Abstract. Objective: To investigate the effects of Paeoniflorin (Pae) on inflammatory mediators and G protein – coupled signaling in fibroblast – like synoviocytes (FLS) from collagen induced arthritic (CIA) rats.

Methods: SD rats were injected with type II collagen. Pae (25, 50, 100 mg·kg⁻¹) was administered to CIA rats. The inflammation of CIA rats was evaluated by paw swelling, arthritis index and histopathology of joints. FLS were isolated and cultured. Interleukin (IL)-1 activity was measured by the ³H-TdR – intake method. Tumor necrosis factor alpha (TNF-alpha), prostaglandin E₂ (PGE₂) and cAMP were measured by radioimmunoassay. Protein kinase A (PKA) was assessed by luminescent kinase assay. Gi was detected by Western blot.

Results: Inflammation in CIA rats was accompanied by hyperplastic synovium, pannus and cartilage erosion in joints. IL-1 activity and Gi expression increased, PGE₂ and TNF-alpha production were enhanced, but cAMP level and PKA activity decreased. Pae significantly suppressed the inflammatory response and inflammatory mediators (IL-1, TNF-alpha and PGE₂) in vivo. Pae inhibited Gi expression and restored cAMP level and PKA activity in FLS of CIA rats in vivo and vitro.

Conclusion: Inflammatory mediators and G protein - coupled signaling were associated with the pathogenesis of synovitis in CIA rats. Pae, as a new monomer, had anti-inflammatory effects on the animal model of CIA in rats, but also had regulatory effects on FLS from CIA rats in vitro. These results highlight Pae as a good candidate for therapeutic intervention in RA.

Key words: Paeoniflorin – Synoviocyte – Arthritis – Signaling – Immunology

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease, characterized by severe synovium inflammation, resulting in destruction of bone and cartilage. Proliferation of the synovium is associated with increased production of inflammatory mediators. The pivotal role of interleukin-1 (IL-1), tumor necrosis factor (TNF)-alpha and prostaglandin E₂ (PGE₂) has been demonstrated in basic research [1–3]. G proteins are biochemical transducers operating allosteric regulatory elements. The G protein- adenylate cyclase (AC) – cyclic adenosine 3', 5'-monophosphate (cAMP) signaling pathway is an important mediator of extracellular signals in fibroblast – like synoviocytes (FLS) of RA and plays crucial roles in cartilage degradation [4]. Therefore, blockade of inflammatory mediators and regulation of G protein – coupled signaling is proposed to be of potential therapeutic benefit in RA.

Paeoniflorin (Pae) is a monoterpenic glucoside and one of the components of total glucosides of paeony (TGP) extract ed from the root of Paeonia lactiflora. TGP was approved for marketing in China in 1998[5]. As a disease-modifying drug, TGP has both anti-inflammatory and immune-regulatory effects and is used in the treatment of RA. TGP ameliorates clinical symptoms and signs of RA, decreased erythrocyte sedimentation rate (ESR) and rheumatic factor (RF). In animal models, TGP inhibits acute inflammation in the rat induced by carrageenan [6], but also suppresses the chronic inflammatory reaction in adjuvant arthritis (AA) rats [7]. TGP contains more than 90% of Pae. The studies in our re-
search group have shown that Pae as well as TGP significantly ameliorates the symptoms in AA rats. Compared with TGP, Pae is a new monomer and has higher solubility. Therefore, the mechanisms of Pae in treatment for RA may well be observed in vivo or in vitro.

Collagen induced arthritis (CIA) is a chronic immune-inflammatory model for RA. The model has been extensively used to elucidate the pathogenic mechanisms of RA and to evaluate anti-arthritic drugs [8, 9]. Compared with AA, CIA is a T-cell and humoral, immune dependent disease and is characterized by onset of inflammation 3 wk after induction. The main pathological features of CIA include edema accompanied by an acute phase response, infiltration into the joint of inflammatory cells, pannus formation, and erosion of cartilage and bone. AA is a largely CD4+ T-cell dependent disease and characterized by onset of inflammation 12 day after induction. CIA shares more features with RA than AA does. In a previous in vitro study, we only found the effect of Pae on the proliferation of mesenteric lymph node (MLN) lymphocytes in AA rats [10]. The present in vitro study utilized FLS from CIA rats to further investigate the therapeutic potential of Pae in RA. Our in vivo studies included induction of CIA in male Sprague–Dawley rats by chicken collagen type II (CC II). When CC II was used, the resultant disease of CIA rats was characterized by severe synovium inflammation resulting in destruction of bone and cartilage. Pae significantly decreased disease severity and joint destruction in chronic CIA. A potential mechanism of action of Pae in vivo was through reduction of inflammatory mediators and regulation of Gi protein–coupled signaling by FLS, since IL-1, TNF-alpha, PGE2 and Gi were inhibited by Pae, cAMP level and PKA activity were enhanced by Pae. In vitro studies as well as in vivo studies showed that Pae suppressed Gi protein expression and restored cAMP level and PKA activity. These findings provide support for the concept that blockade of inflammatory mediators and regulation of Gi protein–coupled signaling by FLS may be an approach for treatment of RA.

