The effect of liver fibrosis on gadolinium retention after administration of gadodiamide, gadobutrol, and gadoxetic acid disodium in rats

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Directed by Professor Yong Eun Chung

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Hyung Cheol Kim

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This certifies that the Doctoral Dissertation of Hyung Cheol Kim is approved.

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In the blessed winter of 2023,

Hyung Cheol Kim

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ABSTRACT

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PURPOSE

To investigate the effect of liver fibrosis on gadolinium retention in the liver, kidney, bone and brain tissues after administration of gadoxetic acid disodium, gadodiamide, and gadobutrol.

MATEIRALS AND METHODS

A total of 120 male Sprague-Dawley rats (200-220 g) were categorized into 60 for the

normal liver group and the remaining 60 for the liver fibrosis group. The liver fibrosis group underwent induction of liver fibrosis by intraperitoneal injection of 150 mg/kg thioacetamide three times a week for 12 weeks. Each group was further divided into subgroups (n=15), and they were intravenously administered with a single dose of gadolinium-based contrast agents (GBCA) including gadodiamide, gadobutrol, gadoxetic acid disodium, and saline. The liver, kidney, bone and brain were harvested at 1 week, 4 weeks and 12 weeks post GBCA administration. Liver fibrosis was investigated by histologic analysis using Metavir scoring system. Gadolinium content in each tissue was measured by inductively coupled plasma mass spectroscopy (ICP-MS). Each subgroup was analyzed in comparison to the saline group to determine the amount of GBCA retention in each organ at 1, 4, and 12 weeks. The analysis was conducted statistically to validate the findings. For the morphological and structural analysis of gadolinium deposition, we conducted observations for liver tissues using Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM-EDX). This study employed t-tests for GBCAs over time, utilized the Mann-Whitney U test to compare Metavir scores for liver fibrosis impact, and applied Spearman's correlation coefficient (ρ) to analyze the relationship between gadolinium retention and liver fibrosis.

RESULTS

Out of the 60 rats in the liver fibrosis group, 55 were diagnosed with liver fibrosis of grade 2 or higher. This showed a statistically significant difference compared to the normal

group ($p<0.001$).

Gadodiamide consistently showed the highest retention in all organs, especially in the liver, irrespective of the presence of liver fibrosis. In contrast, gadoxetic acid generally exhibited lower accumulation levels across all examined organs.

Liver fibrosis increased residual gadolinium concentration significantly in all GBCA administrations ($p<0.05$), except for the 1-week gadodiamide group. Overall, positive correlation with Metavir score was observed for all GBCAs and time points in most of cases.

In the femur, gadolinium retention in liver fibrosis group was significantly smaller than the normal liver group at 1 and 4 weeks for all GBCAs. On the other hand, in kidney tissues, liver fibrosis model represented significantly higher concentration of gadolinium retention at 4 and 12 weeks after gadoxetic acid administration. The concentration of residual gadolinium in the brain tissue had no meaningful pattern between fibrosis models and normal liver groups.

In TEM and SEM-EDX investigation, clear visualization of gadolinium deposition proved challenging. However, SEM-EDX revealed predominant distribution of retained gadolinium in the cytoplasm rather than the nucleus. Subsequently, Flatquad mapping detected gadolinium, yet the specific organelles within the cytoplasm where it was distributed could not be determined

CONCLUSION

Gadolinium retention increased over time in the liver with liver fibrosis, regardless of the GBCA type. Conversely, reduced accumulation was observed in the femur with liver fibrosis. In the kidney, a similar effect was seen only with gadoxetic acid, while no significant changes were noted in the brain.

Key words : gadolinium retention, liver fibrosis, GBCA, gadoxetic acid disodium, gadoxetic acid disodium, gadobutrol

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I. INTRODUCTION

Owing to their exceptionally favorable safety profile and unique paramagnetic properties, the use of gadolinium-based contrast agents (GBCA) has been experiencing a noteworthy surge, with global usage surpassing more than half a million liters in 2018 [1,2]. In addition to the well-known concern of nephrogenic systemic fibrosis, which is typically associated with impaired renal function, recent findings have highlighted a compelling revelation of gadolinium retention in the brain, even in individuals with normal kidney function [3,4]. After exploring the intricate mechanisms underlying this phenomenon, several studies have proposed the potential involvement of active metal transporters [5,6] or cerebrospinal

fluid (CSF) dynamics [7,8] as plausible factors that contribute to gadolinium retention in the brain. Notably, these processes are suggested to occur while maintaining the integrity of the blood-brain barrier (BBB) [7]. However, a more recent and emerging perspective suggests the potential involvement of the glymphatic system [9,10], providing a novel framework for comprehending the intricate dynamics associated with gadolinium retention in the brain. The glymphatic system, which involves the exchange of CSF and interstitial fluid, may play a pivotal role in the distribution and retention of gadolinium in the brain tissues [10]. This evolving understanding highlights the intricate and multifaceted aspects of gadolinium retention, highlighting the need for additional inquiry into the complex interplay between active metal transporters, CSF dynamics, and the glymphatic system in individuals exhibiting normal renal function.

Additionally, the bone, kidney, and other organs can serve as reservoirs for GBCA. Specifically, bone tissue is recognized as the primary reservoir for gadolinium owing to its active binding to the bone matrix facilitated by osteoblasts, wherein it can replace calcium during hydroxyapatite formation [4]. The mechanism by which gadolinium accumulates in the kidneys remains unclear; however, one hypothesis suggests that GBCA molecules may be entrapped through vacuolization in the cytoplasm of proximal tubular epithelial cells [11,12].

Despite ongoing investigations, the mechanisms underlying gadolinium retention in individuals with normal kidney function have not been fully elucidated. Various forms of retained gadolinium are still under investigation, however recent studies have indicated that gadolinium ions, thought to be released from GBCA through transmetalation, accumulate in tissues in both soluble and insoluble forms [13]. Over time, soluble forms may gradually be washed out, whereas insoluble forms persist in tissues, leading to the

establishment of a steady state in the amount of retained gadolinium [14-16]. This complexity highlights the need for further research to comprehensively understand the dynamics of gadolinium retention in different tissues and its implications in individuals undergoing GBCA administration.

Numerous preclinical *in vitro* studies have investigated the potential impact of gadolinium, revealing that it functions as a voltage-gated calcium channel blocker, even at very low concentrations. Additionally, lanthanides, including gadolinium, have been implicated in increasing the expression of inflammatory cytokines [17] and inducing oxidative stress [18]. Complementing these findings, preclinical animal studies have demonstrated neurotoxicity resulting from intraventricular administration of GBCA [19,20].

Given these insights, extensive research has been dedicated to unraveling the potential risks associated with intravenous GBCA administration and the subsequent accumulation of gadolinium in the human body. However, recent preclinical and clinical investigations have not presented conclusive evidence of the harmful effects of gadolinium exposure on brain tissue [21]. Moreover, long-term follow-up studies have failed to identify any notable changes in biomarkers or histological abnormalities [8,13]. Despite these reassuring findings, the potential toxicity associated with gadolinium has not been entirely dispelled, and there remains a valid imperative to minimize the accumulation of gadolinium in the body [13].

Gadoxetic acid (gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid; Gd-EOB-DTPA; Primovist or Eovist, Bayer Healthcare Pharmaceuticals, LLC. Berlin, Germany) stands out as a hepatocyte-specific magnetic resonance (MR) contrast agent distinguished by its linear ionic hydrophilic molecular properties [22]. Owing to its hepatocyte-specific nature, approximately 50% of gadoxetic acid is absorbed by hepatocytes and it is subsequently excreted into the hepatobiliary system. This unique characteristic facilitates

hepatobiliary phase imaging, contributing to enhanced liver-to-lesion contrast [23]. Notably, the dosage of gadoxetic acid is only one-fourth that of other conventional extracellular GBCAs per kilogram, owing to its higher T1-relaxivity (0.025 mmol/kg vs. 0.1 mmol/kg) [24]. This dosage discrepancy serves as a potential explanation for gadoxetic acid administration's lack of association with hyperintensity in the dentate nucleus on unenhanced T1-weighted images, a phenomenon observed with gadodiamide [25,26], despite it being a linear contrast agent with lower kinetic stability.

Recent studies have compared gadoxetic acid with other GBCAs, indicating that when administered at clinically recommended doses of gadoxetic acid disodium, the amount of gadolinium retained is smaller than that observed with gadodiamide and gadobutrol [25,27]. Moreover, a substantial proportion of the retained gadolinium is excreted within the first 4 weeks after injection [27]. These findings highlights the distinct pharmacokinetic profile of gadoxetic acid, suggesting its potential advantages in terms of reduced gadolinium retention and efficient elimination from the body.

Beyond the crucial consideration of the underlying kidney function [28], the types of GBCAs, such as distinction between linear and macrocyclic structures and ionic versus nonionic characteristics [29,30], along with the administered dose of GBCAs, several reported factors contribute to the intricate landscape of gadolinium retention. Notably, the presence of sepsis has been identified as a factor that influences gadolinium retention [31]. Additionally, elevated serum phosphate levels have been linked to increased gadolinium retention, introducing another layer of complexity into the interplay between the factors affecting retention dynamics [32].

Conversely, some factors have been reported to mitigate gadolinium retention in tissues. For instance, diabetes has been associated with reduced gadolinium retention [33].

Furthermore, chelating agent therapy has emerged as a potential mitigating factor, offering insights into strategies for minimizing gadolinium accumulation in various organs [27]. The multifaceted nature of these factors highlights the intricate web of variables influencing gadolinium retention, necessitating a comprehensive understanding of patient-specific conditions and treatment contexts to guide optimal decision-making in clinical practice.

Continued research is essential to unravel the nuanced interactions among these factors and their collective impact on gadolinium retention. This comprehensive understanding is pivotal for refining risk assessment protocols, optimizing contrast agent selection, and tailoring medical interventions to ensure the safety of diagnostic imaging procedures.

MR imaging (MRI) with GBCA, including gadoxetate disodium, is a routine procedure that is often performed in patients with liver fibrosis or cirrhosis. As liver fibrosis progresses, a concurrent decrease occurs in the expression of organic anion transporters [34]. This decrease in transporter expression leads to discernible pharmacokinetic alterations in GBCA within the fibrotic liver milieu [35]. Additionally, the progression of liver fibrosis involves the accumulation of excess collagen in the extracellular matrix, further complicating the tissue environment [36].

In the context of late gadolinium enhancement observed on cardiac MRI, where GBCA binds to the collagen matrix produced by tissue damage and fibrosis [37], a hypothesis has emerged suggesting a potential increase in GBCA retention in fibrotic liver tissue. This hypothesis is based on the analogy between late gadolinium enhancement on cardiac MRI and its association with tissue fibrosis. Despite these intriguing connections, there is a conspicuous absence of dedicated studies investigating the precise impact of liver fibrosis on GBCA retention.

The intricate interplay between liver fibrosis, altered pharmacokinetics of GBCA, and the potential role of collagen in GBCA binding necessitates further research. A comprehensive exploration of how liver fibrosis influences GBCA retention is crucial for enhancing our understanding of the underlying mechanisms and refining the safety protocols and guidelines associated with GBCA-enhanced MRI in patients with liver fibrosis or cirrhosis. Such studies may pave the way for more targeted and personalized approaches to diagnostic imaging, ensuring the utmost safety and efficacy for individuals undergoing these procedures.

The primary objective of this study was to bridge this knowledge gap by comprehensively exploring the influence of liver fibrosis on gadolinium retention after the administration of gadoxetic acid disodium. This investigation will include comparative analyses of gadodiamide and gadobutrol, providing a nuanced understanding of liver fibrosis interactions with different GBCAs and potentially alter the dynamics of gadolinium retention in various tissues. By addressing this critical gap in the literature, we aim to provide valuable insights that may inform clinical practice and enhance our understanding of the multifaceted factors influencing gadolinium retention in diverse patient populations.

