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Effects of chemical forms of gadolinium on the spleen in mice after single intravenous administration

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ABSTRACT

Gadolinium-based contrast agents (GBCAs) are widely used to improve tissue contrast during magnetic resonance imaging. Exposure to GBCAs can result in gadolinium deposition within human tissues and has become a clinical concern because of the potential toxic effects of free gadolinium (Gd^{3+}). Here, we report the impact of a single administration of GBCAs (Omniscan and Gadovist), and Gd^{3+} on mouse tissues. Five-week-old male BALB/c mice were injected intravenously with GBCAs or Gd^{3+}. Seven days after injection, relatively high levels of gadolinium were detected in the spleen (118.87 nmol/g tissue), liver (83.00 nmol/g tissue), skin (48.56 nmol/g tissue), and kidneys (25.59 nmol/g tissue) of the $Gd(NO_3)_3$ (high dose: 0.165 mmol/kg) group; in the bones (11.12 nmol/g tissue), kidneys (7.49 nmol/g tissue), teeth (teeth: 6.18 nmol/g tissue), and skin (2.43 nmol/g tissue) of the Omniscan (high dose: 1.654 mmol/kg) group and in the kidneys (16.36 nmol/g tissue) and skin (4.88 nmol/g tissue) of the Gadovist (high dose: 3.308 mmol/kg) group. Enlargement of the spleen was observed in the Gd^{3+} group ($p < 0.05$), but not in the Omniscan or Gadovist groups. Gd^{3+} caused iron accumulation around the white pulp of the spleen, suggesting that enlargement of the spleen is, at least in part, associated with Gd^{3+} and/or iron accumulation. Our results may help elucidate the relative risks of different types of gadolinium agents, the mechanisms involved, and even recognition of potential toxic effects of GBCAs.

1. Introduction

Gadolinium is a heavy metal belonging to the lanthanide family with strong paramagnetic properties. It is widely used in industrial and medical applications, especially for contrast improvement in magnetic resonance imaging (MRI). GBCAs are an important tool for the diagnosis and management of many neurological diseases. GBCAs are eliminated from the body through urine and, to a lesser extent, the biliary system. In subjects with normal renal function, they are usually eliminated from the blood within approximately 1.5 h and are fully recovered from the urine within 7 d (>90% in the first 12 h) [1]. The only known adverse health effect related to GBCA administration is a rare condition called nephrogenic system fibrosis that occurs in a small subgroup of patients with preexisting chronic or acute kidney failure. Therefore, GBCAs are generally considered safe for individuals with normal renal function [2].

In recent years, it has become clear that exposure to GBCAs can lead to gadolinium deposition in the human brain, despite normal renal function. Additionally, evidence suggests gadolinium deposits in human tissues beyond the brain, including bones, liver, kidneys and skin [3,4]. Furthermore, accumulation of gadolinium in the brain and other tissues has been observed in rats and mice treated with GBCAs [5,6].

GBCAs are divided into linear and macrocyclic agents based on the shape of the organic ligand. The linear GBCAs include gadodiamide (Omniscan), gadopentetate dimeglumine (Magnevist), and gadoversetamide (OptiMARK). The macrocyclic GBCAs include gadobutrol (Gadovist), gadoteridol (ProHance), and gadoterate meglumine (Dotarem). Macrocyclic GBCAs are more stable than linear agents due to the former's cage-like structure, which encloses and tightly binds to gadolinium ion (Gd^{3+}). The rate of gadolinium release was highest for charge-neutral, linear GBCAs (20–21%) followed by charged, linear GBCAs (0.07–1.9%), while negligible amounts of Gd^{3+} were released by macrocyclic GBCAs by incubating the GBCAs in human serum for 15

Abbreviations: GBCAs, Gadolinium-based contrast agents; MRI, magnetic resonance imaging; Gd^{3+}, free gadolinium; ICP-OES, inductively coupled plasma-optical emission spectroscopy.

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d [7].

Gd^{3+} has been shown to be profoundly toxic to many cell types [8–10] and various potential mechanisms underlying the toxic effects of Gd^{3+} accumulation have been postulated, including competition between Gd^{3+} and Ca^{2+}, which results in toxicity in biological systems [11,12]. Recently, we reported that Gd^{3+} can cause an endoplasmic stress response and autophagy in HEK293 cells [13]. To date, few clinical effects have been demonstrated, but Semelka et al. reported that patients might experience skin thickening and bone/chest pain after administration of GBCAs [14]; they identified a new pathologic entity defined as "gadolinium deposition disease".

