Biomedicine & Pharmacotherapy 106 (2018) 149–155

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

$1,25(OH)_2D_3$ promotes chondrocyte apoptosis and restores physical function in rheumatoid arthritis through the NF-κB signal pathway

Run Tian[a], Xiaofang Li[b], Yue Li[a], Kunzheng Wang[a], Chunsheng Wang[a], Pei Yang[a,*]

[a] Department of Orthopaedics, Second Affiliated Hospital of Health Science Center of Xi'an Jiaotong University, Xi'an, Shanxi, 710004, People's Republic of China
[b] Department of Gynaecology and Obstetrics, Second Affiliated Hospital of Health Science Center of Xi'an Jiaotong University, Xi'an, Shanxi, 710004, People's Republic of China

ARTICLE INFO

Keywords:
$1,25(OH)_2D_3$
NF-κB signal pathway
Rheumatoid arthritis
Paw volume
Chondrocyte
Apoptosis

ABSTRACT

We explored the modulatory effect of $1,25(OH)_2D_3$ on chondrocytes and physical function in rats with RA and its mechanism underlying the regulation of NF-κB signal pathway. RA patients and healthy volunteers were selected. Sprague-Dawley (SD) rats were used to establish RA models. The paw volume of rats was estimated. Chondrocytes were isolated from RA rats. The protein levels in both cartilage tissues and chondrocytes were determined using western blotting. Apoptosis was evaluated using TUNEL assay. Serum levels of IL-1β, IL-6, IL-10 and IL-17 were measured by enzyme-linked immunosorbent assay (ELISA). Serum levels of $1,25(OH)_2D_3$ were lower in RA patients than in healthy volunteers. Rats in the RA + VD_3 group were lighter than those in normal and PBS groups, with an increased paw volume, severer joint swelling, higher expression levels of p-IκBα, p-p65, IL-1β, IL-6, and IL-17, and lower expression level of IL-10, while those in RA and RA + VD_3 + NF-κB group differed more significantly. In addition, by comparing RA rats and RA + NF-κB rats, we found that TNF-α stimulation exacerbated RA, increased expression levels of p-IκBα, p-p65, IL-1β, IL-6, and IL-17, and decreased the expression level of IL-10. Compared with RA chondrocytes, chondrocytes from RA + VD_3 rats exhibited lower expression levels of p-IκBα and p-p65, and had more apoptotic cells, while those from RA + NF-κB rats showed an opposite trend. Taken together, $1,25(OH)_2D_3$ accelerates chondrocyte apoptosis and improve physical function in rats with RA by the inhibition of NF-κB signal pathway.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease with a high prevalence and high mortality due to an increased risk of cardiovascular diseases [1]. RA is characterized by consistent synovitis and aggravating cartilage and bones destruction [2,3]. RA affects approximately 0.5–1.1% of population in developed regions, including North America and Northern Europe, while Southern Europe showed a lower prevalence of 0.3% to 0.7% [4,5]. It has been suspected that a complex interplay between environmental factors and genetic factors contributes to the occurrence of RA [6,7]. Moreover, substantial evidence suggests that accumulation of synovial cells is involved in the pathogenesis of RA and leads to pannus formation, cartilage erosion, and joint destruction [8,9].

Interestingly, insufficiency in vitamin D is associated with the pathogenesis of several autoimmune diseases, including RA [10–12]. The active form of vitamin D, $1,25(OH)_2D_3$, has been originally known for its role in calcium and phosphorus homeostasis as well as mineralization [13]. $1,25(OH)_2D_3$, as a steroid hormone, plays a key role in maintenance of autoimmune tolerance and cell functions, such as cell proliferation, differentiation, and apoptosis [10]. A previous study has reported that RA patients significantly differed from age and sex matched controls for $25-OH$ vitamin D levels and the immunomodulatory effect of vitamin D has been demonstrated in animal models of RA [14]. $25(OH)D_3$ insufficiency is common among patients with RA and high disease activity in patients with RA was associated with decreased level of 1,25 dihydroxyvitamin D_3 [15].

