Methods

mSOD1, Als2², and wild type mice were given a pellet of Purina mouse chow containing 42 mg SG/kg of body weight every evening to a total pellet weight of 1.5 g/mouse. The following morning, cages were replenished with 1.2 normal chow pellets, maintaining a daily food intake of 2.5 g. Control-fed mSOD1, Als2-deficient and wild type mice received 2.5g of Purina mouse chow pellets without SG. A battery of behavioral tests were performed on mice to assess general motor function, motor neuron dysfunction, neuromuscular strength, and muscle coordination.

Motor neurons at the lumbar cord level L1 were stained with antibodies against Choline acetyltransferase and detected with FITC secondary antibody. Astrocytosis was detected using GFAP as a marker for activated astrocytes. Quantification was performed counting GABA and GFAP-positive cells, respectively.

Introduction

Dominant mutations in the Cu²⁺/Zn²⁺ superoxide dismutase (mSOD1) gene are the most frequent cause of familial amyotrophic lateral sclerosis (ALS), a progressive and fatal disease characterized by the degeneration of motor neurons in the cortex, brainstem, and spinal cord. One form of mSOD1-mediated ALS occurs when arginine is substituted for glycine at the 37th position of the gene (G37R)1. The etiology of ALS remains largely unknown, but current evidence suggests that motor neuron injury is non-cell-autonomous and involves damage caused by mSOD1 proteins within glia of the central nervous system. The mSOD1 model of ALS is an example of neurodegenerative disease with a primary etiological factor that is genetic.

The recently identified gene ALS2 encodes the protein alsin. Alsin has been shown to have guanine nucleotide exchange factor (GEF) activity for Rab5- and Rho-GTPases, and has been implied to play a role in the development and regulation of neurite and axonal outgrowth2. In humans, loss-of-function mutations in this gene have been linked to autosomal recessive forms of motor neuron disease (MND). Yet, Als2-deficient mouse models have failed to reproduce the classical hallmark symptoms of MND or ALS2. Other forms of ALS, such as ALS-PDC syndrome, have been suggested to be multifactorial diseases caused by gene-environment interactions.

The neurodegenerative disease amyotrophic lateral-sclerosis-parkinsonism dementia complex (ALS-PDC) is characterized by a range of behavioural and neuropathological attributes shared with several neurodegenerative diseases, including ALS, Alzheimer’s Disease (AD), and Parkinson’s Disease (PD). Recent work by our group has shown that sterol glucosides (SG) are neurotoxic to motor neurons and induces an ALS-PDC phenotype in an in vivo model of disease3. Such work provides evidence that neurotoxin can be the sole necessary and sufficient condition for neurodegenerative disease.

We have hypothesized that exposure to SG in combination with a genetic predisposition will act as combined stressors in the events leading to motor neuron degeneration in the central nervous system (CNS) to produce a more pronounced phenotype than either stressor introduced alone.

Motor Skills

Motor neurons in the ventral horn of G37R mSOD1 lumbar cord (L1). Preliminary results for a quantitative analysis showed no significant differences between PGRN injected and sham injected mice in motor neuron numbers. Mutant mice consistently show fewer motor neurons than their wild type littermates. WT PGRN (N=1), G37R Control and G37R PGRN (N=2). This cohort of mice was sacrificed before onset of any classical ALS symptoms including muscle weakness or slowness in movement. Histology shows the presence of CNS pathology and behavior deficits before ALS symptom onset. However, PGRN injection at five months of age where pathology was expected to be well underway was not able to rescue the loss of motor neurons.

Motor neurons and reactive astrocytes in the ventral horn of Als2 KO lumbar cord (L1). Mice exposed to BSSG show a trend towards decreased motor neurons in the anterior horn. Quantitative analysis did not reveal significant differences between genotypes. Als2-deficient animals exposed to BSSG showed a subtle trend towards increased astrocytosis compared to controls. Scale bars: 20 µm.

References


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