Although there is growing evidence of gadolinium deposition after GBCA administration in humans and animals, little information is available on the differences in tissue dynamics and tissue toxicities between Gd^{3+} and GBCAs. Therefore, this study investigated the gadolinium deposition and tissue damage in mice after a single intravenous administration of $Gd(NO_3)_3$ or $GdCl_3$ and two GBCAs, Omniscan and Gadovist.

2. Materials and methods

2.1. Chemicals

$Gd(NO_3)_3$ and $GdCl_3$ were purchased from Wako Pure Chemical Industries (Osaka, Japan) and were dissolved in 100 mM HEPES (pH7.6), and volumes were adjusted with sterilized water. To prepare a 10 mM $Gd(NO_3)_3$ or $GdCl_3$ solution, 10 μL of 1 M $Gd(NO_3)_3$ or $GdCl_3$ solution was added to a 990 μL of sterile isotonic sodium chloride (0.9% sodium chloride solution). The pH of the solution was approximately 7. The injection solution was clear, and there were no insoluble particles after centrifugation at 2000×*g* for 5 min. Omniscan was purchased from Daiichi and Sankyo Inc. (Tokyo, Japan). Gadovist was purchased from Bayer HealthCare Pharmaceuticals (Whippany, NJ, USA). All other chemicals and solvents were of analytical grade.

2.2. Animals and treatments

In all experiments, BALB/c male mice from Japan SLC (Shizuoka, Japan) were used. Mice were kept under standard conditions (24–26 °C with 55–75% humidity) of daylight (12 h light cycle) and provided with commercial feed (Japan Crea, Tokyo, Japan), and *ad libitum* water. All experimental conditions were approved by the Committee for Animal Experiments at Kitasato University, in accordance with the procedures for animal experiments and the guiding principles for the care and use of laboratory animals. After a 7 d acclimation period, mice were randomly divided into seven experimental groups of 4–5 animals each as follows: vehicle (saline), Omniscan (0.165 mmol/kg), Omniscan (1.654 mmol/kg), Gadovist (0.165 mmol/kg), Gadovist (3.308 mmol/kg), $Gd(NO_3)_3$ (0.033 mmol/kg), and $Gd(NO_3)_3$ (0.165 mmol/kg). Each solution was intravenously administered via the tail vein. Seven days after the single administration, the animals were sacrificed and the following tissues were explanted: brain, liver, spleen, kidneys, muscle, bone, and skin. The explanted specimens were weighed, homogenized, and processed for inductively coupled plasma-optical emission spectroscopy (ICP-OES; iCAP 7400 Duo, Thermo Fisher Scientific) or paraffin embedding. To assess the effect of $GdCl_3$ on spleen, a $GdCl_3$ (0.165 mmol/kg) solution was intravenously administered via the tail vein and the explanted specimens were weighed and processed for histology.

2.3. Histology

For histopathological examination, the tissues were fixed in 10% buffered formalin for 24 h and embedded in paraffin after dehydration in graded ethanol as described previously [15]. Paraffin blocks were cut into 5-μm-thick sections (Retoratome REM-710, Yamato Kohki Industrial, Saitama, Japan), rehydrated with gradient ethanol, and stained with hematoxylin-eosin (Sakura Finetek Japan, Tokyo, Japan). Iron was detected based on a Prussian blue reaction using an iron stain kit (Scy-Tek Laboratories, Logan, UT, USA).

2.4. Measurement of gadolinium concentration in mice tissues

Tissues (100 mg) were homogenized in distilled water and then digested with 1 mL of 60% nitric acid (HNO_3) for 1 h at 180 °C. After filling up to 5 ml with 2% HNO_3, gadolinium concentrations in the digested tissue samples were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES; iCAP 7400 Duo, Thermo Fisher Scientific) as described previously [16]. The limit of quantification (LOQ) was determined using a calibration curve obtained from diluted standards. The value for the LOQ was 0.25 ppm.

2.5. Statistical analysis

Quantitative data are expressed as the mean ± standard deviation. The statistical significance ($p < 0.05$) of each variable was estimated using one-way analysis of variance followed by Tukey-Kramer test. Normality and homoscedasticity of the data were tested using the Shapiro–Wilk test and F-test, respectively. R software (ver. 4.0.5) was used for statistical analyses.

