Spatial Mapping of Mineralization with Manganese-Enhanced Magnetic Resonance Microscopy

I. E. Chesnick[1], J. A. Centeno[2], T. I. Todorov[3], and K. Potter[1]

[1]Department of Biophysics, Armed Forces Institute of Pathology, Rockville, MD, United States, [2]Department of Environmental & Infectious Disease Sciences, Armed Forces Institute of Pathology, Washington, DC, United States, [3]Crustal Imaging and Characterization Team, US Geological Survey, Denver, CO, United States

Introduction: It is hypothesized that during intramembranous bone formation, calcium is taken up by osteoblasts through calcium channels, concentrated and excreted within matrix vesicles, and transported to the mineralizing front (1). By employing paramagnetic manganese, a calcium surrogate, the MRM technique can be sensitized to the unique changes in the calcium microenvironment during the mineralization process. In earlier studies, we used high doses of manganese (1 mM) and chronic exposure to establish that osteoblasts take up manganese, and organ-cultured calvariae can process the manganese present in the culture medium into mineralized deposits (2). The putative toxicity of high doses of manganese precludes its use in studying the mineralization process in depth. For this reason, we decided to (i) study the effect of low manganese doses on cell viability and (ii) examine whether low doses are sufficient to cause notable changes in T1 values compared to control cell pellets, and (iii) determine if organ-cultured calvariae, treated with a low dose of manganese (100 μM) for just 24 hours, can sequester sufficient manganese from the culture medium to after measured MRM parameters compared to untreated specimens.

Experimental: For our cell studies, primary osteoblasts were grown to confluence and then treated with 0, 25, 100, 250, 500, 1000 μM $MnCl_2$ for 48 hours. Cell pellets were subsequently subjected to MRM and inductively coupled plasma mass spectrometry (ICP-MS), and cell viability was measured with a MTT assay. For our mineralization studies, calvariae from 16-day chick embryos were treated with 100 μM $MnCl_2$, 3 mM $CaCl_2$, and 1% β-glycerophosphate for 24 hours and MRM images were acquired 48 hours after manganese treatment. Manganese-treated and control calvariae were imaged between two glass plates positioned in a tube filled with tissue culture medium. High-resolution MRM images, with a nominal in-plane resolution of 109 μm, were acquired on a Bruker DMX spectrometer at 9.4T at 37ºC. The following measurements were performed on calvarial specimens: water proton longitudinal (T1) and transverse (T2) relaxation times, and the magnetization transfer ratio (MTR). The accumulation of manganese in organ-cultured calvariae was verified with ICP-MS.

Results and Discussion: There was a steady reduction in water proton T1 values compared to the control cell pellet as the manganese dose was increased. This decline in T1 was attributed to increasing levels of intracellular manganese with higher doses, which was confirmed by ICP-MS. Notably, T2 and MTR values were virtually unaffected by the presence of intracellular manganese for manganese doses below 100 μM, but were significantly reduced at higher doses. At higher doses, cell viability was markedly reduced. For calvariae treated with 100 μM $MnCl_2$ for just 24 hours, the manganese content was sufficient to cause a reduction in the water proton T1 (A), T2 (B), and MTR (C) values compared to the untreated specimen shown in Figure 1. This result was consistent with the inevitable uptake of manganese by the osteoblasts and the processing of manganese into mineral. We are currently exploring the use of manganese to dynamically map those locations with the highest mineralizing activity and to explore the different calcium environments necessary for the production of bone.

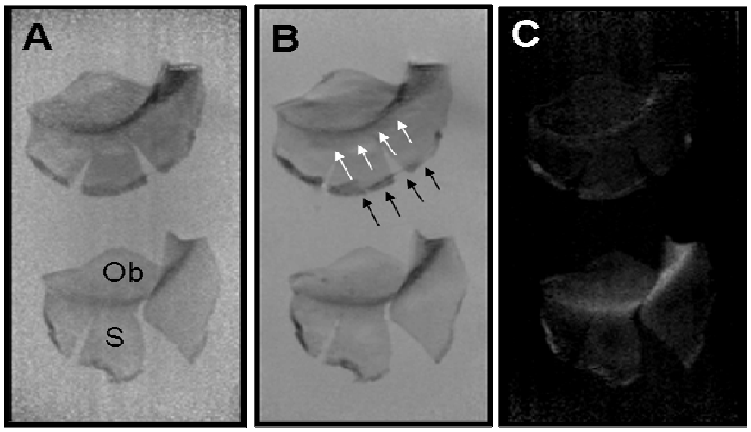

Figure1. Quantitative T1 (A), T2 (B), and MTR (C) maps acquired for organ-cultured calvaria maintained in culture medium supplemented with 100 μM $MnCl_2$, 3 mM $CaCl_2$, and 1% β-glycerophosphate for 24 hours. MRM images were acquired with a nominal in-plane resolution of 109 μm. The calvarial ridge (white arrows), mid-line suture (black arrows), and the superior (S) and orbital (Ob) regions of the calvaria are indicated.

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References: 1.Anderson, H.C., Lab Invest (1989) **60:**320-30. 2.Chesnick, I.E., et al., Magn Reson Imaging (in press).