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Chemical characterization and varying level of the proteoglycans

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ABSTRACT

Proteoglycans are a major component of the animal extracellular matrix, the "filler" substance existing between cells in an organism. Here they form large complexes, both to other proteoglycans, to hyaluronan and to fibrous matrix proteins (such as collagen). Proteoglycans are heavily glycosylated glycoproteins. In this study, we evaluate effect of collagen and proteoglycan on osteoporosis. Rats were treated with ovariectomy (OVX) for 4 weeks to induce osteoporosis. In OVX rats, the levels of collagen and proteoglycan in knee joint cartilage and anterior cruciate ligament were significantly decreased in the OVX group animals compared to the sham group. OVX rats showed decreased levels of magnesium (Mg^{2+}) and calcium (Ca^{2+}), increased concentrations of alkaline phosphatase (ALP) and phosphorus (P) in the serum. We conclude that osteoporosis is closely associated to decreased collagen and proteoglycan levels in knee joint cartilage and anterior cruciate ligament.

1. Introduction

Osteoporosis is often called a silent disease of aging because bone loss occurs without symptoms until microarchitectural deterioration becomes so significant that bone fracture occurs. According to the National Osteoporosis Foundation, in the United States approximately 10 million individuals are estimated to have osteoporosis, and 2 million of these are men (Dobson, Yarnall, Noyce, & Giovannoni, 2013). Osteoporosis (a disease of aging associated with bone loss that often occurs without symptoms until microarchitectural deterioration becomes so significant that bone fracture occurs invariably at the point where a reduction in the bone mass is below what is required for normal bone support), is of great importance in health care (Sontakke & Tare, 2002). Plenty of studies around this disease have been carried out in recent years. Accumulating data have indicated that the loss of estrogen at menopause is a major contributor to pathogenesis of the disease because this hormone is a principal negative regulator of osteoclast activity (Aubin & Bonnelye, 2000; Compston, 1997; Meiner, 1999), and osteoclasts are the chief effector's cells responsible for bone remodeling in osteoporosis (Roodman, 1996).

Type I collagen is the most abundant structural protein found in the extracellular matrix of vertebrates. It assembles into fibrils forming the structural scaffold of bone, skin, and other connective tissues (Trentham et al., 1982). It consists of three polypeptide α-chains folded into a 300-nm-long triple helix with short nonhelical terminal peptides (telopeptides). The collagen component can act as a matrix to promote an early bone deposition and mineralization (de Jonge et al., 2010).

Proteoglycans are a major component of the animal extracellular matrix, the "filler" substance existing between cells in an organism. Here they form large complexes, both to other proteoglycans, to hyaluronan and to fibrous matrix proteins (such as collagen). Proteoglycans are heavily glycosylated glycoproteins (Richmond, Carlson, Register, Shanker, & Loeser, 2000). This means that they are proteins with chains of polysaccharides, a kind of carbohydrate, attached. The specific type of polysaccharides attached to proteoglycans is called glycosaminoglycans (GAGs). In addition to the type of GAG they carry, proteoglycans can be categorized by size. Large molecules include aggrecan, an important component of cartilage, and versican, which is found in the blood vessels and skin. Small molecules present in various connective tissues include decorin, biglycan, fibromodulin, and lumican. Because they are negatively charged, proteoglycans also help to attract positive ions, or cations, such as calcium, potassium, and sodium. They also bind water, and aid in the transport of water and other molecules through the extracellular matrix (Blochberger, Vergnes, Hempel, & Hassell, 1992; Blumberg, Brenner, Budny, & Kresse, 1997).

2. Materials and methods

2.1. Animals and treatments

A total of 24 female white rats were used in this study. Animals were housed in an air conditioned animal room at 23 ± 2 °C,

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with 12 h/12 h light/dark photoperiod, and provided with standard laboratory diet and water ad libitum.

