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Influence of Low Molecular Weight Chondroitin Sulfate on IL-1β, TNF-α and TGF-β, Calcium and Phosphate in the Process of Cartilage Reconstruction of Rabbit*

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ABSTRACT Objective: To investigate the influence of low molecular weight chondroitin sulfate on IL-1β, TNF-α and TGF-β, calcium and phosphate in the process of cartilage reconstruction of rabbit. **Methods:** 36 New Zealand rabbits were randomly divided into 6 groups with 6 rabbits in each group: control group, model group, low-dose of low molecular weight CS group, high-dose of low molecular weight CS group, low-dose of high molecular weight CS group and high-dose of high molecular weight CS group. A defect, 3mm diameter, 3mm depth, was drilled at the condyles of femur of the hind legs by using an electric bone drill. After the next day for animal drug perfusion, 1 time a day. Drawn after 5 weeks. The expression of cytokines, such as IL-1β, TNF-α and TGF-β in synovial fluid was detected by ELISA, determining levels of calcium and phosphorus in serum of rabbit by automatic biochemical analyzer kit. **Results:** Low molecular weight and high molecular weight CS were able to reduce levels of inflammatory cytokines and increase levels of TGF-β in the joint fluid. And compared with that in the model group, there is statistical significance ($P<0.05$). While low molecular weight CS' effect were better than high molecular weight CS ($P<0.05$). However, there was no obvious difference between high and low dose ($P>0.05$). Compared with that in the model group, the groups of CS, which levels of calcium and phosphorus in the serum were relatively low ($P<0.05$), but their levels were higher than that in the control group. However, these indexes had no obvious difference between low molecular weight CS group and high molecular weight CS. **Conclusion:** High and low molecular weight CS can increase the levels of TGF-β and reduce the levels of IL-1β, TNF-α and calcium, phosphate in the serum. For cartilage reconstruction, the above factors may have positive effect.

Key words: Low molecular weight of chondroitin sulfate; Cartilage reconstruction; IL-1β; TNF-α; Phosphate

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Introduction

Chondroitin sulfate(CS) is widely present in the cartilage tissue of humans and animals, which mainly from throat bone of animal, nasal septum, trachea cartilage[1]. High molecular weight CS has a high biological activity, it plays an important role in the biological processes of cell transfer, differentiation, proliferation, recognition and organizational forms[2]. However, due to the selective permeability of cell membranes and other factors, most of the glycosaminoglycan especially the face issues is low bioavailability in the application of clinical[3]. However, there are experiment had proved molecular weight are 3500-5300Da CS, its pharmacological activity is the strongest, it has better efficacy in the aspect of prevention of rheumatic inflammation and wound healing[4].

Articular cartilage is a bright connective tissue, which is covered articular surface. However, mature chondrncytes' division capacity is limited in articular cartilage, so the ability to repair itself is relatively poor after cartilage injury [5-7]. And studies had shown that IL-1β, TNF-α, TGF-β and other factors in the process of cartilage reconstruction has important significance [8-10], while studies have found that the CS plays a role of the improvement on the above mentioned factors[11]. Due to the macromolecular drugs have low bioavailability, therefore, the purpose of the experiment is to investigate the influence of low molecular weight chondroitin sulfate on IL-1β, TNF-β and TGF-β, calcium and phosphate.

1 Materials and Methods

1.1 Materials

1.1.1 Reagents and Drugs Rabbit interleukin 1β (Wuhan ColorfulGene Biological Technology Co., Ltd, Lot number 201306); Rabbit tumor necrosis factor α (Wuhan ColorfulGene Biological Technology Co., Ltd, Lot number 201306); rabbit transforming growth factor β (Wuhan ColorfulGene Biological Technology Co., Ltd, Lot number 201306); Chondroitin sulfate (Provided by Qingdao Better Biotechnology Co. Ltd. Weight-average molecular weight of high molecular: 55426; Weight-average molecular weight of low molecular weight: 4296).

1.1.2 Animals 36 New Zealand rabbits, Adult male, 2.5~3.3 kg,

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purchased from Shandong LuKang Pharmaceutical Co., Ltd. License number: SCXK Lu 20080002.

1.1.3 Experimental instruments Automatic biochemical analyzer CS-800B (Changchun Dirui Medical Technology Co., Ltd); Microplate Reader Stat Fax 2100 (Awareness Technology, Inc).

