

## Original Article

# Decreasing cartilage damage in a rat model of osteoarthritis by intra-articular injection of deoxycholic acid

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Received March 22, 2015; Accepted June 3, 2015; Epub June 15, 2015; Published June 30, 2015

**Abstract:** Background: The aim of this experimental study was to evaluate the effect of intra-articular injection of Deoxycholic acid (DCA) on articular cartilage and subchondral bone following induction of knee Osteoarthritis (OA) in a rat model. Methods: Twenty-four Sprague Dawley rats were randomized divided into 4 groups (n = 6). Eighteen of the 24 rats underwent surgical destabilization of the medial meniscus on the right knee joints to induce OA, were divided into 3 groups: DCA 30 mg/kg group, DCA 120 mg/kg group and OA group. The rats in DCA-treated groups were given intra-articular injections of DCA (30 mg/kg or 120 mg/kg) in the operated knees once per 3 days for 42 days. The rats in OA group given intra-articular injections of vehicle alone in the operated knees under the same conditions. The remaining 6 rats (sham-operation group) received sham operations on the right knee joints. 45 days postoperatively, all of the animals were euthanized for macroscopic, histological and radiographic analysis to evaluate the effect of DCA on OA and to determine its potential mechanisms. Results: The results showed that DCA attenuated the severity of OA by reducing macroscopic observation sores for femoral condyles and histological sores for articular cartilage. DCA also significantly decreased bone destruction and erosion of joint evaluated by radiographic examination. Furthermore, DCA could markedly reduce the release of MMP-1, MMP-3 and IL-1 $\beta$  in serum. Conclusions: Intra-articular injection of DCA is beneficial for knee OA. It might repair and protect OA cartilage by delaying cartilage degeneration and impairing the function of inflammatory mediators. These findings highlight DCA might be a useful therapeutic agent for OA.

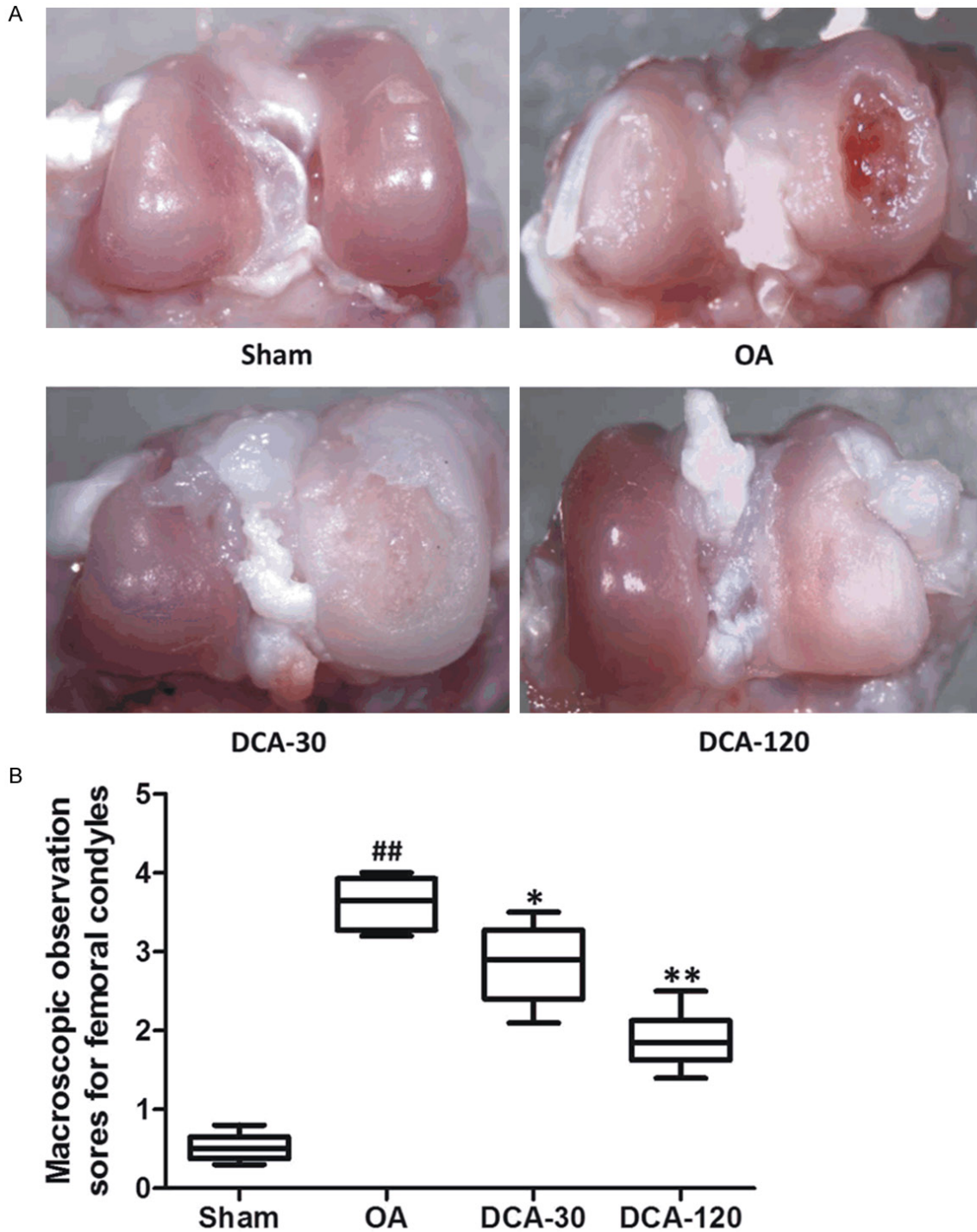
**Keywords:** Deoxycholic acid, osteoarthritis, intra-articular injection, treatment