II. MATERIALS AND METHODS

1. Animals

All experimental procedures conducted in this study received approval from the Department of Laboratory Animal Resources at Yonsei University College of Medicine and the Animal Ethical Committee, ensuring compliance with ethical standards. A total of one hundred and twenty Sprague–Dawley male rats, weighing between 200-220g, were employed for the experiments. The animals were housed in cages and provided a sterile environment with a 12-hour light and 12-hour dark cycle, maintaining a humidity level of 50 ± 10% and a temperature of 22 ± 2°C.

The rats were stratified into two main groups: the normal liver group and the liver fibrosis group, as illustrated in Figure 1. Liver fibrosis was induced in the designated group by administering intraperitoneal injections of 150mg/kg thioacetamide (TAA, Sigma-Aldrich, St Louis, MO) three times a week, with a two-day interval, spanning a period of 12 weeks [38]. Regular examinations were conducted on all rats to monitor signs of distress or ill health, and their weights were recorded before each injection to ensure accurate dosage calculation for TAA.

Both the normal liver group and the liver fibrosis group were further subdivided into four subgroups. Each subgroup (n=15, respectively) received intravenous administration of three types of gadolinium contrast agents: gadodiamide (0.47 mmol/kg), gadobutrol (0.47 mmol/kg), gadoxetic acid disodium (0.12 mmol/kg), and saline (0.47 ml/kg). The injected dose of the gadolinium-based contrast agents was determined based on the allometric dose appropriate for rats [38]. Notably, gadoxetic acid disodium was administered at one-fourth the dosage compared to gadodiamide and gadobutrol, aligning with the clinically

recommended human dose, which is one-fourth (0.025 mmol/kg) of other GBCAs (0.1 mmol/kg). At specified intervals of 1 week, 4 weeks, and 12 weeks after the administration of GBCAs or normal saline, five rats from each group were humanely sacrificed using a CO2 gas chamber, and tissues including the liver, kidney, bone, and brain were harvested, as depicted in Figure 1.

Bone tissues were meticulously collected from the right femur, with attached muscles carefully removed using surgical tools to prevent tissue contamination. To ensure the integrity of subsequent analyses, all surgical tools were diligently cleaned with an ultrasonic cleaner after each tissue harvesting procedure. This comprehensive experimental design and stringent procedural protocol were established to guarantee the reliability and ethical compliance of the study, laying the foundation for robust and meaningful results.

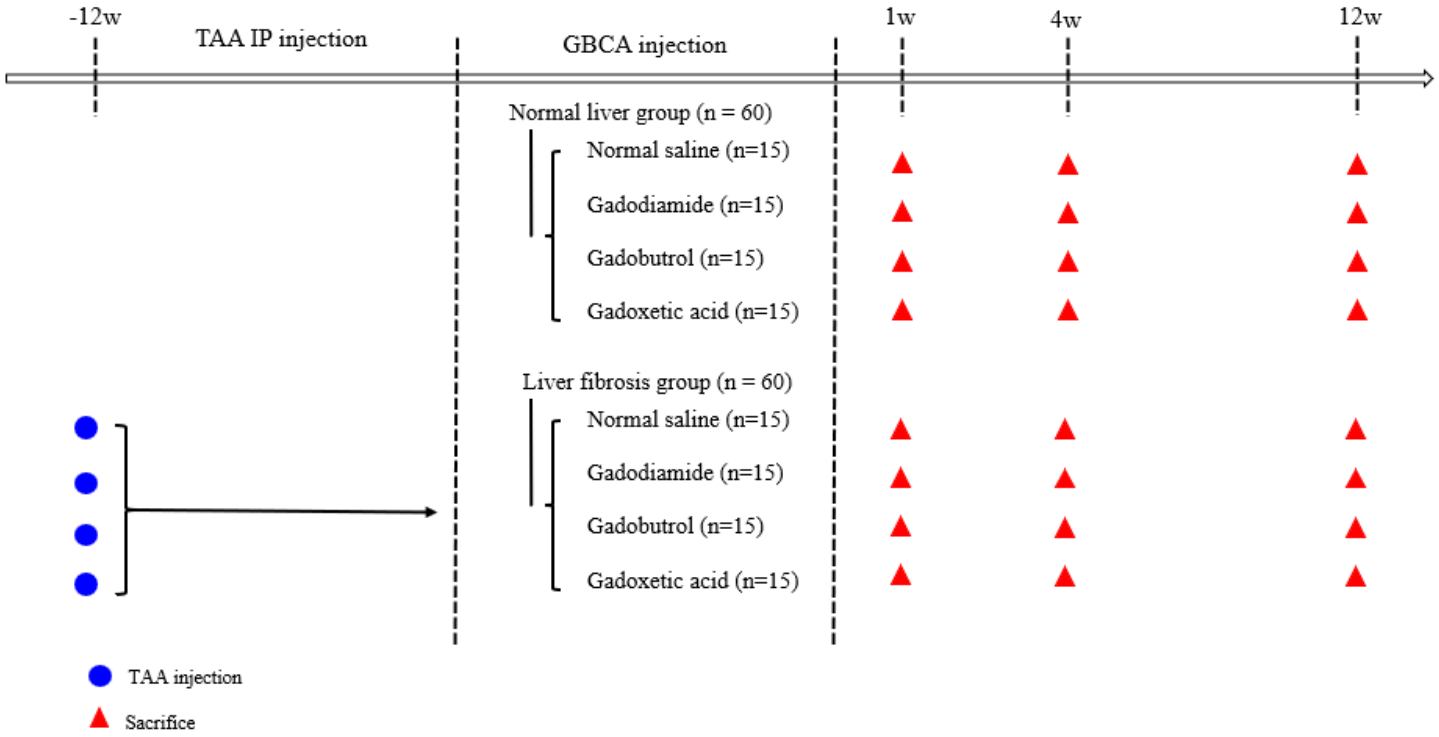


Figure 1 Scheme of the animal experiments. Single dose was defined as the dose recommended on the label: gadodiamide (0.1 mmol/kg), gadobutrol (0.1 mmol/kg), and gadoxetic acid (0.025 mmol/kg). TAA; Thioacetamide, IP; Intraperitoneal

2. Histologic analysis of hepatic fibrosis

The liver tissues obtained from each rat underwent a meticulous preservation process, where they were fixed in 10% neutral buffered formalin for a duration of 24 hours. Subsequently, paraffin blocks were meticulously prepared following standardized procedures. The paraffin blocks were sectioned at a thickness of 4 μm, employing a precision Autostainer (Leica Microsystems, Buffalo Grove, IL), to facilitate downstream analyses.

For a comprehensive assessment of liver morphology and fibrotic changes, Hematoxylin-eosin staining (Gill III, Merck KGaA, Darmstadt, Germany) and Masson trichrome staining were carried out on the paraffin-embedded sections. These staining procedures were executed with precision, adhering to established protocols. The Autostainer was instrumental in ensuring consistency and accuracy throughout the staining process.

To evaluate the degree of liver fibrosis, a crucial aspect of the study, a board-certified pathologist (H.M.K.) conducted assessments using the Metavir scoring system[39,40]. Notably, the pathologist was blinded to the experimental results during the evaluation process, ensuring an unbiased and objective analysis. The Metavir scoring system, a widely recognized and standardized method, provided a systematic framework for grading liver fibrosis. This meticulous approach to tissue processing, staining, and evaluation contributes to the robustness and reliability of the study's histopathological analyses, enhancing the validity and interpretability of the obtained results.

3. Inductively coupled plasma mass spectrometry (ICP-MS)

The quantification of retained gadolinium in tissues was meticulously conducted using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with the NexION300

instrument from PerKinElmer, headquartered in Waltham, MA. To prepare the tissues for analysis, a 24-hour lyophilization process was employed using the Freezone 12 plus system (Labconco, Kansas City, MO). The lyophilized tissues, with an approximate weight ranging from 0.1 to 0.5 g, ensured consistency in the subsequent analytical procedures.

For dissolution and reaction with the acid, the dried tissue samples were carefully reconstituted using a mixed acid solution (HNO3+H2O2). This process involved slow heating to facilitate a thorough reaction with the acid, ensuring optimal dissolution of the samples. The prepared tissues, now in a dissolved form, underwent detailed analysis by ICP-MS, with specific parameters set as follows: radiofrequency power at 1600 W, sampling depth at 8 mm, carrier gas flow at 0.85 L/min, and makeup gas flow at 0.3 L/min.

The instrumental limit of quantification (LOQ) for gadolinium detection was determined to be an average of 0.230 μg/g for liver samples, 0.070 μg/g for femur samples, 0.080 μg/g for kidney samples, and 0.430 μg/g for brain samples. Importantly, these values were approximately 10 times higher than those observed in blank solutions, attesting to the precision and sensitivity of the ICP-MS method utilized in this study.

This meticulous approach in the analytical methodology, from tissue preparation to ICP-MS analysis, ensures the reliability and accuracy of the quantification of retained gadolinium levels in various tissues. The establishment of stringent analytical parameters and the use of advanced instrumentation contribute to the robustness of the study, enhancing the scientific rigor and credibility of the obtained results.

4. Transmission electron microscopy (TEM)

In order to comprehensively investigate the morphological deposition sites of gadolinium,

a detailed and methodical sample preparation protocol was followed. Liver tissues were initially fixed in a solution containing 2% glutaraldehyde, 2% paraformaldehyde, and 0.5% CaCl2 in 0.1 M phosphate buffer (pH 7.4) overnight, ensuring optimal preservation of tissue morphology. Subsequently, the fixed samples underwent two 30-minute washes with 0.1 M phosphate buffer, further preparing them for subsequent processing steps.

The next phase involved additional fixation, where the samples were immersed in a solution of 1% OsO4 dissolved in 0.1 M phosphate buffer for 2 hours. This step aimed to enhance contrast and facilitate detailed visualization during subsequent imaging processes. Following fixation, a systematic dehydration process was initiated using a series of solutions with incrementally increasing ethanol concentrations (in 10% increments), with each concentration step lasting 10 minutes. This meticulous dehydration process ensured the removal of water content from the samples while maintaining tissue integrity.

Once dehydrated, the specimens were embedded using the Poly/Bed812 kit (Polyscience, Inc., Warrington, PA) and polymerized at 65℃ in an electron micro-oven (TD-700, DOSAKA, Kyoto, Japan) for 24 hours. This embedding and polymerization step provided a solid and stable matrix for subsequent sectioning. The resulting blocks were precision-sectioned into ultra-thin slices with a thickness of 80 nm using an ultramicrotome (LEICAEM UC-7, Leica Microsystem, Vienna, Austria).

The ultra-thin sections obtained were then subjected to observation using transmission electron microscopy (TEM) with the JEM-1011 instrument from JEOL (Tokyo, Japan). TEM, known for its high resolution and ability to visualize subcellular structures, played a crucial role in elucidating the morphological deposition sites of gadolinium within the liver tissues. This meticulous sample preparation and imaging approach ensures the reliability and accuracy of the investigation into the ultrastructural aspects of gadolinium

deposition, contributing valuable insights to the study's overall findings.

5. Scanning electron microscopy (SEM) with energy dispersive x-ray spectrometer (EDX)

Additionally, to precisely identify the intra-tissue locations of retained gadolinium, a thorough examination was carried out using SEM and EDX.

For SEM preparation, the samples underwent fixation for 24 hours in Karnovsky's fixative, consisting of 2% Glutaraldehyde and 2% Paraformaldehyde in 0.1M phosphate buffer with a pH of 7.4. Following fixation, the samples were washed twice for 30 minutes in 0.1M phosphate buffer. Subsequently, they were postfixed with 1% OsO4 for 2 hours and subjected to dehydration in an ascending gradual series of ethanol concentrations (ranging from 50% to 100%). A Critical Point Dryer (LEICA EM CPD300) was employed in the drying process. To enhance conductivity, the samples were coated with carbon by ion sputter (LEICA EM ACE600) before being meticulously observed utilizing a field emission Scanning Electron Microscopy (MERLIN, ZEISS).

