의학박사 학위논문

관절 연골의 지연성 가돌리늄 조영증강 자기공명영상 기법: 생체 외 돼지 슬개골 모델에서 여러 가돌리늄 기반 조영제의 비교연구

Delayed Gadolinium-enhanced MR Imaging of Cartilage: A Comparative Analysis of Different Gadolinium-based Contrast Agents in an ex Vivo Porcine Model

2017년 2월

서울대학교 대학원

의학과 영상의학 전공

강 유 선

A thesis of the Degree of Doctor of Philosophy

Delayed Gadolinium-enhanced MR Imaging of Cartilage: A Comparative Analysis of Different Gadolinium-based Contrast Agents in an ex Vivo Porcine Model

관절 연골의 지연성 가돌리늄 조영증강 자기공명영상 기법: 생체 외 돼지 슬개골 모델에서 여러 가돌리늄 기반 조영제의 비교연구

February 2017

Department of Radiology

Seoul National University

College of Medicine

Yusuhn Kang

Abstract

Delayed Gadolinium-enhanced MR Imaging of Cartilage: A Comparative Analysis of Different Gadolinium-based Contrast Agents in an ex Vivo Porcine Model

Yusuhn Kang

Department of Radiology, College of Medicine

The Graduate School

Seoul National University

Introduction: To compare the delayed gadolinium-enhanced magnetic resonance (MR) imaging of cartilage (dGEMRIC) indexes acquired with different gadolinium-based contrast agents (GBCAs), with emphasis on the difference in electrical charge, and to evaluate the feasibility of the use of GBCAs other than gadopentetate dimeglumine with a double negative charge ($Gd\text{-}DTPA^{2-}$) as alternatives at dGEMRIC.

Materials and Methods: Intact porcine patellae (n = 44) were divided into four groups according to GBCA used: double negative gadopentate dimeglumine ($Gd\text{-}DTPA^{2-}$), double negative gadobenate dimeglumine (Gd-

BOPTA^{2-}), single negative gadoterate meglumine (Gd-DOTA^{-}), and nonionic gadobutrol (Gd-BT-DO3A). Patellae in each group were further assigned to control (n = 3) or trypsin-treated (n = 8) groups and were immersed in GBCA solutions prepared at a concentration of 2.5 mmol/L. T1 maps were acquired at 10-minute intervals at 0–120 minutes. The difference between postcontrast R1 and precontrast R1 (ΔR) and the time-ΔR curves were plotted. Patellae were stained with safranin-O to evaluate the proteoglycan content of the cartilage. The intensity of staining was quantified by calculating the relative fraction of red ($r = R/(R^2 + G^2 + B^2)^{1/2}$) from the intensity values of red(R), green(G), and blue(B). A linear mixed-effects model was used to analyze the time-ΔR curves, and Student t tests and Mann-Whitney U tests were used to compare dGEMRIC indexes between groups.

Results: The difference in the estimated slopes of the time-ΔR curves between control and trypsin-treated groups were greatest with Gd-BOPTA^{2-}, followed by Gd-DTPA^{2-}, Gd-DOTA^{-}, and Gd-BT-DO3A, with differences in the estimated slopes of 0.037, 0.022, 0.018, and 0.011, respectively. The slope difference between control and trypsin-treated groups was significantly greater with Gd-BOPTA^{2-} ($P < .001$) and significantly smaller with Gd-BT-DO3A ($P = .004$) in comparison with that with Gd-DTPA^{2-}. Only the GBCAs with double negative charges showed significant differences in both the T1

measured after equilibration of cartilage with GBCA solution and the ΔR at 90 and 120 minutes between the control and trypsin-treated groups. The safranin-O staining intensity (r, relative fraction of red) differed significantly between control (0.77±0.07) and trypsin-treated groups (0.46± 0.05) ($p<.001$).

Conclusion: Double negative GBCAs produced better contrast between normal and degenerated cartilage than did those with a single negative charge and nonionic GBCAs at the same concentration for dGEMRIC. Because Gd-BOPTA^{2-}, a high-relaxivity GBCA, showed higher contrast than did Gd-DTPA^{2-}, Gd-BOPTA^{2-} may be useful as an alternative GBCA for dGEMRIC.

Keywords: Cartilage

Delayed gadolinium-enhanced MR imaging of cartilage

Gadolinium-based contrast agent

Glycosaminoglycan

Student number: 2014-30639

CONTENTS

List of Tables

List of Figures

Figure 3. Box-and-whisker plots of (a,b) T1(Gd) and (c,d) ΔR values between controls and the trypsin-treated group at (a,c) 90 minutes and (b,d) 120 minutes. Boxes show 25th -75th percentile values with median indicated with a line across the box, and whiskers are extended to the minimum and maximum values that are not outliers. P values are from Student's *t*-test or Mann-Whitney U test (asterisk).

Figure 4. Representative histological sections show (a) control and (b) trypsin-treated porcine patellae (safranin O, fast green staining; original magnification, ×10). The control group patellar cartilage shows strong safranin-O staining, whereas the trypsin-treated patellar cartilage is devoid of safranin-O staining, indicating the depletion of glycosaminoglycan. C=cartilage, B= subchondral bone.

INTRODUCTION

The advent of various quantitative magnetic resonance (MR) imaging techniques has enabled visualization of biochemical and biophysical changes in the articular cartilage. Among various imaging methods, delayed gadolinium-enhanced MR imaging of cartilage (dGEMRIC) allows the detection of cartilage lesions at an early stage through quantification of the glycosaminoglycan (GAG) content in the cartilage. This method is based on the assumption that a negatively charged MR imaging contrast agent will distribute in an inverse relationship to the concentration of GAG, which confers a negative charge to the cartilage (1,2). Results of many studies (1–4) have shown that the estimation of GAG concentration based on T1 measurements is in good agreement with biochemical assay and histologic results.

Most of the previously reported studies of dGEMRIC were performed with an intravenous injection of a double dose (0.2 mmol/kg of body weight) of gadopentetate dimeglumine (Gd-$DTPA^{2-}$), a gadolinium-based contrast agent (GBCA) with a double negative charge. However, Gd-DTPA has been

classified as a GBCA with a high risk of nephrogenic systemic fibrosis (5,6). Nephrogenic systemic fibrosis, characterized by thickening and hardening of the skin with hyperpigmentation, is a rare but potentially fatal disorder that may occur in patients with severe renal impairment who receive GBCAs. Gd-$DTPA^{2-}$ accounts for approximately 13% of the unconfounded cases of nephrogenic systemic fibrosis reported in the peer-reviewed literature, and it is contraindicated in patients with severe renal impairment (5,6). Recently, the deposition of gadolinium in the brain associated with prior GBCA administration has garnered much attention (7–12). Reports (7,9) have shown that the accumulation of gadolinium in the dentate nucleus and the globus pallidus is associated with the linear GBCA, Gd-$DTPA^{2-}$, but not with macrocyclic GBCAs. Although the clinical importance of this finding remains to be determined, these findings do suggest that Gd-$DTPA^{2-}$ should be administered with caution.

To our knowledge, few studies of dGEMRIC with contrast agents other than Gd-$DTPA^{2-}$ have been conducted. Li et al (1) compared the results of studies of dGEMRIC with nonionic gadodiamide and an ionic contrast agent (Gd-

$DTPA^{2-}$) and reported that a negatively charged contrast agent should be used for better discrimination of the cartilage status. Zilkens et al (13) used gadoterate meglumine ($Gd\text{-}DOTA^{-}$), a contrast agent with a single negative charge, in their study comparing intra-articular and intravenous dGEMRIC of hip joint cartilage. They suggested that dGEMRIC was feasible when a contrast agent with a single negative charge was used. Bittersohl et al (14) also reported on a study of the knee joint cartilage in which dGEMRIC was performed with $Gd\text{-}DOTA^{-}$. However, to the best of our knowledge, authors of studies of dGEMRIC performed with contrast agents with a single negative charge have not compared them with gadolinium chelates with a double negative charge, so further examination is warranted. Thus, the purpose of our study was to compare the dGEMRIC indexes acquired with different GBCAs, with emphasis on differences in electrical charge, and to evaluate the feasibility of using gadolinium chelates other than $Gd\text{-}DTPA^{2-}$ as alternatives for dGEMRIC.

