MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2020; 26: e920784
DOI: 10.12659/MSM.920784

Received: 2019.10.17
Accepted: 2019.11.26
Available online: 2020.01.22
Published: 2020.03.03

Promotion of G1/S Transition and Inhibition of Inflammatory Cytokine Production by Hydroxypyridinone-Coumarin in Osteoarthritis Rats

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCD 1 Kai Yang
BCD 2 Gang Chen
ADEF 3 Xiongxun Wang

1 Department of Orthopedics, Zhengzhou Central Hospital Affiliated to Zhengzhou University, Zhengzhou, Henan, P.R. China
2 Department of Orthopedics, Binzhou People's Hospital, Binzhou, Shandong, P.R. China
3 Department of Spine Surgery, Xi'an International Medical Center Hospital, Xi'an, Shaanxi, P.R. China

Corresponding Author: Xiongxun Wang, e-mail: chengzf222@sina.com
Source of support: Departmental sources

Background: Osteoarthritis is a joint disorder characterized by articular cartilage degradation leading to joint stiffness and pain. The present study investigated the effect of hydroxypyridinone-coumarin on proliferation of chondrocytes.

Material/Methods: Chondrocyte proliferation was assessed by MTT assay, and distribution of cells in various phases of the cell cycle was determined using flow cytometry. RT-PCR and Western blot assays were used for assessment of mRNA and protein levels, respectively. Osteoarthritis was induced in the rats by injecting monosodium iodoacetate (5 mg/kg) by the intra-articular route. The rats in the treatment groups were intraperitoneally injected with 5, 10, or 15 mg/kg doses of hydroxypyridinone-coumarin alternately for 1 month.

Results: The proliferation of chondrocytes was increased significantly ($P<0.05$) by treatment with hydroxypyridinone-coumarin in a concentration-based manner. The increase in chondrocyte proliferation by hydroxypyridinone-coumarin was maximum at 50 μM. Treatment with hydroxypyridinone-coumarin markedly increased chondrocyte population in S and G2/M phases, with subsequent reduction in G0/G1 phase. The cyclin D1, CDK4, and CDK6 levels in the chondrocytes were increased by treatment with hydroxypyridinone-coumarin. The production of IL-6, TNF-α, and IL-1β in the osteoarthritis rats was markedly suppressed by hydroxypyridinone-coumarin. Treatment of the OA rats with hydroxypyridinone-coumarin markedly reduced the expression of IκB-α and NF-κB p65.

Conclusions: The present study revealed that the proliferative potential of chondrocytes is increased by hydroxypyridinone-coumarin through acceleration of G1/S transition. Moreover, hydroxypyridinone-coumarin treatment reduced inflammatory cytokine production in the osteoarthritis rats. Therefore, hydroxypyridinone-coumarin should be evaluated further for possible use in the treatment of osteoarthritis.

MeSH Keywords: Anti-Inflammatory Agents • Chondrocytes • Cytokines

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/920784

2233 — 5 28

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Osteoarthritis is a disorder of joints characterized by the degeneration of articular cartilage leading to stiffness of joints and acute pain [1,2]. Various factors, including age, trauma, obesity, and genetics, have been found to be responsible for the development of osteoarthritis [3]. The pathogenesis of osteoarthritis is not fully understood [3]. The clinical symptoms of early-stage osteoarthritis include inflammation, edema, joint stiffness, and severe pain [4]. The secondary stage of osteoarthritis leads to difficulty in movement and severely affects quality of life [4]. The small percentage of non-proliferating chondrocytes present in the adult articular cartilage secretes extracellular matrix [5]. The 2 major components of extracellular matrix are collagens and proteoglycans. Tissue homeostasis in cartilage is maintained by chondrocytes through synthesis and breakdown of extracellular matrix [5]. Thus, higher proliferation of chondrocytes plays a vital role in regulating chondrocyte integrity and cellular function [5]. The main inducer of osteoarthritis is interleukin (IL)-1β cytokine, which produces higher levels of catabolic enzymes, leading to metabolic imbalance. The increased production of catabolic enzymes degrades cartilage matrix and interferes with the normal physiological functioning of cartilage [6–8]. Chondrocytes of osteoarthritis patients have been shown to secrete higher levels of IL-1β, TNF-α, and IL-6 [9]. The secretion of inflammatory cytokines is also higher in synovial membranes [10]. These cytokines penetrate into the cartilage, damage its integrity, and lead to the development of osteoarthritis [10].

