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Danshen prevents articular cartilage degeneration via antioxidation in rabbits with osteoarthritis

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SUMMARY

Objective: To evaluate the efficacy of Danshen on histological parameters and antioxidative activity in the articular cartilage of rabbits with osteoarthritis (OA).
Design: Twenty-four rabbits were randomly divided into three groups (control, OA, and Danshen OA; eight rabbits per group). Anterior cruciate ligament transection (ACLT) of the left hind knees was performed in all rabbits in the OA and Danshen OA group for induction of OA. The rabbits in the control group underwent a sham operation. After surgery, 3 g/kg body weight of Danshen granules dissolved in 5 mL distilled water was administered by gastric intubation once per day and over a 6-week period to the Danshen OA group. The same volume of distilled water was administered to the OA and control groups. After 6 weeks, the medial femoral condyles and synoviums of the left hind knees in all three groups were harvested and used for histological and biochemical analyses.
Results: Severe articular cartilage degeneration as well as lower proteoglycan (PG) content were noted in the OA group compared to the Danshen OA group ($P < 0.05$). The glutathione (GSH) levels in the synovium and articular cartilage of the rabbits in the Danshen OA group were significantly higher compared to the OA group ($P < 0.001$). The malondialdehyde (MDA) levels of the synovium and articular cartilage in the Danshen OA group was markedly depleted compared to the OA group ($P < 0.001$).
Conclusion: Danshen can prevent articular cartilage degeneration in OA though the defense against oxidative stress.

Introduction

Osteoarthritis (OA) is the most common joint disease that leads to pain and disability. The prevalence of symptomatic OA in those 60 years old and above was 9.6% in men and 18% in women[1]. It will be the fourth leading cause of disability worldwide by 2020[2], resulting in a large worldwide socioeconomic burden. The etiology and pathogenesis of OA currently remains unclear. Nevertheless, increasing evidence from both experimental and clinical studies suggest that oxidative stress plays a pivotal role in the pathological process of OA[3,4].

Oxidative stress is generated from an imbalance in the production and elimination of reactive oxygen species (ROS)[5]. In order to maintain balance under physiological conditions, ROS are produced and removed in the human body by the cellular antioxidant defense system. If regulated improperly, the excess ROS can inhibit the activity of normal cells by damaging cellular lipid, protein, and DNA content[4]. Previous studies have demonstrated the critical role played by oxidative stress in directly promoting chondrocyte apoptosis, catabolic processes, and matrix degradation[6]. Furthermore, some studies suggest that the telomere shortening and reduced number and function of mitochondria seen in OA chondrocytes are due to oxidative stress[4,7]. According to these studies, the oxidative stress in OA patients is due to either increased lipid peroxidation or decreased antioxidant levels[8–11]. The excess ROS can result in oxidative damage to various components of the joint, including collagen, proteoglycans, and hyaluronan[6,12].

Malondialdehyde (MDA) is one of the most prevalent end byproducts of lipid peroxidation during oxidative stress. As an oxidative biomarker, MDA has been extensively detected in biological and clinical samples in previous studies on oxidative

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stress[13,14]. Glutathione (GSH) is a naturally occurring tripeptide with nucleophilic and reducing properties that play a central role in the antioxidant system of most aerobic cells. Since GSH is a naturally occurring antioxidant with several functions including detoxification of xenobiotics and removal of hydroperoxides and free radicals, GSH concentration has been determined in many studies on oxidative stress[15,16].

Most medicines for OA are administered in order to alleviate the pain temporarily. Since all pain relievers may have gastrointestinal and cardiovascular adverse effects[17], there is a clinical need for disease-modifying OA drugs (DMOADS) for OA patients. However, currently available drugs and therapies are unable to effectively delay the progression of OA. Joint replacement is the only treatment available for end-stage OA[18,19].

Danshen (*Salvia miltiorrhiza*), a traditional Chinese medicine with a number of physiological benefits, is widely used for the treatment of heart disease[20]. The pharmacokinetic and pharmacodynamic studies on the active components of Danshen indicate that Danshen is a compound that contains mainly two types of constituents, lipid soluble diterpenoid quinines (e.g., tanshinone and cryptotanshinone) and water soluble phenolics (e.g., danshensu, rosmarinic acid, salvianolic acids, protocatechuic acid, and protocatechuic aldehyde)[21–23]. Both components are responsible for the pharmacological activities of Danshen. It has been reported that Danshen has antioxidative, anti-inflammatory, vasodilative, antihypertensive, anticoagulant, antibacterial, and anticancer effects[24]. Danshen has been widely used for the treatment of angina pectoris, myocardial infarction, and stroke[20,21].

