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The expression change of β-arrestins in fibroblast-like synoviocytes from rats with collagen-induced arthritis and the effect of *total glucosides of paeony*

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ABSTRACT

Aim of the study: To investigate the expression of β-arrestins in fibroblast-like synoviocytes (FLS) from collagen-induced arthritis (CIA) rats and the effect of total glucosides of paeony (TGP).
Materials and methods: TGP and glucosides of tripterygium wilfordii (GTW) were intragastricly administrated to collagen-induced arthritis (CIA) rats after immunization. The secondary inflammatory reaction was evaluated by hind paw swelling, polyarthritis index and histopathological changes. Antibodies to type II collagen (CII) were determined by enzyme-linked immunosorbent assay (ELISA). Synoviocyte proliferations were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl (MTT) assay. The expression of β-arrestins in synoviocytes from CIA rats was measured by western blot.
Results: The administration of TGP (25, 50, 100 mg/kg) depressed hind paw swelling and decreased the arthritis scores of CIA rats. TGP improved the pathologic manifestations of CIA. Serum anti-CII antibodies level increased significantly in CIA rats, while TGP had no effect on it. Fibroblast-like synoviocytes (FLS) proliferation was inhibited by TGP (50, 100 mg/kg). On d14, d28 after immunization, β-arrestins expression greatly up-regulated in synoviocytes from CIA rats and then returned to baseline levels on d42 after immunization. TGP (50, 100 mg/kg) significantly reduced the expression of β-arrestins.
Conclusion: An inflammatory process in vivo induces an up-regulation of β-arrestins in synoviocytes from CIA rats while TGP can inhibit this change, which might be one of the important mechanisms for TGP to produce a marked therapeutic effect on RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks the joints producing an inflammatory synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints. An inflammatory synovitis is the hallmark of RA (Christodoulou and Choy, 2006) and G protein-coupled receptors (GPCRs)-mediated signaling of fibroblast-like synoviocytes (FLS) in RA models was imbalance (Chen and Wei, 2003; Zhang et al., 2008).

β-Arrestin1 (arrestin 2) and β-arrestin2 (arrestin 3) are cytosolic proteins involved in the termination of signaling by activated GPCRs with G-protein-coupled receptor kinases (GRKs) (Wolfe and Trejo, 2007; Tilley et al., 2009). However, β-arrestins also function as scaffold proteins that interact with several cytoplasmic proteins, which link GPCRs to intracellular signaling pathways (Gurevich and Gurevich, 2006; Dewire et al., 2007). β-Arrestin-mediated regulation of transcription also appears to play important roles in cell growth, apoptosis and modulation of immune functions (Ma and Pei, 2007; Lee et al., 2008). Lombardi et al. observed a profound down-regulation of GRK2, -3, and -6 in splenocytes and mesenteric lymph node cells from adjuvant arthritis (AA) rats at the peak of the inflammatory process. In splenocytes from AA rats on day 18 after induction of the disease, they observed a moderate, but statistically significant, increase in immunodetectable β-arrestin1. On day 45, β-arrestin1 levels in splenocytes had returned to the levels of control rats. A similar time course for β-arrestin-1 expression was obtained in mesenteric lymph node (MLN) cells (Lombardi et al., 2001). However, the changes of β-arrestins expressions in synoviocytes from RA animal models are still not clear.

Paeonia lactiflora Pall, a traditional Chinese herbal medicine, has been shown to possess antispasmodic, anti-inflammatory and analgesic effects, used to treat RA for hundreds of years in China. Total glucosides of paeony (TGP), extracted from roots of *Paeonia lactiflora Pall*, have been marked as medicaments (Pafulin) for the

Abbreviations: TGP, total glucosides of paeony; RA, rheumatoid arthritis; CIA, collagen-induced arthritis; GRK, G-protein-coupled receptor kinases; FLS, fibroblast-like synoviocytes; GTW, glucosides of tripterygium wilfordii; GPCRs, G protein-coupled receptors; AA, adjuvant arthritis; Gs, stimulatory G protein; Gi, inhibitory G protein.

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treatment of RA. Clinical practice confirms that TGP can significantly improve symptoms of RA with little known side effect or adverse reaction. It has been extensively investigated in our laboratory for many years and was confirmed to have anti-inflammatory and immunoregulatory effects (Zhu et al., 2005; Zheng and Wei, 2005; Xu et al., 2007; Chang et al., 2009). Paeoniflorin (Pae), a monoterpene glucoside, is one of the main bioactive components of TGP (Wu and Gu, 2009). Pae could induce the T helper (Th) 1 cells immune tolerance, which then shift to Th2, Th3 cells mediated activities to take effect the anti-inflammatory and immunoregulatory effects (Wu et al., 2007). Glucosides of tripterygium wilfordii (GTW), extracted from the root of Tripterygium wilfordii Hook f (TWHF), are isolated by the Institute of Dermatology of Chinese Academy of Medical Sciences for the first time in the 1970s. It contains trace diterpenes, small quantity of alkaloids, and some pentacyclic triterpenes (Wang and Guo, 1995). In China, GTW was approved by SFDA for marketing as a drug in the treatment of several rheumatic diseases, including RA and systemic lupus erythematosus (SLE) (Tao et al., 1998; Tao and Lipsky, 2000; Zhang et al., 2004). So, in this study, we detected the expression change of β-arrestins in synoviocytes from collagen-induced arthritis (CIA) rats and the effect of TGP, with GTW used as a suitable positive control.

