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

Martin Freitag 1, Nadja Walder 1, Jens Hartmann 1, Heinz Redl 1, Peter Parzer 1, and Bram Stieljes 1

1Quantitative Image-based Disease Characterization, German Cancer Research Center, Heidelberg, Baden-Württemberg, Germany, 2Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Research Center of the AUVA, Vienna, Austria, 3Department of Child and Adolescent Psychiatry, Center for Psychosocial Medicine, Section Disorders of Personality Development, Heidelberg, Germany

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 Ca²⁺-channels [3]. Since damaged neurons lack uptake of manganese [3,5], we hypothesize, that in spinal cord injury MEMRI will depict a loss of T1 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. MnCl₂-injections were administered immediately after surgery. Eighty microliters of a 0.8 M MnCl₂-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 administered immediately after surgery. Eighty microliters of a 0.8 M MnCl₂-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.

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, χ² = 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).

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