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대한영상의학회지

The Effects of Proteoglycan and Type II Collagen on T1ρ Relaxation Time of Articular Cartilage

Proteoglycan과 Type II 콜라겐이 관절연골의 T1ρ 이완시간에 미치는 영향에 대한 연구

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Purpose: To evaluate the effects of proteoglycan and type II collagen within articular cartilage on T1ρ relaxation time of articular cartilage.

Materials and Methods: This study was exempted by the institutional and animal review boards, and informed consent was not required. Twelve porcine patellae were assigned to three groups of control, trypsin-treated (proteoglycan-degraded), or collagenase-treated (collagen-degraded). The T1ρ images were obtained with a 3 tesla magnetic resonance imaging scanner with a single loop coil. Statistical differences were detected by analysis of variance to evaluate the effects of the enzyme on T1ρ relaxation time. Safranin-O was used to stain proteoglycan in the articular cartilage and immunohistochemical staining was performed for type II collagen.

Results: Mean T1ρ values of the control, trypsin-treated, and collagenase-treated groups were 37.72 ± 5.82, 57.53 ± 8.24, and 45.08 ± 5.31 msec, respectively ($p < 0.001$). Histology confirmed a loss of proteoglycan and type II collagen in the trypsin- and collagenase-treated groups.

Conclusion: Degradation of proteoglycans and collagen fibers in the articular cartilage increased the articular cartilage T1ρ value.

Index terms
Osteoarthritis
Proteoglycan
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Cartilage
T1ρ Imaging

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INTRODUCTION

Osteoarthritis, also known as degenerative arthritis, is a very common and significant disease characterized by decreases in proteoglycan (PG) and collagen fibers in the articular cartilage (1, 2). Although many cartilage regeneration treatment methods have been developed due to advances in molecular biology and stem cell research (2, 3), cartilage damage is irreversible, and the only treatment method for severely damaged articular cartilage is arthroplasty with an artificial joint. Thus, early detection of degeneration is important to enable early therapeutic intervention, reduced disability, and improve patient quality of life (1-3).

Recent advances in magnetic resonance imaging (MRI) have made it possible to detect degenerative changes in articular cartilage, which are characterized by changes only in the biochemical component with minimal or no structural changes (4-10). Delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC) is a method to quantify loss of PGs in articular cartilage (5). However, a double dose of intravenous contrast media is required for this type of imaging, and patients must wait 2 hours after the injection before analysis. T2 mapping is a MRI method used to measure T2 relaxation time, which is influenced by the content and orientation of collagen fibers in articular cartilage. Thus, T2 mapping visualizes changes in artic-

ular cartilage collagen fibers but has low sensitivity and lacks specificity to quantify PG loss (7, 9, 11, 12). T1ρ relaxation time is a parameter related to the energy exchange between water molecules and the surrounding environment by spin-lattice relaxation in a rotating frame. Unlike dGEMRIC and T2 mapping, T1ρ imaging does not require contrast media or a long scan time and has better sensitivity than that of T2 mapping. Thus, T1ρ imaging has been proposed as an attractive alternative method to probe biochemical changes in cartilage (7, 13). Several studies have demonstrated that T1ρ relaxation time reflects macromolecules, such as PGs and collagen, in the articular cartilage extracellular matrix (8, 9). Nishioka et al. (8) showed that T1ρ relaxation time varies with degenerative changes in human articular cartilage. Akella et al. (14) showed that T1ρ relaxation time is directly proportional with loss of PGs in articular cartilage. However, it remains unclear whether *in vivo* T1ρ relaxation time reflects the changes in PGs, collagen fibers, or both that result from cartilage degeneration (15, 16).

Thus, the purpose of this study was to evaluate the effects of PGs and type II collagen on T1ρ values of articular cartilage.

MATERIALS AND METHODS

This study was exempted by the institutional and animal review boards, and informed consent was not required.

Cartilage Preparation

Twelve fresh porcine patellae from 1–2-year-old pigs were obtained from a local slaughter house within 6 hours after sacrifice. After dissecting the surrounding soft tissue, the patellae were sagittally hemisected with a mechanical cutter and stored frozen. The cartilage was thawed at room temperature for the investigation. We assigned the specimens to three groups as follows: 1) trypsin-treated (n = 4), 2) collagenase-treated (n = 4), and 3) control (n = 4).

