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Original article

# Artesunate attenuates ACLT-induced osteoarthritis by suppressing osteoclastogenesis and aberrant angiogenesis

Chengjin Zhao[a,b,1], Qingbai Liu[c,1], Kunzheng Wang[d,*]

[a] Xi'an Jiaotong University Health Science Center, Xi'an, 710049, China
[b] Department of Orthopedics, Yan'an University Affiliated Hospital, Yan'an, 716000, China
[c] Department of orthopedics, Lianshui County People's Hospital, Huaian, 223400, China
[d] Department of orthopedics, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710004, China

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ABSTRACT

Artesunate (ART) is a semi-synthetic derivative of artemisinin and used preferentially in treatment of malaria in China. ART has strong anti-inflammatory, anti-tumor, and anti-angiogenic properties. Although the pharmacological effect of ART in osteoarthritis (OA) is unknown, evidence suggests that ART might regulate osteoclastogenesis through NF-κB signaling. In this study we explored the therapeutic effect of ART in an anterior cruciate ligament transection (ACLT)-induced OA model. We found that ART effectively relieved ACLT-induced osteoarthritis, as demonstrated by the improvement expression of pathological genes. We further demonstrated that ART markedly reduced OA-associated osteoclastogenesis and angiogenesis. In addition, ART also regulated inflammatory response and inhibited the activation of Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) signaling in ACLT rats. Taken together our study has identified a novel function of ART and provided a molecular basis for ART potential applications in the treatment of OA and other joint disorders.

## 1. Introduction

Osteoarthritis (OA) a leading cause of physical disability in the elderly and affects approximately 10% of those aged 60 years and older [1]. It is pathologically characterized by the degeneration of articular cartilage with accompanying inflammatory syndrome and alterations in subchondral bone, and it is clinically characterized by persistent pain, stiffness and disability [2–4]. The epidemiology of OA is multifactorial and complex, with genetic, biological, and biomechanical components [5]. In addition, abnormal angiogenesis may also play an important role in the development of OA.

Osteoclast is a type of bone cell that breaks down bone tissue and is likely involved in the pathophysiology of OA [6]. The osteoclast disassembles and digests the composite of hydrated protein and mineral at a molecular level by secreting acid and a collagenase, a process known as bone resorption. Excessive activation of osteoclast is the principal mechanism leading to joint diseases such as OA, rheumatoid arthritis, cartilaginous degeneration and osteoporosis [7]. Currently, OA treatment is based on the use of glucocorticoids, nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics and immunosuppressor. However, these approaches only focus on temporarily alleviating the symptoms rather than treating the pathogenesis of the disease or reversing the process of OA. Instead, some traditional anti-inflammatory drugs such as dexamethasone and methotrexate may facilitate osteoclastogenesis and exacerbate bone destruction. Therefore, seeking a safer, more effective method for treatment of OA is extremely urgent.

Artesunate (ART) is a semi-synthetic derivative of the herbal *Artemisia annua* ingredient artemisinin, which was extensively used for centuries in traditional Chinese medicine and is currently being used as antimalarial drug [8]. According to previous studies, ART exerts a number of pharmacological effects, including anti-viral, anti-inflammatory, anti-tumor, and anti-angiogenic effects [9–11]. In addition, ART also inhibits RANKL-induced osteoclastogenesis by suppressing the NF-κB signaling pathway [12]. However, the application of ART in OA is not well explored.

In the present study, we investigated the efficacy of ART in a rat

*Abbreviations:* OA, Osteoarthritis; ACLT, Anterior cruciate ligament transection; JAK/STAT, Janus Kinase/Signal Transducers and Activators of Transcription; NSAIDs, Nonsteroidal anti inflammatory drugs; CTX-II, Type II collagen; PEG2, Prostaglandin E2; IL, Interleukin; MMP-9, Matrix metalloproteinase-9; VEGF, Endothelial growth factor; HGF, Hepatocyte growth factor; Ang-1, Angiopoietin-1; IGF-1, Insulin-like growth factor-1; TGF-β, Transforming growth factor beta

* Corresponding author. Kunzheng Wang Address: No.157 Xiwu Road, Xincheng District, 710000, Xi'an, Shaanxi, China.
*E-mail address:* docwangzk@163.com (K. Wang).

[1] These authors contribute equally to this work.

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anterior cruciate ligament transection (ACLT)-induced OA model, in an attempt to provide a new strategy for the treatment of OA.

## 2. Materials and methods

### 2.1. Reagents

Artesunate (ART, purity > 98%) was obtained from J & K Scientific Ltd., (Beijing, China).

### 2.2. Experimental animal model and drug treatment

Seventy-two male Wistar rats (eight weeks, body weight of 245–255 g, obtained from the Laboratory Animal Center of Xi'an Jiaotong University Health Science Center, China) were used in present studies. Rats were housed under controlled conditions (25 ± 2 °C, 70% humidity and 12-hlight-dark periods) and fed on regular sterile chow diet and water *ad libitum*. The experimental protocols were performed according to relevant national and international guidelines and were approved by the Animal Experimental Ethical Committee.

ACLT operation was generated essentially as previously described [13]. All rats were randomly divided into following four groups: 1, sham (operation); 2, ACLT; 3, ACLT with DMSO; 4, ACLT with ART. ART at 25 mg/kg/day was injected intraperitoneally for consecutive 10 weeks starting from the first day of operation. All rats were euthanized at week 4, 8 and 10 after experiment completion. Synoviums and serums were collected.

