Biochemical and Biophysical Research Communications 480 (2016) 309–313

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Carvedilol suppresses cartilage matrix destruction

Zhigang Li [a, b, 1], Baoyi Liu [b, 1], Benjie Wang [b], Yupeng Liu [b], Yao Zhang [b], Fengde Tian [b], Borui Li [b], Dewei Zhao [b, *]

[a] Department of Biomedical Engineering, Faculty of Electronic Information and Electrical Engineering, Dalian University of Technology, Dalian, Liaoning, PR China
[b] Department of Orthopaedics, Affiliated Zhongshan Hospital of Dalian University, Dalian, Liaoning, PR China

ARTICLE INFO

Article history:
Received 3 October 2016
Accepted 13 October 2016
Available online 14 October 2016

Keywords:
Carvedilol
NF-κB
Collagen type II
MMP-1
Osteoarthritis (OA)

ABSTRACT

Collagen type II (col II) and aggrecan, the main components of the extracellular matrix (ECM) in human joint cartilage, have been reported to be reduced by chronic production of inflammatory cytokine interleukin (IL)-1β in arthritic joints. Carvedilol, a licensed medicine, has been used for treatment of hypertension, congestive heart failure and coronary disease in clinics. In this study, we investigated the effects of Carvedilol on the expression of col II and aggrecan. Our results demonstrate that treatment with Carvedilol didn't change the expression of aggrecan or col II at mRNA levels in SW1353 chondrocytes. However, the expression of aggrecan and Col II at protein levels were significantly reduced by IL-1β treatment, which were reversed by Carvedilol in a dose dependent manner, suggesting the inhibitory effects of Carvedilol on the expression of aggrecan and Col II are at post-translational modification levels. In addition, it was shown that IL-1β treatment highly induced MMP-1 and MMP-13 expression in SW1353 chondrocytes at both gene and protein expression levels, which were restored by Carvedilol in a dose dependent manner. Mechanistically, exposure to IL-1β increased phosphorylation of IKK-α/β and degradation of IκB-α in SW1353 chondrocytes, which were suppressed by pretreatment with Carvedilol. Administration of Carvedilol inhibited IL-1β-induced translocation of NF-κB p65 from cytosol to nucleus manner. Notably, a luciferase reporter assay showed that IL-1β severely increased NF-κB luciferase activity, which was markedly suppressed by Carvedilol treatment. Our results suggest that Carvedilol might be a potential therapeutic agent for chondro-protective therapy.

1. Introduction

The extracellular matrix (ECM) in human joint cartilage consists of collagens, elastin, proteoglycans and their large aggregates [1]. Among them, collagen type II (col II) and aggrecan are the main components of ECM materials, which play a pivotal role in providing support and tensile strength for the joint [2]. Increasing evidence has shown that the abundant extracellular matrix of articular cartilage has to be maintained by a limited number of chondrocytes. The denaturation and degradation of ECM materials, especially col II in the cartilage and synovial fluid leads to progressive loss of integrity and strength of the cartilage, which is one of the most important pathological characteristics in osteoarthritis (OA). Chronic production of inflammatory cytokine interleukin (IL)-1β in arthritic joints by activated synovial cells and infiltrating macrophages, is considered one of the most potent catabolic factors in arthritis [3]. Overproduction of IL-1β activates chondrocytes, which in turn produce catabolic factors such as matrix metalloproteinases (MMPs), nitric oxide (NO), and other proinflammatory cytokines [4]. MMPs are a large group of enzymes that degrade a wide variety of ECM components. At the basal levels, MMPs take responsible for the normal turn-over of ECM materials. However, under special pathological conditions, chondrocytes highly induce MMPs, which lead to the degradation of ECM components [5]. Among these, MMP-1 and -13 are of particular importance because they are elevated in joint disorders and degrades cartilage collagens [6]. Inhibiting the activity of MMPs has become a potential strategy to prevent and/or treat osteoarthritic symptoms and several other ECM-degradation diseases to some extent.

* Corresponding author. Department of Orthopaedics, Affiliated Zhongshan Hospital, Dalian University, Address: No. 6 Jiefang Street, Dalian, Liaoning, 116001, PR China.
E-mail address: dwzhao0908@163.com (D. Zhao).
[1] Co-first author.

http://dx.doi.org/10.1016/j.bbrc.2016.10.032
0006-291X/

Carvedilol, a non-specific β-adrenergic blocker, has been used for treatment of hypertension, congestive heart failure and coronary disease in clinics [7]. Interestingly, several *in vivo* and *in vitro* studies demonstrated the cardio-protective [8], nephron-protective [9] and hepato-protective effects of carvedilol against various toxicants induced preclinical models. And these effects are independent of its vasodilator and β-adrenergic blocking properties. Notably, increasing evidence has shown that Carvedilol possesses distinctive antioxidant property with both ROS-scavenging and ROS-suppressive effects [10,11]. In addition, Carvedilol also displays its anti-inflammatory properties through reducing pro-inflammatory cytokine production combined with increased anti-inflammatory cytokine (e.g., IL-10) production [12]. However, few studies have examined the therapeutic potential of Carvedilol for cartilage degradation. In this study, we aimed to investigate whether Carvedilol has a protective effect against IL-1β induced expression of MMPs and degradation of col IIand aggrecan.