3. Results

3.1. Tissue weight of mice after single administration of GBCAs and $Gd(NO_3)_3$

Gadovist, like other GBCAs, is injected into the vein for use in diagnostic MRI in humans. The recommended dose of Gadovist is 0.1–0.3 mmol/kg body weight in humans; at these doses, the efficacy and safety of Gadovist have been demonstrated in numerous clinical studies [2]. Therefore, we used low doses (0.165 mmol/kg of Omniscan and Gadovist) and high doses (1.654 mmol/kg of Omniscan and 3.308 mmol/kg of Gadovist) in this study. The $Gd(NO_3)_3$ dose of 0.033 mmol/kg, corresponding to a free or dechelated gadolinium dose of 20%, was used, considering the possible dechelation of a fraction of GBCA when administered (*in vitro* dechelation showed approximately 20% dissociation). The $Gd(NO_3)_3$ dose of 0.165 mmol/kg, corresponding to 100% dechelation of the GBCA, was used to determine the effect of Gd^{3+}. Each solution was intravenously administered via the tail vein. Seven days after the single administration, the animals in all groups did not show any significant body weight loss or behavioral abnormalities (data not shown). Administration of Omniscan and Gadovist did not affect the weights of the brain, thymus, liver, spleen, kidney, and testis (Fig. 1A and B). In contrast, administration of $Gd(NO_3)_3$ (0.165 mmol/kg) led to a significant increase in the weight of the spleen (spleen weight normalized to body weight) compared to that of the vehicle control (Fig. 1C).

3.2. Gadolinium deposition in tissues of mice after single administration of GBCAs and $Gd(NO_3)_3$

To compare gadolinium tissue concentrations of Omniscan, Gadovist, or $Gd(NO_3)_3$ following a single intravenous administration, quantification of gadolinium in acid-hydrolyzed tissue samples was performed using ICP-OES. The Omniscan (1.654 mmol/kg)-exposed mice had elevated levels of gadolinium (teeth: 6.18 nmol/g tissue; kidney: 7.49 nmol/g tissue; bone: 11.12 nmol/g tissue; skeletal muscle: 0.53 nmol/g tissue; skin: 2.43 nmol/g tissue), whereas the values of gadolinium in the brain, thymus, and spleen were lower than that of the LOQ (Table 1). In the Gadovist (3.308 mmol/kg)-exposed group, high levels of gadolinium were detected in the kidney (16.36 nmol/g tissue), and skin (4.88 nmol/g tissue), whereas low levels of gadolinium accumulated in the thymus, liver, testis, bone, and skeletal muscle (Table 1). In the $Gd(NO_3)_3$ (0.165

