NMR IN BIOMEDICINE
NMR Biomed. 2004;**17**:527–531
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/nbm.940

Editorial

Manganese-enhanced magnetic resonance imaging (MEMRI)

Alan P. Koretsky* and Afonso C. Silva

Laboratory of Functional and Molecular Imaging, National Institutes of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA

Received 3 October 2004; Revised 6 October 2004; Accepted 6 October 2004

ABSTRACT: Manganese ion (Mn^{2+}) is an essential metal that participates as a cofactor in a number of critical biological functions, such as electron transport, detoxification of free radicals and synthesis of neurotransmitters. Mn^{2+} can enter excitable cells using some of the same transport systems as Ca^{2+} and it can bind to a number of intracellular sites because it has high affinity for Ca^{2+} and Mg^{2+} binding sites on proteins and nucleic acids. Paramagnetic forms of manganese ions are potent MRI relaxation agents. Indeed, Mn^{2+} was the first contrast agent proposed for use in MRI. Recently, there has been renewed interest in combining the strong MRI relaxation effects of Mn^{2+} with its unique biology, in order to further expand the already broad assortment of useful information that can be measured by MRI. Such an approach has been continuously developed in the past several years to provide unique tissue contrast, to assess tissue viability, to act as a surrogate marker of calcium influx into cells and to trace neuronal connections. This special issue of *NMR in Biomedicine* on manganese-enhanced MRI (MEMRI) is aimed at providing the readers of this journal with an extensive review of some of the most prominent applications of MEMRI in biological systems. Written by several of the leaders in the field, the reviews and original research articles featured in this special issue are likely to offer an exciting and inspiring view of the broad range of applications of MEMRI. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: manganese; imaging; brain; heart; neuronal-tract tracer; neuroarchitecture; contrast agents

BRIEF HISTORY OF MANGANESE AND MAGNETIC RESONANCE

Divalent manganese ion (Mn^{2+}) has played an important role in the history of magnetic resonance. In nearly every case, the principles used in a range of early experiments that relied on Mn^{2+} still rule in present day biological NMR and MRI. Very early after the first successful liquid phase NMR experiments, it became clear that NMR spectral properties were extremely sensitive to exchange processes that could not be measured using other techniques.[1] Elegant experiments that measured the rate of water exchange from the first coordination sphere of paramagnetic ions used Mn^{2+} among other ions.[2] The basis of these experiments was the modulation of the T_2 relaxation of water that occurred due to the unpaired electrons on Mn^{2+} by the exchange rate of water into and out of the first coordination sphere of Mn^{2+}. These early experiments laid much of the foundation for present thinking about the effects of water exchange that guides the design more effective MRI contrast agents.[3]

As magnetic resonance moved from a major chemistry tool to one that could begin to impact biological sciences, once again Mn^{2+} played a critical role. In some of the earliest experiments that enabled quantitative structural information to be obtained from biological molecules, the paramagnetic relaxation enhancement effect of Mn^{2+} binding to nucleic acids or to proteins and their substrates was used to begin to study macromolecular structure with NMR.[4,5] Such enhancement effect was induced by strong dipole–dipole interactions between paramagnetic Mn^{2+} and the specific NMR detectable atom of interest in the biomolecule. The use of dipole–dipole interactions to gauge distances is one of the foundations for present day determination of protein structure by NMR.[6]

The ability of Mn^{2+} to alter water relaxation properties and have exchange influence relaxation times was used to make some of the most unambiguous and quantitative measurements of water exchange through the membrane of an intact cell, the erythrocyte.[7] Mn^{2+} was added to the extracellular space to alter the T_2 of water outside cells with respect to water inside cells, enabling exchange rates to be calculated from T_2 measurements. Today, this thinking is being used in MRI experiments designed to

**Correspondence to:* A. P. Koretsky, Laboratory of Functional and Molecular Imaging, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 10 Center Drive Building 10, Room B1D728, Bethesda, MD 20892-1065, USA.
E-mail: KoretskyA@ninds.nih.gov

