의학석사 학위논문

Several factors contributing to variations of T1 ρ mapping of articular cartilage

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최 원 석

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Several factors contributing to variations of T1 ρ mapping of articular cartilage

by

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ABSTRACT

Introduction: Degenerative arthritis is a very common and important disease that is characterized by decreasing proteoglycan and collagen fibers in the articular cartilage. Early detection of degeneration and prediction of the development of advanced osteoarthritis are important for planning the treatment of osteoarthritis patients. The purpose of this study is to evaluate various factors that contribute to variations in the T1 ρ value of articular cartilage.

Methods: This study was exempted from institutional and animal review board reviews, and informed consent was not required. Twelve porcine patellae were assigned into the following three groups: trypsin-treated (proteoglycan-degraded), collagenase-treated (collagen-degraded), and control groups. T1 ρ imaging was obtained using 3 Tesla magnetic resonance imaging(MRI) scanners with a single loop coil at the three different orientations with respect to the main magnetic field. The T1 ρ relaxation map of articular cartilage was constructed with a homemade mapping program using Mat lab R2013. Significant differences were explored using the ANCOVA test to evaluate the effects of the enzyme and orientation on the T1 ρ relaxation time.

Results: There was a statistically significant difference in the T1 ρ values among the three different enzyme groups ($P<0.001$). However, there was no significant difference in the T1 ρ values among the three different orientations ($P=0.220$).

Conclusions: Degradation of the proteoglycans or collagen fibers in the articular cartilage caused an increase in the T1 ρ value of articular cartilage, but the orientation dependence of the T1 ρ value was not proven.

Keywords: Osteoarthritis, trypsin, collagenase, proteoglycan, collagen type II, T1 ρ

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LIST OF ABBREVIATIONS

MRI = Magnetic resonance imaging

ANCOVA = Analysis of covariance

dGEMRIC = Delayed gadolinium-enhanced magnetic resonance imaging of cartilage

GAGs = Glycosaminoglycans

INTRODUCTION

Osteoarthritis, also known as degenerative arthritis, is a very common and important disease that is characterized by decreases in the proteoglycan and collagen fibers of the articular cartilage (1, 2). Although many types of treatment methods have been challenged in cartilage regeneration by virtue of the advances in the fields of molecular biology and stem cell research (2, 3), cartilage damage is still known as an irreversible process, and the only method for severely damaged articular cartilage is arthroplasty with an artificial joint. Therefore, the early detection of degeneration is important to enable the early therapeutic intervention, reduction of disability, and eventual improvement in the patient's quality of life (1, 2, 3). Furthermore, subclinical biochemical changes in articular cartilage might be a predictive factor of the development of the advanced osteoarthritis; therefore, they could be important when planning treatment for osteoarthritis patients (2, 3). Recent advances in MRI make it possible for degenerative changes in articular cartilage to be characterized by only changes in the biochemical components with minimal or no structural changes (4-10). Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) is an MRI method for quantifying the loss of proteoglycans (PGs) in the articular cartilage. MRI can be used to visualize PGs (5). However, a double dose of intravenous contrast media is needed for imaging, and patients should wait for 2 hours after the injection of intravenous contrast media. T2 mapping is an MRI method for measuring the T2 relaxation time, which is influenced by the

content and orientation of collagen fibers in the articular cartilage. Therefore, T2 mapping could be used to visualize changes in the collagen fibers in articular cartilage, but this method has low sensitivity and a lack of specificity for quantifying proteoglycan loss, which are the drawbacks of T2 mapping (7, 9, 11, 12). The T1 ρ relaxation time is a parameter that is related to the energy exchange between water molecules and the surrounding environment via spin-lattice relaxation in a rotating frame. Unlike dGEMRIC and T2 mapping, T1 ρ imaging does not require contrast media or a longer scan time and is more sensitive than T2 mapping. Although the requirements of specific technical modulation and issues with the specific absorption rate (SAR) are obstacles for the clinical application of T1 ρ imaging, T1 ρ imaging has recently been proposed as an attractive alternative imaging method for probing biochemical changes in cartilage (7,13). According to Nishioka and Stahl et al (8, 9), the T1 ρ relaxation time is influenced by the contents of macromolecules, such as PGs and collagen fibers. Nishioka et al (8) showed that the T1 ρ relaxation time varies with degenerative changes in the articular cartilage with the use of human articular cartilage. Akella et al (14) showed that the T1 ρ relaxation time has a direct and proportional relationship with the loss of the PGs in the articular cartilage. However, T1 ρ imaging does not correlate with dGEMRIC imaging, suggesting that the T1 ρ relaxation time is a complex value that could be influenced by both the PGs and collagen fibers and other molecules (15, 16). Another factor related to the T1 ρ relaxation time is the orientation of collagen fibrils in cartilage because the spin-lock technique cannot completely eliminate the residual dipolar interaction in the organized collagen structure in

