Effect of tensile stress on type Ⅱ collagen and aggrecan expression in rat condylar chondrocytes*★

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Abstract

BACKGROUND: Changes in extracellular of chondrocyte can reflect influence of external force on temporomandibular joint and adaptability of body to external force.

OBJECTIVE: To study the effect of cyclic tensile stress on main extracellular matrix of condylar chondrocyte.

METHODS: The cyclic tensile stress was exposed to the third passage condylar chondrocyte for 0, 1, 6, 12 and 24 hours, respectively, using a Flexcell Strain Unit-5000T system (10% surface elongation, 6 cycles/min). After mechanical loading, total RNA was extracted from the cells harvested from Six-well BioFlex flexible cell Petri Dish, reverse transcribed, and reverse trabscription-PCR was performed to quantify mRNA levels for type Ⅱ collagen and aggrecan.

RESULTS AND CONCLUSION: Compared with the control group (0 hour group), both type Ⅱ collagen and aggrecan mRNA expression was significantly increased after loading for 6 hours ($P < 0.05$), but began to decrease since 12 hours, and significantly decreased at 24 hours ($P < 0.05$). Results showed that cyclical tensile stress stimuli can affect the synthesis of main extracellular matrix of condylar cartilage, *i.e.* the synthesis was gradually enhanced with prolonged stimulation duration, but significantly inhibited in response to further stress stimuli.

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INTRODUCTION

Functional orthopedic is a common method to treat early functional and skeletal malocclusion caused by stomatognathic muscle dysfunction before the peak of growth and development of youth. In the orthopedic treatment, condylar adaptive reconstruction is considered a basis of functional orthopedic treatment, while the condylar chondrocytes are converted to effector cells[1], and its response to orthopedic forces determines the clinical effect. It has been the research focus of domestic and foreign scholars. Condylar chondrocytes play an important role in the generation and degradation of extracellular matrix (ECM), as a unique cellular component in condylar cartilage. ECM consists of type Ⅱ collagen (Col-Ⅱ) and aggrecan (AGG). Col-Ⅱ forms the fibrillar network to provide tensile strength to the cartilage while proteoglycans such as AGG comprising a core protein linked with hyaluronic acid and chondroitin sulfate are immobilized into collagen fibrillar network to provide resistance to compression. Studies have shown that changes of ECM synthesis reflect the situation of temporomandibular joint and the body's adaptability under external loading[2]. Therefore, Col-Ⅱ and AGG synthesis can be used as direct reflection of condylar chondrocyte functional status under mechanical loading. In the present study, we tested dynamic changes of Col-Ⅱ and AGG synthesis to explore the common reaction of condylar chondrocyte to cyclic tensile stress applied by loading system FX-5000T.

MATERIALS AND METHODS

Design

An *in vitro* cell-based stress loading comparison.

Time and setting

The experiment was performed in the central laboratory of Qingdao Municipal Hospital from March to November 2011.

Materials

A total of 20 Sprague-Dawley rats, aged 2 weeks, of either gender, were provided by the Laboratory Animal Center of Qingdao Municipal Hospital. Reagents and equipment used in this study are as follows:

Reagent and instrument	Source
Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS)	HyClone, USA
Total RNA extraction kit, TIANScript cDNA, first chain synthesis kit	Tiangen, Beijing
Flexcell Strain Unint-5000T, Six-well BioFlex flexible cell Petri Dish	Flexcell Company, America
Inverted optical microscope	Olympus, Japan
Gel-Proanalyzer	BIORAD Gel Doc2000, USA

Methods

Condylar chondrocyte isolation[3]

The 20 rats born in 2 weeks were sacrificed and soaked in 75% alcohol for 10 minutes. Bilateral mandibular condyles were sterilely separated and the fibrous layer on the cartilage was removed. The cartilage was cut into tissue blocks, 1 mm×1 mm×1 mm. Condylar chondrocytes were obtained using two-step enzyme digestion method.

Condylar chondrocyte culture

Primary cultures of chondrocytes were maintained in DMEM containing 15% heat-inactivated fetal bovine serum, antibiotics comprising 100 μg/mL penicillin G and 50 μg/mL streptomycin. Cells were incubated at 37 ℃ in a humidified atmosphere in a 95% air 5% CO_2 incubator, and the medium was changed every 2 days. Cell morphology and growth were observed under an inverted microscope. Cells were usually

subcultured at 80%−90% confluence using 0.25% trypsin to dissociate cell monolayers. Briefly, the media was removed, the cells were washed with phosphate buffered saline (PBS), then 2 mL of trypsin was added to make the cells detach from the bottom of the culture flask. In order to terminate the reaction, the medium of an equal volume was added. Then, cell suspension was centrifuged at 1 200 r/min for 5 minutes. An appropriate number of cells in suspension were then transferred to new culture flask. Fresh media was added to each culture flask, and were incubated for the next growth phase.

