의학박사 학위논문

어린 쥐의 뇌내 가돌리늄 침착에 신기능이 미치는 영향

The effect of renal function on the intracranial gadolinium deposition in young rats

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이 논문을 의학박사 학위 논문으로 제출함

2018 년　 8 월

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Abstract

Purpose: To assess the effect of renal function on the signal intensity of brain tissue after multiple administration of linear and macrocyclic Gadolinium-based contrast agents (GBCAs) in young rats.

Materials and Methods: A total of 39 young rats were divided into 3 normal renal function groups (n=6 per group) and 3 decreased renal function groups (n=7 per group) according to the injected GBCAs (gadopentetate dimeglumine [linear GBCA] or gadobutrol [macrocyclic GBCA]) or saline for control groups. One-stage 5/6 nephrectomy was performed to produce rat model of decreased renal function (n=21). Ten intravenous injections of GBCAs were performed over a period of 2 weeks at a dose of 0.9mmol Gd/kg since rats became 6 weeks of age. T1-weighted magnetic resonance image and T1 mapping were performed on a 7 Tesla scanner at intervals of 2 weeks as follows: before the first GBCA administration (precontrast); one day (week 2), and two weeks after the last GBCA administration (week 4). Qualitative and quantitative analysis of T1 signal intensity ratio of deep cerebellar nucleus (DCN) to cerebellar cortex or pons, and quantitative analysis of T1 value of DCN were performed by two radiologists, and those were compared between time points in each group and between groups at each time point using Wilcoxon signed ranks test and 2-way analysis of variance, respectively.

Results: Decreased renal function group with linear GBCA injection was the only group showed statistically significant increase of T1 signal intensity ratio of DCN/cerebellum on

week 2 (P = 0.043), T1 signal intensity ratio of DCN/pons on both week 2 and week 4 (P = 0.043, respectively), and decrease of T1 value of DCN on week 2 (P = 0.043) and week 4 compared to baseline (P = 0.043). Decreased renal function group with linear GBCA injection also showed statistically significant percentage change of T1 value to baseline compared to other groups at week 2 (P = 0.011) and week 4 (P = 0.018). Other groups including all three normal renal function groups and decreased renal function groups with macrocyclic GBCA and saline injection did not show significant change in T1 signal intensity ratio and T1 value of DCN. In rats with decreased renal function with both linear and macrocyclic injections, T1 value of fourth ventricle was markedly decreased at week 2 (P = 0.043) and then slightly increased at week 4 but still low compared to the baseline level (P = 0.043).

Conclusions: Repeated administration of both linear and macrocyclic GBCA to young rats with decreased renal function caused significant decrease of T1 value in fourth ventricle at two weeks after the last contrast injection, suggesting delayed excretion of contrast material from CSF in ventricle. Rats with decreased renal function with linear GBCA injections, not with macrocyclic GBCA injections, showed significant T1 hyperintensity and decreased T1 value of DCN, suggesting deposition of gadolinium. There was no significant change in T1 signal intensity and T1 value of DCN in young rats with normal renal function after both linear and macrocyclic GBCA injections.

차 례

표 및 그림목차

Introduction

Gadolinium-based contrast agents (GBCAs) have been widely used for contrast-enhanced magnetic resonance (MR) imaging which enables detection of a great diversity of pathologic conditions. The safety of GBCAs has been re-focused since the publication of a study on hyperintensity in certain brain regions on non-enhanced T1-weighted images after multiple administrations of GBCAs [1]. Subsequent postmortem and animal studies as well as retrospective human studies revealed that the T1 hyperintensity and high gadolinium concentrations in brain tissue were detected after multiple injections of linear GBCAs, not after injection of macrocyclic GBCAs [2-10]. Unlike nephrogenic systemic fibrosis (NSF), a complication of GBCAs observed only in patients with severely compromised renal function [11-13], the gadolinium deposition in brain after administrations of GBCAs was detected in patients with normal renal function and healthy rats [1, 6, 10, 14]. Although studies on pediatric patients have reported similar results as in adult studies [9, 15, 16], large-scale studies with pediatric patients are still lacking.

Two previous studies with 3 patients with impaired renal function [17] and 25 patients on hemodialysis [5] reported that decreased renal function may increase the rate of gadolinium deposition in the brain after injection of linear GBCAs. However, there has been no human or animal study on the effect of renal function in gadolinium deposition in brain after multiple administration of macrocyclic GBCA.

Hence, the purpose of our study was to assess the effect of renal function on the signal intensity of brain tissue after multiple administration of linear and macrocyclic GBCAs in young rats.

Materials and Methods

Animal Model and Contrast Agents Injection Protocols

All study protocols were approved by the Institutional Animal Care and Use Committee of our institution. We utilized 39 female healthy Wistar rats (ORIENT BIO Inc., Seongnam-si, South Korea), and maintained under standard laboratory conditions (22-24 , 12/12h light/dark cycle). To produce rat model of decreased renal function, 21 rats with 5-week-old age (mean weight, 156.78 ± 5.57g) underwent one-stage 5/6 nephrectomy under isoflurane inhalation anesthesia. In brief, after the right kidney was exposed via a parasagittal incision along the lumbar spine, the proximal renal pedicle was ligated using silk tie and distal renal pedicle was clamped. Right radical nephrectomy was done by cutting of renal pedicle at the middle portion between ligated and clamped renal pedicle. The incision was closed, and then rats were turned back to the opposite site. Left kidney was exposed through a lateral dorsal incision and decapsulated. The renal vessels were clamped and both poles (approximately 2/3 of the functional kidney mass) were resected. Absorbable gelatin sponge (Gelfoam®, Pfizer Inc, NY) was applied to cut surface of left kidney for hemostasis. The vessel clamp was removed and the remained kidney was returned into the renal fossa. To check the renal function of rats who underwent 5/6 nephrectomy, serum creatinine and blood urea nitrogen (BUN) levels were measured using automatic biochemical analyzer (Hitachi 7180, Hitachi. Tokyo, Japan) three times; just before the nephrectomy, one week and two weeks after the nephrectomy. Contrast injections were performed one week after the nephrectomy. For the

normal renal function groups, 18 rats with 6-week-old age (mean weight, 176.19 ± 9.71g at the beginning of the study) were used.

Both groups with normal (n=18) and decreased renal function(n=21) were divided into 3 groups, respectively, to receive injections of the following gadolinium-based contrast agents: saline for control groups, gadopentetate dimeglumine (linear ionic GBCA, Magnevist; Bayer Healthcare, Berline Germany) and gadobutrol (macrocyclic nonionic GBCA, Gadovist; Bayer Healthcare, Berline Germany) (Table 1). Rats with normal renal function are classified according to the injected contrast materials as follows: normal saline in group 1, gadopentetate dimeglumine in group 2, and gadobutrol in group 3. Rats with decreased renal function are also classified according to the injected contrast materials as follows: normal saline in group 4, gadopentetate dimeglumine in group 5, and gadobutrol in group 6.