1.2 Experimental methods

1.2.1 Grouping and Preparation of animal model These animals were randomly divided into 6 groups (6 rabbits in each groups): control group(Group A), model group(Group B), low-dose of low molecular weight CS group(Group C), high-dose of low molecular weight CS group(Group D), low-dose of high molecular weight CS group (Group E) and high-dose of high molecular weight CS group(Group F). Anesthetized via ear margin intravenous with 25% urethane (4mL/kg). After anesthesia, a full-thickness articular cartilage defect, 3mm diameter, 3mm deep, was drilled by bone drill in the trochlear surface of medial femoral condyle, which causes cartilage loss. Flushing with saline and 80 million units of penicillin sodium, arthroscopic reset later, sutures, closing joint of knee. There was no treatment on rabbits in control group. After surgery, inject 80 million units of penicillin sodium, 1 time/d, continuous 3 days, and were injected in the first five days to prevent infection.

1.2.2 Feeding animals after surgery Gavage after surgery, once a day, for 5 weeks (Dose according to the table of commuting equivalent dose rate between body surface area of humans and body surface area of animals to calculate). Low-dose of low molecular weight CS group and low-dose of high molecular weight CS group were fed daily dose of 0.2 g/kg. High-dose of low molecular weight CS group and high-dose of high molecular weight CS group were fed daily dose of 0.4 g/kg. Model group was given the same amount of distilled water to gavage. Control group of normal feeding, without gavage.

1.3 Experimental drawn

Five weeks later, anesthetized via ear margin intravenous with 25% urethane (4mL/kg), then inject 1mL sterile PBS into the joint cavity from patellar bursa, after repeatedly unsteady joint, siphoning synovial fluid, well marked and placed at -80℃ to save backup. Blooding from abdominal aorta, collect serum after centrifugation, well marked and placed at -80℃ to save backup.

1.4 Observing indexes

1.4.1 Examination of inflammatory cytokines in lavage fluid The levels of IL-1β, TNF-α and TGF-β in synovial fluid of rabbit were detected by ELISA according to kit instructions.

1.4.2 Inspection of content of calcium and phosphorus in serum The levels of calcium and phosphorus in serum of rabbit were detected by automatic biochemical analyzer kit.

1.5 Statistical processing

The data were expressed in $\bar{x} \pm s$ and analyzed by one-way analysis of variance with SPSS17.0. $P<0.05$ was considered statistically significant.

2 Results

2.1 The levels of IL-1β and TNF-α in synovial fluid

Table 1 showed that the levels of IL-1β and TNF-α from low molecular weight CS groups and high molecular weight CS groups were lower than that in the model group ($P<0.05$). And each indicator of low molecular weight CS groups were better than that in high molecular weight CS groups ($P<0.05$), Some indicators even close to the control group. However, there was no significant difference between high and low dose($P>0.05$).

2.2 The levels of TGF-β in synovial fluid

Table 1 showed that the levels of TGF-β from low molecular weight CS groups and high molecular weight CS groups were higher than that in the model group ($P<0.05$). And low molecular weight CS groups were better than that in high molecular weight CS groups ($P<0.05$). However, there was no obvious difference between high and low dose($P>0.05$).

Table 1 The levels of IL-1β, TNF-α and TGF-β in synovial fluid of each group($\bar{x} \pm s$, ng/L)

Groups	n	IL-1β	TNF-α	TGF-β
Group A	6	19.63 2.65	121.58 7.53	59.02 2.00
Group B	6	61.04 2.17	540.25 24.93	92.85 2.16
Group C	6	20.31 2.65*#	125.51 3.53*#	127.49 1.91*#
Group D	6	30.79 3.75*▲	203.44 34.05*▲	123.22 3.57*▲
Group E	6	39.36 7.28*	255.71 34.80*	107.50 11.25*
Group F	6	40.95 1.01*	302.01 22.59*	111.08 1.91*

Note: * $P<0.05$, compare with the model group; # $P<0.05$, compare with the low-dose of high molecular weight CS group;
▲ $P<0.05$, compare with the high-dose of high molecular weight CS group.

2.3 The levels of calcium and phosphorus in serum

Table 2 showed that the levels of calcium and phosphorus from low molecular weight CS groups and high molecular weight CS groups were lower than that in the model group($P<0.05$), while these indicators were higher than that in the control group. However, these indicators had no obvious difference between low molecular weight CS group and high molecular weight CS.