For elemental mapping in SEM, the Bruker EDX System was utilized. EDX Mapping was performed on specimen surfaces using a XFlash®5060FlatQUAD at an accelerating voltage of 5kV, with a magnification of x2000 for SEM images, and a 10-minute acquisition time. This process was carried out with the X-flash 6 from BRUKER, employing the ESPRIT 2.1 software for data analysis.

6. Statistical analysis

The evaluation of the concentration of retained gadolinium in the liver, kidney, brain, and bone encompassed a comparative analysis between the normal liver group and the liver

fibrosis group. This assessment was conducted at each time point for all GBCAs through the application of an independent t-test. Additionally, an examination of the retained gadolinium concentration was performed across GBCA types and over time intervals, employing an independent t-test to discern potential variations.

To further elucidate the impact of liver fibrosis, the Metavir score comparison between the normal liver group and the liver fibrosis group was executed using the Mann-Whitney U test. This analytical approach aimed to identify any significant differences in the Metavir scores between these two groups.

In assessing the relationship between gadolinium retention and the severity of liver fibrosis, Spearman's correlation coefficient (ρ) was employed. This correlation analysis was conducted at each designated time point to explore potential associations between gadolinium retention levels and the Metavir score.

All statistical analyses were meticulously executed using the commercial software SPSS v25.0 (IBM Corp., New York, NY). The criterion for statistical significance was established at $p < 0.05$, ensuring a robust and reliable interpretation of the obtained results. This comprehensive statistical framework contributes to the rigor and validity of the study's findings, providing a thorough understanding of the dynamics of gadolinium retention in the context of liver fibrosis.

III. RESULTS

1. Histological analysis of hepatic fibrosis

In the normal liver group, 17 rats were grade 0 and 43 grade 1 rats; none of the rats were classified as grade 2 or higher. Among the 60 rats in the liver fibrosis group, none were grade 0, 5 were grade 1, 15 were grade 2, 17 were grade 3, and 23 were grade 4 (Figure 2). A significant difference in fibrosis grade was observed between the two groups ($p<0.001$). Notably, 5 rats with fibrosis grade 1 in the liver fibrosis group were considered to have failed to develop liver fibrosis and were therefore, excluded from subsequent analyses, except for the assessment of the correlation between the Metavir score and gadolinium retention in the liver. Of these five rats, one was at the 12-week mark after normal saline injection, one at 12 weeks after gadobutrol injection, one at 12 weeks after gadoxetic acid injection, and the remaining two at 4 and 12 weeks after gadodiamide injection.

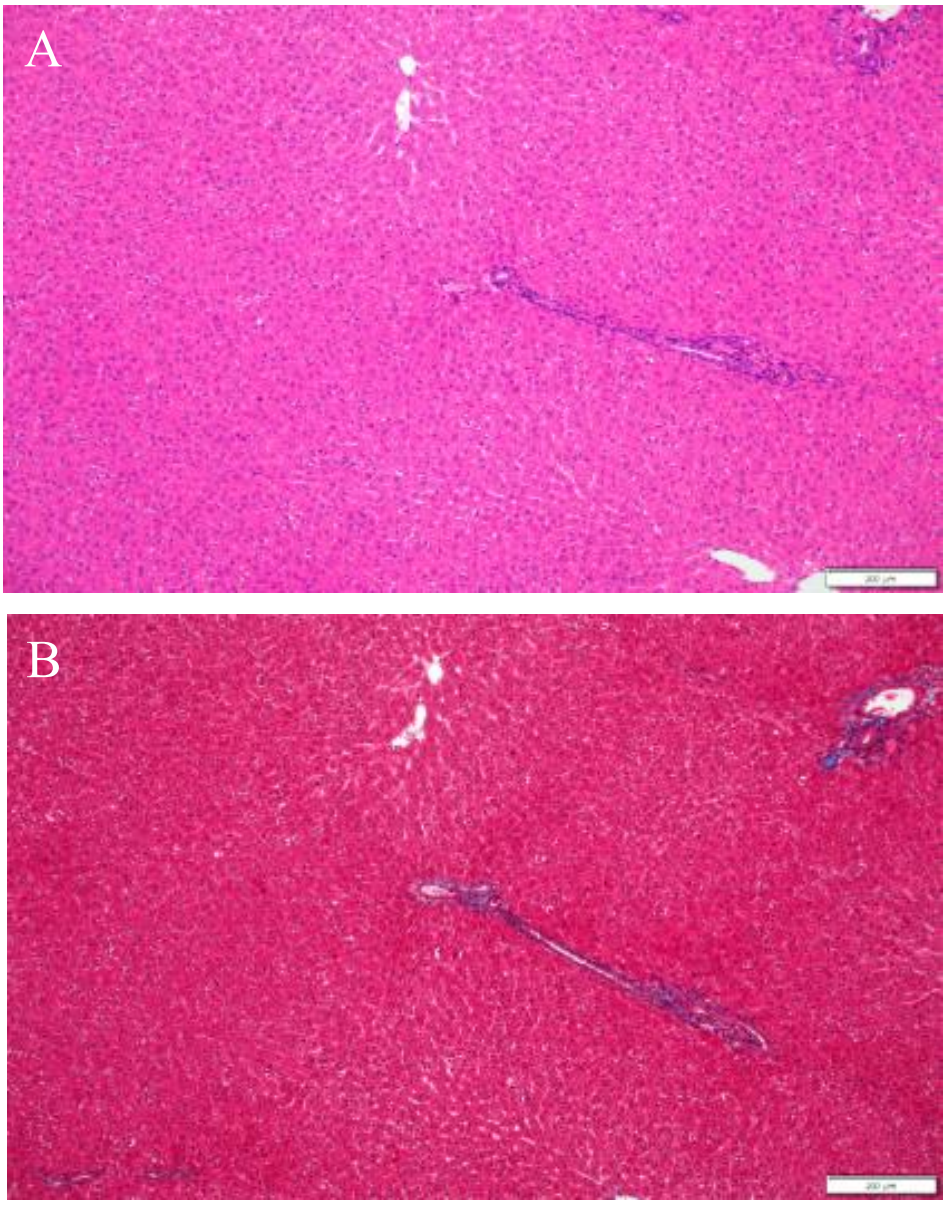


Figure 2 Microscopy examples of liver tissue in normal liver and liver fibrosis model. A; Normal liver with H-E staining, B; Normal liver with M-T staining, C; Fibrotic liver with H-E staining, D; Fibrotic liver with M-T staining, H-E; Hematoxylin-Eosin, M-T; Masson-Trichrome

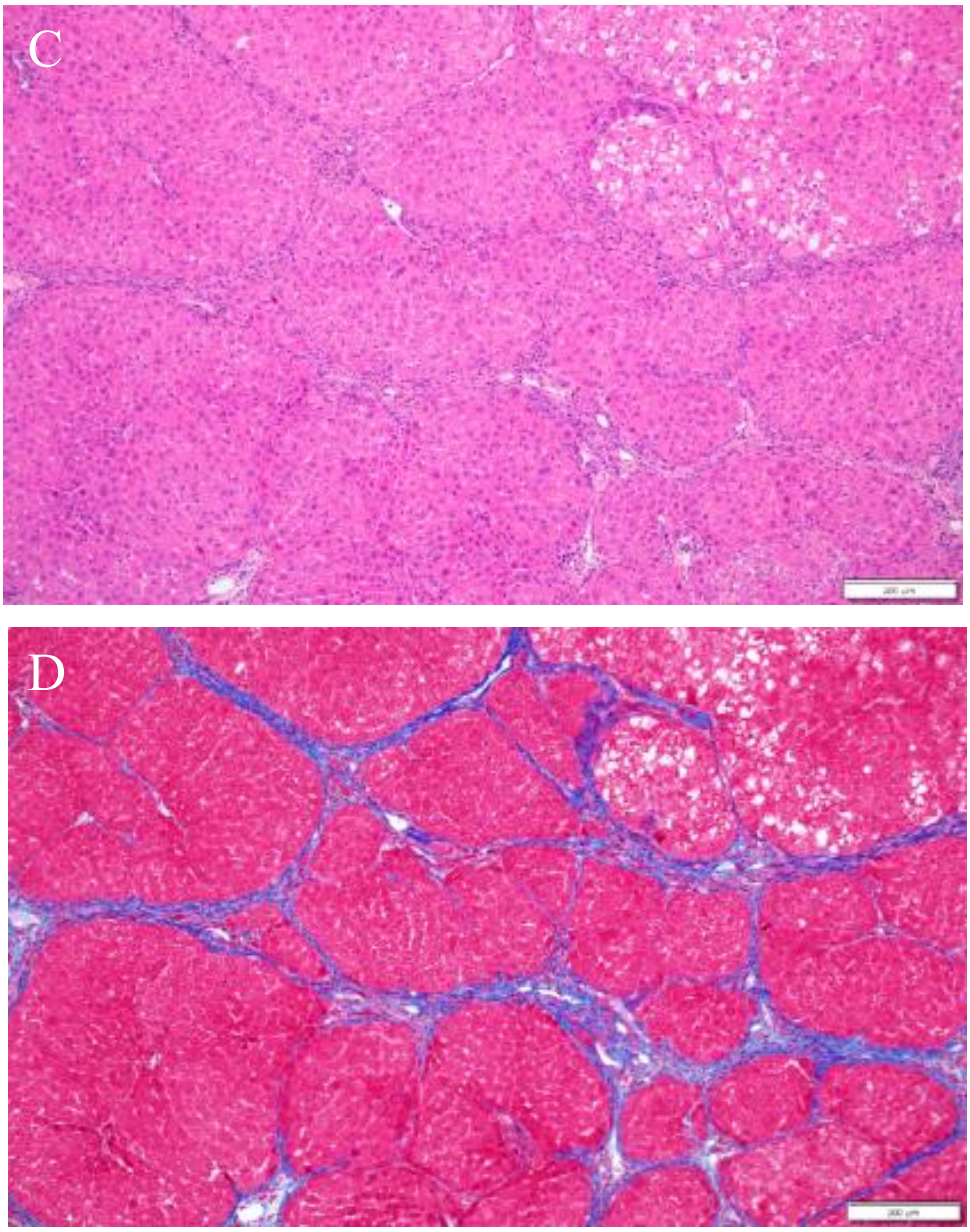


Figure 3 Microscopy examples of liver tissue in normal liver and liver fibrosis model. A; Normal liver with H-E staining, B; Normal liver with M-T staining, C; Fibrotic liver with H-E staining, D; Fibrotic liver with M-T staining, H-E; Hematoxylin-Eosin, M-T; Masson-Trichrome

2. Evaluation of gadolinium retention in the liver, femur, kidney, and brain tissues

Table 1 and figure 3 summarize the average residual gadolinium concentrations in the liver, femur, kidney, and brain tissues of the normal liver and liver fibrosis groups. Rats that received normal saline and retained gadolinium had levels less than the instrumental LOQ in all tissues in both groups.

Table 1. Gadolinium retention depending on the liver fibrosis status.