MATERIALS AND METHODS

Gadolinium-based Contrast Agents

To evaluate the effect of charge on the distribution of gadolinium chelates in normal and degenerated cartilage, we selected three GBCAs: Gd-DTPA (Magnevist; Bayer Schering Pharma, Montville, NJ), Gd-DOTA (Dotarem; Guerbet, Sulzbach, Germany), and gadobutrol (Gd-BT-DO3A, Gadovist; Bayer Schering Pharma, Berlin, Germany). Gd-DTPA and Gd-DOTA dissociate into $Gd\text{-}DTPA^{2-}$ and $Gd\text{-}DOTA^{-}$, conveying a double and a single negative charge, respectively, whereas Gd-BT-DO3A is a nonionic contrast agent. To evaluate the possible use of other double negative GBCAs with a lower risk of nephrogenic systemic fibrosis, a fourth GBCA, gadobenate dimeglumine (Gd-BOPTA, MultiHance; Bracco Imaging SpA, Milan, Italy) was selected. Gd-BOPTA dissociates into $Gd\text{-}BOPTA^{2-}$. Gd-BOPTA, Gd-DOTA, and Gd-BT-DO3A are classified as intermediate- or low-risk agents for nephrogenic systemic fibrosis, whereas Gd-DTPA is a high-risk agent (5,6). The biochemical properties and safety profiles of the four GBCAs used

in our study are summarized in Table 1. All four contrast media were diluted with phosphate-buffered saline to an identical concentration of 2.5mmol/L. The concentration of the gadolinium-saline solution was based on that in a previous study of intra-articular dGEMRIC (17).

Table 1. Biochemical properties and safety profile of gadolinium-based contrast agents

Brand name	**Short name**	**Net charge**	**Chemical structure**	**Molecular weight (g/mol)**	**Osmolality (mOsmol/kg H_2O at 37°C) ***	**R1 Relaxivity ($mM^{-1}s^{-1}$) in water †**	**Conditional Stability at pH = 7.4‡**	**NSF risk‡**
Magnevist	Gadopentetate Gd-DTPA	(-2)	Linear	938	1,960	3.1	18.4	High
Multihance	Gadobenate Gd-BOPTA	(-2)	Linear	1058.2	1,970	4.0	18.4	Intermediate
Dotarem	Gadoterate Gd-DOTA	(-1)	Macrocyclic	753.86	1350	2.8	19.3, 18.8	Low
Gadovist	Gadobutrol Gd-BT-DO3A	0	Macrocyclic	604.7	1603	3.2	15.5	Low

Note- NSF, nephrogenic systemic fibrosis.

* From Ref. (37).

† From Ref. (29).

‡ From Ref. (6).

Sample Preparation

Our study was performed between April 2014 and January 2015 and was exempt from the requirements of the institutional animal care and use committee. Patellae of 5–6–month-old pigs were obtained from a local slaughterhouse within 6 hours after death. The surrounding soft tissue was carefully dissected away from the patellae. The patellae were inspected visually for morphologic changes in the cartilage, and only patellae without gross abnormalities in the cartilage surface were used.

The patellae (n = 44) were assigned to four groups (n = 11 each) according to the different contrast agents that would be used, and within each group, patellae were further assigned to a control (n = 3) or trypsin-treated (n = 8) group. The trypsin-treated group was pretreated with 0.1% (wt/vol) trypsin solution (trypsin from porcine pancreas, Sigma-Aldrich, St Louis, Mo) in calcium-free phosphate-buffered saline for 24 hours at room temperature to deplete the cartilage of GAG content. The control group patellae were kept in a phosphate-buffered saline solution for 24 hours. After the 24-hour immersion, the patellae were rinsed with phosphate-buffered saline to remove

the trypsin solution and were then fixed in a plastic container filled with agarose gel.

Image Acquisition

The MR images were acquired with a 3-T MR imaging unit (TrioTim; Siemens, Erlangen, Germany) with a six-channel cylindrical coil (Stark Contrasts, Erlangen, Germany) tailored for small animals such as mice or rats, with an inner diameter of 7 cm. The coil was placed at the isocenter of the MR unit. Two plastic containers, each containing a porcine patella, could fit in the coil, and so the patellae were imaged two at a time.

A B1 field mapping sequence was performed before T1 mapping for B1 field inhomogeneity correction (14,18). The B1 preimaging was performed with the following parameters: repetition time msec/echo time msec, 1000/14.0; flip angles, 90°, 120°, 60°, 135°, 45°; bandwidth, 260.4 Hz/pixel; field of view, 250 ×250 mm; section thickness, 5 mm; number of sections, 22; matrix, 32 × 32; in-plane resolution, 7.8 × 7.8 mm; number of signals acquired, one; and total acquisition time, 37 seconds. Fast T1 mapping

methods with a three-dimensional spoiled gradient-echo acquisition with variable flip angles have been shown to be equal to inversion-recovery techniques for measurement of the T1 relaxation time (18,19). On the basis of these previous reports, we acquired T1 maps with a dual flip angle three-dimensional gradient-echo sequence with volumetric interpolated breath-hold examination, with the following parameters: 12.3/4.9; flip angles, 4° and 23° (for an estimated T1 of 800 msec); bandwidth, 270 Hz per pixel; field of view, 150 × 60 mm; section thickness, 2.5 mm; number of sections, 30; matrix, 512 × 512; in-plane resolution, 0.3 × 0.3 mm; total acquisition time, 9 minutes, 13 seconds (two acquisitions of 4 minutes 36.5 seconds each).

T1 mapping was performed with the plastic container filled with the prepared gadolinium-saline solution. The total volume of solution surrounding the cartilage was approximately 100mL. The gadolinium-saline solution was stirred before image acquisition was initiated. Postcontrast T1 mapping images were acquired immediately after immersion (0 minutes) and at 10-minute intervals up to 120 minutes after immersion in the gadolinium-saline solution.

Image Analysis

All images were viewed on a picture archiving and communication system workstation (Gx; Infinit Technology, Seoul, Korea). For standardization, all measurements were performed by one researcher (Y.K., with 4 years of experience in musculoskeletal radiology) and reevaluated by another (J.Y.C., with 11 years of experience in musculoskeletal radiology). For T1 measurements, a free-hand region of interest was drawn on the gradient-echo sequence images, with a flip angle of 4° or 23°, and was copied to the T1 map images. The regions of interest were drawn to encompass the whole thickness of the cartilage, from the cartilage-bone interface to the articular cartilage surface (Fig 1). The mean T1 value of the region of interest was used for calculations. The thickness of the cartilage was also measured on the gradient-echo sequence image. We used the T1 value measured after equilibration of cartilage with GBCA solution ($T1_{Gd}$) and the difference between postcontrast R1 and precontrast R1 (ΔR) for data analysis ($\Delta R = 1/T1_{Gd} - 1/T1_0$) on the basis of previous study protocols (20–23). The T1 value acquired immediately

after immersion in the GBCA solution ($T1_0$) was used for calculating the ΔR, which was calculated for each time point at 0–120 minutes. The temporal profiles of the control and trypsin-treated groups were compared for each contrast agent.

