDTA and embedded in paraffin for histological analysis. The paraffin sections were stained with hematoxylin and eosin (H&E).

2.8. Culture of FLS

The rats were anesthetized and sacrificed on day 36 after immunization, and the synovial tissues from the knees joints were excised, minced, digested with type IA collagenase for 2 h, filtered, extensively washed, and cultured in DMEM containing 20% fetal bovine serum (FBS; Sigma), 100 U/ml penicillin, 100 μg/ml streptomycin, and L-glutamine, at 37 °C in 5% CO_2. At confluence, the adherent cells were trypsinized, split in a 1:3 ratio, and re-cultured in medium. The spindle-shaped cells from passages 3 to 5, which consisted of a homogeneous population of synoviocytes, were used in the subsequent experiments.

2.9. Measurement of cAMP

FLSs were obtained using the above-described method. The adherent cells were resuspended in 15% FBS-DMEM, added to 24-well flat-bottomed culture plates at a density of 5×10^5 cells/well, incubated at 37 °C in 5% CO_2 for 48 h, and centrifuged at 2000 rpm for 10 min. The adherent cells were scraped and disrupted by sonication. The cell lysates were harvested to detect the level of cAMP by RIA using the ^{125}I cAMP kit according to the manufacturer's instructions.

2.10. Proliferation assay by MTT

FLSs were obtained using the above-described method. After the cells achieved adherence, the culture medium was replaced, and the cells were resuspended in 200 μl of 15% FBS-DMEM medium containing TGP (0.5, 2.5, 12.5, 62.5, and 312.5 μg/ml) with or without IL-1β (10 ng/ml) at a cellular density of 1.0×10^5 cells/ml in 96-well flat-bottomed culture plates. The cultures were incubated at 37 °C in 5% CO_2 for 48 h. Two hours before termination of the culture, 10 μl of MTT