## Introduction

Osteoarthritis (OA) is the most prevalent form of joint disease and evolves from a local inflammatory response to a chronic process accompanied by progressive degeneration of articular cartilage [1, 2]. The clinical manifestations of OA are pain and musculoskeletal disorder that may lead to disability, deterioration of quality of life and a heavy socioeconomic burden [3, 4]. It is characterized mainly by the degeneration of articular cartilage and subsequent synovial inflammation, bone remodeling and muscle weakness [5]. Though much research has been performed, the concrete causes of OA remain unclear.

At present, OA progression is considered to be regulated largely by an excess of matrix metalloproteinases (MMPs), which contributes to the degradation of the extracellular matrix. Among the various MMPs, collagenases such as MMP-1 and MMP-3 play pivotal roles in OA progression by degrading the extracellular matrix [6-8]. In addition, inflammatory mediator IL-1 $\beta$  has been implicated in the synovial inflammation and cartilage degradation in OA [9]. Elevated IL-1 $\beta$  levels are present in the synovial fluid and cartilage of OA patients, implying a role for IL-1 $\beta$  in the pathogenesis of OA [10].

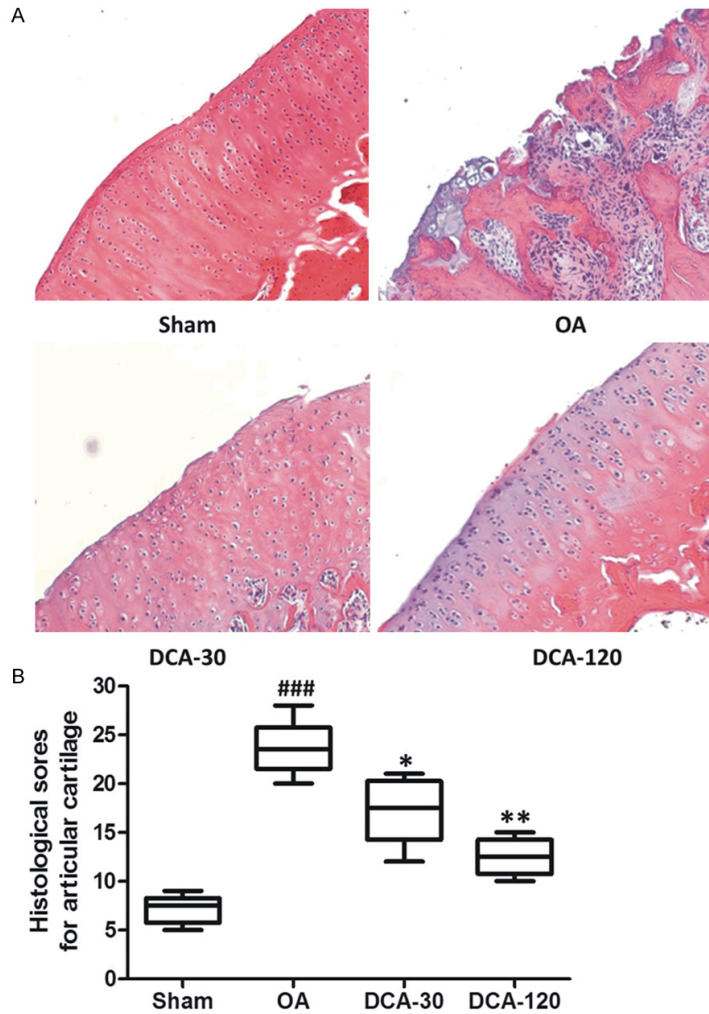
The currently available pharmacological treatments for OA are effective only temporarily and



