MRI Visualization of Rodent Liver Structure and Peritoneal Adhesion With Dialyzate Enhancement

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This study investigated the use of peritoneal dialysis fluid (dialyzate) as a MR contrast agent to visualize the liver structure and peritoneal adhesion in rats at 7 T. Intrapерitoneal injection of dialyzate (~0.1 ml/g) yielded excellent and consistent intraperitoneal enhancement that delineated the liver lobular structure in all rats studied (N = 8). It also allowed the MR detection of peritoneal adhesions that were surgically induced. MR measurements of adhesion surface areas correlated well with the postmortem estimations (R = 0.99; N = 6). Dialyzate persisted in the intraperitoneal cavity for up to 2 days. T1 and T2 values of undiluted dialyzate were found to be 3017.5 ± 35.3 ms and 108.4 ± 2.0 ms, respectively. These findings demonstrated dialyzate-enhanced MRI as a potentially valuable technique to localize certain activities within liver (such as local tumor metastasis) and to monitor therapeutic interventions (e.g., against peritoneal adhesion) in preclinical research using small animal models. Magn Reson Med 59:1170–1174, 2008. © 2008 Wiley-Liss, Inc.

Key words: peritoneal dialysis fluid; dialyzate; MRI; intraperitoneal enhancement; rodent liver; peritoneal adhesion

MRI provides superb soft tissue contrast in abdominal imaging. However, its application in visualizing organs inside the abdominal cavity has been hampered by their structural complexity and difficulty in delineating organ boundaries. The peritoneum is the largest and most complexly arranged serous membrane in the body (1). It is composed of layer of mesothelium supported by a thin layer of connective tissue, forming the lining of the abdominal cavity that covers most of the intraabdominal organs, including liver, spleen, stomach, and cecum. High-resolution MRI of these intra- and retroperitoneal organs in rodent models at high magnetic field is particularly challenging because of the high respiration/heart rates and increased magnetic susceptibility and motion artifacts, thus hampering the MR application in preclinical study of small animals. For example, while rodent liver is a widely used model for in vivo investigation of hepatocellular carcinoma (also called hepatoma) progression, metastasis, and drug interventions (2–5), its relatively complex lobular structure cannot be well depicted by the conventional MR methods. The other example is that MRI thus far could not provide any detection and characterization of postsurgical peritoneal adhesion, which remains an extremely common complication in abdominal and pelvic operations, and a source of considerable morbidity (6).

Peritoneal dialysis fluid (dialyzate) is a clinically accepted medium in treatment of patients in the later stage of chronic kidney failure (7). It is injected into the peritoneal cavity to absorb the wastes that pass from the blood through the peritoneum, and then drained from the body. Typical dialyzate consists of water, electrolytes, and glucose, likely yielding distinct MR relaxation properties. There have been only few studies so far that reported the use of dialyzate to examine the peritoneal dialysis patients without (8) and with (9–11) adding contrast agent such as gadodiamide, and the peritoneal dialysis contact surfaces in rats (12). There has been no report of dialyzate MR relaxation properties. In this study, peritoneal dialysis fluid was characterized, and demonstrated as a contrast agent for abdominal imaging to visualize the liver lobular structure and detect peritoneal adhesion in rodents.

METHODS

All MRI experiments were performed on a 7-T MRI scanner with maximum gradient of 360 mT/m (70/16 PhasorScan; Bruker Biospin GmbH, Germany). A 60-mm quadrature resonator was used for RF transmission and receiving. All animal experiments were approved by the local institutional animal ethics committee. For in vivo imaging, animals were anesthetized with isoflurane/air using 3% for induction and 1.5% for maintenance via a nose cone. Body temperature was maintained at 36.5°C by circulating warm water in a heating pad.

MR Characterization of Dialyzate as a Contrast Agent

Clinically approved 4.25% dextrose peritoneal dialysis fluid (~0.1 ml/g; Fresenius Medical Care, Bad Homburg, Germany) was chosen in this study to enhance the peritoneal cavity for visualizing the abdominal structures. For measurement of MR relaxation properties of this dialyzate, five phantoms were made with this standard dialyzate diluted to volume concentrations of 0%, 10%, 20%, 50%, and 100%, using phosphate-buffered saline (PBS) solution in cylindrical tubes of 4 cm in length and 1 cm in diameter. Their transverse and longitudinal relaxation rates were measured using a multiecho spin echo sequence (TR = 3 s with TEs of 20, 40, 60, 80, 100, 120, 140, 160, 180, and
200 ms) and single-echo spin echo sequence (TE = 10 ms with TRs of 2, 4, 6, 8, and 10 s), respectively.