The cell cycle consists of a sequence of properly regulated events during which DNA is synthesized and cells undergo reproduction. At the initial stage of the cell cycle, replication of DNA and duplication of the chromosomes takes place [11]. In S phase, the DNA replication involves opening of the double-stranded structure, exposing the individual helices to enzymes for the synthesis of new strands [11]. In the M phase, chromosomes segregate, providing a template for the synthesis of more strands [12,13]. The G1 phase of cell cycle is the decisive period during which cells either divide or not [14,15]. Several factors, such as Ser/Thr protein kinases (CDK4 and CDK6), regulate cell cycle progression [16]. Cyclin D1 upregulates the transition from G1 phase to S phase on combining with CDK4 or CDK6 [16]. Heterocyclic compounds exhibit a wide range of biological activities, including anticancer, anti-inflammatory, and immune-modulator effects. The present study investigated the effect of hydroxypyridinone-coumarin on the proliferative potential and cell cycle progression of chondrocytes *in vitro* and on inflammatory cytokine production and NF-κB signalling pathway activation *in vitro* in osteoarthritis rats *in vivo*.

Material and Methods

Chondrocyte isolation from rats and culture

We obtained 4-week-old Sprague-Dawley rats (weight 180–200 g) from the Laboratory Animal Center of the Academy of Military Medical Sciences, Beijing, China, which were used for isolating the chondrocytes. The articular cartilage was carefully excised from the knee joint of rats using isoflurane anaesthesia. The cartilage was washed 3 times in cold PBS and then sliced into thin 1-mm^3 sections. The slices were digested in 0.2% type II collagenase, after which isolation of chondrocytes was performed in an incubator at 37°C. The supernatant obtained from the digested slices after every 3 h was centrifuged for 10 min at 1000 g to isolate the cell pellet. Stainless-steel filters with mesh size 200 were used to filter the cells, which were seeded at 2×10^5 cells/ml density in 6-well plates. The cells were cultured at 37°C in DMEM supplemented with 10% FBS under 5% CO_2. The cultured primary cells were monitored using an inverted microscope.

MTT assay

The chondrocytes were distributed at 1.0×10^4 cells/well density in 96-well plates containing 10% FBS/DMEM. Hydroxypyridinone-coumarin at 5, 10, 15, 20, 25, 30, 40, and 50 μM concentrations was added to the plates and incubation was performed for 48 h. The medium was replaced by fresh medium, and then 20 μl of MTT solution (0.5%) was put into each well of the plates. Following incubation for 4 h at 37°C, 120 μl dimethyl sulfoxide (DMSO) was put into each well of the plate. Absorbance was recorded for each well of the plate at 487 nm using an ELISA reader (ELx800™; BioTek Instruments, Inc., Winooski, VT, USA).

Analysis of cell cycle

The chondrocytes were distributed in 35-mm petri dishes at 2×10^4 cells/ml density. Incubation of the chondrocytes was performed for 48 h with 5, 30, 40, and 50 μM of hydroxypyridinone-coumarin. Then, chondrocytes were digested and subsequently re-suspended in ice-cold PBS. The cell suspension was centrifuged at 1000×*g* for 10 min at room temperature, after which the concentration of cells was adjusted to 2×10^6 cells/ml. The cell plates were fixed using 70% ethyl alcohol for 12 h at 4°C, followed by incubation with DNase-free RNaseA and propidium iodide according to the manufacturer's instructions. The cells were analyzed by a flow cytometer (BD Accuri™ C6; BD Biosciences, Franklin Lakes, NJ, USA).

RT-PCR analysis

The chondrocytes at 2×10^5 cells/well density were distributed in 6-well plates containing 2 ml of medium and incubated

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

for 48 h with 5, 30, 40, and 50 μM of hydroxypyridinone-coumarin. The cells were treated with TRIzol reagent (Invitrogen) in accordance with the manual protocol for isolation of total RNA. The 3-μg RNA samples were used as templates for reverse transcription into cDNA using oligo(dT) primers and SuperScript III RT (Invitrogen). The gene expression was quantified using SYBR Green Master mix according to manufacturer's instructions. The sequence of events involves denaturation at 93°C for 5 min, then 40 cycles of amplification at 93°C for 10 s, followed by quantification at 58°C for 1 min. Data were assessed for relative gene expression using the $2^{-\Delta\Delta Ct}$ method.