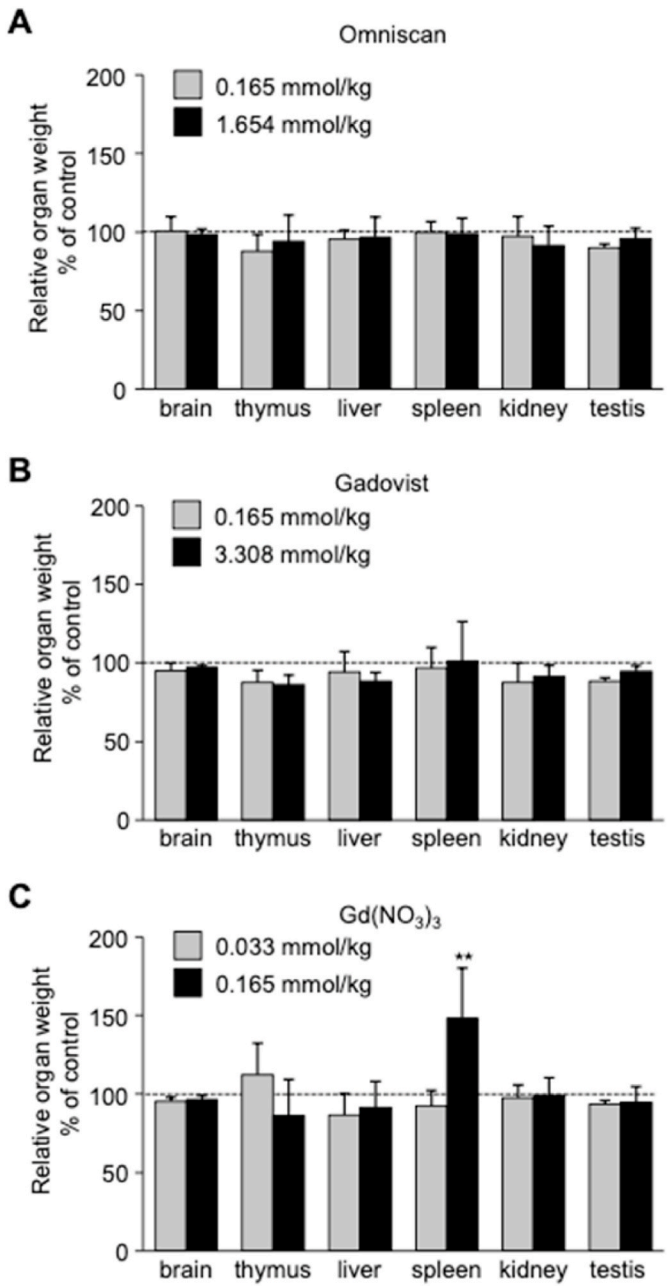

Fig. 1. Relative weights of the brain, thymus, liver, spleen, kidney, and testes after single intravenous administration of (A) Omniscan, (B) Gadovist, and (C) $Gd(NO_3)_3$. Values are means ± standard deviation. The significance of differences between groups is shown as $^{**}p < 0.01$. The error bars represent the standard deviation of measurements within the groups ($n = 8$, except "0.165 mmol/kg $Gd(NO_3)_3$" where $n = 7$).

mmol/kg)-exposed group, gadolinium was found in all tissues surveyed and there were very high levels of gadolinium in the thymus (5.85 nmol/g tissue), liver (83.00 nmol/g tissue), spleen (118.87 nmol/g tissue), kidney (25.59 nmol/g tissue), bone (13.79 nmol/g tissue), skeletal muscle (10.23 nmol/g tissue), and skin (48.56 nmol/g tissue) (Table 1). In the $Gd(NO_3)_3$ (0.033 mmol/kg)-exposed group, gadolinium remained in the teeth (2.03 nmol/g tissue), liver (17.72 nmol/g tissue), and spleen (30.53 nmol/g tissue).

3.3. Histological characteristics of tissues with mice after single administration of GBCAs and $Gd(NO_3)_3$

Next, to investigate the histological characteristics of tissues with high levels of gadolinium, we prepared paraffin sections of the liver, spleen, and kidney after injection of high doses of Omniscan, Gadovist, and $Gd(NO_3)_3$ and stained the sections with hematoxylin and eosin. The livers (Fig. 2A, D, 2G, and 2J), spleens (Fig. 2B, E, 2K, and 2H), and kidneys (Fig. 2C, F, 2I, and 2L) were analyzed by light microscopy and showed no abnormalities compared with vehicle controls.

3.4. Splenic analysis of mice after single administration of $Gd(NO_3)_3$ or chloride

Gross morphological and histological analyses of Omniscan-, Gadovist-, or $Gd(NO_3)_3$-administered mice did not reveal any apparent tissue abnormalities, except for enlargement of the spleen of the Gd $(NO_3)_3$-treated group. Comparative pictures of spleen and the graph of spleen mass for each group are depicted in Fig. 3A and B. Spleen weight increased in $Gd(NO_3)_3$ ($p < 0.05$), and $GdCl_3$ ($p < 0.05$) treatment groups. Similar to the $Gd(NO_3)_3$-exposed spleen, histological analysis of the $GdCl_3$-exposed spleen did not show any changes in white pulp structures compared with those of the control mice (Fig. 3C–E).

We next asked whether enlargement of the Gd^{3+}-exposed spleen was associated with accumulation of blood cells: as the spleen filters blood and removes abnormal blood cells from the bloodstream. To test the deposits of red blood cells in the spleen exposed to Gd^{3+}, the tissue samples were stained with Prussian blue, which reacts with deposits of red blood cells. Prussian blue staining revealed an abundance of iron storage throughout the spleen exposed to Gd^{3+} (Fig. 3F–H). This excess iron was widely distributed around the white pulp (Fig. 3I–K).

4. Discussion

GBCAs have been highlighted because of concerns associated with potential adverse events. Recent studies have found that the GBCAs are deposited in human and animal bodies after both single and repeated administration [17]. In addition, gadolinium release from the GBCA molecule has been proposed as a risk factor for adverse health effects [18]. Therefore, it is important to elucidate the tissue deposition and adverse effects of both GBCAs and Gd^{3+}.