### 2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Equal amounts of RNA were reversely-transcribed to cDNA with SuperScript Reverse Transcriptase Kit (Thermo Fisher Scientific, Waltham, USA). Then the total cDNA was amplified and analyzed by SYBR Green PCR Master Mix (Thermo Fisher Scientific, Waltham, USA) in a Fast Real-time PCR 7500 System (Applied Biosystems, Foster City, USA). The original Ct values were adjusted to GAPDH.

### 2.4. Weight bearing analysis

A pressure sensing walkway measuring (Taimeng Technology, Chengdu, China) was used to monitor paw pressure as previously described [14]. The differential weight bearing of the left versus right hind limb was calculated across gait cycles and expressed as a ratio of the average maximum force left divided by the average maximum force right hind limb.

### 2.5. Western blot

Total protein samples from tissues were prepared with standard protocol. Equivalent amounts of protein samples were separated by 10% SDS-PAGE and transferred to PVDF membranes (Millipore, Billerica, USA). Membranes were then incubated at room temperature with 5% non-fat dry milk dissolved in T-BST. The blots were probed overnight with respectively primary antibodies (Abcam, Cambridge, UK) at 4 °C and then incubated with HRP-conjugated secondary antibodies (Beyotime Biotechnology, Shanghai, China) at room temperature. Membranes were extensively washed several times. Proteins were detected using a ChemiDoc XRS imaging system and Quantity One analysis software (Bio-Rad, Hercules, USA). GAPDH were used as endogenous reference.

### 2.6. Immunohistochemistry

Synovium sections were prepared with standard protocol. The sections were incubated with primary antibodies (VEGF) overnight at 4 °C, followed by incubation with fluorophore-conjugated secondary antibodiy (Invitrogen, Carlsbad, USA) for 1 h. Sections were visualized with a Zeiss LSM-510 fluorescent microscope.

### 2.7. Statistical analysis

All results were presented as mean ± SD. The statistical significance of the studies was analyzed using Student's *t*-test. The difference was considered statistically significant at $P < 0.05$.

## 3. Results

### 3.1. ART relieves ACLT-induced osteoarthritis

To gain insights into the ART's anti-osteoarthritic function, we examined the effect of ART on the expressions of osteoarthritic makers in ACLT rats. ART was administrated intraperitoneally right after ACLT operation for various times. The results showed that ACLT knee displayed marked induction of CTX-II, IL-6, and PGE2 compared to the sham control at 4–10 weeks after surgery. However, ART treatment significantly inhibited the induction of these cytokines, whereas DMSO, the solvent for ART, had no effect [Fig. 1A–C]. In addition, the left to right hind limb average maximum force ratio were significantly increased at 10 weeks after ACLT operation. However, ART treatment effectively corrected this abnormality (Fig. 2). These results strongly indicate that ART may improve ACLT-induced osteoarthritis.

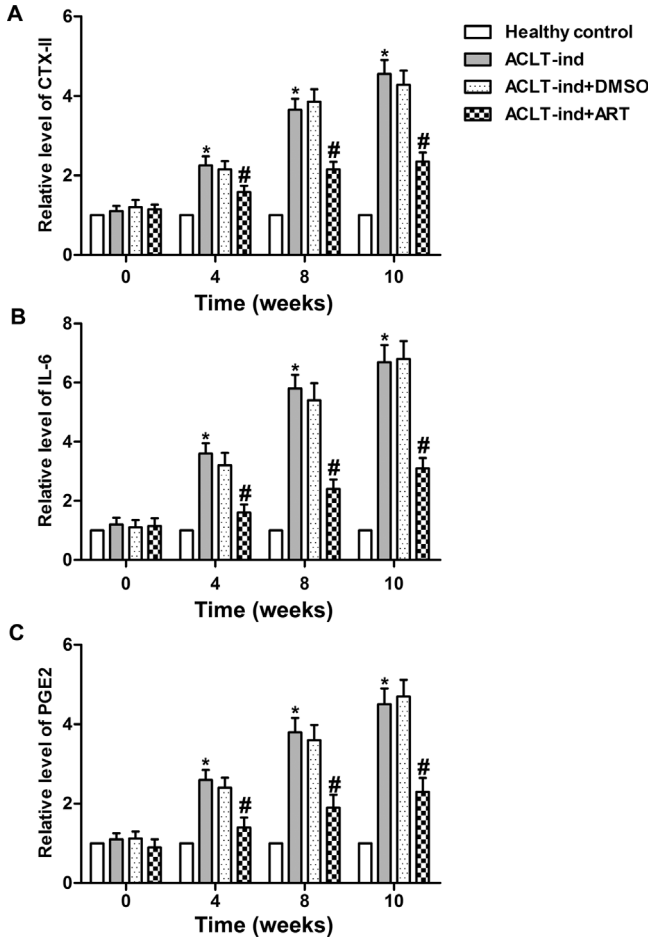


**Fig. 1.** ART relieves ACLT-induced osteoarthritis. Rats operated with ACLT were treated with DMSO or ART for 4, 8 and 10 weeks. (A) The levels of CTX-II (A), IL-6 (B) and PGE2 (C) in knee were assayed by qRT-PCR. n = 10. $^{*}P < 0.05$ versus control. $^{\#}P < 0.05$ versus ACLT.