Animal Procedures

Rodent liver has relatively complex structure, including four lobes (left, median, right, and caudate), though the fundamental structures of rodent and human livers are similar (13). To evaluate if dialyzate could differentiate different liver lobes, intact adult Sprague-Dawley rats (200–300 g, N = 4) were used in this study. In addition, magnetically labeled cells were surgically injected into the main portal vein of inbred male Buffalo rats (200–300 g, N = 4) while the portal pedicle to the right lobe and the right part of the median lobe were clamped, leading to the cell deposition localized to the left side of liver (i.e., left lobe, left part of median lobe, and caudate lobe). Specifically, hepatoma cell line McA-RH7777 (CRL1601; ATCC, Manassas, VA, USA) was first labeled via incubation with 0.9 μm superparamagnetic styrene-divinyl benzene inert

Table 1

<table>
<thead>
<tr>
<th>Dialyzate concentration (% volume in PBS)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁ (ms)</td>
<td>3420.6 ± 58.6</td>
<td>3285.5 ± 44.6</td>
<td>2986.0 ± 12.3</td>
<td>2951.0 ± 10.7</td>
<td>3017.5 ± 35.3</td>
</tr>
<tr>
<td>T₂ (ms)</td>
<td>928.0 ± 34.2</td>
<td>463.2 ± 12.9</td>
<td>316.2 ± 6.0</td>
<td>199.9 ± 2.6</td>
<td>108.4 ± 2.0</td>
</tr>
</tbody>
</table>

FIG. 1. Multislice coronal, axial, and sagittal liver images obtained from a normal intact rat immediately before (a) and after (b) the IP administration of dialyzate solution (~0.1 ml/g). Note that four liver lobes were well delineated after dialyzate administration. Similar enhancement of the liver structure was observed in all four Sprague-Dawley rats studied.
polymer-coated micron-sized iron oxide particles (MPIOs; Bangs Laboratory, Fishers, IN, USA) of concentration $2.5 \times 10^8$ particles/ml for 24 h (14,15). The labeling efficiency was higher than 90% with approximately 15–20 particles per cell, and the viability after labeling was higher than 85% as confirmed using Trypan blue staining. Approximately $2 \times 10^5$ of such MPIO-labeled hepatoma cells in 500 µl PBS were then injected into each inbred Buffalo rat (anesthetized with intraperitoneal [IP] injection of sodium pentobarbital 40 mg/kg) using the procedure described above. Note that the clamping was maintained for 3 min after the cell injection.

To evaluate MRI detection of peritoneal adhesion with dialyzate, adhesion was induced in male Sprague-Dawley rats (150–250 g, $N = 6$) using a surgical procedure of abdominal wall and cecal abrasion similar to that previously described (16). Briefly, animals were anesthetized with IP injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). A 3-cm vertical midline incision was made through the abdominal wall and peritoneum. Both dorsal and ventral surfaces of the cecum were exposed and abraded with a size-15 scalpel blade until blood appeared on the cecal surface in all cases. The parietal peritoneum lateral to the midline incision was also scraped 30 times until petechial hemorrhage was observed. The abdominal incision was closed subsequently in two layers with 4-0 silk sutures. Animals were scanned with MRI at day 21 after the surgical procedure. They were then killed 1 day after MRI. The abdomen was opened through a U-shaped incision for confirmation of the adhesion site. Surface area of peritoneal adhesion was estimated by measuring the representative lengths according to the shape of the adhesion (such as ellipse, rectangle, trapezoid, etc.) by two independent observers.

**In Vivo MRI Experiments**

For liver imaging, each animal was first scanned before dialyzate administration, removed from the magnet, given an IP injection of prewarmed 100% dialyzate ($\sim 0.1$ ml/g), and immediately scanned again. Gradient echo images were acquired by a multislice two-dimensional (2D) flow-compensated sequence with respiratory gating and TR = 1 s, TE = 7.5 ms, flip angle (FA) = 90°, FOV = 5.2 × 5.2 cm, slice thickness = 0.5 mm, acquisition matrix = 192 × 192, resolution = $0.27 \times 0.27 \times 0.5$ mm$^3$, number of averages = 6, and total scan time of approximately 20 min. Note that such high spatial resolution was required in order to visualize the local distribution of MPIO-labeled cells (14,15). To examine the dialyzate clearance from the peritoneal cavity, images were also acquired 1 day and 2 days after the dialyzate administration. In addition, $T_2$-weighted fast spin echo (FSE) images were acquired immediately after and 4 weeks after the cell injection in order to confirm the local tumor growth in the left side of liver only with TR = 2.7 s, TE = 40 ms, echo train length (ESL) = 8, FOV = 5.2 × 5.2 cm, slice thickness = 0.5 mm, acquisition matrix = 192 × 192, resolution = $0.27 \times 0.27 \times 0.5$ mm$^3$, number of averages = 8.