**Figure 1.** Effect of DCA at macroscopic observation in the OA rat model. A. Representative macroscopic observation images of femoral condyles are shown. B. Macroscopic observation scores for femoral condyles in the four groups of experimental rats. Each reported value is a mean  $\pm$  SEM ( $n = 6$ ); ## $P < 0.01$ , compared with the sham-operation group; \* $P < 0.05$  and \*\* $P < 0.01$ , compared with the OA group. Similar results were observed in two separated experiments.

might result in undesirable cardiovascular and gastrointestinal side effects [11-13]. There is

an increasing interest in the use of natural compounds extracted from Traditional Chinese



**Figure 2.** Effect of DCA on histological evaluation in the OA rat model. A. Representative Hematoxylin-eosin (HE) stained sections of articular cartilage are shown. B. Histological scores for articular cartilage in the four groups of experimental rats. Each reported value is a mean  $\pm$  SEM (n = 6); ###P < 0.001, compared with the sham-operation group; \*P < 0.05 and \*\*P < 0.01, compared with the OA group. Similar results were observed in two separated experiments.

Medicine (TCM) for the treatment of OA because they are reported to demonstrate satisfactory clinical efficacy with minimal side effects, compared to routine pharmacological strategies [14]. Deoxycholic acid (DCA) was an important active constituent from animal bile, which has been reported for the treatment of cancer, respiratory and immunologic system diseases [15-17]. However, little is known about its possible use in the treatment of OA. Our group has previously reported that animal bile constituents exhibited obviously inhibitory effect on MMPs in vitro [18]. In continuation of our previous study and development of a novel small

molecule drug to treat OA, we elucidated the therapeutic effect of DCA in an experimental rat model of OA for the first time in this article.

