Organ retention of gadolinium in mother and pup mice: effect of pregnancy and type of gadolinium-based contrast agents

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Abstract
Purpose To investigate the effect of pregnancy and type of gadolinium (Gd)-based contrast agents (GBCAs) on organ retention of Gd in mother and pup mice after maternal administration.

Materials and methods Gd-DTPA-BMA (gadodiamide) or Gd-DOTA (gadoterate dimeglumine) was administered (2.0 mmol/kg of maternal weight) four times to pregnant Balb/c mice from gestational day 16–19, respectively. At 28 days after birth, they were euthanized and their organs (blood, brain, liver, kidney, spleen, and bone) were removed for the measurement of Gd by inductively coupled plasma mass spectrometry.

Results Gd retention in maternal organs was generally lower than that in the organs of non-pregnant mice in both Gd-DTPA-BMA and Gd-DOTA groups. Significantly higher Gd retention was observed in the organs of pups whose mothers were administered Gd-DTPA-BMA as compared to those whose mothers were administered Gd-DOTA. Tissue-to-muscle ratio in the brains of pups was higher than that of mothers.

Conclusion We demonstrated in utero transplacental Gd retention in pups. In various organs in both mothers and pups, Gd retention was consistently higher for Gd-DTPA-BMA than Gd-DOTA administration. Pregnancy affected Gd retention in many maternal organs.

Keywords Gadolinium-based contrast agent · Gadolinium · Retention · Maternal administration · Placenta

Introduction
Gadolinium (Gd)-based contrast agents (GBCAs) are widely used in magnetic resonance imaging (MRI). These agents shorten the T1 effect, i.e., increase the signal intensity on T1-weighted images and improve delineation of lesions within the body. GBCAs are categorized into two main types based on the chemical structure of the chelates, i.e., linear and macrocyclic GBCAs. Macrocyclic GBCAs are more stable than linear agents, i.e., they are less likely to release Gd$^{3+}$ ions, which are toxic to the human body [1–3]. Retained Gd for patients with renal impairments may lead to nephrogenic systemic fibrosis (NSF), a lethal disease marked by joint contractures and skin thickening [4–7]. Recent studies have documented the possibility of Gd retention, even in patients with normal renal function [8].

Use of GBCAs during pregnancy is a contentious issue because of their ability to traverse the placental barrier [9]. Although the adverse effects of GBCA administration during pregnancy have not been reported [10–12], there have been still two issues about Gd retention for mothers and babies. Contrast enhanced MRI is sometimes needed during pregnancy to assess maternal and fetal abnormalities.
Khurana et al. reported a case of renal failure, in which NSF symptoms improved after a successful pregnancy [13]. Maternofetal transmission of Gd after intravenous administration of GBCA during pregnancy has been demonstrated in animal models [14]. These reports suggested a transplacental Gd transfer and a potential association between the pregnancy and symptom resolution; however, the mechanism underlying this relationship has not been known. It also has not been confirmed whether or not the retained Gd crosses the placental barrier in utero and whether or not Gd is retained in the infant’s body after delivery.

In this study, we investigate the effect of pregnancy and type of gadolinium (Gd)-based contrast agents (GBCAs) on organ retention of Gd in mother and pup mice after maternal administration.

Materials and methods

Animals

The Institutional Animal Care and Use Committee of our institution approved all study protocols. Six pregnant Balb/c mice (5 weeks old; mean weight 17 ± 3 g; 15 embryonic days [E15]) and nine non-pregnant female mice (5 weeks old; mean weight 14 ± 2 g) were purchased from Japan SLC, Inc. (Tokyo, Japan). All mice were housed in an approved animal facility at room temperature (27–28 °C) with ad libitum access to food and water.

