Determiniation and progression of toxin-induced neuropathology in the aging mutant SOD1 mouse central nervous system

Grace Lee and Christopher A. Shaw
University of British Columbia, Vancouver, BC, Canada

**BACKGROUND**

Adult onset amyotrophic lateral sclerosis (ALS) poses progressive and irreversible function deficits to the central nervous system due to loss of motor neurons, caused by some poorly characterized, multifactorial etiology. Research focused on sporadic ALS describes the potential causes to be of environmental origin. The discovery of endemic ALS in Guam during the 1950s and the co-occurrence of parkinsonism and dementia led to searches for an environmental cause. The earliest etiologic studies of this neurodegenerative disease cluster pointed to a long latency neurotoxin in cycad seeds now known as seryl glucosides (SG). Hypothetical spinal motor neurons are vulnerable to cycad toxicity in the ageing mouse spinal cord (single stressor), particularly in transgenic mice with a genetic predisposition to ALS (combined stressors). The CNS response to cycad toxins originates in the spinal cord.

**METHODS**

To determine whether a genetic predisposition to ALS could be exacerbated by a toxin that is known to produce a similar phenotype, I combined genetic and environmental models of ALS and tested SG for its potential synergistic properties in combination with the genetic defect. Twenty male mice and an equal number of female mice were given 42 mg of SG per kg of body weight in their daily diet. Half of these animals of each sex harboured the G37R SOD1 mutation for genetic predisposition to ALS, while wild type littermates served as controls. Similarly, an equal number of transgenic and wild type mice of each sex were given 42 mg kg control food pellets. 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, glosis, and end plate innervation were analysed using unpaired two-tailed Student’s t-test and results are presented as means and SDs.

**RESULTS**

(A-D) Macroscopic view of representative lumbar spinal cords of mice in the groups as indicated. Based region defined as ventral horn for motor neuron counting. (E) A significant effect of genotype on the loss of motor neurons was revealed in both sexes by Student’s t-test. Data are means ± SD. ***P < 0.0001 versus control.

(F-G) Light microscopic representative images from the ventral horn of mice stained with Cresyl Violet in the groups as indicated. Motor neurons in each micrograph were classified as apparently normal, chromatolytic, or shrunken. Motor neurons were counted under each classification and plotted against their measured diameters. Size histograms were plotted per each animal group (J-M) and compared to WT controls.

(M) Number of motor neurons classified by morphology and cell diameter per animal group. Both sexes show a shift in size distribution towards smaller cell diameters in experimental groups compared to control mice in both sexes. Student’s t test revealed significant denervation of endplates in both sexes, SG-fed females showing the most significant differences (p < 0.0001 versus control). G37R males were significantly hypoactive compared with WT males. Unpaired Student’s t test revealed a significant effect of genotype in measures of total distance traveled (p < 0.05). Female mice showed a no difference in time spent in the perimeter versus central area of the arena. G37R males fed SG spent significantly less time in the center zone of the arena (p < 0.05).

**ACKNOWLEDGEMENTS**

Thanks to CIHR and the CSHRF organizers for this amazing opportunity. Thanks to Dr. Neil Cashman and the Ludwig Institute for generously providing male G37R breeders. We are grateful for grants from NIH, Pacific Alzheimer Research Foundation, and the Scottish Rite Charitable Foundation of Canada. GL would like to acknowledge husband Arthur Kam for his unending support.

**CONCLUSIONS**

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 motor dysfunction, and point to a dynamic interplay of genes and environment in the etiology of ALS. The environmental agent studied here has cytotoxic effects approaching significance, contributes to disease progression in ALS, and suggest an additive effect of dietary neurotoxin in combination with genetic mutations leading to familial 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 etiology of other neurodegenerative diseases.