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The effects of long-term low selenium diet on the expression of CHST-3, CHST-12 and UST in knee cartilage of growing rats

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ABSTRACT

Objectives: To investigate the effect of low selenium diet on rat´s knee cartilage and expression of chondroitin sulfate (CS) sulfated enzymes in articular and epiphyseal-plate cartilage of rats' femur and tibia.
Methods: Twenty-four SD rats were randomly divided into two groups with six female and six male in each group: control group (selenium 0.18 mg/kg), and low selenium group (selenium 0.02 mg/kg). After 109 days, the rats were sacrificed. The ultrastructural changes in chondrocytes of rat knee cartilage were observed by transmission electron microscopy (TEM). The morphology and pathology changes of knee cartilage were examined by hematoxylin-eosin (HE) and toluidine blue (TB) staining. The localization and expression of enzymes involved in CS sulfation, including chondroitin 6-O-sulfotransferase 1 (CHST-3), chondroitin 4-O-sulfotransferase 2 (CHST-12) and uronyl 2-O-sulfotransferase (UST) were examined by immunohistochemical staining and semi-quantitative analysis.
Results: In low selenium group, ultrastructural changes of chondrocytes were observed in articular cartilage of femur (AF), articular cartilage of tibia (AT), epiphyseal-plate cartilage of femur (EF) and epiphyseal-plate cartilage of tibia (ET); however, no significant changes in chondrocytes number were observed in the above AF, AT, EF or ET. Moreover, reduced thickness of cartilage layer in AF, EF and ET was detected along with reduced staining areas of sulfated glycosaminoglycan in EF and ET in low selenium group. In addition, positive staining rate of CHST-3 was lower in AF, AT and EF, while positive staining rates of CHST-12 and UST were lower in AF, AT, EF and ET in low selenium group when compared with control group.
Conclusions: Low selenium undermines the ultrastructure of chondrocytes, inhibits the normal development of cartilage and the expression of CS sulfated enzymes.

1. Introduction

Under physiological circumstances, selenium is an essential micronutrient in human and animal body, which plays a very important role in maintaining health. Selenium is a component of selenium-containing protein, which maintains the catalytic function of the enzyme [1]. Low selenium condition may lead to disease, such as Kashin-Beck disease (KBD) associated with selenium [2], Keshan disease [3], immune dysfunction [4], liver disease [5], thyroid disease [6], cardiovascular disease [7], diabetes [8], psoriasis [9], cancer [10] and reproductive system diseases [11]. The degradation of extracellular matrix (ECM) and cartilage, especially the reduction and destruction of proteoglycans (PGs), have been identified in low selenium-associated KBD [12].

Aggrecan is the main type of PGs providing the negative charge of the ECM in articular cartilage. Moreover, it consists of core protein chain, where different types of polysaccharide glycosaminoglycan (GAG) side chains and oligosaccharides are covalently attached. In cartilage ECM, aggrecan exists in an aggregate composed of several aggrecan molecules that bind to a single filament of hyaluronan [13]. Chondroitin sulfate (CS), one of the two GAGs involved in aggrecan composition, is a linear polysaccharide consisting of repeating disaccharide units of glucuronic acid (GlcA) and N-acetylgalactosamine (GalNAc) [14]. Previous studies have shown that the destruction of GAG can reduce the synthesis of aggrecan [15], and the presence of PGs contributes to the absorption of water. Therefore, loss of PGs may lead to a lack of fluid pressurization and inadequate mechanical function. The normal cartilage function is gradually inhibited, which caused physical illness and degenerative joint disease [16,17]. Our previous

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study has shown the inadequacy of chondroitin sulfate sulfotransferase including carbohydrate sulfotransferase 3 (CHST-3), carbohydrate sulfotransferase 12 (CHST-12) and uronyl 2-O-sulfotransferase (UST) in KBD adult patients, which may cause the damage of the KBD articular cartilage [16]. Even though low selenium has been reported as one of the causes of KBD, the metabolic changes of CS sulfation patterns in the articular cartilage of KBD patients by low selenium have not been reported. We hypothesized that low selenium may be closely related to the dysfunction of the sulfatase for CS modification, which alters certain specific sites of CS.

Therefore, our present study was aimed to investigate the effects of low selenium diet on the sulfotransferases including CHST-3, CHST-12 and UST in rats' articular cartilage. The ultrastructural changes of chondrocytes in both control and low selenium groups were compared by transmission electron microscope (TEM). Hematoxylin & Eosin (HE), toluidine blue (TB) and immunohistochemistry (IHC) stainings were used to investigate the morphological and pathological changes of cartilage, as well the differences of PG and sulfotransferases. Combined with the results of previous crowd experiments, a clear explanation of the effects of low selenium on the CS sulfation and cartilage damage would provide new ideas for the prevention and treatment in the low selenium-related knee diseases.