We recently reported that Gd^{3+} exposure reduced the viability of several cell lines and activated autophagy *in vitro* [13]. The present study elucidates the tissue deposition of linear and macrocyclic GBCAs, as well as Gd^{3+}, and their effect on the deposited tissues of mice. GBCAs are intravenous drugs used in diagnostic imaging procedures to enhance the quality of MRI; the recommended single dose of both Omniscan and Gadovist is 0.1–0.3 mmol/kg for diagnostic purposes [19,20]. Dose extrapolation from humans to animals necessitates consideration of body surface area; the dose used in mice is recommended to be approximately 12-fold higher than that used in human [21]. However, we used the doses of the GBCAs mg/kg conversion in this study because the toxic doses of the GBCAs were unknown in humans. In addition, Omniscan and Gadovist were administered by injection into a vein with the recommended dose or 10-fold or 20-fold higher than the recommended dose, in the current study, as maximal doses. Single administration of Omniscan and Gadovist had no significant impact on tissue weight, even in the high dose-administered groups (Fig. 1A and B). Meanwhile, the normalized spleen weight of mice in the 0.165 mmol/kg $Gd(NO_3)_3$ group was greater than that of the control group (Fig. 1C). However, we did not observe a significant difference in spleen weight of mice in the 0.033 mmol/kg $Gd(NO_3)_3$ group. We cannot accurately determine the toxicity of 0.033 mmol/kg $Gd(NO_3)_3$ from these finding alone. Further investigations and validations are needed to assess the degree of $Gd(NO_3)_3$ toxicity.

GBCAs are deposited mainly in the brain, kidney, liver, spleen, bone,

Table 1
Average concentrations of gadolinium in tissues after single intravenous administration of Omniscan, Gadovist, and $Gd(NO_3)_3$. Total tissue gadolinium concentration measured via ICP-OES. Values are expressed as nmol/g tissue. Data represent mean ± standard deviation $n = 8$, except "0.165 mmol/kg $Gd(NO_3)_3$" where $n = 7$.

	nmol/kg	brain	teeth	thymus	liver	spleen	kidney	testis	bone	muscle	skin
Saline		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Omniscan	0.165	0.35 ± 0.89	1.47 ± 0.75	<LOQ	<LOQ	<LOQ	1.15 ± 2.09	<LOQ	1.51 ± 0.29	<LOQ	<LOQ
	1.654	<LOQ	6.8 ± 1.41	<LOQ	0.77 ± 0.14	<LOQ	7.49 ± 1.86	0.36 ± 0.07	11.1 ± 13.6	0.53 ± 0.15	2.43 ± 0.69
Gadovist	0.165	<LOQ	0.40 ± 0.08	<LOQ	<LOQ	<LOQ	0.75 ± 0.39	<LOQ	<LOQ	<LOQ	<LOQ
	3.308	<LOQ	1.14 ± 0.22	0.57 ± 0.26	0.34 ± 0.05	0.53 ± 0.14	16.4 ± 14.7	0.85 ± 0.28	0.72 ± 0.14	0.48 ± 0.36	4.88 ± 0.89
$Gd(NO_3)_3$	0.033	<LOQ	2.03 ± 0.95	<LOQ	17.7 ± 0.1	30.5 ± 11.2	4.83 ± 3.21	0.27 ± 0.16	4.56 ± 1.39	0.54 ± 0.32	1.36 ± 0.29
	0.165	<LOQ	1.49 ± 0.26	5.85 ± 2.07	83.0 ± 20.5	119 ± 59	25.6 ± 6.0	0.53 ± 0.56	13.8 ± 10.1	10.3 ± 22.2	48.6 ± 43.3

