In Vitro Cell Labeling for Manganese Enhanced Magnetic Resonance Imaging

I. Aoki[1,2], Y. Takahashi[3], K-H. Chuang[1], T. Igarashi[3], A. C. Silva[1], C. Tanaka[2], R. W. Childs[3], A. P. Koretsky[1]

[1]Laboratory of Functional and Molecular Imaging, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, United States, [2]Department of Medical Informatics, Meiji University of Oriental Medicine, Hiyoshi, Kyoto, Japan, [3]Hematology Branch, National Heart, Lung and Blood Institute, NIH, Bethesda, MD, United States

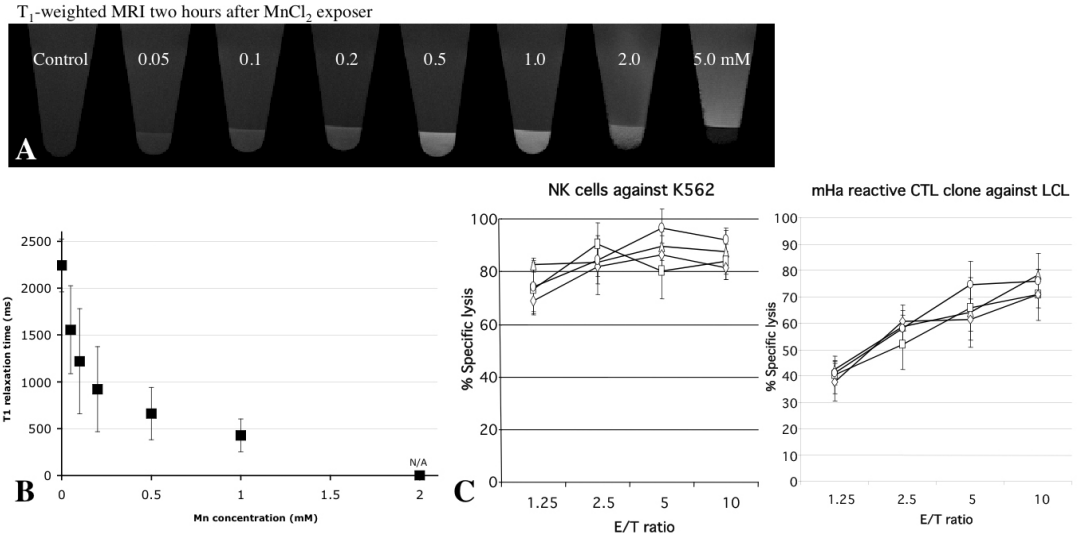
Introduction

Labeling isolated cells in vitro with dextran-coated iron oxide particles has been used successfully to track a wide variety of cells when re-introduced into an animal by MRI (1, 2). The ability of adoptively infused tumor specific T-cells and NK cells to traffic to the tumor microenvironment may be a critical determinant of their therapeutic efficacy (3). Non-invasive tracking of tumor antigen specific T cells could have additional diagnostic value, allowing for the detection of metastasis not previously identified by other radiographic modalities. Manganese chloride ($MnCl_2$) alters signal intensities due to changes in T_1 relaxation time on MRI, thus providing increased signal as apposed to iron oxide, which enhances by T_2 mechanisms and leads to signal loss. Mn^{2+} is an essential heavy metal that is known to readily enter most cells. Therefore, we tested the hypothesis that peripheral blood lymphocytes (PBL) and EBV transformed B-cells (EBV-LCL) would label with $MnCl_2$ to a level that would allow their detection by MRI. Further, we evaluated whether Mn^{2+} caused any toxicity or inhibition of function of antigen-specific T cells and K562-cytotoxic NK cell populations.

Materials and Methods

Following incubation for an hour with 0.05 - 5.0 mM $MnCl_2$ solution, cell viability and the % of apoptotic cells were measured by flow cytometry after staining with Annexin V and Propidium iodide. MRI acquisitions were performed in an 11.7-Tesla, 31 cm bore magnet (Magnex Scientific Ltd., UK) interfaced to a Bruker Avance console (Bruker Medical GmbH, Germany). Pelleted $MnCl_2$ labeled cells in saline were placed in a 27 mm diameter bird-cage coil (Bruker). Conventional spin-echo (TR/TE = 300/7.5 msec), inversion recovery (TR/TE/TI = 16000/5.7/10, 300, 600, 1000, 1500, 2000, 3000, 4000 msec), and multi-echo RARE (TR/TE = 12000/10, 20, 30, 40, 50, 60, 70, 80 msec) were obtained for generating T_1 weighted MRI, T_1 maps, and T_2 maps, respectively. Two and twenty four hours after $MnCl_2$ labeling, NK cells cytotoxic to K562 cells and a CD3+ T-cell clone specific for a minor histocompatibility antigen (mHa) were evaluated by Cr51 release for cytotoxicity against their relevant targets. All statistical analyses were performed using StatView (SAS Institute). A probability value of less than 0.05 was considered significant for each analysis.

Results and Discussion



There were three major findings in this study: 1) Significant signal enhancement was observed in normal human peripheral blood lymphocytes (Fig. A) and EBV-LCL 2 and 24 hours after incubation with 0.05-1.0 mM $MnCl_2$ on the T_1-weighted MRI. In addition, both T_1 and T_2 relaxation times were decreased significantly depending on the concentration of the manganese solution, although T_1 decreased more rapidly (Fig. C). Signal intensities of T_1-weighted MRI gradually increased with higher concentrations of $MnCl_2$. These results indicate that $MnCl_2$ allowed MRI cell visualization by functioning as a T_1 contrast agent. 2) No apoptosis or necrosis of lymphocytes was observed up to 0.5 mM $MnCl_2$. 3) The killer activity of both NK cells and cytotoxic T-cells were not significantly affected up to the 0.5 mM $MnCl_2$ concentration (Fig. C). In conclusion, this is the first report to describe the use of $MnCl_2$ to label lymphocytes to a level sufficient for their detection by MRI. There were no toxic effects of labeling cells for an hour with up to 0.5mM $MnCl_2$. Animal studies testing whether $MnCl_2$ labelled tumor-specific T cells can be tracked in vivo by MRI will be pursued.

References

1. Yeh TC, Zhang W, Ildstad ST, Ho C., Intracellular labeling of T-cells with superparamagnetic contrast agents., Magn Reson Med. 1993 Nov;30(5):617-25.
2. Bulte, J., Duncan, I., and Frank, J. In vivo magnetic resonance tracking of magnetically labeled cells after transplantation. J. Cereb. Blood flow Metab. 22, 899-907.
3. Childs R, Chernoff A, Contentin N, Bahceci E, Schrump D, Leitman S, Read EJ, Tisdale J, Dunbar C, Linehan WM, Young NS, Barrett AJ. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N Engl J Med. 2000 Sep 14;343(11):750-8.