International Immunopharmacology 52 (2017) 218–226

Contents lists available at ScienceDirect

International Immunopharmacology

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

Baicalin suppresses IL-1β-induced expression of inflammatory cytokines via blocking NF-κB in human osteoarthritis chondrocytes and shows protective effect in mice osteoarthritis models

Chunhui Chen, Chuanxu Zhang, Leyi Cai, Huanguang Xie, Wei Hu, Te Wang, Di Lu, Hua Chen*

Department of Orthopaedic Surgery, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325000, China

ARTICLE INFO

Keywords:
Osteoarthritis
Inflammation
Baicalin
Chondrocyte
NF-kB

ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease with an inflammatory component that drives the degradation of cartilage extracellular matrix. Baicalin, a predominant flavonoid isolated from the dry root of *Scutellaria baicalensis* Georgi, has been reported to have anti-inflammatory effects. However, the anti-inflammatory effects of baicalin on OA have not been reported. Our study aimed to investigate the effect of baicalin on OA both in vitro and in vivo. In vitro, human OA chondrocytes were pretreated with baicalin (10, 50, 100 μM) for 2 h and subsequently stimulated with IL-1β for 24 h. Production of NO and PGE2 were evaluated by the Griess reaction and ELISAs. The mRNA expression of COX-2, iNOS, MMP-3, MMP-13, ADAMTS-5, aggrecan and collagen-II were measured by real-time PCR. The protein expression of COX-2, iNOS, MMP-3, MMP-13, ADAMTS-5, p65, p-p65, IκBα and p-IκBα was detected by Western blot. The protein expression of collagen-II was evaluated by immunofluorescence. Luciferase activity assay was used to assess the relative activity of NF-kB. In vivo, the severity of OA was determined by histological analysis. We found that baicalin significantly inhibited the IL-1β-induced production of NO and PGE2, expression of COX-2, iNOS, MMP-3, MMP-13 and ADAMTS-5 and degradation of aggrecan and collagen-II. Furthermore, baicalin dramatically suppressed IL-1β-stimulated NF-κB activation. In vivo, treatment of baicalin not only prevented the destruction of cartilage but also relieved synovitis in mice OA models. Taken together, these results suggest that baicalin may be a potential agent in the treatment of OA.

1. Introduction

Osteoarthritis (OA), the most common joint disorder in the aging population, is characterized by the progressive degeneration of articular cartilage and subchondral bone sclerosis, accompanied by joint swelling, pain and stiffness, resulting in joint dysfunction [1–3]. Many different factors, including heredity, obesity, joint injury and joint deformation contribute to the initiation and progression of OA [4]. Although the explicit etiology and pathogenesis of OA are poorly understood, inflammation and inflammatory response have been regarded as critical factors that initiate and accelerate the development of OA [5]. It widely accepted that inflammatory cytokines are vital mediators in the disturbed metabolism and enhanced catabolism of tissue in the OA joint [6]. Among the inflammatory cytokines involved in OA, interleukin-1β (IL-1β) has been considered to play a critical role in the pathological development of OA [7]. A number of studies demonstrated that IL-1β down-regulates the synthesis of major extracellular matrix (ECM) components collagen-II and proteoglycans by inhibiting anabolic activities of chondrocytes [8]. Additionally, IL-1β could stimulate chondrocytes to release several proteolytic enzymes, among which are the matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) [9]. It is well known that MMPs and ADAMTS exert pivotal roles in cartilage ECM degradation during the development of OA because they are responsible for the degradation of collagen-II and proteoglycans in articular cartilage [10]. Moreover, stimulating chondrocytes with IL-1β could induce the release of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), which directly stimulates the excessive production of prostaglandin E2 (PGE2) and nitric oxide (NO), respectively [11]. In patients with OA, the level of IL-1β is elevated in the synovial fluid, synovial membrane, subchondral bone and cartilage [12]. Consequently, there is reason to believe that inhibition of IL-1β and IL-1β-induced inflammatory mediators may attenuate the progression of OA.