3 Discussions

Chondroitin sulfate is widely present in the cartilage tissue of

Table 2 The levels of calcium and phosphorus in serum of each group ($\bar{x}\pm$ s, mmol/L)

	Group A	Group B	Group C	Group D	Group E	Group F
Ca	2.19 0.53	3.81 0.13	2.92 0.38*#	2.81 0.31*#	3.09 0.69*#	3.12 0.43*#
P	1.02 0.11	2.81 0.13	1.59 0.34*#	1.88 0.52*#	1.93 0.27*#	1.83 0.27*#

Note: * P<0.05, compare with the model group; # P<0.05 compare with the control group.

humans and animals, and its structure is more complex. Its molecular structure is dominated by D-glucuronic acid and a 2-acetamido-2-deoxy-sulfate-D-galactose, and relative molecular mass (Mr) is (10-50)×10^3. According to a sulfuric acid group at different locations in the N-acetyl-D-galactosamine, so chondroitin sulfate can be divided into different isomers, including chondroitin sulfate A (CS-A), chondroitin sulfate B(CS-B), chondroitin sulfate C(CS-C) [12]. Chondroitin sulfate functions are reflected by composition of proteoglycans. It can be broadly divided into two categories, including structural function and regulation function,and the CS is widely used in orthopedics[13].

IL-1β can up-regulate expression of mRNA of matrix metalloproteinase, and cause matrix degradation of cartilage[14]. TNF-α is an inflammatory cytokines, which can mediate cartilage injury. It not only can selectively inhibit the generation of cartilage collagen, and inhibit the proteoglycan synthesis, but also can promote degradation of cartilage matrix [15-18]. TGF-β is a cytokine to promote cartilage growth[19]. It not only can promote the proliferation of chondrocytes, but also can increase the synthesis of osteocyte proteoglycans and collagen. This experimental results showed that the low molecular weight CS group, which the levels of IL-1β and TNF-α in synovial fluid are less than the high molecular weight CS group and model group. However, its levels of TGF-β in synovial fluid are higher than that in the high molecular weight CS group and model group. This showed that the low molecular weight CS is better than the high molecular weigh CS for the reduction of levels of IL-1β, TNF-α and exaltation of levels of TGF-β in the process of cartilage restoration. For cartilage repair, it may also be affected by these factors, so as to achieve a better effect.

Calcium and phosphorus are mainly adjusted by three kinds of active substances in the metabolic process of organism, including active vitamin D, calcitonin and parathyroid hormone. However, when the levels of blood calcium and phosphorus decreased, it will stimulated the three active substances, thus promoting bone decalcification and making the levels of blood calcium and phosphorus returned to normal or even increased. Although the levels of blood calcium and phosphorus have increased at this point, the bones have been undernourished[20]. The experimental results show that the CS groups' levels of blood calcium and phosphorus were lower than that in the model group. This may suggest that the CS groups, what bone nutritional status is good, but they are not as good as the control group.

This experiment investigated the influence of low molecular weight chondroitin sulfate on IL-1β, TNF-α and TGF-β, calcium and phosphate in the process of cartilage reconstruction of rabbit, for studies and application of low molecular weight CS to provide a basis in future.

References

[1] Ma YH, Chen SE, Cang RX. A Survey of Studies on Chondroitin Sulfate[J]. China Animal Husbandry & Veterinary Medicine, 2007, 34(12): 138-140

[2] Deepa SS, Kalayanamitra K, Ito Y, et al. Novel Sulfated Octaand Deeasaccharides from Squid Cartilage Chondroitin Sulfate E: Sequencing and Application for Determination of the Epitope Structue of the Monoclonal Antibody MO-225[J]. Biochemistry, 2007, 46(9): 2453-2465

[3] Ofman D, Slim GC, Watt DK, et al. Yorke. Free radical induced oxidative depo1ymerisation of cboudroitin sulphate and dermatan sulphate [J]. Carbohydrale Polymers, 1997, 33:47-56

[4] Yang XB, You SY, Qi DQ, et a1. A preparation of low molecular weight chondroitin [P]. China, 2006,10146273. 2. 2007-7-l1

[5] O'Driscoll SW. Current concepts review the healing and regeneration of articular cartilage [J]. The journal of Bone and Joint Surgery, 1998, 80: 1795-1812

[6] Dai YK, Gu YH, Ma Y. Advance in Therapy of Acupuncture Combined with Medicine for Promoting the Repair of Cartilage Injury [J]. Acta Chinese Medicine And Pharmacology, 2010, 38(6): 114-116

[7] Xu ST, Ge BF, Xu YK. The utility of orthopedics (Third Edition)[M]. Beijing: People's Military Medical Press, 2008: 42-48

