The inhibitory effects of rosmarinic acid on catabolism induced by IL-1β in rat chondrocyte

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The effects of rosmarinic acid (RosA) on osteoarthritis (OA) was investigated in rat chondrocytes. Chondrocytes were isolated from rat cartilage, incubated with RosA in the presence of interleukin-1beta (IL-1β) (10 ng/ml). The production of IL-6, as well as the mRNA level of aggrecan (ACAN) and collagen 2 (COL2), were assessed. The gene and protein expression of A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4, ADAMTS-5 were also measured. RosA inhibited the production of IL-6 as well as the gene and protein expression of ADAMTS-4 and ADAMTS-5. In addition, RosA abolished IL-1β-induced inhibition of ACAN and COL2 gene expression. Our results suggest that RosA can inhibit extracellular matrix (ECM) degradation in OA, thus, RosA may be a possible agent in the treatment of OA.

Key words: rosmarinic acid, osteoarthritis, chondrocyte, interleukin-1β

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INTRODUCTION

Osteoarthritis (OA) is a prevalent disease in elderly people which always results in the loss of joint function (Kevorkian et al., 2004; Neogi, 2013; Glyn-Jones et al., 2015). Currently, there is lack of disease-modifying drugs for OA and nonsteroidal anti-inflammatory drugs (NSAIDs) are still the main agents to treat the disease (Tonge et al., 2014). Therefore, it is necessary to develop novel agents for the treatment of OA.

In normal condition, chondrocytes help to maintain the integrity of the extracellular matrix (ECM). However, during OA chondrocytes secrete excessive matrix proteins including matrix metalloproteinases (MMPs), A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS). MMPs can digest collagen 2 (COL2) and ADAMTS can digest aggrecan (ACAN) (Martel-Pelletier et al., 2001; Troebner & Nagase, 2012). ADAMTS-4 and ADAMTS-5 are considered important aggrecanases in the progression of OA (Verma & Dalal, 2011; Yang et al., 2017).

Inflammatory cytokine interleukin-1-beta (IL-1β) also plays a critical role in ECM degradation. IL-1β can induce increased production of MMPs and ADAMTS in OA leading to cartilage degradation.

Rosmarinic acid (RosA) is an ester of caffeic acid with many biological properties (Petersen & Simmonds, 2003). Increasing evidence shows that RosA exerts anti-inflammatory, anti-cancer and antioxidant effects (Wu et al., 2015; Cao et al., 2016; Jin et al., 2017; Eftekhar et al., 2018). Recently, RosA was identified as a potential complementary therapeutic agent in OA treatment (Connelly, 2014). However, the effects of RosA on ADAMTS are poorly understood. In this study, we investigated the effects of RosA on ADAMTS, ACAN and COL2 in rat chondrocytes.

MATERIAL AND METHODS

Reagents. RosA, dimethyl sulfoxide (DMSO), recombinant rat IL-1β and MITT (3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were purchased from Gibco BRL (Grand Island, NY, USA).

Cell culture. The study was approved by Institutional Animal Care and Use Committee of Zhejiang University (Hangzhou, China). Two-week-old Sprague-Dawley (SD) rats were used as the source for chondrocytes culture. The cartilage from the knee joints of the rats was digested with 0.2% collagenase II and the chondrocytes were isolated as previously described (Chen et al., 2012). Then, the chondrocytes were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. The experiments were conducted during cell passages 3 to 4.

Cell viability. The chondrocytes were seeded in the 96-well plates (5×103/well) with RosA at different concentrations for 24 h and 48 h, the culture medium was removed and MITT solution (5 mg/ml) was added (20 μl/well). Then, the solution was removed and DMSO (150 μl/well) was added. The optical density (OD) at 570 nm was determined using a microplate reader (Bio-Rad, Hercules, CA, USA).

Measurement of IL-6 production. Chondrocytes were seeded in six-well plates (1×105/well), cells were serum starved overnight and incubated with different concentrations of RosA for 1 h in the presence of IL-1β (10 ng/ml) for 24 h. The culture medium was collected and subjected to IL-6 production assay by ELISA kit (R&D Systems, Minneapolis, MN, USA). The chondrocytes were collected for gene expression analysis.