GBCAs and their administrations

Gd-DTPA-BMA (gadodiamide, linear chelate, 0.5 mol/L; Omniscan, Daiichi-Sankyo Co. Ltd., Tokyo, Japan) and Gd-DOTA (gadoterate meglumine, macrocyclic chelate, 0.5 mol/L; Magnescope, Terumo Co., Tokyo, Japan) were used in this study. Six pregnant mice were randomly divided into two groups to receive injections of one of the two GBCAs: Gd-DTPA-BMA (n = 3) and Gd-DOTA (n = 3), respectively. GBCAs were injected on four consecutive days (E16 to E19) via the tail vein at a dose of 2.0 mmol/kg. Six non-pregnant mice in the two control groups Gd-DTPA-BMA (n = 3) and Gd-DOTA (n = 3) also received GBCAs injections on four consecutive days. Three non-pregnant mice received injections of 100 µL saline on four consecutive days to show the limit of detection (LOD) and limit of quantification (LOQ) for our measurements.

Study design

After delivery at E19, mouse pups were fed with their mother’s milk and housed in the same cages for 21 days. At postnatal day-21 (P21), the pups were separated from their mothers and were fed with standard food and water for the next seven days. Two pups in each mother were selected and they were sacrificed at P28 and mothers were also sacrificed on the same day. Non-pregnant mice in the control group were treated in the same manner, i.e., they were sacrificed at 28 days after the last GBCA administration.

Tissue collection

Samples of brain, spleen, kidney, liver, bone, femoral muscle, and blood were collected from six mothers and nine non-pregnant mice. These tissues were snap frozen and stored at −80 °C before analysis by inductively coupled plasma mass spectrometry (ICP-MS).

Measurement of Gd by inductively coupled plasma-mass spectrometry

Standard solutions of Gd were prepared at four different concentrations: 0, 25, 50, and 100 ng/g in 3% nitric acid. Prior to quantitative analysis of Gd retention in each sample, the tissues were weighed and digested in a microwave oven (MLS-1200 MEGA, Milestone Inc. Shelton, CT) in acid-treated PFA vials with ultra-pure nitric acid (HNO₃, 0.5 mL) and hydrogen peroxide (H₂O₂, 0.1 mL) with specific eight-sequences of a microwave program for 125 min. After digestion, samples were transferred to acid-treated 15 mL polypropylene (PP) tubes, and ultra-purified water was added to attain a total volume of 10 mL. The concentrations of Gd in each sample were determined by a stable Gd isotope (¹⁵⁸Gd) using ICP-MS (the ELAN® DRC II instrument, PerkinElmer Inc, Waltham, MA). In each of the samples, the concentration of Gd (ng/g) was calculated from the calibration line based on standard solutions.

Data analysis

To investigate the effect of pregnancy, we compared Gd retentions between maternal and non-pregnant mice, and evaluated the difference of Gd retentions between the Gd-DTPA-BMA and Gd-DOTA groups. Gd retentions in pup organs were also examined. To show tendencies of Gd deposition in mother mice and pup mice, we calculated organ-to-muscle ratios.

Statistical analysis

Statistical analysis was carried out using the t test for each pair and multiple t test followed by the Bonferroni correction for multiple comparison. All data were analyzed using Prism version 6.0 (GraphPad software, San Diego, CA) and expressed as mean ± standard deviation. p value of less than 0.05 was considered statistically significant.
### Results

Gd retentions in each organ of maternal and non-pregnant mice, and pups were shown in Table 1.

