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Study on the effects of gradient mechanical pressures on the proliferation, apoptosis, chondrogenesis and hypertrophy of mandibular condylar chondrocytes in vitro

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ABSTRACT

Objective: To investigate the effects of gradient mechanical pressure on chondrocyte proliferation, apoptosis, and the expression of markers of chondrogenesis and chondrocyte hypertrophy.
Methods: Mandibular condylar chondrocytes from 5 rabbits were cultured in vitro, and pressed with static pressures of 50 kPa, 100 kPa, 150 kPa and 200 kPa for 3 h, respectively. The chondrocytes cultured without pressure (0 kPa) were used as control. Cell proliferation, apoptosis, and the expression of aggrecan (AGG), collagen II (COL2), collagen X (COL10), alkaline phosphatase (ALP) were investigated. Ultrastructures of the pressurized chondrocytes under transmission electron microscopy (TEM) were observed.
Results: Chondrocyte proliferation increased at 100 kPa and decreased at 200 kPa. Chondrocyte apoptosis increased with peak pressure at 200 kPa in a dose-dependent manner. Chondrocyte necrosis increased at 200 kPa. The expression of AGG increased at 200 kPa. The expression of COL2 decreased at 50 kPa and increased at 150 kPa. The expression of COL10 and ALP increased at 150 kPa. Ultrastructure of the pressurized chondrocytes under TEM showed: at 100 kPa, cells were enlarged with less cellular microvillus and a bigger nucleus; at 200 kPa, cells shrank with the sign of apoptosis, and apoptosis cells were found.
Conclusions: The mechanical loading of 150 kPa is the moderate pressure for chondrocyte: cell proliferation and apoptosis is balanced, necrosis is reduced, and chondrogenesis and chondrocyte hypertrophy are promoted. When the pressure is lower, chondrogenesis and chondrocyte hypertrophy are inhibited. At 200 kPa, degeneration of cartilage is implied.

1. Introduction

Disc displacement of temporomandibular joint (TMJ) is an abnormal anatomic relationship between disc and mandibular condyle (Dolwick, 1995). Though many clinical studies reported the cor-relation of disc displacement and hypoplasia of mandible (Nebbe, Major, & Prasad, 1998; Yamada et al., 1999; Nakagawa, Sakabe, Nakajima, & Akasaka, 2002; Gidarakou, Tallents, Kyrkanides, Stein, & Moss, 2003; Gidarakou, Tallents, Kyrkanides, Stein, & Moss, 2004; Flores-Mir, Nebbe, Heo, & Major, 2006), the cause-effect sequence was still on controversial. However, many animal studies (Ali, Sharawy, O'Dell, & al-Behery, 1993; Bryndahl, Eriksson, Legrell, & Isberg, 2006; Bryndahl, Warfvinge, Eriksson, & Isberg, 2011; Legrell & Isberg, 1998, 1999; Li et al., 2014) reported that unilateral or bilateral disc displacement of TMJ induced mandibular asymmetry or retrognathia in growing animals.

The mandibular condyle performs as an important growth site in the maxillofacial development (Bryndahl et al., 2006, 2011). The growth modification of condylar cartilage directly influences the morphology and function of mandible. Endochondral ossification drives the mandibular advancement (Tang & Rabie, 2005), and mechanical stress may be the most important single environmental factor affecting joint homeostasis (Sun, 2010).

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When anterior disc displacement (ADD) of TMJ occurs, the stress on the posterior condylar articular surface increases (del Pozo, Tanaka, & Tanaka, 2003; Nishio et al., 2009). Mechanical stress may affect the morphology and metabolism of articular cartilage and chondrocyte in vivo (Wu, Herzog, & Epstein, 2000; Hasler, Herzog, Leonard, Stano, & Nguyen, 1998; Herzog, Clark, & Longino, 2004) and vitro (Kessler & Grande, 2008; Maeda et al., 2005). Functional stress on mandibular condyle stimulates the growth modification of condyle (Tang & Rabie, 2005). Overloaded (Wu et al., 2000) or insufficient (Hasler et al., 1998; Herzog et al., 2004) stress on cartilage of articular surface may induce dysfunctional remodeling.

Endochondal ossification is the pattern of mandibular condylar growth, and has been categorized into two phases: chondrogenesis and osteogenesis (Inoue, Nebgen, & Veis, 1995). Chondrocyte proliferation, differentiation and hypertrophy are included in the phase of chondrogenesis, Chondrocyte hypertrophy, as the terminal phase of chondrogenesis, is critical for the osteogenesis. Aggrecan (AGG) is an essential constituent of cartilage extracellular matrix (ECM). AGG sustains the osmotic pressure within the ECM, and is responsible for the functional properties of cartilage (Bernhard & Panitch, 2012). Collagen II (COL2) and collagen X (COL10) are the major types of collagen in condylar cartilage ECM. COL2 is cartilage-specific collagen in ECM, which is secreted by maturing chondrocytes (Chen, Carrington, Hammonds, & Reddi, 1991). COL10 is exclusively synthesized by hypertrophic chondrocytes, which is the sign of hypertrophy of chondrocyte (Salo, Hoyland, & Ayad, 1996). ALP is one of the terminal differentiation markers of chondrogenesis (Mrugala et al., 2009), which is associated with bone formation during endochondral ossification (Tuckermann, Pittois, Partridge, Merregaert, & Angel, 2000). Hence, as the terminal proteins, AGG and COL2 could be selected as the suitable markers of chondrogenesis, and COL10 and ALP would be the markers of chondrocyte hypertrophy.