The patellae were placed in a vial containing 1 mg trypsin (Sigma, St. Louis, MO, USA) in 1 mL phosphate buffered saline (PBS) for the trypsin-treated group, in a vial containing 135 units collagenase (Sigma) in 1 mL PBS for the collagenase-treated group, and in PBS without enzymes for the control group. We expected the trypsin and collagenase to degrade the PGs and collagen, respectively. All three groups were incubated for 6 hours at 37°C with gentle agitation. Each patella was placed in a container half-filled with agarose gel. The flat surface of the hemisected patella was fixed to an agar plate using adhesive. Perfluorocarbon was used as the buffer, which filled the empty space in the container.

Acquisition of T1ρ Imaging by MRI

T1ρ imaging was obtained with a 3 tesla MRI scanner (Trio; Siemens Medical Solutions, Erlangen, Germany) with a single loop coil. The specimen was loaded into the coil ensuring that one of the articular surfaces was perpendicular to the B_0 direction of the magnetic field. Coronal two-dimensional T1ρ-weighted images were obtained using the spin-lock technique and spiral image acquisition. The acquisition parameters were: field of view = 6 × 6 cm, matrix = 128 × 128, effective in-plane spatial resolution = 0.47 × 0.47 mm, slice thickness = 3 mm, repetition time/echo time = 3500/3.08 ms, number of excitation = 2, spin-lock time (TSL) = 0/10/30/50/80 ms, flip angle = 30°, and spin-lock frequency = 500 Hz. Acquisition time was 1 min 30 sec for each TSL.

Reconstructing the T1ρ Map

T1ρ maps were reconstructed by fitting image intensity pixel-by-pixel to the equation below using the mono-exponential fitting algorithm: $S(TSL) = S_0 \times \exp(-TSL / T1\rho)$, where TSL is spin-lock time, and S is the signal intensity of the T1ρ-weighted image with a given TSL. The articular cartilage T1ρ map was constructed with a homemade mapping program using Mat Lab R2013 (MathWorks, Natick, MA, USA). After obtaining the T1ρ map result as a DICOM file, the T1ρ value of each patellar cartilage was measured using a Picture Archiving Communication System (M-view, Infinitt, Seoul, Korea). The mean T1ρ value of the patellar cartilage was evaluated by placing the region of interest in the patellar cartilage using a freehand drawing technique (Fig. 1B). The program also provided a T1ρ map as a color mapping file (Fig. 1A).

Histological Examination

All patellae were sagittally sawed with a 3–4 mm thickness thin plate using a mechanical cutter immediately after MRI scanning. The thin plates of cartilage were fixed in neutral buffered formalin at room temperature. After decalcification and paraffin embedding, the cartilage plates were cut into tiny fragments. The plates were stained with Safranin-O for PGs, and

A B

Fig. 1. Reconstruction of T1ρ map and measurement of T1ρ value.
A. Image shows result of T1ρ map using color scale.
B. Image shows positioning of region of interest to calculate the T1ρ value of full-thickness cartilage. Region of interest was placed on the patellar cartilage with a freehand drawing technique.

Table 1. Results of T1ρ Value of Articular Cartilage for Each Group

Group	T1ρ Value (msec)	Mean T1ρ Value (msec) ± Standard Deviation	*p*-Value of ANOVA Test
Trypsin-treated group	56.04	57.53 ± 8.24	0.000
	50.01		
	54.82		
	69.25		
Collagenase-treated group	48.70	45.08 ± 5.31	
	44.74		
	49.01		
	37.59		
Control group	42.03	37.72 ± 5.82	
	43.30		
	31.53		
	34.01		

Note.—ANOVA = analysis of variance

immunohistochemical staining was used to detect type II collagen. Safranin-O staining was performed with a 0.1% Safranin-O solution, and with a 0.2% aqueous solution of Fast Green used as a counter-stain. The tissue sections for type II collagen immunohistochemistry were deparaffinized in xylene and rehydrated in a reverse-graded ethanol series. The sections were immunohistochemically stained with rabbit polyclonal antibodies against rabbit type II collagen (Abcam, Cambridge, UK) and goat anti-rabbit IgG/biotin (Abcam) using the manufacturer's standard protocol (17).

Statistical Analysis

Analysis of variance was used to determine the effect of the enzymes on T1ρ relaxation time. Two-sided *p*-values < 0.05 were considered significant. All analyses were performed using SPSS for windows ver. 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