Surgery of animals was performed under pentobarbital sodium (50 mg/kg body weight, i.p.) anesthesia. The rats underwent either bilateral laparotomy (sham group, $n = 12$) or bilateral oophorectomy (OVX group, $n = 60$). Four weeks after recovering from surgery, the OVX rats were randomly divided into two groups: vehicle-treated (OVX), OVX with tanshinone (20 mg/kg). Rats in the sham and OVX control groups were administered with the same volume of distilled water. Vehicle and tanshinone were all administered orally through an oral gavage. The treatments started 4 weeks after the surgery and lasted for 15 weeks.

Before sacrifice, each rat was individually housed in a metabolic cage for 24 h without providing food before euthanization, and urine samples were collected using separators. After laparotomy using anesthesia with diethyl ether, blood samples were taken from the abdominal aorta; serum was then prepared by centrifugation of the collected blood. Femurs were isolated from adjacent tissues, wrapped in saline-soaked gauze bandages to prevent dehydration, and stored at −20 °C until examination for biomechanical testing and structural analysis. All animals were treated according to the Guide for Care and Use of Laboratory Animals with the approval of the Institutional Ethics Committee of China regarding the animal experiments.

2.2. Biochemical analysis

Collagen and proteoglycan levels were measured with colorimetric method.

Serum ALP, P, Mg^{2+} and Ca^{2+} levels were measured by colorimetric method using commercial kits.

2.3. Statistical analysis

Statistical analysis was carried out using SPSS version 10.0 for Windows software (SPSS, Chicago, IL, USA). Results were presented as mean ± SD. $p < 0.05$ was considered statistically significant.

3. Results and discussion

Osteoporosis is the leading cause of serious morbidity and functional loss in the elderly (Inzerillo & Zaidi, 2002). It is a bone disease characterized by reduced bone mass and deterioration of bone microarchitecture (Goltzman, 2002; Tannirandorn & Epstein, 2000). Biochemical markers of bone turnover are products released from osteoblasts and osteoclasts or collagen breakdown products (Swaminathan, 2001) and can be used as molecular tools both for the detection of high bone turnover, as well as for monitoring the efficacy of the administered drug (Riggs, 2000).

The results of the present study employing a OVX rat animal model indicate that collagen cross-links coupled with structural changes are a major contributor to bone strength, in line with previously published reports in animal models and human tissue.

The levels of collagen and proteoglycan in knee joint cartilage were significantly decreased in the OVX group animals compared to the sham group ($p < 0.01$) (Fig. 1).

The levels of collagen and proteoglycan in anterior cruciate ligament were significantly decreased in the OVX group animals compared to the sham group ($p < 0.01$) (Fig. 2).

The levels of collagen and proteoglycan in knee joint cartilage were significantly decreased in the OVX group animals compared to the sham group ($p < 0.01$) (Fig. 3).

The levels of collagen and proteoglycan in anterior cruciate ligament were significantly decreased in the OVX group animals compared to the sham group ($p < 0.01$) (Fig. 4). Our results indicated that loss of collagen and proteoglycan were significant in osteoporosis diseases. Decreased collagen and proteoglycan levels were closely associated with the development of osteoporosis diseases.

A decrease of bone mineral density and early onset of osteoporosis have been reported in young patients (Anapliotou et al., 1995; Filosa et al., 1997; Molyvda-Athanasopoulou et al., 1999; Soliman, El Banna, Fattah, ElZalabani, & Ansari, 1998; Wonke, Hoffbrand, Bouloux, Jensen, & Telfer, 1998). Among the mineral nutrients, Mg^{2+}