2. Materials and methods

2.1. Sample collection and groups

24 unweaned (18-day-old) Sprague-Dawley rats (Charles River, Wilmington, MA, USA, body weight 30 ± 3 g, SPF class, 12 male and female, respectively) were raised in four polypropylene cages under controlled environment conditions (12 h light-dark cycle, temperature 22 ± 2 °C, humidity 65 ± 4%). Before the experiment, all healthy rats were randomly divided into 2 groups (control diet and Se-deficient diet, 6 males and females in each group, respectively) according to AIN-93M formula [18,19]. Diets were supplied by Trophic Animal Feed High-tech Co., Jiangsu, China. All animals were obtained from the Animal Experimental Center of Xi'an Jiaotong University. The Medical Animal Research Ethics Committee in Xi'an Jiaotong University approved the experimental procedures in the present study.

2.2. Collection of cartilage samples

All rats were fed for 109 days and sacrificed. The knee cartilage samples were collected after the rats were sacrificed. For TEM observation, the cartilage tissue from each rat was cut into pieces on ice, and then collected into 1.5 ml Eppendorf tubes where has 2.5% glutaraldehyde immobilized solution. For HE, TB and IHC staining, the knee from each rat was collected into 20 ml of 4% paraformaldehyde in phosphate buffered saline (PBS). Finally, the samples were stored at 4 °C refrigerator for future analysis.

2.3. TEM observation

Ultrastructure changes (n = 4 from each group) were observed with TEM (H-7650, Hitachi, Japan). After fixation in 2.5% glutaraldehyde solution, the samples were treated with 1% osmium tetroxide solution for two hours and then propylene oxide for 10 min, subsequently embedded in epoxy resin. Finally, slices with 50–70 nm were cut from each sample by ultramicrotome (Sweden LKB-V). The slices were observed by TEM with uranyl acetate and lead citrate staining.

2.4. HE and TB stainings

The cartilage samples were decalcified in 10% (w/v) ethylene-diamine-tetra-acetic acid disodium salt for four weeks after fixed in paraformaldehyde. Then, all the samples were embedded in paraffin and cut in serial six-μm-thick sections in a coronal plane. The sections were deparaffinized by immersing them in xylene, rehydrated with a series of gradually decreasing concentrations of ethanol solution (100-80%).

After deparaffinization, the sections were stained with HE or with 0.1% (w/v) TB dye solution. After washed with running water, the sections were dehydrated by a series of gradually increasing concentrations of ethanol solution (80–100%) and xylene, and then covered by coverslips.

2.5. IHC staining and quantification

The deparaffinized sections (n = 6 from each group) were incubated with primary antibodies [CHST-3 (18242-1-AP, Proteintech Group Inc., Rosemount, IL, USA), CHST-12 (15341-1-AP, Proteintech Group Inc., USA) and UST (ab137624, abcam)] with the dilution of 1:50 in phosphate buffered saline (PBS) containing 1% bovine serum albumin at 4 °C overnight, streptavidin-peroxidase-conjugated secondary antibody was used to detect CHST3, 12 and UST according to the previously described [16]. Finally, the sections were visualized using a diaminobenzidine kit according to the manufacturer's protocols and counterstained with hematoxylin.

2.6. Image and data acquiring

The variation in topology was controlled by taking photos of parallel zones of articular cartilage and epiphyseal-plate cartilage from femur and tibia, respectively. Five randomly selected fields in four zones [articular cartilage of femur (AF), articular cartilage of tibia (AT), epiphyseal-plate cartilage of femur (EF) and epiphyseal-plate cartilage of tibia (ET)] from one section of the rats (n = 6) were chosen for each subjects of the groups. Photos were taken with Olympus BX51 fluorescence microscope (Olympus, Japan) at 100× magnification. For HE staining, pathological changes of knee cartilage were observed, and Image J (NIH, USA) was used for quantifying the cell numbers of articular cartilage and epiphyseal-plate cartilage. For TB staining, Image J (NIH, USA) was used for quantifying intensive staining area; the threshold for the selection of intensive staining area is 155. The average percentage of intensive staining area represents for cartilage was calculated both in low selenium and control groups. In addition, the maximum (Max) and minimum (Min) thickness values (μm) for the articular cartilage and epiphyseal-plate cartilage in each picture was measured by Photoshop (Adobe, USA). The thickness of cartilage was calculated as: the thickness of cartilage = (Max + Min) / 2. For IHC staining, the percentages of positively stained cells were counted. The positive staining rate was calculated as: the positive staining rate = (positively stained cells)/(all cells) × 100%.

2.7. Statistical analysis

SAS 9.3 was used for the data entry and analysis of HE, TB staining results and positive staining rate. Since the data from low selenium and control groups were not in accordance with normal distribution by the test for normality and equal variance, the difference between the low selenium and the control groups was analyzed by rank sum test and two groups were calculated as median (P25, P75). Statistical significance was accepted when $P < 0.05$.

3. Results

3.1. Ultrastructural changes of chondrocytes

In low selenium group, TEM images showed that the chromosomes were mildly aggregation and marginalization in the irregular nucleus when compared with control group. In addition, organelles such as Golgi, endoplasmic reticulum were reduced or disappeared, but the cytoplasmic glycogen was significantly increased in low selenium group