Ten intravenous injections of GBCA were performed over a period of 2 weeks (5 daily and sequential injections per week) since rats became 6 weeks of age under isoflurane inhalation general anesthesia as described in Figure 1. The daily dose of GBCAs (0.9mmol Gd/kg) was 1.5 times equivalent dose to the usual human dose of GBCAs after adjusting for body surface area as recommended by the Food and Drug Administration. The control groups were injected with 0.9 ml/kg of normal saline per injection similar to the amount of GBCA injection.

Table 1. Groups classified according to the renal function and the injected contrast materials

		Injected Contrast Material		
		Normal saline	gadopentetate dimeglumine (linear GBCA)	Gadobutrol (macrocyclic GBCA)
Renal function	Normal renal function (n=18: n=6 per group)	Group 1	Group 2	Group 3
	Decreased renal function (n=21: n=7 per group)	Group 4	Group 5	Group 6

Figure 1. Timing of contrast injection, magnetic resonance imaging acquisition in all groups and timing of nephrectomy and measurement of serum creatinine (Cr) and blood urea nitrogen (BUN) in groups of rats with decreased renal function.

A, Groups of rats with normal renal function, group 1, 2 and 3, performed 10 GBCA injection, over a period of 2 weeks (5 daily and sequential injections per week) since rats became 6 weeks of age, and three MR scans at intervals of 2 weeks; before the first GBCA administration (precontrast), one day after (week 2) and two weeks after the last GBCA administration (week 4).

B, Groups of rats with decreased renal function, group 4, 5 and 6, also performed GBCA injections and MR scans in the same manner as in the groups of rats with normal renal function. Nephrectomy was performed when rats became 5 weeks of age. Serum Cr and BUN were measured three times; just before the nephrectomy, one week and two weeks after the nephrectomy.

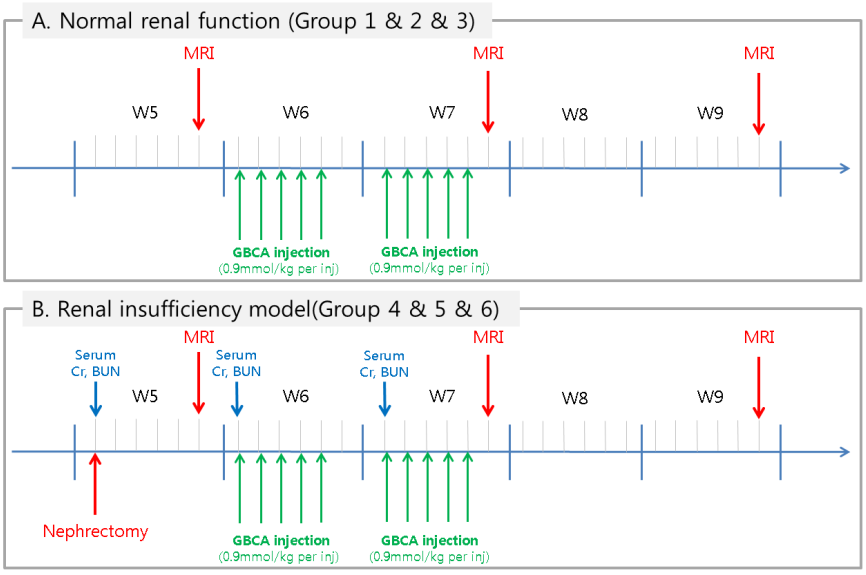


Image Acquisition and Image Analysis

Images were obtained using a 7.0 Tesla MR system (PharmaScan, Bruker Medical Systems, Karlsruhe, Germany) three times at intervals of 2 weeks as follows: (1) before the first GBCA administration (precontrast scan); (2) one day after the last GBCA administration (two weeks after first contrast injection: week 2); <3> two weeks after the last GBCA administration (four weeks after first contrast injection: week 4, Figure 1). Animals were maintained under anesthesia using 1% isoflurane in a 1:2 mixture of O2:N2O during MR examination with respiratory monitoring. Axial scans corresponding to coronal images in the neuroanatomic axis were obtained through the whole rat brain. The imaging protocol included T1-weighted fast spin echo image [repetition time (TR) = 500ms; echo time (TE) = 6ms; 2 averages; slice thickness = 1mm; 24 axial slices; matrix = 256 × 256; acquisition time = 3 minutes 12 seconds] and T1 mapping (TR = 875.7ms; TE = 12.2ms; TI = 900, 1500, 2500, 4000, 7000ms; 1 average; slice thickness = 1mm; multi spin echo RARE factor = 4; 24 axial slices; matrix = 128 × 128; acquisition time = 17 minutes 53 seconds).

Qualitative and quantitative analysis of T1 signal intensity and quantitative analysis of T1 value of deep cerebellar nucleus (DCN) were performed by two radiologists under blinded condition to the result of each other. Three point scoring scale of T1 signal intensity of DCN relative to that of adjacent cerebellar cortex was used for qualitative analysis as follows: 0 point for no demonstrable difference in signal intensity; 1 point for suspicious

increase of signal intensity in DCN relative to adjacent cerebellar cortex; 2 points for definite increase of signal intensity in DCN relative to adjacent cerebellar cortex.

Quantitative analysis of T1 signal intensity of DCN was performed by free-hand drawing of region of interest (ROI) at the DCN on T1-weighted images and calculating the ratio of mean DCN signal to the mean cerebellar cortex or pons signal. Drawing of ROIs and measurement of the mean signal intensity of the ROIs were done three times for each side of DCN and pons, and three times for cerebellar cortex (Figure 2a). The mean values of six measurements of signal intensity of DCN and pons, and three measurements of signal intensity of cerebellar cortex were used to calculate DCN/cerebellar cortex ratio and DCN/pons ratio in each rat.

