Manganese Oxide (MnO) Nanoparticles As a New T1 Contrast Agent for MRI

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Purpose

A contrast agent that exhibits positive contrast has a number of advantages in MRI. We have developed biocompatible water dispersed manganese oxide (MnO) nanoparticles that produce positive T_1 contrast effect *in vivo* and that can be used similarly to SPIO for applications in molecular and cellular imaging. To investigate the mechanism of contrast enhancement for the MnO nanoparticles, the relaxation measurements were carried out on various sized MnO, compared with those of Fe_3O_4 nanoparticles, MnO nanoparticles coated with SiO_2, and $MnCl_2$ solution.

Methods

Preparation of biocompatible water-dispersed MnO nanoparticles: Uniform-sized MnO nanoparticles were synthesized by the thermal decomposition of Mn-oleate complex as described (1), and the resulting MnO nanoparticles were then encapsulated by PEG-phospholipid shell to endow them with biocompatibility (2). Various sized MnO nanoparticles (7, 15, 20, and 25 nm) were synthesized. To confirm that free Mn^{2+} ions are not leached out from the MnO nanoparticles, we conducted elemental analysis by ICP-AES and MRI experiments for the supernatants of MnO nanoparticles dispersion that are centrifugated after incubation for 7 days at room temperature. Three cycles of centrifugation and aspiration were done, and the Mn concentrations and the corresponding relaxation rates ($1/T_1$) of the supernatants (S1, S2, S3) and the precipitate (P) obtained after the third centrifugation were measured. To further investigate the contrast enhancement of the MnO nanoparticles, the surface of the MnO nanoparticles were coated with SiO_2 ($MnO\text{-}SiO_2$), and various sized Fe_3O_4 nanoparticles were synthesized.

MRI Relaxation Properties and Contrast Enhancement: We measured T_1 and T_2 relaxation times of the size-tuned water dispersed MnO, $MnO\text{-}SiO_2$, and Fe_3O_4 nanoparticles, and $MnCl_2$ solutions at a 3.0T clinical MRI scanner. A Look-Locker sequence was used to measure T_1, and multiecho turbo spin echo sequence was used to measure T_2. The images were fitted into a 3-parameter function to calculate T_1 values, and Levenberg-Margardt method to calculate T_2 values using Matlap program. We derived the following three specific relaxivities based on different expression of Mn concentration: r_A1 ($mM^{-1}s^{-1}$) based on the molar concentration of manganese atom measured by ICP-AES, r_N1 ($M^{-1}s^{-1}$) based on the number of MnO nanoparticles, and r_S1 ($m{\cdot}s^{-1}$) based on the surface area of MnO nanoparticles. The specific relaxivities were compared with those of Fe_3O_4, $MnO\text{-}SiO_2$ nanoparticles synthesized in the laboratory, and commercial $MnCl_2$.

Distribution in Blood and Urine: The T_1 values of the blood samples of mouse at each time points (at 30-min, 1-hr, 3-hr, 6-hr, 1-day, 3-day, 7-day, 14-day and 24-day after the MnO injection) were measured along with the corresponding Mn contents by ICP-AES analysis. Urine was collected for initial 6 hours after injection to mouse, which was then subjected to T_1 measurements and ICP-AES measurement for estimating the Mn content.

Cytotoxic evaluation of the human cell lines in vitro. The human cancer cell lines (PC-3, U-87MG, MCF7, Huh7, MRC5, NCI H460, HEK 293, and HL-60) were exposed to various concentrations of the MnO nanoparticles (450, 45, 4.5, 0.45, and 0.045 μg/l) under 5% CO2 atmosphere at 37 °C for 72 hours to assess the cell viability by SRB (sulforhodamine B) or WST-8 assay. The IC_{50} was obtained for each cell type by plotting a concentration-effect curve.

