Manganese Enhanced MRI (MEMRI) of Rat Endometrial Cysts

T. C-C. Hu[1], R. E. Haimbach[2], A. R. Olzinski[1], T. R. Schaeffer[3], R. N. Willette[1], A. C. Sulpizio[2], B. M. Jucker[1]

[1]Investigative Biology, GlaxoSmithKline, King of Prussia, PA, United States, [2]Urogenital Biology, GlaxoSmithKline, King of Prussia, PA, United States, [3]Laboratory of Animal Science, GlaxoSmithKline, King of Prussia, PA, United States

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 (Mn^{2+}) 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 Mn^{2+} 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 mls 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-Mn^{2+} infusion) once the endometrial cyst reached a steady-state of growth (4.5 months post-surgery). There were two Mn^{2+} infusion concentrations used, 8.14±0.92 and 4.05±0.55 nmoles/min/g BW. The animals were anaesthetized using a mixture of oxygen, and isoflurane (~1.5-2%). Respiratory signal was monitored and a T_1-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 Mn^{2+} 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 Mn^{2+} over a period of time (Figure 2). The time course of signal intensity enhancement suggests Mn^{2+} 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 T_1-weighted images). Further analysis of cyst fluid content is warranted.

Conclusions

This study demonstrates that T_1-weighted MRI enhancement in the endometriosis rat model in the presence of Mn^{2+} 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 endometrial lesions in a rat model and studying possible kinetics with content.

Figure 1. Examples of short-axis T_1-weighted MEMRI rat endometrial cyst images (1a) baseline *in vivo* 4.5 months post-surgery, (1b) zoomed in endometrial cyst image of 1a, (2a) 1day post-Mn^{2+} infusion image, and (2b) zoomed in image of 2a.

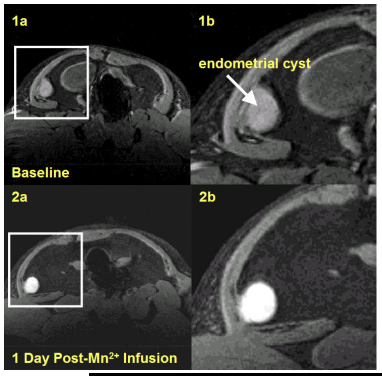

Figure 2. Time course signal intensity enhancement at two manganese infusion concentration 8.14±0.92 and 4.05±0.55 nmoles/min/g.

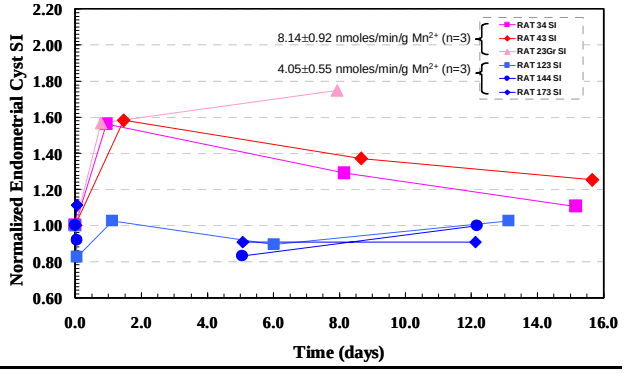

Table 1 Normalized signal intensity enhancement time course due to Mn^{2+}. Body weight (BW), 1-Day, 1-Week, and 2-Week post-Mn^{2+} infusion. Values are expressed in mean±SD. * P<0.01

Mn^{2+} Conc (nmoles/min/g)	BW, g	Baseline SI	1 Day Post-Mn SI	1 Wk Post-Mn SI	2 Wks Post-Mn SI
8.14±0.92 (n=3)	338 ± 48	1.00 ± 0.00	1.57 ± 0.01*	1.47 ± 0.25*	1.18 ± 0.10
4.05±0.55 (n=3)	334 ± 43	1.00 ± 0.00	1.02 ± 0.10	0.88 ± 0.04	0.98 ± 0.06

References

1. Igarashi, M, Ikuma, K et al. Fertility and Sterility 2003; 80:1065-1066.
2. Akoum, A, Lawson, C, et al. Molecular Human Reprod 2001; 7: 859-866.
3. Ota, H, Igarashi, S, et al. Fertility and Sterility 1999; 72: 129-134.
4. Narita, K, Kawasaki, F, Kita, H. Brain Res 1990;510:289-295.
5. Fujioka, M, Taoka, T et al Ann Neurol 2003; 54: 732-747.

Acknowledgment

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