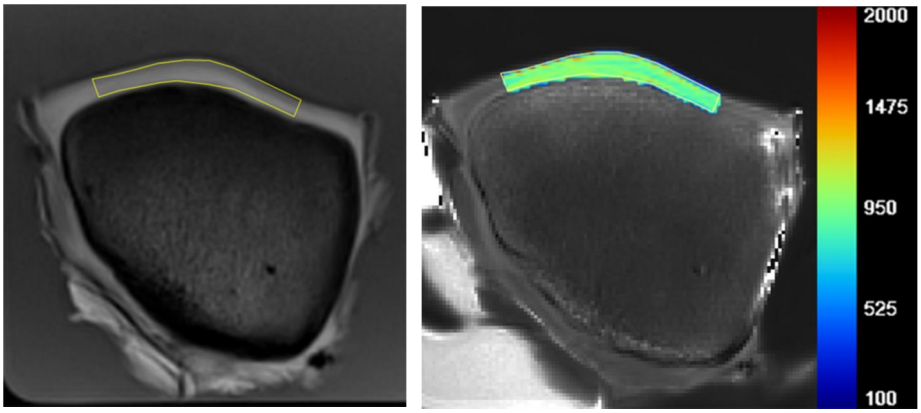


Fig. 1a **Fig. 1b**

Figure 1. To measure the mean T1 value of cartilage, a free-hand region-of-interest (ROI) was drawn manually on **(a)** the gradient echo sequence with a flip angle of 4° or 23°, and copied to **(b)** the T1 map image. The ROIs were drawn so as to encompass the whole thickness of the cartilage, from the cartilage-bone interface to the articular cartilage surface.

Histologic Sample Preparation and Analysis

Safranin-O staining with fast green counterstaining was performed in both control and trypsin-treated patellae to confirm the depletion of GAG in the articular cartilage after trypsin digestion. The intensity of safranin-O staining was measured by using software (ImageJ; National Institutes of Health, Bethesda, Md) by one researcher (Y.K., with 4 years of experience in musculoskeletal radiology). A rectangular region of interest was placed to encompass the whole thickness of the cartilage, from the surface to the cartilage-bone interface. The color of a given pixel was based on the intensity of red, green, and blue, with the intensity value ranging from 0 to 255. The integrated intensity of red (R), green (G), and blue (B) within the selected region of interest was measured, and the relative fraction of red (r) was calculated by using the equation $r = R/(R^2 + G^2 + B^2)^{1/2}$, on the basis of previous studies (24–26). The calculated relative fraction of red (r) was considered the intensity of safranin-O staining.

Statistical Analysis

A linear mixed-effects model was used to investigate the ΔR, and the effect of time, contrast agent, and trypsin on ΔR. The ΔR was considered the dependent variable and modeled as a linear function of time (which was considered a continuous variable). The model included the interaction terms of (a) time and contrast agent, (b) time and trypsin, and (c) time, contrast agent, and trypsin, as fixed effects. The interaction terms were the primary effect of interest, because they represented the relationship between the variable and ΔR over time. Because all of the subjects started off with a ΔR of 0 at 0 minutes, the constant term was excluded from the fixed-effects and random-effects equation. The $T1_{Gd}$ and ΔR of the control and trypsin-treated group for a given GBCA at 90 minutes and 120 minutes were compared by using either a two-sample Student t test or a Mann-Whitney U test according the results of the Shapiro-Wilk test for normality of data. The time points 90 minutes and 120 minutes were selected for comparison according to previous dGEMRIC studies that also used these time points (1,18, 20 ,21, 27–29). The safranin-O staining intensity of the control and trypsin-treated group was

compared with the Student t test. Statistical analyses were performed with software (STATA version 14.0; Stata, College Station, Texas, and SPSS for Windows version 18.0; SPSS, Chicago, Ill). P values less than .05 were considered to indicate a significant difference.

RESULTS

The thickness of the porcine patella cartilage used in our study was on average 1.47 ± 0.23 mm. The time-ΔR curves of the control and trypsin-treated groups acquired with the four different GBCAs are shown in Figure 2 and the results of linear mixed model analysis are shown in Table 2. The estimated slopes of the time-ΔR curves over the time period of 0-120minutes were acquired with the linear mixed model. The difference between the estimated slopes of the control and trypsin-treated group were greatest for Gd-BOPTA^{2-} followed by Gd-DTPA^{2-}, Gd-DOTA^{-}, and Gd-BT-DO3A with a difference of 0.037, 0.022, 0.018 and 0.011, respectively. The slope difference between control and trypsin-treated group were significantly greater in Gd-BOPTA^{2-} ($p<0.001$), and significantly smaller in Gd-BT-DO3A ($p=0.004$) compared to Gd-DTPA^{2-}. However the slope difference between control and trypsin-treated group in Gd-DTPA^{2-} and Gd-DOTA^{-} did not differ significantly ($p =0.262$).

Table 3 and Figure 3 show the T1(Gd) and ΔR of the control and trypsin-treated group at 90 and 120 minutes, acquired with the four different GBCAs.

Only the double-negatively charged GBCAs, Gd-BOPTA^{2-} and Gd-DTPA^{2-}, showed significant differences in both T1(Gd) and ΔR at 90 and 120 minutes between the control and trypsin-treated groups. A tendency toward a lower T1(Gd) and higher ΔR in trypsin-treated group was noted in both Gd-DOTA^{-}, and Gd-BT-DO3A, but the difference was not statistically significant.

Representative histological sections with safranin-O staining are shown in Figure 4. The control group patellae showed strong safranin-O staining, whereas the trypsin-treated (GAG-depleted) patellae were devoid of safranin-O staining. The relative fraction of red (*r*) was 0.77 ± 0.07 and 0.46 ± 0.05 in the control and trypsin-treated group, respectively, and the difference was statistically significant ($p < 0.001$).

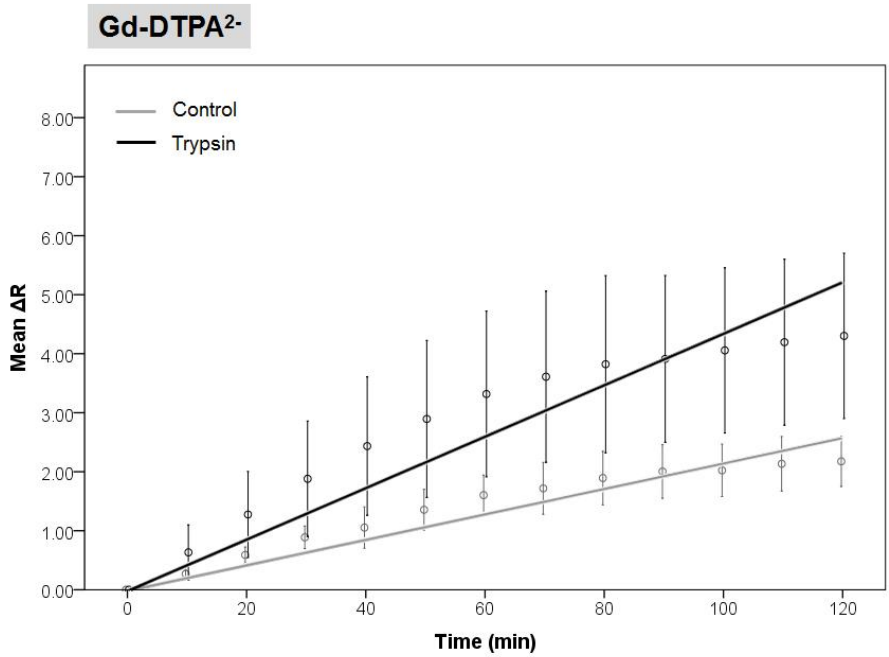

Gd-DTPA2-
Control
Trypsin
Mean ΔR
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
20
40
60
80
100
120
Time (min)

