Convertible manganese contrast for molecular imaging

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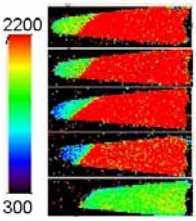
INTRODUCTION: Manganese has a long history as an MRI contrast agent. The paramagnetic Mn^{2+} species has an R_1 ~ 6 mm^{-1} s^{-1}, and gives rise to altered contrast in T_1 weighted imaging sequences. Manganese in the form of an insoluble metal carbonate or metal oxide nanoparticle, however, has little R_1 relaxivity, <0.1 mm^{-1} s^{-1}, with R_2 relaxivity ~ 3 mm^{-1} s^{-1} (1). Additionally, depending on the size of the insoluble particle, there can be significant magnetic susceptibility effects, rendering pixels containing particles dark under T_2 or T_2^* weighted imaging. Solubilization of metal carbonates and oxides is negligible at a physiological pH of 7.0 with solubility products ~10^{-11}. In acidic pH, metal carbonates and oxides dissolve slowly. When cells are labeled with nano- or microparticles for cell labeling, nearly all particles become incorporated in endosomes or lysosomes (2), compartments with pH ~ 5. Thus, we hypothesized that solubilization of inorganic manganese carbonate ($MnCO_3$) and manganese oxide (Mn_3O_4) would occur slowly in the lysosomes of cells. This would result in the conversion of the insoluble manganese particles to ionic Mn^{2+}, which would then lead to T_1 contrast, enabling all of the techniques of manganese enhanced MRI to be accomplished, such as tracing active neural pathways in the brain (3). Furthermore, in the case of neural track tracing, contrast would be converted from dark at its source to bright along an active neural pathway. Here, the use of manganese oxide and carbonate as convertible contrast agents is demonstrated in cultured cells and for neural track tracing in the rodent brain.

MATERIALS AND METHODS: 325 mesh $MnCO_3$ and Mn_3O_4 were manually ground using a mortar and pestle, then used without size sorting. Under electron microscopy, particles were in the size range of 200 nanometers to 5 microns. To investigate the ability of cells to endocytose particles and then dissolve them, $MnCO_3$ in PBS was added to confluent culture dishes of mouse fibroblasts (~ 5 million cells, 10 ml growth medium) at 1650, 165, 33 and 8.25 µg and allowed to incubate 24 hours. Controls received only PBS. To test whether particles could dissolve in growth medium alone, 33 µg of particles were incubated in either growth medium only or PBS only (no cells). Cells were then washed to remove free particles, trypsinized and centrifuged in eppendorf tubes. Cell tubes underwent rapid T_1 mapping at 11.7 T using a multi-slice Look-Locker imaging sequence (4). In plane resolution was 200 microns, with 1 mm slice thickness. To investigate the ability of the dissolved manganese from the particles to trace neural pathways, either $MnCO_3$ or Mn_3O_4 nanoparticles was injected directly into the right somatosensory cortex (S1) of 11, six week old rats at a concentration of 660 µg in 2 µl PBS. Previous reports have demonstrated the ability of brain cells to endocytose free particles, in vivo (5). Rats were returned to the animal facility and imaged ten days later. These animals displayed normal activity over the ten days of housing. T_1 maps were acquired of the entire rat brain using the same multi-slice Look-Locker imaging sequence with 100 micron in plane resolution, 1 mm slice thickness. T_1 maps were calculated in Matlab.

RESULTS: Cells in culture endocytosed $MnCO_3$ nanoparticles following 24 hour incubation, as visualized with light microscopy. Figure 1 shows T_1 maps of the cell pellet and media for the different amounts of added $MnCO_3$. All T_1 measurements are averaged from the entire cell pellet and the entire media above the cell pellets. As cells carrying more particles were heavier, heterogeneous cell pellets were created, with lower T_1 cells at the bottom of the tubes. The T_1 of control cells was 1595 ms and the T_1 of the medium was 2980 ms. With the addition of 8.25 µg of $MnCO_3$, the T_1 of the cells dropped 5% to 1420 ms, with no significant change in the T_1 of the media. Increasing the quantity of $MnCO_3$ to 33 µg resulted in a 28.5% decrease in T_1, with an 11% decrease in the T_1 of the media. Further increasing the $MnCO_3$ amount to 165 µg resulted in a 52% decrease in the T_1 of the cells, with a 14% decrease in the media T_1. 1650 µg resulted in undetectable signal for the cells, presumably due to very short T_2 relaxation times, and a 50% drop in the T_1 of the media. The T_1 of the media and PBS incubated with particles were indifferent from control media and PBS without particle incubation, meaning that particles did not dissolve on their own. Animals which had been injected with $MnCO_3$ and Mn_3O_4 particles into S1 ten days prior to MRI displayed marked T_1decreases in the injected side of the brain. Injection sites were dark due to short T_2^* effects caused by the particles. In 8 out of 11 animals, marked decreases in T_1 were observed in the thalamus ipsilateral to the injection, as expected. These T_1 decreases averaged 155 ms and were present in both $MnCO_3$ and Mn_3O_4 injected animals. The standard deviation of T_1 values for the left thalamus was 37 ms, making the differences observed in the right thalamus significant. For the 3 animals where contrast was not observed in the thalamus, the injection site was not observed either, meaning the initial injection of particles failed. In one $MnCO_3$ injected animal, contrast was observed not only in the thalamus, but also in the secondary somatosensory cortex (Figure 2).

