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A study on role of triiodothyronine (T3) hormone on the improvement of articular cartilage surface architecture

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ABSTRACT

The present study was aimed to investigate the effect of triiodothyronine (T3) on the improvement of articular cartilage surface architecture at *in vitro* level. The T3 hormone was applied to neo-tissues in the range of 50, 100, 150 and 200 ng/ml for 5 weeks. At the end of the treatment, biochemical and histological evaluation was carried out in the neo-tissues. T3 hormone application significantly increased the collagen production in neo-cartilage tissues. The properties of tensile and compressive were significantly increased compared to the controls. However, T3 hormone application also induced hypertrophy. At the higher dose concentration of T3 hormone application, tensile and compressive properties were tremendously increased 4.3 and 4.6 fold respectively. Taking all these data together, it suggested that the T3 hormone application could be a potential agent to increase the functional properties such tensile and compressive in neo-tissues.

1. Introduction

The thyroid hormone triiodothyronine (T3) is secreted from the parathyroid glands. It has been reported to regulate the development of cartilage and chondrocytes (Williams et al., 1998; Shao et al., 2006). The proliferation of hypertrophic chondrocytes has been promoted by the T3. In cell receptors, T3 is derived from T4 by the removal of iodine by enzyme action (Miura et al., 2002). Thyroid hormones have been known to upregulate Sox9 expression in chondrocytes plate growth (Studer et al., 2012), and T3 has been known to upregulate Runx2 expression (Wang et al., 2010).

Several therapies have been proposed to avoid joint replacement in the patient suffering from articular cartilage (De Windt et al., 2013). Anti-inflammatory and pain-relieving treatments have been known to reduce joint space, which increases the lubrication and degradation of the matrix (Elmorsy et al., 2014). Most of the therapies are very limited to active cartilage repair and osteoarthritis environment. Cartilage tissue engineering method has been known as a potential method for the focal cartilage therapy, and it has been known to delay the total joint arthroplasty.

Development of neocartilage and mechanical properties has been attained through the application of exogenous compound (Makris et al., 2013). The good biochemical features and increased mechanical properties increased through the development of neocartilage. Administration of thyroid hormone has been reported to promote the production of cartilage matrix in the osteoarthritic model (Kudo et al., 2011). Therefore, the present study was aimed to investigate the effect of T3 hormone application on articular chondrocytes.

2. Materials and methods

2.1. Materials

Dulbecco Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, and trypsin-EDTA were obtained from Welgene (Daegu, South Korea). Type II collagenase and triiodothyronine (T3) were purchased from Sigma-Aldrich (St. Louis, MO 63178 USA).

2.2. Isolation of chondrocyte

Bovine joints were obtained from the Research Centre (Fudan University, 138 Yixueyuan Rd, Xuhui Qu, Shanghai Shi, China). The articular chondrocytes were isolated from the distal femurs within 24 h. Collagenase enzyme was used for the tissue digestion. DMEM was used for the cell washing, and cells were made in stock and stored at −80 °C for the further use.

Abbreviations: PTH, Parathyroid hormone; GAG, Glycosaminoglycan; T3, Triiodothyronine; DMEM, Dulbecco's Modified Eagle Medium; SD, Standard Deviation

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2.3. In vitro neocartilage constructs

Scaffold-free constructs were made by culturing 1×10^5 cells. The scaffold was made according to the previously reported method (Elder and Athanasiou, 2008). Cells were provided chondrogenic medium and transferred to 48 well plates following the formation of constructs.

2.4. Treatment

The T3 hormone was applied in the range of 50, 100, 150 and 200 ng/ml for 5 weeks. Controls were maintained without treatment. At the end of the treatment, samples were collected for the investigation.

2.5. Total DNA estimation

Total DNA was estimated in the constructs according to the previously reported method (Rawal et al., 1977). Briefly, a known volume of the nucleic acid extract was made up to 2 ml with perchloric acid (1N), and it was mixed with diphenylamine (2 ml). This was kept in a boiling water bath for 15 min, and the blue color developed was read at 640 nm in a spectrophotometer. The DNA content was expressed as µg.

2.6. Estimation of total glycosaminoglycan

Total glycosaminoglycan (GAG) content was estimated in the constructs using Blyscan Glycosaminoglycan Assay kit (Invitrogen, USA). Briefly, place tissue and papain extraction reagent to the volume of 1 ml, and kept in boiling water bath at 65 °C for overnight digestion of tissue. Tubes were centrifuged for 15 min at 10000*g*, and glycosaminoglycan was measured by measuring absorbance peak is broad and most microplate readers 675 nm (Heinegard, 2009).

2.7. Estimation of total collagen

Chloramine-T hydroxyproline assay was used to determine the total collagen in the constructs (Woessner Jr, 1961). The method is based on the acid hydrolysis of tissue extract samples to form the hydroxyproline and hydrolysates. Hydroxyproline oxidized to form a reaction intermediate, which further in the reaction, forms a chromophore and it was measured at 560 nm.

