Metabolic magnetic resonance progression markers of neurodegeneration in the transgenic G93A-SOD1 mouse model of amyotrophic lateral sclerosis

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Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a selective loss of motor neurons in spinal cord, brainstem, and motor cortex. So far, the underlying mechanisms for the selective cell death of motor neurons are still uncertain [1]. For an independent evaluation of putative therapeutic approaches, sensitive and specific progression markers for the disease are a prerequisites. In the most prevalent ALS animal model, the transgenic G93A-SOD1 mouse [2], progressive neuronal degeneration of brain stem motor nuclei was recently visualized by T_2-weighted MRI [3]. In this study, we report the application of high-field high-resolution ^{1}H-MRS to evaluate metabolic alterations in G93A-SOD1 mice and to define potential metabolic ALS progression markers.

Methods

Tissue from five G93A-SOD1 and five control mice was measured 34, 75, 90, and 120 days postpartum. Time points were chosen to reflect disease stages either long before the onset of clinical symptoms (34 days), right before the disease onset (75 days), at the average disease onset (90 days), and at the terminal stage of ALS (120 days). For high-resolution ^{1}H-MRS, the central nervous system (CNS) of the mice was divided into brain stem, cerebellum, cortex, and spinal cord. Metabolites were extracted with perchloric acid and neutralized samples were lyophilized over night. The dried powder was dissolved in 600 µl deuterium oxide (D_2O) and ^{1}H-MR spectra (Fig. 1, right) were recorded on a Bruker DRX 600 spectrometer (Bruker Biospin, Karlsruhe, Germany) operating at 14.1 Tesla (600 MHz proton resonance frequency). For each spectrum between 64 and 512 scans were accumulated depending on the wet weight of the investigated sample.

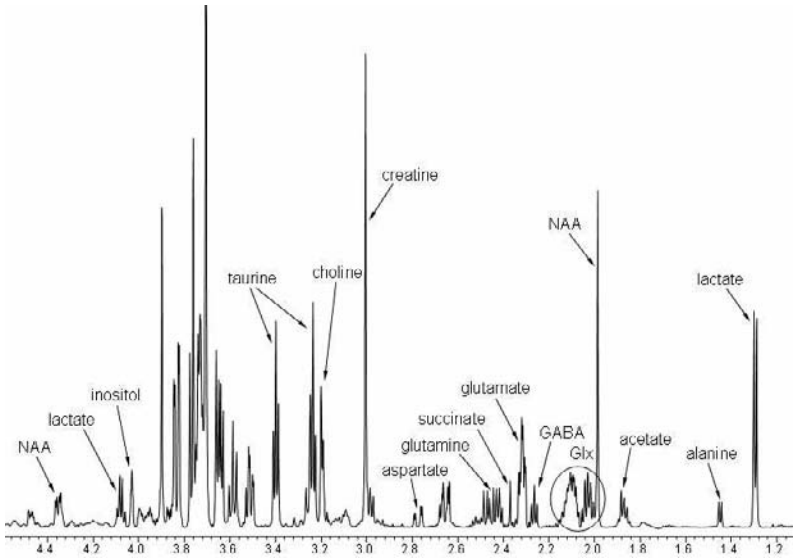

Fig. 1: High-resolution ^{1}H-MR spectrum of G93A-SOD1 mouse cortex.

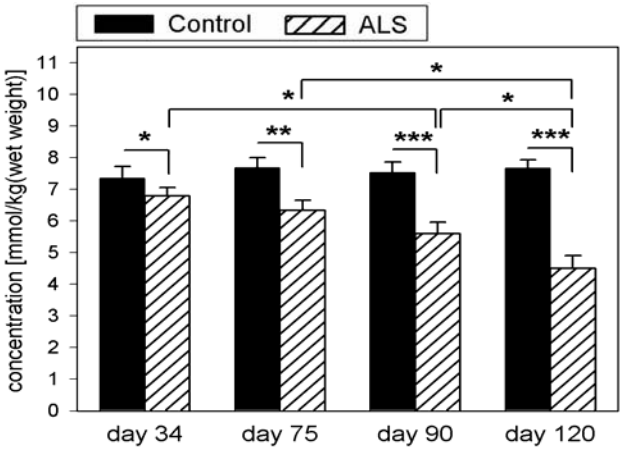

Fig 2: Concentrations of NAA in the spinal cord

Results

In spinal cord and brain stem, age-dependent concentration effects were exclusively found in G93A-SOD1 mice with either loss of aspartate, GABA, glutamate, glutamine, inositol, and NAA (NAA shown in Fig. 2, left) or increase of succinate and lactate. Concentration changes of GABA, glutamine and NAA occurred at very early time points. The first significant differences between G93A-SOD1 and control mice were found for NAA 34 days postpartum, while GABA and glutamine start to decrease 75 days postpartum. In some cases even individual time points can be separated by means of NAA (shown in Fig. 2), GABA, and glutamine concentrations. In the cortex and cerebellum, only the concentration of taurine decreased age-dependent in both animal groups. In addition, in spinal cord and brain stem we observed significant correlations between the concentrations of NAA and lactate as well as between the mitochondrial citric acid cycle intermediate succinate and lactate.

Conclusions

In summary, high-resolution ^{1}H-MRS identified multiple age-dependent metabolic alterations mainly in spinal cord and brain stem of G93A-SOD1 mice. Hereby, neurochemical changes can be detected before the onset of clinical signs and even before significant neuronal cell loss occurs. Hence, functional metabolic properties of neurons can be characterized and prognostic markers for neurodegeneration in ALS and for the evaluation of novel therapies may be obtained under *in vivo* conditions.

References

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