[8] Chen Y, Liu Q. The change of the levels and significance of IL-1β, TNF-α of repair of the rabbit cartilage damage [J]. National Medical Frontiers of China, 2011, 6(17): 13-14

[9] Chen B, Xie XM, Li JL. Experimental study of moxibustion treatment on KOA cartilage injury of rabbit and expression regulation of TNF-α, TGF-β and IGF-1 [J]. Jiangsu Journal of Traditional Chinese Medicine, 2009, 41(6): 69-71

[10] Fu JJ. Expression and significance of connective tissue growth factor and transforming growth factor and transforming growth factor β1 in articular cartilage during repair after full-thickness cartilage defect of knee in young and adult rabbit model [J]. Chongqing: Third Military Medical University of Chinese P.L.A, 2009: 1-53

[11] Fu X, Lin L, Wei XL, et al. Application of Glucosamine and Chondroitinsulfate in the Treatment of the Knee Joint with Osteoarthritis in Rabbit Model[J]. Chinese Journal of Sports Medicine, 2008, 27(5): 588-592

[12] Shen BJ, Dou Y. The Application of Chondroitin Sulfate Products in Australia[J]. Food and Drug, 2009, 11(1): 4

[13] Zhang Q, Ling PX, Chen L. Application of Chondroitin Sulfate in Orthopedics[J]. Food and Drug, 2010, 12(4): 292-293

[14] Xiu ZB. Research Progress of the Correlation between Articular Cartilage repair and Cytokines [J]. Medical Recapitulate, 2009, 15

(20): 3045-3047

[15] Gao YX, Jiang RX. Domestic research progress of pathogenesis of osteoarthritis [J]. The Journal of Traditional Chinese Orthopedics And Traumatology, 2005, 17(4): 55

[16] Xiang C. Apoptosis in chondrocyte and osteoarthritis [J]. Foreign Medical Sciences, 2003, 26(2): 110-113

[17] Sun JL, Fan JZ. The Model Development and Histo-pathology of Rabbit Osteoarthritis of the Knee[J]. Chinese Journal of Rehabilitation, 2005, (20): 78-80

[18] Neidel J, Sihulze M, Sova L, et al. Practical significance of cytokine determination in joint fluid in patients with arthroses or rheumatoid arthritis[J]. Z Orthop Ihre Grenzgeb, 1996, 134(4):38l-385

[19] Li XH, Wu YC, Zhang XY, et al. Effects of TGF-β, IGF-1 on cartilage tissue [J]. Journal of Inner Mongolia Medical University, 2006, 28: 197

[20] Wang MC, He SH. Determination of the Content of Ca, P and the Activity of Alkaline Phosphatase (ALP) in Little Han Sheep with Pregnancy Toxemia [J]. Journal of Anhui Agricultural Sciences, 2006, 34(23): 6197-6200

低分子硫酸软骨素在兔软骨修复过程中对 IL-1β、TNF-α 和 TGF-β 及钙磷的影响 *

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摘要 目的:探讨低分子量硫酸软骨素(CS)在兔软骨修复过程中对于 IL-1β、TNF-α 和 TGF-β 及血液中钙磷含量的影响。**方法:**36 只成年新西兰大白兔,随机分成 6 组,分别为对照组、模型组、低分子量 CS 低剂量组、低分子量 CS 高剂量组、高分子量 CS 低剂量组、高分子量 CS 高剂量组,每组 6 只。通过在实验兔股骨髁关节面部位,钻出直径 3mm,深度 3mm 的缺孔,造成其关节软骨的缺损。术后次日给予药物进行灌胃,每日 1 次,5 周后取材。采用酶联免疫法测定关节液中 IL-1β、TNF-α 和 TGF-β 的含量,同时用全自动生化分析仪及其配套的试剂盒来测定兔血清中钙,磷含量。**结果:**低分子量 CS 和高分子量 CS 都能够减少关节液中炎性因子的含量和增加 TGF-β 的含量, 且与模型组相比具有统计学意义 (P<0.05); 同时低分子量 CS 与高分子量 CS 相比效果较好(P<0.05);而高低剂量之间无统计学意义(P>0.05)。与模型组相比,给予 CS 的组别,其血清中的钙磷含量相对较少(P<0.05),但都高于对照组。**结论:**高、低分子量的 CS 都可以增加 TGF-β 的含量,降低 IL-1β、TNF-α 和血清中钙、磷的含量。而对于软骨修复,这可能是通过对上述因子的影响,从而产生积极的作用。

关键词:低分子量硫酸软骨素;软骨修复;IL-1β;TNF-α;磷

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