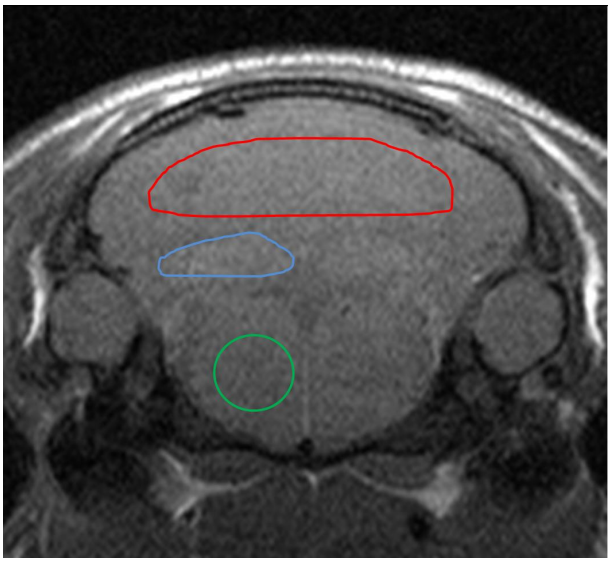
Quantitative analysis of T1 value of DCN was performed using the mean value of measurements of DCN T1 values. Above mentioned each free-hand drawing of ROI at DCN on T1-weighted image was copied and pasted on T1 map using software (AsanJ, BII Laboratory, Asan Medical Center) to measure mean value of T1 value using the same size and shape of ROI at the same location as on T1-weighted image (Figure 2b). Therefore, measurement of the mean T1 value of the ROIs was also done three times for each side of DCN. The mean value of six T1 value measurements was calculated in each rat.

Additionally, T1 value of fourth ventricle was measured by free-hand drawing of ROI at fourth ventricle, tree times for each side of lateral recess of fourth ventricle. The mean values of six measurements was calculated in each rat at each time point.

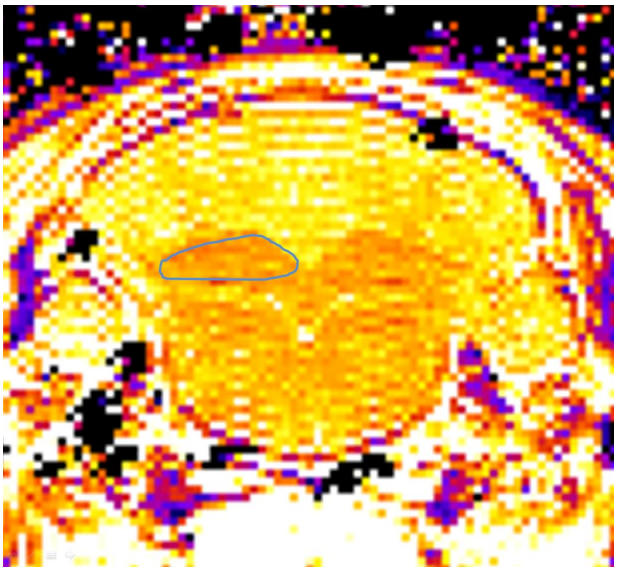
Figure 2. Examples of positioning of region of interest (ROI) by free-hand drawing for quantitative analysis

At DCN (blue), cerebellar cortex (red) and pons (green) on T1-weighted image (a) and on T1 map (b).

(2a)



(2b)



Statistical Analysis

Data for quantitative variables were presented as the mean ± standard deviation (SD) value. The time course of mean ± SD of scored points for qualitative analysis, and the time courses of DCN/cerebellar cortex and DCN/pons T1 signal intensity ratios, and that of T1 values for quantitative analyses of each group were plotted using box plot graphs. The differences in the average of T1 signal intensity ratios and T1 values between three time points (precontrast scan, two and four weeks after first contrast injection) within each group were assessed using Wilcoxon signed ranks test.

The mean ± SD of respective percent change to baseline of DCN/cerebellar cortex and DCN/pons T1 signal intensity ratios and that of T1 values at each time point were also plotted using box plot graphs. The differences in the percentage change to baseline of T1 signal intensity ratios and T1 values between groups at each time point were assessed using 2-way analysis of variance followed by the post hoc Bonferroni test for multiple comparisons.

The time course of mean T1 value of fourth ventricle of each group was plotted. The differences in the mean T1 value of fourth ventricle between three time points within each group were assessed using Wilcoxon signed ranks test.

All analyses were performed using SPSS software (version 20.0; IBM-SPSS Inc., Chicago, IL, USA). A P value below 0.05 was considered to indicate significant difference.

Results

A total of 34 rats survived during study period and completed the entire protocol of our study. Four rats died during or shortly after nephrectomy probably as a result of complication of surgery or anesthesia. Remaining one rat which also underwent nephrectomy died during the last MR examination at four weeks after first contrast injection probably due to anesthetic complication. Finally 18 rats with normal renal function (6 rats for group 1 – 3, respectively) and 16 rats with decreased renal function (6 rats for group 4; 5 rats for group 5 and group 6, respectively) were enrolled in our study.

In 16 rats with decreased renal function, the mean values of serum BUN and creatinine levels were elevated more than 2 times compared to preoperative levels as follows: (1) serum BUN level at preoperative stage, 18.42 ± 2.40 mg/dL; at one week after the nephrectomy, 52.10 ± 12.04 mg/dL ; and two weeks after the nephrectomy, 54.76 ± 7.69 mg/dL, (2) serum creatinine level at preoperative stage, 0.30 ± 0.03 mg/dL; at one week after the nephrectomy, 0.72 ± 0.12 mg/dL ; and two weeks after the nephrectomy, 0.65 ± 0.06 mg/dL.

Qualitative Analysis of T1 Signal Intensity of DCN

On visual assessment of serial follow-up of T1-weighted images at the level of DCN, only group 5 (decreased renal function with gadopentetate dimeglumine injections) showed suspicious increase in T1 signal intensity in DCN (Figure 3).

Qualitative analysis of T1 signal intensity of DCN using 3 points scoring scale revealed that all groups except group 3 (normal renal function with gadobutrol injection) had tendency toward increasement of T1 signal intensity after injection of contrast or saline (Figure 4). Group 5 (decreased renal function with gadopentetate dimeglumine injection) showed the most prominent T1 signal increase, and it was the only group with the mean of score on week 4 exceeding 1.0 point.

Figure 3. T1-weighted images at DCN level of representative changes of each group at three time points (before contrast injection, two weeks and four weeks after first contrast injection). Suspicious increase in T1 signal intensity in DCN was noted in group 5, rats with decreased renal function with gadopentetate dimeglumine injections (arrows). Note increased T1 signal intensity due to the contrast material remaining in the ventricle only in the rats with decreased renal function (group 5 and group 6, dashed arrows) on week 2 and week 4, that is a couple of days later and two weeks after the last contrast injection.