**Materials and methods**

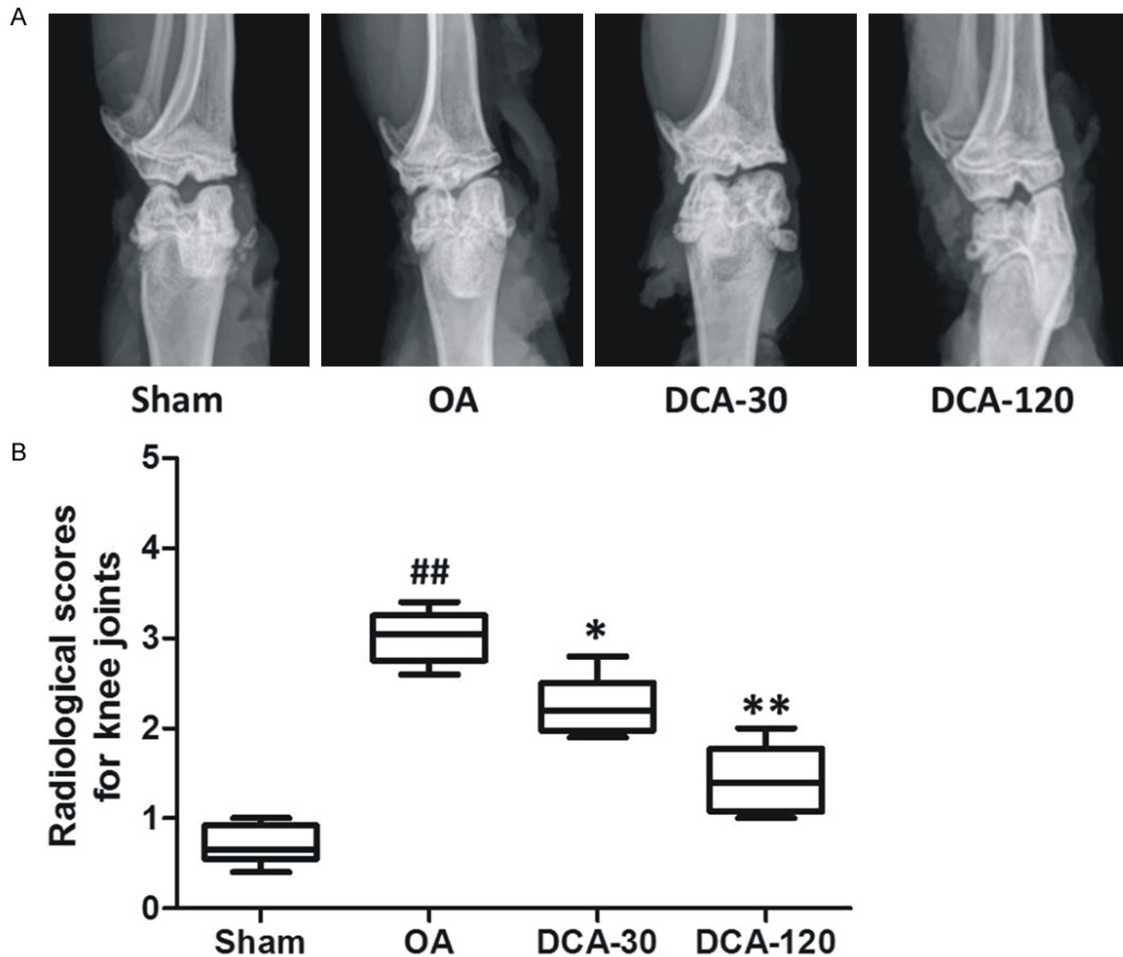
*Experimental animal model and drug treatment*

Twenty-four adult male Sprague Dawley rats (8 weeks old, 240-270 g in weight) were purchased from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). All experiments were conducted with the approval of Soochow University Animal Care and Use Committee. Eighteen of the 24 rats underwent surgical destabilization of the medial meniscus (DMM) on the right knee joints to induce OA as described previously by Glasson et al [19] and were randomly divided into 3 groups (n = 6 per group). The remaining 6 rats (sham-operation group) received sham operations on the right knee joints, which involved opening the articular cavity and resuturing it without transecting the medial meniscotibial ligament. After surgery, all animals were returned to their cages, the limbs were not immobilized. Treatments began 4 weeks after surgery. Drugs were given once per 3 days for 42 days. The rats in DCA-treated (DCA, purity above 98%, Sigma-Aldrich, St. Louis, MO, USA)

groups were given intra-articular injections of DCA (30 mg/kg or 120 mg/kg) in the operated knees once per 3 days for 42 days. The rats in OA group were given intra-articular injections of 50  $\mu$ L vehicle alone in the operated knees under the same conditions. In sham-operation group, no other procedures were conducted. All rats were sacrificed 3 days after the last injection.