T1ρ Relaxation Time

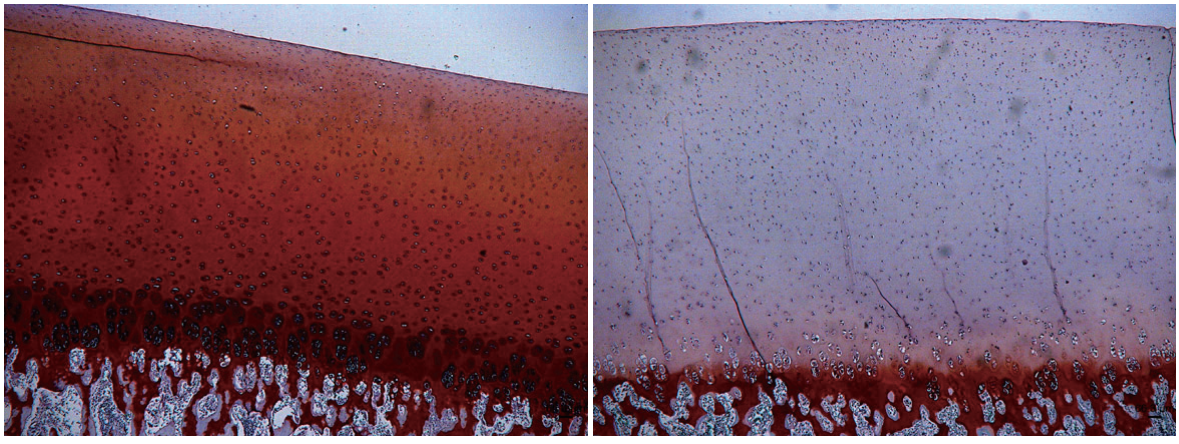
Mean T1ρ values of the control, trypsin-treated, and collagenase-treated groups were 37.72 ± 5.82, 57.53 ± 8.24, and 45.08 ± 5.31 msec, respectively ($p < 0.001$). The mean T1ρ value of the trypsin-treated group was significantly higher than that of the control and collagenase-treated groups ($p < 0.001$). The difference in the T1ρ values between the control and the collagenase-treated groups was small, but significant ($p = 0.008$). Table 1 summarizes the T1ρ values for each group.

Comparison of T1ρ Mapping by Histology and Immunohistochemistry

Loss of PGs was observed in the trypsin-degraded group and was confirmed by microscopy in the Safranin-O stained specimens. The histological samples of normal articular cartilage showed a dense and homogeneous distribution of PGs (Fig. 2A), whereas those of Safranin-O stained trypsin-degraded articular cartilage showed observable decreases in PGs (Fig. 2B). The color-mapped T1ρ data, wherein a color scale represents the T1ρ value, illustrated high T1ρ values in trypsin-degraded articular cartilage compared with those of control articular cartilage. Similarly, loss of type II collagen was confirmed by microscopy and immunohistochemical staining with a rabbit polyclonal antibody against type II collagen. The histological samples of normal articular cartilage showed a dense and homogeneous distribution of collagen II fibers (Fig. 3A), whereas those of the collagenase-degraded articular cartilage showed observable decreases in collagen II fibers on the immunohistochemically stained specimens (Fig. 3B).

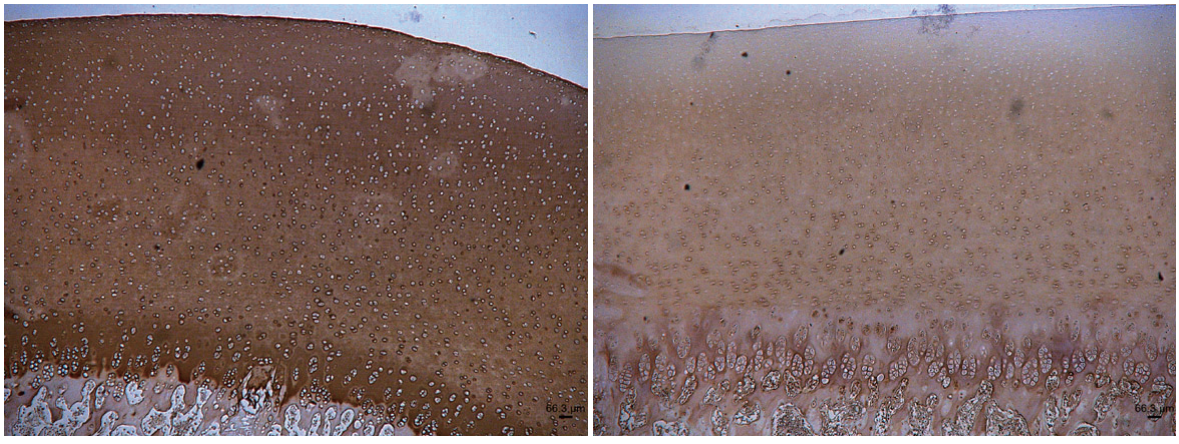
DISCUSSION

Our results show that 3-T MRI and T1ρ mapping can be used to quantify loss of PGs and collagen II fibers. Because cartilage



A B

Fig. 2. Safranin-O staining for proteoglycans.
A. In Safranin-O staining, control cartilage showed a dense and homogeneous distribution of proteoglycans.
B. In Safranin-O staining, trypsin degraded articular cartilage showed observable decreases of proteoglycans.