2.8. Histopathological analysis

Tissue samples were prepared for the histological analysis according to Muthuviveganandavel et al. (2008). Briefly, tissues were fixed with neutral formalin (10%) and embedded in paraffin. Tissues were sectioned with the use of a microtome to obtain 4–5 µm-thick paraffin sections. Finally, sections were dewaxed and stained with hematoxylin and eosin (H & E).

2.9. Immunohistochemical analysis

Safranin-O/Fast Green and picrosirius red were used analyze the GAG and collagen distribution respectively (Leong and Wright, 1987). Mineralization was analyzed by using alizarin red staining probe. All the slides were fixed in acetone. Fixation process was carried out for at least 20 min at 4 °C. Hydrogen peroxide (2%) was used to quench all the endogenous peroxidases. Bovine serum albumin (BSA) (1%) was used for the blocking. Slides were incubated with the anti-mouse monoclonal primary antibody (1:500) was used for 60 min, and then horseradish peroxidase (HRP)-conjugated secondary antibody (1:300) was applied for 40 min.

2.10. Data analysis

Results were expressed as mean ± SD. The difference between treated and control group has been evaluated using Student's *t*-test. A $p < 0.05$ was considered statistically significant.*P < 0.05, **P < 0.01 and ***P < 0.001.

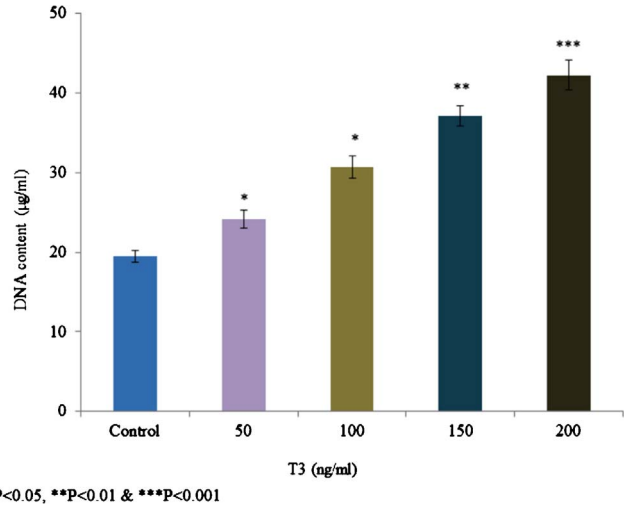

Fig. 1. Effect of the T3 hormone on DNA content. The T3 hormone was treated to *in vitro* chondrocytes. Total DNA content was expressed as ug/ml. The experiment was repeated thrice. *P < 0.05, **P < 0.01 and ***P < 0.001.

3. Results

3.1. Effect of T3 hormone on DNA content

Total DNA content was estimated in control and T3 hormone-treated samples and expressed as µg/ml. Total DNA content was found to be 19.45 µg/ml in control. T3 hormone treatment significantly increased total DNA content 24.15, 30.66, 37.12 and 42.25 µg/ml at 50, 100, 150 and 200 ng/ml respectively (Fig. 1, *P < 0.05, **P < 0.01, ***P < 0.001).

3.2. Effect of T3 hormone on glycosaminoglycan

Glycosaminoglycan (GAG) content was estimated in control and T3 hormone-treated samples and expressed as µg/ml. GAG content was found to be 621.55 µg/ml in control. T3 hormone treatment significantly increased GAG content 691.83, 755.32, 811.91 and 915.63 µg/ml at 50, 100, 150 and 200 ng/ml respectively (Fig. 2,

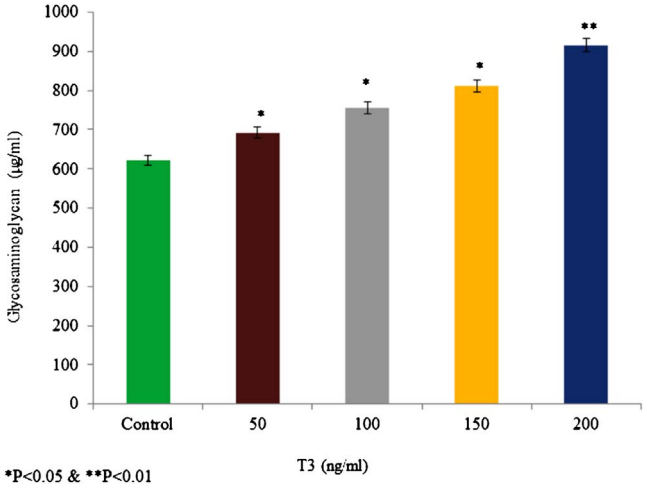

Fig. 2. Effect of the T3 hormone on GAG content. The T3 hormone was treated to *in vitro* chondrocytes. GAG content was expressed as ug/ml. The experiment was repeated thrice. *P < 0.05 and **P < 0.01.