Assessment of Pathologic Mouse Spinal Cord Recovery using High-Resolution Diffusion and ASL-based Perfusion Imaging

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Introduction: The combined use of diffusion/perfusion imaging may greatly improve the characterization of spinal cord (SC) pathologies by allowing the detection of functional impairments, white matter tract disruption, deficient tissue blood supply and extensions of the lesion from the primary site of injury (2nd injury). This combination may additionally play an important role in the evaluation of functional recovery or regenerative therapeutic strategy [1]. Nowadays, diffusion tensor imaging (DTI) is a common tool for SC injury (SCI) investigation, whereas the possibility of assessing SC perfusion by MRI has only been demonstrated very recently in a study where accurate measurements of healthy mice SC blood flow (SCBF) have been obtained using pulsed arterial spin labeling (PASL) [2]. Despite these promising results, the low sensitivity of ASL and therefore its ability to detect vascular deficits or blood flow variations in follow-up studies was questionable and remained to be demonstrated. In this study we applied high-resolution DTI and ASL on a SC mouse pathological model (hemisection), highlighting then the promising potentiality of diffusion/perfusion imaging to help in the characterization of SC pathologies.

Methods: *Hemisection model:* Experiments were performed in mice (C57Bl/6J, age 14 weeks, 25 g, anaesthesia: air+isoflurane (~1.4%), n=3). The hemisection injury was realized according to the following procedure: the dorsal and lateral surfaces of the C3-C5 spinal cord were exposed following laminectomy. After aperture of the dura, a first transversal incision of the dorsal part of the C4 left spinal matter was made with microsissors and was then completed laterally using a microscalpel. Specific details of the hemisection model can be found in [3].

Imaging: Experiments were performed on an 11.75T vertical MR system (Bruker, AV 500WB). High sensitivity was obtained by the use of a small transmitter/receiver volumic coil (∅ 2cm, length 3cm), well adapted to the mouse SC imaging. An optimized 4-shot SE-EPI sequence [4] (matrix 128x128, FOV=1.28x1.28cm^2, slice thickness 0.75 mm) with OVS pulses was used for all the images acquisition. High-resolution diffusion imaging was obtained using a standard Stejskal-Tanner sequence (TR/TE=4500/15.2 ms, δ/Δ =2.3 ms/6.8 ms, b-values {0,700} s/mm², 7 slices and 6 diffusion-encoding directions, NEX=15, acq. time=30 min) [5]. DTI metrics (FA, diffusivities) were obtained by reconstructing the diffusion tensor with the manufacturer software. High-resolution quantitative perfusion imaging was obtained by PASL, using a presaturated-FAIR sequence [6] (TE=10.7ms, inversion time TI=1.3s, recovery time τ=3.5s, 40 ΔM averages, 2 slices, acq. time=25 min/slice). Due to the short transmitter coil, partial RF coverage of the mice only could be achieved during the gl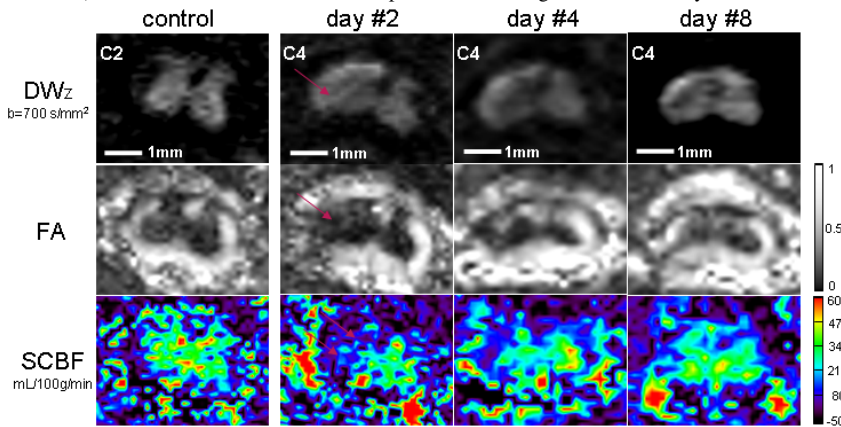obal inversion (labeling experiment). This led to fresh blood spins entering the imaging slice after a time Δ_c, called coil inflow time, modifying then the classical presat-FAIR model for SCBF quantification. In our experimental conditions, Δ_c was measured to 2.9±0.2 s, leading to the following equation for SCBF quantification [6]: $\Delta M= 2M_0.\alpha_0.(SCBF/\lambda).[(e^{-T1.R1app} - e^{-T1.R1a}) / (R1^a - R1^{app})]$. Absolute quantitative SCBF maps were obtained by solving this equation with λ =0.9 ml/g (water blood/tissue partition coefficient) and $R1^a=1/2.1s^{-1}$ (blood longitudinal relaxation rate). M_0 (equilibrium magnetization), α_0 (inversion efficiency) and $R1^{app}$ (SC tissue apparent longitudinal relaxation rate) were determined with a slice-selective inversion recovery prescan. DTI metrics and SCBF values were evaluated in ROIs selected in the SC ventral and dorsal gray matter (vGM, dGM) and in the lateral SC white matter (lWM). Measurements were performed at the injury level (C4) and at a distal level ("control" level, C2), 2 days, 4 days and 8 days after the injury.