2. Materials and methods

2.1. Cell culture

The human chondrosarcoma cell line SW1353 cells were obtained from American type culture collection (ATCC HTB-94, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 60 U/mL penicillin, 60 μg/mL streptomycin, and 2 mM glutamine at 37 °C. SW1353 cells were pretreated with the vehicle (DMSO, 0.01%, v/v) or Carvedilol (10 and 50 μM) for 24 h and then stimulated with IL-1β (10 ng/mL) for 24 h.

2.2. RNA isolation and real-time PCR

Total intracellular RNA from cultured SW1353 cells was isolated using Qiazol (Qiagene, USA) in accordance with the manufacturer's instructions. 2 μg of total RNA was then used to synthesize cDNA by using the iScript cDNA synthesis kit (Bio-rad, USA). Quantitative real-time polymerase chain reaction (qRT-PCR) was carried out using SYBR Green qPCR Master Mix in the StepOne Plus Real time PCR System (Roche AG, Basel, Switzerland). Gene expression at mRNA levels was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the $_{\Delta\Delta}$Ct method.

2.3. Western blot analysis

After indicated treatment, cell lysate was prepared by using cell lysis buffer (Cell signaling, USA). Equal amounts (10 μg) of total protein extract were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and were electro-transferred onto Immobilon-P Membrane (Millipore, USA). Then membranes were blocked in TBS containing 10% non-fat dry milk and 0.5% Tween-20 for 2 h at RT, followed by being probed with the indicated primary antibodies overnight at 4 °C and with the horseradish peroxidase linked secondary antibodies for 2 h at RT. The blots were developed with Immobilon Western Chemiluminescent HRP Substrate (Millipore, USA) and the bands on the films were scanned and analyzed by Image J software (LabWorks Software, USA).

2.4. Nuclear protein extraction

Extraction of nuclear proteins from SW1353 cells was performed using a compartmental protein extraction kit (Thermo Scientific, USA) in accordance with the manufacturer's instructions. Extracted nuclear protein was prepared for western blot analysis to determine the level of p65. Lamin B was used as internal control of nuclear protein.

2.5. Luciferase reporter gene assay

NF-κB activity was measured using the luciferase reporter gene assay by transfecting SW1353 cells with 1 μg reporter gene (either pNF-κB-luc or negative control; Qiagen) using lipofectamine 2000 in accordance with the manufacturer's instructions. The transfection efficiency was determined with β-galactosidase. 24 h after transfection, SW1353 cells were pretreated with the vehicle (DMSO, 0.01%, v/v) or Carvedilol (10 and 50 μM) and then stimulated with IL-1β (10 ng/mL) for 24 h. Luciferase and β-gal activities were measured using commercial kits from Promega. Transcriptional activity was determined as luciferase activity normalized to β-galactosidase activity and compared with unstimulated controls.

2.6. Immunofluorescence

The patterns of p65 nuclear translocation were determined by the immunofluorescence assay. After the indicated treatment, cells were fixed with 4% paraformaldehyde for 10 min at room temperature (RT), followed by permeabilization with 0.4% Triton X-100 for 10 min on ice. Then cells were blocked with 5% normal goat serum in PBST for 1 h at RT and incubated with primary antibody against p65 diluted in TBS (1:500) for 2 h at RT, followed by Alexa-594 conjugated secondary antibodies for 1 h at RT.

2.7. Statistical analysis

Experimental data are shown as arithmetic mean ± SD. Statistical analysis was evaluated using one-way Analysis of variance (ANOVA) followed by Dunnett's analysis. $P < 0.05$ was considered as significantly different.

3. Results

Firstly, we detected the effects of Carvedilol on the expression of aggrecan and Col II in IL-1β-stimulated SW1353 chondrocytes. SW1353 chondrocytes were pretreated with the vehicle (DMSO, 0.01%, v/v) or Carvedilol (10 and 50 μM) for 24 h and then stimulated with IL-1β (10 ng/mL) for another 24 h. Results in Fig. 1A demonstrate that neither the expression of aggrecan nor the expression of Col II at mRNA levels was significantly changed. However, the expression of aggrecan and Col II at protein levels were significantly reduced by IL-1β treatment in SW1353 chondrocytes, which appeared to be reversed by Carvedilol in a dose dependent manner. These results suggest that Carvedilol regulates the expression of aggrecan or Col II at post-translational modification levels, but not at transcriptional levels. Matrix metalloproteinases (MMPs) have proteolytic activities assisting in degradation of ECM and basement membrane at protein levels. Among the MMPs, MMP-1 and MMP-13 are considered to be of particular interest due to their roles in cartilage degradation. We then investigated the ability of Carvedilol to modulate IL-1β-mediated expression of MMP-1 and MMP-13 *in vitro*. As shown in Fig. 2A, B, real time PCR results indicate that IL-1β treatment (10 ng/mL) highly induced MMP-1 and MMP-13 expression in SW1353 chondrocytes, which were reversed by Carvedilol in a dose dependent manner. Also, the expressions of MMP-1 and -13 at protein levels in SW1353 cells were determined by western blot analysis. And the result indicates that levels of MMP-1 and -13 detected in SW1353 cells treated with IL-1β alone increased by 5.1- and 5.9-folds compared to levels detected in untreated chondrocytes (Fig. 2C, D). However, pretreatment of cells with Carvedilol