Western blot assay

The chondrocytes cultured in culture flasks were treated with 5, 30, 40, and 50 μM of hydroxypyridinone-coumarin for 48 h. The cells were then scrapped from the medium, washed 2 times with PBS, and subsequently suspended in RIPA buffer (30 μl). The lysate was centrifuged to collect the supernatant, in which the protein concentration was determined by bicinchoninic acid (BCA) protein assay kit in accordance with the manufacturer's instructions. The 20-μg protein samples were subjected to electrophoresis using 8–12% SDS-polyacrylamide gels and subsequently transferred to the PVDF membranes. The membranes were blocked by incubation with 5% skimmed milk in TBST solution. Incubation of the membranes was performed overnight with primary antibodies against CDK6, CDK4, cyclin D1, IκBα, and NF-κB p65 at 4˚C. After washing with PBS, the membranes were incubated with secondary antibodies conjugated to HRP for 2 h. Enhanced chemiluminescence detection was used for visualization of the protein bands.

Animals

Twenty-five male Wistar rats (age 10–12 weeks, weight 285–405 g) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences, Beijing, China. The rats were housed individually in plastic boxes under a 12-h light/dark cycle. The temperature in the animal house was maintained 23±2°C and humidity was controlled at 55±10%. All the rats were provided free access to food and water *ad libitum*. The study was approved by the Animal Ethics Committee, Zhengzhou University, Henan China [ZU/08/16]. The experimental protocols involving animals were conducted in accordance with the guidelines of the Animal Ethics Committee, Chinese Academy of Science.

Establishment of osteoarthritis rat model

The rats were divided randomly into 5 groups of 5 each and then anesthetized using 2% isoflurane anaesthesia. The rats in 4 groups (untreated control group and 3 treatment groups) were administered a single dose of monosodium iodoacetate (5 mg/kg) through the intra-articular route. The rats in the treatment groups were intraperitoneally injected with 5, 10, and 15 mg/kg doses of hydroxypyridinone-coumarin alternately for 1 month starting on day 2 of MIA administration. The normal control and untreated groups received equal volumes of normal saline.

Determination of cytokine levels

The rats were anaesthetized on day 45th of monosodium iodoacetate administration using mebumal sodium (1.1% solution). Blood was collected from rat carotid arteries and subsequently centrifuged for 20 min at 3500×g. The obtained supernatant was frozen at –78°C for measurement of cytokine production. Commercially available ELISA kits supplied by Santa Cruz Biotechnology were used for measurement of IL-6, TNF-α, and IL-1β content in the supernatant in accordance with the manufacturer's instructions.

Statistical analysis

The expressed data are the mean±SD of 3 experiments performed independently. The data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. Statistical analysis of the data was performed using SPSS 16.0 software. The differences were regarded as statistically significant at $P<0.05$.

Results

Hydroxypyridinone-coumarin promotes chondrocyte viability

The chondrocyte viability was determined after 48-h treatment with 5–50 μM hydroxypyridinone-coumarin (Figure 1A). The hydroxypyridinone-coumarin treatment significantly ($P<0.05$) increased the viability of chondrocytes in a dose-dependent manner. Treatment of the chondrocytes with 5, 10, 15, 20, 25, 30, 40, and 50 μM of hydroxypyridinone-coumarin increased viability by 5.63%, 8.23%, 13.47%, 17.45%, 20.59%, 24.33%, 32.72%, and 39.54%, respectively. The effect of 50 μM hydroxypyridinone-coumarin on chondrocyte viability was also determined at 12 h, 24 h, 48 h, and 72 h (Figure 1B). The chondrocyte viability increased significantly ($P<0.05$) with the increase in hydroxypyridinone-coumarin treatment duration from 12 h to 48 h.