*Macroscopic observation*

After the treatment, the rats were sacrificed and femoral condyles were collected for macro-



**Figure 3.** Effect of DCA at radiographic analysis in the OA rat model. A. Representative radiographic images of rat knee joints are shown. B. Radiological scores for knee joints in the four groups of experimental rats. Each reported value is a mean  $\pm$  SEM (n = 6); ###P < 0.01, compared with the sham-operation group; \*P < 0.05 and \*\*P < 0.01, compared with the OA group. Similar results were observed in two separated experiments.

scopic observation. The cartilage degradation on the surface of femoral condyles was observed under dissecting microscope and the degree of degradation was graded on a scale of 0-4 as follows: 0 = surface smooth with normal color; 1 = surface rough with minimal fibrillation or a slight yellowish discoloration; 2 = cartilage erosion extending into superficial or middle layers; 3 = cartilage ulceration extending into deep layers; 4 = cartilage depletion with subchondral bone exposed [20]. The examination was performed by two independent observers who were kept unaware of the treatment groups.

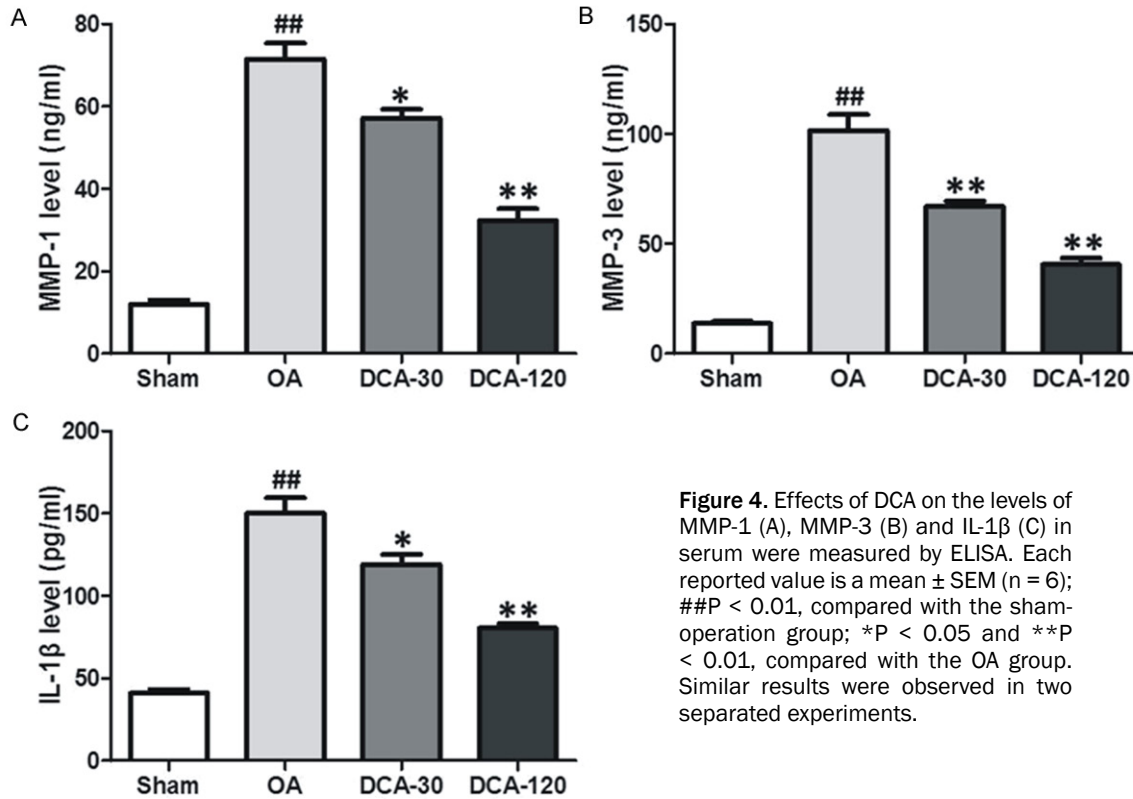
#### *Histological examination*

After macroscopic observation, isolated specimens were prepared for further histological

analysis. The specimens were decalcified (10% EDTA), embedded in paraffin, cut into 5- $\mu$ m sections, and stained with hematoxylin and eosin (H&E). The histological evidence of articular cartilage was assessed according to the scoring system by Kikuchi et al and eight parameters [21], namely loss of superficial layer, erosion of cartilage, fibrillation and/or fissures, loss of proteoglycan, disorganization of chondrocytes, loss of chondrocytes, exposure of subchondral bone, and cluster formation. All sections were graded by two independent observers that were kept unaware of the treatment groups.