2. Materials and methods

2.1. Experimental animals

Sprague–Dawley (SD) rats (male, 140 ± 20 g, Grade II, Certificate No. 006) were obtained from the Animal Department of Anhui Medical University (Hefei, Anhui Province, China). All rats were housed under standard laboratory conditions at 24 °C. During the experimental period, tap water and commercially available food were given freely. The lighting duration in the breeding room was 12 h (7:00 am to 7:00 pm). All experiments were approved by Ethics Review Committee for Animal Experimentation of Institute of Clinical Pharmacology, Anhui Medical University.

2.2. Materials and reagents

Mouse anti-β-arrestins antibody was obtained from BD Biosciences Pharmingen. SuperSignal west femto maximum sensitive substrate was purchased from Pierce (Rockford, IL). Bacillus Calmette Guerin (BCG) from Shanghai Biochemical Factory; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H tetrazolium bromide (MTT) from Sigma Chemical Co. (St. Louis, MO, USA); Dulbecco's modified Eagle's medium (DMEM) from Gibco Co. (CA, USA). Other chemicals used in these experiments were analytical grade from commercial sources.

2.3. Drug

TGP (yellow power) was provided by the Chemistry Lab of Institute of Clinical Pharmacology of Anhui Medical University (Hefei, Anhui Province, China). GTW was purchased from Shanghai Fudan Fuhua Medicine Company (Shanghai, China). Both TGP and GTW were suspended in 0.5% sodium carboxymethylcellulose (CMC-Na) respectively before use.

2.4. Induction of CIA

CIA was induced by immunizing rat with native Chicken type II collagen (CCII) that had been dissolved overnight at 4 °C in 0.1 M acetic acid (4 mg/ml) and emulsified with an equal volume of incomplete freund's adjuvant (IFA, Sigma). Rats were injected i.d. twice with 200 μg emulsifying agent. The first injection was made in a hind metatarsal footpad; the second, 7 days later, was made into the proximal one-third of the tail (Joosten et al., 1994). The day of the first immunization was defined as day 0.

2.5. Treatment of CIA

After the onset of arthritis, animals were divided into six groups randomly. Rats with CIA were given intragastrically TGP (25, 50, 100 mg/kg) and GTW (40 mg/kg) once per day from d14 to d28 after immunization. Normal and CIA model rats were given an equal volume of vehicle (CMC-Na) at the same time.

2.6. Arthritis assessment

Rats were inspected daily for signs of arthritis by two independent observers who were not aware of the treatment. Noninjected hind paw volume was detected with YLS-7A toe volume meter (Equipment station of Academy of Medical Sciences, Shandong, China, 2004). Paw swelling (Δml) was calculated by subtracting the paw volume at d0. The arthritic severity in each paw was evaluated by 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 ankle or wrist joints; 3, paws with severe inflammation of the entire paws; and 4, paws with deformity or ankylosis. The cumulative score for all four paws of each mouse was used as polyarthritis index with a maximum value of 16 (Hughes et al., 1994).

2.7. Measurement of anti-CII antibodies

On day 14 and day 28, blood was collected from the rat by cardiac puncture under ether anesthesia. The serum level of immunoglobulin G (IgG) antibodies to type II collagen (CII) was determined using an enzyme linked immunosorbent assay (ELISA) kit (R&D Systems, USA), according to the manufacturer's instructions (Wang et al., 2007).

2.8. Histological examination

Rats were sacrificed by cervical dislocation after ether anesthesia on d42 after immunization. The hind paws and knees were fixed in 10% phosphate-buffered formalin, decalcified in 10% ethylenediamine tetraacetic acid (EDTA) for 14 days at 4 °C, then embedded in paraffin. Serial paraffin sections (5 mm) were stained with hematoxylin and eosin (H&E). All sections were evaluated histologically by two independent observers, and the gradation of arthritis was scored, as described previously (Chen and Wei, 2003): 0, normal joint; I, normal synovium with occasional mononuclear cells; II, definite arthritis, a few layers of flat to rounded synovial lining cells and scattered mononuclear cells; III, clear hyperplasia of the synovium with three or more layers of loosely arranged lining cells and dense infiltration with mononuclear cells; IV, severe synovitis with pannus and erosions of articular cartilage and subchondral bone.

2.9. Preparation and culture of FLS

CIA rats were anaesthetised and sacrificed. Synovial tissues obtained from knee joints of CIA rats were minced and digested with type IA collagenase for 3 h, filtered, extensively washed, and then cultured in DMEM containing 20% fetal bovine serum (FBS, Sigma) at 37 °C in a humidified atmosphere of 5% CO_2. At confluence, adherent cells were trypsinized, split in a 1:3 ratio, and recultured in medium (Kim et al., 2007). The spindle-shaped cells of passages 3 were used in subsequent experiments, during which time they were a homogeneous population of FLS.