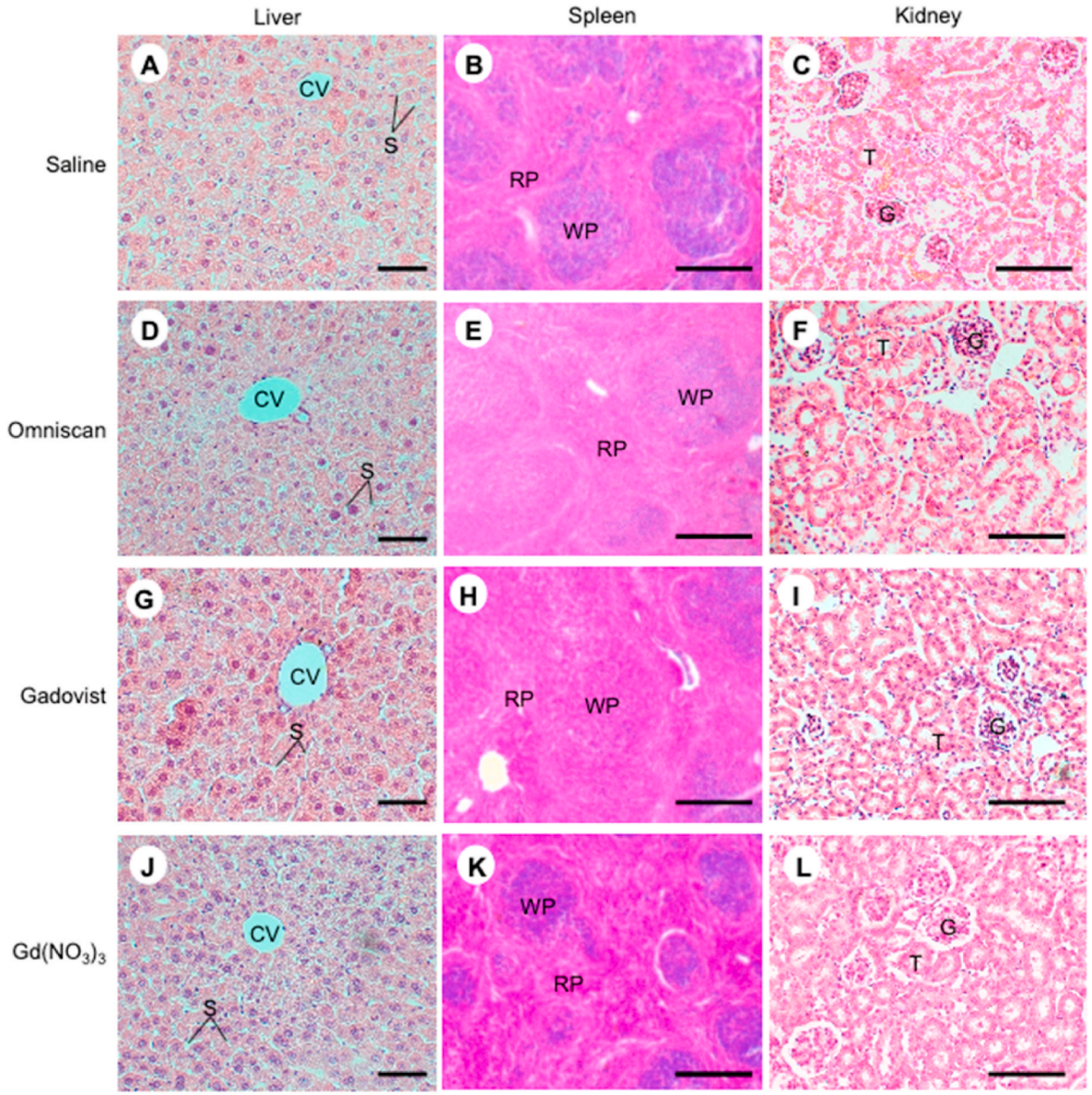

Fig. 2. Representative photomicrographs from light microscopy (hematoxylin-eosin stain) of liver, spleen, and kidney are shown for, (*A–C*), control group mice, (*D–F*), Omniscan-exposed mice, (*G–I*), Gadovist-exposed mice, and (*J–L*), $Gd(NO_3)_3$-exposed mice. Scale bars = 100 μm, liver and kidney; Scale bars = 100 μm, spleen. (CV) central vein, (S) sinusoids, (RP) red pulp, (WP) white pulp, (G) glomeruli, and (T) tubules. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and skin [22,23]. In the present study, the highest gadolinium content was found in the femur (Omniscan) and in the kidneys (Gadovist) (Table 1). A high gadolinium content was detected in the spleen, liver, skin, and kidney of the $Gd(NO_3)_3$ group. These results are consistent with previous studies showing high gadolinium retention in the bone in the case of Omniscan [24], kidney in the case of Gadovist [25], and