Although the mechanism for antioxidant activity remains unclear, Danshen is believed to function as an active oxygen inhibitor[25–27]. It scavenges the oxygen free radicals generated by a myocardial ischemic reperfusion injury as effectively as superoxide dismutase[28]. Another study demonstrated the mechanisms by which Danshen protect endothelial cells against oxidative stress[29]. Additionally, it has been reported that Danshen could prevent the occurrence of oxidative stress in the eye and aorta of diabetic rats without affecting their hyperglycemic state[30].

OA has been experimentally induced in animals through a variety of techniques including joint immobilization, joint structure destabilization by surgery, and intra-articular injection of an agent. It has been determined that simple anterior cruciate ligament transection (ACLT) can lead to a progressively degenerative OA of the knee in the New Zealand rabbit[31]. Another study demonstrated that measured changes on the femoral condyle (specifically the medial femoral condyle) were good indicators of cartilage degeneration[32]. The ACLT model in rabbits is a reproducible and effective OA model. The cartilage lesions of this OA model and their response to therapy can be graded according to an adapted histological and histochemical grading system.

Therefore, we hypothesized that Danshen supplementation at the initial stage of OA, may prevent the further degeneration of articular cartilage through its antioxidative activity. The purpose of this study was to evaluate the effects of Danshen on histological parameters and GSH and MDA concentrations in the articular cartilage of the medial femoral condyles of New Zealand rabbits with early OA induced by ACLT.

Materials and methods

Drugs and reagents

Danshen granules are a type of pure Danshen product that are manufactured in compliance with good manufacturing practices (GMP) (Shaanxi Aoxing Pharmaceutical Co. Ltd. China; approval number: Z61021162 approved by the State; specification: 10 g × 10 bags; one bag is equivalent to the original crude drugs 10 g). As a clinical drug, Danshen granules have been widely used for the treatment of angina pectoris in China. The Glutathione (GSH) Colorimetric Kit (ApoGSH™) was a product of BioVision Inc. (USA). The OxiSelect™ Thiobarbituric Acid Reactive Substance (TBARS) Assay Kit for Malondialdehyde (MDA) Quantitation was purchased from Cell Biolabs, Inc. (USA).

Experimental animals

Twenty-four healthy mature New Zealand White rabbits (weighing 2.2–2.8 kg; aged 9–10 weeks; 12 males and 12 females) were obtained from the Animal Experimental Center at Xi'an Jiaotong University. The rabbits were randomly divided into three groups (eight rabbits per group) designated as the control, OA, and Danshen OA groups, respectively and fed sterilized food and redistilled water. Each rabbit was housed in a single clean cage (measuring 60 × 60 × 45 cm). Environmental conditions were kept at a temperature of 20 ± 2°C and humidity of 60% ± 5%, with good ventilation, under 4 watts of light intensity per square meter and for 12 h per day. In the animal operation room, all the rabbits were anesthetized with sodium pentobarbital (at a dose of 30 mg/kg body weight through the ear edge vein). ACLT of the left hind knees was performed under an arthroscope in all the rabbits in OA and Danshen OA groups in order to induce OA[31]. The same surgical techniques were imitated under arthroscope with the exception of ACLT in all of the rabbits of the control group. After surgery, all rabbits were permitted activity in the cages without immobilization. The rabbits in the Danshen OA group were postoperatively treated with 3 g/kg body weight of Danshen granules dissolved in 5 mL distilled water by gastric intubation once per day over a 6-week period. The dose (3 g/kg body weight) was determined based on the dose used in humans, as well as on other literature involving rats[30]. The rabbits in both the control and OA groups were given the same volume of distilled water. The body weights of the rabbits were monitored once a week until sacrifice. The dose of Danshen administered was adjusted every week on the basis of their body weights. During housing, rabbits were monitored twice daily for their health status. No adverse events were observed. Handling and care of the animals were in accordance with the policies of the Animal Experimental Center of Xi'an Jiaotong University. The Committee on Animal Experimentation at the Xi'an Jiaotong University approved this study. All sections of this report adhere to the Animal Research Reporting *in Vivo* Experiments (ARRIVE) Guidelines for reporting animal research.

Tissue collection

Six weeks after surgery, all the rabbits were sacrificed by the intracardiac injection of T-6I euthanasia solution in three groups. The medial femoral condyles and the synovium from the infrapatellar fat pad were harvested (except for the synovium at the incision site) from all the rabbits. The trimmed tissues blocks were rinsed briefly with ice-cold saline solution and quickly blotted dry. The tissues were immediately frozen in liquid nitrogen and stored at −80°C until use.

Histological analysis

In each group, the medial femoral condyle tissue was fixed in 10% buffered formalin at room temperature for 72 h and decalcified with 10% EDTA solution (pH = 7.4). The EDTA was changed on a 48 h/48 h/72 h cycle for approximately 10 weeks until complete

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