EARLY EVIDENCE OF DISEASE ONSET BY IN VIVO MRI IN A MODEL OF ALS

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Introduction

Amyotrophic lateral sclerosis (ALS) is a severe form of motor neuron diseases leading rapidly to death. The rat bearing the human gene for mutant SOD1 is a model of the disease which pathophysiology is still under investigation. In this study, the progression of peripheral nervous system damage was monitored in the hSOD-1G93A rat model by in vivo MRI. Both T2 relaxation time and DTI parameters were analyzed from the preclinical onset to advanced stage of degeneration.

Methods

Animal model: Female Sprague-Dawley rats, genetically modified (SOD, n=15) to express mutated (G93A) human SOD-1 gene were used together with non transgenic littermates (NTG, n=6). Animals were included in the study starting from 60 days of age. The weight and the motor score of each animal were routinely monitored.

in vivo MRI: Imaging was performed on a 3T scanner (Biospec, Bruker Biospin) with animals under gas anesthesia and constant respiratory control. Serial MRI exams were done along the sciatic nerves with the acquisition of T2 maps with a multi echo sequence (8 echoes, TE=10 to 80ms, TR=4000ms, in plane resolution of 179×287μm^2 and slices of 1.4 mm thickness. DTI data were acquired with a diffusion-weighted echo planar imaging (EPI) sequence with a b value of 1000 s/mm^2 in 6 directions and two reference images without diffusion gradient (TR= 3000ms, TE=31.7ms, spatial resolution = 219×275 μm^2, slice thickness of 1.4mm). From diffusion tensor maps, fractional anisotropy (FA), mean diffusivity (MD), transversal (TD) and longitudinal (LD) diffusivities were measured in along the sciatic nerve. At each MRI points, 2 animals per group (SOD and NTG) were sacrificed and nerves were removed. This imaging protocol was (2-5 times) repeated every 2 weeks till animals reached advance stage (130-200 days old) with clear motor deficits.

Morphopathological: Ultrathin sections (100 to 120 nm thick) were stained with uranyl acetate and lead citrate and examined by electron microscopy. For each nerve, at least 5 non-overlapping images were acquired. The number of myelinated and degenerating nerve fibers and the transverse sectional areas were quantified using ImageJ and fiber density was then calculated and compared to MRI data.

Results

Disease follow-up was successfully performed in both SOD (n=6) and NTG (n=3) groups. While first motor deficits were observed at 110 days old SOD rats, initial T2 changes of the sciatic nerve were found at asymptomatic stage. Compared to age-matched control rats (NTG), the sciatic nerve was progressively enlarged and hyperintense on T2-weighted images (Figure A). Such differences were particularly emphasized on T2 values of the sciatic nerve (Figure B) whereas radial diffusivity (figure C) increased and fractional anisotropy decreased at advanced stage of the disease (>120days old, severe muscle weakness). Morphopathological analysis demonstrated an increased number of degenerating fibers starting from pre-symptomatic stage which correlated with T2 increase. Axonal degeneration, reduction of myelinated nerve fibers and endoneurial oedema were observed starting from sub-clinical stage.

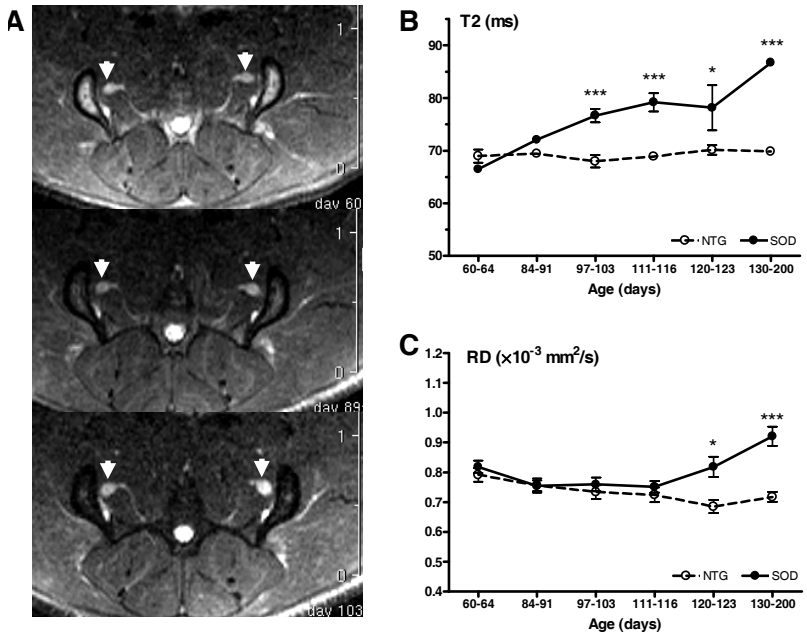

Figure: (A) Cross section of the sciatic nerve, T2 weighted, acquired in a SOD rat at 60, 89 and 103 days of age (top to bottom) showing the progressive hypertrophy and hyperintensity of the nerves. T2 relaxation time (B) and radial diffusivity (C) progression with age measured in sciatic nerves in both groups (NTG and SOD). (* p<0.05, *** p<0.001).

Conclusion

MRI allowed a quantitative in vivo follow-up of disease progression in the hSOD-1G93A rat model. Moreover, T2 relaxation time was highly sensitive to the first signs of nerve alterations as detected at asymptomatic stage. At this early phase, T2 increase correlated to degenerating nerve fibers associated to endoneurial oedema. Standard notions of disease onset based on physical disability should be reconsidered by including in vivo MRI to improve pharmacological studies.