Fig. 2a

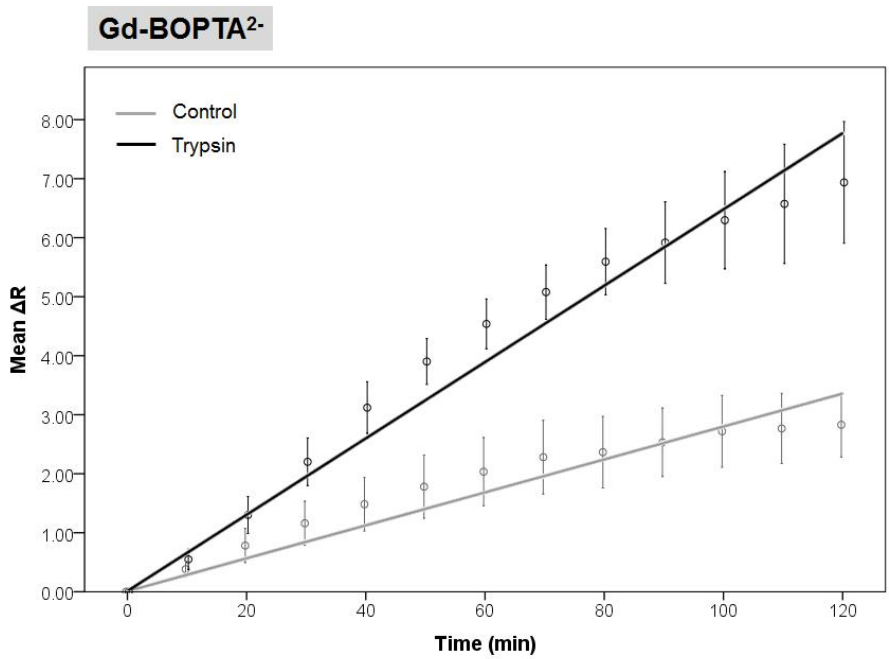

Gd-BOPTA2-
Control
Trypsin
Mean ΔR
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
20
40
60
80
100
120
Time (min)

Fig. 2b

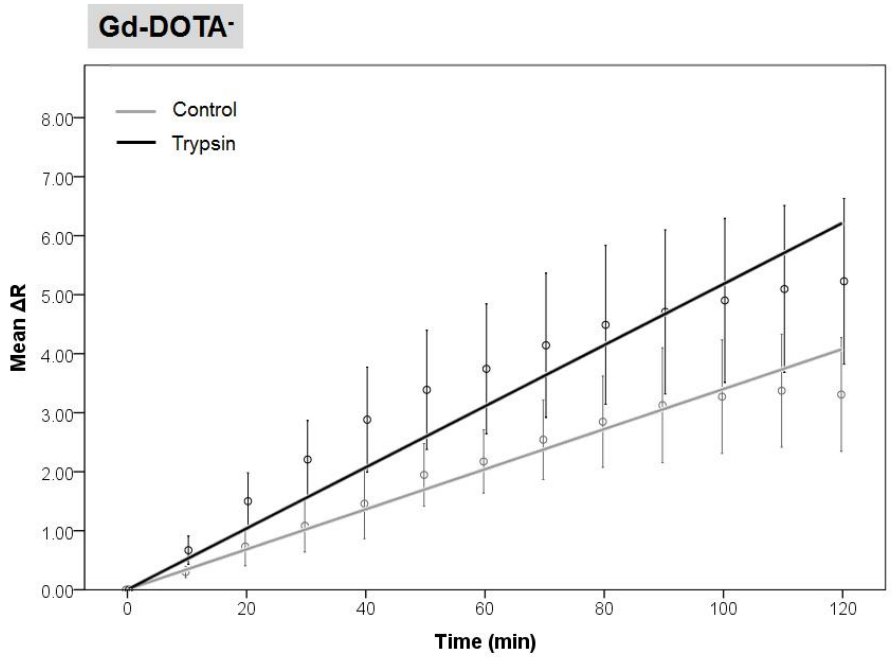

Gd-DOTA-
Control
Trypsin
Mean ΔR
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
0
20
40
60
80
100
120
Time (min)

Fig. 2c

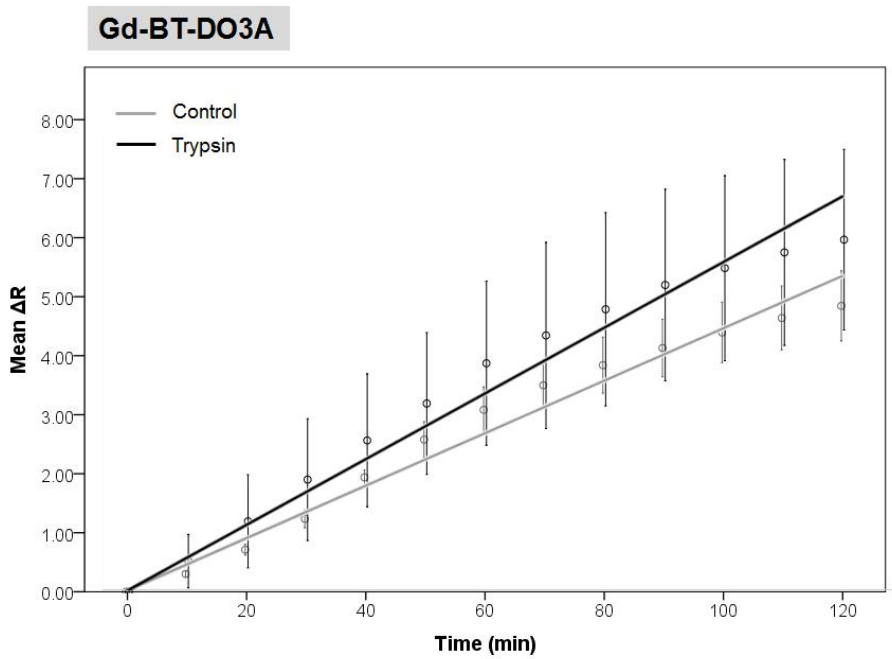

Gd-BT-DO3A
Control
Trypsin
Mean ΔR
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
0
20
40
60
80
100
120
Time (min)

Fig. 2d

Figure 2. The time-ΔR curves of control and trypsin-treated porcine patellar cartilage acquired with the four different gadolinium-based contrast agents, (a) Gd-DTPA^{2-}, (b) Gd-BOPTA^{2-}, (c) Gd-DOTA^{-}, and (d) Gd-BT-DO3A, are shown. The curves were modeled with linear mixed model analysis, and error bars show mean and standard deviation of control and trypsin-treated group at each time point. The largest difference in slope between contrast and trypsin-treated group was observed with Gd-BOPTA^{2-}, followed by Gd-DTPA^{2-}, Gd-DOTA^{-}, and Gd-BT-DO3A.

Table 2. Estimated slope of the time-ΔR curves in the four different groups

Contrast agent	Control group	Trypsin-treated group	Slope difference (Trypsin - Control)	Increment of slope difference with reference to Gd-DTPA^{2-}	P value*
Gd-DTPA^{2-}	0.021	0.043	0.022		
Gd-BOPTA^{2-}	0.028	0.065	0.037	0.015 [0.008, 0.221]	<0.001
Gd-DOTA^{-}	0.034	0.052	0.018	-0.004 [-0.011, 0.003]	0.262
Gd-BT-DO3A	0.045	0.056	0.011	-0.011 [-0.018, -0.003]	0.004

Note – Data are estimated slopes of time-ΔR curves calculated with linear mixed model analysis, expressed in ΔR/min. Numbers in brackets are 95% confidence intervals.

* P values are for increment of slope difference (Trypsin-Control) with reference to Gd-DTPA^{2-}.