Baicalin, whose chemical structure is shown in Fig. 1, is a predominant flavonoid isolated from the dry root of *Scutellaria baicalensis* Georgi (Huang-Qin, a medicinal plant). It is considered to be one of the

* Corresponding author at: Department of Orthopaedic Surgery, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, 109 Xueyuan Xi Road, Wenzhou 325000, China.
E-mail address: chenhuafey@126.com (H. Chen).

http://dx.doi.org/10.1016/j.intimp.2017.09.017
Received 18 April 2017; Received in revised form 4 September 2017; Accepted 18 September 2017
Available online 22 September 2017

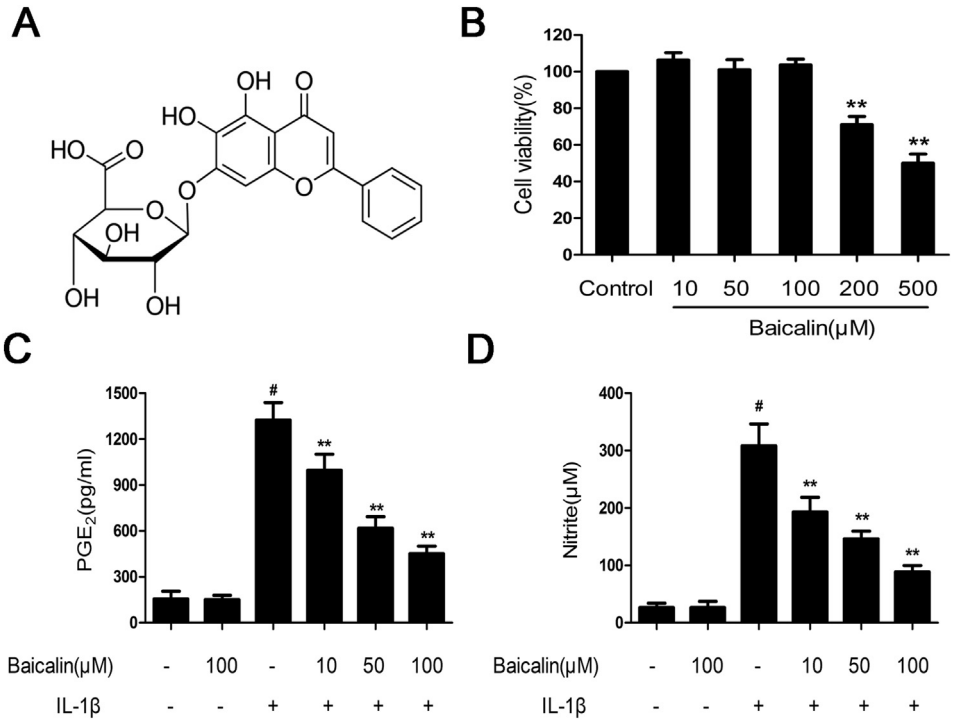

Fig. 1. The molecular structure of baicalin, effect of baicalin on human OA chondrocyte viability and IL-1β-induced PGE2 and NO production in human OA chondrocytes. The cells were cultured with increasing concentrations of baicalin (0, 10, 50, 100, 200 or 500 μM) for 24 h, followed by the CCK-8 analysis for cell viability (B). Human OA chondrocytes were pre-treated for 2 h with various concentrations of baicalin (10, 50, 100 μM) and then stimulated or not stimulated with IL-1β (10 ng/ml) for 24 h. The levels of PGE2 were determined using ELISA (C). The nitrite levels in the culture medium were assessed by Griess reaction (D). The values are mean ± SD of three independent experiments. $^{\#}P < 0.05$ compared with control group, $^{*}P < 0.05$, $^{**}P < 0.01$ compared with IL-1β group.