Results and Discussion

Uniform water-dispersed MnO nanoparticles: Fig. 1a shows the TEM images of uniform and biocompatible water-dispersed MnO nanoparticles with various sizes. They had the antiferromagnetic property at room temperature, which means that they do not exert the susceptibility artifacts in MRI, contrasting with SPIO-based T_2 agent. Neither T_1 contrast enhancement in MRI, nor the Mn content ICP-AES was observed from all of the supernatants. This indicates that the nanoparticles are highly stable and no appreciable leaching-out occurs from the MnO dispersions. The Mn content of the blood was maximum at 1hr and sharply reduced to the baseline value by 6 hours after injection, which was concordant to the blood T_1 measurement. The Mn content estimated by MRI T_1 measurement and ICP-AES was negligible in the urine sample.

Relaxation properties: As shown in Figure 1b, MnO nanoparticles with particle sizes of 7, 15, 20 and 25 nm at the MnO concentration of 5 mM clearly showed bright signal enhancement in T_1-weighted MRI, showing an ability of the MnO nanoparticles as a T_1 contrast agent for MRI. The MnO nanoparticles shortened both T_1 and T_2 as shown in Table 1. The smaller the size of the nanoparticles is, the brighter the signal is in T_1-weighted MRI, which indicates increasing T_1 shortening effect as the size of the nanoparticles decreases. The r_A1 was higher for the smaller sized MnO nanoparticles, r_N1 was higher for the larger sized, and r_S1 is nearly the same for the various sized nanoparticles at the same concentration. The contrast enhancement seems to be attributed to the Mn^{2+} exposed at the surface of the nanoparticles, which is also confirmed by the MnO size-independent contrast as well as significantly reduced contrast effect observed in $MnO\text{-}SiO_2$ compared to the MnO nanoparticles (Table 2).

In vitro cytotoxic evaluation for human cell lines: No appreciable toxicity with the MnO concentration below 0.82 mM was observed in human normal and cancer cell lines such as lung fibroblast, embryonic kidney, and glioblastoma cells, otherwise 82 μM of Mn concentration in hepatoma, large cell lung cancer, breast adenocarcinoma, prostate adenocarcinoma, and leukemia cells.

Conclusion

This is the first reported nanoparticulated T_1 MRI contrast agent that has adequate specific relaxivities and tolerable cytotoxicity to be used for MRI, which will have significant implications for applications in biomedical research and molecular and cellular imaging. Biocompatibility and water dispersibility in various sized form will expand its application to various organs. The nanoparticulate form will particularly be useful for modifying the surfaces to be labeled with the targeting agents (e.g., disease-specific antibody, (stem) cells, genes, and drugs) for molecular and cellular imaging or with other imaging probes (e.g., fluorescence material for optical imaging) for multi-modality imaging purposes.

Figure 1. TEM and MRI

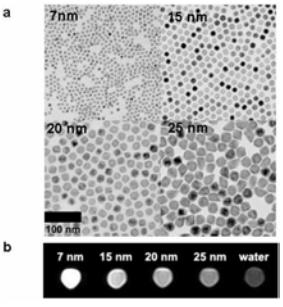

Table 1. Relaxation properties of the MnO nanoparticles.

	Longitudinal Relaxation				Transverse Relaxation			
Size	**T_1†**	**r_A1**	**r_N1**	**r_S1**	**T_2†**	**r_A2**	**r_N2**	**r_S2**
nm	ms	$mM^{-1}s^{-1}$	$\mu M^{-1}s^{-1}$	$m{\cdot}s^{-1}$	ms	$mM^{-1}s^{-1}$	$\mu M^{-1}s^{-1}$	$m{\cdot}s^{-1}$
7	481	0.37	3	33	85	1.74	14	154
15	624	0.18	15	34	95	0.57	46	121
20	707	0.13	25	33	120	0.52	99	102
25	752	0.12	46	39	132	0.44	165	139

†: 5mM

Table 2. T_1 values

Agents	T_1 (ms)	T_2 (ms)
MnO (5mM)	484	94
$MnO\text{-}SiO_2$ (5mM)	1157	130
$MnCl_2$ (0.15mM)	468	39
Fe_3O_4 (0.4mM)	434	74

References

1. Park, J. *et al.* Nature Mater. 3, 891-895 (2004); **2.** Dubertret, B. *et al. Science* 298, 1759-1762 (2002).