Establishment of chondrocyte mechanical stimulation models

The chondrocytes were randomly divided into five groups: 0, 1, 6, 12 and 24 hours groups. Cyclic tensile stress was applied on the cells in each group. Briefly, cell suspension at 1×10^5/L was seeded on Bioflex 6-well culture plates, with 2 mL per well. The medium was changed every 48 hours. At 80%−90% confluency, serum-free medium was used for 24 hours, and cell synchronization was made. The FX-5000T was used to expose chondrocytes to tensile stress that 10% surface elongation and a frequency of 10 cycles per minute, each cycle including 3-second stretch /3-second relaxation.

Analysis of Col-Ⅱ and AGG mRNA levels

Total RNA was extracted with RNA extraction kit from each group cells according to the manufacturer's protocol. The collected samples were stored at −80°C or samples of total RNA (1.5−5 μg) were reverse transcribed using TIANScript RT Kit according to the instruction of the production and semiquantitative PCR was performed to detect the expression of Col-Ⅱ and AGG mRNA.

Primers are as follows:

Primer	Sequence (5'−3')	Size (bp)
Type Ⅱ collagen	Forward: GCC TCG CGG TGA GCC ATG ATC	472
	Reverse: CTC CAT CTC TGC CAC GGG GT	
Aggrecan	Forward: GAT GTC CCC TGC AAT TAC CA	230
	Reverse: TCT GTG CAA GTG ATT CGA GG	
β-actin	Forward: CCC ATC TAT GAG GGT TAC GC	150
	Reverse: TTT AAT GTC ACG CAC GAT TTC	

Amplification conditions were as follows: PCR was done for 30 cycles (an initial denaturation at 95 ℃ for 1 minute, denaturation at 95 ℃ for 30 seconds, annealing at 58°C for 30 seconds, extension at 72 ℃ for 30 seconds and a final elongation step of 5 minutes at 72 ℃) for Col-Ⅱ[4]. As for AGG, there were 50 cycles (95 ℃ for 5 minutes, 95℃ for 10 seconds, 60 ℃ for 10 seconds, 72 ℃ for 10 seconds and a final elongation step of 5 minutes at 72 ℃). Then PCR products were identified by 2% agarose gel electrophoresis (80 V, 60 minutes). Finally, the ratio of target bands and β-actin bands were calculated by Gel-Proanalyzer.

Main outcome measures

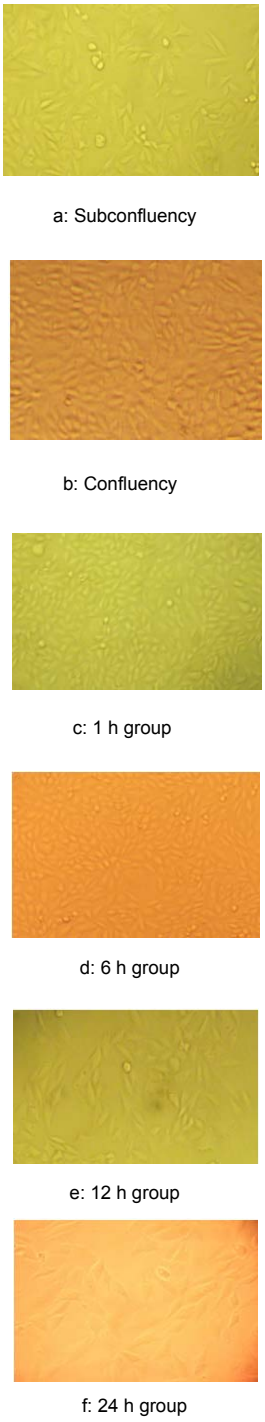
Col-Ⅱ and AGG mRNA expression in condylar chondrocytes loaded tensile stress stimulation of different time by reverse transcription-polymerase chain reaction.