Abbreviations used: MEMRI, manganese-enhanced MRI.

measure water exchange between different compartments in tissues *in vivo* with MRI and a variety of relaxation agents.[8] A final example is that binding of Mn^{2+} to particular biological molecules has enabled NMR detection of specific compartmentalization of molecules in biological structures, allowing, for example, the study of phospholipid asymmetry in membranes[9] and the compartmentalization of phosphates in yeast.[10] In all of these cases, the indirect detection of the presence of Mn^{2+} via its relaxation effects on NMR signals yielded useful new insights.

The impact of Mn^{2+} as a contrast agent can be found at the earliest stage of MRI. In 1973, Paul Lauterbur published the seminal paper entitled, 'Image formation by induced local interactions: examples employing nuclear magnetic resonance'.[11] This paper laid down the basic strategy that has evolved into modern MRI. The notion to image water distribution at first seemed to be limited, because water density only varies by a small degree in biological tissues. However, there was some evidence already that the magnetic resonance relaxation times of water were different in different tissues, and that those might be altered by pathology.[12] To demonstrate that relaxation times could affect the intensity of his zeugmatographs, Lauterbur used a paramagnetic ion to alter the longitudinal relaxation time of water.[11] Thus, Lauterbur demonstrated not only the feasibility of MRI, but also a strategy to alter contrast with exogenous agents. The agent he chose was $MnSO_4$ thus, the manganese ion was the first MRI contrast agent and many of the issues associated with relaxation in complex biological media were worked out, inspired by understanding relaxation effects of Mn^{2+} in blood and tissue.[13,14]

Problems with toxicity slowed the development of Mn^{2+} as a useful MRI contrast agent.[15] However, interest in Mn^{2+} in MRI has been sustained for three reasons. First, because manganese exposure is toxic and causes neurological deficits there is an active literature using MRI to localize sites of accumulation of Mn^{2+} in the brain.[16,17] The second reason for continued interest in Mn^{2+} in MRI is due to the development of an FDA approved, chelated Mn^{2+} contrast agent, manganese dipyridoxaldiphosphate (MnDPDP) for application to liver and other organs.[18] The Mn^{2+} is released from this chelate due to trans-metallation with zinc and some of the contrast detected in liver, pancreas, and heart is probably due to enhancement from the released Mn^{2+} opening up interesting possibilities for using MnDPDP as a 'slow release' agent of Mn^{2+}. The third reason is the renewed interest in using the biological properties of Mn^{2+} to guide new types of MEMRI experiments. Indeed it is this third area that is the major focus of this special issue. Figure 1 plots the number of references that were found on a PUBMED literature search using 'manganese' and 'MRI' as keywords. This search is not inclusive of all publications, yet it clearly demonstrates a continuing and growing interest in the possibilities of MRI enhancement with Mn^{2+}, indicating that this is a good time for a special issue on MEMRI!

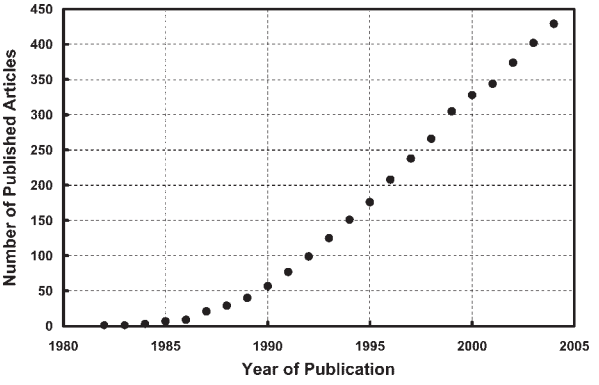

Figure 1. Cumulative number of published articles related to Mn^{2+} and MRI. The data was obtained by performing a search on the PUBMED database using the keywords 'manganese' and 'magnetic resonance imaging'. Search performed on 6 October 2004

