Manganese-enhanced MRI of spinal cord injury in rat

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Introduction

The architecture of spinal cord (SC) neural circuitry under normal and pathological conditions is traditionally evaluated using histology and immunohistochemistry. These are highly invasive techniques that require sacrificing groups of animals at different time points for ex vivo tissue processing and elaborate analysis of many sections. This prohibits studying the same animal over time. Novel imaging applications may provide new opportunities for in vivo mapping of SC circuitry in longitudinal studies. Manganese-enhanced MRI (MEI) has been previously used for neuronal tract tracing (1). In these studies, we applied this technique to visualize the fiber connectivity in normal SC and to detect the viable fibers in injured SC in rat animal model of SC injury (SCI).

Materials and Methods

All MRI scans were performed on a 9.4 T horizontal Varian scanner (Varian Inc., Palo Alto, CA) using a homebuilt inductively coupled coil (2). Under isoflurane anesthesia administrated via a facemask, the coil was implanted subcutaneously in Sprague Dawley rats adjacent to the SC at thoracic level. The contrast agent manganese (Mn; 4 mmol/L of aqueous $MnCl_2$ solution in volume of 10 nL) was slowly delivered into the SC using a pulled glass pipette tapered on a 1 µL Hamilton syringe and positioned using a micropositioner attached to a stereotaxic frame. Experiments were conducted on a normal cord (Mn is injected at the T8 level), and hemi-sectioned and injured cords (Mn was bilaterally injected 3 mm rostral to the injury at the T8 level). Hemisectioning completely transected the fiber connections on the right side of the cord. For this case, conventional neural tracer Biotinylated dextran amine (10 % BDA; 10,000 MW conjugated to lysine) was also diluted in the Mn solution, and the final mixture was administered bilaterally rostral to the cut. After the delivery of Mn, high-resolution, T1-weighted axial images were acquired repetitively using standard spin echo (SE) sequence with the parameter values: T_R/T_E = 1000/13 ms, FOV=15 mm, image matrix = 128 X 128, slice thickness = 1 mm and NEX=2. The data was processed using the vendor-supplied software (VNMRJ1.1C) to detect the spatiotemporal spread of the Mn enhancement in SC.

Results and Discussion

The feasibility of administering the contrast agent manganese (Mn) into a normal SC was demonstrated in Figs. 1 and 2. Spatiotemporal spread of Mn was observed in neuronal projections both rostral and caudal to the injection site due to its axonal transportability. In studies with the hemi-sectioned cord, Mn was delivered bilaterally into the SC section rostral to the hemi-section in conjunction with a histological neuronal tract tracer. On MEI and histology obtained caudal to the cut, labeling was observed in SC tissue contra-lateral to the cut side, but not ipsilateral, as shown in Figs. 3 and 4. This indicates that Mn is transported in live fibers, but it fails to cross fibers that are disconnected by the hemi-section. In experiments with a contusion-type SCI model, we delivered Mn rostral to the contusion in the chronic phase of the injury, and detected labeling at the injury epicenter and below, as shown in Figs. 5 and 6. This implied the presence of live neuronal fibers transporting Mn across the injury. In conclusion, these results together provide an initial evidence of an in vivo imaging methodology for tracking the reorganization of neuronal connectivity in an injured SC.

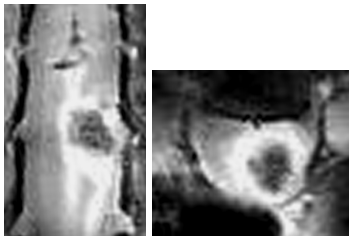

Figure 1. *In vivo* MEIs of normal rat spinal cord in coronal and axial views.

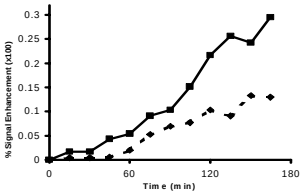

Figure 2. Temporal profiles of mean signal enhancement in two ROIs selected in WMs of slices 3 mm away from the injection site, rostral (solid line) and caudal (dashed-line).

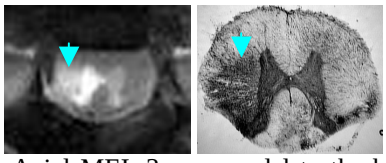

Figure 3. Axial MEI, 3 mm caudal to the hemisection performed on the right side of the SC. The signal enhancement is on the contra lateral left side of the cut.

Figure 4. BDA labeling agreeing with the MEI in Fig. 3.

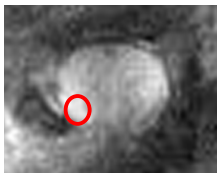

Figure 5. Axial MEI of an injured SC on postinjury day 7. The circle indicates the selected ROI.

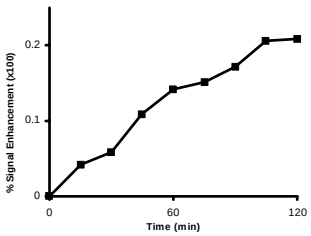

Figure 6. Temporal profile of the mean signal enhancement in the selected ROI of the injured SC in Fig. 5.

References

1. Leergaard, T.B., et al. Neuroimage, 2003. **20**(3): p. 1591-600.
2. Bilgen M. Magn Reson Med 2004;52(4):937-940.