Screening for manganese-binding proteins in the mouse brain

J. A. Gleave[1], and B. J. Nieman[1]
[1]Mouse Imaging Centre, Toronto, Ontario, Canada

Introduction: Manganese enhanced magnetic resonance imaging (MEMRI) is used in small animals due to its unique T_1 contrast. Systemic administration of Mn^{2+} results in signal enhancement in the hippocampus, pituitary gland, olfactory bulb, and cerebellum[1]. The paramagnetic Mn^{2+} ions enter excitable cells via voltage-gated calcium channels, much like Ca^{2+}, and once inside the cells appear to be retained through interactions with proteins that sequester other divalent ions[2]. Identifying the proteins that Mn^{2+} interacts with intracellularly will increase our understanding of the mechanisms involved in MEMRI and may suggest candidate Mn^{2+}-based reporter genes.

Purpose: To develop a screen for Mn^{2+}-binding proteins in the mouse brain that may be responsible for the T_1 contrast seen in MEMRI we combined methods of native-protein gel electrophoresis and MEMRI to isolate proteins from tissue extracts.

Methods: *Sample preparation:* Protein was extracted from the hippocampus, cerebellum, olfactory bulb, pituitary gland, and cortex of male C57BL/6J mice, 30 days post-natal. Extracted tissue was frozen at -20°C until use. 12µg of extracted protein was run on 4-20% native tris-glycine gradient gels (Lonza) at 125V for 100 min with NativeMark™ unstained protein standard (Invitrogen). After the electrophoresis was complete, gels were soaked in a 0.24mM $MnCl_2$ solution for 10 minutes and subsequently rinsed with distilled water. *MRI acquisition:* A 7.0 Tesla MRI scanner (Varian Inc.) was used to acquire an image of the gel (2D gradient-echo, TE/TR = 3.5/150ms, 90° flip angle, 10-20 averages, 10cm x 20cm, matrix size = 256 x 512, 12-25 mins) using a 13cm diameter coil. *Gel staining and band isolation:* After MRI the gel was stained with SimplyBlue SafeStain (Invitrogen) and an image of the gel was acquired on a light box. Protein bands corresponding to those on the MRI image were extracted and then processed for protein identification using liquid chromatography/mass spectrometry (LC/MS).

Results and Discussion: Distinct protein bands, reminiscent of a stained Western blot, are evident in the MR image of the gel after soaking in $MnCl_2$ (Fig 1, top). Different proteins/protein complexes were highlighted in tissue samples from different brain regions. These corresponded to a subset of bands in the optical image of the gel, which after non-specific protein staining showed a few distinct bands on a background protein "smear" (Fig 1). The apoferritin band of a control protein sample (a protein ladder, Fig 1, lane 4) was highlighted in the "Mn-stained" MR image (IgM pentamer, B-phycoerythrin, lactate dehydrogenase, and bovine serum albumin were not). The MRI isolated protein bands from each tissue extract have been isolated for identification with LC/MS.

Conclusion: The combination of gel electrophoresis and MRI in tissue extracts may provide a powerful tool for isolation of proteins/protein complexes that interact with paramagnetic ions such as Mn^{2+}. Identification of these proteins will improve our interpretation of MRI contrast and may provide new reporter gene candidates.

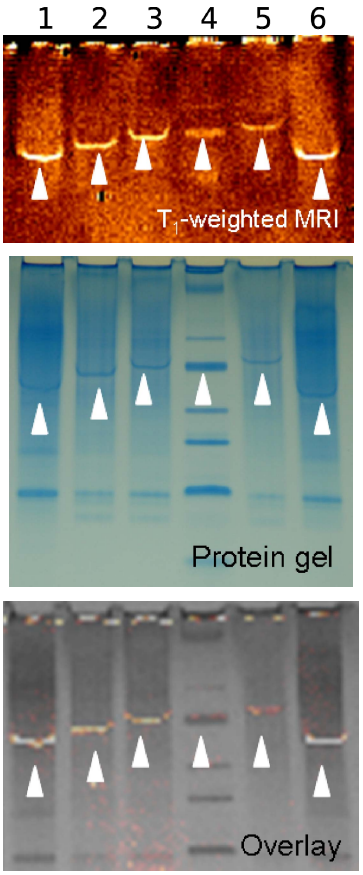

Figure 1. MRI and optical images of native-protein gel electrophoresis. Protein extract from 1. cerebellum 2. hippocampus 3. olfactory bulb 4. ladder 5. pituitary gland 6. cortex.

Reference:

1. Watanabe, T., Natt, O., Boretius, S., Frahm, J. & Michaelis, T. In vivo 3D MRI staining of mouse brain after subcutaneous application of MnCl2. *Magn Reson Med* **48**, 852-859 (2002).
2. Silva, A.C. & Bock, N.A. Manganese-enhanced MRI: an exceptional tool in translational neuroimaging. *Schizophr Bull* **34**, 595-604 (2008).

Acknowledgement: This study was conducted with the support of the Ontario Institute for Cancer Research through funding provided by the Government of Ontario.