A B

Fig. 3. Immunohistochemical staining for type II collagen.
A. In immunohistochemical staining with antibodies against type II collagen, control cartilage showed a dense and homogeneous distribution of collagen II fibers.
B. In immunohistochemical staining with antibodies against type II collagen, collagenase degraded articular cartilage showed observable decreases of collagen II fibers.

damage is irreversible, quantification and early detection of osteoarthritis is important. Our results agree with those of previous studies. Duvvuri et al. (18) suggested that T1ρ measurements are selectively sensitive to PG content and can potentially distinguish early degenerative changes in cartilage associated with osteoarthritis. Li et al. (19) demonstrated that the T1ρ value is significantly but moderately correlated with PG content and reflects the degree of histological cartilage degeneration. Nishioka et al. (8) revealed that the T1ρ value reflects cartilage glycosaminoglycan content and indicates *in vivo* cartilage degeneration. They also insisted that a noninvasive diagnosis and evaluation of cartilage degeneration can be facilitated using the T1ρ value. Thus, similar with a previous study, our results suggest that T1ρ MRI has the potential to provide noninvasive imaging biomarkers for cartilage degeneration by measuring articular cartilage T1ρ values, which are affected by PG content. This process could enable early detection and treatment of degenerative arthritis, which could prevent invasive treatments, such as arthroplasty with an artificial joint.

A few controversial studies have reported a relationship between the articular cartilage T1ρ value and collagen fiber content. Menezes et al. (20) reported a relationship between T1ρ value and collagen concentration. However, Duvvuri et al. (18) revealed that the T1ρ value does not increase in collagen-degraded bovine patellae. Li et al. (19) also reported no correlation between the T1ρ value and collagen content in degenerated human cartilage. In our study, the T1ρ value increased in the collagen-degraded specimens but less than that in the PG-degraded specimens. We postulate that changes in the T1ρ value based on collagen content are small in articular cartilage; thus, the controversial results reported by previous studies may have been dependent on the study population or design. Our results suggest that both PG and collagen contents should be considered at the time of application in clinical practice.

Our study had several limitations. First, the number of specimens was small. However, we showed significant differences in T1ρ values among the three different enzyme groups. Second, quantitative biochemical assays were not performed to assess loss of PG and type II collagen in articular cartilage. The histological staining only showed the qualitative loss of macromolecules. We revealed the feasibility of T1ρ mapping using a clinical MRI scanner to quantify and evaluate cartilage degeneration; therefore, further studies, including an evaluation of *in vivo* animal joints, should be conducted to apply this process in clinical practice.

In conclusion, degradation of articular cartilage PG and collagen fiber content increased the T1ρ value of articular cartilage.

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Proteoglycan과 Type II 콜라겐이 관절연골의 T1ρ 이완시간에 미치는 영향에 대한 연구

최원석 · 류혜진 · 홍성환 · 최자영

목적: 본 연구는 관절연골 내에 존재하는 proteoglycan과 type II 콜라겐이 관절연골의 T1ρ 이완시간에 미치는 영향에 대해서 알아보고자 한다.

대상과 방법: 본 연구에는 총 12개의 돼지 슬개골이 사용되었고, trypsin-분해군, collagenase-분해군, 대조군의 3개의 군으로 분류되었다. 대조군을 제외한 실험군에는 각각 trypsin 또는 collagenase를 이용하여 proteoglycan 또는 type II collagen을 분해하였다. 이후 3 테슬라 자기공명영상 촬영기와 single loop 코일을 이용하여 관절연골의 T1ρ 이완시간을 얻은 후, analysis of variance 방법을 이용하여 각 군에서 T1ρ 이완시간의 차이를 비교하였다. 실험에 사용된 돼지 슬개골에는 proteoglycan에 대한 Safranin-O 염색, type II collagen에 대한 면역염색이 시행되었다.

결과: 관절연골 면의 평균 T1ρ 이완시간 값은 대조군에서 37.72 ± 5.82 msec, trypsin-분해군에서 57.53 ± 8.24 msec, 그리고 collagenase-분해군에서 45.08 ± 5.31 msec였고, 이는 통계적으로 유의한 차이를 보였다($p < 0.001$). Safranin-O 염색에서는 proteoglycan의 결손을, type II collagen에 대한 면역염색에서는 collagen의 결손을 확인하였다.

결론: 관절연골 내 proteoglycan과 collagen 섬유의 소실에 따라 관절연골의 T1ρ 이완시간 값은 증가하였다.

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