		Gadodiamide			Gadobutrol			Gadoxetic acid		
		Normal liver group	Liver fibrosis group	p-value	Normal liver group	Liver fibrosis group	p-value	Normal liver group	Liver fibrosis group	p-value
Liver	1week	25.030±3.828	24.051±3.250	0.673	7.033±2.079	15.669±3.421	0.002	0.750±0.057	3.561±0.750	0.001
	4weeks	2.353±0.566	7.981±2.194	0.025	0.165±0.070	1.463±0.362	<0.001	0.102±0.032	1.272±0.598	0.002
	12weeks	0.388±0.216	1.552±0.216	<0.001	0.025±0.006	0.757±0.407	0.004	0.013±0.006	0.521±0.388	0.020
Femur	1week	23.021±3.434	4.928±1.717	<0.001	1.857±0.343	0.566±0.197	<0.001	0.541±0.038	0.286±0.076	0.001
	4weeks	18.226±4.127	4.235±0.591	<0.001	0.382±0.076	0.159±0.013	0.002	0.343±0.038	0.178±0.076	0.002
	12weeks	6.397±3.644	3.682±1.017	0.175	0.273±0.045	0.604±0.903	0.434	0.140±0.057	0.140±0.038	0.941
Kidney	1week	136.280±42.251	260.350±136.674	0.113	303.784±34.550	176.216±53.660	0.042	11.612±5.355	17.876±4.191	0.075
	4weeks	33.196±15.364	34.480±4.083	0.865	17.151±8.483	19.886±6.925	0.593	0.668±0.229	1.342±0.369	0.011
	12weeks	18.130±13.119	24.795±8.375	0.387	1.030±0.649	5.768±6.881	0.163	0.210±0.070	0.515±0.134	0.013
Brain	1week	1.755±0.111	1.335±0.318	0.456	0.267±0.159	0.388±0.261	0.399	0.064±0.025	<LOQ	0.001
	4weeks	0.789±0.121	0.738±0.025	0.443	0.057±0.025	<LOQ	<0.001	0.013±0.006	0.013±0.025	0.977
	12weeks	0.769±0.114	0.560±0.051	0.011	0.324±0.324	0.121±0.242	0.313	0.114±0.184	<LOQ	0.229

Each value shows residual gadolinium (nmol/g) in the liver, femur, kidney, and brain measured by ICP-MS. Sixty rats were assigned as normal liver group and fifty-five were assigned as liver fibrosis group (5 rats were considered as failed to induce liver fibrosis and were not included in the analysis).

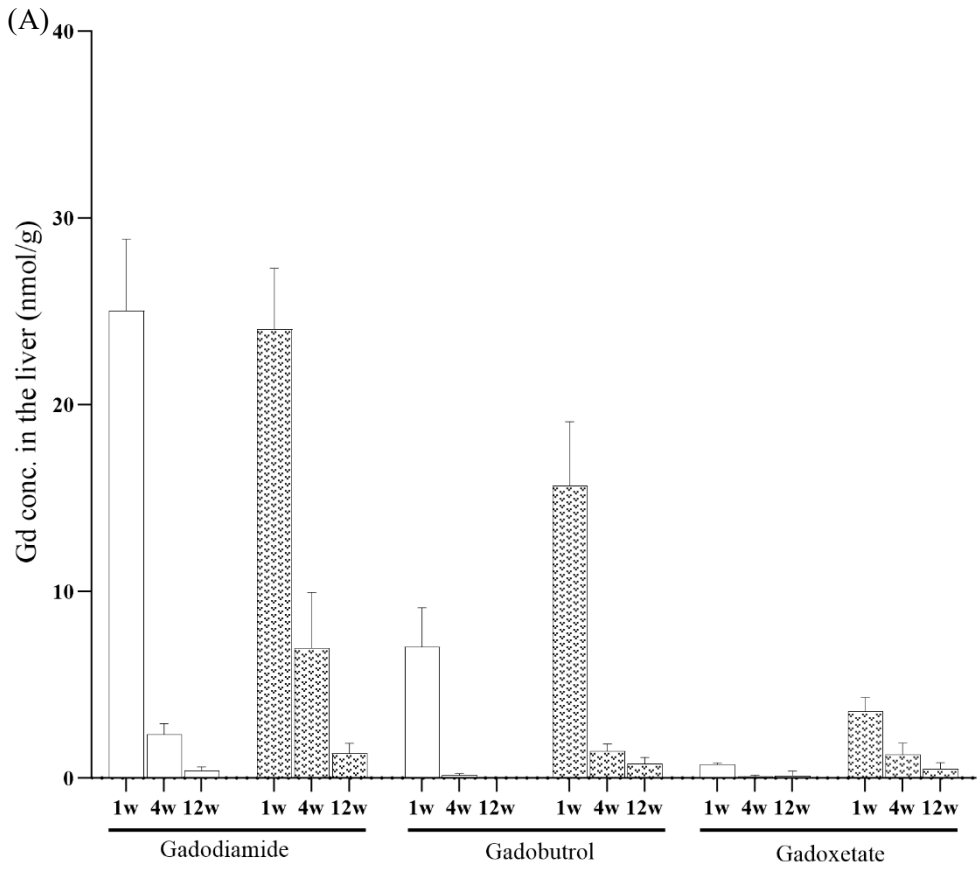
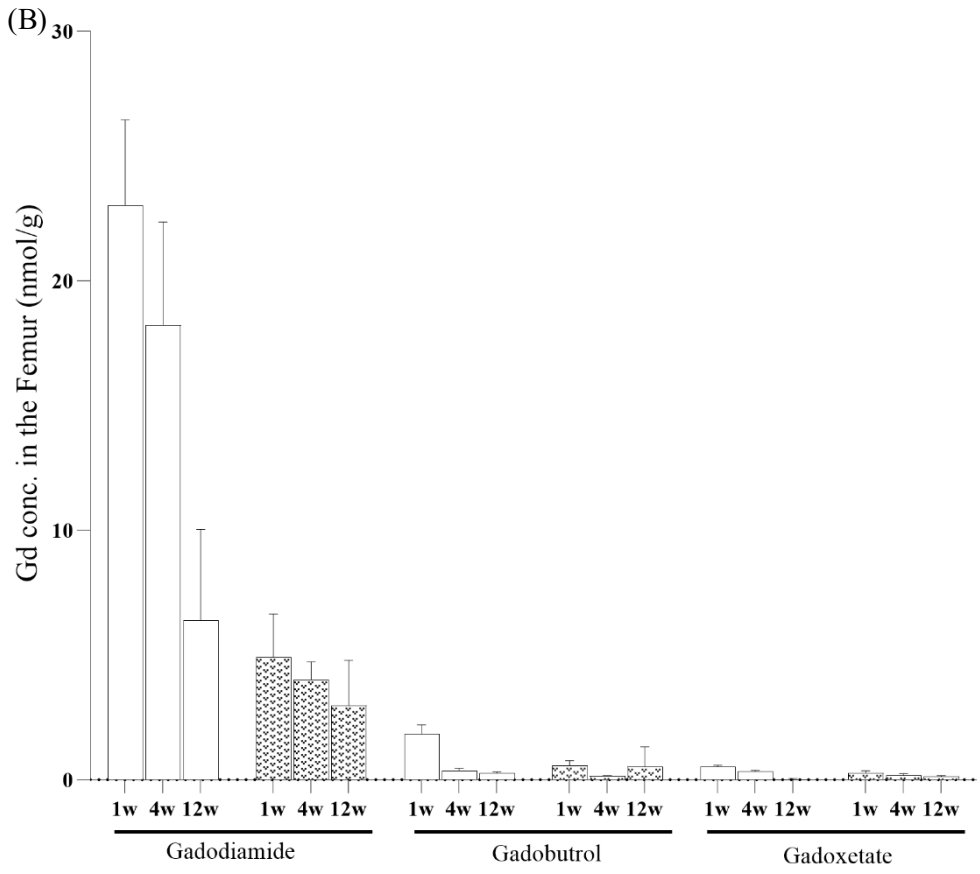


Figure 3. The concentration of gadolinium retention depending on the liver fibrosis status. The graphs shows the concentration of residual gadolinium (nmol/g) in the liver (A), femur (B), kidney (C), and brain (D) measured by ICP-MS. Sixty rats were assigned as normal liver group and fifty-five were assigned as liver fibrosis group (5 rats were considered as failed to induce liver fibrosis and were not included in the analysis). Whiskers represents standard deviation. Single-dose gadodiamide: 0.1 mmol/kg, gadobutrol: 0.1 mmol/kg, gadoxetic acid: 0.025 mmol/kg.



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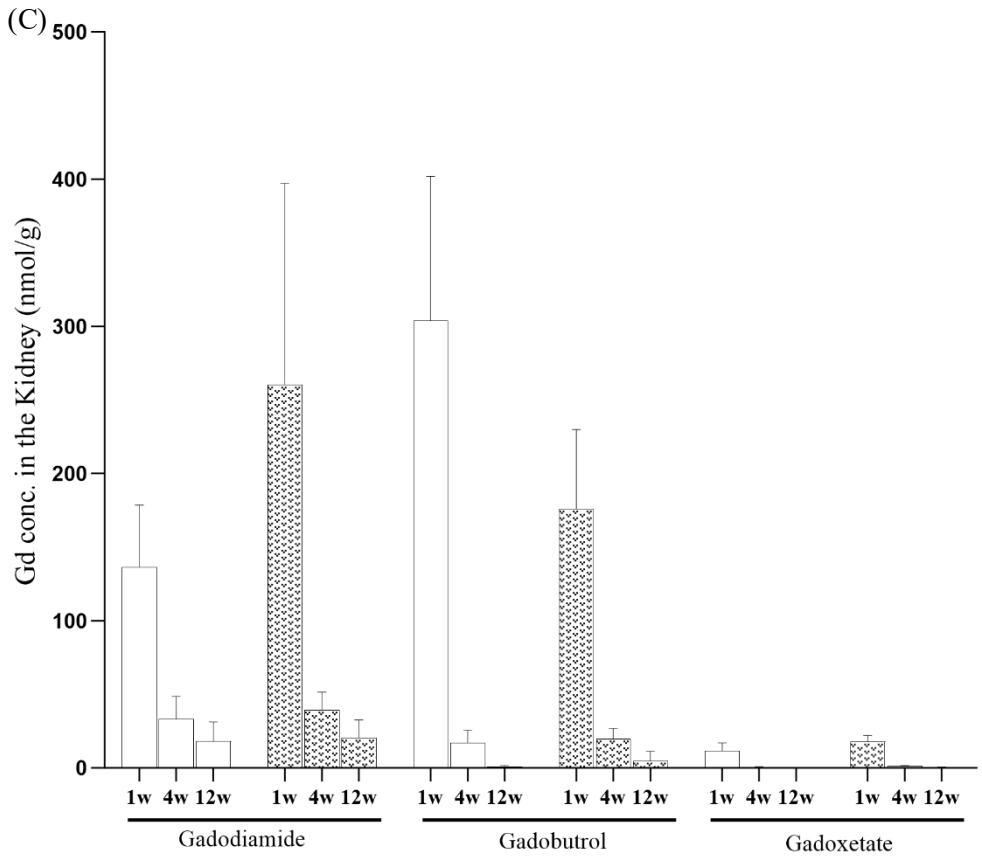