#### *Radiographic evaluation*

At the end of study, the right knee joints of experimental rats were subject to radiographic



**Figure 4.** Effects of DCA on the levels of MMP-1 (A), MMP-3 (B) and IL-1 $\beta$  (C) in serum were measured by ELISA. Each reported value is a mean  $\pm$  SEM (n = 6); ##P < 0.01, compared with the sham-operation group; \*P < 0.05 and \*\*P < 0.01, compared with the OA group. Similar results were observed in two separated experiments.

analysis using a mammographic imager (Siemens Medical Solutions, Germany). The joint destruction and bone erosion were scored on a scale of 0-4 in a blind manner: 0 = no osteophytes; 1 = doubtful osteophytes; 2 = minimal osteophytes, possibly with narrowing, cysts, and sclerosis; 3 = moderate or definite osteophytes with moderate joint space narrowing; 4 = severe with large osteophytes and definite joint space narrowing [22].

#### ELISA for measurement of MMP-1, MMP-3 and IL-1 $\beta$ in serum

Blood was obtained from the eyeball vein of rats before sacrificing, and centrifuged at 3000 rpm for 15 min. The serum was collected and stored at -70°C until assayed. The levels of MMP-1, MMP-3 and IL-1 $\beta$  in serum were measured by ELISA kits (R&D Systems Inc., USA) according to the manufacturer's instructions. All samples from animals in each experimental group were assayed in duplicate.

#### Statistics

Data were presented as the mean  $\pm$  SEM. Statistical analyses were performed using SPSS 16.0. Statistical comparisons were per-

formed using the Mann-Whitney U test. P < 0.05 was considered significant.

## Results

### Effect of DCA at macroscopic observation in the OA rat model

As shown in **Figure 1A**, the cartilage on the femoral condyles in the sham-operation group was macroscopically normal, with a glistening, smooth surface, and no cartilage defect or osteophyte was observed. In the OA group, general characteristics of OA, including erosion and osteophyte formation, were seen on the side of the femoral condyles after surgery. In the DCA-treated groups, less bone wear was observed after DCA treatment, compared with the OA group. Accordingly, as scored by macroscopic observation, DCA treatment could significantly decrease the degree of cartilage degradation (**Figure 1B**).

### Effect of DCA on histological evaluation in the OA rat model

As shown in **Figure 2A**, the sham-operation of rats revealed no significant histological changes in the articular cartilages, whereas the OA

group and other treated groups developed different degrees of OA-like degenerative changes. A significant decrease in the severity of cartilage degradation was observed in DCA-treated groups, particularly in the 120 mg/kg group. Histological scores in the DCA-treated group were significantly lower than in the OA group (**Figure 2B**).

### *Effect of DCA at radiographic analysis in the OA rat model*

In order to observe the bone changes of knee joints after DCA treatment, radiographic examination was performed on OA rat treated with DCA and vehicle in this study. As shown in **Figure 3A**, the surface of knee joints was smooth in the sham-operation group. In contrast, obvious osteophyte, incomplete and thickening articular surface with sclerosis and deformation were observed in the OA group. The DCA treated groups markedly prevented bone erosions at the operated knee joint, no obvious sclerosis and osteophyte formation were observed. Accordingly, radiological scores in the DCA-treated group were significantly lower than in the OA group (**Figure 3B**). The results suggested that DCA treatment was an effective therapeutic approach to reduced joint destruction in OA rats.

### *Effects of DCA on the levels of MMP-1, MMP-3 and IL-1 $\beta$ in serum were measured by ELISA*

Compared to sham-operation group, OA group showed higher level of MMP-1 and MMP-3 in serum ( $P < 0.01$ ) (**Figure 4A** and **4B**). The levels of MMP-1 and MMP-3 in serum were significantly decreased by DCA treatment in a dose-dependent manner compared with those in OA group. Moreover, inflammatory mediator IL-1 $\beta$  involved in cartilage destruction was also significantly inhibited by DCA treatment (**Figure 4C**).

