Visualization of the optic nerve in several laboratory animal models using manganese-containing contrast agents

M. Thuen[1], T. E. Singstad[2], T. B. Pedersen[1], Y. Mørch[3], G. Skjåk-Bræk[3], O. Haraldseth[1], M. Berry[4], A. Sandvig[5], C. Brekken[1]

[1]Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway, [2]Department of Medical Imaging, St. Olavs Hospital HF, Trondheim, Norway, [3]Department of Biotechnology, Norwegian University of Science and Technology, Trondheim, Norway, [4]Neural Damage and Repair, Centre for Neuroscienceical Science, GKT School of Biomedical Sciences, King's College, London, United Kingdom, [5]Institute for Experimental Medical Research, Ullevål University Hospital, Oslo, Norway

Introduction: Axons in the mammalian peripheral nervous system regenerate after injury, while those in the central nervous system (CNS) do not. In contrast, spontaneous regeneration in the CNS occurs in some lower vertebrates, for example fish and amphibians (1). Manganese (Mn^{2+}) is paramagnetic and acts as an MRI contrast agent mainly by shortening the T_1 relaxation time. In addition, Mn^{2+} is a calcium analogue that is taken up by Ca^{2+}-channels and transported along axons. Here, we show that MRI using $MnCl_2$ as a contrast agent is a viable method for *in vivo* visualization of the optic nerve (ON) in rats, mice, frogs and fish. However, cellular toxicity remains a challenge, and new methods for administrating Mn^{2+} to reduce toxicity would be of interest. We demonstrate that two other Mn^{2+}-based contrast agents, the clinically approved contrast agent mangafodipir (Teslascan®) and Mn^{2+}-containing alginate spheres, can be used to visualize the retina and ON in rats.

Materials and Methods: MRI of the retina and ON were obtained 24h after unilateral intravitreal $MnCl_2$-injection (150nmol) in rats (Fischer, n=5), mice (C57/BL, n=5), frogs (Rana Pipiens, n=5) and fish (Salvenlinus Alpinus, n=5). In rats, the $MnCl_2$-injection was repeated 20d later, and MRI performed after 24h. In addition, 6 rats were injected with either mangafodipir (Teslascan®, 0.3nmol, n=4), or Mn^{2+}-containing alginate gel spheres (10-15 spheres in 0.9% NaCl, n=2) and underwent MRI 24h later. MRI was performed at 2.35T using a Bruker Biospec Avance DBX-100 (Bruker Biospin AG, Ettlingen, Germany) with a 72mm volume coil for transmission and an actively decoupled quadrature rat head surface coil for receive-only. Water-cooled BGA-12 (200mT/m) gradients were used. For scanning, animals lay prone in a dedicated animal bed within the magnet. A 3D data set was obtained using a T1-weighted 3D low flip angle gradient-echo sequence (FLASH) with TR=15ms and TE=4.2ms, and a flip angle of 25º. For rats, mice and fish, the acquisition matrix was 256×256×128 and the voxel resolution either 195×195×156µm^3 (rats and fish), or 156×156×125µm^3 (mice). 8 averages were used and the total acquisition time was 65.5min. For frogs, the acquisition matrix was 128×128×64 and the voxel resolution 234×195×234µm^3. 16 averages were used and the total acquisition time was 32.8min. All 3D data sets were analyzed using ParaVision 3.0.1 (Bruker).

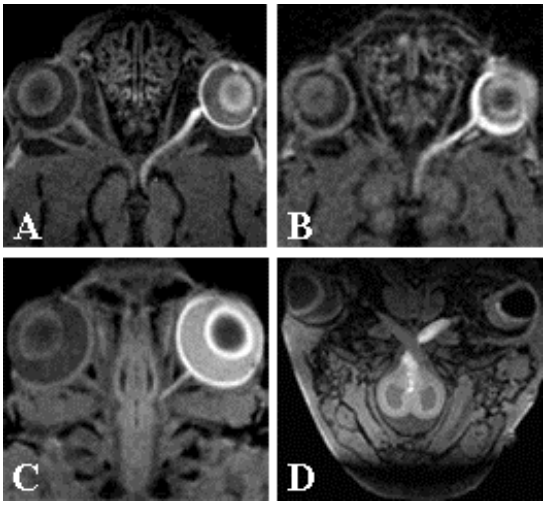

Figure 1: MRI of the retina and ON 24h after intravitreal Mn^{2+}-injection in a rat (A), mouse (B), frog (C) and fish (D).

Results and discussion: MRI 24h after intravitreal Mn^{2+}-injection produced a clear enhancement of the retina and ON in rats, mice, frogs and fish (figure 1), and no differences in enhancement were found between the first and second Mn^{2+}-injection in rats. These findings demonstrate that Mn^{2+}-enhanced MRI can be used in longitudinal studies, and promises to be an effective method for studying axon regeneration in different species at varying times after ON-transection (2). MRI 24h after intravitreal injection of mangafodipir showed the retina and ON clearly even though the Mn^{2+}-dose was greatly reduced compared to that of $MnCl_2$ (figure 2 A, B). MRI 24h after intravitreal injection of Mn^{2+}-containing alginate spheres produced a clear enhancement of the retina and ON, with the individual alginate spheres visible in the vitreous body (figure 2 C). The unique Mn^{2+}-binding properties of alginate makes it a novel probe for localized Mn^{2+}-delivery, and the slow-release of Mn^{2+} from mangafodipir and alginate may have an advantage in reducing cellular toxicity that can be a problem when using $MnCl_2$.

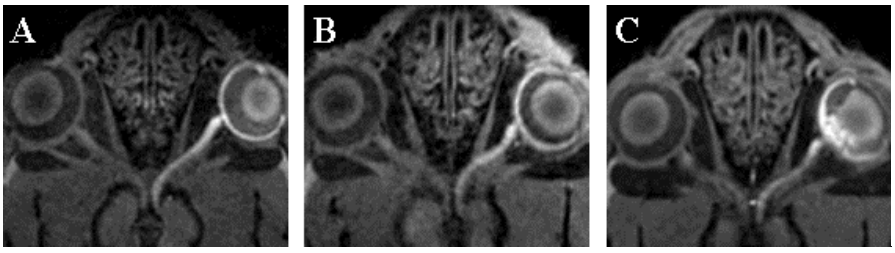

Figure 2: MRI of the rat retina and ON 24h after intravitreal injection of $MnCl_2$ (A), mangafodipir (B) and Mn^{2+}-containing alginate spheres (C).

Conclusions: $MnCl_2$ is a viable contrast agent for Mn^{2+}-enhanced MRI of the retina and ON in rats, mice, frogs and fish, and can be used in longitudinal studies to investigate the genetic and molecular basis for the differences in CNS regeneration between these species. In the search for new methods for administrating Mn^{2+}, we demonstrate that Mn^{2+}-containing alginate spheres and the clinically approved contrast agent mangafodipir, also can be used to visualize the retina and ON.

Reference: (1) Jacobson M. Developmental neurobiology. Plenum Press, New York, 1978. (2) Thuen M et al. Manganese-enhanced MRI of optic visual pathway and optic nerve injury in adult rats, 2004. Submitted.