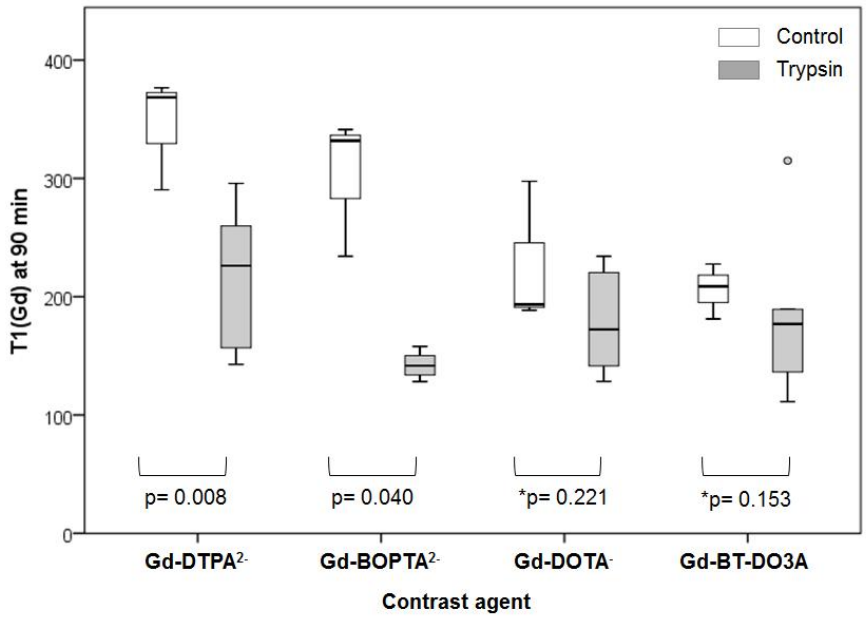

Control
Trypsin
T1(Gd) at 90 min
400
300
200
100
0
p= 0.008
p= 0.040
*p= 0.221
*p= 0.153
Gd-DTPA2-
Gd-BOPTA2-
Gd-DOTA-
Gd-BT-DO3A
Contrast agent

Fig. 3a

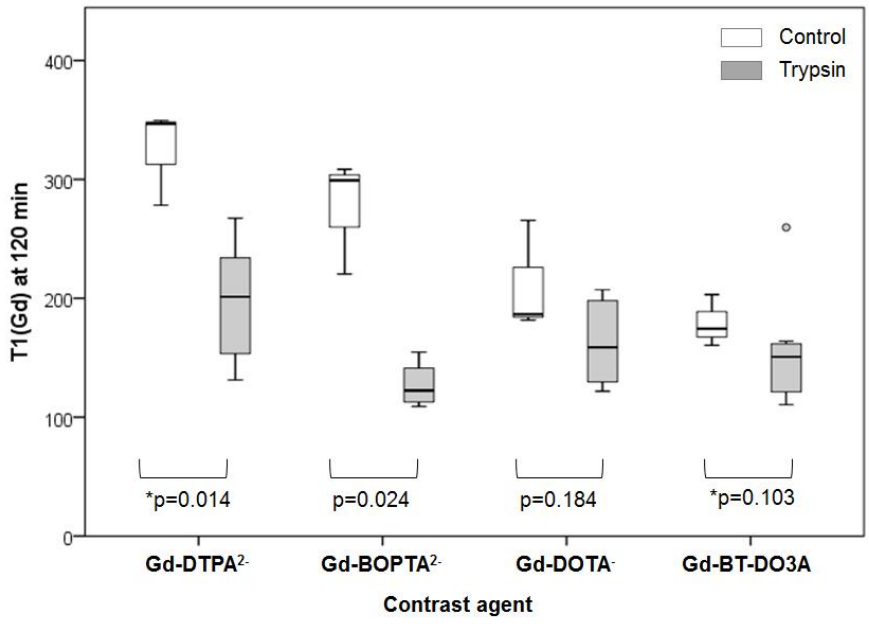

Control
Trypsin
T1(Gd) at 120 min
400
300
200
100
0
*p=0.014
p=0.024
p=0.184
*p=0.103
Gd-DTPA2-
Gd-BOPTA2-
Gd-DOTA-
Gd-BT-DO3A
Contrast agent

Fig. 3b

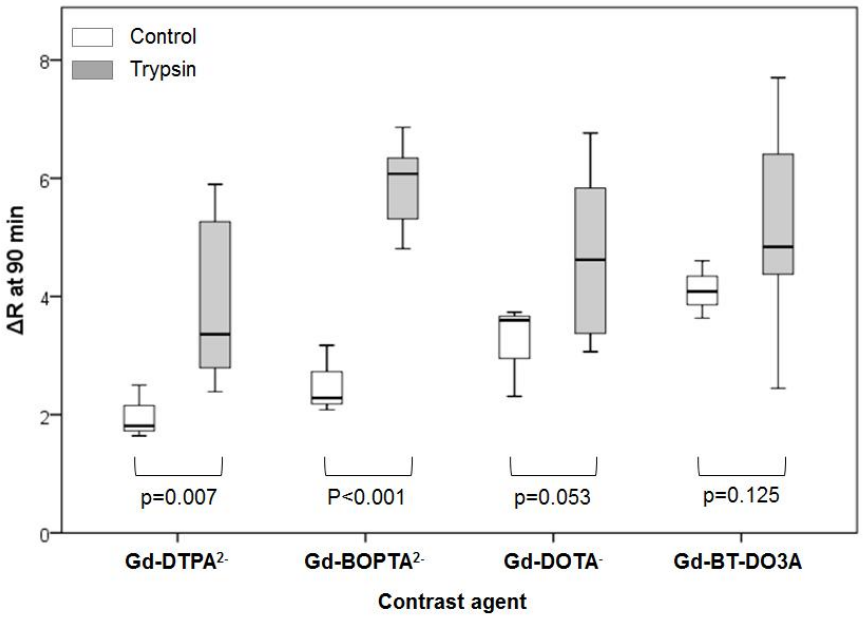

Control
Trypsin
ΔR at 90 min
0
2
4
6
8
p=0.007
P<0.001
p=0.053
p=0.125
Gd-DTPA2-
Gd-BOPTA2-
Gd-DOTA-
Gd-BT-DO3A
Contrast agent

Fig. 3c

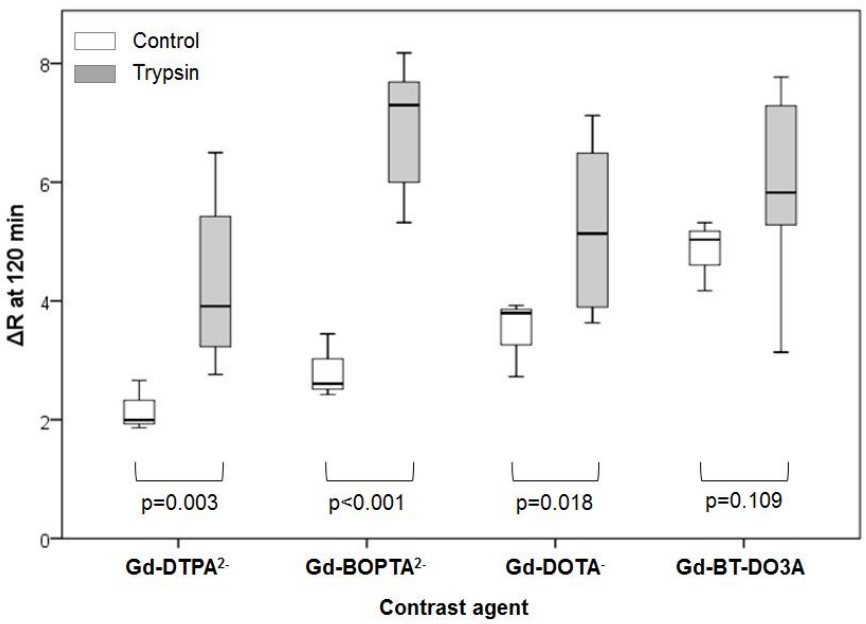

Control
Trypsin
ΔR at 120 min
0
2
4
6
8
p=0.003
p<0.001
p=0.018
p=0.109
Gd-DTPA2-
Gd-BOPTA2-
Gd-DOTA-
Gd-BT-DO3A
Contrast agent

Fig. 3d

Figure 3. Box-and-whisker plots of (a,b) T1(Gd) and (c,d) ΔR values between controls and the trypsin-treated group at (a,c) 90 minutes and (b,d) 120 minutes. Boxes show 25th -75th percentile values with median indicated with a line across the box, and whiskers are extended to the minimum and maximum values that are not outliers. P values are from Student's *t*-test or Mann-Whitney U test (asterisk).

