Monitoring therapeutic effects of rhEPO in a rat model of spinal cord injury using MEMRI

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Introduction

Numerous emerging therapy concepts are being discussed for the early treatment of spinal cord injury (SCI) including the application of recombinant human Erythropoietin (rhEPO). There is strong evidence that it harbors neuroprotective and neuroregenerative qualities in the central nervous system [1,2]. To evaluate the outcome of new treatment concepts in SCI, a reproducible and reliable imaging method is urgently needed. It has been shown earlier that manganese enhanced magnetic resonance imaging (MEMRI) provides details about spinal cord function and injury in animal models [3,4]. Manganese acts as an excellent contrast agent for neuronal tissue as it is imported and processed into the neuron by voltage-gated Ca^{2+}-channels [3]. Since damaged neurons lack uptake of manganese [3,5], we hypothesize, that in spinal cord injury MEMRI will depict a loss of T1w contrast that is partially reversible under rhEPO therapy.

Materials and Methods

Eighteen rats were anesthetized with intraperitoneal injections of Ketasol (Ketaminhydrochloride, 100 ml/mg) and Rompun (Xylazinehydrochloride, 2%). A laminectomy was carried out on each rat at the level of the 11th thoracic vertebra (TH11) and a contusion injury was induced using the Infinite Horizon Impactor (Precision Systems and Instrumentation, LLC, Lexington, KY). This device applies standard-force contusion injuries to the spinal cords of mice or rats. $MnCl_2$-injections were administered immediately after surgery. Eighty microliters of a 0.8 M $MnCl_2$-solution were manually injected into the cisterna magna via the membrana atlanto-occipitalis using a 27-gauge needle. Nine rats received a single dose of rhEPO, nine received a saline solution as placebo treatment. Physical examination was performed by observers blind to the animals' grouping on day 3 post injury, using the locomotor rating scale by Basso, Beattie and Bresnahan (=BBB-score [6]). It allows assessment of each hind-limb separately, using a scale from 0 to 21, where 0 denotes total paraplegia and 21 denotes full function of the hind-limb. All rats were euthanized after locomotion testing on day 3. For MRI, the excised vertebral columns were stored in 15 mL polypropylene-tubes with a diameter of 23 mm, to avoid motion artifacts. The tubes were filled with formaldehyde solution to conserve samples. MRI was performed at room temperature on a clinical 1.5-T-scanner (Siemens Symphony, Erlangen, Germany) with a dedicated custom-made animal volume resonator using a 3D-FLASH imaging pulse sequence with the following parameters: TR/TE 14.0/5.22 milliseconds, flip angle 30°, 28 partitions, partition thickness: 0.5 mm, field of vision (FOV) 80 mm, matrix size 512 x 512, voxel size 0.15 x 0.15 mm, 16 averages. Imaging was performed perpendicular to the spinal cord. Imaging time was 30 minutes per sample.

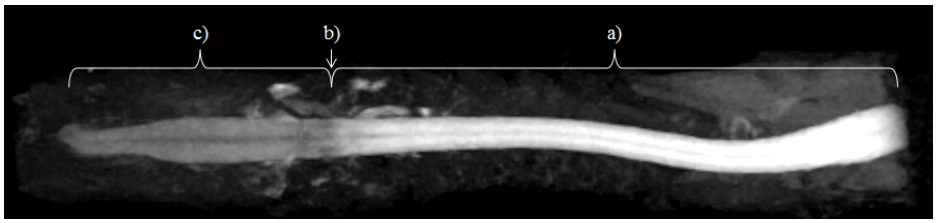

Fig.1: Postmortem T1-weighted manganese enhanced magnetic resonance imaging of an extracted spinal cord (rat). The spinal cord has been stored and measured in a tube containing formaldehyde. a) Homogeneous contrast uptake is seen above the lesion epicenter (b). Below, c) a decrease of signal intensity is observed.

Results

Fig. 1 represents a typical example of an extracted spinal cord depicted using MEMRI. As shown in Fig. 2 there is an increased signal intensity in the rhEPO group compared to controls and the group difference is significant ($p = 0.0001$, $\chi^2 = 17.99$). The physical examination results show increased summed BBB-scores in the treated animals and the group difference is significant ($p = 0.0049$, degrees of freedom = 15, $t = 3.29$) (Fig. 3).

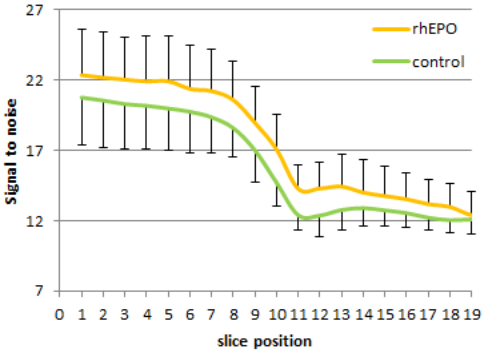

Fig. 2: SNR within the spinal cord in the two groups. Slices are running from cranial (1) to caudal (19), the lesion epicenter was located around slice number 10. Error bars represent the standard deviation within both groups.

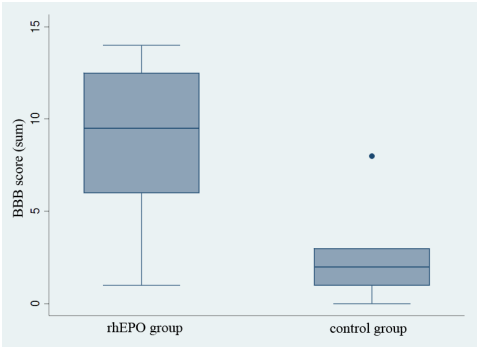

Fig. 3: Boxplot of physical examination using the BBB-scoring system. The rhEPO treated animals show higher values as a sign of therapy response. The BBB-scores of both hind-limbs have been summed to one number per animal.

Discussion

Considering the physical performance as quantified using the BBB-score, the rhEPO treated group showed significantly increased values indicating a clear therapy effect. This effect was also seen in MEMRI as an increased uptake of manganese in this group resulting in a significantly higher SNR in the spinal cord. Thus, MEMRI may serve as a semi-quantitative imaging approach to monitor spinal cord injury and functional recovery under rhEPO therapy. The proposed technique may also be applied in future in vivo studies, as it has previously been shown in a mouse model [3].

References

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