Hydroxypyridinone-coumarin promotes chondrocyte cell cycle progression

The chondrocytes were treated with hydroxypyridinone-coumarin for 48 h and cell cycle distribution was analyzed by flow cytometry (Figure 2). Treatment with 5, 30, 40, and 50 μM

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

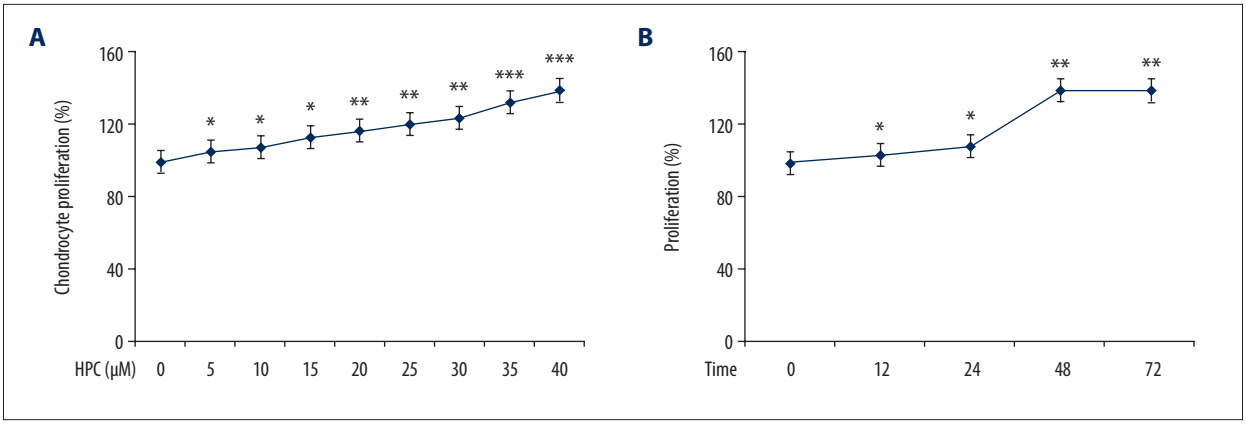

Figure 1. Effect of hydroxypyridinone-coumarin on chondrocyte viability. (**A**) The chondrocytes were treated with 5–50 μM of hydroxypyridinone-coumarin and viability was assessed by MTT assay. (**B**) The chondrocyte viability was assessed by MTT assay at 12 h, 24 h, 48 h, and 72 h. * P<0.05, ** P<0.02 and *** P<0.01 *vs.* untreated chondrocytes.