OVERVIEW OF THE SPECIAL ISSUE

This special issue of *NMR in Biomedicine* on manganese-enhanced MRI (MEMRI) is due to a recent resurgence of interest in the use of Mn^{2+} as an MRI contrast agent. The search for strategies to get more detailed physiological, biochemical and molecular biological information from MRI has helped motivate this interest. This along with the rise in importance of animal imaging to enable the easy exploration of the possibilities of a broad range of contrast strategies is fueling the rise of molecular imaging in the radiological imaging community. Mn^{2+} is an essential heavy metal playing a key role as cofactor in a number of important enzymes such as manganese superoxide dismutase and glutamine synthetase.[19,20] A rich biology has evolved to absorb and transport Mn^{2+}, much of which is just beginning to be understood at a molecular level. To date three uses of $MnCl_2$ for MEMRI have been developed. As a tissue contrast agent after systemic administration, as a surrogate marker for calcium influx and as a non-invasive neuronal track tracer. The purpose of this special issue is to provide a broad review of some of these most prominent applications of MEMRI in biological systems. The issue is composed of seven review papers, and two original research articles. Written by several of the leaders in the field, these reviews and original research were organized to present a clear and systematic explanation of the development, approaches, issues and applications associated with MEMRI as a tool to characterize biological systems.

One of the first questions that come to mind when one decides to learn and apply MEMRI in his/her own research is 'How to do it?' Silva *et al.* review a number of issues associated with optimization of MEMRI. The

ever increasing sensitivity and stability of MRI systems has been of fundamental help in the detection of relaxation effects of Mn^{2+} in organs. Significant attention needs to be given to the route and dose of administration, which vary with the major experimental goal of the study.

A major drawback to the use of Mn^{2+} as a contrast agent is its cellular toxicity, and thus a critical issue for the development of MEMRI for use in humans is to use as low a dose as possible. Mn^{2+} is an essential heavy metal and we require a daily intake. However, it is well known that chronic exposure to manganese leads to a neurological disorder resembling Parkinson's disease. Indeed, toxic effects in animals discouraged the early development of Mn^{2+} as an MRI contrast agent.[15] Crossgrove and Zhang write a timely essay on the toxicology of Mn^{2+} upon overexposure.

There have been three major classes of applications of MEMRI reported in the past several years. One exciting application of MEMRI is to use Mn^{2+} as a contrast agent to better define tissue architecture. Owing to the toxic effects of manganese there have been many MRI studies of brain that have used relaxation changes to monitor the distribution of Mn^{2+} in the animal and human brain after acute and chronic administration.[16,17] Such work has demonstrated the potential of Mn^{2+} to give detailed views of brain structures. Rather than use MRI to follow distribution of Mn^{2+} after systemic administration, recent work has demonstrated that detailed cytoarchitecture can be obtained from the rodent brain, not only to identify specific regions of the brain, such as amygdala, but also contrast can be achieved that enables detection of neuronal cell layers in olfactory bulb, hippocampus, cerebellum and cortex.[21–23] Watanabe *et al*. contribute a paper reviewing their recent work in using MEMRI to visualize neuronal architecture in the rodent brain, with particular emphasis on the morphology and dynamics of Mn^{2+}-induced MRI signal enhancements, as well as on the physiological mechanisms underlying cerebral Mn^{2+} uptake and distribution.