Quantitative real-time polymerase chain reaction (PCR). Total RNA from the chondrocytes was isolated using TRIzol reagent (Invitrogen, Carlsbad, California). cDNA was synthesized using reverse transcriptase (Promega, Madison, WI, USA). Quantitative real-time PCR was performed using SYBR Green (Life Technologies, Eggenstein-Leopoldshafen, Germany) in a 7500 Real Time PCR system (Applied Biosystems, Foster City, CA, USA). The expression of target genes were normalized to GAPDH. The primer sequences used were designed in Primer3 and are listed in Table 1.
was isolated using TRIzol reagent according to the manufacturer’s protocol (Invitrogen, Carlsbad, CA, USA) and reversed-transcribed using the Moloney murine leukemia virus reverse transcriptase cDNA synthesis kit (Promega, Madison, WI, USA). cDNA samples were amplified and quantified by qPCR using the iCycler apparatus system (Bio-Rad). The primers for amplification of each gene were as follows: for ADAMTS-4: 5′-CATCCTACGCCGGAAGAGTC-3’ and 5′-AAGCGAAGCGCTTGTTTCTG-3’; for ADAMTS-5: 5′-CCCAAATACGCAGGTGTCCT-3’ and 5′-ACACACGGAGTTGCTGTAGG-3’; for COL2: 5′-TCAAGTCGCTGAACAACCAG-3’ and 5′-GTCTCCGCTCTTCCACTCTG-3’; for ACAN: 5′-GCAGCACAGACACTTCAGGA-3’ and 5′-CCCACTTTCTACAGGCAAGC-3’; for GAPDH: 5′-TGTGAACGGATTTGGCCGTA-3’ and 5′-TGAACTTGCCGTGGGTAGAG-3’. GAPDH was used as an internal control. The real-time PCR data were quantified using the 2−ΔΔCT method.

Western blotting analysis. The protein was extracted from the chondrocytes, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinyl fluoride (PVDF) membranes. The membranes were incubated with primary antibodies (ADAMTS-4/5, COL2 and β-actin) (Cell Signaling Technology, Beverly, MA, USA). After washing the membranes were incubated for 1 h at room temperature with HAP-conjugated secondary antibodies. Finally, the membranes were incubated with the electroencephalographic substrate and exposed to X-ray film (Kodak). All of the assays were performed in triplicate.

Statistical analysis. Data are expressed as the mean±standard deviation (SD) and were analyzed statistically using one-way ANOVA. The Dunnett-t method was used as the post-test in the ANOVA. Differences were considered significant when p values were lower than 0.05. Data analysis was performed using SPSS 15.0.

RESULTS

Effect of RosA on cell viability

First, we assessed the effect of RosA on cell viability using MTT assay. RosA at a concentration of 100 μM did not show toxicity to the chondrocytes (Fig. 1).

RosA decreased the production of IL-6

The production of IL-6 in the culture media was investigated by ELISA. IL-1β stimulation resulted in up-regulation of the production of IL-6, whereas RosA at the concentration of 50 and 100 μM inhibited the production of IL-6 (Fig. 2).

RosA inhibited the IL-1β-induced expression of ADAMTS-4 and ADAMTS-5

In order to investigate the effects of RosA on ADAMTS in the presence of IL-1β, the gene and protein expression of ADAMTS-4 and ADAMTS-5 in the chondrocytes were measured. IL-1β stimulation significantly increased the expression of ADAMTS-4 and ADAMTS-5 in the chondrocytes. RosA at the concentration of 50 and 100 μM significantly reduced ADAMTS-4 and ADAMTS-5 expression in IL-1β-induced chondrocytes (p<0.05) (Fig. 3).