cartilage (17). Wang et al (17) found that the collagen orientation in nasal cartilage can influence the T2 and T1 ρ values. However, to the best of our knowledge, few researchers have investigated the factors that could change the T1 ρ relaxation time of the articular cartilage, such as PGs, collagen fibers, and the orientation of articular cartilage. Therefore, the purpose of this study is to evaluate various factors that contribute to the variations in the T1 ρ relaxation time of articular cartilage.

MATERIALS AND METHODS

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

Cartilage preparation

A total of 12 fresh porcine patellae of 1- to 2-year-old pigs were obtained from the local slaughterhouse within 6 hours after sacrifice. After dissecting the surrounding soft tissues, the patellae were sagittaly hemisected with a mechanical cutter and stored while frozen. Before the degradation with enzyme, cartilage was thawed at room temperature. We assigned the specimens into the following three groups: (a) trypsin-treated (n=4), (b) collagenase-treated (n=4), and (c) control (n=4).

The patellae were placed in a vial containing 1 mg trypsin (Sigma, St. Louis, MO, USA) in 1 ml of PBS for the trypsin-treated group, in a vial containing 135 units of collagenase (Sigma, St. Louis, MO, USA) in 1 ml of PBS for the collagenase-treated group, and in a vial of PBS without any enzyme for the control group. We expected that trypsin and collagenase would degrade the proteoglycans and collagen, respectively. All three groups were incubated for 6 hours at 37℃ with gentle agitation. After degradation, each patella was placed in a container that was half-filled with agarose gel. The flat surface of the hemisected patella was fixed to the agar plate with adhesive. Perfluorocarbon (PFC) was used as buffer, which filled the empty space of the container.

MRI acquisition and T1 ρ relaxation time acquisition

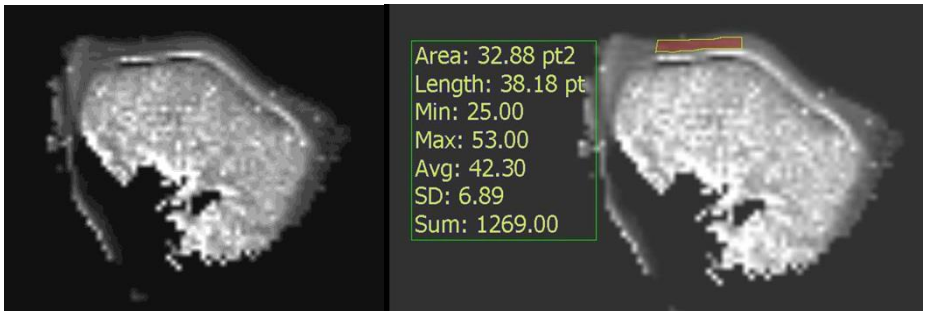
The T1 ρ imaging was obtained with a 3 Tesla MRI scanner (Trio, Siemens Medical Solutions, Erlangen, Germany) with a single loop coil. The specimen was loaded into the coil, and we ensured that one of the articular surfaces was perpendicular to the magnetic field B0 direction, which was used as the reference angle, i.e., the angle between the B0 and a line perpendicular to one of the subchondral bone plates is 0° (Fig. 1a). Because the collagen fibrils in the deep layer of cartilage are oriented perpendicular to the subchondral bone plate, the subchondral bone plate was used as a surrogate for the collagen fibril orientation. To observe the orientation dependency of the T1 relaxation time, specimens were scanned at the following three orientations with respect to the main magnetic field (B0): 0°, 90°, and 56°. Coronal 2D T1ρ-weighted images were obtained using spin-lock techniques and spiral image acquisition. The acquisition parameters were as follows: FOV=6x6cm, matrix=128x128, effective in-plane spatial resolution=0.47x0.47mm, slice thickness=3 mm, TR/TE =3500/3.08 ms, NEX=2, time of spin-lock (TSL) =0/10/30/50/80ms, flip angle=30° and spin-lock frequency= 500 Hz. The acquisition time was 1 min 30 sec for each TSL.