effective and safe drugs widely used in Asia for the treatment of a variety of diseases, such as brain diseases, hepatic disorders, inflammatory diseases and so on. Furthermore, it has been reported that baicalin has multiple biologic functions, including anti-inflammatory [13], anti-oxidant [14], anti-microbial [15] and anti-tumor properties [16]. Previous studies showed that baicalin Inhibited lipopolysaccharide (LPS)-induced inflammation through signaling nuclear factor-kappa B (NF-κB) pathway in HBE16 airway epithelial cells [17]. Besides, baicalin attenuated LPS-induced inflammation and apoptosis of cow mammary epithelial cells by regulating NF-κB and heat shock protein 72 (HSP72) [18]. Also, baicalin protected keratinocytes from toll-like receptor-4 (TLR-4) mediated DNA damage and inflammation following ultraviolet irradiation [19]. In addition to the in vitro studies, baicalin was found to relieve joint inflammation in collagen-induced arthritis rats through the NF-κB pathway [20]. Moreover, baicalin attenuated inflammation in mice with ovalbumin (OVA)-induced asthma by inhibiting NF-κB [21]. However, the anti-inflammatory effect of baicalin in OA remains unknown. Therefore, in this study, we investigated the anti-inflammatory effect and the underlying mechanism of baicalin on IL-1β-stimulated human OA chondrocytes.

2. Materials and methods

2.1. Chemicals and reagents

Baicalin (purity > 98%), recombinant human IL-1β, collagenase type II and dimethylsulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The total volume of intraperitoneal injection in the animals was 200 ml and the total volume added in cell culture was 0.5 ml. Cell-Counting Kit-8 (CCK-8) was purchased from Dojindo (Kumamoto, Japan). Primary antibodies against COX-2, iNOS, collagen-II, MMP-3, MMP-13 and ADAMTS-5 were purchased from Abcam (Cambridge, MA, USA). Primary antibodies against p65, p-p65, IkBα and p-IkBα were purchased from Cell Signaling Technology (Beverly, MA, USA). Goat anti-rabbit and goat anti-mouse horseradish peroxidase conjugates were purchased from Bio-Rad Laboratories (Calif., USA). Fetal bovine serum (FBS), bovine serum albumin (BSA), Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 medium and 0.25% trypsin-ethylenediaminetetraacetic acid (trypsin–EDTA) were purchased from Gibco (Life Technologies Corp. Carlsbad, Calif., USA). TRIzol reagent was purchased from Invitrogen (Carlsbad, Calif., USA). QuantiTect Reverse Transcription kit was purchased from Qiagen (Valencia, CA). SYBR Green Master Mix was purchased from Bio-Rad Laboratories (Calif., USA). ELISA kit of PGE2 was purchased from R & D systems (Minneapolis, MN, USA). Griess reagent was purchased from Beyotime Institute of Biotechnology (Shanghai, China).

2.2. Primary human chondrocytes culture

Articular cartilage samples collection was according to the terms of the Medical Ethical Committee of the Second Affiliated Hospital, Wenzhou Medical University and following the guidelines of the Declaration of Helsinki and Tokyo. OA human cartilage tissues were obtained from six OA patients (aged 52–65 years, three men and three women) who underwent total knee replacement surgery at the Second Affiliated Hospital of Wenzhou Medical University. The OA patients met the American College of Rheumatology (ACR) classification criteria for the diagnosis of osteoarthritis [22]. Full ethical consent was obtained from all patients. The human chondrocytes were isolated and cultured as described previously [23]. In brief, cartilage was separated from underlying bone and connective tissues, obtained cartilage tissues were cut into $1 \times 1 \times 1\ mm^3$ pieces and washed three times with PBS. After that, the joint cartilage pieces were treated with 0.25% trypsin–EDTA solution to be digested. After removing 0.25% trypsin–EDTA, they were digested in 0.2% collagenase type II for 5 h at 37 °C. Then centrifuged at 1000 rpm for 5 min and the supernatant was discarded. The inner cell mass was obtained and suspended in DMEM/F12 with 10% FBS and 1% antibiotic mixture (Penicillin and Streptomycin). Finally, cells were plated at a density of 1×10^5 cells/ml in 6-well plate and incubated in a humidified atmosphere of 5% CO_2 at 37 °C. The media were changed every 2–3 days. Cells were passaged when at 80 to 90% confluence using 0.25% trypsin–EDTA solution. Only passage 1 to 2 were used in our study to avoid the phenotype loss. The cell viability experiment was aimed to assess the potential toxicity of baicalin on the chondrocytes and it was evaluated using the CCK-8 according to the manufacturer's instructions. However, in other