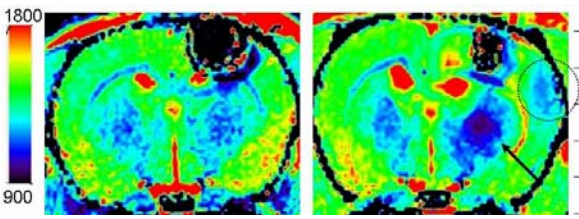
DISCUSSION: There is interest in creating switchable or activatable MRI contrast agents that respond to changes in cell physiology. Here we describe the use of inorganic $MnCO_3$ and Mn_3O_4 as convertible MRI contrast agents, that is, agents that can be converted from low R_1 relaxivity, high R_2^* relaxivity agents, to high R_1 relaxivity, low R_2^* relaxivity agents. As an insoluble nanoparticle, the inorganic manganese particles have low R_1 relaxivity and exhibit significant R_2^* relaxivity. We have demonstrated that cells can endocytose inorganic manganese based particles and can dissolve them to form ionic Mn^{2+}. Solubilization only occurred upon internalization of particles into low pH compartments of the cell, such as endosomes and lysosomes. The resultant Mn^{2+} species had high R_1 relaxivity and caused cells to turn bright on T_1 weighted imaging sequences after 24 hour incubation with the particles (Figure 1). Additionally, the decomposition of the nanoparticle decreases the susceptibility induced R_2^* relaxivity. A similar gadolinium based contrast agent that relies on low pH has been reported (6). The decrease in the T_1 of the medium indicated that some manganese leaked into the media, likely due to cell death. However, this change was minimal for the lower particle amounts. We also demonstrated that the converted manganese could be transported by neurons in the rat brain in tract tracing experiments. Ten days after injection of manganese particles into S1 of rats, substantial T_1 decreases were observed in the thalamus and in one case, secondary somatosensory cortex. Thalamus and secondary somatosensory cortex are known anterograde targets of S1. In all animals, the area of dark contrast in S1 was decreased ten days later, further demonstrating the decomposition of the original, susceptibility generating nanoparticles. It is unlikely that particles dissolved outside of cells in CSF, as the T_1 of both growth medium and PBS following 24 hour incubation with only particles were identical to control media and PBS incubated without particles. An important result is that the manganese enhanced effect was robust even ten days after administration. Most manganese tract tracing experiments lose signal after only a few days (3) which demonstrates the potential of having a slow release form of manganese for track tracing experiments. Advances in particle coating and composition can improve bioavailability, cellular targeting and toxicity.

References: 1) Wisner, et al, Acad Radiol, 2, 140-7 (1995); 2) Shapiro, et al, MRM, 53, 329-38 (2005); 3) Lee and Koretsky, Curr Pharm Biotechnol, 5, 529-37 (2004); 4) Chuang and Koretsky, MRM 55, 604-11 (2006); 5) Shapiro, et al, Neuroimage, 32, 1150-7 (2006); 6) Himmelreich, et al, Neuroimage, 32, 1142-9 (2006).



µg $MnCO_3$	T_1 cells	T_1 medium
0	1595	2980
8.25	1420	3030
33	1140	2695
165	925	2580
1650	X	1520

Figure 1: T_1 maps of cell pellets and media for different amounts of added $MnCO_3$. Slight heterogeneity was observed in the cell pellets, likely due to differential sedimentation of cells with more internalized particles. Scale bar and all T_1 measurements are in milliseconds.



Location	T=0	T=10 days
S2 (R)	1390	1250
S2 (L)	1425	1469
Thal (R)	1147	1060
Thal (L)	1161	1205
CSF	2950	3030

Figure 2: T_1 maps of rat brain immediately after (left) and ten days after (right) injection of 660 micrograms $MnCO_3$ into right somatosensory cortex. Note the highly decreased T_1 of the ipsilateral thalamus (black arrow) ten days after injection, as well as decreased T_1 in the secondary somatosensory cortex (dotted circle). The T_1 of the contralateral side, as well as that of CSF, were nearly unchanged. Scale bar and all T_1 measurements are in milliseconds.