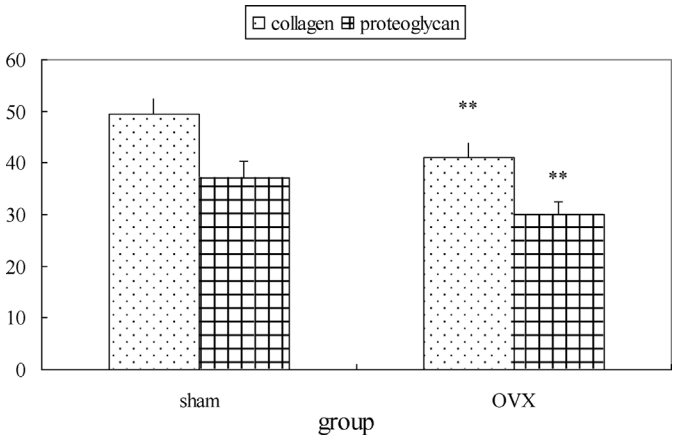

Fig. 1. The levels of collagen and proteoglycan in knee joint cartilage (2-week).

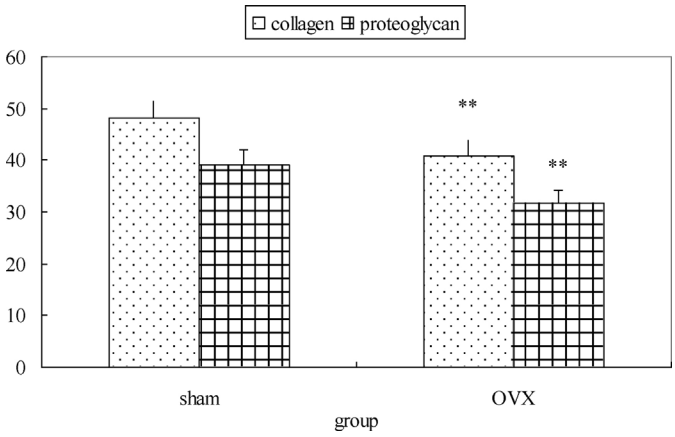

Fig. 2. The levels of collagen and proteoglycan in anterior cruciate ligament.

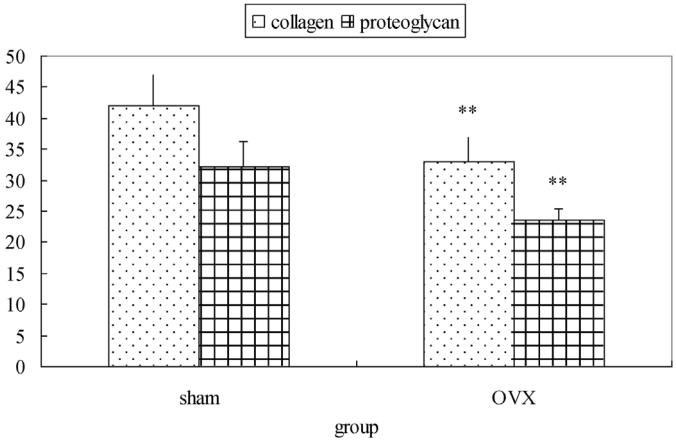

Fig. 3. The levels of collagen and proteoglycan in knee joint cartilage.

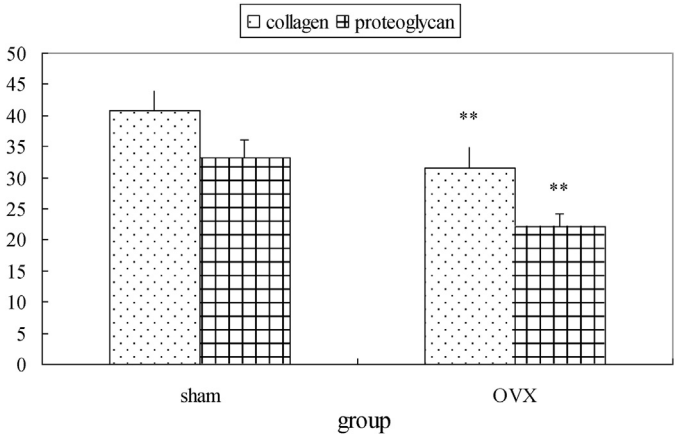

Fig. 4. The levels of collagen and proteoglycan in anterior cruciate ligament.