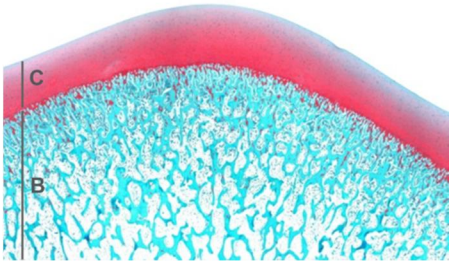
Table 3. T1(Gd) and ΔR values at 90 and 120 minutes

Contrast agent		T1(Gd) at 90 minutes	P value	ΔR at 90 minutes	P value	T1(Gd) at 120 minutes	P value	ΔR at 120 minutes	P value
Gd-DTPA^{2-}	Control	345 ± 48	0.008	2.00 ± 0.46	0.007	325 ± 40	0.014*	2.18 ± 0.42	0.003
	Trypsin	215 ± 59		3.91 ± 1.41		197 ± 49		4.30 ± 1.40	
Gd-BOPTA^{2-}	Control	302 ± 60	0.040	2.53 ± 0.59	< 0.001	276 ± 48	0.024	2.83 ± 0.54	< 0.001
	Trypsin	141 ± 11		5.92 ± 0.69		127 ± 17		6.94 ± 1.03	
Gd-DOTA^{-}	Control	226 ± 62	0.221*	3.23 ± 0.79	0.053	211 ± 47	0.184	3.49 ± 0.66	0.018
	Trypsin	178 ± 42		4.71 ± 1.39		163 ± 36		5.23 ± 1.41	
Gd-BT-DO3A	Control	205 ± 23	0.153*	4.13 ± 0.49	0.125	179 ± 22	0.103*	4.84 ± 0.59	0.109
	Trypsin	178 ± 62		5.20 ± 1.62		155 ± 47		5.97 ±1.53	

Note - Values are means ± standard deviation. T1(Gd) values are expressed in milliseconds.

P values are of Student's t-test unless otherwise specified.

* P values are of Wilcoxon rank-sum test.



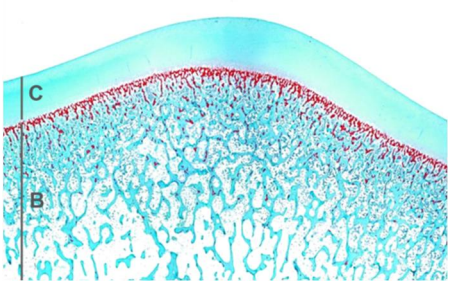


Fig. 4a **Fig. 4b**

Figure 4. Representative histological sections show (a) control and (b) trypsin-treated porcine patellae (safranin O, fast green staining; original magnification, ×10). The control group patellar cartilage shows strong safranin-O staining, whereas the trypsin-treated patellar cartilage is devoid of safranin-O staining, indicating the depletion of glycosaminoglycan. C=cartilage, B= subchondral bone.

DISCUSSION

The results of our study show that GBCAs with a double negative charge allow better discrimination of normal from trypsin-treated (GAG-depleted) cartilage than do those with a single negative charge and nonionic GBCAs at the same concentration. Between the two GBCAs with double negative charges that we used in our study, Gd-BOPTA^{2-} produced greater contrast between normal and GAG-depleted cartilage.

The greater contrast between normal and GAG-depleted cartilage with Gd-BOPTA^{2-} than that with Gd-DTPA^{2-} may be attributed largely to the higher relaxivity of Gd-BOPTA^{2-}. The relaxivity of Gd-BOPTA^{2-} and Gd-DTPA^{2-} in water at 37°C and 3 T is 4.0 $mM^{-1}s^{-1}$ and 3.1 $mM^{-1}s^{-1}$, respectively (16). With the same concentration of GBCA distributed in the cartilage, Gd-BOPTA^{2-} would result in a lower $T1_{Gd}$ and higher ΔR than those with Gd-DTPA^{2-}. Because only a small amount of GBCA distributes in normal GAG-rich cartilage, the difference in $T1_{Gd}$ and ΔR that results from the relaxivity differences would be relatively small, whereas in GAG-depleted cartilage, the difference of $T1_{Gd}$ and ΔR that results from relaxivity differences would be

considerable. This would result in greater contrast between normal and GAG-depleted cartilage in dGEMRIC studies with high-relaxivity GBCAs. Our study results suggest the possibility of using Gd-BOPTA^{2-} as an alternative to Gd-DTPA^{2-} for discrimination of normal from degenerated cartilage.

Authors of two previous dGEMRIC studies (13,14) conducted with Gd-DOTA^{-} suggested that dGEMRIC imaging is feasible with GBCAs with a single negative charge. In our study, the estimated slope of the time ΔR curve was significantly larger in GAG-depleted cartilage compared with that in normal cartilage when Gd-DOTA^{-} was used for imaging. However, single measurements of $T1_{Gd}$ acquired with Gd-DOTA^{-} at 90 and 120 minutes did not show a statistically significant difference between normal and trypsin-treated cartilage. These results indicated that Gd-DOTA^{-}, with its single negative charge, should be used with caution for discrimination of healthy from degenerated cartilage. The reduced contrast between normal and trypsin-treated cartilage noted with Gd-DOTA^{-} in dGEMRIC studies may be attributable to both the charge and the chemical structure of the GBCA. The negative charge of the GBCA is known to be the major determinant of its

distribution in the cartilage, in accordance with the GAG concentration (1,30). Because Gd-DOTA^{-} has a weaker negative charge compared with Gd-DTPA^{2-}, its repulsion from normal GAG-rich cartilage may be weaker than that with Gd-DTPA^{2-}. This may have resulted in higher distribution of Gd-DOTA^{-} in normal GAG-rich cartilage than that with Gd-DTPA^{2-}. The macrocyclic structure of Gd-DOTA^{-} may also influence the distribution in the cartilage. We did not assess the influence of the macrocyclic structure on the diffusion of the contrast media into cartilage in our study, so this requires further investigation.

Although not statistically significant, a difference was seen in the time ΔR curve of the normal and GAG-depleted cartilage in the nonionic Gd-BT-DO3A dGEMRIC study. A more rapid increase in ΔR and a higher tissue concentration of nonionic Gd-BT-DO3A were noted in the GAG-depleted cartilage than were seen in the normal cartilage. These findings are consistent with the results of a previous study. Li et al (1) compared $T1_{Gd}$ values measured with nonionic and ionic GBCAs in patients with osteoarthritis and in healthy control subjects. Contrary to the expectations of Li et al, the

distribution of the nonionic GBCA was not constant throughout individuals with osteoarthritis or control subjects. The diffusivity of GBCAs, both ionic and nonionic, has been reported to be faster in GAG-depleted cartilage than in intact cartilage (31). The abundance of GAG in normal cartilage may physically hinder the diffusion of Gd-BT-DO3A, resulting in the contrast between normal and GAG-depleted cartilage. This also may account for the results of our study.

Our experimental model was based on an intra-articular dGEMRIC condition, and the porcine patellae were immersed in a diluted GBCA solution. Authors of several studies (13, 30, 32–34) have investigated the feasibility of dGEMRIC after intra-articular GBCA injection. Authors of recent studies (13, 33, 34) have shown a 15- to 45-minute delay after intra-articular injection to be sufficient in dGEMRIC studies of the hip joint. The shorter postinjection delay reported in the intra-articular dGEMRIC studies of the hip compared with intravenous dGEMRIC studies of the knee may have been the result of the injection technique and the difference in cartilage thickness. The porcine patellar cartilage included in our study had a thickness of 1.47 ± 0.23mm,

which is somewhat thinner than the reported thickness of human knee and hip cartilage (2.08–3.05 mm in the knee and 2.83–2.97 mm at the hip, according to the measured location) (35,36). A shorter equilibrating time would be expected with thinner cartilage, but, contrary to expectations, in our study the time ΔR curve of normal cartilage did not reach equilibrium by 120 minutes. A number of factors may be responsible for this discrepancy with the results of previous reports, including the absent wash-out effect in our ex-vivo model, the large volume of the equilibrating solution, and the lack of stirring. Although more invasive, intra-articular administration of GBCAs may have advantages over intravenous administration such as a shorter imaging time delay, lower systemic exposure, and better depiction of cartilage morphologic abnormalities. For example, an adult patient weighing 60 kg would receive a total of 12 mmol of Gd-$DTPA^{2-}$ when undergoing an intravenous injection for dGEMRIC (0.2 mmol/kg of body weight), whereas in an intra-articular dGEMRIC condition, a 10-mL intra-articular injection of 2.5 mmol/L solution would result in a total of 0.025 mmol administered. As more concerns are raised regarding the risk of nephrogenic systemic fibrosis (5, 6, 15, 37) and

gadolinium deposition in the brain (7–12), intra-articular dGEMRIC may be an important alternative to intravenous dGEMRIC in the future.

