

Original Article

Baicalein inhibits inflammatory and catabolic gene expression in interleukin-1 β -induced human chondrocytes

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Received September 21, 2015; Accepted December 6, 2015; Epub February 15, 2016; Published February 29, 2016

Abstract: Baicalein is a flavone compound isolated from *Scutellaria baicalensis* Georgi (Huang Qin) which exerts multiple biological effects including anti-inflammatory, anti-apoptotic and anti-tumor. However, there is little known about the effects of baicalein on cathepsins and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) in osteoarthritis (OA). In the present study, we investigated whether baicalein exerts inhibitory effects on ADAMTS and cathepsins. Chondrocytes were cultured in the absence or in the presence of interleukin-1 β (IL-1 β) (10 ng/ml) and with or without baicalein (5-50 μ M). Nitric oxide (NO) production was determined by Griess reaction. Gene expressions of ADAMTS and cathepsins, inducible nitric oxide synthase (iNOS) were detected by real-time quantitative PCR. We found that baicalein inhibited the IL-1 β -induced gene expression of ADAMTS-4, ADAMTS-5, cathepsin K and cathepsin B. In addition, baicalein also suppressed NO production as well as iNOS mRNA level. Our results indicate that baicalein may be considered a possible agent in the treatment of OA.

Keywords: Baicalein, cathepsin, chondrocyte, osteoarthritis

Introduction

Osteoarthritis (OA) is a degenerative joint disorder with cartilage degradation, osteophyte formation and synovial inflammation [1]. Matrix metalloproteinases (MMPs) have been considered as the main mediator in the degradation of cartilage, leading to extracellular matrix (ECM) components, including type II collagen and aggrecan degradation [2, 3]. Additionally, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) and cysteine cathepsins also play pivotal roles in cartilage degradation [4, 5]. ADAMTS-4 and ADAMTS-5 are responsible for aggrecan degradation while cathepsins are capable to cleave type II collagens as well as aggrecan.

Baicalein, isolated from *Scutellaria baicalensis* Georgi (Huang Qin), is a flavone compound which exerts multiple biological effects. Previous studies have confirmed the pharmacological effects of baicalein including anti-inflammatory, anti-apoptotic and anti-tumor [6-8].

Moreover, recently studies have demonstrated that baicalein inhibited expression of MMPs including MMP-3 and MMP-13 which are two important catabolic enzymes in OA [9]. However, there is little known about the effects of baicalein on cathepsins and ADAMTS.

In the present study, we investigated whether baicalein exerts inhibitory effects on ADAMTS and cathepsins. We demonstrated that baicalein inhibited ADAMTS and cathepsins expression. In addition, we also found that baicalein inhibits inflammation mediator in OA.

Materials and methods

Chondrocytes culture

Human cartilages were obtained from OA patients undergoing knee joint replacement. In brief, cartilage was minced, then digested with 0.25% trypsin for 30 min and followed by 2 mg/ml collagenase II for 6 h at 37°C. Cells were cultured in Dulbecco's modified Eagle medium

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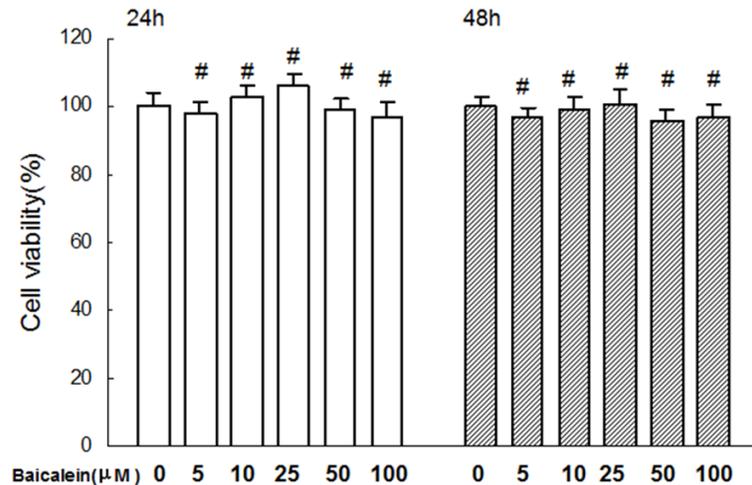