#### Table 1 Gd\(^{3+}\) concentrations in maternal, non-pregnant, and pup mouse organs

<table>
<thead>
<tr>
<th>Samples</th>
<th>GBCAs</th>
<th>Brain</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Liver</th>
<th>Bone</th>
<th>Blood</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td>Gd-DTPA-BMA</td>
<td>120.5 ± 19.5</td>
<td>5674.3 ± 699.4</td>
<td>3034.2 ± 678.3</td>
<td>1764.0 ± 579.5</td>
<td>2672.6 ± 977.8</td>
<td>22.1 ± 6.8</td>
<td>350 ± 157.5</td>
</tr>
<tr>
<td></td>
<td>Gd-DOTA</td>
<td>5.7 ± 1.2</td>
<td>1335.7 ± 237.7</td>
<td>419.3 ± 78.7</td>
<td>267.0 ± 40.1</td>
<td>192.7 ± 87.2</td>
<td>4.7 ± 0.6</td>
<td>53.0 ± 8.5</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>Gd-DTPA-BMA</td>
<td>371.9 ± 23.3</td>
<td>13676.3 ± 2281.1</td>
<td>3851.1 ± 851.8</td>
<td>4350.5 ± 821.3</td>
<td>8824.3 ± 467.2</td>
<td>52.3 ± 10.9</td>
<td>833.1 ± 231.0</td>
</tr>
<tr>
<td></td>
<td>Gd-DOTA</td>
<td>37.7 ± 14.2</td>
<td>5120 ± 4145.2</td>
<td>1308.6 ± 170.8</td>
<td>1560.7 ± 336.8</td>
<td>817.3 ± 165.5</td>
<td>30.0 ± 2.8</td>
<td>280.1 ± 72.6</td>
</tr>
<tr>
<td>Pup</td>
<td>Gd-DTPA-BMA</td>
<td>73.5 ± 35.5</td>
<td>51.3 ± 20.4</td>
<td>41.0 ± 9.9</td>
<td>76.8 ± 32.6</td>
<td>612.5 ± 112.8</td>
<td>4.4 ± 1.7</td>
<td>47.8 ± 45.6</td>
</tr>
<tr>
<td></td>
<td>Gd-DOTA</td>
<td>11.0 ± 3.2</td>
<td>55.8 ± 34.7</td>
<td>&lt;LOD</td>
<td>6.8 ± 0.8</td>
<td>&lt;LOQ</td>
<td>1.2 ± 1.6</td>
<td>15.2 ± 1.5</td>
</tr>
</tbody>
</table>

Gd retention in the maternal and non-pregnant mice organs

Gd retention in the maternal organs were consistently higher in the Gd-DTPA-BMA group compared to the Gd-DOTA group (Fig. 1: brain and kidney, \(p < 0.001\); spleen, liver, bone and blood, \(p < 0.01\); muscle, \(p < 0.05\)).

In the Gd-DTPA-BMA group, Gd retention in the brain (\(p < 0.001\)), kidney (\(p < 0.01\)), liver (\(p < 0.01\)), bone (\(p < 0.001\)), and blood (\(p < 0.001\)) were lower in the maternal mice compared to the non-pregnant mice. In the Gd-DOTA group (Fig. 2b), differences in Gd retentions between the maternal and non-pregnant organs were also observed, except for kidney (spleen and blood, \(p < 0.001\); liver and bone, \(p < 0.01\); brain and muscle, \(p < 0.05\)).

Gd retention in pup organs

Two pups of each of the three mothers were used for assessment of Gd retention. Gd retention was detected in all mouse pups. Significant differences in Gd retentions in brain (\(p < 0.01\)), spleen (\(p < 0.0001\)), liver (\(p < 0.001\)),
bone ($p < 0.0001$), and blood ($p < 0.01$) were observed between the Gd-DTPA-BMA and Gd-DOTA groups, respectively, while no significant difference was observed in kidney and muscle (Fig. 3).

The organ-to-muscle ratios of Gd retention in the pup brains (Gd-DTPA-BMA: $2.0 \pm 0.2$, Gd-DOTA: $0.7 \pm 0.0$) were significantly higher than those in the maternal brain (Gd-DTPA-BMA: $0.4 \pm 0.1$ ($p < 0.001$), Gd-DOTA: $0.1 \pm 0.0$ ($p < 0.05$)). The organ-to-muscle ratio in bone of pups with in utero exposure to Gd-DTPA-BMA ($20.7 \pm 6.8$) showed the highest value among maternal and pup bones ($0.9 \pm 0.1–8.6 \pm 3.6$) (Fig. 4).

