Comparison of Genetic and Environmental Murine Models of Amyotrophic Lateral Sclerosis in Fixed Spinal Cords Using MR Microscopy

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Introduction

Amyotrophic lateral sclerosis (ALS) is a chronic and progressive disease marked by degeneration of the motor neurons that control voluntary muscles, as well as variable involvement of descending motor tracts and other neurons. However, the cellular mechanisms responsible for cell death leading to neurodegeneration are not well understood (1). Genetic and environmental factors, alone and in combination, may be integral to the development of ALS. The familial form of ALS (fALS) is observed in 5-10% of ALS patients, and approximately 20% of fALS patients have mutations in the gene coding for the antioxidant enzyme, superoxide dismutase (SOD) (2). The sporadic form of ALS (sALS) is thought to arise from the action of environmental toxins alone or in synergy with various susceptibility genes. With regard to animal models of ALS, a genetic model that over-expresses human mutant SOD mimics several of the features of fALS (3), while an environmentally induced model of ALS-parkinsonism-dementia complex (ALS-PDC), which presents components that are indistinguishable from classical sALS, can be introduced through the consumption of a neurotoxin found in the cycad seeds local to Guam (4). The focus of the present study is to utilize histology and MR microscopy (MRM) findings from mSOD mice to determine the extent of neurodegeneration in the excised spinal cord. These findings were compared to similar data from a previously published study conducted on cycad-fed mice (5).

Methods

mSOD samples: Hemizygous transgenic mice over-expressing human mutant SOD1 protein (G93A mSOD) and wild type control mice were obtained from the Jackson Laboratory (strain B6SJL-TgN 1Gur) or locally bred from progenitor stock. mSOD mice were monitored closely following the onset of behavioral motor symptoms and sacrificed at a pre-determined end-point based on behavioral markers. mSOD mice (n= 7) and controls (n= 6) were sacrificed and perfused transcardially with phosphate buffered saline (1xPBS) followed by 4% PFA. Lumbar-sacral cords were dissected sand stored in 4% PFA for MRM and histological evaluation.

MR imaging: Following a PBS wash of at least 12 hrs, individual spinal cord samples were immersed in a fluorocarbon and placed within an Alderman-Grant coil having a length/diameter of 3.5/0.5 cm. Using a 17.6-T magnet, T2*-weighted datasets were generated with a 3D gradient-echo sequence (NEX=8; TE/TR=7.5/150 ms). Although parameters varied to accommodate cord length, a resolution of 39x31.25x31.25 µm was achieved for all mSOD samples, typically in ~3 hours.

Image analysis: MRI datasets were examined using Amira 3.1.1 visualization software (TGS, Inc.). Based on automatic signal intensity thresholding and cross-referenced against traditional histological atlases, Anatomical regions (white matter, grey matter, ventral and dorsal horns) of the excised cords were segmented from 80 transverse images, segmented tissues were rendered into 3D reconstructions to provide volumetric data from each of the regions (Fig 1).

Immunohistochemistry: Fixed spinal cords from mSOD (n=5) and control mice (n=5), as well as cords from cycad-fed (n=5) and control mice (n=5), were sectioned in the transverse plane at 50 µm on a cryostat. We used the following primary antibodies: rat polyclonal antibody against glial fibrillary acidic protein (GFAP, 1:200) to stain for reactive astrocytes and a rat polyclonal antibody against F4/80 antigen (Serotec Ltd., 1:1000) to stain for microglia.

Results and Discussion

mSOD mice showed significantly decreased volumes of total grey (-16%) and white matter (-20%) in lumbar spinal cord segments (Table 1). Overall, ventral horn analysis showed a decreased volume (-11%) but did not reach significance. There was no change in dorsal horn volumes seen between groups. Spinal cord sections from mSOD and wild-type mice labeled for GFAP are shown in Fig. 2. GFAP expression was seen in wild-type mice (Fig. 2A) but was significantly more pronounced in mSOD mice and localized primarily to the ventral horn (VH) (Fig. 2B). Spinal cord sections labeled with antibodies against F4/80 demonstrate immunoreactivity against F4/80 both in WT and mSOD mice, but mSOD mice showed significantly increased labeling in the lumbar spinal cord (Fig. 2D). Cycad-fed mice showed significantly increased GFAP labeling compared to controls indicating increased numbers of astrocytes in the spinal cord compared to controls. Cycad-fed mice showed increased OX-6 immunoreactivity compared to wild types, suggesting that reactive microglia are present in cycad-fed mice.

When comparing these mSOD results to previous MR studies of cycad-fed mice (5), 3D MR-based volumetrics revealed significant decreases in the grey matter of both ALS models (Table 1), which corresponds well to immunohistologically-measured losses of motor neurons. Histological findings in both models showed reduced motor neuron counts and increased proliferation of astrocytes and microglia. However, mSOD mice showed significantly decreased volumes in white matter while cycad-fed mice did not. Although grey matter volume measurements indicate similarities between the genetic and toxic models of ALS, white matter volumetrics point to potential differences in the extent of white matter involvement in these two murine models, at least as a function of temporal stage of degeneration. These apparent variations in the extent of degeneration in descending tracts may hint at significant differences in underlying pathological processes. These findings also underscore the utility of MRM in the analysis of progressive neurodegeneration.

Acknowledgments and References

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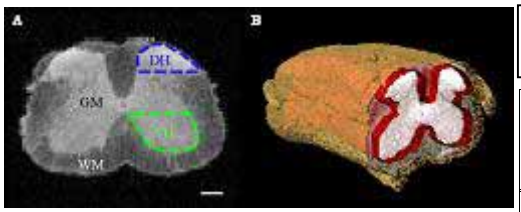

Fig 1. MRM images of mSOD lumbar spinal cords.
A: GM = Grey matter; WM = White matter; Green=ventral horn (VH), Blue=dorsal horn (DH). Scale bar = 500µm.
B: 3D isosurface reconstruction illustrates GM segmentation (red) and WM segmentation (gold).

Table 1: Volumetric data of mSOD & cycad-fed mice. *Cycad-fed results previously published in (5)

Region	Genetic model of ALS (mSOD)	Environmental model of ALS (cycad-fed)*
Total grey matter volume	-16% ($p<0.05$)	-21%
Total white matter volume	-20% ($p<0.05$)	0% ($p<0.01$)
Ventral horn volume	-11%	-20%
Dorsal horn volume	0%	0%

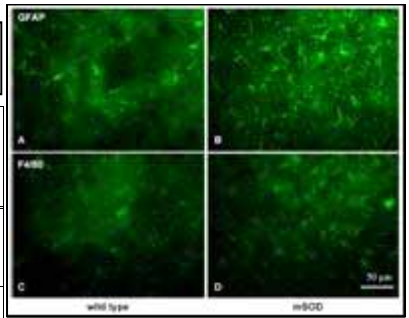

Fig 2. Immunoreactivity of V. Horn
WT (A & C) and mSOD (B&D) VHs were labeled for GFAP (green; A, B), and F4/80, (green; C, D). Scale bar = 50 µm.