In vivo and In vitro High Field Imaging of Rat Spinal Cord Injury

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Introduction

An increasing number of animal models for spinal cord diseases has become available over the past years. Despite the availability of a variety of imaging techniques most research groups still use histopathological analysis for tissue damage quantification exclusively. This is a very time consuming method and it often provides only a rough estimate of the lesion volume, since – due to time constraints – mostly not all slices are analyzed quantitatively. Only a very limited number of MR studies [1,2] has been performed on SCI models, most of them suffering from a limited spatial resolution.

In vitro and in vivo MR imaging methods provide the potential to improve information obtained by histopathology in various ways. In vitro MRI enables a rapid evaluation of the whole sample at one specific time point while maintaining a large part of the quality of the histopathological data. In vivo MR data yields information about lesion evolution in a single animal. High magnetic fields serve both techniques by providing a better signal-to-noise ratio (SNR).

Objects and Methods

Animal preparation

Adult female Fischer 344 rats received contusions of the midthoracic spinal cord using an IH Impactor (applied force: 2 N). Two weeks after contusion the rats were anesthetized with 2% isoflurane with Carbogen (95 % oxygen and 5 % carbon dioxide) as respiratory gas.

MR imaging

All MR imaging experiments were conducted on a Bruker 750 WB system (Bruker Biospin, Rheinstetten, Germany) at 17.6 Tesla.

For in vitro imaging experiments a gradient system with 1 T/m was used with a 5 mm linear birdcage resonator as transmit- and receive coil. 3D gradient echo data sets were obtained with a spatial resolution of 35x35x54 µm³ at an echo time of 13.5 ms and a repetition time of 50 ms in a total scan time of 5.5 hours. For detection of cysts 2D spin echoes with TE=7.5ms and TR=2s showed the best contrast. The spatial resolution was 23x23x300 µm³.

In vivo imaging experiments were conducted using an animal gradient system with 200 mT/m and 57 mm inner diameter. Since rat spinal cord imaging in wide bore magnets is not possible with conventional imaging hardware due to space constraints a homebuilt probehead and a linear surface coil was constructed to offer a maximum of space for the animal. To avoid artefacts caused by blood flow and respiratory motion a combination of ECG triggering and breath gating was used. A multi-slice 2D gradient echo was used with an echo time of 4 ms and a repetition time around 200 ms depending on the heart rate. The acquisition of 3D data sets was not possible at a reasonable resolution due to time constraints because of the ECG triggering. Instead two interleaved multi-slice data sets were acquired to cover a full 3D volume. The spatial resolution was at least 78x78x370 µm³ in axial slices and 156x98x370 in sagittal slices.

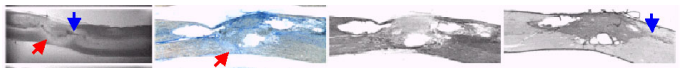

Figure 1: sagittal slice of an in-vitro MRI scan (a) with the corresponding Nissl, GFAP and Collagen type III sections (b-d)

Histology

At 4 and 8 weeks post injury, the animals were sacrificed by transcardiac perfusion with 4% paraformaldehyde. The spinal cords were excised, post fixed over night and cryoprotected in 30% sucrose. Spinal cord samples from the 4 week time point were used for ex vivo MRI scanning before histological analysis. Finally, sagittal 35mm thick sections were processed for histochemical (Nissl) and immunohistochemical staining using antibodies against GFAP (astroglia) and collagen type III (fibrous scar).

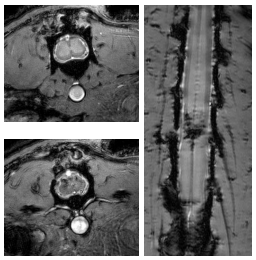

Figure 2: axial and coronal in vivo gradient echo images ; upper left: healthy area; lower left: center of the contusion.

Results

In vitro MRI data of the thoracic part of the spinal cord showed in normal tissue that gray and white matter could be easily distinguished whereas in the contusion area the contrast disappeared. The radius of the spinal cord around the injured tissue was reduced compared to healthy areas. Around the contusion both in cervical and caudal direction cysts of various sizes were visible in the T2-weighted images. Figure 1 shows a sagittal view of a cyst of the thoracic portion of the spinal cord and the corresponding histology sections. Corresponding features are shown with the blue and and red arrows.

For in vivo lesion characterization axial images of lower thoracic part of the spinal cord could be acquired at a very low artefact level in a total scan time of 2 x 15 minutes for two interleaved multi-slice data sets covering a full 3D volume. Figure 2 shows two axial and one coronal image of an injured spinal cord. The center of the injury can be recognized as a hypointense area in the coronal image. The overall diameter in the center of the contusion is reduced compared to healthy regions, the shape of the cord is distorted which can be clearly seen on the lower left image. Also a small hypointense area inside the spinal cord that may originate from clotted blood can be identified.

Figure 3: sagittal view of the spinal cord of an injured animal. The contusion can clearly be seen in the center of the image where the shape of the spinal cord is distorted.

Discussion

The study showed that it is possible to directly visualize spinal cord contusion injuries at a very high spatial resolution as compared to studies performed at lower field strengths. Specific pathological changes that can be represented in histological sections are visible in MRI data to a limited extent. These pathological features are partially visible in the in-vivo images, but in a reduced quality.

[1] Fraidakis et al., Exp Neurol, 153 (2), 299-312 (1998)
[2] Gareau et al., Magn Reson Med, 45, 159-63 (2001)