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Expression and function of β-arrestin 2 stimulated by IL-1β in human fibroblast-like synoviocytes and the effect of paeoniflorin

Hua-Xun Wu, Jing-Yu Chen, Qing-Tong Wang, Wu-Yi Sun, Li-Hua Liu, Ling-Ling Zhang, Wei Wei *

Institute of Clinical Pharmacology, Anhui Medical University, Key Laboratory of Anti-inflammatory and Immunopharmacology of Education Ministry of China Key Laboratory of Antiinflammatory and Immunopharmacology, Ministry of Education, China, Hefei, 230032, China

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ABSTRACT

The aim of this study was to explore the expression and function of β-arrestin 2 in human fibroblast-like synoviocytes (FLS) stimulated by IL-1β and the effect of paeoniflorin (Pae). We isolated and cultured human FLS, which were stimulated by IL-1β. The FLS proliferations were detected by [3H] thymidine incorporation. The level of cAMP stimulated by IL-1β on different times was investigated by radioimmunoassay, and the activity of PKA was measured by luminescent kinase assay. The expression of β-arrestin 2 was measured by western blot. We found that the human FLS proliferation increased apparently in 24 h, and the activities of PKA and cAMP accumulation increased significantly in 6 h after stimulated by IL-1β, while cAMP accumulation and the activities of PKA decreased especially in 24 h when the expression of β-arrestin 2 increased significantly. Decreased cAMP accumulation and the increased expression of β-arrestin 2 may reveal a positive correlation with the FLS proliferation. Pae (10^{-5}, 10^{-6}, 10-7 mol•L^{-1}) in vitro could suppress the FLS proliferation and the high expression of β-arrestin 2. The expression of β-arrestin 2 may have a positive correlation with the human FLS proliferation, while the activities of PKA and cAMP accumulation have a negative correlation with the proliferation. The increased β-arrestin 2 may down-regulate cAMP-PKA signaling pathway and promote FLS proliferation. Pae may suppress the expression of β-arrestin 2 and up-regulate cAMP-PKA signaling, which may be one of the mechanisms for the effects of Pae on inhibiting human FLS proliferation.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune and inflammatory disease with chronic synovial inflammation and progressive articular damage in multiple joints [1]. Fibroblast-like synoviocytes (FLS), the key effector cells in the inflamed joint, release a lot of pro-inflammatory and matrix-degrading effector molecules [2], which have the capacity to provide chemotactic and activation signals to resident parenchymal cells and infiltrating immunocytes and the destruction of the involved joints [3–5]. Various cytokines are known to be involved in RA, particularly pro-inflammatory cytokines such as TNF-α, IL-1β, and IFN-γ, that are produced by macrophages, dendritic cells, and T cells [6]. IL-1β is considered as one of the most important cytokines in the pathogenic process of inflammations in rheumatoid arthritis [7]. IL-1β can induce RA progression through mediators such as cyclooxygenase-2 (COX-2), which increases prostaglandin E2 (PGE2) production followed by synovial inflammation, and matrix metalloproteinases (MMPs) [8–10].

G-protein-coupled receptors (GPCRs) constitute a family of seven-transmembrane domain proteins that transmit signals to the interior of the cell by activating a variety of signaling pathway [11]. Recently, more and more studies suggested that alteration of GPCRs, G proteins and their signaling was involved in RA. Previously, we reported that GPCR signaling was associated with the pathogenesis in collagen-induced arthritis (CIA) and adjuvant arthritis (AA) rats. In synoviocytes from CIA rats, cAMP level decreased, mRNA expression of Gαi increased and mRNA expression of Gαs decreased [12,13].

β-Arrestins (β-arrestin 1 and β-arrestin 2) are a family of scaffolding proteins that are well-known negative regulators of GPCR signaling [14]. Upon GPCR activation, β-arrestins translocate to the cell membrane and bind to the agonist-occupied receptors. This uncouples these receptors from G proteins and promotes their internalization, thus causing desensitization [15,16]. Our previous studies found that compared to the normal group, the expression of β-arrestins in the synovial tissue of CIA rats increased significantly, which was in accord with the inflammatory course of CIA [17]. Li et al. found that β-arrestin 2 expressions significantly increased in joint tissue of CIA mice, and β-arrestin 2 proteins and mRNA levels

Abbreviations: RA, rheumatoid arthritis; AA, adjuvant arthritis; CIA, collagen-induced arthritis; FLS, fibroblast-like synoviocytes; GPCRs, G-protein-coupled receptors; Pae, paeoniflorin; PBMC, peripheral blood mononuclear cells; MLN, mesenteric lymph nodes; 7TMRs, 7 transmembrane receptors.