NF-κB, a protein complex, is found in almost all animal cell types and involved in cellular responses including stress, cytokines, free radicals, heavy metals, ultraviolet irradiation, oxidized low density lipoprotein (LDL), and bacterial or viral antigens [16,17]. The involvement of NF-κB signal pathway in the occurrence and development had been proved in RA [18,19]. Furthermore, NF-κB functions as a potential therapeutic target in osteoarthritis and RA [20,21]. Collectively, considering that RA is an autoimmune disease and the implication of $1,25(OH)_2D_3$ and NF-κB signal pathway in bone/joint development and

* Corresponding author at: Department of Orthopaedics, Second Affiliated Hospital of Health Science Center of Xi'an Jiaotong University, No.157, Xiwu road, Xi'an, Shanxi, 710004, People's Republic of China.
E-mail address: yangpei2502@163.com (P. Yang).

https://doi.org/10.1016/j.biopha.2018.06.061
Received 10 April 2018; Received in revised form 11 June 2018; Accepted 13 June 2018
0753-3322/ © 2018 Published by Elsevier Masson SAS.

cell function, we hypothesized that $1,25(OH)_2D_3$ may influence chondrocytes apoptosis and physical function in RA by regulating NF-κB signal pathway. The aim of this study is to provide underlying mechanism involving protective effect of $1,25(OH)_2D_3$ against RA, and thus offering theoretical basis.

2. Materials and methods

2.1. Subjects

From 2014 June to 2016 February, 45 RA patients (18 males and 27 females) admitted in rheumatology department of Second Affiliated Hospital of Health Science Center of Xi'an Jiaotong University were included in current study with age ranging from 31 to 67 years. The inclusion criteria for RA patients were as follows: (1) conforming to standard of classification proposed by American College of Rheumatology in 1987; (2) no history of vitamin D, glucocorticoid, immunosuppressors and tumor necrosis factor antagonist before inclusion; (3) normal function of liver and kidney. Patients were excluded from current experiment if they were pregnant or in lactation period, or combined with disease related with immune system or joints. Meanwhile, 24 health volunteers taking physical examination within the same period were included as health controls with age ranging from 32 to 66 years. This study was conducted based on the protocols proposed by the commitment of Second Affiliated Hospital of Health Science Center of Xi'an Jiaotong University. All patients signed written informed consents prior to the study.

2.2. Animal grouping and treatment

A total of 60 clean grade SD rats, half male and half female, were purchased from animal center in the Third Military Medical University (license #, SCXK (YU)2007-0005) with weight ranged from 200 ± 20 g. One week later, rats were grouped into normal group (normal rats, no treatment), NC group (negative control group, normal rats fed with normal saline), RA group (rats with RA, feed without $1,25(OH)_2D_3$), RA + VD_3 group (rats with RA, fed with $1,25(OH)_2D_3$), RA + NF-κB group (rats with RA, stimulated by TNF-α), and RA + VD_3 + NF-κB group (rats with RA, feed with $1,25(OH)_2D_3$ and stimulate with activator of NF-κB signal pathway, TNF-α (10 ng/mL)). Each group had 10 rats. The rats were fed with 5 μg/kg of $1,25(OH)_2D_3$ (an active form of vitamin D_3, D1530-1MG, sigma, St. Louis, MO, USA) through stomach tube, once a day. TNF-α stimulation was performed by a intraperitoneal injection of 10 μg/kg TNF-α, once a week. Animal treatment lasted for 28 days. Animal experiments were conducted in strict accordance with the approved animal protocols and guidelines.

2.3. Establishment of RA rat models

Then, 10 mg chicken type II collagen (CCII, Sigma) and 5 ml glacial acetic acid (0.1 mol/L, Sinopharm Chemical Reagent Co., Ltd) were mixed, which was then emulsified with equal volume of complete Freund's adjuvant (CFA, Sigma) with a final density of 1 g/L. Rats were then anesthetized with chloral hydrate (0.3 mL/100 g, Shanghai Yunzhan chemical Limited). Then the paws of right hindlimb of rats were subcutaneously injected with 1 ml type II collagen solution with multiple injection points. One week later, rats were injected with the same solution using the above mentioned method. Rats in the NC group were injected with same volume of normal saline.