T1 ρ relaxation time acquisition

T1 ρ maps were reconstructed by fitting the image intensity pixel-by-pixel to the equation below using the mono-exponential fitting algorithm: S

(TSL) = S_0xexp (-TSL/T1 ρ), where TSL is the time of spin-lock and S is the signal intensity of the T1 ρ-weighted image with a given TSL. A T1 ρ map of the articular cartilage was constructed using a homemade mapping program in Matlab R2013 (MathWorks, MA, USA). After obtaining the T1 ρ map result as a DICOM file, the T1 ρ value of the cartilage of each patella was measured using the Picture Archiving Communication System (M-view, Infinite, Korea). The mean T1 ρ value of the cartilage of each patella was evaluated by placing a region of interest in the patellar cartilage using a freehand drawing technique (Fig. 1b). The measured angle between one of the articular surfaces and the magnetic field, B0, was evaluated using the Cobb angle technique (Fig. 1c). The program also provided us with a T1 ρ map as a color mapping file (Fig. 2).

(A) (B)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Length: 38.18 pt
Min: 25.00
Max: 53.00
Avg: 42.30
SD: 6.89
Sum: 1269.00

(C)

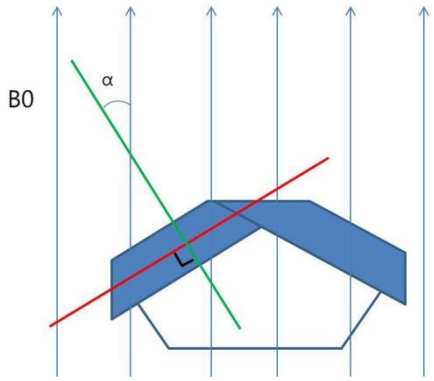
B0
α

Figure 1.

Measurement and image analysis of the specimens.

(a) Image showing that the left side of the articular surface is perpendicular to the magnetic field B0 direction, which was used as the reference angle, i.e., the 0° orientation of the specimen.

(b) Image showing the positioning of the region of interest to calculate the T1 ρ relaxation time of full-thickness cartilage. The region of interest was placed on the patellar cartilage using a freehand drawing technique.

(c) Image showing the use of the Cobb angle to calculate the measured angle between a line perpendicular to the one of the articular surface and the magnetic field B0 direction.

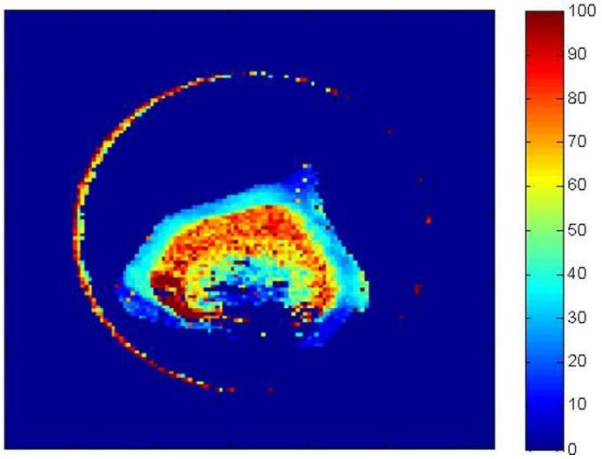

Figure 2.

Image showing the result of the T1 ρ map using a color scale.

Statistical Analysis

To determine the effect of the enzyme and orientation on the T1 ρ relaxation time, an analysis of covariance (ANCOVA) was used. Using the ANCOVA test, we could compare the difference in the T1 ρ values of the cartilage among the three different groups without affecting the orientation. We also compared the difference in the T1 ρ values of the cartilage for the three different orientations without affecting the enzyme. An additional subgroup analysis was performed to evaluate the orientation-dependency of the T1 ρ relaxation time in each enzyme group with analysis of variance (ANOVA). Two-sided P values of < 0.05 were considered to indicate statistical significance. All analyses were performed using SPSS for Windows version 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

T1 ρ value evaluation

The mean T1 ρ relaxation time of the trypsin-treated group was 57.53 ± 8.24 msec for 0°, 58.20±6.48 msec for 56°, and 55.99±6.48 msec for 90°. In the collagenase-treated group, the T1 ρ value was 45.08±5.31 msec for 0°, 50.04±1.96 msec for 56°, and 46.70±4.09 msec for 90°. The T1 ρ value of the control group was 37.72±5.82 msec for 0°, 42.81±4.31 msec for 56°, and 39.74±4.84 msec for 90°. With respect to the measured angle, the mean measured angles for 0°, 56°, and 90° in the three groups were as follows: in the trypsin-treated group, 0°, 54.8°, and 89.46°, respectively; in the collagenase-treated group, 0.8°, 56.83°, and 89.4°, respectively; and in the control group, 2.5°, 58.23°, and 89.05°, respectively. Table 1 summarizes the T1 ρ value of for each chemical and angle.