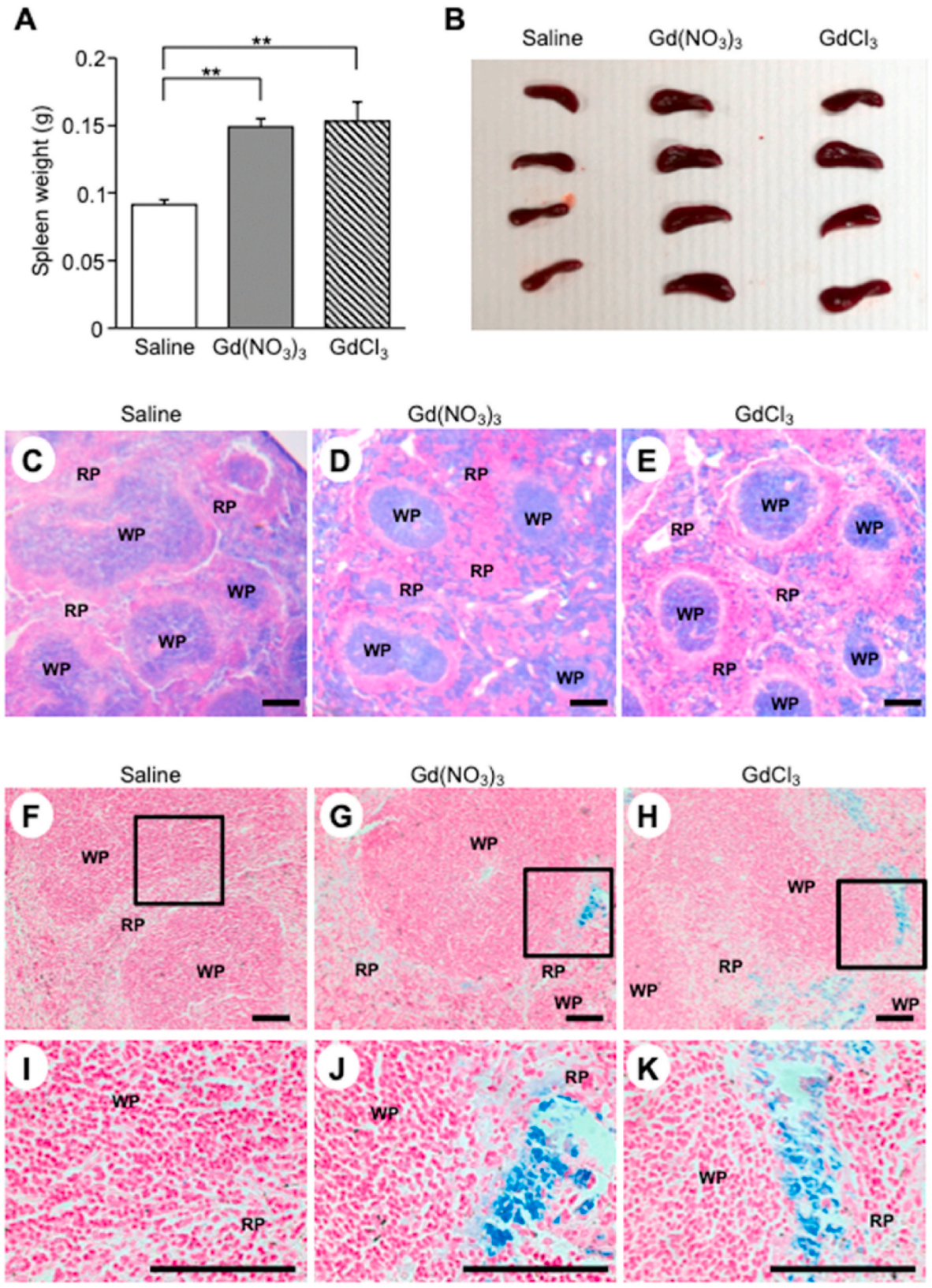

Fig. 3. (A) Spleens from control, $Gd(NO_3)_3$, and $GdCl_3$-exposed mice are 1.5-fold larger by wet weight than control spleens, which is evident on (B) gross examination. Spleen weights normalized to body weight of each mouse. Bar graph data are mean values on ± standard deviation; $*p < 0.05$; n = 4 mice per group. (C–E) Hematoxylin-eosin staining of cross-sectioned spleens of $Gd(NO_3)_3$ or $GdCl_3$-treated mice. Scale bars = 200 μm. Histological structure of spleen stained with Prussian blue of (F) control mice, and (I) the expanded image, (G) $Gd(NO_3)_3$-exposed mice, and (J) the expanded image, and (H) $GdCl_3$-exposed mice, and (K) the expanded image. Scale bars = 100 μm. (RP) red pulp, (WP) white pulp. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