Figure 1. Effect of baicalein on chondrocytes viability. Cells were cultured in 96-well plates and incubated with baicalein (5-50 μM) for 24 h or 48 h. Cell viability was determined using the MTT assay. # $P > 0.05$ compared with cells without baicalein.

(DMEM) with 10% FBS and antibiotics (100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin) at 37°C under a humidified 5% CO_2 atmosphere. The study was approved by the local ethic committee and written informed consent was obtained from each participant.

MTT assay for cell proliferation

Chondrocyte proliferation was assessed in the presence of different concentrations of baicalein (0, 5, 25, 50 μM) by MTT assay. Cell proliferation was examined at 24 h and 48 h after baicalein treatment. A concentration of baicalein ranging exerted non-cytotoxic activities and was used in experiments.

Baicalein treatment and gene expressions of ADAMTS-4, ADAMTS-5, cathepsin K, cathepsin B and inducible nitric oxide synthase (iNOS) by real-time quantitative PCR

Cells were serum-starved overnight, then cells were pre-treated with baicalein (0, 5, 25, 50 μM) for 1 h prior to incubated with IL-1 β (10 ng/ml) for 24 h. Then, cells were collected for PCR and supernatants were collected for nitric oxide (NO) detection.

For real-time quantitative PCR, total RNA was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). 1 μg of total RNA was reverse transcribed using the Moloney murine leukemia virus reverse transcriptase cDNA syn-

thesis kit (Promega, Madison, WI, USA). The quantification of gene-expression levels for ADAMTS-4, ADAMTS-5, cathepsin K, cathepsin B and iNOS were carried out by quantitative real-time PCR using iQTM SYBR Green supermix PCR kit with the iCycler apparatus system (Bio-Rad). GAPDH was used as endogenous control. Primer sequences are as follows: for ADAMTS-4: F: 5'-CCTGGCAAGGACTATGATGCTGA-3'; R: 5'-GGGCGAGTGTGGTCTGG-3'; for ADAMTS-5: F: 5'-GCA-GAACATCGACCAACTCTACTC, R: 5'-CCAGCAATGCCACCGAAC-3'; for cathepsin K: F: 5'-CACTGGGAGCTATGGAAGAA-GAC-3'; R: 5'-GAGAAGCCTCAAGTTATGGATGGA-3'; for cathepsin B: F: 5'-GCAGCCTCAGC-CACCCAGAT-3'; R: 5'-CCACCATTACAGCCGTCCC-CACAC-3'; for iNOS: F: 5'-CCTTACGAGGCGAAG-AAGGACAG-3'; R: 5'-CAGTTTGAGAGAGGAGGC-TCCG-3'; for glyceraldehyde 3-phosphate dehydrogenase (GAPDH): F: 5'-CTGCTCCTCTG-TTCGACAGT-3'; R: 5'-CCGTTGACTCCGACCTTC-AC-3'. The real-time PCR data were quantified using the ΔCT method with the formula: $n = 100 * 2^{-(\Delta\text{CT targeted gene} - \Delta\text{CT GAPDH})}$.

Determination of nitric oxide (NO) production

NO was determined by Griess reaction. Specimen and standard were mixed with 100 μL Griess reagent and the absorbance was measured at room temperature.

Statistical analysis

All experiments were performed in triplicate. Results were expressed as mean \pm standard deviation (SD). Statistical analyses were performed with SPSS 12.0 by Paired-Samples T test. Statistical significance was set at $P < 0.05$.

