Determination of disease progression with early exposure to steryl glucosides in the mSODG93A mouse model of amyotrophic lateral sclerosis

Grace Lee, Oliver T.H. Lam, and Christopher A. Shaw

University of British Columbia, Vancouver, BC, Canada

INTRODUCTION

Missense mutations in the gene encoding superoxide dismutase 1 (SOD1) are reported to be one cause of adult-onset familial amyotrophic lateral sclerosis (FALS). FALS causes injuries to the motor system that lead to progressively debilitating and irreversible motor deficits, which result from a poorly understood, multifactorial neurodegenerative process. FALS makes up only 10% of all ALS cases, while sporadic cases of unknown cause(s) occur with vastly greater incidence. The earliest report of environmental causes of neurodegenerative disease pointed to a long latency neurotoxin in cacao seeds responsible for the high incidence of ALS-parkinsonism dementia complex among the Chomorro people of Guam during the 1950s and 1960s. We have previously shown that an ALS-PDC phenotype can be demonstrated by feeding mice cacao seed flour as well as several neurotoxic steryl glucosides isolated from this flour. To determine whether environmental toxins accelerate disease onset, we studied the influence of dietary exposure of β-sitosterol-β-D-glucoside (BSSG) in mice over expressing the human SOD1 G37R breeders for this project in a timely manner. This work has been made possible by grants from NIH, Pacific Alzheimer Research Foundation, and the Scottish Rite Charitable Foundation of Canada.

CLINICAL PHENOTYPE

Male mice exhibit deficits in grip endurance and rotarod performance when exposed to SG. Wild type male mice show a decline in rotarod performance after continued exposure to SG (C). Wild type and mutant SOD1 males show deficits in grip endurance with SG exposure (B). By repeated measures ANOVA analysis, no significant phenotype was observed after SG exposure in the leg extension scores (A) or measures of body weight (D).

CONCLUSION & ACKNOWLEDGEMENTS

These results indicate that a genetic predisposition to a severe ALS phenotype yields a CNS that has heightened sensitivity to environmental insults that contribute to targeted motor neuron loss, and point to a dynamic interplay of genes and environment in the aetiology of ALS. The environmental mouse model of ALS-PDC developed in this study may be used to further elucidate the role of gene-environment dynamics and its application in the aetiology of other neurodegenerative diseases. We would like to thank Dr. Neil Cashman and the Ludwig Institute for Cancer Research for generously providing sufficient male mSODG93A breeders for this project in a timely manner. This work has been made possible by grants from NIH, Pacific Alzheimer Research Foundation, and the Scottish Rite Charitable Foundation of Canada.

METHODS

Transgenic mSOD1G93A line 29 and wild-type littermates were fed 42 mg kg body weight as part of their daily diet commencing at 3 weeks of age. Indications of motor neuron dysfunction were measured with hind limb clasping reflex scores. Gait abnormalities and aberrant open field activity were monitored for a subgroup of parameters. Mice were euthanized and spinal cord and brain tissues were processed to be frozen for cryosectioning and lumbar cord sections were immunostained to visualize motor neurons, astrocytes, and microglia.

Top panels show distribution of GFAP-positive cells in lumbar cord. Bottom panels show increasing microgliosis in the ventral lumbar horn with introduction of neural insult. P values and percent decrease are compared to WT control except where indicated. ChAT-positive cell counts show that the neurotoxin exerts more deleterious effects in the male wild type lumbar cord where motor neuron loss is greater for WT SG. SOD1 mutation exerts such a predominant deleterious effect that the effect of the neurotoxin seems relatively insignificant in comparison.

Top panels show distribution of GFAP-positive cells in lumbar cord. Bottom panels show increasing astroglial activity in the ventral lumbar horns with introduction of neural insult. P values and percent decrease are compared to WT control. The neurotoxin increases astrogliosis when introduced to wild types. SOD G93A mutation exerts a more deleterious effect in markedly increasing astrocytic proliferation. Astrocytes show thickened cell bodies and processes. The neurotoxic effects seemed relatively insignificant compared to the effect of the SOD1 mutation in both sexes.

Mass ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001, compared to control-fed wild type mice.

Gait dynamics in control chow-fed and SG-fed (42 mg/kg body weight cumulative dose) mice walking on a treadmill belt at a speed of 25 cm/s.

Neurotoxin increases microgliosis when introduced to wild types. Microglia exhibited the resting type morphology: small cells with a round nucleus and a narrow cytoplasmic process. In contrast, dietary exposure to SG did not affect female mice in the tests performed. Wild type female mice performed better than SOD1 G93A animals in rotarod performance (F) and tests for grip endurance (F) as expected.

Leg extension deficits were observed in SOD1G93A animals after 12 trials of testing (G). Wild type and mutant SOD1 males show deficits in grip endurance with SG exposure (B). By repeated measures ANOVA analysis, no significant phenotype was observed after SG exposure in the leg extension scores (A) or measures of body weight (D).