## Discussion

Osteoarthritis (OA) is the most prevalent form of joint disease and evolves from a local inflammatory response to a chronic process accompanied by progressive degeneration of articular cartilage [1, 2]. The clinical manifestations of OA are pain and functional disability that can limit the normal activities of daily life and lead to depression and isolation [3, 4]. At present,

Nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and hyaluronan have been clinically used for the treatment of OA in the clinic. However, they fail to reverse cartilage damage, always result in undesirable cardiovascular and gastrointestinal side effects [11]. Thus, there is a continuing need for novel better agents with which to treat OA.

DCA was an important active constituent from animal bile, which has been reported for the treatment of cancer, respiratory and immunologic system diseases [15-17]. However, the effect of DCA on OA severity in vivo has not yet been studied at present. Our group has previously reported that animal bile constituents exhibited obviously inhibitory effect on MMPs in vitro [18]. In continuation of our previous study and search for a novel small molecule drug to treat OA, we elucidated the beneficial effect of DCA on an OA model in rats for the first time in this article.

In our study, we investigated the protective effect of DCA against cartilage degradation in a rat OA model induced by DMM, which is a commonly used method of identifying OA disease-modifying therapies by intra-articular administration. The results showed that DCA attenuated the severity of OA by reducing macroscopic observation scores for femoral condyles and histological scores for articular cartilage (**Figures 1, 2**). DCA also significantly decreased bone destruction and erosion of joint evaluated by radiographic examination (**Figure 3**). Notably, DCA significantly reduced the degree of OA-like lesions at a dose of 120 mg/kg.

It is well established that MMPs, especially MMP-1 and MMP-3, play vital roles in the progression of cartilage degradation in OA due to their ability to cleave diverse components of the extracellular matrix [6-8]. There are many research groups have confirmed that inhibition of MMPs delayed the progression of cartilage degradation in vitro and in vivo [23]. MMP-1 breaks down fibrillar collagens in an irreversible way, while MMP-3 degrades a variety of matrix substrates, including type II collagen and aggrecan [24]. Many studies have reported that the MMP-1 level is upregulated in the synovium, synovial fluid and cartilage of OA patients, indicating that MMP-1 has a vital role in the pathological degradation of articular cartilage [25, 26]. MMP-3 may have utility as a prognostic

biomarker of OA progression. Patients with higher MMP-3 levels are more likely to suffer from progression of OA over a 30-month period than patients in the lower tertile [27]. Therefore, effects of DCA on the levels of MMP-1 and MMP-3 in serum were measured by ELISA in this paper. As shown in **Figure 4A** and **4B**, DCA significantly decreased the levels of MMP-1 and MMP-3 in serum in a dose-dependent manner.

The role of inflammatory mediators IL-1 $\beta$  in the pathogenesis of osteoarthritis has drawn more and more attention in recent years. IL-1 $\beta$  has been implicated in the synovial inflammation and cartilage degradation in OA [9]. Elevated IL-1 $\beta$  levels are present in the synovial fluid and cartilage of OA patients, implying a role for IL-1 $\beta$  in the pathogenesis of OA [10]. In this study, the levels of IL-1 $\beta$  in serum were also measured. Our results indicate that the levels of IL-1 $\beta$  in serum were significantly inhibited in the DCA-treated group (**Figure 4C**).

Taken together, these results confirm the therapeutic potential of DCA for treatment of OA. However, the exact mechanism of action of DCA needs to be further investigated.

### Conclusions

Intra-articular injection of DCA is beneficial for knee OA. It might repair and protect OA cartilage by delaying cartilage degeneration and impairing the function of inflammatory mediators. These findings highlight DCA might be a useful therapeutic agent for OA.

### Acknowledgements

This work was supported by the grants from the Medical Research Projects of Health Department of Jiangsu Province (No. Z201304), National Natural Science Foundation of China (No. 81202348 and No. 81202394) and Jiangsu Province National Natural Science Funds (No. BK2012171), and Medical Research Projects of Health Department of Guangxi Zhuang Autonomous Region (No. Z2011291).

### Disclosure of conflict of interest

None.

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