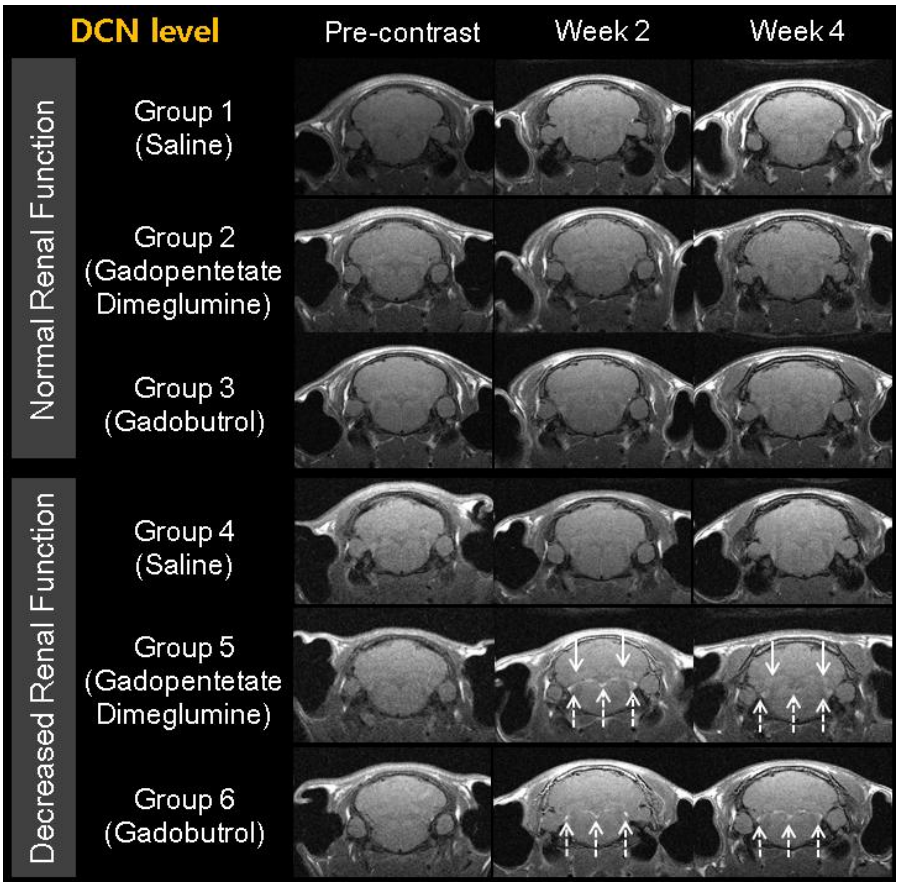
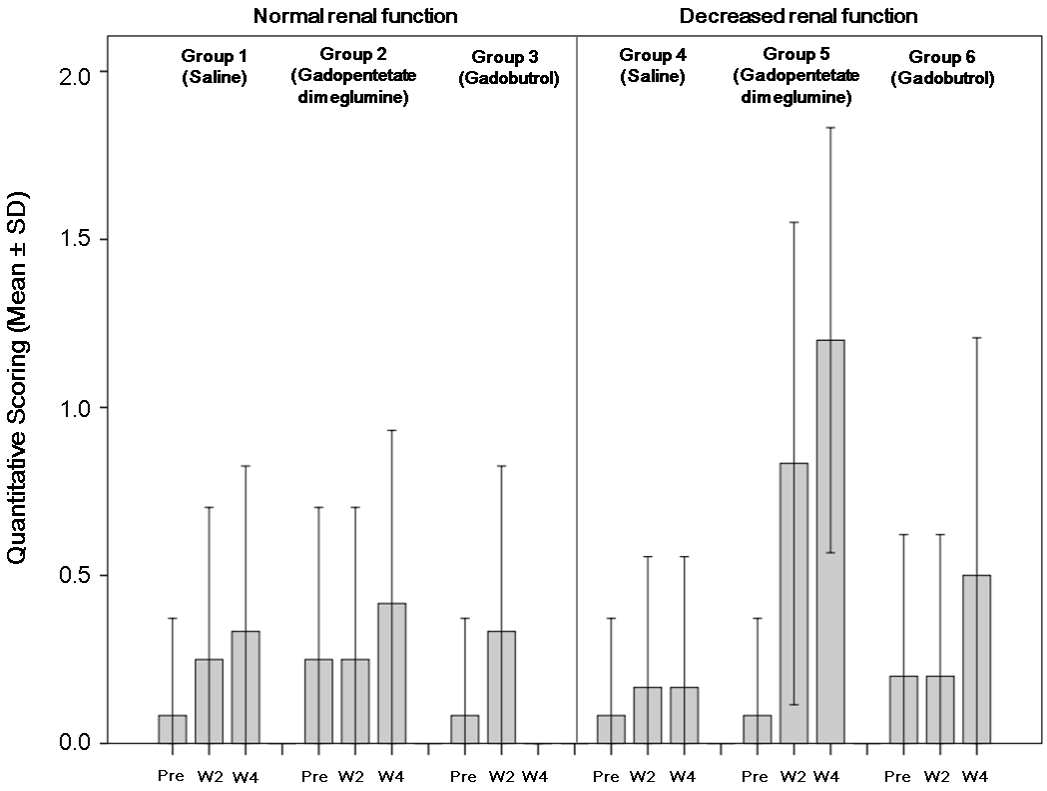


Figure 4. Temporal changes of scored points for T1-weighted hyperintensity of DCN in each group evaluated qualitatively by radiologists (mean ± SD). Each box plot represents mean scores before contrast injection (Pre), two weeks (W2) and four weeks (W4) after first contrast injection.



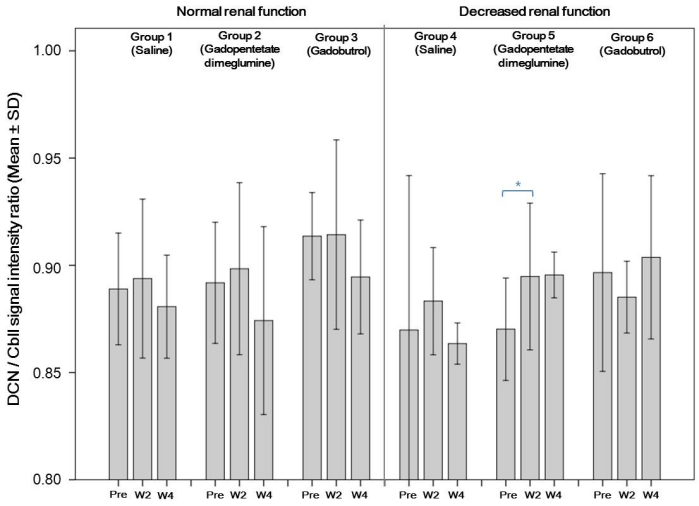
Quantitative Analysis of T1 Signal Intensity of DCN

Group 5 was the only group showed statistically significant increase of T1 signal intensity ratio of DCN/cerebellum on week 2 ($P = 0.043$), which remaining stable on week 4; the mean of T1 signal intensity ratios of group 5 was 0.8702 on week 0, 0.8948 on week 2, and 0.8955 on week 4 (Figure 5a). Group 5 also showed statistically significant increase of T1 signal intensity ratio of DCN/pons on both week 2 and week 4 ($P = 0.043$, respectively; Figure 5b). The comparison of % change to baseline between groups at week 2 and week 4, however, showed no statistically significant difference in increase of T1 signal intensity ratio between groups at the same time points ($P > 0.05$; Figure 5c and 5d).