A second major class of applications of MEMRI takes advantage of Mn^{2+} as a biological calcium analog to study increases in local brain or cardiac function. Functional increases in calcium influx in either the brain or the heart lead to a concomitant increase in the local concentration of Mn^{2+} that leads to specific MRI signal enhancement in the area of activation, thus providing a functional MRI method able to map tissue activation *in vivo* independently of the surrogate hemodynamic changes. When the ability of Mn^{2+} to accumulate in active regions of the brain was first demonstrated it was referred to as activity-induced manganese-enhanced MRI (AIM-MRI).[24] Work in heart since the earliest days of development of MEMRI indicates than changes in Mn^{2+} influx in cardiac tissue may be an excellent marker of cell viability and ionotropic state of the heart.[25–29] Aoki *et al.* describe recent experiments to use AIM MRI to map functional neuronal activity in the rodent brain and Michael Wendland reviews his group's efforts to develop $MnCl_2$ and MnDPDP as markers of cell viability during ischemia, giving particular emphasis to the hypothesis that Mn^{2+} uptake can be interpreted in terms of cardiac function.

A third class of experiments performed with MEMRI exploits the fact that once inside cells in a specific brain region, Mn^{2+} will move along appropriate neuronal pathways in an anterograde direction. This property of Mn^{2+} was used by Pautler *et al*. to develop an MRI approach to trace neuronal projections.[30] Such MEMRI based tracing of neuronal tracts *in vivo* has constituted an exciting application of Mn^{2+} in MRI, and two contributions demonstrate the potential of this approach. One by Robia Pautler details some of the current MEMRI tract-tracing methodologies and major biological applications of MEMRI tract-tracing. The other contribution, by Van der Linden *et al.*, describes the elegant MEMRI experiments her group has performed to map the connections of the song centers of songbirds, and to monitor and study the seasonal plasticity of these regions.

Two original research articles close this special issue of NMR in Biomedicine. The first of these articles, by Wadghiri *et al.* presents data on the use of MEMRI to study early brain development (postnatal days 2–15) of genetically-modified mice, with emphasis on patterning of the mouse cerebellum. The second research article, by Hu *et al.*, addresses the use of MEMRI as a tool to study the myocardium in a mouse model of permanent occlusion of the coronary artery.

CONCLUSION

Manganese has always played an important role in magnetic resonance, with early applications in chemistry that laid the foundation for extensions to biological systems. Presently there are three major ways to productively use MEMRI with $MnCl_2$. First, simple systemic administration of Mn^{2+} leads to interesting and useful anatomical MRI contrast. The accumulation of Mn^{2+} is enabling analysis of anatomical structures by MRI that would otherwise be difficult to detect. The biological basis for the movement of Mn^{2+} into tissues and its final distribution needs to be more fully determined and this may lead to opportunities for new imaging strategies. Second, one well established way for Mn^{2+} to enter cells is on voltage-gated calcium channels. This has enabled work with AIM-MRI to probe activity in brain and heart and the general strategy should be useful for a number of other tissues. Further work needs to be performed to clarify exactly which channels Mn^{2+} can move through to enable AIM-MRI to become a quantitative surrogate of calcium influx. There are no widely used noninvasive imaging techniques that monitor the influx of this important second messenger and therefore, there are many opportunities to study the quantitative control of

Ca^{2+} influx in intact functioning tissues. Third is the ability of MEMRI to trace neuronal connections, which is opening up numerous possibilities for non-invasively imaging of functionally specific neural networks. This should enable changes in the brain of an individual animal to be studied before and after a broad range of perturbations such as learning, plasticity, injury and repair. The combination of the ability to control the accumulation of Mn^{2+} in one region of the brain based on activity and then image the connections from that area should open novel strategies to study functional connectivity in the brain with MEMRI.[31]

A major challenge for the further development of MEMRI is to increase detection sensitivity, so that lower doses of Mn^{2+} can be used. Presently the doses used in animals are higher than what can be used in humans. Most of the intensity changes being used in MEMRI experiments are on the order of 50–100%. Functional MRI experiments, so widely used to map activity in the brain, routinely rely on 2–5% changes in MRI intensities. Increasing sensitivity to Mn^{2+} to this level will make the broad range of information available from MEMRI in animal models available to help diagnosis, stage, and determine treatment efficacy for human disease. In the meantime the articles presented here clearly demonstrate the growing range of exciting biology that is now accessible to MRI due to MEMRI.