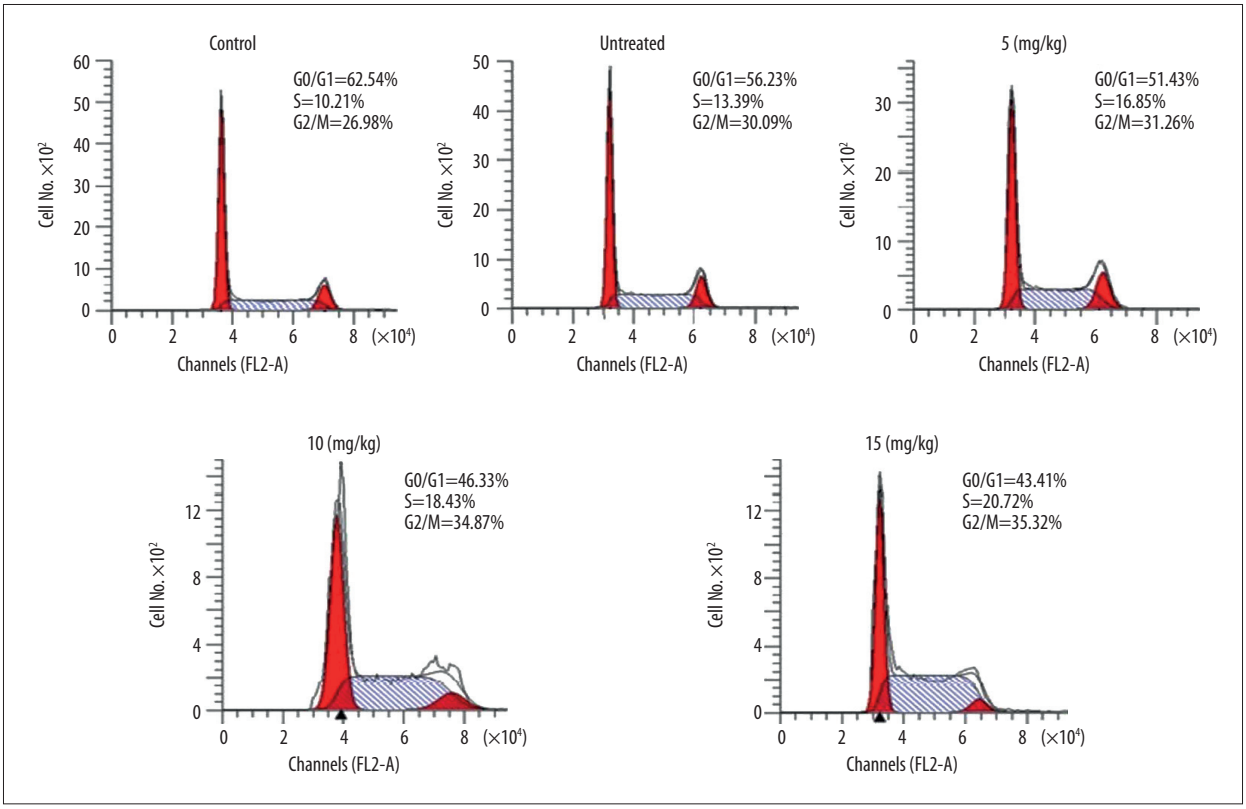

Figure 2. Effect of hydroxypyridinone-coumarin on chondrocyte cell cycle distribution. The hydroxypyridinone-coumarin treatment of chondrocytes with 5, 30, 40, and 50 μM for 48 h was followed by PI staining. Distribution of chondrocytes in various phases as assessed by flow cytometry.

hydroxypyridinone-coumarin significantly (P<0.05) reduced the population of chondrocytes in G0/G1 phase. The percentage of chondrocytes in S phase was increased by treatment with hydroxypyridinone-coumarin. The percentage of chondrocytes was also increased by hydroxypyridinone-coumarin treatment in the G2/M phase.

Hydroxypyridinone-coumarin increased cyclin protein expression in chondrocytes

The chondrocytes were exposed to hydroxypyridinone-coumarin for 48 h and cyclin protein levels were determined by Western blotting (Figure 3A). Treatment with 5, 30, 40, and 50 μM of hydroxypyridinone-coumarin markedly promoted the

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

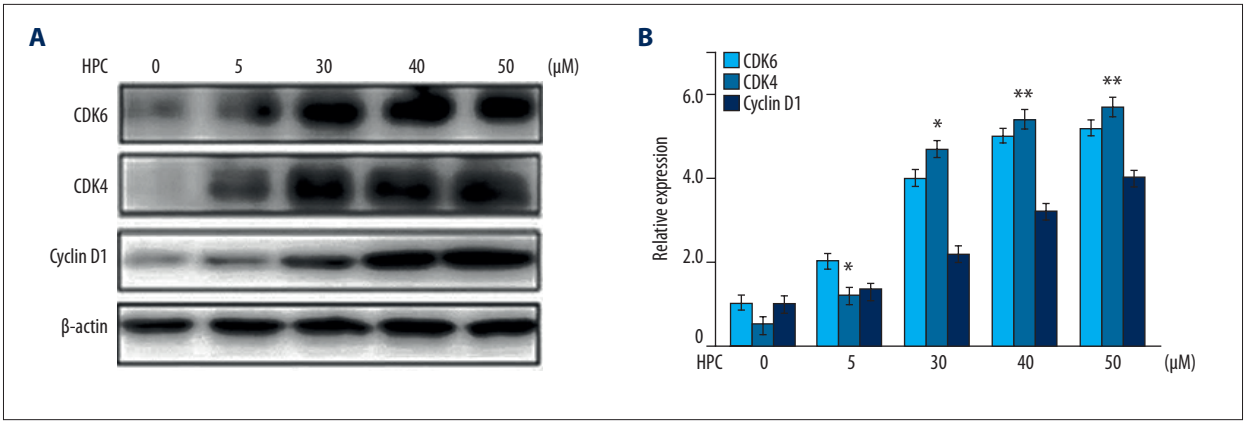

Figure 3. Effect of hydroxypyridinone-coumarin on cyclin protein and mRNA expression in chondrocytes. The chondrocytes were exposed to various hydroxypyridinone-coumarin concentrations for 48 h. The level of CDK6, CDK4, and cyclin D1 (**A**) protein and (**B**) mRNA were assessed by Western blot and RT-PCR assay. * P<0.05 and ** P<0.02 *vs.* untreated chondrocytes.