Results

Effect of baicalein on cell viability

Effect of baicalein on human chondrocytes viability was examined at concentrations of 0, 5,

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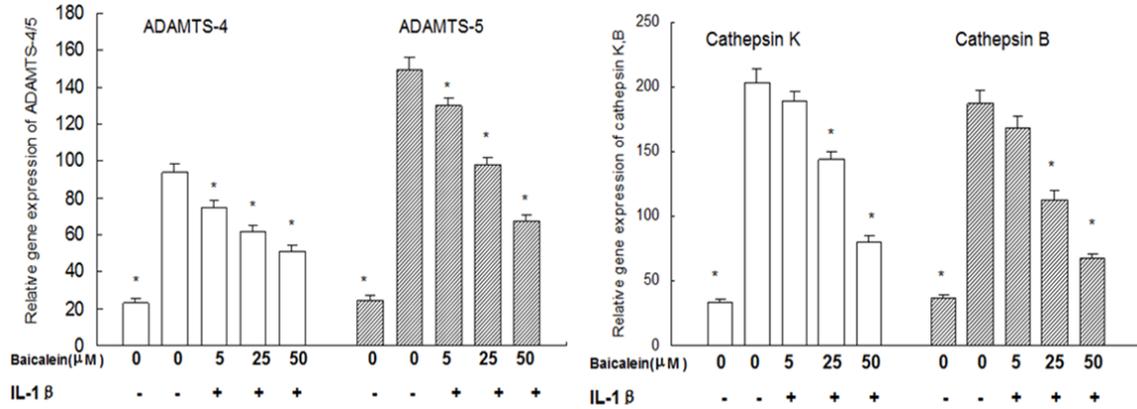


Figure 2. Effects of baicalein on gene expressions of ADAMTS-4, ADAMTS-5 and cathepsin K, B in IL-1 β -induced human chondrocytes. Cells were cultured in the absence or in the presence of IL-1 β (10 ng/ml) and with or without baicalein. mRNA level was detected by real-time quantitative PCR. Values are the mean and SD. * $P < 0.05$ compared with cells stimulated with IL-1 β alone.

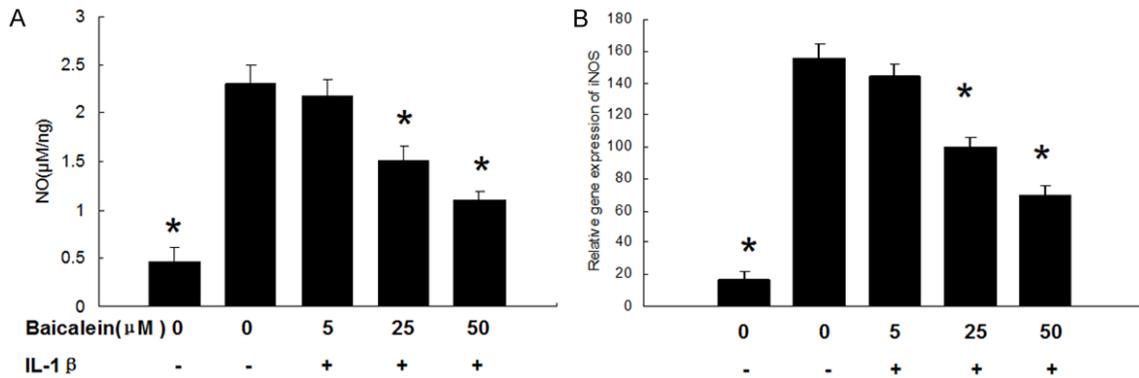


Figure 3. Effects of baicalein on NO synthesis (A) and gene expressions of iNOS (B) in IL-1 β -induced chondrocytes. Supernatants were collected and NO production was detected by Griess reaction. mRNA level of iNOS was detected by real-time quantitative PCR. Data represent mean \pm SD. * $P < 0.05$ compared with cells stimulated with IL-1 β alone.

25, 50 μ M after 24 h and 48 h culture. Baicalein ranging from 5 to 50 μ M did not show significant toxicity (Figure 1).