spleen in the case of Gd^{3+} [26]. However, the high gadolinium concentration in the skin after Gadovist injection has not been previously reported. The risk of urinary contamination of gadolinium cannot be ruled out in this study. Further investigation is required to dissect the deposition of gadolinium in the skin after a single gadobutrol injection. Additionally, we evaluated different doses of Gadovist (3.308 mmol/kg) and Omniscan (1.654 mmol/kg) in this study. Assuming that the gadolinium concentration in the tissues of the Omniscan-treated group was half than in the tissues of the Gadovist-treated group, it can be surmised that the Omniscan-treated group had a higher gadolinium concentration in the tissues. While the overall amount of gadolinium in the target tissues of Omniscan was similar to that of Gadovist, Omniscan had higher levels of gadolinium in the teeth and femur than did Gadovist. These findings raise the possibility that free Gd^{3+} ions are released from Omniscan and they replaced calcium in the bone. The more stable macrocyclic GBCA, Gadovist deposited low levels of Gd in the teeth and femur. These results suggest that the differences in Gd levels in the teeth and femur between Omniscan and Gadovist can be attributed to their intrinsic stability. Although the analytical method employed here (ICP) could not distinguish between chelated and non-chelated Gd^{3+}, administration of $Gd(NO_3)_3$ clearly indicates that the spleen, liver, and skin are the target tissues for Gd^{3+}.

Histopathological analysis showed that high-dose Omniscan, Gadovist, and $Gd(NO_3)_3$ did not show any remarkable tissue alterations (Fig. 2A–L), suggesting that the harmful effects appear to be minimal even if an excessive dose is given as a single administration of GBCA. One possibility is that Omniscan and Gadovist do not dechelated to a significant degree to damage the tissues upon a single administration. Another possibility is that most of the agent is excreted before dechelation. The number of exposures necessary to elicit these effects on the tissue is currently unknown. This issue needs to be addressed in a future study. The impact of Gd^{3+} on tissues remains controversial. The transient lymphocyte depopulation of the periarteriolar lymphatic sheath areas of the spleen was found to occur after a single administration of gadolinium acetate at a dose of 0.03 mmol/kg, 24 h post-dosing in rats and rapidly repopulated with lymphocytes at 48 h post-dosing [26]. On the other hand, nucleated cells in the red pulp of the spleen were reported in the case of oral exposure to a daily intake of 0.57 mmol/kg $GdCl_3$ over a period of 90 w [27]. Given that a similar effect was observed between $Gd(NO_3)_3$ and $GdCl_3$ in the spleen (Fig. 3A–C), we can exclude the possibility of a consequence of the gadolinium counter-ion. We have not yet explained how spleen enlargement occurs following a single administration of Gd^{3+}. Free Gd^{3+} ions are not present in the physiological milieu. Gd^{3+} forms insoluble precipitates with anions including phosphate, carbonate, acetate, and hydroxide [28]. They might be taken up by the reticuloendothelial system. A possible explanation for spleen enlargement is that phagocytic cells of the reticuloendothelial system and consequent storage of gadolinium in tissues, such as the liver and spleen, which are part of this system, are responsible. In fact, excess iron was widely distributed around the white pulp in the Gd^{3+}-exposed spleen (Fig. 3D). These observations support the hypothesis that significant accumulation of gadolinium in specific tissues is associated with adverse health effects, particularly with respect to spleen toxicity. Gd^{3+} also binds to proteins and nucleic acids. Therefore, another possibility of spleen enlargement is that there is an abundance of undesirable components produced by Gd^{3+} in the spleen. More studies are needed to identify the interplay between GBCA retention and spleen toxicity mediated by Gd^{3+}.

There were some limitations to this study. First, we could not determine whether the retained Gd was free Gd, its chelated form, or other metabolites, because the tissue samples were digested with nitric acid and analyzed with ICP-OES. Second, other target tissues for Gd retention, such as bone, thymus, testis and skin, which lower Gd retention, were not evaluated. Third, we could not accurately to determine the toxicity of 0.033 mmol/kg $Gd(NO_3)_3$, Omniscan, and Gadovist. Assessment of various toxicological parameters and biomarkers would be needed to investigate the potential toxicity of low-level Gd retention.

In summary, we demonstrated for the first time that differences exist in the distribution of gadolinium retained in tissues when comparing different GBCA compounds and Gd^{3+} after a single administration. We also found that Gd^{3+} was substantially deposited in the spleen and it caused an enlarged spleen. Characterization of the effects of Gd^{3+} could improve our understanding of the potential pathology of GBCAs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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