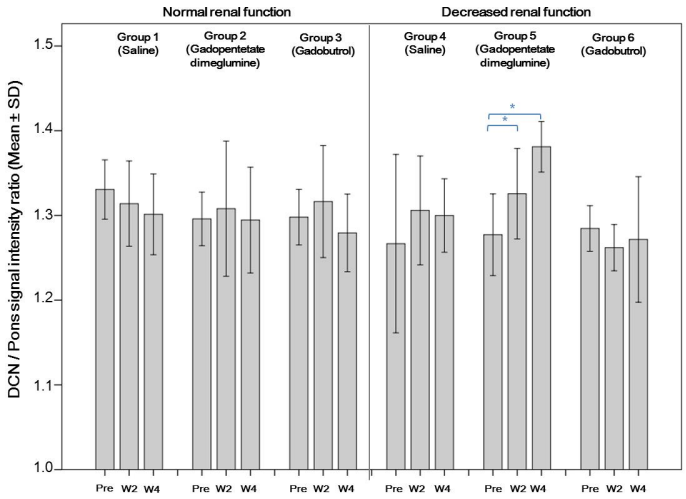
Figure 5. Temporal change of T1 signal intensity ratio and the percentage change of T1 signal intensity ratio to baseline

Temporal changes of T1signal intensity ratio of DCN/cerebellum (a) and DCN/pons (b) of each group evaluated quantitatively, and the percentage changes of T1 signal intensity ratio to baseline of DCN/cerebellum (c) and DCN/pons (d) at each time points, the week 0 (pre-contrast scan), week 2 and week 4 (mean ± SD). * $P < 0.05$

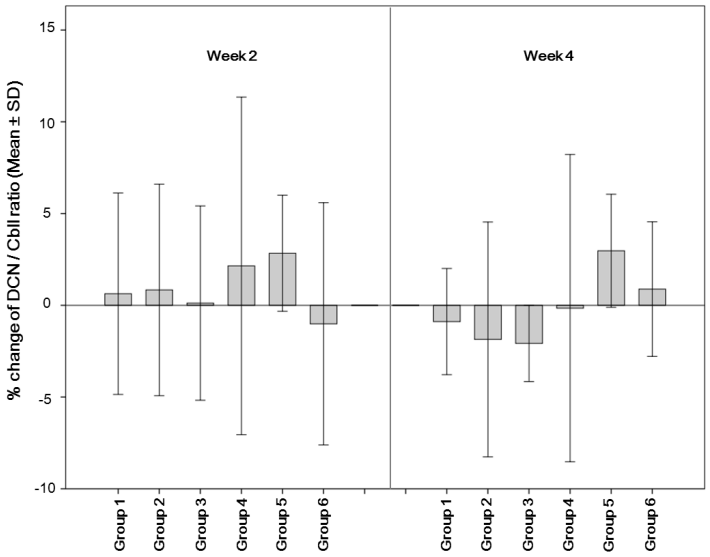
(a)



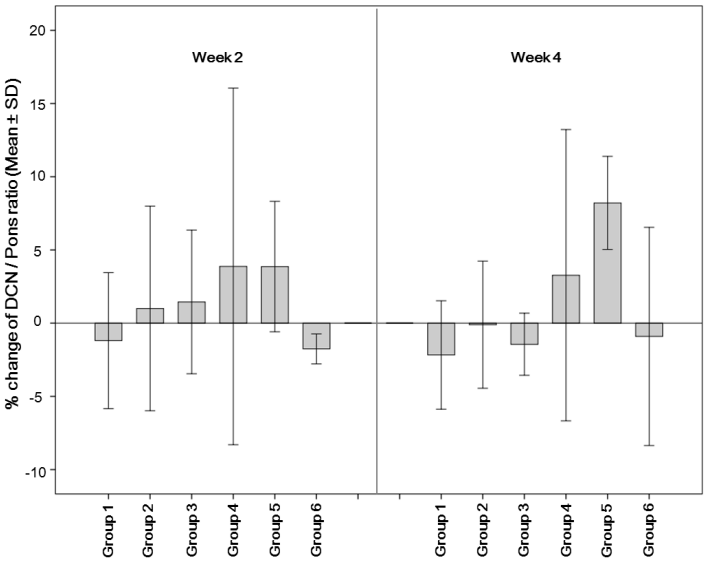
(b)



(c)


Week 2
Week 4
% change of DCN / Cbll ratio (Mean ± SD)
15
10
5
0
-5
-10
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6

(d)

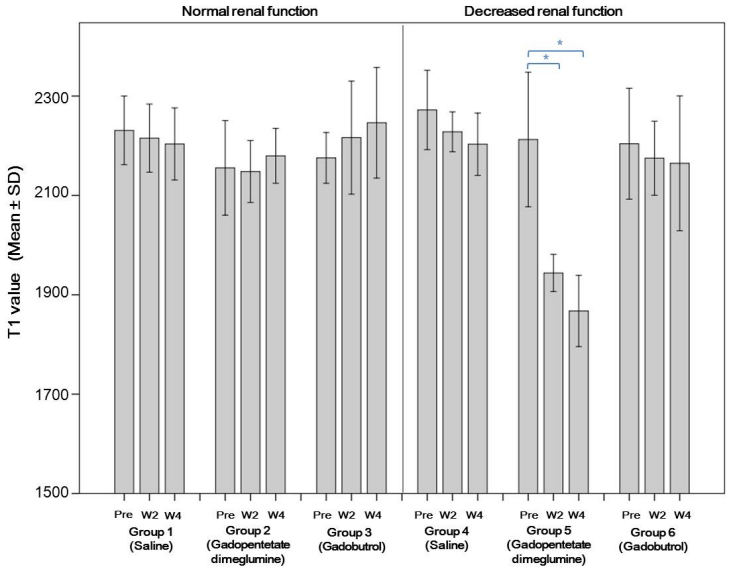

Week 2
Week 4
% change of DCN / Pons ratio (Mean ± SD)
20
15
10
5
0
-5
-10
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6