Statistical analysis

Data were expressed as mean±SD and analyzed using SPSS 17.0 software. One-way analysis of variance was used for intragroup comparison. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Chondrocyte morphology (Figure 1).



a: Subconfluency

b: Confluency

c: 1 h group

d: 6 h group

e: 12 h group

f: 24 h group

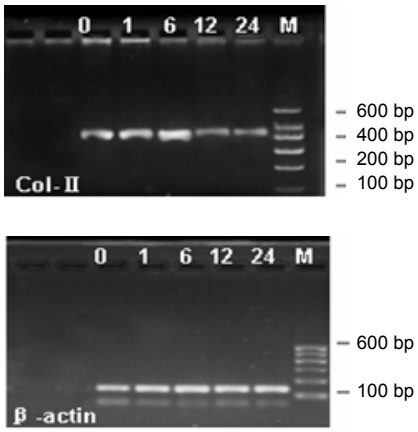
Figure 1 Morphology of condylar chondrocytes (×200)

The micrography showed that cells grown in primary culture underwent distinct morphological changes with respect to

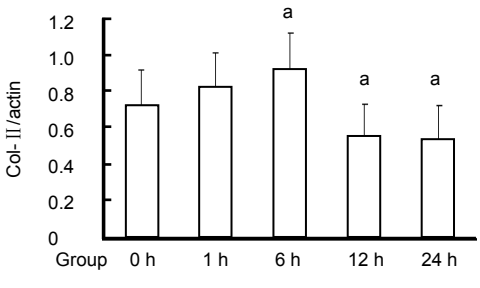
shape, size, and density of the cells. At 48 hours after initial plating, the cells attached to the plates were round and refractile. And then, most of cells became enlarged, polygonal and spread out. Small round cells were scattered throughout the cultures. By the seventh day, the cultures had reached confluence and displayed the characteristic “cobblestone” morphology. After loading, condylar chondrocytes increased in size and shape. At the same time, fusiform and spindle-shaped cells were present. However, the cells did not appear typical phenomenon arranged along the direction of the force after the cyclic tensile stress.

Expression of type Ⅱ collagen mRNA after cyclic stretch

Agarose gel electrophoresis analysis of Col-Ⅱ and β-actin is shown in Figure 2. Compared with the control group (0 hour group), Col-Ⅱ mRNA expression was increased after 1-hour loading; after 6-hour cyclic tensile stress Col-Ⅱ mRNA expression increased significantly ($P < 0.05$); after 12 and 24 hours of loading, Col-Ⅱ mRNA expression reduced remarkably ($P < 0.05$).



a: Electrophoresis diagram analysis of Col-Ⅱ mRNA expression



$^{a}P < 0.05$, *vs.* 0 h

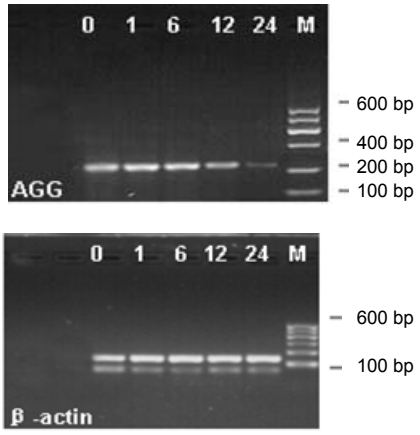
b: Quantitative analysis of Col-Ⅱ mRNA

Col-Ⅱ: type Ⅱ collagen

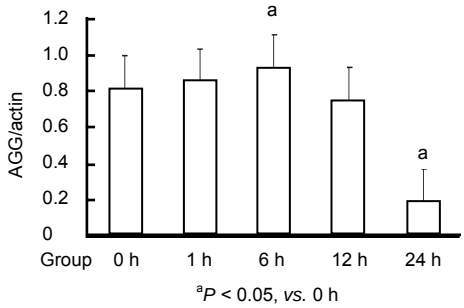
Figure 2 Type Ⅱ collagen mRNA expression after cyclic tensile stress at different time points

Expression of AGG mRNA after cyclic stretch

Figure 3 shows that AGG mRNA expression was increased after 1-hour loading but there were no significant differences between loading and control group; after 6-hour cyclic tensile stress AGG expression increased significantly ($P < 0.05$); after 12 and 24 hours of loading, AGG mRNA expression reduced significantly ($P < 0.05$).



a: Electrophoresis diagram analysis of AGG mRNA expression



$^{a}P < 0.05$, *vs.* 0 h

b: Quantitative analysis of AGG mRNA

Figure 3 Aggrecan (AGG) mRNA expression after cyclic tensile stress at different time points

