High-field in vivo 1H-MR Spectroscopy of the injured mouse spinal cord. Feasibility and potentiality.

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Introduction

^{1}H-MR Spectroscopy (MRS), which is extensively used to characterize neurological diseases, has not yet been applied to mouse spinal cord (SC) pathologies although it could be a valuable asset, in addition to the traditional anatomic and diffusion MR imaging, to better describe the pathological consequences and to evaluate possible regenerative strategies.
In this study, we examined (i) whether single-voxel spectroscopy at high-field would be able to provide information on the metabolic status of an injured mouse spinal cord model (compression) and (ii) whether longitudinal follow-ups would allow monitoring post-traumatic metabolic evolution.

Materials and Methods

Experiments were conducted on healthy control and injured mice (C57Bl/6J, 20-25 g, 1.5% isoflurane anesthesia). The spinal cord compression was induced at the C3 level by transitorily inflating a balloon inserted in the epidural space (pressure 2.5 bar, duration 10 s). Following the compression, mice suffered from left fore-limb paralysis.
MRI/MRS acquisitions were performed on an 11.75T Bruker system, using a transmitter/receiver volume coil. Sagittal, coronal and axial images were initially acquired to precisely locate the spectroscopic voxel of interest (2.1x1.3x2.8 mm^3 (7.6µl)). First order shims were then performed on a (2.1mm)^3-voxel, followed by Point RESolved Spectroscopy (PRESS) acquisitions (TR/TE 2000/12 ms, 2048 points, VAPOR water suppression, 16 ppm spectral width, 512 averages, acquisition synchronized with breath motion and total acquisition time 17 minutes). Data were processed using an in-house-developed software running under IDL and the AMARES/jMRUI time domain fitting algorithm.
As a preliminary step for feasibility and sensitivity studies, spectra were acquired in healthy mice, in the brain, cerebellum and spinal cord (C3). Then, MR experiments were performed on injured mice spinal cord 2, 8 and 14 days post-injury (dpi). Prior each MR exam, the developed fore-limb force was measured using a grip-strength apparatus (Bioseb).

Results

Typical spectrum of healthy mouse SC is presented in fig. 1a. The three major resonances of N-acetyl-aspartate (NAA, 2.02 ppm), creatine (Cr, 3.02 ppm) and choline compound (Cho, 3.2 ppm) could clearly be identified. When repeating the experiments at different time points (data not shown), the reproducibility of the SC metabolic ratios was found equal to 80%.
Fig. 2 shows the major MRS metabolic ratios of normal mice (n=3) in the brain, cerebellum and spinal cord. The standard deviations of the metabolic ratios represent 6 and 35% of the mean, in the brain and SC, respectively.
A typical post-traumatic spectrum is given in fig. 1b. Increase of the choline peak integral was observed along with a slight decrease of the creatine, slight increase of NAA integral and large increase of lipids.
Evolution of the metabolic ratios and developed force, illustrated in fig. 3, first demonstrated an important change after the injury (0 dpi vs. 2 dpi), and then a slight recovery toward normal values 8 dpi for the force and 15 dpi for the metabolic ratios.

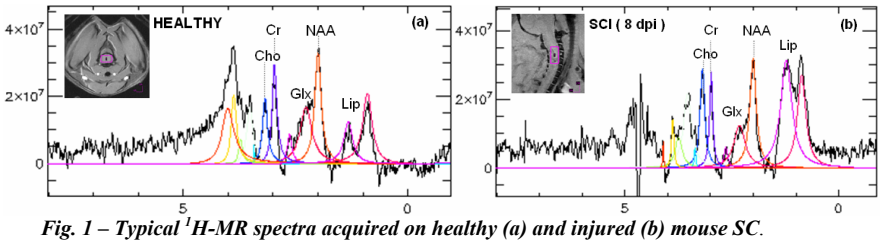

Fig. 1 – Typical ^{1}H-MR spectra acquired on healthy (a) and injured (b) mouse SC.

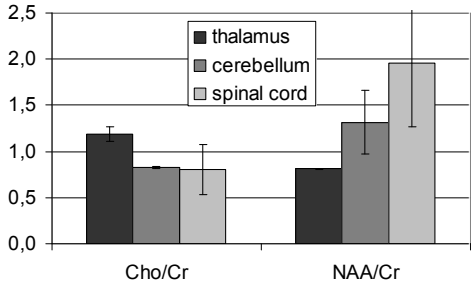

Fig. 2 – Major metabolic ratios (a.u.) in the brain, cerebellum and C3 spinal cord (control mice).

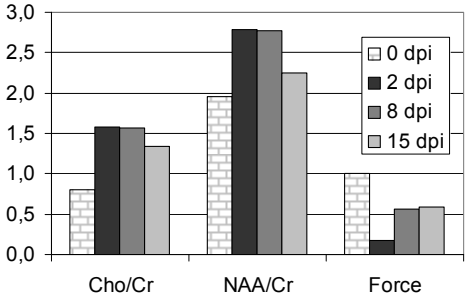

Fig. 3 – Pre (0 dpi) and post-injury (2, 8 and 15 dpi) SC metabolic ratios (a.u.) and normalized fore-limb force.

Discussion

In this work, mouse SC single-voxel spectroscopy has been demonstrated to be feasible despite challenges in terms of signal to noise ratio and susceptibility effects due to the SC size and bony structure, respectively.
The PRESS technique employed in this study was sensitive and robust enough to detect metabolic changes related to the pathology and statistical differences have been observed pre and post-injury ($p<0.05$). Characteristic post-injury features that could be observed were: increase of the choline compound, which may be attributed to cellular proliferation and membrane integrity alteration and decrease of the Cr peak, which may be attributed to a failure in the energy metabolism. The large increase of lipids peak integral may be attributed to membrane breakdown.
Variations of the metabolic ratios with time were finally observed. Although a delayed response, both SC metabolism and motor functionality were correlated ($R^2=0.72$) and tended to indicate an endogeneous repair.
Future studies are required to link the observed metabolite changes with histological analyses and with known physiologic post-traumatic mechanisms.
Finally, with a scan time of 17 minutes, different SC regions may be analyzed within a single session or multimodal protocol, combining MRI and MRS, may be performed.
Both strategies will provide complementary information that should help in the diagnosis and prognosis.
The potentiality of such combination is currently under investigation.
Further work should also include optimization of the voxel geometry to the shape of the spinal cord, improved shim to reach the quality obtained in the brain and improved quantification techniques.

Conclusion

In this preliminary study, we successfully applied localized ^{1}H-MRS to healthy and traumatic mouse SC. Significant MR spectral differences were observed between SCI and normal controls. These findings strongly suggest that *in vivo* ^{1}H-MRS could provide new criteria valuable for our understanding of the pathogenesis mechanisms and could offer new ways to assess the response to regenerative strategies.

Acknowledgements: this work received the support of CNRS (Centre National de la Recherche, UMR 6612) and ANR (Agence National de la Recherche, ANR-09-BLAN-0295-01). The authors acknowledge P. Decherchi and T. Marqueste (ISM, UMR 6633, CNRS) for the surgery and force measurement.