Quantitative Analysis of T1 value of DCN

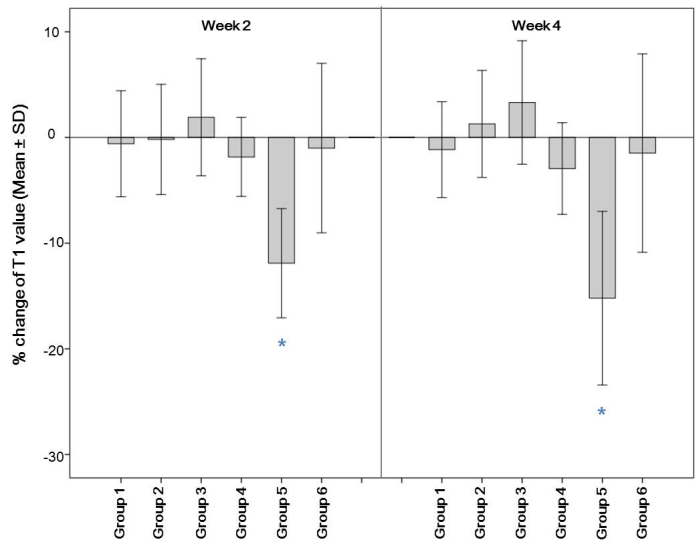
Group 5 was the only group showed statistically significant decrease of T1 value of DCN on week 2 ($P = 0.043$) and week 4 compared to baseline ($P = 0.043$); the mean of T1 values of group 5 was 2212.503 on week 0, 1943.895 on week 2 and 1867.441 on week 4 (Figure 6a). Group 5 also showed statistically significant percentage change of T1 value to baseline compared to other groups at week 2 ($P = 0.011$) and week 4 ($P = 0.018$; Figure 6b). Group 6 also showed decrease of T1 value on week 2 and week 4 compared to baseline, but it was statistically not significant. Group 2 showed decrease of T1 value on week 2, but it increased on week 4, without statistical significance.

Figure 6. Temporal changes of T1 value of DCN (a) of each group evaluated quantitatively, and the percentage changes of T1 value of DCN to baseline (b) at each time points (mean ± SD). * P < 0.05

(a)



(b)



Additional Findings in Ventricles

A notable additional finding was that the increased T1 signal due to the contrast material remaining in the fourth ventricle was noted in the rats with decreased renal function (group 5 and group 6) at week 2 and week 4, that is one day after and two weeks after the last contrast injections, respectively (Figure 3 and Figure 7). At the just caudal portion of the DCN level, remaining contrast material in the fourth ventricle was also noted in rats with normal renal function (group 2 and group 3), but it was less prominent than rats with decreased renal function, and only at week 2, not at week 4 (Figure 7).

On week 2 (one day after the last contrast injection), marked decrease of T1 value of fourth ventricle was noted on group 5 (from 2262.010 ± 35.895 to 1664.400 ± 73.309, P = 0.043) and group 6 (from 2278.005 ± 99.088 to 1732.419 ± 64.333, P = 0.043), and subtle decrease was noted on group 2 (from 2257.167 ± 124.918 to 2171.017 ± 100.818, P = 0.028) and group 3 (from 2227.702 ± 117.440 to 2138.333 ± 83.351, P = 0.028), suggesting the remaining of contrast material in ventricle (Figure 8). Group 2 and group 3 recovered their T1 value of fourth ventricle on week 4 (two weeks after the last contrast injection) near the baseline level suggesting washout of contrast material (2241.368 ± 102.153 and 2257.167 ± 124.918, respectively). Although the T1 values of the fourth ventricle were slightly increased in group 5 (1944.600 ± 101.238) and group 6 (1937.213 ± 55.138) at week 4, these were still low compared to the baseline level (P = 0.043), suggesting that the contrast material was

slightly washed out but still remained in ventricle of rats with decreased renal function with both linear and macrocyclic injections, even two weeks after the last contrast injection.

Figure 7. T1-weighted images at the fourth ventricle level of representative changes of each group at three time points: before contrast injection (pre-contrast), two weeks (week 2) and four weeks (week 4) after the last contrast injection. Note definitely increased T1 signal intensity probably due to the contrast material remaining in the ventricles in rats with decreased renal function (group 5 and group 6, dashed arrows) on week 2 and week 4, and relatively less prominent increased T1 signal intensity in ventricles in rats with normal renal function (group 2 and group 3, dashed arrows) on only week 2.