* Corresponding author. Tel.: +86 551 516 1208.
E-mail addresses: wuhuaxun@yahoo.cn (H.-X. Wu), cjy.anyi@gmail.com (J.-Y. Chen), hfwqt727@163.com (Q.-T. Wang), liulihuawomen@hotmail.com (L.-H. Liu), llzhang@ahmu.edu.cn (L.-L. Zhang), wwei@ahmu.edu.cn (W. Wei).

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increased in FLS of CIA mice. β-Arrestin 2 knockout mice influenced severity in collagen antibody-induced arthritis. These observations suggest that β-arrestin 2 regulates FLS inflammation and influences experimental arthritis severity [18]. β-Arrestin 2 regulates the GPCR–G-protein–cAMP signaling, which is be closely related to the FLS proliferation in murine model of RA. But the relationships among β-arrestin 2 and cAMP–PKA and human FLS proliferation stimulated by IL-1β are not clear.

Paeoniflorin (Pae), a monoterpene glucoside, is one of the main bioactive components of the root of *Paeonia lactiflora* Pall. Pae is considered low toxic, and we found that intravenous and intraperitoneal LD50 of Pae were 3530 mg kg^{-1} and 9530 mg kg^{-1} respectively in mice. Our previous studies showed that Pae had anti-inflammatory effects on CIA rats, and also could regulate FLS function from CIA rats. Pae could significantly suppress inflammatory response by inhibiting synoviocytes proliferation and production of inflammatory mediators (such as IL-1, PGE2, TNF-α) [19], and Pae could up-regulate cAMP level in human FLS stimulated by IL-1β.

To further confirm the relationships among β-arrestin 2 and cAMP–PKA and human FLS proliferation stimulated by IL-1β and the effect of Pae in this paper, we measured β-arrestin 2 protein, FLS proliferation and cAMP–PKA levels in human FLS on different times stimulated by IL-1β, and then investigated the effects of Pae on β-arrestin 2 protein level and human FLS proliferation.

2. Materials and methods

2.1. Drugs and reagents

Pae [$C_{23}H_{28}O_{11}$, MW: 480.45] was extracted from the roots of *P. lactiflora* Pall and purified by methods of solvent extraction and column chromatography in the Chemistry Lab of Institute of Clinical Pharmacology of Anhui Medical University (Hefei, Anhui Province, China). The dried and powdered roots of *P. lactiflora* Pall were extracted with 70% ethanol under reflux. The concentrated extract was dissolved in water and tandem passed through a macroporous resin column. First, the column was washed with water until no Molisch reaction, and then with 40% ethanol. Concentration of the 40% eluate under reduced pressure gave the total paeony glycoside. The yellow powder was subjected to silica gel column chromatograph and then eluated with EtOAc/MeOH (20/1). The pure compound was yielded after the concentration of the collected eluate containing only Pae. The purity of Pae is above 95% as determined by HPLC assay (LC-10AD, Shimadzu Co., Japan). The structure of Pae was identified by physicochemical properties and spectroscopic analysis. Pae was suspended in distilled water at required concentration respectively before use.

The following reagents were obtained commercially: IL-1β (peprotech), anti-β-arrestin 2 and anti-β-actin antibodies (Santa Cruz Biotechnology, Inc), SuperSignal west femto maximum sensitive substrate Pierce (Rockford, IL), [125I] cAMP radioimmunoassay (RIA) kit (Shanghai University of Traditional Chinese Medicine, China), luminescent kinase assay kit (Promega, Inc), DMEM (Gibco Co), fetal bovine serum (FBS) (HyClone Co). Other chemicals used in these experiments were of analytical grade from commercial sources.

2.2. Cell isolation and culture

Synovial tissues were obtained from five patients with fracture of neck of femur at joint replacement surgery. FLS were isolated from individual tissues with tissue transplantation method and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum, penicillin (200 U ml^{-1}), and streptomycin (200 ng ml^{-1}). Cell lines were used from the third to ninth passage, when they were a homogeneous population of fibroblast-like cells. This study was approved by the ethics committee of Anhui Medical University. All procedures involving specimens obtained from human subjects were performed with informed consent from each patient.