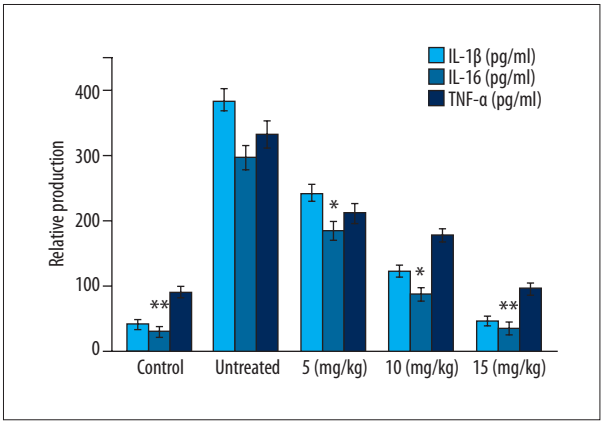

Figure 4. Effect of hydroxypyridinone-coumarin on cytokine level in OA rat serum. The OA rats were administered 5, 10, and 15 mg/kg doses of hydroxypyridinone-coumarin. The levels of IL-6, TNF-α, and IL-1β in the rat serum were assessed by enzyme-linked immunosorbent assay (ELISA). * P<0.05 and ** P<0.02 *vs.* untreated chondrocytes.

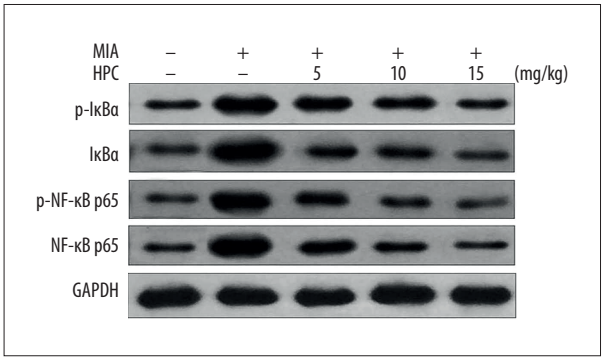

Figure 5. Effect of hydroxypyridinone-coumarin on the NF-κB pathway in OA rats. The rats were treated with 5, 10, and 15 mg/kg doses of hydroxypyridinone-coumarin and levels of IκBα and NF-κB p65 were determined by Western blotting.

expression of CDK6, CDK4, and cyclin D1 proteins in chondrocytes. The RT-PCR assay also showed that hydroxypyridinone-coumarin treatment of chondrocytes significantly increased the levels of CDK6, CDK4, and cyclin D1 mRNA (Figure 3B).

Hydroxypyridinone-coumarin inhibits level of cytokines in OA rat serum

The level of inflammatory cytokines in OA rats treated with hydroxypyridinone-coumarin was markedly lower in comparison to the untreated rats (Figure 4). Treatment of the OA rats with 5, 10, and 15 mg/kg doses of hydroxypyridinone-coumarin markedly suppressed IL-6, TNF-α, and IL-1β levels in the serum.

Hydroxypyridinone-coumarin inhibits NF-κB signalling pathway activation

Treatment of the OA rats with hydroxypyridinone-coumarin markedly suppressed the levels of IκB-α and NF-κB p65 (Figure 5). The expression of activated IκBα and NF-κB p65 was also suppressed in the OA rats by treatment with hydroxypyridinone-coumarin, whereas the levels of IκB-α and NF-κB p65 were markedly higher in the untreated OA rats.

Discussion

Osteoarthritis (OA) is the most commonly detected joint disorder in aged people caused by the degeneration of cartilage [17,18]. The loss of articular cartilage integrity is associated with extracellular matrix breakdown and chondrocyte death [17–19]. The present study investigated the effect of hydroxypyridinone-coumarin on chondrocyte viability and cell

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

cycle progression *in vitro* and inflammatory cytokine level *in vivo* in an OA rat model.