Table 1. Results of the T1 ρ value for each enzyme and angle.

Chemicals	Angles (°)	T1 ρ value (msec)	*p*-value*
Trypsin-treated group	0	57.53 ± 8.24	0.880
	54.8	58.20±6.48	
	89.46	55.99±6.48	
Collagenase-treated group	0.8	45.08±5.31	0.252
	56.83	50.04±1.96	
	89.4	46.70±4.09	
Control group	2.5	37.72±5.82	0.392
	58.28	42.81±4.31	
	89.05	39.74±4.84	

* *p*-value of the ANOVA test

Statistical analysis

Using the ANCOVA test, we could estimate the T1 ρ relaxation time of each enzyme group. The estimated T1 ρ values were 57.22 ± 6.52 msec for the trypsin-treated group, 42.81 ± 4.31 msec for the collagenase-treated group, and 42.81 ± 4.31 msec for the control group (Table 2). There was a statistically significant difference in the T1 ρ values among the three groups ($P<0.001$) (Fig. 3). With respect to the orientations, the estimated T1 ρ values were 46.75 ± 10.41 msec for 0°, 50.96 ± 7.99 msec for 56°, and 48.14 ± 8.60 msec for 90° (Table 3). The T1 ρ value tended to increase at 56° compared with 0° or 90°, but the difference was not statistically significant ($P=0.220$) (Fig. 4). In the subgroup analysis in each enzyme group, there was also no significant difference in the T1 ρ values among the three orientations ($P=0.880$ (trypsin), 0.252 (collagenase), 0.392 (control)) (Table 1).

Table 2. Estimated T1 ρ values in the three different enzyme groups.

Chemicals	Estimated T1 ρ value (msec)	*p*-value*
Trypsin-treated group	57.22 ± 6.52	<0.001
Collagenase-treated group	47.25 ± 4.25	
Control group	40.09 ± 8.95	

* *p*-value of the ANCOVA test

Table 3. Estimated T1 ρ values at three different orientations.

Angles (°)	Estimated T1 ρ value (msec)	*p*-value*
0	46.75 ± 10.41	0.220
56	50.96 ± 7.99	
90	48.14 ± 8.60	

* *p*-value of the ANOVA test

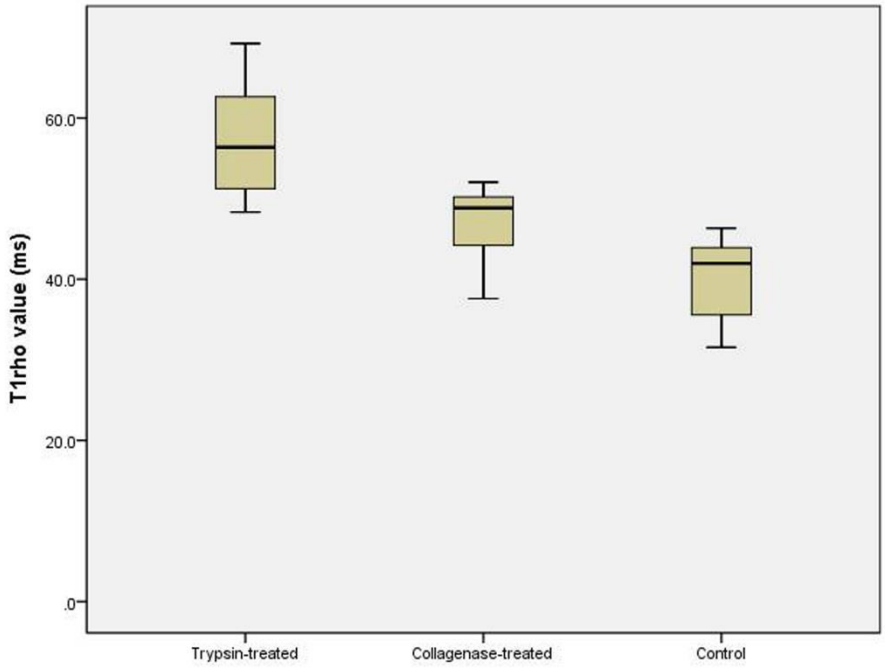

Figure 3.

