Reduced T2 reveal therapeutic effect of the antioxidant Vitamin E in the G93A-SOD1 Mouse Model of ALS

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Introduction

Amyotrophic lateral sclerosis (ALS) is a human neurodegenerative disorder that progressively leads to paralysis and death due to the loss of motor neurons in brainstem, motor cortex and spinal cord. About 5-10% of ALS cases are inherited (familial), the majority of cases has no genetic component (sporadic). In familial ALS 15-20% of all cases are associated with mutations in the gene coding for the Cu/Zn superoxide dismutase 1 (SOD1). However, the pathological mechanisms that cause a selective motor neuron degeneration still remain unclear. *In vivo* Magnetic resonance imaging (MRI) provides an excellent tool to study disease progression in the G93A-SOD1 mouse model of ALS [1, 2], even before first clinical symptoms of the disease are present [3]. The T_2 enhancement in the brainstem nuclei correlated with the development of dendritic vacuoles in motor neurons, which is a characteristic sign of neurodegeneration. However, the underlying mechanisms for the vacuolisation are still unclear but seem to be induced by the mutant SOD1. In our study, we investigated the effects of a preclinical antioxidant treatment with vitamin E on T_2 relaxation time in order to evaluate if T_2 is a sensitive non-invasive biomarker for the assessment of therapeutic approaches in the G93A-SOD1 mouse model of ALS.

Animal Handling and MR Methods

At day 30 after birth transgenic and wildtype mice were separated and housed in individual cages with a temperature- and humidity-controlled environment. At that time, Vitamin E treatment was started by feeding the animals with Vitamin E-enriched food (450 mg/kg, Evion 100, Merck, Darmstadt). MRI was applied in the brainstem of vitamin E-treated G93A-SOD1 mice (n = 15), untreated transgenic G93A-SOD1 mice (n = 12), Vitamin E-treated wild-type mice (n = 11) and untreated wild-type mice (n = 7). MRI data were acquired on a Biospec 47/40 scanner (Bruker BioSpin, Ettlingen, Germany) at 4.7 Tesla. Mice were anaesthetized through continuous inhalation of 1.2-1.5% isoflurane (in 70:30 N_2O:O_2) and fixed in a stereotactic head holder. For anatomical orientation, five contiguous T_2-weighted sagittal slices were acquired using a RARE sequence [4]. Imaging parameters were: TR 2100 ms, TE 20 ms, TE_{eff} 81 ms, slice thickness 600 µm, interslice distance 200 µm, FOV 30 mm x 30 mm, matrix size 256 x 256, RARE factor 8, 16 averages. With the same geometrical orientation and slice parameters as before, maps of the spin–spin relaxation time (T_2) were generated using a multi slice multi echo (MSME) sequence with the following parameters: TR 4000 ms, 16 echo images, TE equally spaced from 10 to 161 ms, 5 slices, slice thickness 600 µm, FOV 28.1 mm x 25.6 mm, matrix size 256 x 128, 2 averages. MR experiments were performed every 20 days between day 40 and 120 after birth.

Results

In the motor brainstem of vitamin E-treated and untreated G93A-SOD1 mice the hypoglossal and facial nucleus appeared as hyperintensities around day 80, while the trigeminal nucleus appeared at day 100 (Fig. 1). Both vitamin E-treated and untreated G93A-SOD1 mice revealed a significant increase of transversal relaxation time in the facial, trigeminal, hypoglossal nucleus starting around day 80 after birth, compared to wild-type controls, before the first clinical symptoms appear around day 90 as hind limb muscle paralysis (Fig. 2). However, an analysis of the age-dependent T_2 increase in treated and untreated transgenic G93A-SOD1 mice revealed a statistically significant difference in the progression of the disease in favour of a preclinical intervention with vitamin E (Fig. 3).

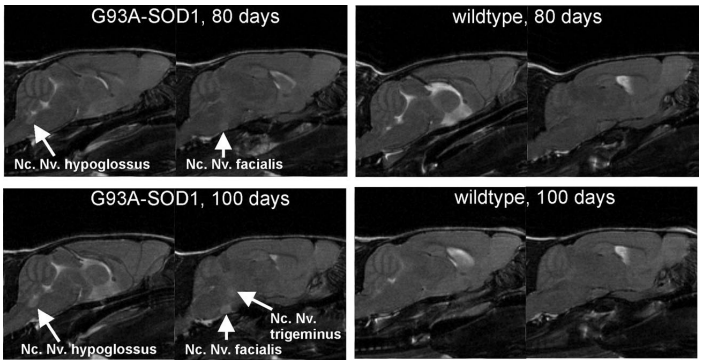

Fig.1: *Sagittal T_2-weighted MR sections of G93A-SOD1 mice and control mice.*

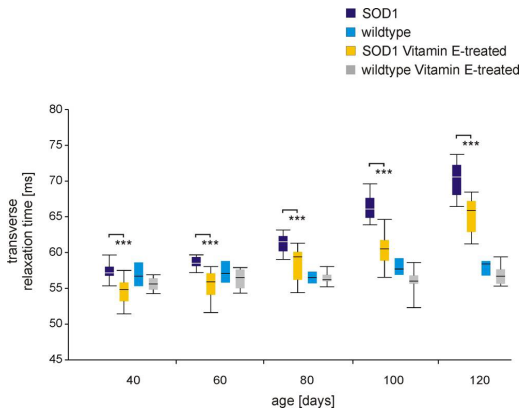

Fig.2 *Age-dependent change of T_2 in nucleus Nc. XII of G93A-SOD1and wild-type mice with or without Vitamin E treatment*

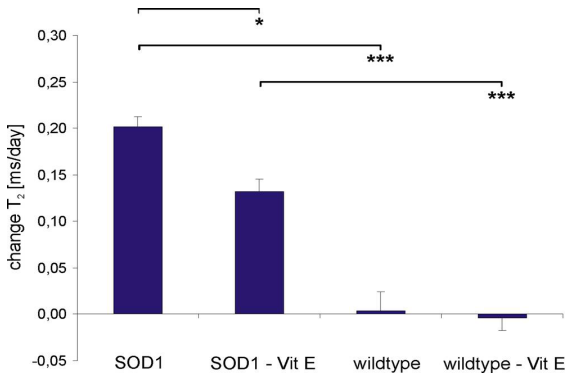

Fig. 3: *Vitamin E treatment leads to a significantly reduced increase in T_2 in nucleus Nc. XII of G93A-SOD1 mice compared to controls*

Conclusion

Overall, a clear benefit of Vitamin E treatment for a significantly reduced ALS progression was shown in the presented study by means of MRI and validated by histology. In more general terms, it was shown that the measurement of the relaxation time T_2 enables for a longitudinal non-invasive evaluation of therapeutic approaches in the G93A-SOD1 mouse model of ALS.

References

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