Manganese Enhanced MRI (MEMRI) of Rat Endometrial Cysts

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Introduction

Endometriosis, the presence of a viable endometrium outside the uterine cavity, is an estrogen-dependent condition that affects 5 million American women. Our laboratory has established a rat model of endometriosis in which the growth of the tissue is monitored non-invasively using MRI. In this model, the endometrial cyst has a fluid-filled interior which is comprised of various proteins including adhesion molecules (1), cytokines (2), and superoxide dismutase (3). Previously, manganese ion (Mn2+) has been used as an intracellular contrast agent that enters viable cells via voltage gated calcium channels (4). Proteins such as Mn-superoxide dismutase have been implicated in the recruitment of Mn2+ from elsewhere (5). Therefore, we examined the potential for using manganese as a molecular contrast agent for endometrial cyst MRI signal enhancement.

Methods

Eight-week-old female Sprague-Dawley rats were pre-screened for at least 3 estrous cycles. Only consistent 4-day cyclers were used for uterine auto-transplantation. Surgery was performed on estrus confirmed by vaginal lavage. Using aseptic technique, a 5x5 mm patch prepared from the distal end of the right uterine horn was sewn with the endometrial side against the right peritoneal wall across a visible blood vessel. Before the three layer closure, 5 ml of sterile saline was used to generously hydrate the abdominal tissues.

Manganese-Enhanced MRI (MEMRI) was performed at various time points (1 day, 1, 2 weeks post-Mn2+ infusion) once the endometrial cyst reached a steady-state of growth (4.5 months post-surgery). There were two Mn2+ infusion concentrations used, 8.14±0.92 and 4.05±0.55 nmoles/min/g BW. The animals were anesthetized using a mixture of oxygen, and isoflurane (~1.5-2%). Respiratory signal was monitored and a T1-weighted Fast Low Angle SHot (FLASH) sequence was used. All MRI images were acquired on a 4.7T Bruker BioSpec MRI spectrometer (Billerica, MA) using a half-birdcage surface coil.

Short-axis images were acquired using a FLASH sequence. A pilot coronal image of the rat abdomen was obtained. This pilot coronal image provided a clear view of the endometrial cyst location. The short axis slices covered the entire endometrial cyst. The imaging parameters were as follows: matrix dimensions, 256x256; TE/TR, 4.5/400 ms; slice thickness, 1.0 mm; FOV, 4.5 cm; 4 averages; 20 slices. Fat-suppression was used to minimize the lipid chemical shift artifact and enhance the quality of endometrial cyst images. Image analysis was performed using ANALYZE software (AnalyzeDirect, KS); the ROI tools were used to select the areas of interest (cyst volume) and signal intensity values were recorded and analyzed.

Results

The fat-suppressed MRI pulse sequence provided high quality images for the observation of the endometrial cysts (Figure 1). In addition, the infusion of Mn2+ in the endometriosis rat model clearly delineated and enhanced the cyst fluid cavity. These data suggest that the endometrial cyst has the potential to trap and retain Mn2+ over a period of time (Figure 2). The time course of signal intensity enhancement suggests Mn2+ was retained for ~2 weeks. Table 1 shows the normalized signal intensity enhancement over 2 weeks time. There is a small window for signal intensity enhancement between 8.14±0.92 and 4.05±0.55 nmoles/min/g BW infusion. This suggests there is room for improvement for both the infusion protocol and MRI pulse sequence (i.e. inversion recovery to optimizing T1-weighted images). Further analysis of cyst fluid content is warranted.

Conclusions

This study demonstrates that T1-weighted MEMRI enhancement in the endometriosis rat model in the presence of Mn2+ provides clear signal enhancement which may be beneficial for analyzing the cyst volume and content. There was a relative narrow concentration versus contrast enhancement window suggesting further improvement of imaging optimization could be accomplished. In conclusion, MEMRI may be used as a possible method for enhancing detection of smaller endometriol lesions in a rat model and studying possible kinetics with content.

Figure 1. Examples of short-axis T1-weighted MEMRI rat endometrial cyst images (1a) baseline in vivo 4.5 months post-surgery, (1b) zoomed in endometrial cyst image of 1a, (2a) 1 day post-Mn2+ infusion image, and (2b) zoomed in image of 2a.

Figure 2. Time course signal intensity enhancement at two manganese infusion concentration 8.14±0.92 and 4.05±0.55 nmoles/min/g.

Table 1 Normalized signal intensity enhancement time course due to Mn2+. Body weight (BW), 1-Day, 1-Week, and 2-Week post-Mn2+ infusion. Values are expressed in mean±SD. * P<0.01

<table>
<thead>
<tr>
<th>Mn2+ Conc (nmoles/min/g)</th>
<th>BW, g</th>
<th>Baseline SI</th>
<th>1 Day Post-Mn SI</th>
<th>1 Wk Post-Mn SI</th>
<th>2 Wks Post-Mn SI</th>
</tr>
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<tbody>
<tr>
<td>8.14±0.92 (n=3)</td>
<td>338 ± 48</td>
<td>1.00 ± 0.00</td>
<td>1.57 ± 0.01*</td>
<td>1.47 ± 0.25*</td>
<td>1.18 ± 0.10</td>
</tr>
<tr>
<td>4.05±0.55 (n=3)</td>
<td>334 ± 43</td>
<td>1.00 ± 0.00</td>
<td>1.02 ± 0.10</td>
<td>0.88 ± 0.04</td>
<td>0.98 ± 0.06</td>
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</tbody>
</table>

References

1. Igarashi, M, Ikuma, K et al. Fertility and Sterility 2003; 80:1065-1066.

Acknowledgment

The authors thank S. Lenhard, K. Maniscalco, J. Bray, and M.T. Li for stimulating discussions.