Graph showing the estimated T1 ρ values in the three enzyme groups. There was a significant difference in the estimated T1 ρ values in the three groups ($P<0.001$).

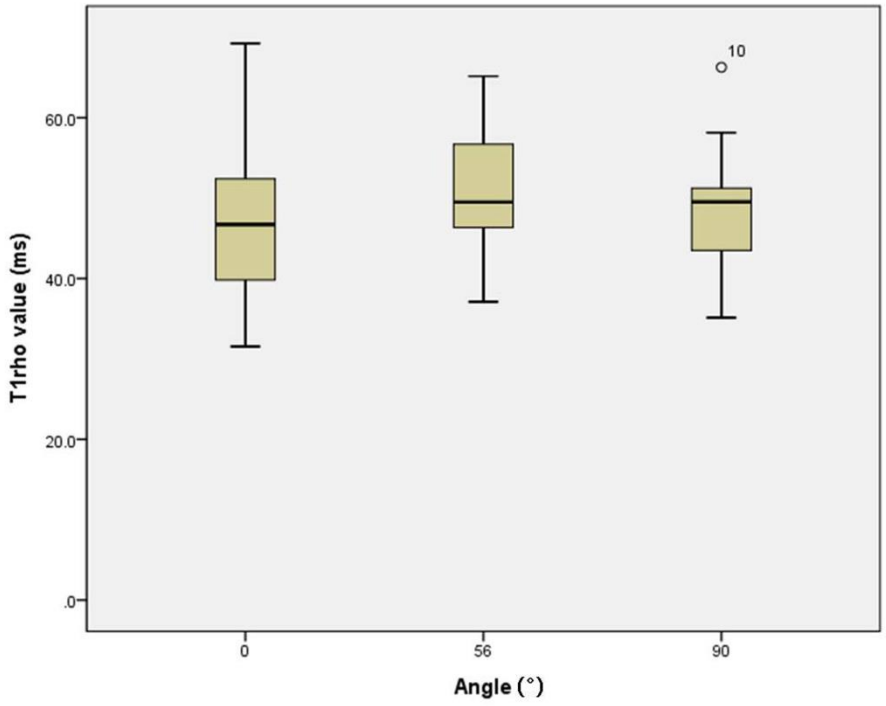

Figure 4.

Graph showing the estimated T1 ρ values at different orientations of cartilage. There was no significant difference in the estimated T1 ρ values among the three orientations (P=.220).

DISCUSSION

According to the results of our study, quantification of the loss of the proteoglycans and collagen II fibers can be obtained using a 3 Tesla MRI scanner and a T1 ρ mapping program. Because cartilage damage is still known as an irreversible process, the quantification and early detection of osteoarthritis is important. Our results are concordant with the findings of previous studies. Duvvuri et al (18) suggested that the T1 ρ measurements are selectively sensitive to the proteoglycan content and could potentially distinguish the early degenerative changes in cartilage that are associated with osteoarthritis. Li et al (19) demonstrated that the T1ρ value had a significant but moderate correlation with the proteoglycan content and could reflect the degree of the histologic degree of cartilage degeneration. Nishioka et al (8) reported that the T1 ρ values reflect the GAG content of the cartilage and can indicate the cartilage degeneration in vivo. They also indicated that the noninvasive diagnosis and evaluation of cartilage degeneration could be facilitated by the use of T1 ρ values. Similar to a previous study, our study could suggested that T1 ρ MR imaging has great potential to provide noninvasive imaging biomarkers for cartilage degeneration by measuring the T1 ρ values of articular cartilage that is affected by the contents of proteoglycan. This process could enable the early detection and treatment of degenerative arthritis patients, which could prevent invasive treatments such as arthroplasty with artificial joints.