Fig 1: Diffusion/perfusion imaging of a mouse SC hemisection model.
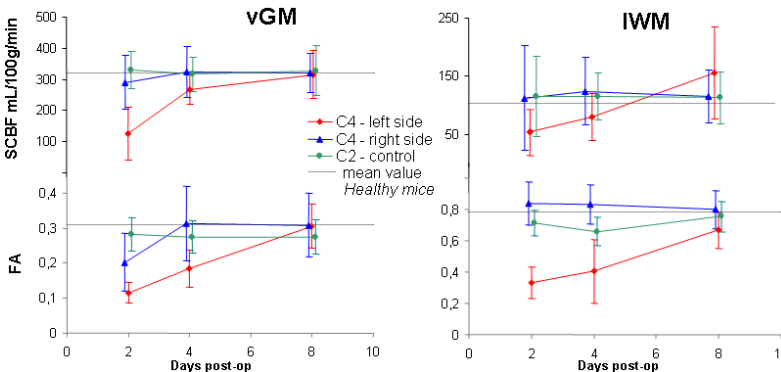
Fig 2: Temporal evolution of FA and SCBF values in hemisected/control SC

Results: High resolution SC diffusion-weighted (DW$_Z$) images, FA and SCBF maps for one mouse are shown on Fig. 1. At the C2-control level, the typical butterfly shape of the SC GM structures (DWz image) is clearly visible on the FA map (low FA values, FA=0.27±0.05) and on the SCBF map (high perfusion values 320±65 mL/100g/min). The surrounding WM (high FA values, 0.71±0.08) presents low perfusion values (120±65 mL/100g/min). At day #2, combined diffusion/perfusion images acquired at the C4 level revealed that the hemisection injury affected both lateral left WM and left GM (fig1. arrows). The lesion was characterized by hypersignal (resp. hyposignal) of the DWz image (resp. FA map) in the left WM, and by hyposignal of the SCBF map in the left vGM and dGM. Images acquired at days #4 and #8 highlighted a progressive recovery of both FA values in WM and perfusion values in GM. This evolution was confirmed by quantitative measurements (fig. 2, red curves, C4-level left side). At day #2, SCBF and FA values were measured to 126±86 mL/100g/min and 0.11±0.03 in the vGM and to 54±40 mL/100g/min and 0.33±0.09 in the lWM. These values, measured respectively ~3 and ~2 times lower than the corresponding values at the C2-control level (green curves) or in healthy mice (gray curves. SCBF ~ 320 and 100 mL/100g/min, FA ~ 0.3 and 0.78, for vGM and WM [2, 5]), progressively increase with time (day #4), and converge to control values (day #8).

Discussion: High resolution (100x100μm^2/pixel) SC diffusion and perfusion images were obtained in this study. Sensitivity and image quality of both DTI and ASL allowed to accurately detecting the lesion affecting lWM (FA maps) and gray matter (SCBF maps). Moreover, the follow-up of quantitative diffusion and perfusion measurements performed in lesion and control sites, permitted to highlight both tissue and vascular progressive recovery (fig2, FA and SCBF values (red curves), converging to healthy mice standard values (gray curves)). Although not focused on the complete description of the hemisection model, this study highlighted promising potentiality of high resolution diffusion/perfusion imaging to help in the characterization of SC pathologies. Further investigations will be focused on compression models which are more adapted to SCI pathology. In order to perform a complete characterization of the model, diffusion/perfusion imaging follow-up studies will be correlated with functional motor tests.

References: [1] Xiaowei et al., Spinal Cord (2006). [2] Duhamel et al., MRM (2008) [3] Decherchi et al, Neuroscience (2000) [4] Callot et al., Magma (2007) [5] Callot et al., NMR Biomed (2008) [6] Pell et al, MRM (1999)