Acknowledgments

The authors are especially grateful to the Editorial Advisory Board of *NMR in Biomedicine*, and in particular to Professor Mathias Hoehn, for their kind invitation to edit this Special Issue of *NMR in Biomedicine*. Many thanks to Professor John R. Griffiths, Editor-in-Chief, and to Professor Chris Boesch, Reviews Editor, for their trust in our amateur editorial skills. Special recognition goes to all the authors of the articles featured in this issue, for their generous commitment to contribute, and for their careful observation of all the deadlines and specific requirements. Support for the work comes from the Intramural Research Program of the National Institute for Neurological Disorders and Stroke at NIH, directed by Drs Eugene Major and Henry McFarland.

REFERENCES

1. Johnson CS. Chemical rate processes and magnetic resonance. *Adv. Magn. Res.* 1965; **1**: 33–102.
2. Connick RE, Poulson RE. Effect of paramagnetic ions on the nuclear magnetic resonance of O-17 in water and the rate of elimination of water from molecules in the first coordination sphere of cations. *J. Chem. Phys.* 1959; **30**: 759–761.
3. Lauffer RB. Magnetic resonance contrast media: principles and progress. *Magn. Reson. Q.* 1990; **6**: 65–84.
4. Mildvan AS, Cohn M. Magnetic resonance studies of the interaction of the manganous ion with bovine serum albumin. *Biochemistry* 1963; **338**: 910–919.
5. Eisinger J, Fawaz-Estrup F, Shulman RG. Binding of Mn^{2+} to nucleic acids. *J. Chem. Phys.* 1965; **42**: 43–53.
6. Wuthrich K. NMR studies of structure and function of biological macromolecules (Nobel Lecture). *J. Biomol. NMR* 2003; **27**: 13–39.
7. Fabry ME, Eisenstadt M. Water exchange between red cells and plasma. Measurement by nuclear magnetic relaxation. *Biophys. J.* 1975; **15**: 1101–1110.
8. Yankeelov TE, Rooney WD, Li X, Springer CS Jr. Variation of the relaxographic 'shutter-speed' for transcytolemmal water exchange affects the CR bolus-tracking curve shape. *Magn. Reson. Med.* 2003; **50**: 1151–1169.
9. Michaelson DM, Horwitz AF, Klein MP. Transbilayer asymmetry and surface homogeneity of mixed phospholipids in cosonicated vesicles. *Biochemistry* 1973; **12**: 2637–2645.
10. Castro CD, Koretsky AP, Domach MM. Performance trade-offs in *in situ* chemostat NMR. *Biotechnol. Prog.* 1999; **15**: 185–195.
11. Lauterbur PC. Image formation by induced local interactions: examples employing nuclear magnetic resonance. *Nature* 1973; **242**: 190–191.
12. Damadian R. Tumor detection by nuclear magnetic resonance. *Science* 1971; **171**: 1151–1153.
13. Lauterbur PC, Mendonca-Dias M, Rudin A. Augmentation of tissue water proton spin-lattice relaxation rates by *in vivo* addition of paramagnetic ions. In *Frontiers of Biological Energetics*, Dutton P, Leigh JS, Scarpa A (eds). Academic Press: New York, 1978; 752–759.
14. Kang YS, Gore JC. Studies of tissue NMR relaxation enhancement by manganese. Dose and time dependences. *Invest. Radiol.* 1984; **19**: 399–407.
15. Wolf GL, Baum L. Cardiovascular toxicity and tissue proton T_1 response to manganese injection in the dog and rabbit. *AJR Am. J. Roentgenol.* 1983; **141**: 193–197.