The proliferative activity of chondrocytes has been found to be very low during osteoarthritis; therefore, promotion of proliferation is believed to play a vital role in the treatment of osteoarthritis [20]. The present study investigated changes in chondrocyte viability after treatment with hydroxypyridinone-coumarin. The study showed that hydroxypyridinone-coumarin treatment significantly increased the proliferation of chondrocytes *in vitro* compared to untreated cells. The DNA is synthesized in the S phase of the cell cycle and becomes 4N during the G2/M phases [21,22]. The increase in S to G2/M phase transition rate facilitates cell cycle progression and promotes proliferation activity [22]. In the present study, hydroxypyridinone-coumarin treatment increased the population of chondrocytes in S to G2/M phases, with a subsequent decease in the population of chondrocytes in the G1/G0 phases. Therefore, hydroxypyridinone-coumarin promoted G1/G0 to S phase transition in the chondrocytes. The 2 major check-points for regulation of interphase transition in the cell cycle are G1/S and G2/M. The activity of cyclins is positively regulated by their interaction with DKs [23,24]. The increased expression of CDK6, CDK4, and cyclin D1 plays a vital role in up-regulation of G1/S transition [23,24]. In the present study, hydroxypyridinone-coumarin treatment markedly upregulated the expression of CDK6, CDK4, and cyclin D1 proteins and mRNA in chondrocytes. Thus, hydroxypyridinone-coumarin increased proliferation and promoted G1/G0 to S phase transition in chondrocytes. Osteoarthritis is characterized by joint inflammation, articular cartilage degeneration, and synovial tissue hyperplasia [25]. The inflammation leads to joint pain through neuronal activation [25]. Inflammatory cytokines such as IL-1β, TNF-α, and IL-6 are overexpressed in chondrocytes obtained from osteoarthritis patients [9]. The present study showed that treatment of osteoarthritis rats with hydroxypyridinone-coumarin suppressed the production of IL-1β, TNF-α, and IL-6 cytokines. The NF-κB pathway is considered to be an important target for the treatment of various inflammation-associated disorders [26]. It is reported that stimulation of chondrocytes with IL-1β leads to activation of the NF-κB pathway [26–28]. The results from the present study revealed that hydroxypyridinone-coumarin suppressed the levels of IκB-α and NF-κB p65 expression in osteoarthritis rats. The levels of phosphorylated IκBα and NF-κB p65 were also reduced in osteoarthritis rats after treatment with hydroxypyridinone-coumarin.

Conclusions

In summary, hydroxypyridinone-coumarin increases chondrocyte viability by promoting the G1/S transition in cell cycle progression of chondrocytes *in vitro*. Moreover, the production of cytokines and activation of NF-κB were downregulated in the osteoarthritis rats by treatment with hydroxypyridinone-coumarin. Therefore, hydroxypyridinone-coumarin appears to be a promising treatment of osteoarthritis.

Conflict of interest

None.

References:

1. Wattanachai T, Yonemitsu I, Kaneko S, Soma K: Functional lateral shift of the mandible effects on the expression of ECM in rat temporomandibular cartilage. Angle Orthod, 2009; 79: 652–59
2. Gentili C, Cancedda R: Cartilage and bone extracellular matrix. Curr Pharm Des, 2009; 15: 1334–48
3. Hu H, Yang B, Li Y et al: Blocking of the P2X7 receptor inhibits the activation of the MMP-13 and NF-κB pathways in the cartilage tissue of rats with osteoarthritis. Int Mol Med, 2016; 38: 1922–32
4. Sinkov V, Cymet T: Osteoarthritis: Understanding the pathophysiology, genetics, and treatments. J Natl Med Assoc, 2003; 95: 475–82
5. Brondello JM, Philipot D, Djouad F et al: Cellular senescence is a common characteristic shared by preneoplasic and osteo-arthritic tissue. Open Rheumatol J, 2010; 4: 10–14
6. Goldring MB: Osteoarthritis and cartilage: The role of cytokines. Curr Rheumatol Rep, 2000; 2: 459–65
7. Largo R, Alvarez-Soria MA, Díez-Ortego I et al: Glucosamine inhibits IL-1beta-induced NFkappaB activation in human osteoarthritic chondrocytes. Osteoarthritis Cartilage., 2003; 11: 290–98
8. Fan Z, Bau B, Yang H et al: Freshly isolated osteoarthritic chondrocytes are catabolically more active than normal chondrocytes, but less responsive to catabolic stimulation with interleukin-1beta. Arthritis Rheum, 2005; 52: 136–43
9. Pelletier JP, Martel-Pelletier J, Abramson SB: Osteoarthritis, an inflammatory disease: Potential implication for the selection of new therapeutic targets. Arthritis Rheum, 2001; 44: 1237–47
10. Pelletier JP, Fernandes JC, Jovanovic DV et al: Chondrocyte death in experimental osteoarthritis mediated by MEK 1/2 and p38 pathways: Role of cyclooxygenase-2 and inducible nitric oxide synthase. J Rheumatol, 2001; 28: 2509–19
11. Grimmer C, Balbus N, Lang U et al: Regulation of type II collagen synthesis during osteoarthritis by proly-4-hydroxylases: possible influence of low oxygen levels. Am J Pathol, 2006; 169: 491–502
12. Tsuji K, Bandyopadhyay A, Harfe BD et al: BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. Nat Genet, 2006; 38: 1424–29
13. Sherr CJ, Roberts JM: Living with or without cyclins and cyclin-dependent kinases. Genes Dev, 2004; 18: 2699–711
14. Planas-Silva MD, Weinberg RA: The restriction point and control of cell proliferation. Curr Opin Cell Biol, 1997; 9: 768–72
15. Zetterberg A, Larsson O: Kinetic analysis of regulatory events in G1 leading to proliferation or quiescence of Swiss 3T3 cells. Proc Natl Acad Sci USA, 1985; 82: 5365–69
16. Zhang M, Xie R, Hou W et al: PTHrP prevents chondrocyte premature hypertrophy by inducing cyclin-D1-dependent Runx2 and Runx3 phosphorylation, ubiquitylation and proteasomal degradation. J Cell Sci, 2009; 122: 1382–89
17. Heinegård D, Bayliss MT, Lorenzo P: Biochemistry and metabolism of normal and osteoarthritic cartilage. In: Osteoarthritis. Brandt KD, Doherty M, Lohmander LS (eds.), Oxford University Press, New York, NY, 1998; 74–84

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

18. Pritzker KPH: Pathology of osteoarthritis. In: Osteoarthritis. Brandt KD, Doherty M Lohmander LS (eds.), Oxford University Press, New York, NY, 1998; 50–61
19. Kim HA, Blanco FJ: Cell death and apoptosis in osteoarthritic cartilage. Curr Drug Targets, 2007; 8: 333–45
20. Chan BY, Fuller ES, Russell AK et al: Increased chondrocyte sclerostin may protect against cartilage degradation in osteoarthritis. Osteoarthritis Cartilage, 2011; 19: 874–85
21. Hwang SG, Song SM, Kim JR et al: Regulation of type II collagen expression by cyclin-dependent kinase 6, cyclin D1, and p21 in articular chondrocytes. IUBMB Life, 2007; 59: 90–98
22. Li TF, Chen D, Wu Q et al: Transforming growth factor-β stimulates cyclin D1 expression through activation of β-catenin signaling in chondrocytes. J Biol Chem, 2006; 281: 21296–304
23. Susaki E, Nakayama K, Yamasaki L, Nakayama KI: Common and specific roles of the related CDK inhibitors p27 and p57 revealed by a knock-in mouse model. Proc Natl Acad Sci USA, 2009; 106: 5192–97
24. Cheng A, Solomon MJ: Speedy/Ringo C regulates S and G2 phase progression in human cells. Cell Cycle, 2008; 7: 3037–47
25. Konttinen YT, Sillat T, Barreto G et al: Osteoarthritis as an autoinflammatory disease caused by chondrocyte-mediated inflammatory responses. Arthritis Rheum, 2012; 64: 613–16
26. Csaki C, Mobasheri A, Shakibaei M: Synergistic chondroprotective effects of curcumin and resveratrol in human articular chondrocytes: Inhibition of IL-1beta-induced NF-kappaB-mediated inflammation and apoptosis. Arthritis Res Ther, 2009; 11: R165
27. Chen YJ, Tsai KS, Chan DC et al: Honokiol, a low molecular weight natural product, prevents inflammatory response and cartilage matrix degradation in human osteoarthritis chondrocytes. J Orthop Res, 2014; 32: 573–80
28. Ding Q, Zhong H, Qi Y et al: Anti-arthritic effects of crocin in interleukin-1β-treated articular chondrocytes and cartilage in a rabbit osteoarthritic model. Inflamm Res, 2013; 62: 17–25

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]