For peritoneal adhesion imaging, each animal was given an IP injection of prewarmed 100% dialyzate ($\sim 0.1$ ml/g) after overnight fasting to reduce intestinal motion during MRI scan. Sagittal gradient echo images were obtained using a similar gradient echo sequence with FOV = 6.0 × 6.0 cm, slice thickness = 1.2 mm, number of averages = 2, and total scan time of approximately 7 min. The boundary of the peritoneal adhesion near cecum was manually segmented and smoothed for each animal in the multislice image data set obtained after the dialyzate administration. The corresponding adhesion area was computed from the

**FIG. 2.** Coronal $T_2$-weighted gradient echo and $T_2$-weighted FSE images obtained from an inbred male Buffalo rat without (a,b) and with dialyzate (c,d) immediately after the injection of $2 \times 10^8$ MPIO-labeled hepatoma cells into the main portal vein while the portal pedicle to the right lobe and the right part of the median lobe were clamped. The cells were found to localize in the left side of the liver as expected. Four weeks after cell injection, gradient echo and FSE liver images were obtained from the same rat without (e,f) and with dialyzate (g,h). Tumor metastasis was found mainly in the left side of liver.

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estimated length of adhesion boundary on each slice and the center-to-center slice spacing by a blinded observer. A total of two normal rats ($N = 2$) without any surgical procedure for adhesion were also scanned with identical protocols after IP injection of prewarmed 100% dialyzate as controls.

RESULTS

Table 1 lists the dialyzate relaxation properties measured. The dialyzate was clearly shown to be a medium of long longitudinal and transverse relaxation times. $T_1$ and $T_2$ values of undiluted dialyzate were found to be $3017.5 \pm 35.3$ ms and $108.4 \pm 2.0$ ms at 7 T, respectively, distinctly differing from those of most soft tissues in abdominal region. As the dialyzate volume concentration decreased with PBS dilution, the transverse relaxation rate ($R_2 = 1/T_2$) was found to decrease linearly while the longitudinal relaxation rate ($R_1 = 1/T_1$) remained largely the same. Figure 1 shows a typical set of pre- and postinjection images, clearly demonstrating the IP administered dialyzate as a useful contrast agent to depict the liver lobular structures. The four lobes were not discernible without dialyzate, but were well and consistently delineated in the images acquired after dialyzate administration in all eight animals (four intact rats and four rats injected with hepatoma cells). Such IP enhancement was found to be visible for up to 2 days. Among the rats with the cell injection, MPIO-labeled hepatoma cells were observed in all four animals (Fig. 2a and c). They were localized mainly in the left side of the liver, as shown from the liver lobular structure delineated by dialyzate in Fig. 2c. Four weeks after the cell injection, tumor nodules were clearly seen in the $T_2$-weighted FSE images (Fig. 2f). With dialyzate enhancement, they were found to occur predominantly in the left side of liver (Fig. 2h). Such metastasis is largely expected as the hepatoma cells were initially deposited in the left side of the liver only because of the clamping. Note that the certain signal decrease was observed within the peritoneal cavity between liver lobes in the FSE images (Fig. 2d and h), likely caused by the dialyzate fluid motion induced by respiration and heart beating.

Figure 3 shows the typical sagittal images of rats with and without peritoneal adhesion obtained after the dialyzate administration. For the rats surgically induced for peritoneal adhesion, IP dialyzate enhancement has clearly depicted the peritoneal adhesion sites at cecum in five out of six animals studied. The postmortem inspection confirmed the successful adhesion inductions in five out of the six animals (Fig. 4). Figure 5 plots the adhesion surface areas determined by in vivo dialyzate-enhanced MRI and postmortem measurement. Good correlation ($R = 0.99$) was observed.

DISCUSSION AND CONCLUSIONS

Our experimental results demonstrated the intraperitoneal dialysis fluid as a useful MRI contrast medium of long $T_1$ and $T_2$ values. While previous MRI studies employed dialyzate mainly to monitor the complications of continuous ambulatory peritoneal dialysis such as peritoneal leak in patients (8–11), the current work illustrated that IP administration of dialyzate solution provided an excellent IP enhancement that could be used to visualize the rodent liver lobular structure and delineate peritoneal adhesion.
Such relatively easy procedure enables MRI to accurately examine the abdominal structures (both IP and retroperitoneal organs) and abnormalities, providing a valuable technique to locate certain activities in these structures (such as local tumor metastasis) and to monitor therapeutic interventions (i.e., against peritoneal adhesion) in future preclinical research. Furthermore, peritoneal dialyzate enhancement may also be potentially applicable in clinical detection and evaluation of postsurgical peritoneal adhesion.

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REFERENCES


FIG. 5. Surface areas of the peritoneal adhesions estimated by in vivo MRI and postmortem measurements in the six animals studied. One animal yielded no adhesion as confirmed by postmortem inspection.