16. London RE, Toney G, Gabel SA, Funk A. Magnetic resonance imaging studies of the brains of anesthetized rats treated with manganese chloride. *Brain Res. Bull.* 1989; **23**: 229–235.
17. Lucchini R, Albini E, Placidi D, Gasparotti R, Pigozzi MG, Montani G, Alessio L. Brain magnetic resonance imaging and manganese exposure. *Neurotoxicology* 2000; **21**: 769–775.
18. Federle M, Chezmar J, Rubin DL, Weinreb J, Freeny P, Schmiedl UP, Brown JJ, Borrello JA, Lee JK, Semelka RC, Mattrey R, Dachman AH, Saini S, Harms SE, Mitchell DG, Anderson MW, Halford HH, III, Bennett WF, Young SW, Rifkin M, Gay SB, Ballerini R, Sherwin PF, Robison RO. Efficacy and safety of mangafodipir trisodium (MnDPDP) injection for hepatic MRI in adults: results of the U.S. Multicenter phase III clinical trials. Efficacy of early imaging. *J. Magn. Reson. Imag.* 2000; **12**: 689–701.
19. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.* 1995; **11**: 376–381.
20. Wedler FC, Denman RB. Glutamine synthetase: the major Mn(II) enzyme in mammalian brain. *Curr. Top. Cell Regul.* 1984; **24**: 153–169.
21. Lin YJ. MRI of the rat and mouse brain after systemic administration of $MnCl_2$. Ph.D. Dissertation, Carnegie Mellon University, Pittsburgh, PA, 1997; 1–149.
22. Watanabe T, Natt O, Boretius S, Frahm J, Michaelis T. *In vivo* 3D MRI staining of mouse brain after subcutaneous application of $MnCl_2$. *Magn. Reson. Med.* 2002; **48**: 852–859.
23. Aoki I, Wu YJ, Silva AC, Lynch RM, Koretsky AP. *In vivo* detection of neuroarchitecture in the rodent brain using manganese-enhanced MRI. *Neuroimage* 2004; **22**: 1046–1059.
24. Lin YJ, Koretsky AP. Manganese ion enhances T_1-weighted MRI during brain activation: an approach to direct imaging of brain function. *Magn. Reson. Med.* 1997; **38**: 378–388.
25. Hollis DP, Nunnally RL, Jacobus WE, Taylor GJ. Detection of regional ischemia in perfused beating hearts by phosphorus nuclear magnetic resonance. *Biochem. Biophys. Res. Commun.* 1977; **75**: 1086–1091.
26. Brady TJ, Goldman MR, Pykett IL, Buonanno FS, Kistler JP, Newhouse JH, Burt CT, Hinshaw WS, Pohost GM. Proton nuclear magnetic resonance imaging of regionally ischemic canine hearts: effect of paramagnetic proton signal enhancement. *Radiology* 1982; **144**: 343–347.

27. Wendland MF, Saeed M, Lund G, Higgins CB. Contrast-enhanced MRI for quantification of myocardial viability. *J. Magn. Reson. Imag.* 1999; **10**: 694–702.
28. Brurok H, Skoglund T, Berg K, Skarra S, Karlsson JO, Jynge P. Myocardial manganese elevation and proton relaxivity enhancement with manganese dipyridoxyl diphosphate. *Ex vivo* assessments in normally perfused and ischemic guinea pig hearts. *NMR Biomed.* 1999; **12**: 364–372.
29. Hu TC, Pautler RG, MacGowan GA, Koretsky AP. Manganese-enhanced MRI of mouse heart during changes in inotropy. *Magn. Reson. Med.* 2001; **46**: 884–890.
30. Pautler RG, Silva AC, Koretsky AP. *In vivo* neuronal tract tracing using manganese-enhanced magnetic resonance imaging. *Magn. Reson. Med.* 1998; **40**: 740–748.
31. Pautler RG, Koretsky AP. Tracing odor-induced activation in the olfactory bulbs of mice using manganese-enhanced magnetic resonance imaging. *Neuroimage* 2002; **16**: 441–448.

