Disease progression with early toxin-induced neuropathology in the ageing mutant SOD1 mouse model of ALS
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RESULTS

BACKGROUND

Familial adult onset amyotrophic lateral sclerosis (FALS) is the result of injuries to the central nervous system that lead to progressively debilitating and irreversible motor deficits. The loss of motor neurons results from a poorly understood, multifactorial neurodegenerative process. Mutations in the gene encoding superoxide dismutase 1 (SOD1) are one cause of FALS. In contrast, sporadic ALS occurs with a vastly greater incidence occurs from unknown etiologies. Among suspected causes are various environmental toxins. An early report of environmental causes of neurodegenerative disease, including a form of ALS, pointed to a long latency neurotoxin in cyanid seeds amongst the Chomorro people of Guam. Both washed cyanid as well as isolated water insoluble steryl glucosides (5G) similar to that found in cyanid seeds reproduced an ALS-PDC phenotype in an in vivo model.

METHODS

To determine whether environmental agents such as those from cyanid accelerate disease onset in an otherwise genetic late-onset condition, we combined two in vivo models of ALS, testing dietary SGs for their potential synergistic properties in combination with genetic predisposition to adult onset ALS in the G37R mouse. Male and female mice were given 42 mg of SG per kg of body weight in their daily diet. A cohort of these animals harboured the G37R SOD1 mutation for genetic predisposition to ALS, while wild type littermates served as controls. Comparisons of motor dysfunction between groups were made by using unpaired two-tailed Student’s t tests (95% confidence intervals) using PRISM 3.02 software (GraphPad, San Diego) and repeated measures two-way ANOVA. Assessment of motor neuron loss, gliosis, and end plate preservation were analyzed using unpaired two-tailed Student’s t test and results are presented as means and SDs.

RESULTS

Morphological changes are evident in Nissl-stained motor neurons in the ventral horn of the lumbar cord. (a) Control-fed wild types (i) 5G-fed wild types (c) Control-fed G37R mice (g) 5G-fed G37R mice. Neuron density in the ventral horn of the transgenic mouse (e) is reduced compared with (a), (b,d,e) Representative higher power micrographs of the ventral horn in the corresponding groups. Morphologically abnormal cells are evident and shrunken (d), chromatolytic (f), and ghost cell appearances (h) are observed among apparently healthy cells (b), although not limited to these corresponding experimental groups. Scale bars: 60μm (upper panels); 30μm (lower panels).

Changes in cell diameters of different cell morphologies between groups are illustrated by a cumulative distribution functions. Data are broken down further into sexes. There was a significant leftward shift of soma diameters of healthy neurons in all experimental groups of female (p<0.001) and male (p<0.0001) mice, demonstrating a reduction in soma size compared to wild type controls. A significant reduction in soma size was also observed for chromatolytic neurons of 5G-fed G37R mice of both sexes (p<0.05). Motor neurons with shrunken soma did not show any changes in cell size across groups.

Green fluorescent GFAP-positive cell distribution and number in lumbar spinal sections. (a,b) Control-fed wild types; (d,e) 5G-fed wild types; (g) control-fed G37R, (h) 5G-fed G37R mice. Wild type cords show astroglia clusters in left and right ventrolateral sides (h,e); cells have a small cell body with long thin processes (j). GFAP grey matter shows uniform astroglial proliferation (g) with significantly increased numbers compared to controls. Control-fed G37R mice showed activated astrocyte morphology (h,i) or cells with increased branching of thin processes (h,j). Treatment with 5G in mutant animals did not change the intensity of astroglia (compare h,j with m), but all astrocytes assumed the activated morphology (n). Graph presented as percent of GFAP labelling compared to wild type controls ± S.E.M. Both sexes showed significant main effects of genotype on GFAP proliferation and a significant diet × genotype interaction. Scale bars: 300μm (left); 60μm (middle); 30μm (right).

CONCLUSIONS/ACKNOWLEDGEMENTS

The current results showed that dietary exposure to SGs alone was sufficient to produce a disease phenotype, and in combination with the G37R genotypes produced additive, not synergistic, degenerative effects.

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