With respect to collagen, there have been few controversial reports on the

relationship between the T1 ρ values of the articular cartilage and the contents of collagen fiber. Menezes et al (20) reported a relationship between the T1 ρ values and the collagen molecule concentration. However, Duvvuri et al (18) revealed that the T1 ρ value is not increased in a collagen-degraded bovine patella produced by collagenase, and Li et al (19) showed that there is no significant correlation between the T1 ρ value and collagen contents in degenerated human cartilage. In our study, the T1 ρ relaxation time increased in collagen-degraded specimens, but this decrease was less than that observed in proteoglycan-degraded specimens. We postulated that the T1 ρ changes according to the collagen contents are small in articular cartilage so previous studies have shown controversial results that depended on the study population or study design. Based on our results, we should consider the contents of both proteoglycans and collagen when applying the current results in other studies or clinical practice. In terms of the orientation-dependency, our results are inconsistent with those of previous studies. Wang et al (17) showed that the collagen orientation in nasal cartilage can influence the T2 and T1 ρ values. Native perpendicular tissues demonstrated significant T1 ρ dispersion at 0° and less dispersion at 55°. Li et al also demonstrated that both the mean T1 ρ and T2 at 54.7° were significantly higher than those measured at 90° and 0°, with T1 ρ showing a lower increase compared with the T2 values. Similarly, our study result showed a tendency of less dispersion of T1 ρ around 56°, but there was no statistical significance. We thought that the total number of specimens was too small to reveal a statistically significant difference among the three different angles.

Our study had several limitations. First, the number of specimens in our study was small. Despite the small specimen number, we observed statistically significant differences in the T1 ρ values among the three different chemical groups. However, we only observed a trend towards, but not significant differences in the T1 ρ values among the three different orientations. We hope that further study with a larger number of specimens could reveal the statistically significant orientation dependence of T1 ρ value due to the anisotropy effect. Second, there was measurement error during setup in the angle between the articular surface and magnetic field B0 direction. Therefore, we should use the mean values of the angle between articular surface and the magnetic field B0 direction. Further study with a more delicate angle control system would likely provide a better result. Third, we could not perform histologic examinations, such as safranin-O for staining for proteoglycan and immunohistochemical staining for type II collagen. Therefore, we did not know the actual contents of the proteoglycan and type II collagen in the specimen and could not evaluate the correlation between the T1 ρ value and the histologic result. Additional histologic confirmation could make our results more reliable. Finally, with respect to the current study, as an ex vivo study, we only found the feasibility of T1 ρ mapping with clinical MRI scanner for the quantification and evaluation of cartilage degeneration. Therefore, further study, including in vivo animal joints, should be conducted to apply this process in clinical practice.

In conclusion, degradation of proteoglycans or collagen fibers in the articular cartilage caused an increase in the T1 ρ value of the articular

cartilage, but the orientation dependence of the T1 ρ value was not proven.

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국문 초록

서론: 퇴행성 관절염은 관절연골의 구성성분인 proteoglycan 과 collagen 섬유가 소실되는 과정으로 세계적으로 수백만 명이 이환되어있는 매우 흔하고 중대한 질병이다. 최근에는 퇴행성 관절염을 조기에 진단하고 골관절염의 진행을 예측하는 것이 환자의 치료계획을 수립하는데 중요할 것으로 생각되고 있다. 본 연구에서는 관절연골에서 T1 ρ 이완시간에 영향을 줄 수 있는 다양한 요소들에 대해서 분석하였다.

방법: 본 연구에는 총 12 개의 돼지 슬개골이 사용되었고, trypsin-분해군, collagenase-분해군, 대조군의 3 개의 군으로 분류되었다. 대조군을 제외한 실험군 에는 각각 trypsin 과 collagenase 를 이용하여 효소반응이 시행되었다. 3 테슬라 자기공명영상 촬영기와 single loop 코일을 이용하여 T1 ρ mapping 이 시행되었고, Mat lab R2013 프로그램을 이용하여 관절연골의 T1 ρ 평균이완시간이 계산되었다. T1 ρ 이완시간에 영향을 미치는 인자의 분석에는 공분산 분석법을 통해 비교하였다. 추가로 분산분석법을 이용하여 각 효소반응 군에서 각도에 따른 T1 ρ 이완시간의 차이를 비교하였다.

결과: 관절연골 면의 평균 T1 ρ 이완시간 값을 비교하였을 때 각기 다른 효소로 처리한 3 개의 군에서 통계적으로 유의한 차이를 보였다 (P<0.001). 그러나 각기 다른 각도의 의한 T1 ρ 이완시간 값은 통계적으로 유의한 차이를 보이지 않았다 (P=0.220).

결론: 관절연골 내 proteoglycan 과 collagen 섬유의 소실에 따라 관절연골의 T1 ρ 이완시간 값은 증가하였다. 그러나 proteoglycan 과 collagen 섬유의 방향에 따른 T1 ρ 이완시간 값은 통계적으로 유의한 차이를 보이지 않았다.

주요어 : 퇴행성 관절염, proteoglycan, collagen, collagen 분해효소, 트립신, T1 ρ

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