DISCUSSION

Condylar cartilage, one of the major growth sites, is fibrous cartilage that covers the surface of the mandibular condyle, which consists of chondrocytes (3%), ECM (24%) and water (70%−80%). Condylar chondrocytes, as main function components of articular cartilage, play an important role in producing or degrading the ECM, which belongs to terminally differentiated cells[5]. ECM includes collagens and proteoglycans. Col-Ⅱ is the most abundant protein in the cartilage, and forms the fibrillar network with other collagens (such as Ⅸ, Ⅲ, Ⅺ collagen) to provide resistance to compression; the proteoglycans are polysaccharide complexes that trap water and affect the viscoelastic properties of the cartilage, helping the cartilage resist compressive forces too[6-7]. It is known that the body’s condylar chondrocytes are always in certain stress conditions, including tensile stress, compressive stress and shear stress[8-12]. Different intensity and time of stress will produce different effects on ECM synthesis. Huang *et al* observed that after 0.5 Hz, 10% elongation of cyclical tensile stress to the articular cartilage of the pig for three hours, Col-Ⅱ and AGG synthesis reached a peak; after 12 hours, the expression of these two substances returned back to normal levels[13]. Valhmu *et al* reported that AGG mRNA synthesis of primary chondrocytes increased under the pressure of 0.1 MPa

for 1 hour and decreased back to the basic level at 4 hours[14]. Fujisawa found that high frequency (2 s/cycle) can inhibit proteoglycan synthesis, with the frequency of the lower (120 s/cycle, 240 s/cycle) and its inhibition is also reduced[15]. Functional orthopedics was the main method to treat patients with mandibular hypoplasia and deformities in the field of orthodontics. Temporomandibular joint is the only joint that keeps relatively active reconstruction ability for all one's life[16-19]. The condylar cartilage as a major growth center is a major functional area of orthopedic treatment which directs response to the clinical therapy. Previous studies have proved that during mandibular movement, condyle is subjected to repetitive compression and the mandibular condylar chondrocytes can detect and respond to this biomechanical environment by altering their metabolism, and further to maintain their decomposition and anabolic balance and normal physiological function of articular cartilage[20-23]. The present study demonstrated that with the different time of loading Col-Ⅱ and AGG mRNA expression also significantly changed. Col-Ⅱ and AGG mRNA began to increase in 1 hour group, which can be considered that early tensile stress can stimulate ECM synthesis; after 6-hour loading, Col-Ⅱ and AGG mRNA expression was significantly increased, showing that suitable tensile stress increased the Col-Ⅱ and AGG secretion; after 12-hour loading, Col-Ⅱ and AGG mRNA began to drop; up to 24 hours, the expression was significantly reduced, indicating that tensile stress of too long inhibits the synthesis of ECM.

In summary, cyclic tensile stress can stimulate major changes in condylar cartilage extracellular matrix (Col-Ⅱ and AGG) synthesis. Tensile stress of appropriate time can promote the synthesis of ECM which is profit for the proliferation of condylar chondrocytes and there was a peak between 6 and 12 hours. On the other hand long time loading inhibits the synthesis of ECM and was not conducive to cartilage remodeling. We hope that these experimental results can provide new ideas and pathways for present treatment of functional orthopedics.

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张应力刺激大鼠髁突软骨细胞Ⅱ型胶原和聚集蛋白聚糖的表达**★

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摘要

背景：研究表明软骨细胞外基质合成的变化反映了外力对于颞下颌关节的影响和机体对于外力的适应性。

目的：观察周期性张应力对髁突软骨细胞主要细胞外基质合成的影响。

方法：采用FX-5000T应力加载系统对第3代大鼠髁突软骨细胞分别施加0，1，6，12和24 h的周期性张应力，应力刺激强度为10% 1 Hz。加力完成后收集加力细胞，提取总RNA反转录成cDNA，应用RT-PCR技术检测软骨细胞主要细胞外基质Ⅱ型胶原和聚集蛋白聚糖的表达变化情况。

结果与结论：与对照组(0 h 组)相比，加力6 h时Ⅱ型胶原和聚集蛋白聚糖表达均显著增加($P < 0.05$)；加力12 h时Ⅱ型胶原和聚集蛋白聚糖表达均开始下降；当加力至24 h时二者表达量均显著降低($P < 0.05$)。结果表明：周期性张应力可以影响髁突软骨细胞主要细胞外基质的合成,随加力时间的延长基质合成逐渐增强；进一步延长加力时间,基质的合成受到明显抑制。

关键词：张应力；功能矫形；髁状突软骨；Ⅱ型胶原；聚集蛋白聚糖

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本期专题：神经缺损动物模型的构建②

4　低频电刺激对坐骨神经损伤大鼠不同类型骨骼肌萎缩及内源性胰岛素样生长因子1表达的影响

张　玮(江西中医学院体育教学部,江西省南昌市　330004)