2.3. Human synoviocyte stimulation

Human FLS were cultured in 20% FBS–DMEM with IL-1β (10 ng ml^{-1}) in 96-well flat-bottomed culture plates at 1×10^5 cells ml^{-1} and incubated at 37 °C, 5% CO_2. FLS proliferation was measured after FLS were stimulated with IL-1β (10 ng ml^{-1}) for 3 h, 6 h, 12 h, 24 h, and 48 h by [3H] thymidine incorporation, and normal human FLS were regarded for 0 h.

Human FLS were cultured in 20% FBS–DMEM with IL-1β (10 ng ml^{-1}) in 24-well flat-bottomed culture plates at 2×10^5 cells ml^{-1} and incubated at 37 °C, 5% CO_2. Intracellular cAMP level and PKA activity were measured after FLS were stimulated with IL-1β (10 ng ml^{-1}) for 3 h, 6 h, 12 h, 24 h, and 48 h and normal human FLS were regarded for 0 h.

Human FLS were cultured in 20% FBS–DMEM with IL-1β (10 ng ml^{-1}) in 6-well flat-bottomed culture plates at 5×10^6 cells ml^{-1} and incubated at 37 °C, 5% CO_2. The expression of β-arrestin 2 was measured by western blot for 3 h, 6 h, 12 h, 24 h, and 48 h after stimulated by IL-1β, and normal human FLS were regarded for 0 h.

Human FLS were cultured in 20% FBS–DMEM with Pae (1×10^{-5} mol L^{-1}, 1×10^{-6} mol L^{-1}, 1×10^{-7} mol L^{-1}, 1×10^{-8} mol L^{-1}, 1×10^{-9} mol L^{-1}) and IL-1β (10 ng ml^{-1}) in 96-well flat-bottomed culture plates at 1×10^5 cells ml^{-1} and incubated at 37 °C, 5% CO_2 for 24 h. FLS proliferations were measured by [3H] thymidine incorporation.

Human FLS were cultured in 20% FBS–DMEM with Pae (1×10^{-5} mol L^{-1}, 1×10^{-6} mol L^{-1}, 1×10^{-7} mol L^{-1}, 1×10^{-8} mol L^{-1}, 1×10^{-9} mol L^{-1}) and IL-1β (10 ng ml^{-1}) in 6-well flat-bottomed culture plates at 5×10^6 cells ml^{-1} and incubated at 37 °C, 5% CO_2 for 24 h. The expression of β-arrestin 2 was measured by western blot.

2.4. Proliferation assay by [3H]-TdR

[3H] thymidine incorporation was used for detecting the FLS proliferation. FLS (1×10^5 cells ml^{-1}) were added to 96-well flat-bottomed culture plates at 1×10^4 cells/well in DMEM with 20% FBS and incubated at 37 °C, 5%CO_2 for 24 h. 100 μl DMEM containing IL-1β (10 ng ml^{-1}) and (or) Pae (1×10^{-5} mol L^{-1}, 1×10^{-6} mol L^{-1}, 1×10^{-7} mol L^{-1}, 1×10^{-8} mol L^{-1}, 1×10^{-9} mol L^{-1}) was added to each well. Then the cells were incubated at 37 °C, 5% CO_2, and then incubated with [3H]-TdR (1 μCi/well) at 37 °C and 5% CO_2 for 6 h prior to harvest. Subsequently, the cells were trypsinated,

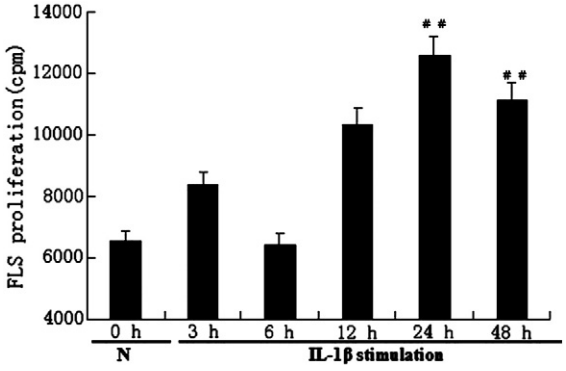

Fig. 1. FLS proliferation induced by IL-1β on different times. 0 h represents normal group without IL-1β stimulation, and 3 h, 6 h, 12 h, 24 h, and 48 h represent IL-1β stimulation groups on different times. Data shown are mean ± SD (n = 5). ##P<0.01 compared with normal group (0 h).