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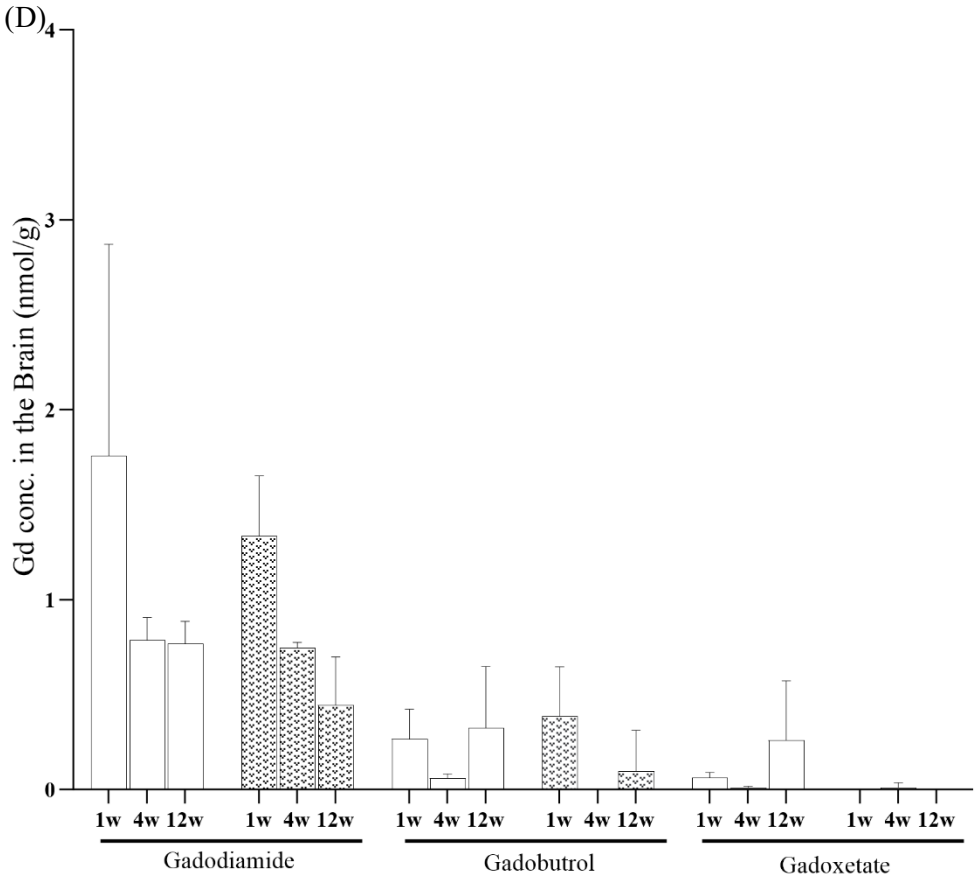

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Gadodiamide was mostly retained in both groups and at all time points in all organs (all $p<0.05$), except in the kidney tissues 1 week after GBCA injection. In contrast, the concentration of retained gadolinium was lower with gadoxetic acid than with gadobutrol in both groups and at all time points, with or without statistical significance.

The amount of gadolinium significantly decreased over time after GBCA administration in the liver tissues. However, the difference was not statistically significant between some time points in the femur and kidney tissues, although the residual gadolinium tended to decrease over time (Table 2). In some brain tissues, a significantly higher amount of residual gadolinium was observed at later time points than at earlier time points. However, this might be meaningless because the concentration of residual gadolinium was below the decimal point and could have been contaminated on account of various errors during the experimental and measurement stage.

In terms of the effect of liver fibrosis, the residual gadolinium concentration in the liver fibrosis group was significantly higher than in the normal liver group after the administration of all types of GBCAs ($p<0.05$), except for the 1-week gadodiamide group ($p=0.673$). Furthermore, a significant positive correlation was observed between the Metavir score and the concentration of residual gadolinium in all GBCAs at all time points, except at 1 week after gadodiamide administration (Figure 4–5, Table 3).

Table 2. Independent t-test results of residual gadolinium over time (1week vs. 4weeks and 4weeks vs. 12weeks) after GBCA administration.

		Gadodiamide		Gadobutrol		Gadoxetic acid	
		Normal liver	Fibrotic liver	Normal liver	Fibrotic liver	Normal liver	Fibrotic liver
Liver	1week	25.030±3.828	24.051±3.250	7.033±2.079	15.669±3.421	0.750±0.057	3.561±0.750
	4weeks	2.353±0.566	7.981±2.194	0.165±0.070	1.463±0.362	0.102±0.032	1.272±0.598
	12weeks	0.388±0.216	1.552±0.216	0.025±0.006	0.757±0.407	0.013±0.006	0.521±0.388
	p-value (1week vs. 4weeks)	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
	p-value (4weeks vs. 12weeks)	<0.001	0.013	0.010	0.014	0.004	0.040
Femur	1week	23.021±3.434	4.928±1.717	1.857±0.343	0.566±0.197	0.541±0.038	0.286±0.076
	4weeks	18.226±4.127	4.235±0.591	0.382±0.076	0.159±0.013	0.343±0.038	0.178±0.076
	12weeks	6.397±3.644	3.682±1.017	0.273±0.045	0.604±0.903	0.140±0.057	0.140±0.038
	p-value (1week vs. 4weeks)	0.082	0.325	<0.001	0.010	<0.001	0.048
	p-value (4weeks vs. 12weeks)	0.001	0.277	0.030	0.365	<0.001	0.281
Kidney	1week	136.280±42.251	260.350±136.674	303.784±34.550	176.216±53.660	11.612±5.355	17.876±4.191
	4weeks	33.196±15.364	34.480±4.083	17.151±8.483	19.886±6.925	0.668±0.229	1.342±0.369
	12weeks	18.130±13.119	24.795±8.375	1.030±0.649	5.768±6.881	0.210±0.070	0.515±0.134
	p-value (1week vs. 4weeks)	0.004	0.022	0.003	0.003	0.010	<0.001
	p-value (4weeks vs. 12weeks)	0.135	0.036	0.013	0.007	0.008	0.005
Brain	1week	1.755±0.111	1.335±0.318	0.267±0.159	0.388±0.261	0.064±0.025	<LOQ
	4weeks	0.789±0.121	0.738±0.025	0.057±0.025	<LOQ	0.013±0.006	0.013±0.025
	12weeks	0.769±0.114	0.560±0.051	0.324±0.324	0.121±0.242	0.114±0.184	<LOQ
	p-value (1week vs. 4weeks)	0.124	0.014	0.042	-	0.008	-
	p-value (4weeks vs. 12weeks)	0.817	0.056	0.139	-	0.268	-

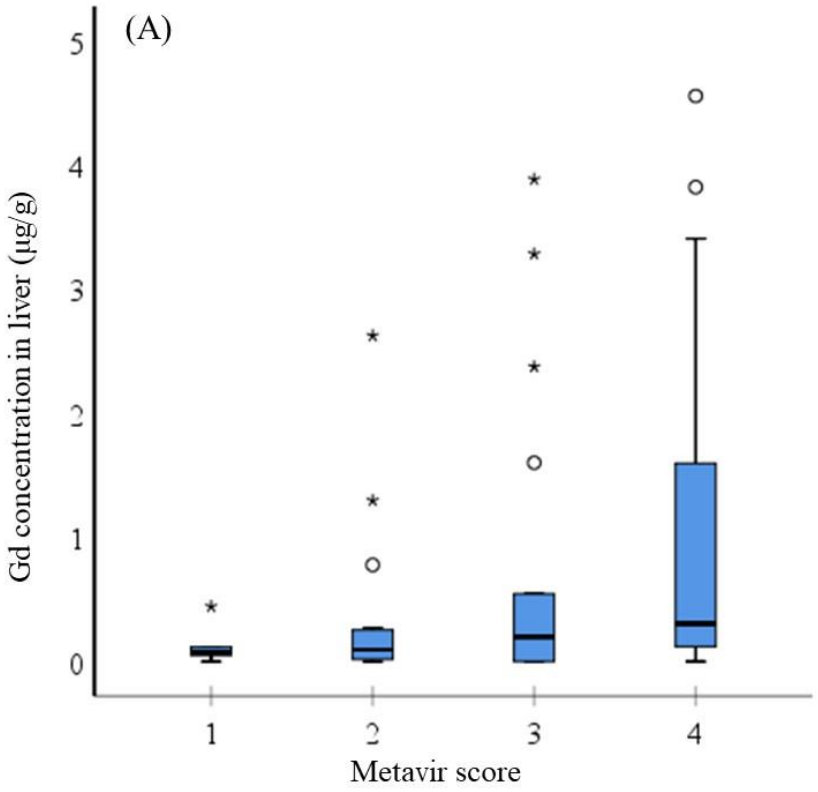
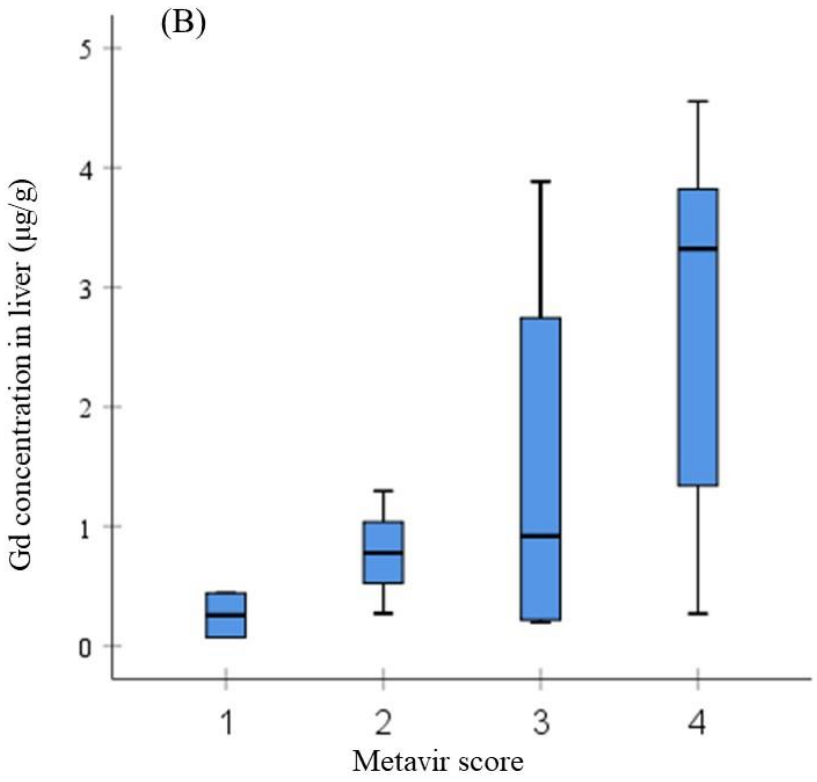


Figure 4. Box plots between fibrosis (Metavir score) and retained gadolinium concentration in liver tissue for all contrast agent (A) and gadodiamide (B).



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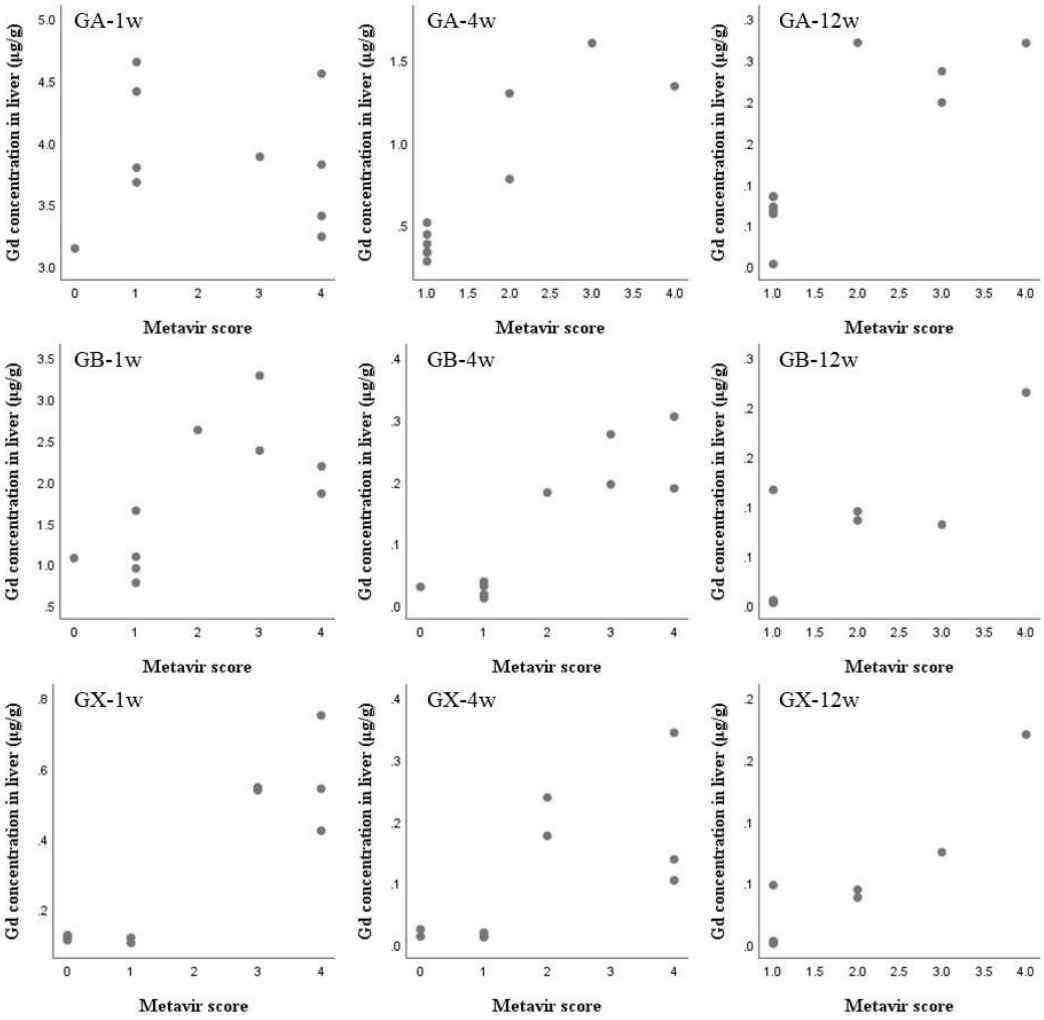