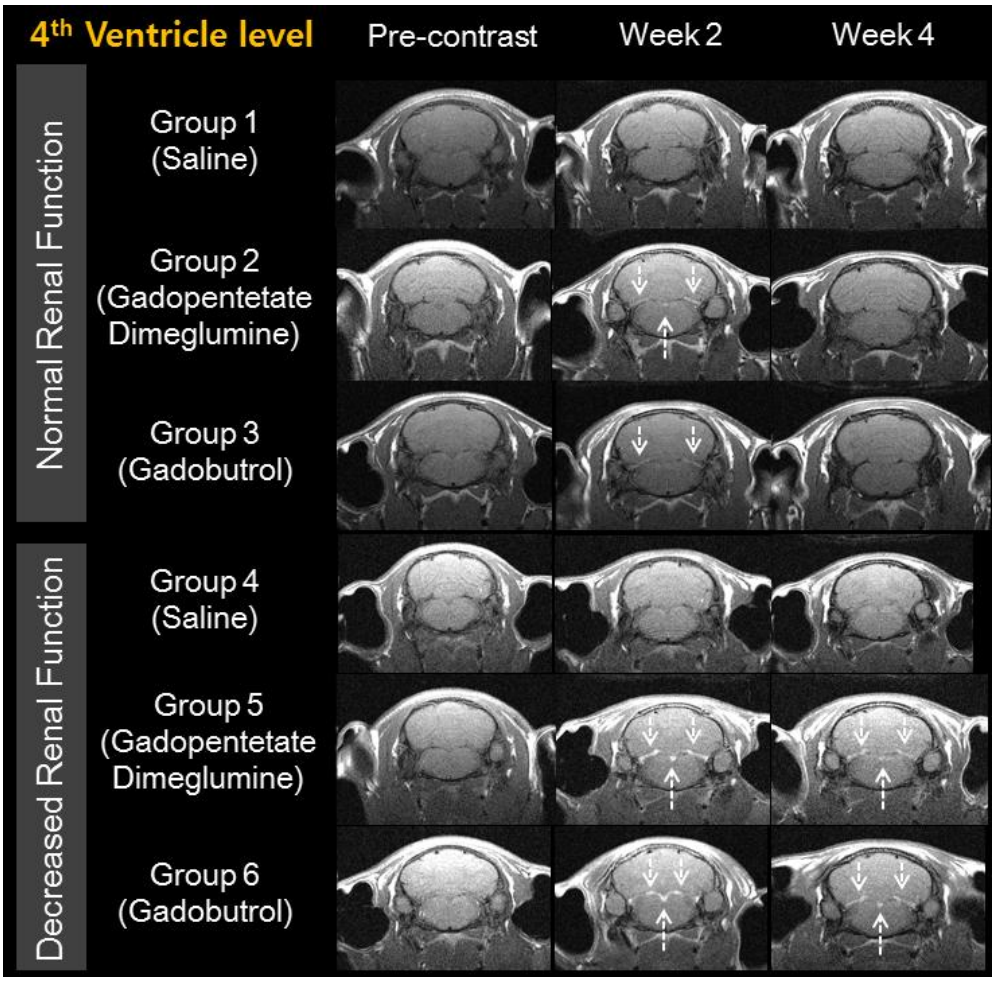
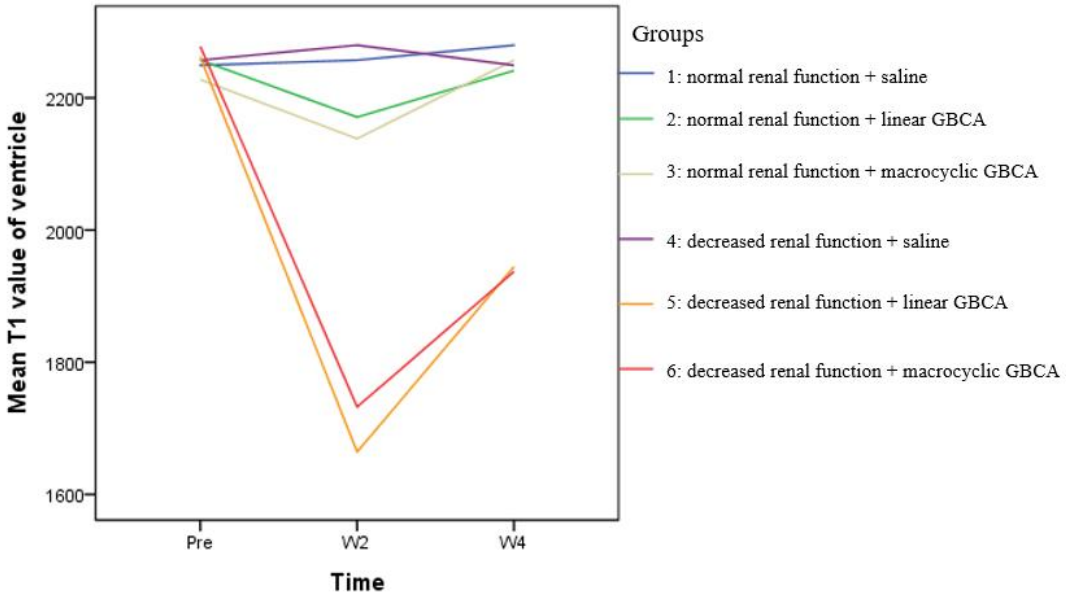


Figure 8. Mean T1 value of fourth ventricles in each group at each time point. The T1 values of fourth ventricle were markedly decreased at week 2 and slightly increased at week 4 in rats with decreased renal function with both linear (group 5) and macrocyclic GBCA injections (group 6). T1 value in rats with normal renal function with linear (group 2) and macrocyclic GBCA injections (group3) also decreased at week 2, but this was less than in group 5 and group 6, and increased to baseline level at week 4.



Discussion

After repeated injection of linear and macrocyclic GBCAs in young rats with decreased renal function, only linear GBCA caused significant signal increase in T1-weighted signal intensity ratio and decrease in T1 value of DCN, suggesting the deposition of gadolinium. There was no significant increase in T1-weighted signal intensity ratio and decrease in T1 value of DCN in young rats with normal renal function after both linear and macrocyclic GBCA injections.

Hyperintensity on precontrast T1-weighted image after multiple doses of GBCAs within the dentate nucleus was observed in human research as well as animal studies, and its association with the deposition of gadolinium had been demonstrated by postmortem and animal studies [4, 6, 18]. There is controversy as to whether gadolinium deposits on globus pallidus, since no significant increase in signal intensity was noted in globus pallidus in some studies with rats and pediatric patients [6, 16]. The increase of signal intensity in globus pallidus was not observed as well in our pilot study before a full-scale study, therefore, only DCN was evaluated in this study.

The degrees of deposition of gadolinium in brain tissue are different depending on the molecular structure of GBCAs; previous studies have shown that the greatest T1 signal increase in non-ionic linear agents, less prominent increase in ionic linear agents, and no significant T1 signal increase in macrocyclic agents [3-8, 10, 14, 19]. Whereas NSF occurred

only in patients with severely compromised renal function [11-13], the gadolinium deposition in brain after linear GBCA administration was observed in human and animal with normal renal function [1, 6, 10, 14]. However, in this study, there was no significant change in T1 signal intensity and T1 value after linear GBCA injections in rats with normal renal function. Two hypotheses can be considered as the cause of this issue; first, our study was conducted using 7 Tesla MR system, whereas previous studies used MR systems with field strength of 3 Tesla or less. T1 relaxivities of GBCAs are dependent on field-strength. The prolongation of T1 relaxation at a higher field goes along with a lower relaxivity. Therefore, the difference between T1 signal intensities on precontrast and postcontrast scans can be smaller at ultra-high field strengths relative to lower fields [20, 21]. Second, our study was conducted with young rats, 6-week-old age at the time of first contrast injection. There has been no study on gadolinium deposition in brain tissue with young rats. We can assume that multiple administrations of linear GBCAs in young rats with normal renal function may cause less or no deposition of gadolinium in brain tissue than in adult rats. To confirm this hypothesis, further study using the same study protocol with adult and young rats is required.

Unlike rats with normal renal function, a significant increase in T1 signal intensity and a decrease in T1 value were observed in rats with decreased renal function after multiple administrations of linear GBCA in this study. In addition, delayed gadolinium excretion and remained contrast enhancement in the fourth ventricle, even two weeks after last GBCA injection, was noted only in rats with decreased renal function after both linear and

macrocyclic GBCAs, whereas it was noted only two weeks after last GBCA injection in rats with normal renal function in our study. It is thought that decreased renal function caused delayed excretion of GBCAs from CSF allowing the linear GBCAs which have lower stability to dissociate to form other gadolinium-containing compounds. This assumption can be supported by results from previous studies. Previous studies reported that macrocyclic GBCAs are cleared from the brain as intact chelates, whereas linear GBCAs dissociate to three forms as follows: intact chelate, insoluble gadolinium-based inorganic salts, and a water-soluble gadolinium macromolecular fraction. And the authors reported that it is highly unlikely that the intact chelate is the cause of T1 hyperintensities [10, 22, 23]. Since CSF can be a potential pathway of entry into the brain tissue of GBCAs presumably through "glymphatic system" [23-25], dissociated gadolinium-containing compounds from retained linear GBCAs in ventricular system may be the potential cause of the T1 hyperintensity and decrease in T1 value which were only observed in rats with decreased renal function in our study.