Several limitations of our study should be addressed. First, the GBCAs used differ in biochemical properties other than charge. Consequently, the difference in $T1_{Gd}$ and ΔR noted between the GBCAs cannot be fully attributed to the difference in electric charge. It would be ideal to compare compounds that differ only in electrical charge, but this is not feasible because these compounds are not commercially available or readily synthesized. Second, after the cartilage was treated with trypsin, the trypsin solution was not washed out of the cartilage nor was an inhibitor used, and therefore the trypsin remaining in the cartilage may have continued to degrade the cartilage over the time of the experiment. However, this condition was identical regardless of the GBCA used, and would not have influenced the comparison between GBCAs. Third, the ratio of the volume of gadolinium solution to that of the cartilage was greater than what would result from a typical intra-articular dGEMRIC study. This may have resulted in different diffusion kinetics of the GBCA. Fourth, the gadolinium-saline solution could not be

stirred between MR imaging acquisitions, because the MR images were consecutively acquired. This may have resulted in concentration inhomogeneity of the gadolinium-saline solution. Finally, our experimental study was performed in an ex vivo model at room temperature, and results may differ in an in vivo situation. The increase in temperature would be expected to alter the Brownian motion and viscosity of the GBCAs. In addition, in an in vivo setting, the washout of the GBCAs from cartilage may have an effect on the $T1_{Gd}$ value and the optimal timing of imaging. In addition, in an in vivo setting of osteoarthritis, the GAG would not be fully lost as in our experimental model, and the dGEMRIC results with the various GBCAs may differ. Our study results only suggest the possibility of discriminating normal from degenerated cartilage with alternative GBCAs, Gd-BOPTA and Gd-DOTA. To use alternative GBCAs in dGEMRIC studies in a clinical setting, further in vivo investigations are necessary.

In conclusion, GBCAs with a double negative charge produced better contrast between normal and degenerated cartilage than did those with a single negative charge and nonionic GBCAs at the same concentration at

dGEMRIC. Gd-BOPTA^{2-}, a high-relaxivity GBCA, showed higher contrast than did Gd-DTPA^{2-}, thus Gd-BOPTA^{2-} may be useful as an alternative to Gd-DTPA^{2-} at dGEMRIC. The high relaxivity of Gd-BOPTA^{2-} may enable the use of a lower dose of GBCA, which requires further investigation. When alternative GBCAs are used at dGEMRIC, the $T1_{Gd}$ and ΔR values could not be directly compared with $T1_{Gd}$ and ΔR values obtained with Gd-DTPA^{2-} because of relaxivity differences.

REFERENCES

1. Li W, Scheidegger R, Wu Y, et al. Delayed contrast-enhanced MRI of cartilage: comparison of nonionic and ionic contrast agents. Magn Reson Med. 2010;64(5):1267-73.

2. Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. Magn Reson Med. 1999;41(5):857-65.

3. Bashir A, Gray ML, Burstein D. Gd-DTPA2- as a measure of cartilage degradation. Magn Reson Med. 1996;36(5):665-73.

4. Trattnig S, Mlynarik V, Breitenseher M, et al. MRI visualization of proteoglycan depletion in articular cartilage via intravenous administration of Gd-DTPA. Magn Reson Imaging. 1999;17(4):577-83.

5. Thomsen HS, Morcos SK, Almen T, et al. Nephrogenic systemic fibrosis and gadolinium-based contrast media: updated ESUR Contrast Medium Safety Committee guidelines. Eur Radiol. 2013;23(2):307-18.

6. Haneder S, Kucharczyk W, Schoenberg SO, Michaely HJ. Safety of

magnetic resonance contrast media: a review with special focus on nephrogenic systemic fibrosis. Top Magn Reson Imaging. 2015;24(1):57-65.

7. Radbruch A, Weberling LD, Kieslich PJ, et al. Gadolinium retention in the dentate nucleus and globus pallidus is dependent on the class of contrast agent. Radiology. 2015;275(3):783-91.

8. Kanda T, Fukusato T, Matsuda M, et al. Gadolinium-based Contrast Agent Accumulates in the Brain Even in Subjects without Severe Renal Dysfunction: Evaluation of Autopsy Brain Specimens with Inductively Coupled Plasma Mass Spectroscopy. Radiology. 2015;276(1):228-32.

9. Kanda T, Osawa M, Oba H, et al. High Signal Intensity in Dentate Nucleus on Unenhanced T1-weighted MR Images: Association with Linear versus Macrocyclic Gadolinium Chelate Administration. Radiology. 2015;275(3):803-9.

10. Ramalho J, Castillo M, AlObaidy M, et al. High Signal Intensity in Globus Pallidus and Dentate Nucleus on Unenhanced T1-weighted MR Images: Evaluation of Two Linear Gadolinium-based Contrast Agents.

Radiology. 2015;276(3):836-44.

11. Radbruch A, Weberling LD, Kieslich PJ, et al. High-Signal Intensity in the Dentate Nucleus and Globus Pallidus on Unenhanced T1-Weighted Images: Evaluation of the Macrocyclic Gadolinium-Based Contrast Agent Gadobutrol. Invest Radiol. 2015;50(12):805-10.

12. Karabulut N. Gadolinium deposition in the brain: another concern regarding gadolinium-based contrast agents. Diagn Interv Radiol. 2015;21(4):269-70.

13. Zilkens C, Miese F, Kim YJ, et al. Direct comparison of intra-articular versus intravenous delayed gadolinium-enhanced MRI of hip joint cartilage. J Magn Reson Imaging. 2014;39(1):94-102.

14. Bittersohl B, Hosalkar HS, Miese FR, et al. Zonal T2* and T1Gd assessment of knee joint cartilage in various histological grades of cartilage degeneration: an observational in vitro study. BMJ Open. 2015;5(2):e006895.

15. Ersoy H, Rybicki FJ. Biochemical safety profiles of gadolinium-based extracellular contrast agents and nephrogenic systemic fibrosis. J Magn

Reson Imaging 2007;26(5):1190–1197.

16. Rohrer M, Bauer H, Mintorovitch J, Requardt M, Weinmann HJ. Comparison of magnetic properties of MRI contrast media solutions at different magnetic field strengths. Invest Radiol 2005;40(11):715–724.

17. Li W, Lazarus M, Koh J, Gliwa E, Prasad PV. Intra-articular dGEMRIC in patients scheduled for MR arthrography of the hip joint. J Arthritis 2013;2:110.

18. Andreisek G, White LM, Yang Y, Robinson E, Cheng HL, Sussman MS. Delayed gadolinium-enhanced MR imaging of articular cartilage: three-dimensional T1 mapping with variable flip angles and B1 correction. Radiology. 2009;252(3):865-73.

19. Mamisch TC, Dudda M, Hughes T, Burstein D, Kim YJ. Comparison of delayed gadolinium enhanced MRI of cartilage (dGEMRIC) using inversion recovery and fast T1 mapping sequences. Magn Reson Med. 2008;60(4):768-73.

20. Trattnig S, Burstein D, Szomolanyi P, Pinker K, Welsch GH, Mamisch TC. T1(Gd) gives comparable information as Delta T1 relaxation rate in

dGEMRIC evaluation of cartilage repair tissue. Invest Radiol. 2009;44(9):598-602.

21. Williams A, Mikulis B, Krishnan N, Gray M, McKenzie C, Burstein D. Suitability of T(1Gd) as the dGEMRIC index at 1.5T and 3.0T. Magn Reson Med. 2007;58(4):830-4.