Figure 5. Scatter plots between degree of liver fibrosis (Metavir score) and retained gadolinium concentration in liver tissues. All cases of gadolinium retention concentration had positive correlation to the degree of fibrosis with statistical significance except 1-week gadodiamide cases. GA; Gadodiamide, GB; Gadobutrol, GX; Gadoxetic acid, 1w; 1 week, 4w; 4 weeks, 12w; 12 weeks after GBCA administration models.

Table 3. Spearman's correlation coefficient (ρ) between degree of liver fibrosis (Metavir score) and retained gadolinium concentration in liver tissue and their p-values depending on each GBCA and week(s) after single dose GBCA administration.

GBCA	Week(s)	N	Spearman's coefficient (ρ)	*P*-value
Gadodiamide	1	10	0.065	0.859
	4	10	0.870***	0.001
	12	10	0.802**	0.005
Gadobutrol	1	10	0.692*	0.027
	4	10	0.850***	0.002
	12	10	0.658*	0.039
Gadoxetic acid	1	10	0.719*	0.019
	4	10	0.688*	0.028
	12	10	0.775**	0.009

All cases of gadolinium retention concentration had positive correlation to the degree of fibrosis with statistical significance except 1-week gadodiamide cases. *P<0.05, **P<0.01, *** P<0.005, GBCA; Gadolinium based contrast agent.

In the femur, gadolinium retention in the liver fibrosis group was significantly lower than that in the normal liver group at 1 and 4 weeks for all GBCAs (all $p<0.005$). At 12 weeks, no statistical difference was observed in the retained GBCAs between the fibrotic and normal liver groups.

In contrast, in the kidney tissues, the liver fibrosis group showed significantly higher concentrations of gadolinium retention at 4 and 12 weeks after gadoxetic acid administration. However, no difference was observed between the fibrotic and normal liver groups after the administration of gadodiamide and gadobutrol, except in the 1-week gadobutrol group.

In brain tissues, the concentration of residual gadolinium was not significantly different between the normal group and liver fibrosis group, except at 4 weeks for gadobutrol ($p<0.001$), 12 weeks for gadodiamide ($p=0.011$), and 1 week for gadoxetic acid ($p=0.001$).

3. Visualization of gadolinium deposition locations within cells using TEM and SEM-EDX

We used two distinct electron microscopy techniques to meticulously examine patterns of gadolinium retention within the liver. Despite our diligent efforts, achieving a clear visualization of the gadolinium deposition proved to be a considerable challenge. (Figure 6)

Nevertheless, in our persistent endeavors, detailed observations revealed that gadolinium was predominantly distributed within the cytoplasm rather than in the nucleus of hepatic cells. This insight was discerned through the application of SEM-energy-dispersive X-ray spectroscopy (EDX). (Figure 7)

Subsequently, the implementation of flatquad mapping allowed the detection of gadolinium. (Figure 8) However, identifying the specific organelles within the cytoplasm where gadolinium was distributed is challenging.

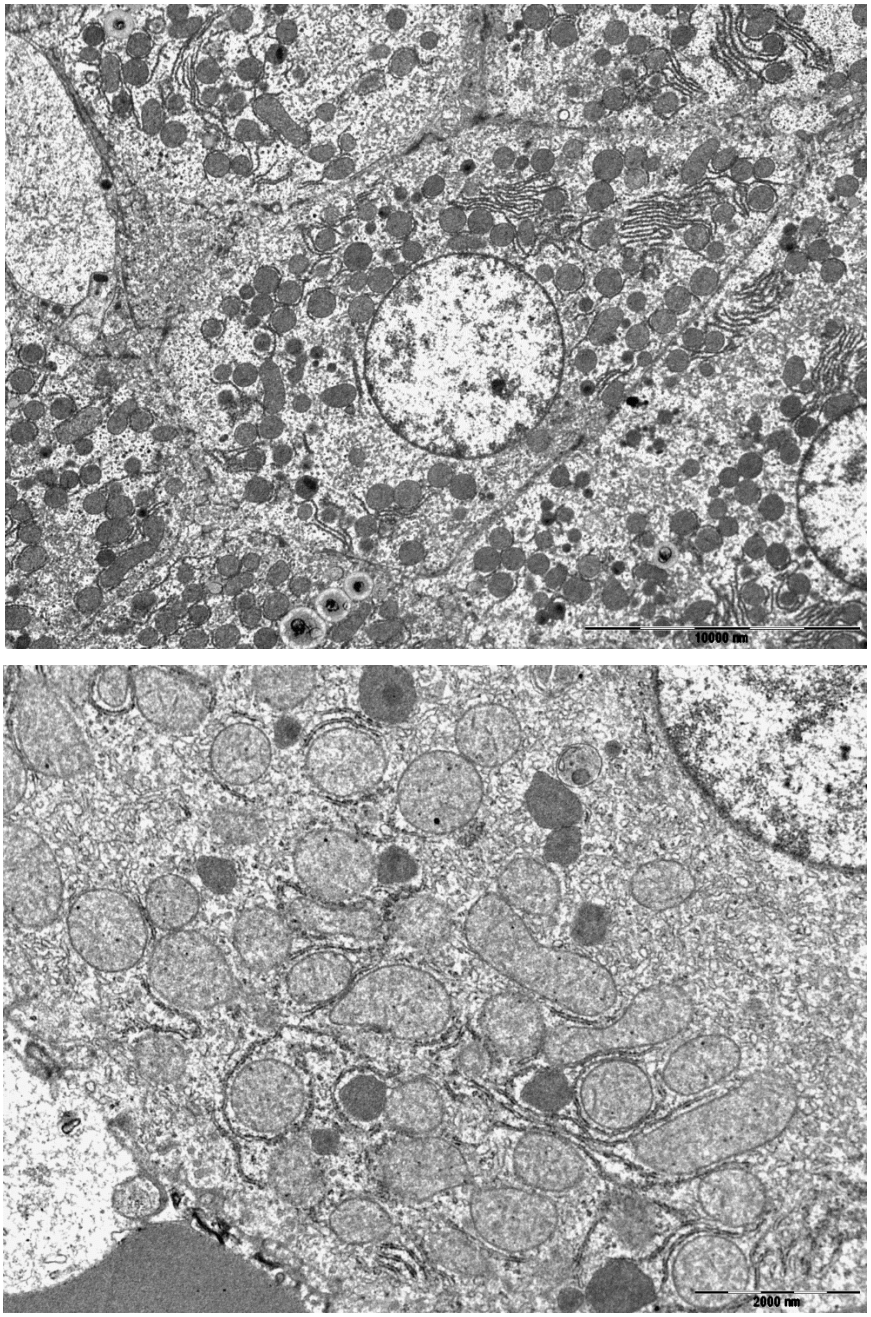
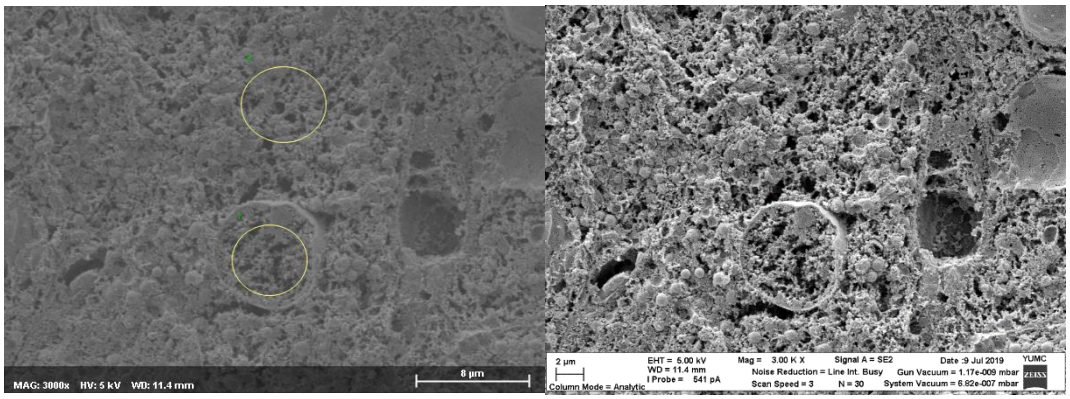
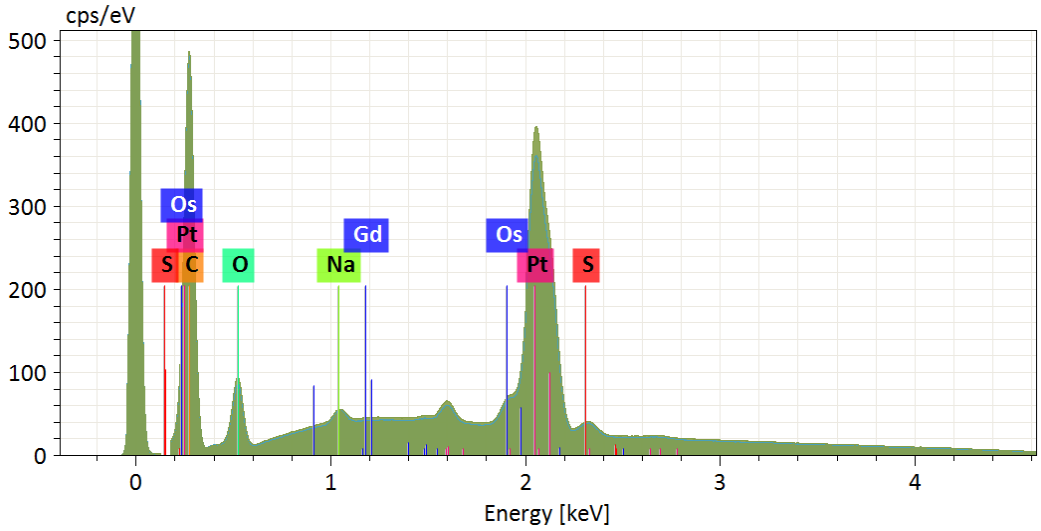


Figure 6. The cellular morphology of the liver captured through Transmission Electron Microscopy (TEM). No distinct gadolinium deposition is observed.





