Manganese-enhanced MRI of the spinal cord for in vivo assessment of damage and functional improvement following spinal cord injury in mice treated with 95CDL Antibodies

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Introduction

Recent developments in treatment of spinal cord injury (SCI) in mice have shown promising results of functional recovery using CD95L antibody treatment (1). We have shown that manganese enhanced MRI (MEMRI) of the spinal cord (SC) can be used as a method to visualize and quantify spinal cord injury in mice (2). Here we describe how this protocol can be used to evaluate the efficacy of novel drugs directed against SCI. We studied the differences of manganese uptake in the SC in SCI mice and compared saline treated- and CD95L antibody treated mice. We correlated clinical evaluation of SCI mice with findings of follow up SC MEMRI.

Methods

Animal preparation: 12 C57BL/6 mice were anesthetized using 3% isoflurane mixed with oxygen with a flow rate of 0.5 l/min. During trauma induction, isoflurane dose was lowered to 1.5% to maintain anesthesia. Mice were laminectomized at level Th 8/9 and the SC was transected leaving the ventral funiculus intact. 6 mice received placebo- and 6 mice CD95L antibody treatment. 3 hours after injection, 250 nl 0.8 mM $MnCl_2$ was injected bilaterally in the lateral ventricle using an oocyte injector. Imaging was performed 3 days after injection of $MnCl_2$. This protocol was repeated after 6 weeks of treatment. *Behavioral testing:* All animals were clinically tested once weekly using the BBB score and a grid walking test. *Imaging:* MRI experiments were performed on a 1.5 T clinical scanner (SIEMENS symphony, Erlangen, GER) and a dedicated animal volume resonator using a 3D-FLASH imaging sequence. Parameters: TR/TE 14.0/5.22 ms, flip angle 30º, 28 partitions, partition thickness: 0.5 mm, FOV 80 mm, matrix size 512 x 512, voxel size 0.15 x 0.15 mm, 32 averages. Imaging was performed perpendicular to the SC. 60 minutes imaging time. *Data processing:* Images were evaluated using the scanner software package (Syngo, SIEMENS, Erlangen, GER). The SC was outlined and mean signal was calculated. A second ROI was placed within the FOV outside the animal contours for noise measurement. SNR along the spinal cord surrounding the lesion epicenter was measured. We calculated the area under the curve (AUC) before and after treatment and tested the value of the change in AUC (Δ AUC) as a measure of clinical improvement**.** This measure was correlated with clinical parameters of SCI.

Results:

In the clinical tests for the evaluation of SCI, the antibody- and placebo-treated groups differed significantly in both BBB and grid walk ranks (Wilcoxon rank sum test; $p = 0.0011$) indicating that the treatment was effective. In saline-treated animals Mn^{2+} uptake did not significantly increase after therapy (linear regression analysis of relative SNR: $p = 0.018$, slope -0.269, 95% confidence interval: -0.048 to -0.49, n=6). The anti-CD95L antibody-treated animals showed a significant increase of Δ AUC (linear regression analysis: $p = 0.0005$, slope 1.150, 95% confidence interval: 0.831 to 1.470, n=6). A gradual decrease of SNR along the rostro-caudal axis of the spinal cord was observed at the pre-treatment time point, reaching baseline levels at the lesion site, similar to the curve of the injured untreated animals. After treatment a strong increase in relative SNR was found, indicating recovery of perseverated functional neurons at the level of injury and beyond (see fig. 1 for comparison of treated and untreated animals). The SNR level at the lesion site and beyond, however, never reached uninjured levels indicating residual damage after treatment. To test the correlation of MEMRI with clinical scores we ranked the 12 animals based on Δ AUC as well as on their BBB score assuming that preservation of functional neurons would lead to both increased Mn^{2+} uptake and improved motor function. In Fig. 2 the combination of MEMRI and BBB score rank orders is shown. It states a highly significant correlation between the two rankings (Kendall's $\tau = 0.9394$, $p = 0.000005$).

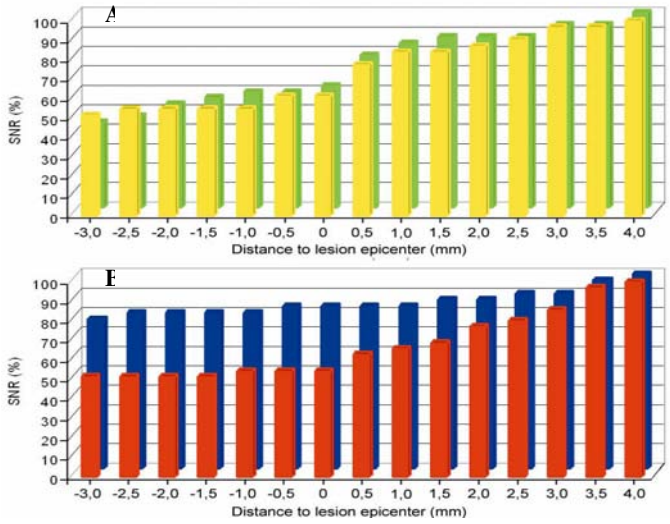

Fig. 1 SC Manganese enhancement before and after treatment. (A) Un-treated animal before (yellow) and after (green) treatment. **(B)** Treated animal before (red) and after (blue) treatment.

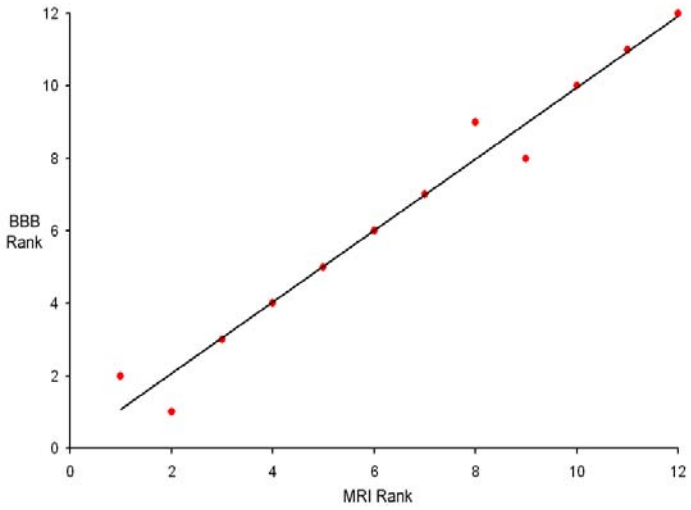

Fig.2 Correlation of clincal and MEMRI derived functional parameter. BBB rank is the clinical score, MRI rank based on AUC before and after treatment.

Discussion

We report of an in vivo method for functional spinal cord imaging for monitoring of treatment effects in mice using MEMRI. MEMRI-derived parameters correlate strongly with the clinical status as evaluated using locomotor test. Thus, MEMRI of the SCI can be used as an objective measure of improved function after treatment for SCI. Furthermore, it offers visual evaluation of the SCI and its changes under therapy.

References

(1) Demjen, D et al. Nat Med. 2004 Apr; 10 (4): 389-95

(2) Stieltjes et al. ISMRM 2005