2.4. Pathological observation and assessment

Activities, hair, excrement and appetites of rats in each group were observed and recorded. Rat weight was measured every week in a regular manner. Joints swellings of each rat were observed and the toe volume was assessed using toe volume measurer. According to the degree and area of redness and swelling of joints, arthroncus and arthrentasis, 5-level scoring [22] was carried out, in which the highest score for arthritis was 4 points and the highest sum of score of four cases of arthritis was 16 points. When animal treatment finished, rats were sacrificed by cervical dislocation and pathological changes of rat joint were observed. The changes of joints were evaluated after detection of X ray.

2.5. Cell isolation, identification and grouped

RA rat models were sacrificed by intravenous injection of pentobarbital sodium (30 mg/kg). After disinfection, rats were placed in a supine position on the sterile operate table. The skins of their lower limbs were shaved along the groin and the ligament around were disconnected to isolate the attached tissues. The keel joints were exposed and the joint cartilage tissues were obtained using a scalpel. The obtained tissues were washed in PBS for three times and cut into small pieces of $1mm^3$ size using an eye scissor. After another PBS wash for three times, the tissues were centrifuged and removed from supernatant, and then mixed with 2 ml of 0.2% II collagenase and digested in an incubator for 30 min at 37 °C. The digested solution was under centrifugation for 5 min prior to cell collection. After that, cell suspension was prepared by adding DMEM/F12 culture medium containing 10% fetal bovine serum (FBS) and inoculated in a culture flask at 37 °C and with 5% CO_2 for cell culture. Hematoxylin-eosin (HE) staining was carried out for chondrocyte shape. In order to identify chondrocytes successfully isolated from joint cartilage tissues, we stained glycosaminoglycans secreted by chondrocytes using toluidine and detected the expression of Type II collagen using immunohistochemistry. RA chondrocytes in logarithmic phase were classified into five groups. RA group (RA chondrocyte, no treatment), NC group (RA chondrocytes, maintained in culture medium supplemented with PBS), RA + VD_3 group (RA chondrocytes, maintained in culture medium supplemented with $1,25(OH)_2D_3$), RA + NF-κB group (RA chondrocytes, maintained in culture medium supplemented with 10 ng/ml TNF-α), and RA + VD_3 + NF-κB group (RA chondrocytes, maintained in culture medium supplemented with $1,25(OH)_2D_3$ and 10 ng/ml TNF-α).

2.6. Western blotting

The isolated proteins from chondrocyte and cell groups were added with loading buffer for boiling for 10 min at 95 °C using BCA kit (Beyotime Biotechnology, Shanghai, China). Then 30 μg samples was added in each plate and undergone electrophoresis to isolate the protein by 10% polyacrylamide gel. Voltage for electrophoresis was transferred from 80 V to 120 V. Wet transfer was conducted under 100 V for 45–70 min to transfer proteins to PVDF membrane. Rabbit anti mouse primary antibodies for IκBα, P-IκBα, P65, P-P65, Caspase-3, Bax and Bcl-2 (1:1000) purchased from Cell Signaling (USA), antibody for β-actin (1:3000) purchased from Becton, Dickinson and Company (USA), were added and incubated overnight at 4 °C. The samples were washed with TBST for three times, each for 5 min, followed by adding goat anti rabbit secondary antibodies (Biomart, Shanghai), followed by incubation at room temperature for one hour. Color was developed and Bio-rad Gel Dol EZ analyzer (GEL DOC EZ IMAGER, Bio-rad, California, USA) was used for image observation. Image *J* software was used to analyze the grey values of target bands. Experiments were conducted for three times with the mean value obtained.

2.7. TUNEL assay

After 1×10^6 cells collection and suspension in PBS, cells were put on polylysine slide, and dried before being fixed in 4% paraformaldehyde for 25 min. PBS wash was conducted for 2 × 5 min and Triton X-100 was added for reaction for 5 min. Washed in PBS for 2 × 5 min,

Download English Version:

https://daneshyari.com/en/article/8524854

Download Persian Version:

https://daneshyari.com/article/8524854

Daneshyari.com