Atomic concentration [%]

Spectrum	Carbon	Oxygen	Sodium	Sulfur	Gadolinium	Osmium	Platinum
1	61.54	15.61	1.78	1.10	0.00	1.36	18.62
2	58.96	15.97	2.17	1.42	0.36	1.43	19.69
Mean	**60.25**	**15.79**	**1.97**	**1.26**	**0.18**	**1.40**	**19.16**
Sigma	**1.83**	**0.25**	**0.28**	**0.23**	**0.25**	**0.05**	**0.76**
SigmaMean	**1.29**	**0.18**	**0.19**	**0.16**	**0.18**	**0.04**	**0.54**

1: nucleus 2. cytoplasm

Figure 7. The nucleus and cytoplasm of the liver observed through Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM-EDX). While not morphologically evident, it is notable that gadolinium is predominantly distributed in the cytoplasm rather than the nucleus.

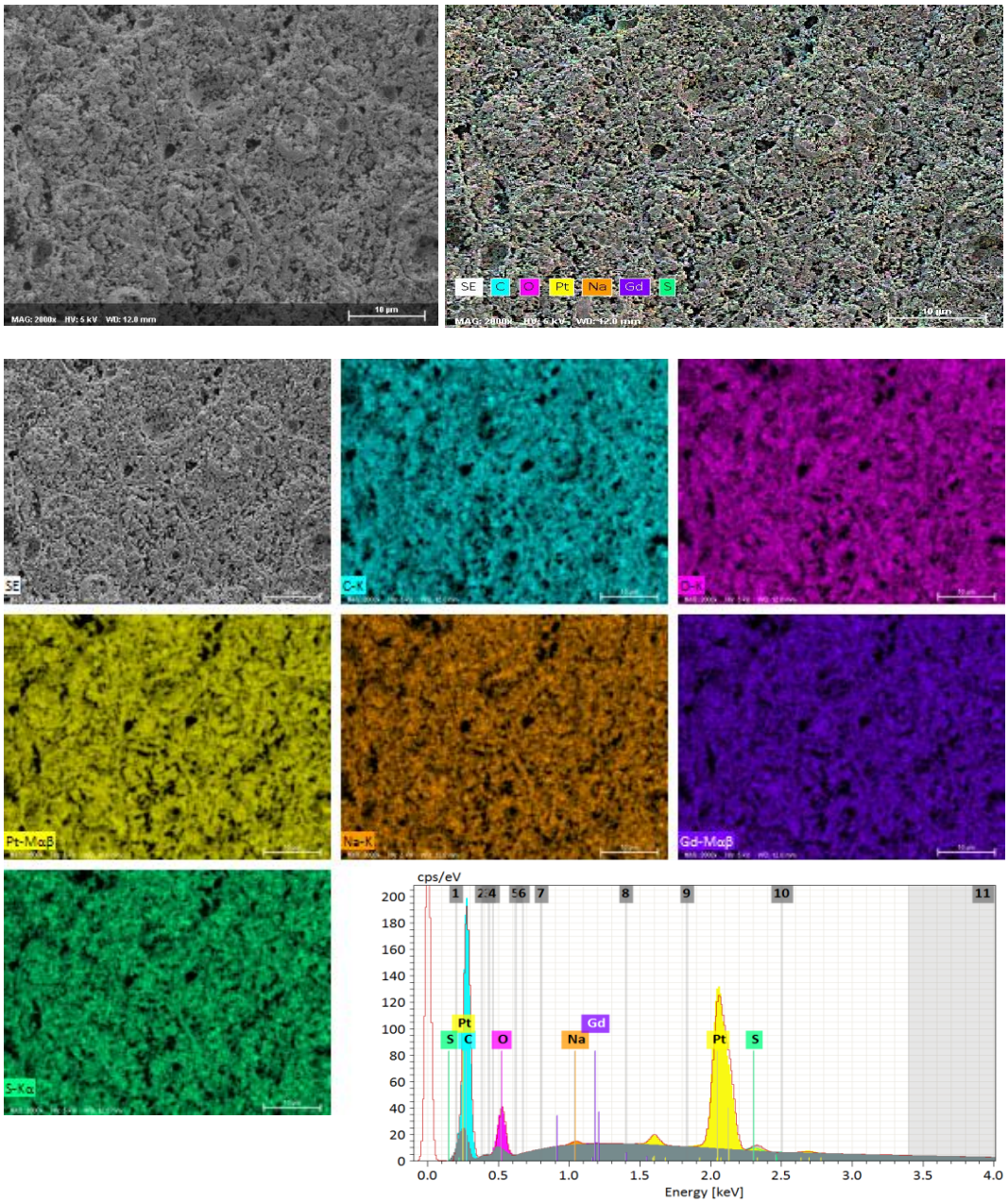


Figure 8. Results of flatquad mapping (5Kv-55kcps-10min-2) conducted using SEM-EDX. The specific regions within the cytoplasm where gadolinium is detected are not conclusively determined.

IV. DISCUSSION

Our findings provide intriguing insights into the effects of liver fibrosis on gadolinium retention in various tissues over different time intervals. Specifically, our results indicated that liver fibrosis might induce elevated gadolinium retention in the liver as compared to the control group, a phenomenon consistently observed up to the 12-week mark. Surprisingly, in the femur, liver fibrosis seemed to have a contrasting effect, resulting in decreased gadolinium retention for up to 4 weeks after administration of all types of GBCA.

In the kidney, a notable increase in gadolinium retention was observed only 12 weeks after the administration of gadoxetic acid in the liver fibrosis group as compared to that in the control group. However, liver fibrosis did not significantly influence the amount of gadolinium retained in the brain across all types of GBCAs at any time point. This intriguing tissue-specific variation highlights the complex dynamics of gadolinium retention in liver fibrosis.

Consistent with our expectations, the analysis of gadolinium retention across GBCAs and time points demonstrated that the highest accumulation occurred with gadodiamide, followed by gadobutrol and gadoxetic acid. Notably, the retained gadolinium levels decreased over the 12-week observation period. This temporal evolution of gadolinium retention emphasizes the need for a comprehensive understanding of the dynamics of gadolinium distribution in different tissues and temporal aspects of its retention. These findings provide valuable insights into the complexities of gadolinium retention and its modulation by liver fibrosis.

Gadodiamide, classified as a linear-type GBCA, exhibits the highest retention in all tissues owing to its low complex stability [14]. This finding is consistent with previous studies [27,29,30].

Gadodiamide has a linear ligand form; therefore, it has lower complex stability than GBCA, which has a macrocyclic form, and thus shows higher gadolinium retention, as shown in previous studies [14,27]. In contrast, despite having a linear ligand, gadoxetic acid consistently demonstrated the least gadolinium retention compared to the other GBCAs across all time intervals. Although the literature suggests that gadoxetic acid possesses the highest thermodynamic stability among linear GBCAs, its overall stability remains lower than that of macrocyclic GBCAs on account of its lower kinetic stability [41]. This unique characteristic may have contributed to lower retention levels.

Interestingly, our study aligns with a few others that have reported that gadoxetic acid is the least retained in tissues, even when compared to macrocyclic GBCAs [14,27]. Notably, these studies shared the common approach of administering gadoxetic acid at only a quarter of the dose of other GBCAs. This dose adjustment was necessitated by the recommended dose in the label of gadoxetic acid (0.025 mmol/kg), which is one-fourth compared to that of other GBCAs (0.1 mmol/kg), owing to its high T1 relaxivity [42,43]. The evident consequence of administering a smaller amount of gadolinium-containing contrast agent is reduced retention in tissues, emphasizing the importance of considering the administered dose in interpreting retention levels, similar to the dose-dependent relationship of brain gadolinium retention in previous studies [29,44].

Another plausible explanation for the lower retention of gadolinium after gadoxetic acid administration is its dual excretion pathways through the kidney and hepato-biliary systems [16,45]. This dual excretion mechanism results in a shorter half-life of gadolinium in the blood (1 h) than that of other GBCAs (1.5–2 h). This shorter blood half-life, combined with dual excretion routes, contributes to a more efficient elimination of gadolinium from the body, thereby reducing the overall amount of gadolinium retained

in tissues [45].

As temporal progression unfolded after GBCA administration, a discernible reduction in residual gadolinium was observed across all organs, irrespective of the specific GBCA type. Notably, this trend was more accentuated in the case of gadodiamide administration. Such observations align with findings from prior investigations, both in the context of liver studies [27] and across various organs [30], substantiating the generalizability of this temporal trend.

In particular, within the liver and kidney tissues, a significant decrease in residual gadolinium was observed between the first and fourth weeks postadministration. However, subsequent to this initial reduction, the levels of residual gadolinium plateaued, which is consistent with the patterns observed in earlier studies. This temporal pattern suggests an initial rapid elimination phase followed by a more stable state of gadolinium retention.

Conversely, notable exceptions to this typical trend were observed, particularly in the case of gadoxetic acid administration and in brain tissue. The deviation from the anticipated pattern may be attributed to the relatively small amount of gadolinium retained in these tissues, potentially leading to larger relative errors in the measurements. This underscores the intricacies involved in quantifying gadolinium retention, especially when dealing with lower concentrations, and it emphasizes the importance of considering potential variations in measurement precision.

In this comprehensive investigation, the findings revealed a higher level of gadolinium retention in the liver fibrosis group than in the normal liver group, with a notable association with the degree of liver fibrosis. Moreover, a positive correlation was consistently identified between the amount of gadolinium retained in the liver and various

GBCAs. Although the precise mechanisms underlying the relationship between liver fibrosis and gadolinium retention remain unclear, we propose the following three plausible hypotheses:.

Liver fibrosis is a wound-healing response to liver damage caused by diverse factors, leading to the accumulation of excessive collagen in the extracellular matrix [46]. This expansion of the extracellular space may create a conducive environment for gadolinium retention, extending the indwelling time of the GBCAs [36]. Moreover, previous studies have shown that lanthanides, including gadolinium, tend to bind to collagen proteins for several weeks and promote collagen proliferation [47,48]. The progressive nature of fibrosis is intricately associated with the augmented accumulation of collagen in the extracellular matrix, notably within spaces, such as the space of Disse. In this evolving fibrotic environment, the dynamics of free Gd^{3+} ions become pivotal, offering an increased opportunity for these ions to bind to collagen. Consequently, the likelihood of gadolinium accumulation increases as fibrosis progresses. Therefore, this mechanism may contribute to the increased amount of gadolinium retained in the liver.

In contrast to other extracellular agents, gadoxetic acid exhibits distinctive behavior as it is actively taken up by hepatocytes through the organic anion transporter in a complex form. This unique characteristic suggests that tissue retention, facilitated by collagen binding in its ionic form, may be less pronounced than that of its counterparts. However, our liver fibrosis model revealed that the development of pericentral and periportal fibrosis can be attributed to cellular toxicity [49], ultimately leading to cholestasis, a phenomenon reminiscent of other chronic liver diseases [37]. This observation forms the basis of the second hypothesis.

Given that gadoxetic acid is excreted through both renal and biliary routes, gadolinium

retention may be influenced more by cholestasis than by collagen binding in this particular context. This hypothesis is further supported by a previous study on copper excretion through the biliary pathway, analogous to gadoxetic acid, which demonstrated tissue accumulation with progression of as liver fibrosis [50].

The third hypothesis centers on metallothionein, a protein renowned for its role as a heavy metal ion scavenger. As hepatic fibrosis progresses, a discernible decline occurs in serum albumin levels, coupled with a reduction in zinc concentrations bound to albumin [51,52]. This phenomenon results in zinc deficiency, a condition observed in various hepatic disorders including alcoholic liver disease, chronic hepatitis, and liver failure. Importantly, zinc deficiency impedes the expression of metallothionein, a cysteine-rich protein that plays a pivotal role in scavenging free radicals and sequestering potentially harmful heavy metal ions [53,54].