Therefore, the chondrogenesis and hypertrophy of mandibular condylar chondrocytes may be influenced by mechanical pressure. In the present study we hypothesized that: (i) chondrogenesis and hypertrophy of the chondrocytes would be promoted under a moderate pressure, and (ii) chondrogenesis and hypertrophy would be inhibited under a pressure lower or higher than the moderate pressure. To validate this hypothesis, cell proliferation, apoptosis, necrosis, and the expression of AGG, COL2, COL10 and ALP were detected after mandibular condylar chondrocytes cultured under static pressures in vitro.

2. Materials and methods

2.1. Cell isolation and culture

The protocol for chondrocyte isolation was identical to that of a previously reported method (Huang et al., 2015). Condylar cartilage was harvested from five 2-week-old New Zealand white rabbits. The cartilage tissue was washed with phosphate-buffered saline (PBS) and sliced into pieces less than 1 mm^3. Then, the cartilage was digested with 0.25% trypsin (Gibco, New York, USA) for 30 min, and subsequently with 0.1% collagenase (Serva, Heidelberg, Germany) at 37 °C in a humidified atmosphere of 5% CO2. The chondrocytes were collected at 4 and 12 h after digested by centrifugation. The condylar chondrocytes were resuspended and replated in culture 6-well or 96-well plates with a suitable density of 2×105 cells/ml. P2 of condylar chondrocytes was used in this study.

The study was conducted according to the Guidelines for Animal Experimentation of Shanghai Jiao Tong University School of Medicine.

2.2. Application of static pressure

The pressure-loading system was described in our previous study (Huang et al., 2015). P2 of condylar chondrocytes was plated at a density of 2×10^5 cells/ml in 2 ml of medium. After 72 h of culture, the chondrocytes reached 70–80% confluence and then static pressure was applied. In addition, the growth medium was replaced 12 h before applied static pressure. Static pressures were applied at 50 kPa, 100 kPa,150 kPa and 200 kPa for 3 h, respectively. Chondrocytes cultured at 0 kPa were chosen as control. All specimens were harvested immediately after pressure loading finished.

2.3. Cell proliferation assay

Cell proliferation was quantified with CCK-8 (Dojindo, Kumamoto, Japan) method. P2 of chondrocytes plated in flat-bottomed 96-well plates at 2×10^5 cells/ml in 100 μl of medium. After 72 h of culture and 3 h pressure (0 kPa, 50 kPa, 100 kPa, 150 kPa and 200 kPa) applied, CCK-8 (10 μl in100 μl of medium per-well) was added to each well. After incubation at 37 °Cfor 4 h, the optical density (OD) value of each well was determined by using a microplate reader (Thermo, Rockford, USA) at 450 nm. The percentage of cell proliferation was determined as follows: cell proliferation = 100% × (OD in pressurized group/OD in the control group).

2.4. Apoptosis assay

Apoptosis was analyzed using the Invitrogen Annexin V-FITC apoptosis detection kit (BD, USA). The chondrocytes in the pressurized and control groups were harvested and stained with FITC-conjugated anti-Annexin V antibody and propidium iodide (PI). The stained chondrocytes were then detected by flow cytometry (BD, USA).

2.5. Real-time polymerase chain reaction

The total RNA was isolated from the chondrocytes using the Trizol reagent (Invitrogen, Carlsbad, CA). The RNA concentrations were determined using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE). The RNA was used for synthesizing complementary DNA (cDNA) with using a reagent kit (Takara, Japan) according to the manufacturer's instructions. Real-time polymerase chain reaction was performed on a real-time PCR (PCR) system (Bio-Rad, USA) with SYBR Premix Ex TaqTM (Takara, Dalian, China). Primer sequences for aggrecan (AGG), collagen II (COL2),

Table 1
Primer sequences for Real-time PCR.

Gene	Primers(F = forward,R = reverse)
AGG	F: CTCTGGGCAGGCAGACATAG R: CCCCATTCTTGTGGAGACCA
COL2	F: GGGTCCTTTGGCTGTTCAGA R: TTCTCCCCAGAAACACACCG
COL10	F: GGCTTCCCAGTGGCTGATAG R: TTTTGCTCTCTCTGGGTGGC
ALP	F: ATGGGATGGGTGTCTCCACA R: CCACGAAGGGGAACTTGTC
β-actin	F: TTATTGACTGCCGAGACCGC R: TACGAGTCCTTCTGGCCCAT