Effects of baicalein on gene expressions of ADAMTS-4, ADAMTS-5, cathepsin K and cathepsin B

The effects of baicalein on gene expression of ADAMTS-4, ADAMTS-5, cathepsin K and cathepsin B were investigated by real-time quantitative PCR. IL-1 β -stimulation increased the transcript levels of ADAMTS-4 and ADAMTS-5 and baicalein abolish this effect ($P < 0.05$). In addition, cathepsin K and cathepsin B were also elevated after treatment of IL-1 β , and baicalein showed inhibitory effects on cathepsin K and cathepsin B gene expression (Figure 2).

Effects of baicalein on IL-1 β -stimulated NO synthesis and gene expression of iNOS

Similarly, IL-1 β significantly increased NO production in supernatants. Baicalein inhibited NO production in a dose-dependent manner ($P < 0.05$). In addition, IL-1 β -stimulated gene expression of iNOS was also inhibited by baicalein ($P < 0.05$) (Figure 3).

Discussion

In the present study, we showed that baicalein inhibited the gene expressions of ADAMTS-4, ADAMTS-5, cathepsin K and cathepsin B. In addition, we also found that baicalein inhibited NO production and iNOS in IL-1 β -induced chondrocytes.

It is well known that ADAMTS (also known as aggrecanases) play an important role in OA via cleaving the aggrecan, and aggrecan depletion leading to cartilage degradation. IL-1 β can up-regulate the expression of ADAMTS-4 and ADAMTS-5 in chondrocytes. Previous studies showed that inhibition of ADAMTS-4 and ADAMTS-5 can prevent cartilage degradation in OA [10, 11]. In this study, we demonstrated that IL-1 β increased both ADAMTS-4 and ADAMTS-5 gene expression. And baicalein inhibited the enhanced expression of ADAMTS-4 and ADAMTS-5 in chondrocytes. Thus baicalein may exert protective effect on cartilage via inhibiting the aggrecanases-mediated degradation.

In the present study, we also found that baicalein inhibited the expression of cathepsin K and cathepsin B in IL-1 β -induced chondrocytes. Cathepsins of the cysteine protease family have been known to involve in ECM degradation in OA. Especially, cathepsin K was proved to degrade cartilage matrix via cleaving type I and II collagens as well as aggrecan, which were main components of ECM [12]. Previous studies demonstrated that inhibition of cathepsin K can reduce cartilage degradation in experimental OA [13, 14]. Cathepsin B is also considered as a catabolic factor in cartilage degradation [15]. Elevated expression of cathepsin B in experimental OA has been reported [16, 17]. There is little known about the effects of baicalein on the expression of cathepsin K and B. We found that IL-1 β stimulation can increased gene expressions of cathepsin K and B in human chondrocytes and baicalein could abolish this effect. Taken together, our results indicate that baicalein may inhibit cartilage degradation in OA by down-regulating gene expressions of various catabolic enzymes including ADAMTS and cathepsins.

In the present study, we also found that baicalein suppressed the production of NO as well as iNOS gene expression in IL-1 β -stimulated chondrocytes. NO was involved in OA via suppressing proteoglycan synthesis and inducing chondrocytes apoptosis [18, 19]. Fan et al. demonstrated that baicalein inhibited NO and iNOS expression via regulating NF- κ B pathway [20]. We speculated that baicalein could modulate the inflammatory process in arthritis.

In conclusion, we found that pretreatment of chondrocytes with baicalein could inhibit IL-1 β -

induced gene expressions of ADAMTS-4, ADAMTS-5, cathepsin K and cathepsin B. In addition, baicalein blocked NO production and suppressed iNOS mRNA level. Our results indicate that baicalein may possess chondroprotective effect in OA.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (8120-1429) and the Project Supported by Zhejiang Provincial Natural Science Foundation of China (LY14H060002).

Disclosure of conflict of interest

None.

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