Several studies have corroborated the decrease in metallothionein expression in the context of liver fibrosis [55,56]. This implies that the scavenging capacity of metallothionein for Gd^{3+} ions in the liver may be compromised by its reduced expression. Consequently, this impairment in metallothionein function can contribute to the enhanced accumulation of gadolinium in liver tissues. This hypothesis sheds light on the intricate molecular mechanisms linking hepatic fibrosis, metallothionein expression, and gadolinium retention. However, further in-depth investigations of the pathophysiological mechanisms underlying the effect of liver fibrosis on gadolinium retention are warranted to gain a comprehensive understanding of these complex interactions.

After exploration of the specific tendencies between the liver fibrosis and normal liver groups in various organs, an intriguing observation emerged, particularly in the femur tissues, setting it apart from other organs. Contrary to the pattern observed in the liver

tissues, the liver fibrosis groups exhibited lower gadolinium retention than the normal liver groups in femur tissues, a trend consistent across all GBCAs utilized in our study. Although the precise mechanism underlying this phenomenon remains elusive, to the best of our knowledge, chronic inflammation is a potential key player.

Chronic inflammation has been implicated in the induction of impaired bone metabolism through dysbiosis, leading to the onset of bone diseases such as osteoporosis [57-59]. In the context of osteoporosis development, the rate of new osteoblastic bone formation decreases, resulting in bone remodeling that contains less gadolinium [60]. Furthermore, the bone tissue affected by osteoporosis experiences mineral loss, which triggers the release of trace metals. Gadolinium was anticipated to follow a mechanism similar to that observed in previous studies on lead (Pb) [61].

Examination of gadolinium retention in kidney tissues between the normal liver model and the liver fibrosis model revealed distinctions, albeit only evident at 4- and 12-week intervals after gadoxetic acid administration. Intriguingly, no significant differences in liver fibrosis were observed among the other GBCAs in this context. Notably, the amount of Gd retained in the liver was notably lower, ranging from one-tenth to one-twentieth of the amount retained in the kidney. Given this considerable discrepancy, the effect of liver fibrosis on the quantity of gadolinium retained in the kidneys is challenging to discern.

However, a noteworthy exception emerged in the case of gadoxetic acid, where gadolinium retention in the kidneys was significantly higher in the liver fibrosis group. Unlike other GBCAs, gadoxetic acid follows a unique excretion pathway, traversing not only the renal but also the hepatobiliary system. Considering this distinctive excretion pattern, renal excretion may be augmented by cholestasis induced by liver fibrosis. Consequently, these altered dynamics could contribute to higher retention of gadolinium in the kidney,

emphasizing the importance of considering specific GBCA characteristics and pathways in the context of hepatic conditions.

In the brain tissue, discerning a specific trend in gadolinium retention based on the presence of liver fibrosis has proven elusive. The quantity of gadolinium retained in the brain tissue, a fraction of that retained in the liver or bone, emphasizes the formidable challenges posed by the BBB. The BBB imposes a substantial hindrance, making it inherently more difficult for gadolinium to access the brain parenchyma than other abdominal organs or bones.

Recent studies have proposed several hypotheses regarding the pathways through which gadolinium species may access the brain parenchyma [62]. These include (1) a direct access route to the brain parenchyma through the blood-CSF barrier in the choroid plexus epithelium, (2) permeation through the periarterial pial-glial basement membrane, and (3) a limited but direct route through the BBB. The quantification of gadolinium retention in the brain depends on the efficiency with which gadolinium species navigate these intricate pathways.

To unravel the precise localization of gadolinium in the liver tissue, we employed electron microscopy techniques, specifically TEM and SEM-Flatquad. These methods were coupled with ICP-MS to validate the presence of gadolinium in the samples. However, similar to findings from previous studies [27,63], our TEM observations did not reveal electron-dense particles in either normal or fibrotic liver tissues.

In the case of SEM-Flatquad, although the numerical data indicated a very small peak of gadolinium in both normal and fibrotic liver samples, the specific spatial distribution could not be discerned from the mapping results. This deviation from previous studies suggests potential differences in our experimental conditions, such as the administration

of a single dose of GBCA, resulting in relatively small retention amounts 1 week postadministration, possibly reaching the detection limit.

This discrepancy underscores the need for a more nuanced approach in future research. Specifically, acquiring tissue samples at shorter intervals after excessive GBCA administration in liver fibrosis models is imperative. Thus, we aimed to capture the dynamic phase of gadolinium retention and visualize its accumulation in fibrotic liver tissue more effectively using the refined techniques of TEM and SEM-Flatquad. This strategic adjustment in the study design holds promise for unveiling the finer details of gadolinium distribution within liver tissues, contributing to a more comprehensive understanding of contrast agent dynamics and their implications for hepatic conditions.

Our study had several inherent limitations that warrant careful consideration. First, as is common in many animal studies, the findings presented here may not precisely mirror the complexities of clinical situations in humans; rather, they may serve as indicators. Variables such as GBCA clearance may exhibit substantial differences between humans and animals, and organ-specific interactions with GBCAs may also diverge. Despite these absolute discrepancies, notably, the results of our study align with those of a previous human study [64], where distinctions between linear GBCA and macrocyclic GBCA, as well as differences between brain and bone tissues, exhibited similar tendencies.

The second limitation pertained to the relatively small number of rats in each group. Despite utilizing a total of 120 animals, they were distributed across the normal liver and liver fibrosis groups and were further categorized into three different GBCAs and four distinct time spans within each group. Ultimately, the animals were allocated to 12 subgroups, each comprising five animals. Although this number precludes the use of parametric methods, these limitations are common constraints in prospective animal

experiments.

Another consideration is the use of TAA to induce liver fibrosis, which may exhibit physiological characteristics distinct from those of liver fibrosis induced by viral hepatitis or alcoholic liver disease. Nonetheless, recent studies have highlighted that TAA-induced liver cirrhosis closely mimics clinical chronic liver damage in humans, both physiologically and histologically [65,66]. This model manifested prominent regenerative nodules with periportal and lobular distribution [66]. Despite these advantages, TAA-induced liver fibrosis may not fully replicate the varied physiological nuances of different types of chronic liver diseases in humans.

In conclusion, although our study provides valuable insights into the dynamics of gadolinium retention in the context of liver fibrosis, these limitations highlight the need for cautious interpretation and warrant further exploration. Future research should address these limitations by refining study designs, increasing sample sizes, and exploring diverse models of liver fibrosis to enhance the generalizability and robustness of the findings.

V. CONCLUSION

The influence of liver fibrosis on gadolinium retention manifested as a consistent escalation in the liver, irrespective of the GBCA type or time range of administration. This robust effect emphasizes the significant impact of liver fibrosis on the biodistribution dynamics of Gd in hepatic tissues. Interestingly, this effect extends beyond the liver and affects the gadolinium retention in other vital organs.

In the context of bone tissues, a noteworthy deviation from the trend was observed in the liver. Liver fibrosis results in reduced gadolinium retention in the bone tissue. This intriguing finding suggests that systemic changes induced by liver fibrosis may contribute to altered gadolinium dynamics in the liver as well as in the distant skeletal structures.

Examination of the impact on renal tissues revealed a pattern akin to that of the liver, with increased gadolinium retention in the presence of liver fibrosis. However, this phenomenon was observed only in conjunction with gadoxetic acid, highlighting the nuanced interplay between liver fibrosis, GBCA type, and renal gadolinium retention. The specificity of this effect emphasizes the importance of considering the unique characteristics of individual contrast agents when assessing their interactions with hepatic conditions.

In contrast, no significant differences were noted in the brain, suggesting a relative resilience of the BBB to systemic changes induced by liver fibrosis. This observation aligns with the intricate nature of the brain's protective barriers, highlighting its distinct response compared to other organs in the context of liver fibrosis.

In summary, our study revealed a complex inter-organ relationship, illustrating how liver fibrosis influences gadolinium retention in diverse tissues. These findings provide a

foundation for further exploration of the underlying mechanisms governing these interactions, ultimately contributing to a more comprehensive understanding of the effects of liver fibrosis on contrast agent dynamics across various organs.

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ABSTRACT(IN KOREAN)

Gadodiamide, Gadobutrol, Gadoxetic acid를 주입한 랫드에서 간 섬유화가 가돌리늄의 장기조직 내 침착에 미치는 영향

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자기공명영상장치의 조영제로 널리 사용되는 가돌리늄 조영제의 조직내 축적은 다양한 장기에서 일어날 수 있으나 그 조건과 유해성에 대해 잘 알려져있지 않다. 본 연구에서는 간 조직 내 축적되는 가돌리늄이 간 섬유화에 의해 어떤 영향을 받는지 연구하고 간 조직 내 어디에 가돌리늄이 축적되는지 확인하고자 하였다.

120마리의 랫드 중 60마리에게 thioacetamide를 복강 내로 주입, 간섬유화를 유발하여 60마리의 간섬유화 그룹과 60마리의 정상간 그룹을 만들고 이를 각각 15마리씩 4개의 소그룹으로 분류하여 gadodiamide, gadobutrol, gadoxetate disodium 및 생리식염수를 정주한 후 1주, 4주 및 12주 후에 간, 신장, 뼈 및 뇌 조직을 모두 채취하였다. 얻어진 조직들은 Metavir 분류를 사용한 조직학적 분석으로 간 섬유화 정도를 점수화하였다. 이 후 이 조직들에 대해 유도결합플라즈마 질량분광법 (inductively coupled plasma mass spectroscopy, ICP-MS)을 사용하여 조직 내 축적된 가돌리늄의 양을 정량화 하였다. 또한 투과전자현미경 (Transmission electron microscopy, TEM)과 주사전지현미경 (Scanning Electron Microscope, SEM)을 사용, 가돌리늄이 축적된 간 조직을 관찰하여 가돌리늄이 축적된 조직의 위치를 살펴보고자 하였다.

60마리의 간 섬유화 그룹 중 Metavir 점수가 2인 경우 15마리, 3인경우 17마리, 4인경우 23마리로 측정되었고 0인경우는 없었고 1인경우는 5마리로 측정되어 비교적 간 섬유화가 잘 유발 된 것으로 보였다.

정상간내 축적된 가돌리늄과 섬유화된 간내 축적된 가돌리늄의 양을 비교한

결과, gadodiamide를 정주한 후 1주후 얻은 간 조직 외에 모든 결과에서 섬유화된 간 내 축적된 가돌리늄의 양이 정상간보다 통계적으로 유의하게 높았다. 또한 가돌리늄 조영제의 종류에 따라, gadodiamide는 간섬유화 정도에 상관 없이 가장 많은 간 내 가돌리늄 축적을 보였으며 gadoxetic acid는 전반적으로 가장 적은 간 내 가돌리늄 축적을 보였다. 반면 대퇴골 조직내 가돌리늄 축적은 간섬유화 그룹에서 정상간 그룹보다 유의하게 낮은 수치를 보였다. 신장 조직에서는 gadoxetic acid 정주 후 4주와 12주 랫드들에서 간섬유화 그룹이 정상간 그룹보다 유의하게 높은 조직내 가돌리늄 축적이 일어났음을 확인하였다. 뇌 조직의 잔류 가돌리늄 농도는 간 섬유증 그룹과 정상간 그룹간의 유의한 차이가 없었다. 전자현미경을 이용한 관찰에서는 아주 작은 가돌리늄 특이 피크가 관찰되었으나 그 위치를 특정지을 수는 없었다.

결론적으로 간 조직내 가돌리늄의 축적은 가돌리늄 조영제의 종류에 상관없이 섬유화된 간에서 정상간보다 많았으며 따라서 만성간질환 등에 의한 간섬유화 환자들에 대해서는 가돌리늄 조영제 축적에 더욱 주의를 기울일 필요가 있다고 사료된다.

핵심되는 말 : 가돌리늄 침착, 간 섬유화, 가돌리늄 조영제