22. Tiderius CJ, Olsson LE, Leander P, Ekberg O, Dahlberg L. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) in early knee osteoarthritis. Magn Reson Med. 2003;49(3):488-92.

23. Watanabe A, Wada Y, Obata T, et al. Delayed gadolinium-enhanced MR to determine glycosaminoglycan concentration in reparative cartilage after autologous chondrocyte implantation: preliminary results. Radiology. 2006;239(1):201-8.

24. Huang Q, Opstelten D, Samman N, Tideman H. Experimentally induced unilateral tooth loss: histochemical studies of the temporomandibular joint. J Dent Res. 2002;81(3):209-13.

25. Moyer JT, Priest R, Bouman T, Abraham AC, Donahue TL. Indentation properties and glycosaminoglycan content of human menisci in the deep

zone. Acta Biomater. 2013;9(5):6624-9.

26. Villegas DF, Hansen TA, Liu DF, Donahue TL. A quantitative study of the microstructure and biochemistry of the medial meniscal horn attachments. Ann Biomed Eng. 2008;36(1):123-31.

27. Burstein D, Velyvis J, Scott KT, et al. Protocol issues for delayed Gd(DTPA)(2-)-enhanced MRI (dGEMRIC) for clinical evaluation of articular cartilage. Magn Reson Med. 2001;45(1):36-41.

28. Bengtsson Mostrom E, Lammentausta E, Finnbogason T, Weidenhielm L, Janarv PM, Tiderius CJ. Pre- and postcontrast T1 and T2 mapping of patellar cartilage in young adults with recurrent patellar dislocation. Magn Reson Med. 2014.

29. Williams A, Gillis A, McKenzie C, et al. Glycosaminoglycan distribution in cartilage as determined by delayed gadolinium-enhanced MRI of cartilage (dGEMRIC): potential clinical applications. AJR Am J Roentgenol. 2004;182(1):167-72.

30. Bashir A, Gray ML, Boutin RD, Burstein D. Glycosaminoglycan in articular cartilage: in vivo assessment with delayed Gd(DTPA)(2-)-

enhanced MR imaging. Radiology. 1997;205(2):551-8.

31. Gillis A, Gray M, Burstein D. Relaxivity and diffusion of gadolinium agents in cartilage. Magn Reson Med. 2002;48(6):1068-71.

32. Tiderius CJ, Jessel R, Kim YJ, Burstein D. Hip dGEMRIC in asymptomatic volunteers and patients with early osteoarthritis: the influence of timing after contrast injection. Magn Reson Med. 2007;57(4):803-5.

33. Bittersohl B, Hosalkar HS, Werlen S, Trattnig S, Siebenrock KA, Mamisch TC. Intravenous versus intra-articular delayed gadolinium-enhanced magnetic resonance imaging in the hip joint: a comparative analysis. Invest Radiol. 2010;45(9):538-42.

34. Bittersohl B, Hosalkar HS, Kim YJ, et al. T1 assessment of hip joint cartilage following intra-articular gadolinium injection: a pilot study. Magn Reson Med. 2010;64(4):1200-7.

35. Wyler A, Bousson V, Bergot C, et al. Hyaline cartilage thickness in radiographically normal cadaveric hips: comparison of spiral CT arthrographic and macroscopic measurements. Radiology.

2007;242(2):441-9.

36. Cohen ZA, McCarthy DM, Kwak SD, et al. Knee cartilage topography, thickness, and contact areas from MRI: in-vitro calibration and in-vivo measurements. Osteoarthritis Cartilage. 1999;7(1):95-109.

37. Khawaja AZ, Cassidy DB, Al Shakarchi J, McGrogan DG, Inston NG, Jones RG. Revisiting the risks of MRI with Gadolinium based contrast agents-review of literature and guidelines. Insights Imaging. 2015;6(5):553-8.

국문초록

목적: 관절연골의 지연성 가돌리늄 조영증강 자기공명영상 (dGEMRIC) 에서 전하가 다른 여러 가돌리늄 기반 조영제를 통해 얻은 측정값을 비교해보고 Gd-DTPA^{2-} 외의 조영제를 사용한 dGEMRIC 검사에서도 정상연골과 변성연골의 구분이 가능함을 보여줌으로써, Gd-DTPA^{2-}를 다른 조영제로의 대체 가능성을 알아보고자 한다.

대상과 방법: 도살된 돼지에서 적출된 슬개골(n=44)을 4개의 가돌리늄 조영제군 (각, n=11)에 배당하였고, 하나의 조영제군 안에서는 정상연골 모델인 대조군(n=3)과 변성연골 모델인 트립신처리군 (n=8)으로 나누었다. 실험에 사용한 조영제는 비이온성 MRI 조영제 (Gd-BT-DO3A, Gadovist), 1가 음이온 조영제 (Gd-DOTA^{-}, Dotarem), 2가 음이온 조영제 (Gd-BOPTA^{2-}, Multihance; Gd-DTPA^{2-}, Magnevist) 4가지로, 모두 인산완충식염수에 희석하여 2.5mmol/L의 농도로 준비하였다. 준비된 돼지 슬개골을 희석한 조영제 용액에 침수시킨 직후인

0분에서부터 120분까지 10분 간격으로 T1 map 영상을 획득하였다. 획득한 T1 map에서 측정한 조영증강 후 T1 값과 조영증강 전 T1 값을 이용하여 ΔR ($\Delta R = 1/T1_{Gd} - 1/T1_0$) 값을 계산하였고, 시간에 따른 ΔR 값의 변화를 분석하였다. 슬개골은 safranin-O 염색을 시행하여 관절연골 내의 glycosaminoglycan content를 확인하였다. Safranin-O 염색의 강도를 정량화하기 위하여 빨간색(R), 녹색(G), 파란색(B)의 강도 수치를 이용하여 붉은 색의 상대 강도 ($r = R/(R^2 + G^2 + B^2)^{1/2}$)를 계산하였다. 시간에 따른 ΔR 값의 변화 양상을 분석하는데 선형 혼합 모형 분석을 이용하였으며, 그룹간 T1값, ΔR값의 비교에는 Student t test 와 Mann-Whitney U test 를 이용하였다.

결과: 정상연골 대조군과 변성연골 트립신처리군의 시간-ΔR 곡선의 기울기의 차이는 Gd-BOPTA^{2-}(0.037)를 이용하였을 때 가장 컸으며, Gd-DTPA^{2-}(0.022), Gd-DOTA^{-}(0.018), Gd-BT-DO3A(0.011) 순으로 기울기의 차이가 작아졌다. Gd-DTPA^{2-}를 기준으로 하였을 때, Gd-BOPTA^{2-}에서는 기울기의 차이가 통계적으로 유의미하게 커졌으며($p<.001$), Gd-BT-DO3A 에서는

유의미하게 작아졌다 (P = .004). 조영제 침수 90분후와 120분후 영상에서 얻은 단일 T1 값과 ⊿R 값의 경우, 2가의 음전하를 띄는 조영제에서만, 대조군과 트립신처리군 간에 통계적으로 유의한 차이가 있었다. Safranin-O 염색 강도 (r, 빨간색의 상대강도)는 대조군(0.77±0.07) 과 트립신처리군 (0.46± 0.05) 사이에 통계적으로 유의한 차이가 있었다 (p<.001).

결론: 동일 농도로 dGEMRIC 검사를 시행하였을 때, 2가의 음전하를 띄는 조영제가, 1가의 음전하를 띄는 조영제나 비이온성 조영제에 비해서 정상연골과 변성연골을 구분함에 있어서 더 우수하였다. 2가의 음전하를 띄는 조영제 중에서도 자기이완율이 높은 조영제인 Gd-BOPTA^{2-} 가 Gd-DTPA^{2-}에 비해서 정상연골과 변성연골 사이의 대조도가 높아, dGEMRIC 검사에서 대체 조영제로 사용될 수 있는 가능성이 있다.

.....

주요어: 관절연골, 관절연골의 지연성 조영증강 자기공명영상(dGEMRIC), 가돌리늄 기반 조영제,

글리코사미노글리칸

학번: 2014-30639