THE HYALURONAN CONTENT OF HUMAN KNEE OSTEOARTHRITIC CARTILAGE: TOPOGRAPHICAL STUDY AND EFFECT OF MECHANICAL STRESS

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INTRODUCTION:
Osteoarthritis (OA) is a group of degenerative disease of diarthrodial joints that are associated with remodeling of articular cartilage, subchondral bone and synovial inflammation. The load-bearing properties of cartilage are essential factors in the regulation of cartilage metabolism, although the mechanisms have not been determined (1). Hyaluronan (HA) is one of the major space-filling molecule in the extracellular matrix of articular cartilage, which has a viscoelastic function to prevent from the load-bearing and in clinical HA used as a viscosupplementation device to treat OA-related joint pain.

The purpose of this study was to 1) clarify the distribution of the HA content and to 2) compare the relationship between mechanical axis and the HA distribution in human knee OA cartilage.

METHODS:
CARTILAGE SAMPLES
Cartilage samples were obtained from 32 knees of 28 patients (average age: 75.3 years; age range: 60-83 years) undergoing total knee replacement surgery. Full-depth sections of cartilage from four different areas, excluding osteophytes, were taken (Fig. 1). These areas were included the 1) weight-bearing area of the medial femoral condyle (MFC), 2) weight-bearing area of the lateral femoral condyle (LFC), 3) non-weight-bearing area of the patellar groove (PG), and 4) non-weight-bearing area of the lateral posterior femoral condyle (LPC). Articular cartilage from these areas was shaved from the bone while on ice, and the wet weight of the cartilage was measured.

HPLC METHOD
The cartilage samples were digested with 2 ml. of a 2.5% solution of a protease mixture derived from Streptomyces griseus (actinase E) at 55°C for 24 hours. The concentration of HA was also analyzed according to the method of Takazono et al. (2) with some modifications. To 150 ml of a 10-fold dilution of the actinase E-treated supernatant, hyaluronidase (2.5 TRU) was added, followed by the addition of 25 ml of 100 mM sodium acetate buffer (pH 6.0) and incubated at 37°C for 16 hours. The filtrate, which contains the unsaturated tetrasaccharides of HA (delta Tetra-HA) and the unsaturated hexasaccharide of HA (delta Hexa-HA) derived from HA was analyzed by the HPLC system. The area of each peak corresponding to delta Tetra-HA and delta Hexa-HA was calculated.

All knees were graded radiographically for OA using the Kellgren/Lawrence (KL) classification. To assess the mechanical axis of the leg, the coronal radiological femorotibial angle (FTA) was measured prior to surgery.

STATISTICAL ANALYSES
Non-repeated measures ANOVA was employed for the significance test for among over three groups, and multiple comparison test was applied thereafter using Tukey test analysis between two groups. A p value of < 0.05 was regarded as significant. All analyses were done using JMP 5.12.

RESULTS
All knees were graded radiographically for OA using the KL classification. The KL classification of the 32 knee joints from 28 patients was as follows: grade 1, n = 3; grade 2, n = 7; grade 3, n = 16; and grade 4, n = 6.

The HA content of cartilage: The HA levels (ng/mg wet weight) of cartilage obtained from the weight-bearing area of the MFC showed a significant decrease with advancing stage of OA. Cartilage obtained from the other regions (LFC, PG, LPC) did not show significant changes in HA content (Fig. 2).

Effect of mechanical axis on the HA content of cartilage: When the HA content of cartilage obtained from the weight-bearing area of the MFC were compared to the FTA, there were a significant negative correlation between the FTA and cartilage HA content (p = 0.0062, r = -0.47, Fig. 3). There were no significant alterations in the HA content of the weight-bearing area of the LFC with advancing OA. We examined the HA ratio which is lateral content was divided for medial in each individual. As a noteworthy event, the ratio showed that When this was performed, the HA ratio increased until about 190 degree FTA as peak with a sharp drop at > 190 degree, reflecting a decrease in HA content in the LFC. R binary of this spline curve is 0.71, and this curve and graph are considerably high correlation (Fig. 4).

REFERENCES:

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