Targeted Imaging of Breast Cancer Brain Metastasis using Antibody Labeled Manganese Oxide (MnO) Nanoparticles

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Purpose

The brain tumor has a high morbidity and mortality in world-wide and early diagnosis as well as treatment at the molecular and cellular level is an arising concept for treating the patient, in which molecular and cellular imaging technology is expected to play a central role (1, 2). Due to the complexity of the brain associated with unique capillary structure, anatomic structure, and metabolism, molecular and cellular imaging for the brain requires a contrast agent that does not destroy the anatomic background with a clear marginal detectability of the lesion. A complete ability to manipulate a platform of contrast agents along with the comprehensive understanding of the contrast mechanism is required as a solid foundation for the further development. In this work, we developed a platform for non-invasive MRI method that enables us to monitor molecular and cellular therapy in treating the brain tumor using manganese oxide (MnO) nanoparticles, a new positive T_1 MR contrast agent developed in our laboratory, and optimized conjugation of monoclonial antibody for Her-2/neu receptor in breast cancer brain metastasis to MnO nanoparticles as targeted MRI contrast agent (3).

Methods

Preparation of Functionalization of MnO nanoparticles: MnO nanoparticles conjugated with Her-2/*neu* receptor antibody (*Herceptin*, Roche Pharma Ltd.) were prepared by reacting maleimido group derivatized MnO nanoparticles with the antibody. During the preparation of water-dispersible MnO nanoparticles, maleimido-PEG phospholipid was added to produce MnO nanoparticles derivatized with maleimide groups that can react with the sulfhydryl group in *Herceptin*. Maleimido derivatized MnO nanoparticles were then conjugated with thiolated *Herceptin via* stable thioether bond.

*Animal Preparation:*The Orthotopic animal model of breast cancer brain metastasis was made by inoculating the MDA-MB-435 human breast cancer cells (2 x 105 cells/5 μl of HBSS) into the specific pathogen-free male balb/c-nu mice (6 wks old) brain. The tumor was grown for 12-17 days until MRI examination. *Herceptin* functionalized MnO nanoparticles (20mg of Mn/kg of body weight) or MnO nanoparticles (20mg of Mn /kg of body weight) were injected *i.v.* for 4 animals. For MRI, the animals were anesthetized by breathing 2% isoflurane into oxygen-enriched air with a facemask and the rectal temperature was maintained at 36 ± 1°C. To investigate the time course of distribution of the MnO nanoparticles, MRI was performed before, and 1-hr, 3-hr, 9-hr, and 24-hr after administration of the MnO nanoparticles. After MRI, H&E staining was done on the excised tissue samples.

*In vivo MRI***:** All *in vivo* MRI were carried on a 4.7T/30 MRI System (Bruker-Biospin, Fallanden, Switzerland) equipped with a 20 cm gradient set capable of supplying up to 100mT/m in 200 μsec rise-time. A birdcage coil (72 mm i.d.) (Bruker-Biospin, Fallanden, Switzerland) was used for excitation, and actively decoupled from a 20 mm diameter saddle-shaped surface coil (homebuilt), which was used for receiving the signal for brain imaging. High-resolution 3D MnO nanoparticle contrast enhanced MRI was obtained from each mouse brain using a fast spin-echo T_1-weighted MRI sequence (TR/TE= 300/12.3 ms, NEX =1, echo train length = 2, 140 μm 3D isotropic resolution) to evaluate the contrast

Results and Discussion

The functionalized MnO nanoparticles by Her-2/*neu* receptor antibody could selectively target the epidermal growth factor receptor (EGFR) that are expressed at the cell surfaces of the breast cancer in the orthotopic animal model of breast cancer brain metastasis. As shown in Figure 1, the breast cancer cells were selectively enhanced in T_1-weighted MRI because the functionalized MnO nanoparticles with the EGFR specific antibody were delivered and accumulated at the EGFR of the cell surfaces of the breast cancer. As the BBB is destructed as a result of the tumor formation in this animal model, both the functionalized and non-functionalized MnO nanoparticles entered the tumor site initially, but only the functionalized MnO are accumulated at the tumor site for an extended time due to the presence of the antibody conjugated to the MnO nanoparticles. The non-functionalized MnO nanoparticles enhanced both the tumor and the normal brain tissues. As a T_1 contrast agent, the MnO nanoparticles did not disrupt the background anatomic image, and the clear detection of the tumor margin was observed, which is important in brain MRI.

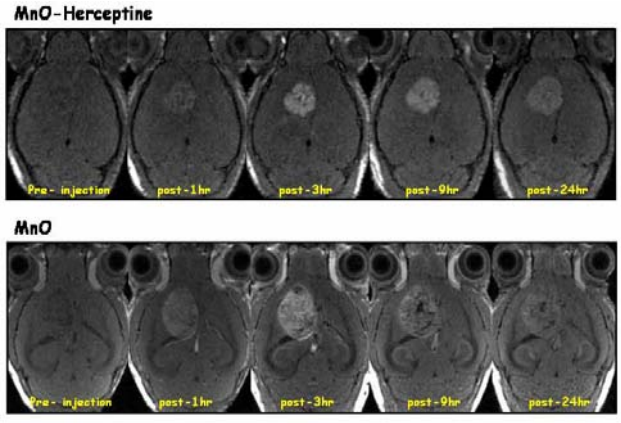

Figure 1. A series of MRI images of the mouse brain bearing the breast cancer brain metastatic tumor. The functionalized MnO with Herceptin was injected *i.v.* (20mg of Mn/kg of body weight) and the breast cancer cells were selectively enhanced in T_1-weighted MRI (top row). The bottom row shows a control mouse that was injected MnO.

Conclusion

We have developed a strategy to selectively image the disease specific biomarker using functionalized MnO nanoparticles for applications in molecular and cellular imaging. The current result suggests a promising application of the MnO nanoparticles for molecular imaging and targeted therapy in the brain tumor.

Reference

1. Rehemtulla A., et al. Mol Imaging 1(1):43-55 (2002); 2. Spencer AM, et al, J Cell Biochem Suppl 39:25-35(2002); 3. Artemov D., et al. MRM 49:403-408 (2003).