Cao Y et al reported that hemodialysis patients showed greater increase in T1 signal intensity in dentate nucleus after linear GBCA administration than patients with normal renal function, and suggested renal function may affect the gadolinium deposition in brain [5]. The results of our animal study support previous findings by Cao Y et al.

Rats with normal renal function, although less than Rats with decreased renal function, also showed T1 hyperintensity in the fourth ventricle presumably by retained GBCAs after one day after the last GBCA injection. In previous study with healthy rats, Jost et al reported that both linear and macrocyclic GBCAs were almost completely cleared from the CSF after 48 hours of a single GBCA injection with a dose of 1.8 mmol Gd/kg body weight [25]. Even though the lower dose with 0.9 mmol Gd/kg body weight was used in our study, we performed 10 times of daily injections in our study, which may cause the prolonged retention of GBCAs in the ventricle one day after the last injection in our study.

This study has several limitations. Since this study was conducted only with young rats, it was not possible to confirm whether the cause of the different results from previous studies with adult rats was the different age group of subjects. Further study using the same study protocol in both adult and young rats is needed. This study did not measure the gadolinium concentration directly in the brain tissue of rats, but indirectly estimated the gadolinium deposition through the measurements of T1 signal intensity and T1 value on MR images. The use of the pre-clinical 7 tesla MR system in this study is a limitation that signal intensity changes in T1-weighted images may not be as clear as in previous studies using conventional MR systems with 3 tesla or less. A follow-up period of only four weeks is also a limitation of this study. The schedule of contrast injection of this study, daily injection of high doses of contrast medium for two weeks, was different from clinical situation. Injecting

small doses of contrast agents at intervals of several weeks or months like actual clinical practice may have different results than those of this study.

Conclusion

Repeated administration of both linear and macrocyclic GBCA to young rats with decreased renal function caused significant decrease of T1 value in fourth ventricle at two weeks after the last contrast injection, suggesting delayed excretion of contrast material from CSF in ventricle. Rats with decreased renal function with linear GBCA injections, not with macrocyclic GBCA injections, showed significant T1 hyperintensity and decreased T1 value of DCN, suggesting deposition of gadolinium. There was no significant change in T1 signal intensity and T1 value of DCN in young rats with normal renal function after both linear and macrocyclic GBCA injections.

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국문요약

연구목적

어린 쥐의 신기능이 반복적인 선형 및 거대고리형 가돌리늄-기반 조영제 (Gadolinium-based contrast agent [GBCA]) 주입 후의 뇌내 신호강도에 미치는 영향을 확인하고자 함.

연구재료와 연구방법

총 39 마리의 어린 쥐를 주입한 GBCA (gadopentetate dimeglumine [선형 GBCA] 혹은 gadobutrol [거대고리형 GBCA]) 혹은 생리식염수)에 따라 정상 신기능 3 군 (각 군당 6 마리씩) 및 신기능 저하 3 군 (각 군당 7 마리씩)으로 분류하였다. 신기능 저하를 유도하기 위하여 쥐 21 마리에 대하여 5/6 신장절제술을 시행하였다. 쥐가 6 주령이 되었을 때 GBCA 를 0.9mmol Gd/kg 의 용량으로 2 주간 주당 5 일간 매일 주입하여 총 10 회 정맥 주입하였다. 7 Tesla 자기공명스캐너를 이용하여 T1 강조영상 및 T1 mapping 을 2 주간격으로 총 3 회 (첫 GBCA 주입 전 [조영전], 마지막 GBCA 주입 후 1 일 [실험 2 주째] 및 2 주째 [실험 4 주째]) 촬영하였다. 두명의 영상의학과 의사가 deep cerebellar nucleus (DCN)과 소뇌 피질 혹은 교뇌의 T1 신호강도 비의 정성 및 정량 분석과 DCN 의 T1 값의 정량 분석을 시행하였다. 이에 대한 각 군내에서 자기공명영상 촬영 시점에 따른, 그리고 각 시점에서 군에 따른 유의미한 차이가 있는지를 각각 Wilcoxon signed ranks test 와 이원 분산분석을 통해 비교하였다.

연구결과

선형 GBCA 를 주입한 신기능저하군에서만 조영제 투입전과 비교하여 DCN/소뇌 T1 신호강도비가 실험 2 주째에, DCN/교뇌 T1 신호강도비가 실험 2 주 및 4 주째에 통계학적으로 유의미하게 상승하였고 (각 $P = 0.043$), DCN 의 T1 값이 실험 2 주 및 4 주째에 유의미하게 감소하였다 (각 $P = 0.043$). 조영제 투입전에 대한 DCN 의 T1 값의 변화율은 다른 군들과 비교하여 선형 GBCA 를 주입한 신기능저하군에서만 실험 2 주째 ($P = 0.011$) 및 4 주째에 ($P = 0.018$)에 유의미한 차이를 보였다. 정상신기능을 가진 세 군과 비정상신기능 군에서 거대고리형 GBCA 및 생리식염수를 투입한 군에서는 유의미한 DCN 의 T1 신호강도비 및 T1 값의 변화를 보이지 못했다. 신기능저하군에서 선형 및

거대고리형 GBCA를 주입하였을 때 모두 실험 2주째 및 4주째에 제4뇌실에서 측정한 T1 값이 유의미하게 감소하였다 (각 P = 0.043).

결론

신기능이 저하된 어린 쥐에서 선형 및 거대고리형 GBCA를 반복 주입하였을 때 제4뇌실내의 gadolinium 배출 지연을 시사하는 제4뇌실의 T1 value의 감소가 실험 2주째 및 4주째에 관찰되었다. DCN의 가돌리늄 침착을 시사하는 T1 신호강도증가 및 T1 값의 감소는 선형 GBCA를 주입한 신기능저하군에서만 관찰되었고, 거대고리형 GBCA를 주입한 신기능저하군에서는 관찰되지 않았다. 정상신기능을 가진 어린 쥐에서는 선형 및 거대고리형 GBCA 주입시 유의미한 DCN의 T1 신호강도 및 T1 값의 차이를 보이지 않았다.