Materials and Methods

Animal

Male Sprague–Dawley (SD) rats (male, 150–180 g) were purchased from Shanghai BK Experimental Animal Center (Grade II, Certificate No D-65). All rats were acclimatized under standard laboratory conditions. During the experimental period, all rats were housed five per cage and fed laboratory feed and water, and were kept on a 12h dark vs 12h light cycle at a constant temperature of 20 ± 5°C. Inbred C57BL/6 J mice (For the measurement of IL-1 activity) weighing 16–20 g were also obtained from this Animal Centre. The experimental protocols in this study were discussed and passed by the Ethics Review Committee for Animal Experimentation of Institute of Clinical Pharmacology, Anhui Medical University.

Drug and reagents

Pae [C23H28O11, MW: 480.45, purity: ≥95 % (HPLC), LD50: 9,530 mg·kg−1] was obtained from Xuancheng Baicao Plants Industry and Trade CO., LTD (Anhui, China). Pae is extracted and purified by methods of solvent extraction and column chromatography. The structure of Pae is identified by physicochemical properties and spectroscopic analysis (Figure 1). Pae was suspended in distilled water at required concentration respectively. CC II was obtained from the Institute of Bencao Biological Medicine in Shanghai.

The reagents followed were needed including HEPES (Sigma Co), RPMI1640 powder (Gibco Co, USA), Bacillus Calmette Guerin (BCG, Shanghai Biological Products Factory, No 20051001), 125I-TNFα radioimmunoassay (RIA) kit (Beijing Biotinge Biomedicine Company), 125I-PGE2 RIA kit (Suzhou Medical College), Anti-Gi antibody (1:2500; Santa Cruz Biotechnology, Inc), 125I-cAMP RIA kit (Institute of Medical Academy of China) and luminescent kinase assay kit (Promega, Inc). The RPMI-1640 medium was supplemented with HEPES 10 mmol·L−1, L-glutamine 2 mmol·L−1, 2-mercaptoethanol 50 μmol·L−1, penicillin sodium 100 kU·L−1, streptomycin 100 mg·L−1 and 10% new born bovine serum. PH was adjusted to 7.2.

Induction of CIA

Chicken type II collagen was dissolved in acetic acid 0.1 mmol·L−1 and emulsified with an equal volume of complete Freund’s adjuvant (CFA) in ice-bath. The final concentration of CC II and BCG were both 1 g·L−1. Emulsion 1 ml was injected at three sites (the base of the tail, back and left hind paw of rats). On d7, rats were given a booster injection. Control group received the injection of an equal volume of 0.1 mmol·L−1 acetic acid at the same location [11].

Treatment of CIA

Before the onset of arthritis, animals were divided into five groups randomly, and there were fifteen rats each group. In each group, the rats with CIA were administrated with Pae (25, 50, 100 mg·kg−1) from day 16 to 23 after immunization. For the groups of normal and CIA model, rats were given an equal volume of distilled water at the same time.

Arthritis assessment

Right hind paw volume was determined with MK-550 volume meter (Muromachi Kikai Co Japan, 1995) before immunization (basic value, day 0) and repeated at day 16, day 20, day 24, day 28 [12]. The weight of CIA rats was weighed day 0, day 16, day 20, day 24 and day 28. Weight (Ag) was expressed by the difference value of the weight at day 16, day 20, day 24 or day 28 and the weight at day 0. Arthritis index was inspected daily by two independent observers who were not aware of the treatment. The arthritis severity was graded on a scale of 0–4: 0 = no swelling, 1 = isolated phalanx joint involvement, 2 = involvement of the entire region down to the ankle, 3 = involvement of entire paw, including ankle. The maxi-