**Discussion**

In our study, we demonstrated that Gd retentions in the maternal organs were generally lower compared to non-pregnant mice, and that Gd retentions in both mothers and pups were consistently higher after Gd-DTPA-BMA administration compared to Gd-DTPA administration.

We suspected that differences in Gd retentions between the maternal and non-pregnant mice may be due to the physiological increase in glomerular filtration rate (about 50%) and renal plasma flow (up to 80%) during pregnancy [16]. We also observed Gd retention in pup organs after maternal administration of GBCAs, suggesting that fetal tissue may be an additional, unfortunate route of Gd elimination from the mother. These results may partially explain Khurana’s report, in which NSF symptoms improved after pregnancy [13].

Gd retentions in pups from mothers injected with GBCAs during pregnancy varied with the type of GBCAs administered. When the mother was injected with Gd-DTPA-BMA, pup retention in bone was markedly higher than that in the other organs. Gd-DTPA-BMA would be related to bone synthesis process with high affinity to the bone [17], and bone metabolism in the pups would be more rapid than that in the mothers. This may be the reason why maternal Gd retention in bone was significantly lower than that of non-pregnant mice.

Interestingly, there was no difference in Gd retention in pup kidney between the two kinds of GBCAs. For the maternal kidney, there was a statistically significant difference between them. This may be due to the characteristics of fetal circulation, in which the placenta transfers oxygen, nutrients, and other substances into the fetal circulation via the umbilical vein, prior to directly entering the fetal liver.
The fetal liver may trap Gd at this time, which may explain the decreased retention in kidney [18].

Gd was found in both maternal and pup brain exposed to GBCAs, and this finding was consistent with previous reports, both clinical [19, 20] and laboratory [21]. The higher organ-to-muscle ratios of Gd retention in pup brain than those in the maternal brain may be attributable to the immaturity of the fetal blood brain barrier (BBB) [22]. Nevertheless, even with an adult BBB, Gd entered brain tissue, and was retained 28 days after the final injection.

This study had several major limitations. The first was that pups were fed maternal breast milk for the first 21 days after their birth. It was shown that less than 0.1% of the total injected dose of GBCA may be eliminated through breast milk, and this elimination may occur within the first 24 h of injection. The infant gastrointestinal tract may absorb less than 1% of the GBCA ingested [10]. Considering that information, it was highly unlikely that a nursing infant would be harmed by ingesting breast milk from a mother who has received a clinical dose of GBCA. However, with repeated high doses to the mother, as was the case in our study, there may be minimal contamination of pup organs by Gd ingested after birth through maternal breast milk.

Secondly, the injection dose of GBCAs was a 20-fold higher than a standard clinical dose, and four consecutive injections of GBCAs from E16 to E19 were administered. Oh et al. reported minimal retention of Gd in fetal tissues and in the amniotic fluid compared to the maternal injection dose in a Japanese macaque model. From these results, they concluded the use of macrocyclic GBCAs during pregnancy was safe [15]. However, they used only macrocyclic GBCA (gadoteridol). There should also be further investigation on the effect of GBCAs on fetuses to evaluate their safety.

The third limitation was the small number of mice. We studied three mothers in each GBCA group and two pups from each mother, or a total of six pups in each GBCA group. Because we measured Gd retentions in six organs of each mouse, the total number of samples was quite large. We thought that our data sufficiently showed the differences in Gd retention between two types of GBCAs. We did not evaluate the effect of Gd retention on the pups’ normal development. Intensive observation, including behavioral studies, would be needed to assess the effect of Gd dose and retention on normal development.

Conclusion

We demonstrated in utero transplacental Gd retention in pups. In various organs in both mothers and pups, Gd retention was consistently higher for Gd-DTPA-BMA than Gd-DOTA administration. And we concluded pregnancy affected Gd retention in many maternal organs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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