MRI detection of inhibition of fibrous scar formation after spinal cord injury: monitoring and evaluation of new regenerative strategies

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Introduction

Magnetic resonance imaging is routinely used to evaluate the level and extent of spinal cord injuries (SCI) in humans. After a lesion of the spinal cord, a scar forms within a week consisting of a fibrous and a glial component. The collagen rich fibrous component has been shown to impede axonal regeneration in the rat model of SCI[(1)] by forming a physical and chemical barrier for regenerating axons. New treatment approaches to pharmacologically suppress the fibrous scar formation in the lesioned area are now available which are indeed showing promising results for an effective treatment of the injury. These new treatments are now evaluated in an animal model of SCI. As in humans, MRI should be a well suited tool to follow the therapeutic approach in the animal model, especially for monitoring individual progress. The differentiation of the components of the lesion scar is therefore of paramount importance for the assessment of the treatment success.

Methods

In 14 adult Wistar rats the dorsal cortico-spinal tract was transected with a scoutern wire knife. Six animals received a combination of injections and sustained release of prolyl 4-hydroylase inhibitor BPY-DCA (dicarboxylic acid derivative of bipyridine; 30mM) and solid 8-Br-cAMP to suppress formation of the fibrous scar. Control animals received corresponding vehicle-treatment.

MR-imaging was performed on a 7T BioSpec animal scanner (Bruker Biospin, Ettlingen, Germany), equipped with a gradient insert with gradient strength of 400 mT/m and home-built rf-coils; we used a 10 cm Helmholtz coil for transmission and a 2 cm diameter surface coil for signal detection. To minimize breathing artifacts, the animal was placed in supine position on the animal bed with the surface coil beneath the spinal cord through a hole in the animal bed. Further artifact suppression was achieved by positioning FOV saturation slices through the lung.

Our imaging protocol consisted of localizer scans for animal positioning and slice position reference, a set of 2D SE images (T1-, PD-, and T2-weighted), a 3D-SE and Gd-DTPA enhanced 2D T1-w. Imaging parameters:

T1: MSSE, TE/TR=15/800ms, slTh=1mm, NEx=4, FOV=3cm; matrix 256^2. PD/T2 : 16 echos MSME, TE/TR=16*12.5/3000ms, slTh=1mm, NEx=1, FOV=3cm ; matrix 128^2. 3D-SE: DSE, TE/TR=15 ; 30/ 1000ms, NEx=1, FOV=2.5x2x1cm, matrix=256x128x32. T1/Gd : MSME TE/TR=12.5/1000ms, slTh=1mm, NEx=1, FOV=3cm, matrix=256^2.

The animals were scanned 1 and 8 days post spine lesion; after the second scan the animals were sacrificed, and perfusion fixated. The spinal cord was paraffin embedded for preparation of 10µm parasagittal sections. The fibrous scar was detected by applying an antibody against collagen type IV, whereas the glial scar was identified by staining GFAP positive astrocytes.

Results

With the help of suppression bands to saturate unwanted magnetization from moving tissue of the lung it was possible to acquire high resolution images of the lesioned area without using a breathing trigger. The lesion could be clearly identified for each subject at both scanning time points, 1 and 8 days post lesion induction. Posttraumatic edema is visible as hyperintensity in T2-w images. The native imaging modalities allow a good differentiation between lesion and surrounding tissue, but a further differentiation of the scar tissue is only possible with Gd-DTPA enhanced images. From the histological images we know that the fibrous scar is located in the center of the scar surrounded by the glial scar. Our main finding is that the scar containing the collagenous fibers when compared to a glial scar is showing a more pronounced accumulation of the contrast agent and is therefore detectable on T1-w images.

Our therapeutic intervention to enable axonal regeneration leads to transient inhibition of the collagenous scar formation up to 12 days after treatment onset. Due to the more selective accumulation of the contrast agent in the collageneous scar it is possible to follow the development of the scar formation and even differentiate treated from untreated animals with MR-imaging. In the untreated animals we always detect an enrichment in the center of the lesion whereas in the treated group only minimal accumulation in the glial scar is detectable.

Discussion

With MRI it is possible to detect the lesion in the model of experimental spinal cord injury and to follow the individual development of the lesion and the scar formation longitudinally. With contrast agent enhanced imaging it is further possible to differentiate the scar tissue and thus to follow the individual progress of the therapeutic approach. This non-invasive monitoring of the suppression of fibrous barrier formation allows to assess axonal regeneration and thereby evaluate efficiency of a new and promising treatment strategy for spinal cord injury.

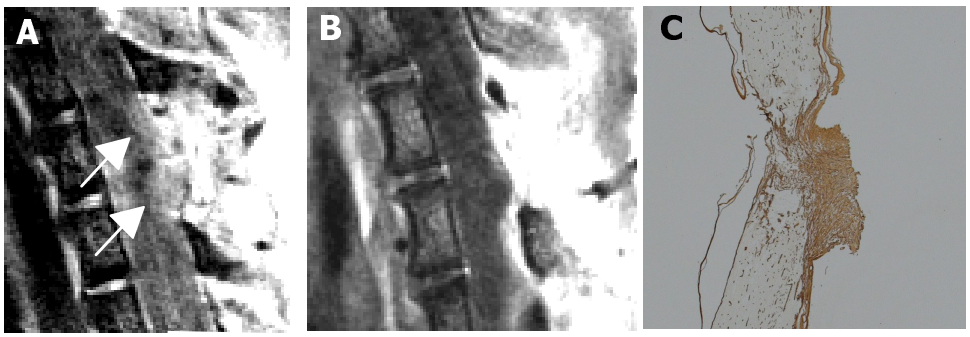

Fig. 1: Gd-DTPA enhanced MR images of spinal cord lesions, untreated (A) and treated (B) animals, contrast agent enrichment in the spinal cord (arrows in (A)) corresponds histologically to the fibrous scar. An example of the Anti-Collagen IV staining is shown in (C).

(1) Hermanns S., Klapka N., Mueller HW. Restor Neurol Neurosci **19**, 139-148 (2001)