RosA increased the gene expression of ACAN and COL2 in chondrocytes

IL-1β significantly decreased the mRNA level of ACAN and COL2. RosA at the concentration of 50 and 100 μM in combination with IL-1β significantly increased ACAN and COL2 gene expression in chondrocytes (p<0.05) (Fig. 4).
DISCUSSION

In this study, we assessed the effects of RosA on the expression of anabolic and catabolic mediators. Our results showed that RosA can antagonize the catabolic effects of IL-1β in chondrocytes.

OA is a multifactorial disease mainly characterised by articular cartilage destruction. It is well-known that COL2 and ACAN are the main components of the cartilage ECM. In the normal cartilage, there is a balance between ECM anabolism and catabolism. However, in OA the balance is disturbed in favor of the catabolism. Depletion of AGAN and COL2 leads to cartilage degradation in OA (Mankin & Lippold, 1970). Previous studies showed that ADAMTS-4 and ADAMTS-5 are responsible for ACAN depletion in osteoarthritic cartilage (Gendron et al., 2007). It is well-known that these two aggrecanases cleave the aggrecan core protein at the aggrecan-specific Glu373-Ala374 bond in the aggrecan interglobular domain (IGD) region (Abbaszade et al., 1999; Tortorella et al., 2001). Thus, ADAMTS-4 and ADAMTS-5 received much attention in research aiming to find a new potential agent for OA treatment (Apte, 2016; Verma et al., 2016).

In the present study, we also investigated the effects of RosA on ADAMTS-4 and ADAMTS-5 expression and our results showed that RosA can inhibit the gene and protein expression of ADAMTS-4 and ADAMTS-5 in chondrocytes. In addition, we examined the effects of RosA on the expression of ACAN and COL2, and we found that RosA can upregulate the gene expression of ACAN and COL2. Together, these results suggest that RosA treatment can inhibit the degradation of cartilage ECM, leading to the suppression of articular cartilage degradation.

It is established that OA is also an inflammatory disease and that inflammation plays an important role in the progression of OA (Berenbaum, 2013). Previous studies showed that several pro-inflammatory cytokines, chemokines and prostaglandins (PGEs) were involved in OA (Robinson et al., 2016; Scanzello, 2017). These inflammatory mediators induced the inflammatory reaction in chondrocytes, leading to synovial inflammation and articular cartilage degradation. As one of the most important inflammatory factors in OA, IL-1β is involved in synovial inflammation, apoptosis of chondrocytes and ECM catabolism, furthermore, IL-1β could induce the expression of aggrecanases in OA (Koshy et al., 2002; Daheshia & Yao, 2008). In the present study, we treated the chondrocytes with IL-1β, as expected, the expression of ADAMTS-4 and ADAMTS-5 was increased after incubation with IL-1β, and the production of IL-6, an inflammatory cytokine, was also upregulated by IL-1β. RosA is known to exhibit anti-inflammatory activities, Rui and others (Rui et al., 2017) reported that RosA inhibited the expression of IL-6 in 3T3-L1 adipocytes. Our results showed that RosA can inhibit the secretion of IL-6. Therefore, the protective effects of RosA on chondrocytes may be partly associated with its anti-inflammatory properties. However, Eo and others (Eo et al., 2017) reported that RosA can induce COX-2, another inflammatory cytokine, in rabbit chondrocytes. Thus, the role of RosA in OA is still unclear.

In conclusion, we found that RosA inhibited IL-1β-upregulated expression of ADAMTS-4, ADAMTS-5 and IL-6. We speculate that RosA exerts its role in OA via inhibiting matrix-degrading enzymes and inflammatory factors. Our results indicate that RosA may possess chondroprotective effect in OA.

Competing Interests

The author(s) declare that they have no competing interests.

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REFERENCES


Figure 4. Effects of RosA on ACAN and COL2 gene expression in IL-1β induced chondrocytes.

Cells were cultured in the absence or in the presence of IL-1β (10 ng/ml) and with or without RosA. mRNA level was measured by real-time quantitative PCR.Values are presented as means and SDs. *p<0.05 compared with cells stimulated with IL-1β alone. The experiment was performed in three replicates.
in dextran sulphate sodium (DSS)-induced mice via dual inhibition of NF-κB and STAT3 activation. Sci Rep 7: 46252.