推荐理由:周围神经损伤后,神经及其所支配的骨骼肌功能的恢复是当今运动创伤康复及外科领域中较为棘手的问题之一。已有实验表明,外周神经损伤后骨骼肌会发生退行性萎缩,电刺激治疗周围神经损伤具有确切的疗效。胰岛素样生长因子1具有广泛的生物学效应,可促进骨形成、蛋白合成、肌肉糖摄取、神经生存及髓鞘合成。近年来研究发现胰岛素样生长因子1与骨骼肌的损伤修复有密切联系。

实验旨在探讨低频电刺激对去神经骨骼肌不同类型肌纤维萎缩的防治效果,并观察失神经肌肉组织中内源性胰岛素样生长因子1的表达情况。

5　伴有脊髓损伤先天性脊柱侧凸实验模型的稳定性评估

陆　明(解放军第三〇六医院骨科,北京市100101)

推荐理由:人体的许多系统性疾病都能引发脊柱侧弯畸形,不同病因所致的脊柱侧弯都拥有原发病本身的特点,同时继发脊柱侧弯畸形变,但大多数脊柱侧弯患者的特点为特发性和先天性脊柱侧弯。以往研究的客观临床资料展现出脊柱侧弯和侧弯发展过程中“病因与症状,各种临床症状相互平衡所形成的拥有鲜明论证特点的《脊柱侧弯疾病的发展示意图》”。

作者通过建立合并脊髓瘫痪综合征的重度脊柱侧弯实验动物模型,引入对出现脊髓瘫痪综合征的病理过程时蛛网膜下腔脑脊液流体动力学通过性试验和脊髓血管造影试验来验证和重现这一病理过程,并对所建动物模型进行评价。

6　皮质脊髓束半横断损伤模型大鼠皮质脊髓束的超微结构改变

刘　苏(南通大学附属医院康复科,江苏省南通市　226001)

推荐理由: Wallerian变性是指受损神经纤维远侧端及其部分近侧端轴突及所属髓鞘发生变性、崩解和被吞噬细胞吞噬的过程,这是神经纤维分子水平的病理学改变。有关皮质脊髓束受损后的超微结构的变化国内报道甚少。

实验观察了大鼠皮质脊髓束损伤后Wallerian变性过程中皮质脊髓束的超微结构改变特征,为进一步研究皮质脊髓束损伤和修复机制提供形态学基础。

7　脊髓损伤模型大鼠神经修复与法舒地尔和RhoA基因沉默的干预

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推荐理由:中枢神经系统损伤后,除原发机械性损伤外,也会发生出血、缺血及其引发的一系列生物化学、细胞毒素物质、代谢产物、自由基等造成神经细胞再灌注损害、兴奋毒性、坏死、凋亡、炎性反应。其中中枢神经系统髓磷脂上存在大量的神经生长抑制因子包括髓鞘相关糖蛋白及少突胶质细胞髓鞘糖蛋白等抑制因子通过激活RhoA/ROCK通路致使生长锥塌陷,轴突再生受到抑制。法舒地尔是一种Rho激酶抑制剂,主要通过激活RhoA/ROCK受体通路,抑制Rho激酶的释放。

故实验通过Rho激酶抑制剂法舒地尔,以及RNA干预介导的RhoA基因沉默治疗大鼠脊髓损伤并比较其疗效。

8　高压氧前处理脊髓损伤大鼠前角运动神经元的凋亡

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推荐理由:脊髓损伤后对缺血耐受性较差,缺血缺氧可导致受损脊髓的二次损伤,并且损伤常常发生在受损后几小时到3 d。Chu等报道缺血缺氧可诱发多种因子引起DNA损伤和修复。

高压氧可提高血液中血红蛋白的氧饱和度,有报告称适度的高压氧可以预防二次损伤。

高压氧前处理可以提高中枢神经对缺血缺氧的耐受性,而其发生机制与线粒体对缺血再灌注的调节相关。在缺血再灌注过程中活性氧自由基的大量产生可导致线粒体内膜的腺嘌呤核苷酸移位酶的氧化巯基释放而导致其通透性增加,在早期的研究中显示高压氧前处理可上调超氧化物歧化酶及过氧化氢酶的活性。然而既往的研究多停留在脊髓缺血后高压氧的保护作用,而高压氧对直接的脊髓损伤后神经元的保护作用鲜有报道。

实验拟用高压氧前处理的手段探讨对脊髓损伤后的前角运动神经元的保护。

详见: www.crter.org/Html/2012_05_15/2_64024_2012_05_15_204170.html