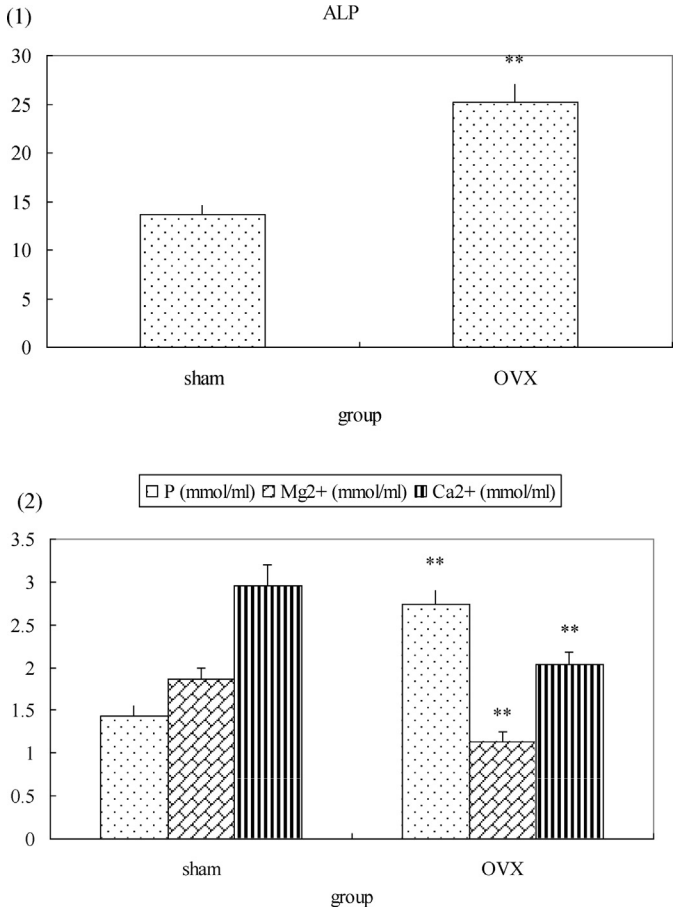

Fig. 5. The levels of ALP, P, Mg^{2+} and Ca^{2+} in serum.

is essential for physiological function in various tissues and organs. In particular, Mg^{2+} deficiency is well known as a potential risk factor of osteoporosis (Stending-Lindberg, Tepper, & Leichter, 1993), as well as a cause of impaired parathyroid hormone (PTH) secretion, which in turn dysregulates Ca^{2+} homeostasis (Rude, Oldham, Sharp, & Singer, 1978). In addition, in the relationship between Ca^{2+} and bone metabolism, PTH secretion is increased due to Ca^{2+} deficiency-induced hypocalcemia, and serum Ca^{2+} level is rapidly restored by PTH stimulating osteoclasts in the bone (Campbell, 2011). Therefore, fracture risk factors, such as increased bone turnover and bone loss, are influenced by excessive PTH secretion (LeBoff et al., 1999; Sahota, Masud, San, & Hosking, 1999). Frequent transfusions and use of desferrioxamine can lead to lower serum Ca^{2+}, Mg^{2+} and higher P and PTH levels (Anapliotou et al., 1995). All of these factors also contribute to the development of osteoporosis.

Fig. 5 shows serum ALP, P, Mg^{2+} and Ca^{2+} levels in different groups animals. It could be found that serum ALP and P levels were significantly higher in the OVX group than in the sham group ($p < 0.05$). Serum Mg^{2+} and Ca^{2+} levels were significantly lower in the OVX group than in the sham group ($p < 0.05$). This result indicates that increased serum ALP, P and decreased serum Ca^{2+} and Mg^{2+} levels were important factors to deteriorate osteoporosis diseases.

In short, to examine collagen, proteoglycan levels in knee joint cartilage and anterior cruciate ligament, serum ALP, P, Ca^{2+}, and Mg^{2+} changes are very useful for clinical diagnosis and therapy of osteoporosis diseases.

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